Apolipoprotein A4-1/2 polymorphism and response of serum lipids to dietary cholesterol in humans

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Abstract The response of serum lipids to dietary changes is to some extent an innate characteristic. One candidate genetic factor that may affect the response of serum lipids to a change in cholesterol intake is variation in the apolipoprotein A4 gene, known as the APOA4-1/2 or apoA-IVGln360His polymorphism. However, previous studies showed inconsistent results. We therefore fed 10 men and 23 women with the APOA4-1/1 genotype and 4 men and 13 women with the APOA4-1/2 or -2/2 genotype (carriers of the APOA4-2 allele) two diets high in saturated fat, one containing cholesterol at 12.4 mg/MJ, 136.4 mg/day, and one containing cholesterol at 86.2 mg/MJ, 948.2 mg/day. Each diet was supplied for 29 days in crossover design. The mean response of serum low density lipoprotein cholesterol was 0.44 mmol/l (17 mg/dl) in both subjects with the APOA4-1/1 genotype and in subjects with the APOA4-2 allele [95% confidence interval of difference in response, −0.20 to 0.19 mmol/l (−8 to 7 mg/dl)]. The mean response of high density lipoprotein cholesterol was also similar, 0.10 mmol/l (4 mg/dl), in the two APOA-4 genotype groups [95% confidence interval of difference in response, −0.07 to 0.08 mmol/l (−3 to 3 mg/dl)]. Thus, the APOA4-1/2 polymorphism did not affect the response of serum lipids to a change in the intake of cholesterol in this group of healthy Dutch subjects who consumed a background diet high in saturated fat. Knowledge of the APOA4-1/2 polymorphism is probably not a generally applicable tool for the identification of subjects who respond to a change in cholesterol intake.—Weggemans, R. M., P. L. Zock, S. Meyboom, H. Funke, and M. B. Katan. **Apolipoprotein A4-1/2 polymorphism and response of serum lipids to dietary cholesterol in humans.** ***J. Lipid Res.*** **2000.** 41: **1623–1628.**

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The response of serum lipids to dietary cholesterol varies between subjects. In some subjects, the response of serum lipids to an increased cholesterol intake is considerable, whereas in others the response is small. The response to dietary cholesterol is to some extent reproducible within a subject and is in part an innate characteristic of a subject (1). There are a large number of candidate genetic factors that may affect the response (2). Identification of these genetic factors may contribute to the development of new tests to predict whether a subject with high serum lipid levels will benefit from a diet low in cholesterol. This may contribute to a more efficient treatment of subjects with high serum lipid levels. In addition, knowledge of genetic factors that determine the response of serum lipids to diet will help to gain insight into the mechanisms by which diet affects serum lipid levels.

One of the candidate genetic factors that may affect the response of serum lipids is the apolipoprotein A4 (APOA4) gene, which encodes the apoA-IV protein. ApoA-IV is synthesized in the intestine (3). While the precise function of apoA-IV is still unknown, some studies suggest that it plays a role in the absorption of dietary fat (4) and in the metabolism of high density lipoprotein (HDL)-cholesterol and triglyceride-rich particles. In vitro studies showed that apoA-IV activates lecithin:cholesterol acyltransferase (5) and regulates the activity of cholesteryl ester transfer protein (6) and lipoprotein lipase (7). One polymorphism in the APOA4 gene, the APOA4-1/2 polymorphism, is caused by a G-to-T substitution in exon 3 of the gene, which causes the glutamine-to-histidine substitution at position 360 in the apoA-IV protein (8). The apoA-IV-2 isoform has more α-helical structure, is more stable in solutions, and is more hydrofobic than the apoA-IV-1 isoform. These distinctive features are associated with a higher affinity for phospholipid surfaces and increased catalytic efficiency of the lecithin:cholesterol acyltransferase activation in vitro (9, 10). In addition, carriers of the apoA-IV-2 isoform have lower activity of plasma cholesteryl ester transport protein, higher apoA-IV concentrations (11), and a slower apoA-IV catabolic rate in vivo (12).

Abbreviations: apoA-IV, apolipoprotein A-IV; APOA4, apolipoprotein A-IV-encoding gene; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein.

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Studies of the effect of the APOA4-1/2 polymorphism on the response of serum lipids to diet are not consistent. Some studies showed that the APOA4-2 allele attenuates the response of low density lipoprotein (LDL)-cholesterol to dietary cholesterol (13) or dietary cholesterol plus fat (14), whereas other studies did not show a difference between subjects with the APOA4-1/1 genotype and those with the APOA4-2 allele in terms of response of LDL-cholesterol to dietary cholesterol plus fat (15, 16) or to dietary fat (17). These results may indicate that the APOA4-1/2 polymorphism affects the response of serum LDL-cholesterol to dietary cholesterol, but not to dietary fat.

