Apolipoprotein B gene polymorphisms and serum lipids: meta-analysis of the role of genetic variation in responsiveness to diet[1–3]

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ABSTRACT

Background: The genetic variance determining plasma lipid and lipoprotein concentrations may modify individual responsiveness to alterations in dietary fat and cholesterol content.

Objective: The aim was to examine the role of apolipoprotein (apo) B DNA polymorphisms in responsiveness of plasma lipids and lipoproteins to diet.

Design: A controlled dietary intervention study was conducted in 44 healthy, middle-aged subjects with a 3-mo baseline, a 1-mo fat-controlled, a 1-mo high-fat, and a 1-mo habitual diet period. We also conducted a meta-analysis of all published dietary trials, including our own.

Results: In our own dietary study, the apo B *Xba*I restriction-site polymorphism affected the responsiveness to diet of the plasma LDL-cholesterol concentration ($P < 0.05$, repeated-measures analysis of variance). Especially during the high-fat diet, homozygous absence of the *Xba*I restriction site (X^-/X^-) was associated with a greater increase in LDL cholesterol (44 ± 5%) than was X^+/X^+ (27 ± 7%) or X^+/X^- (40 ± 5%). The high-fat diet also induced a larger increase in plasma LDL cholesterol in subjects with the R^-/R^- genotype (homozygous absence of the *Eco*RI restriction site) (59 ± 10%) than in those with the R^+/R^- (39 ± 6%) or R^+/R^+ (36 ± 4%) genotype. The M^+/M^+ genotype (homozygous presence of the *Msp*I restriction site) was also more responsive (41 ± 3% increase in LDL cholesterol) than the M^+/M^- genotype (27 ± 10% increase). The meta-analysis supported the finding of the significant role of the *Eco*RI and *Msp*I polymorphisms, but not that of the *Xba*I polymorphism.

Conclusions: The present study indicated that the apo B *Eco*RI and *Msp*I polymorphisms are associated with responsiveness to diet. *Am J Clin Nutr* 2000;71:713–24.

KEY WORDS Apolipoprotein B, cholesterol, diet, meta-analysis, human, polymorphism, restriction endonuclease, restriction-fragment-length polymorphism, restriction-site polymorphism, plasma lipids

INTRODUCTION

Epidemiologic studies have shown an increase in the risk of atherosclerosis and coronary artery disease with increasing serum total and LDL-cholesterol concentrations (1–3). The primary treatment for hypercholesterolemia is a reduction of dietary fat and cholesterol intake and a replacement of dietary saturated fats by unsaturated fats. However, the responsiveness of plasma lipids to dietary changes seems to vary notably from one individual to another (4). The metabolic basis for the variation is not well known, but a considerable part of it may be controlled genetically, potentially by the genes encoding apolipoproteins, lipid-processing enzymes, lipid transfer proteins, and receptors involved in the regulation of lipoproteins. Except for the mutations of the LDL receptor gene (ie, familial hypercholesterolemia), no other single genetic marker of insufficient responsiveness to dietary modification has been uniformly identified. For example, the studies on the role of apolipoprotein (apo) E polymorphism as a significant modifier of dietary responsiveness have yielded contradictory results (5–21).

The genetic variation in apo B, the almost exclusive apoprotein of LDL particles, may play a major role in modifying the response to diet. The variability in the response of serum lipid concentrations has been shown to relate directly to the responsiveness of the LDL apo B production rate to dietary cholesterol (22). Furthermore, the polymorphisms of the apo B gene are associated with plasma total and LDL-cholesterol concentrations (23–26). Several studies elucidated the role of the apo B polymorphism in response to diet (7, 20, 27–37) and showed an association in some (7, 20, 27–30, 32, 35, 36) but not all cases (31, 33, 34, 37).

We wanted to examine the role of apo B DNA polymorphisms in the response to diet with a dietary trial design. Because dietary intervention studies in large groups of human subjects are laborious, each study permits only a limited number of comparisons and limited power to obtain conclusive results on genotype and

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environmental (ie, diet) interactions (38). An alternative to repeating a study with a large sample size is to combine evidence from other studies. Furthermore, systematic reviews help to sort out contradictory results. The technique of testing the statistical significances of combined data is commonly called meta-analysis (39). We carried out such an analysis of several dietary intervention studies by combining their effects and significances into overall estimates and then testing for combined significance.