We therefore tested the effect of the APOA4-1/2 polymorphism on the response of serum LDL-cholesterol to dietary cholesterol in a controlled experiment.

MATERIALS AND METHODS

Subjects

The Ethics Committee of the Division of Human Nutrition and Epidemiology (Wageningen University, Wageningen, The Netherlands) approved the study protocol. We recruited 200 subjects through advertisements in local newspapers and university and public buildings. We explained the aims and protocol of the study to the subjects. All subjects gave their written informed consent. We screened the subjects, mostly students living in or near the city of Wageningen, for the APOA4-1/2 polymorphism and identified 24 carriers of the APOA4-2 allele. We selected these 24 carriers and 47 subjects with the APOA4-1/1 genotype for a medical screening. The medical screening consisted of a medical questionnaire, hemocytometry, and the assessment of triglycerides and total cholesterol in serum and of protein and glucose in urine after a 12-h fast. We excluded one subject with serum triglyceride levels exceeding 3.0 mmol/l, two subjects with disease of the gastroinstestinal tract, one subject with glucosuria, and two subjects with proteinuria. All other subjects were apparently healthy, as indicated by the medical questionnaire. None of them had anemia, glucosuria, or proteinuria and none were taking medications known to affect blood lipids. During the period between the medical screening and the beginning of the dietary trial, 13 subjects withdrew. Nineteen carriers of the APOA4-2 allele and 33 subjects with the APOA4-1/1 genotype started the dietary trial. Two carriers of the APOA4-2 allele dropped out during the dietary trial, one for personal reasons and one because of appendicitis. Seventeen carriers of the APOA4-2 allele, 16 Caucasians and one Hispanic, and 33 subjects homozygous for the APOA4-1 allele, 32 Caucasians and 1 Hispanic, completed the dietary trial (**Table 1**).

The two genotype groups had similar baseline characteristics, except that the APOE4 allele and APOA4-347T allele were more common in subjects with the APOA4-1/1 genotype than in those with the APOA4-2 allele (**Table 2**).

All subjects who completed the dietary trial received a financial reward.

Design

The dietary trial was designed to detect a significant difference ($P < 0.05$) in response of LDL-cholesterol between subjects with the APOA4-1/1 genotype and subjects with the APOA4-2 allele with a power of 80% if the real population effect exceeded 0.27 mmol/l (10 mg/dl). This power calculation was based on a within-subject standard deviation of 0.27 mmol/l (10 mg/dl). In other studies at our laboratory, the within-subject standard deviation was 0.35 mmol/l (13 mg/dl) (18). We expected that the four blood collections per period, instead of two, would decrease the within-subject standard deviation by about 0.08 mmol/l (3 mg/dl) (19).

The dietary trial consisted of two periods of 29 days, during which each subject consumed a diet low in cholesterol and a diet high in cholesterol in crossover design. We included a 6-day washout period between the two periods (**Fig. 1**).

One group of 26 subjects (18 APOA4-1/1, 8 APOA4-1/2 or -2 /2; 7 men, 19 women) first received a diet low in cholesterol and then a diet high in cholesterol; the other group of 24 subjects (15 APOA4-1/1, 9 APOA4-1/2 or -2/2; 7 men, 17 women) received the diets in reverse order. In this way, bias due to the order in which the diets were consumed or to drift of variables over time was eliminated (20). All subjects participated simultaneously. None of the subjects and staff, except for one investigator (R.M.W.), were aware of the APOA4 and APOE genotypes.

TABLE 1. Selection of subjects with the APOA4-1/1 genotype and carriers of the APOA4-2 allele

	APOA4-1/1	APOA4-1/2 or 2/2
Subjects recruited	176	24
Excluded after genetic screening	129	0
Excluded after medical screening[a]	4	2
Withdrawal before the trial	10	3
Subjects entering the trial	33	19
Drop out during the trial	0	2
Subjects finishing the trial	33	17

[a] The medical screening consisted of a medical questionnaire, hemocytometry, and the assessment of triglycerides and total cholesterol in serum and of protein and glucose in urine after a 12-h fast.