SUBJECTS AND METHODS

Our dietary intervention study

Study subjects and study design

The subjects were selected from among the kitchen and technical staff of the Oulu University Central Hospital. The details of this study were reported previously (6, 40). Briefly, at the first stage, 200 employees were screened for their apo E phenotype. Of this cohort, 21 subjects with apo E phenotype 4 (genotypes *E3/4* or *E4/4*) volunteered for the dietary trial, and these subjects were matched for age, sex, and body weight with 23 individuals having apo E phenotype 3 (genotype *E3/3*). All the subjects were healthy and were taking no medication regularly. Their body mass indexes (BMIs; in kg/m^2) ranged from 19 to 29 and their ages from 21 to 54 y, the mean age being 38 y.

Baseline diet (the subjects' habitual diet) was analyzed by 7-d food consumption records. All study subjects were advised about how to prepare food diaries and all food diaries returned were also checked by an experienced dietitian. This food record information was used to calculate the nutrient content of the baseline diets. The intervention diets (low fat and high fat) were designed on the basis of the regular hospital meals. The meals were analyzed chemically for 7 d to confirm the calculated contents of nutrients in the hospital diets. All food during both intervention periods (including breakfast, lunch, dinner, and snacks, 7 d/wk, for 1 mo plus 1 mo in sequence) was delivered from the hospital kitchen to be consumed by the study subjects. The switch-back diet was again the subjects' habitual diet. The percentage distribution of energy during the baseline diet was 17% from protein, 46% from carbohydrate, and 37% from fat and the daily cholesterol intake was 480 mg on average. According to the food diaries, none of the study subjects were following any extreme baseline diets. The percentage distribution of energy during the low-fat diet was 20% from protein, 55% from carbohydrate, and 24% from fat and the daily cholesterol intake was 240 mg on average. During the high-fat diet, 18% of energy was derived from protein, 45% from carbohydrate, and 36% from fat, and daily cholesterol intake was 420 mg.

The study consisted of a 3-mo baseline period; a 1-mo intervention period with a fat-controlled, low-cholesterol diet; a 1-mo intervention period with a high-fat, high-cholesterol diet; and, finally, a 1-mo switch-back period. The most appropriate energy intake was chosen on the basis of the subjects' food consumption records and daily physical activity. The mean daily energy intakes were 12.6 MJ for men and 8.4 MJ for women. All study subjects volunteered for the study, which was approved by the Ethical Committee of the University of Oulu.

Laboratory methods

Analyses of the apo B restriction-fragment-length polymorphisms (RFLPs) were performed with DNA samples prepared by using the salting-out method described by Miller et al (41) involving the following sites: *Xba*I RFLP in exon 26 (the third base in codon 2488 causing a cytosine to thymidine change without changing the amino acid sequence), *Eco*RI RFLP in exon 29 (the first base in codon 4154 causing a guanosine to adenine change and changing glutamic acid to lysine), *Msp*I RFLP in exon 26 (the second base in codon 3611 causing an adenine to guanosine change and changing arginine to glutamine), *Bsp* 1261I RFLP in exon 4 (the second base in codon 71 causing a cytosine to thymidine change and changing threonine to isoleucine), and a 3-codon insertion-deletion polymorphism (alanine-leucine-alanine) in the signal peptide region of the human apo B gene.

The methods for determining the *Xba*I and *Eco*RI DNA polymorphisms were published earlier (42). The *Msp*I, *Bsp* 1261I, and signal peptide polymorphisms were measured by using specific oligonucleotide primers and polymerase chain reaction amplification of digested DNA fragments as reported previously (43–45). The alleles were designated as + or −, ie, X^+/X^+ refers to the homozygous presence of the apo B *Xba*I restriction site, X^+/X^- refers to the heterozygous presence of the restriction site, and X^-/X^- refers to the homozygous absence of the restriction site. The *Eco*RI, *Msp*I, and *Bsp* 1261I restriction sites were designated as alleles *R*, *M*, and *B*, respectively. The more frequent allele of the apo B signal peptide (insertion of alanine-leucine-alanine codons) was designated as *I* and the rare allele (deletion of alanine-leucine-alanine codons) as *D*.