TABLE 2. Baseline characteristics of subjects with the APOA4-1/1 genotype and carriers of the APOA4-2 allele

	APOA4-1/1	APOA4-1/2 or 2/2[a]
Men/women (n)	10/23	4/13
Age (years)	24 ± 9	24 ± 13
Body mass index (kg/m^2)	23 ± 2	22 ± 3
Total cholesterol (mmol/l)[b]	4.8 ± 0.9	4.6 ± 0.8
Triglycerides (mmol/l)[c]	1.1 ± 0.4	1.1 ± 0.4
Smokers (n)	2	3
Users of oral contraceptives (n of women)	9	6
APOE genotype (n)		
E2/2	1	0
E3/2	3	4
E3/3	22	12
E4/2	1	1
E4/3	6	0
APOA4-347 genotype (n)		
A/A	18	13
A/T	12	4
T/T	3	0

[a] The subject with the APOA4-2/2 genotype was a man with the APOA4-347A/T genotype and the APOE2/4 genotype.

[b] To convert serum lipid values from mmol/l to mg/dl multiply mmol/l by 38.67.

[c] To convert serum triglyceride values from mmol/l to mg/dl multiply mmol/l by 88.54.

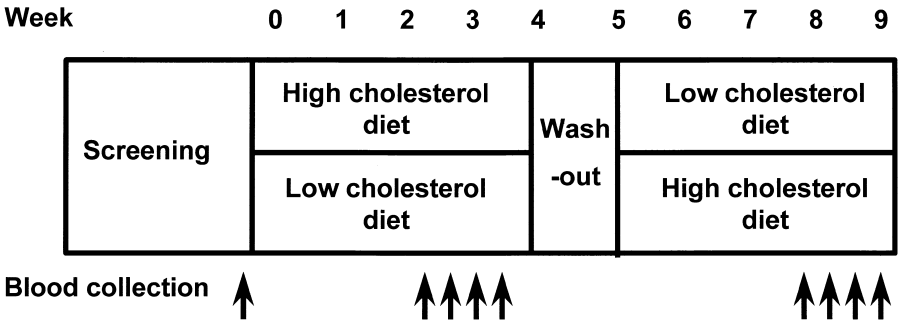

Fig. 1. Design of the controlled dietary trial.

Diets

Before the trial, the habitual energy intake of the subjects was estimated by a food-frequency questionnaire (21, 22). The study diets were formulated at 18 levels of energy intake, ranging from 7 to 24 MJ/day, so that each subject received a diet that met his or her energy needs. Body weights were recorded twice per week and, if necessary, energy intake was adjusted to maintain a stable weight.

The diets consisted of conventional foods and 29 different menus were provided over the course of each period. The nutrient composition of the low and high cholesterol diet was similar, except for dietary cholesterol (**Table 3**).

Dietary cholesterol was added in the form of eggs and egg yolk powder. The egg yolk powder was used for baking bread and preparing salad dressings and deserts. Egg white powder and groundnut oil were used in the diet low in cholesterol to adjust for the added fat and protein from eggs and egg yolk powder in the high-cholesterol diet. Because the response of serum lipids to dietary cholesterol may be enhanced by a background diet high in saturated fat (23–25), both diets were high in saturated fat.

All food items were weighed or counted out for each subject. On weekdays at noon, hot meals were served and consumed in the dining room for metabolic studies at the Division of Human Nutrition and Epidemiology. All other food was supplied daily as a package. Food for the weekend and guidelines for its preparation were provided on Fridays. Approximately 90% of the energy intake was from supplied foods, and the remaining 10% was from foods chosen by the subjects from a list of "free-choice" food items without cholesterol or fat.

Subjects were urged not to change their selection of the free-choice food items throughout the study and not to change their smoking habits or physical activities. The participants kept diaries in which they recorded their daily selection of free-choice food items, any sign of illness, medication used, phase of the menstrual cycle, and any deviations from their diets and the protocol. According to these diaries, adherence to the diets and protocol was excellent.

Duplicate portions of each study diet were collected every day for an imaginary participant with a daily energy intake of 11 MJ, stored at −20°C and pooled and analyzed after the study. The energy and nutrient content of each subject's selection of the free-choice food items were calculated and combined with the analyzed values of the duplicate portions.