For lipid analyses, all blood samples were collected after subjects fasted overnight (12 h). The baseline blood samples were drawn twice with a 2-mo interval, and the baseline values were means of these 2 measurements. The low-fat diet period lasted for 1 mo and the high-fat diet period lasted for 1 mo; blood samples were drawn weekly. Only the mean of the 2 values from the last 2 wk were used for analysis. The switch-back blood samples were drawn twice with a 1-mo interval and the mean of these 2 measurements was used for analysis.

The plasma cholesterol and triacylglycerol concentrations were analyzed enzymatically (46, 47) by using a Gilford Impact 400E Clinical Chemistry Analyser (Gilford Instruments Laboratories, Oberlin, OH). The plasma HDL-cholesterol concentration was determined after precipitation of the plasma sample with heparin-manganese (48). LDL-cholesterol concentration was calculated according to the Friedewald formula (49). The concentrations of apo B and apo A-I were determined with the liquid-precipitate technique by using the nephelometric method (Turbox-Kit; Orion Diagnostica, Espoo, Finland).

In our laboratory, each series of measurements contained an internal stardard to control the accuracy of the method, and the interassay CV for, eg, plasma total cholesterol concentration, was 4%. The intraassay variation was controlled by several parallel determinations of the same sample and the intraassay CVs for plasma total cholesterol, triacylglycerols, and HDL cholesterol were 2%, 5%, and 1%, respectively.

Statistical analyses

The study subjects in the different apo B genotypic subgroups (n = 44) did not differ significantly in their BMIs, whereas their sex and age distributions differed significantly (**Table 1**). Therefore, the lipid values (**Table 2**) were age- and sex-adjusted by linear regression as proposed by Siervogel et al (50). The results are expressed as means ± SEMs. The dietary intervention–induced changes in plasma lipids and lipoproteins and the effects of apo B

TABLE 1
Demographic data of study subjects in dietary intervention trials[1]

Apo B genotype	No. of subjects with apo E3/apo E4 phenotype[2]	Age	BMI
		y	*kg/m²*
All (*n* = 22 F, 22 M)	23/21	37.9 ± 1.2[3]	23.4 ± 0.4
*Xba*I			
X^-/X^- (*n* = 13 F, 6 M)	10/9	40.7 ± 1.6	23.2 ± 0.6
X^+/X^- (*n* = 6 F, 10 M)	9/7	37.9 ± 1.8	23.7 ± 0.7
X^+/X^+ (*n* = 3 F, 6 M)	4/5	32.1 ± 2.4	23.6 ± 0.9
*Eco*RI			
R^+/R^+ (*n* = 11 F, 16 M)	15/12	36.2 ± 1.4	23.8 ± 0.5
R^+/R^- (*n* = 7 F, 6 M)	7/6	39.7 ± 2.1	23.5 ± 0.7
R^-/R^- (*n* = 4 F)	1/3	43.7 ± 3.7	21.1 ± 1.3
*Msp*I			
M^+/M^+ (*n* = 19 F, 20 M)	22/17	37.9 ± 1.2	23.5 ± 0.4
M^+/M^- (*n* = 3 F, 2 M)	1/4	38.0 ± 3.5	23.6 ± 1.2
Signal peptide			
I/I (*n* = 14 F, 9 M)	15/8	37.6 ± 1.6	23.0 ± 0.5
I/D (*n* = 7 F, 13 M)	8/12	38.1 ± 1.8	24.1 ± 0.6
D/D (*n* = 1F)	0/1	41.6	21.9
*Bsp*I			
B^+/B^+ (*n* = 12 F, 10 M)	13/9	38.0 ± 0.1	23.1 ± 0.6
B^+/B^- (*n* = 9 F, 12 M)	10/11	37.7 ± 0.1	24.0 ± 0.6
B^-/B^- (*n* = 1 F)	0/1	41.6	21.9

[1] The apolipoprotein B (apo B) genotype groups are as follows: X^- and X^+, absence and presence of the *Xba*I restriction site; R^- and R^+, absence and presence of the *Eco*RI restriction site; M^- and M^+, absence and presence of the *Msp*I restriction site; *I* and *D*, insertion and deletion of the signal peptide; B^- and B^+, absence and presence of the *Bsp*I restriction site.

[2] Apo E3, apo E phenotype E3,3; apo E4, apo E phenotype E4,3 (*n* = 17) or E4,4 (*n* = 4).