TABLE 3. Composition of the low cholesterol and high cholesterol diets

	Low Cholesterol Diet	High Cholesterol Diet
Energy (MJ/day)	11	11
Protein (% of energy)	14.9	15.4
Fat (% of energy)	37.8	39.6
Saturated fat	16.7	17.5
Monounsaturated fat	11.1	11.7
Polyunsaturated fat	7.7	8.2
Carbohydrates (% of energy)	46.2	43.8
Alcohol (% of energy)	1.3	1.3
Cholesterol		
mg/MJ	12.4	86.2
mg/day	136.4	948.2
Fiber		
g/MJ	3.0	3.1
g/day	33.0	34.1

Blood collection and biochemical analyses

All participants were assigned a random number that was used for labeling blood and serum tubes. In this way, the laboratory technicians did not know the subject's diet sequence or genotype. Blood samples were taken after a 12-h fast on days 22, 24, 27, and 29 of each dietary period. We took a number of measures to reduce within-subject variation in serum cholesterol. Subjects remained standing while waiting for the blood collection. During the two dietary periods, venipunctures were performed by the same technicians, in the same location, at the same time on the same days of the week and with each subject always in the same position, which was either sitting or lying. Serum was obtained by low speed centrifugation between 0.5 to 1 h after venipuncture, stored at −80°C, and analyzed enzymatically for total cholesterol, HDL-cholesterol, and triglycerides (26). All samples from one subject were analyzed within the same run. The coefficient of variation within runs was 0.5% for total cholesterol, 1.2% for HDL-cholesterol, and 0.7% for triglycerides. The mean bias with regard to target values of serum pools (Cholesterol Reference Method Laboratory Network) was −0.07 mmol/l (−3 mg/dl) for total cholesterol and −0.02 mmol/l (−1 mg/dl) for HDL-cholesterol. LDL-cholesterol was calculated (27).

Genotyping

DNA was isolated from fresh blood by a "salting-out" procedure (28). The DNA was amplified for the assessment of the APOA4-1/2, APOA4-347A/T and APOE2/3/4 polymorphisms by mutagenically separated polymerase chain reactions (MS-PCR) (29). In each MS-PCR, the normal and mutant alleles were amplified in the same reaction tube, using allele-specific primers that differ in length. The MS-PCR-products were made visible by UV light on an agarose gel. We used 17 duplicate samples as a quality control measure for the assessment of the genotypes. The investigators who assessed the genotypes did not know which samples were the duplicates. The genotypes of all 17 duplicate samples agreed.

Statistical analyses

The four values of serum lipids obtained for each subject at the end of each dietary period were averaged and then used for the calculation of the individual differences in serum lipid levels between the diets. Differences in response of serum lipids to dietary cholesterol between the subjects with the APOA4-1/1 genotype and subjects with the APOA4-2 allele were analyzed by a two-tailed Student's *t*-test. We used the General Linear Models (GLM) procedure of the SAS program to check the effect of potential confounders, such as gender, body mass index (BMI), age, APOE2/3/4 polymorphism and APOA4-347A/T polymorphism on differences in response between subjects with the various APOA4 genotypes (30).

RESULTS

Overall, the switch from a diet low in cholesterol to a diet high in cholesterol increased levels of serum total cholesterol by 0.55 ± 0.32 mmol/l (21 ± 12 mg/dl) (mean ± SD) or 12%, levels of LDL-cholesterol by 0.44 ± 0.32 mmol/l (17 ± 12 mg/dl) or 17%, and levels of HDL-cholesterol by 0.10 ± 0.13 mmol/l (4 ± 5 mg/dl) or 6%.

The mean difference in response between subjects with the APOA4-1/1 genotype and subjects with the APOA4-2 allele was 0.01 mmol/l (0 mg/dl) for total cholesterol and HDL-cholesterol and 0 mmol/l (0 mg/dl) for LDL-cholesterol (**Table 4**).

Adjustment for either gender, BMI, age, baseline cholesterol level, change in body weight during the trial, APOE2/3/4 polymorphism, or APOA4-347A/T polymorphism did not materially affect the difference in response of serum lipids between the APOA4 genotype groups. The largest effect of adjustment was that for the APOE2/3/4 polymorphism; the adjusted response of LDL-cholesterol was 0.02 ± 0.09 mmol/l (1 ± 3 mg/dl) (estimated mean ± SE) larger in subjects with the APOA4-1/1 genotype than in subjects with the APOA4-2 allele.