[3] $\bar{x}$ ± SEM.

genotypes especially were tested with respect to the intraindividual variation during the dietary intervention periods. This was done by using a layered design in the form of repeated measures across time [repeated-measures analysis of variance (ANOVA)]. The top layer in the model was the between-subject layer, in which the effect of having a certain apo B genotype (eg, X^-/X^-, X^+/X^-, or X^+/X^+) was tested with respect to the interindividual variation. The bottom layer was the within-subject layer, in which the repeated-measures factor for the diet periods (baseline, fat controlled, high fat, and switch back) was tested with respect to the variation from one dietary period to another. For the significant effects revealed by repeated-measures ANOVA, a further paired Student's *t* test (or signed-rank test when appropriate) and Tukey's test were performed to evaluate the respective *P* values. For most of the analyses, the JMP statistical software program (SAS Institute Inc, Cary, NC) was used.

Meta-analysis

All published reports of dietary interventions conducted to investigate the effect of apo B DNA polymorphisms on plasma lipid responsiveness among healthy adults between 1980 and 1998 were identified by literature searches (National Center for Biotechnology Information at the National Library of Medicine, database coverage being Medline and Premedline; 51). Including our present study (6), 14 eligible reports were found (7, 20, 27–37). Five reports turned out to be publications of the North Karelia Study (7, 27–30) and were analyzed as one study in our meta-analysis. All the dietary interventions were conducted with use of solid food diets and the details of the dietary interventions were available. These details included the dietary fat content; the amounts of saturated, polyunsaturated, and monounsaturated fatty acids and dietary cholesterol; the duration of the trials; and the respective plasma lipid and lipoprotein values. Data on the apo B *Xba*I, *Eco*RI, *Msp*I, signal peptide, and *Bsp* 1261I polymorphisms were available in most studies. The effects of dietary modification, ie, the change from a low-fat or low-cholesterol diet or a high-polyunsaturated, low-saturated fatty acid diet to a high-fat or high-cholesterol or low-polyunsaturated, high-saturated fatty acid diet, on plasma total, LDL-cholesterol, HDL-cholesterol, triacylglycerols, apo B, and apo A-I concentrations were investigated.

The 3 genotypes, −/−, −/+, and +/+, of each apo B polymorphism are indicated by the number of restriction sites *P* (*P* = 0, 1, 2). For each genotype, we were provided with the response to diet, sample size, variable mean, and SD (or variance) for both the low-fat and high-fat diets and for the male (M) and female (F) groups. We set up a new group called "all" (A) in each genotype group by combining the data for males and females and by computing the averages of variables and variances weighted by sample sizes and degrees of freedom, respectively.

From the data of each diet study (indexed by *i* where necessary), we first computed the effect size and the pooled SD for the 9 *P* by *X* subgroups (*P* = 0, 1, 2; *X* = M, F, A). We determined the response to diet, Δ_X^P, as a difference between the high-fat (*H*) and low-fat (*L*) values of variables and the corresponding variances s^2 for the subgroups from the following:

$$\left(s_X^P\right)^2 = \left(s_{L,X}^P\right)^2 + \left(s_{H,X}^P\right)^2 - 2r_{LH}s_{L,X}^P s_{H,X}^P \quad (1)$$

where r_{LH} is the correlation coefficient of the high-fat and low-fat variables. The correlation coefficients were available in some of the

TABLE 2
Plasma lipid and lipoprotein values during the dietary trial according to apolipoprotein (apo) B genotype[1]