TABLE 4. Mean levels of serum total, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol (± SD) during the low cholesterol and high cholesterol diets, difference in response, and 95% confidence interval (CI) in subjects with the APOA4-1/1 genotype (N = 33) and carriers of the APOA4-2 allele (N = 17)

APOA4 Genotype	Low Cholesterol Diet	High Cholesterol Diet	Difference in Response (95% CI)
	mmol/l[a]		
Total cholesterol			
1/1	4.53 ± 0.84	5.08 ± 0.97	0.01 (−0.19 to 0.20)
1/2 or 2/2	4.46 ± 0.57	5.00 ± 0.64	
LDL cholesterol			
1/1	2.62 ± 0.72	3.06 ± 0.85	0.00 (−0.20 to 0.19)
1/2 or 2/2	2.54 ± 0.47	2.98 ± 0.63	
HDL cholesterol			
1/1	1.52 ± 0.32	1.63 ± 0.33	0.01 (−0.07 to 0.08)
1/2 or 2/2	1.52 ± 0.20	1.62 ± 0.19	

[a] To convert serum lipid values from mmol/l to mg/dl multiply values in mmol/l by 38.67.

DISCUSSION

We found that the APOA4-1/2 polymorphism did not affect the response of serum lipids to an increased intake of cholesterol against a background diet high in saturated fat in Dutch subjects with normal cholesterol levels. The average response of serum total cholesterol to dietary cholesterol, 0.55 mmol/l (21 mg/dl), was in line with responses estimated from prediction equations (25, 31, 32). There were no significant differences between subjects with the APOA4-1/1 genotype and subjects with the APOA4-2 allele in the potentially confounding factors of gender, BMI, age, and baseline level of total cholesterol. In addition, the intake of total fat, fatty acids, and cholesterol during the trial was the same in the two groups, as was the average change in body weight. In the present study, however, the APOA4-347T allele and APOE4 allele, which may enhance the response of serum lipids to diet (33–35), were more prevalent in subjects with the APOA4-1/1 genotype than in subjects with the APOA4-2 allele. However, this did not lead to a larger response in subjects with the APOA4-1/1 genotype than in those with the APOA4-2 allele.

We did not assess other genetic polymorphisms than the APOA4-1/2 and -347A/T and APOE polymorphisms. Therefore, we cannot exclude the possibility that one of these other genetic polymorphisms biases our results. However, because most of these other candidate polymorphisms are not closely linked to the APOA4-1/2 polymorphism (2), the various genotypes of these polymorphisms are likely to be randomly distributed over the subjects with the various APOA4 genotypes and may thus not bias the results of the present study. In addition, the APOA4-347A/T polymorphism, which is linked to the APOA4-1/2 polymorphism (36), did not bias the present results.

In contrast with the present study, other studies found that the APOA4-2 allele attenuated the response of serum LDL-cholesterol significantly (13, 14) or not significantly (15, 17), whereas one other study found that the APOA4-2 allele enhanced the response (16) (**Fig. 2**) (data for the calculation of 95% confidence intervals: personal communication with R. B. Weinberg and J. M. Ordovas, 1999).

In the study by Mata et al. (14), men with the apoA-IV-2 isoform had smaller responses of LDL-cholesterol to a decrease in the intake of cholesterol plus saturated fat than did men with the apoA-IV-1/1 phenotype. In another study (15), which used in part some of the data of Mata et al. (14), the differences between men with the various apoA-IV phenotypes were somewhat smaller. One explanation for the different findings in the study of Mata et al. and the present study is that in the study by Mata et al. not only the intake of cholesterol, but also the intake of fat, was changed (14). This may indicate that the APOA4-1/2 polymorphism affects the response of LDL-cholesterol to a change in the intake of fat. However, people who overrespond to dietary cholesterol also tend to overrespond to dietary fat (37). In addition, in a study by Jansen et al. (17), responses of LDL-cholesterol were slightly smaller in men with the APOA4-2 allele than in men with the APOA4-1/1