Apo B genotype (n = 44)	Total cholesterol	Triacylglycerol	HDL cholesterol	LDL cholesterol	Apo B	Apo A-I
	mmol/L	*mmol/L*	*mmol/L*	*mmol/L*	*g/L*	*g/L*
*Xba*I						
X^-/X^- (n = 19)						
Basal diet	5.01 ± 0.21	0.84 ± 0.12	1.48 ± 0.06	3.15 ± 0.17	0.76 ± 0.05	1.18 ± 0.06
Low-fat diet	4.24 ± 0.18	1.07 ± 0.12	1.25 ± 0.05	2.51 ± 0.15	0.69 ± 0.05	1.05 ± 0.05
High-fat diet	5.50 ± 0.22	0.79 ± 0.11	1.62 ± 0.05	3.53 ± 0.17	0.82 ± 0.05	1.23 ± 0.05
Switch-back diet	5.12 ± 0.21	0.84 ± 0.12	1.50 ± 0.06	3.24 ± 0.17	0.73 ± 0.05	1.23 ± 0.06
X^+/X^- (n = 16)						
Basal diet	5.25 ± 0.22	1.18 ± 0.13	1.63 ± 0.06	3.08 ± 0.18	0.79 ± 0.05	1.32 ± 0.07
Low-fat diet	4.52 ± 0.20	1.33 ± 0.14	1.36 ± 0.05	2.56 ± 0.16	0.66 ± 0.06	1.10 ± 0.05
High-fat diet	5.78 ± 0.23	1.10 ± 0.12	1.71 ± 0.06	3.57 ± 0.19	0.78 ± 0.05	1.34 ± 0.06
Switch-back diet	5.51 ± 0.23	1.22 ± 0.13	1.55 ± 0.07	3.41 ± 0.19	0.73 ± 0.06	1.27 ± 0.06
X^+/X^+ (n = 9)						
Basal diet	5.93 ± 0.30[2]	0.91 ± 0.18	1.58 ± 0.08	3.94 ± 0.24[2]	0.69 ± 0.07	1.23 ± 0.09
Low-fat diet	5.28 ± 0.27	1.20 ± 0.18	1.34 ± 0.07	3.49 ± 0.21	0.72 ± 0.07	1.11 ± 0.07
High-fat diet	6.32 ± 0.31	1.14 ± 0.16	1.59 ± 0.08	4.22 ± 0.25	0.85 ± 0.07	1.20 ± 0.07
Switch-back diet	6.03 ± 0.30[3]	1.10 ± 0.17	1.54 ± 0.09	4.00 ± 0.25[3]	0.81 ± 0.08	1.23 ± 0.08
*Eco*RI						
R^+/R^+ (n = 27)						
Basal diet	5.49 ± 0.18	1.04 ± 0.11	1.58 ± 0.05	3.44 ± 0.15	0.74 ± 0.04	1.25 ± 0.05
Low-fat diet	4.72 ± 0.17	1.24 ± 0.11	1.32 ± 0.04	2.83 ± 0.14	0.68 ± 0.04	1.08 ± 0.04
High-fat diet	5.91 ± 0.19	1.07 ± 0.09	1.65 ± 0.04	3.78 ± 0.15	0.80 ± 0.04	1.27 ± 0.04
Switch-back diet	5.65 ± 0.18	1.13 ± 0.10	1.51 ± 0.05	3.63 ± 0.15	0.76 ± 0.04	1.24 ± 0.05
R^+/R^- (n = 13)						
Basal diet	4.93 ± 0.26	0.84 ± 0.15	1.49 ± 0.07	3.06 ± 0.21	0.77 ± 0.06	1.19 ± 0.08
Low-fat diet	4.26 ± 0.24	1.10 ± 0.15	1.22 ± 0.06	2.55 ± 0.20	0.68 ± 0.06	1.05 ± 0.06
High-fat diet	5.44 ± 0.27	0.80 ± 0.13	1.59 ± 0.06	3.48 ± 0.22	0.77 ± 0.06	1.21 ± 0.06
Switch-back diet	5.10 ± 0.26	0.84 ± 0.15	1.47 ± 0.07	3.25 ± 0.21	0.71 ± 0.06	1.21 ± 0.07
R^-/R^- (n = 4)						
Basal diet	5.01 ± 0.46	1.02 ± 0.28	1.59 ± 0.13	2.97 ± 0.39	0.81 ± 0.11	1.33 ± 0.14
Low-fat diet	4.41 ± 0.43	1.17 ± 0.28	1.51 ± 0.10	2.37 ± 0.36	0.71 ± 0.11	1.22 ± 0.10
High-fat diet	5.96 ± 0.48	0.92 ± 0.24	1.82 ± 0.11	3.72 ± 0.39	0.96 ± 0.10	1.41 ± 0.11
Switch-back diet	5.16 ± 0.47	0.98 ± 0.26	1.78 ± 0.13	2.94 ± 0.38	0.81 ± 0.11	1.34 ± 0.12
*Msp*I						
M^+/M^+ (n = 39)						
Basal diet	5.23 ± 0.15	0.97 ± 0.09	1.54 ± 0.04	3.26 ± 0.13	0.76 ± 0.03	1.22 ± 0.04
Low-fat diet	4.53 ± 0.14	1.20 ± 0.09	1.28 ± 0.03	2.71 ± 0.12	0.70 ± 0.04	1.08 ± 0.03
High-fat diet	5.81 ± 0.16	0.99 ± 0.08	1.64 ± 0.04	3.73 ± 0.13	0.82 ± 0.03	1.27 ± 0.04
Switch-back diet	5.42 ± 0.15	1.03 ± 0.09	1.51 ± 0.04	3.46 ± 0.13	0.76 ± 0.04	1.24 ± 0.04
M^+/M^- (n = 5)						
Basal diet	5.64 ± 0.42	1.03 ± 0.25	1.71 ± 0.11	3.47 ± 0.35	0.70 ± 0.10	1.38 ± 0.12
Low-fat diet	4.78 ± 0.39	1.13 ± 0.25	1.55 ± 0.09	2.73 ± 0.32	0.57 ± 0.10	1.14 ± 0.09
High-fat diet	5.49 ± 0.44	0.88 ± 0.22	1.72 ± 0.10	3.37 ± 0.35	0.70 ± 0.09	1.22 ± 0.10
Switch-back diet	5.58 ± 0.43	1.01 ± 0.24	1.67 ± 0.12	3.45 ± 0.36	0.67 ± 0.10	1.27 ± 0.11
Signal peptide						
I/I (n = 23)						
Basal diet	5.17 ± 0.20	0.88 ± 0.11	1.50 ± 0.05	3.28 ± 0.17	0.78 ± 0.05	1.16 ± 0.05
Low-fat diet	4.41 ± 0.18	1.07 ± 0.11	1.28 ± 0.04	2.65 ± 0.15	0.68 ± 0.05	1.06 ± 0.04
High-fat diet	5.60 ± 0.20	0.84 ± 0.10	1.62 ± 0.05	3.61 ± 0.16	0.79 ± 0.04	1.23 ± 0.05
Switch-back diet	5.31 ± 0.20	0.92 ± 0.11	1.47 ± 0.06	3.42 ± 1.17	0.75 ± 0.05	1.21 ± 0.05
I/D (n = 20)						
Basal diet	5.45 ± 0.21	1.09 ± 0.12	1.63 ± 0.06	3.33 ± 0.17	0.73 ± 0.05	1.34 ± 0.06
Low-fat diet	4.76 ± 0.19	1.34 ± 0.12	1.34 ± 0.05	2.82 ± 0.16	0.69 ± 0.05	1.12 ± 0.05
High-fat diet	6.01 ± 0.21	1.14 ± 0.11	1.68 ± 0.05	3.82 ± 0.18	0.83 ± 0.05	1.31 ± 0.05
Switch-back diet	5.63 ± 0.21	1.16 ± 0.12	1.59 ± 0.06	3.52 ± 0.18	0.74 ± 0.05	1.28 ± 0.06
D/D (n = 1)						
Basal diet	4.52	0.95	1.41	2.69	0.79	1.13
Low-fat diet	3.84	1.17	1.40	1.92	0.61	1.04
High-fat diet	4.94	0.86	1.68	2.88	0.83	1.09
Switch-back diet	4.69	0.99	1.50	2.75	0.86	1.28

(*Continued*)

TABLE 2 (*Continued*)

Apo B genotype (n = 44)	Total cholesterol	Triacylglycerol	HDL cholesterol	LDL cholesterol	Apo B	Apo A-I
	mmol/L	*mmol/L*	*mmol/L*	*mmol/L*	*g/L*	*g/L*
*Bsp*I						
B^+/B^+ (n = 22)						
Basal diet	5.22 ± 0.20	0.83 ± 0.07	1.50 ± 0.05	3.34 ± 0.17	0.77 ± 0.05	1.17 ± 0.07
Low-fat diet	4.46 ± 0.19	1.07 ± 0.12	1.30 ± 0.05	2.67 ± 0.15	0.67 ± 0.05	1.03 ± 0.04
High-fat diet	5.71 ± 0.21	0.90 ± 0.10	1.63 ± 0.05	3.68 ± 0.17	0.81 ± 0.04	1.25 ± 0.05
Switch-back diet	5.36 ± 0.21	0.92 ± 0.11	1.49 ± 0.06	3.46 ± 0.17	0.74 ± 0.05	1.19 ± 0.05
B^+/B^- (n = 21)						
Basal diet	5.39 ± 0.21	1.13 ± 0.09	1.61 ± 0.05	3.26 ± 0.17	0.74 ± 0.05	1.32 ± 0.06
Low-fat diet	4.69 ± 0.19	1.32 ± 0.12	1.32 ± 0.05	2.78 ± 0.16	0.67 ± 0.05	1.14 ± 0.04
High-fat diet	5.88 ± 0.21	1.06 ± 0.11	1.66 ± 0.05	3.74 ± 0.17	0.80 ± 0.04	1.29 ± 0.05
Switch-back diet	5.57 ± 0.21	1.15 ± 0.12	1.56 ± 0.06	3.49 ± 0.17	0.75 ± 0.05	1.29 ± 0.05
B^-/B^- (n = 1)						
Basal diet	4.52	0.95	1.41	2.69	0.79	1.13
Low-fat diet	3.84	1.17	1.40	1.92	0.61	1.04
High-fat diet	4.94	0.86	1.68	2.88	0.83	1.25
Switch-back diet	4.69	0.99	1.50	2.75	0.86	1.28