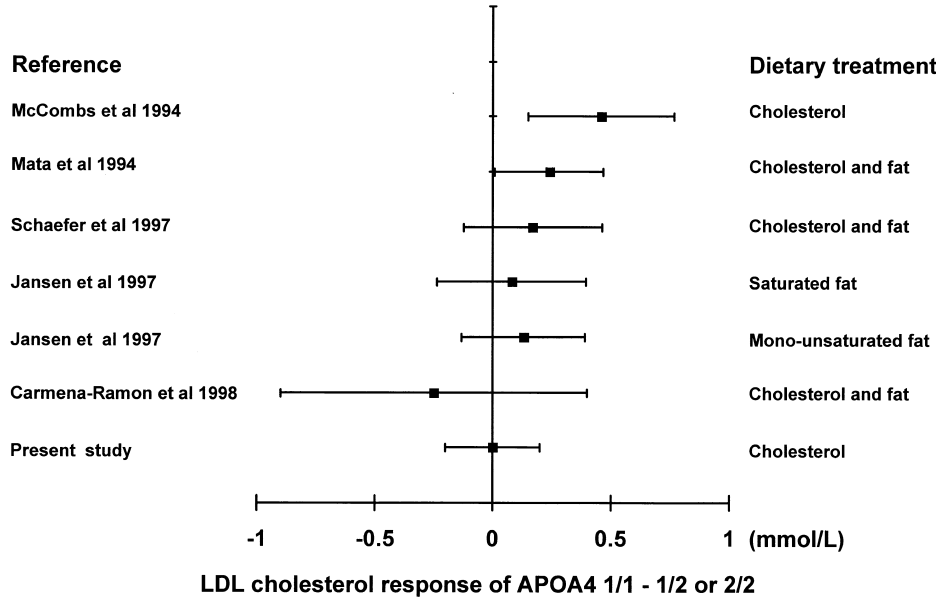

Fig. 2. Differences in response of serum low density lipoprotein (LDL)-cholesterol to dietary cholesterol and/or fat between subjects with the apolipoprotein (APO) A4-1/1 genotype or apoA-IV-1/1 phenotype and subjects with the APOA4-2 allele or apoA-IV-2 isoform and 95% confidence intervals of the difference in LDL-cholesterol response in six studies.

genotype when carbohydrates were replaced by saturated fat or monounsaturated fat. Nonetheless, it remains possible that the APOA4-1/2 polymorphism affects the response of LDL-cholesterol to a change in the intake of both cholesterol and fat.

Another explanation for the different findings are differences in subject characteristics. The subjects in the study by Mata et al. (14) were middle-aged and moderately hyperlipemic, whereas the subjects in the present study were young and had normal cholesterol levels at baseline. However, responsiveness to dietary cholesterol does not differ between older and younger people (38), it is if anything more marked in people with higher cholesterol levels (39). In addition, in one study with subjects with familial hypercholesterolemia the response of serum LDL-cholesterol to an increased intake of cholesterol plus fat was not attenuated but somewhat enhanced by the APOA4-2 allele (16). It might be that the effect of mutations in the LDL-receptor on the response of cholesterol overshadowed the effects of the APOA4-1/2 polymorphism in these subjects.

McCombs et al. (13) showed that 11 young and normolipemic subjects with the apoA-IV-2 isoform had a smaller response of LDL-cholesterol than did 12 subjects with the apoA-IV-1/1 phenotype to an increased cholesterol intake. These results differed significantly from those in the present study [95% confidence interval for difference in response 0.03 to 0.46 mmol/l (1 to 18 mg/dl)].

A possible explanation for the difference in results between the studies by McCombs et al. (13) and Mata et al. (14) and the present study is that the APOA4-1/2 polymorphism affects the response of LDL-cholesterol in men only and not in women. In the study by McCombs et al. (13), 74% of the subjects were men, whereas in the study by Mata et al. (14) the APOA4-1/2 polymorphism affected the response in men, but not in women. In the present study, 28% of the subjects were men and only four of them had the APO4-1/2 or -2/2 genotype. Because of this small number we did not have sufficient power to analyze the effects of the APOA4-1/2 polymorphism in men only. The response of LDL-cholesterol was -0.04 ± 0.11 mmol/l (2 ± 4 mg/dl) (mean $\pm$ SE) ($P = 0.73$), smaller in women with the APOA4-2 allele than in those with the APOA4-1/1 genotype, whereas it was 0.17 ± 0.19 mmol/l (7 ± 7 mg/dl) ($P = 0.39$) larger in men with the APOA4-2 allele than in those with the APOA4-1/1 genotype.

Another explanation for the difference in results is that we used a background diet high in saturated fat to enhance the response of serum cholesterol to dietary cholesterol. It is possible that the effect of the high saturated fat diet overwhelmed the effect of the APOA4-1/2 polymorphism on cholesterol metabolism and response. However, we do not think this is likely, because levels of total cholesterol on the low cholesterol, high saturated fat diet were still fairly low [mean, 5.06 mmol/l (196 mg/dl)] and increased by 11% on addition of cholesterol to the high saturated fat diet.

In the present controlled dietary trial the lipid response to dietary cholesterol was not affected by the APOA4-1/2 polymorphism in 37 women and 13 men with normal cholesterol levels, who were on a background diet high in saturated fat. This suggests that the APOA4-1/2 polymorphism may not be a generally applicable tool for the identification of subjects who respond to dietary cholesterol.

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