[1] $\bar{x}$ ± SEM; values are adjusted for sex and age. The apo B genotype groups are as follows: X^- and X^+, absence and presence of the *Xba*I restriction site; R^- and R^+, absence and presence of the *Eco*RI restriction site; M^- and M^+, absence and presence of the *Msp*I restriction site; *I* and *D*, insertion and deletion of the signal peptide; B^- and B^+, absence and presence of the *Bsp*I restriction site. Diet-induced changes in plasma lipids and lipoproteins were significant in all subgroups, $P < 0.001$ (repeated-measures ANOVA).

[2] Significantly different from the other genotype groups, $P < 0.05$ (Tukey's test).

[3] Significant effect of the apo B *Xba*I genotype on diet-induced responses, $P = 0.015$ (repeated-measures ANOVA).

diet response studies, but if not given, the correlation coefficient was fixed to the most probable value and tested for its sensitivity.

We compared the response effects pairwise between the 3 genotypes (P, Q = 0, 1, 2), ie, the pairs $P \rightarrow Q$: $0 \rightarrow 1$, $1 \rightarrow 2$, and $0 \rightarrow 2$, in each of the groups X = M, F, and A, separately. The scaled effect size (standardized score or z score) of each study i was as follows:

$$z_i^{PQ} = \frac{\Delta_i^Q - \Delta_i^P}{s_i^{PQ}} \quad (2)$$

where the pooled SD is as follows (52):

$$s_i^{PQ} = \sqrt{\frac{(N_i^P - 1)(s_i^P)^2 + (N_i^Q - 1)(s_i^Q)^2}{N_i^P + N_i^Q - 2}} \quad (3)$$

obtained from the sample sizes N_i^P and N_i^Q.

Next, we combined the data from all studies, again for each group X = M, F, and A, separately. The asymptotic distribution of the effect sizes from different studies was normal with a variance (52) equal to

$$(S_i^{PQ})^2 = \frac{N_i^P + N_i^Q}{N_i^P\, N_i^Q} + \frac{(z_i^{PQ})^2}{2(N_i^P + N_i^Q)} \quad (4)$$

Then, the mean weighted z scores over the different studies emerged as

$$z^{PQ} = \frac{\sum_i w_i^{PQ} z_i^{PQ}}{\sum_i w_i^{PQ}} \quad (5)$$

where the weights were

$$w_i^{PQ} = \frac{1}{(S_i^{PQ})^2} \quad (6)$$

The sample estimate of the weighted mean variance $(S^{PQ})^2$ can be obtained from

$$\frac{1}{(S^{PQ})^2} = \sum_i \frac{1}{(S_i^{PQ})^2} \quad (7)$$

Finally, the estimates z^{PQ} and S^{PQ} were used to evaluate the two-tailed 100 $(1 - \alpha)$% CIs and the corresponding significance probabilities of meta-analysis (52) separately for each of the 3 genotype pairs. The significance level was chosen to be $\alpha = 0.05$ (95% CI).

The analysis was carried out separately for men and women, but because no significant differences were found between the groups, the results of the combined group "all" are presented. Because testing for equality of the means of the genotype pairs separately was considered simpler and more sensitive than the ANOVA of all 3 genotypes, we calculated the differences in the diet-induced changes of plasma lipids (z scores) and the respective P (probability) values between separate genotype groups pairwise.

RESULTS

The fat content of the diet consumed in our own study was determined first by analyzing the regular hospital meals chemically for 1 wk (courtesy of the Agricultural Research Centre, Jokioinen, Finland). According to results of calculations made from the daily records and the chemical analysis, the fat content of the diet determined by these 2 methods did not differ significantly. The intervention diets were based on the regular hospital meals; the low-fat diet was prepared by limiting the amount of dairy products and adding margarine, polyunsaturated salad dressings, and vegetables; and the high-fat diet was prepared by increasing the amount of fatty dairy products, adding cold meats, and limiting vegetables. Thus, the fat contents of the intervention diets were easy to determine reliably by calculations made according to the daily records. The fat contents of diets in all

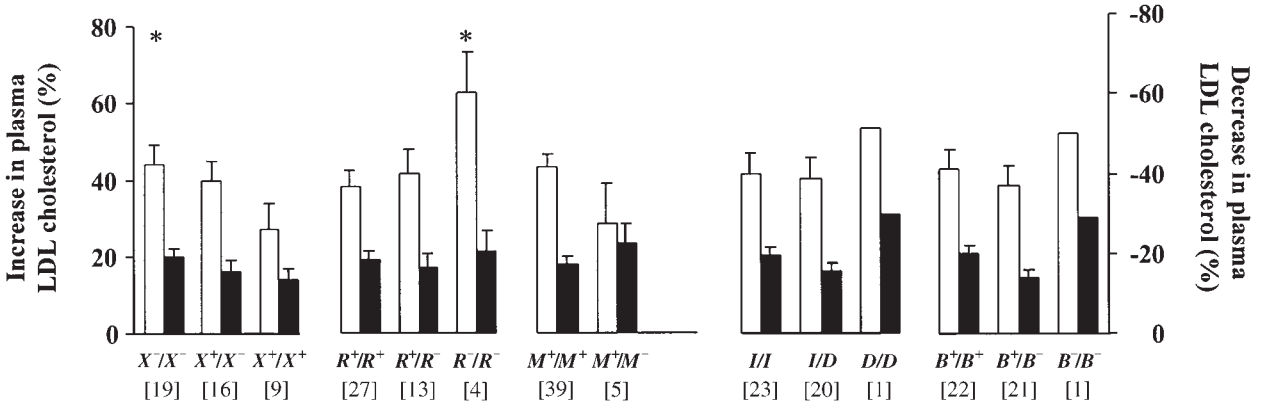

FIGURE 1. Percentage changes in plasma LDL-cholesterol concentrations in the different apolipoprotein B genotype groups (n = 44). *X* refers to the *Xba*I polymorphism, *R* refers to the *Eco*RI polymorphism, *M* refers to the *Msp*I polymorphism, *I* and *D* refer to a signal peptide insertion or deletion, and *B* refers to the *Bsp*I polymorphism; the genotypes are explained in more detail in Methods. Open bars indicate the percentage increase in the plasma LDL-cholesterol concentration (change from the low-fat diet to the high-fat diet). Solid bars indicate the percentage decrease in the plasma LDL-cholesterol concentration (change from the baseline diet to the low-fat diet). *Significantly larger change in X^-/X^- subjects than in X^+/X^- and X^+/X^+ subjects ($P < 0.05$); significantly larger change in R^-/R^- subjects than in R^+/R^+ and R^+/R^- subjects ($P < 0.05$). Number of study subjects in brackets.

other studies included in the meta-analysis were determined as indicated in the original reports.

Our dietary intervention study

Distribution of apo B polymorphisms

The allele frequencies for the rare alleles were 39% for X^+, 24% for R^-, 6% for M^-, 26% for B^-, and 25% for *D*. The genotype variants are presented in Table 1. For all the polymorphisms studied, genotype distributions were not significantly different from the Hardy-Weinberg prediction.

Baseline lipid values

Of the subjects screened for the dietary intervention study (n = 200), lipid values were available for 187 subjects. Among them, the X^+ allele tended to associate with higher plasma total cholesterol concentrations, the age- and sex-adjusted mean plasma total cholesterol concentration being 5.79 ± 0.20, 5.40 ± 0.11, and 5.25 ± 0.12 mmol/L for the X^+/X^+, X^+/X^-, and X^-/X^- subjects, respectively. The M^- and B^- alleles were associated with both high plasma total and LDL-cholesterol concentrations. The M^+/M^- subjects had plasma total and LDL-cholesterol concentrations of 5.79 ± 0.19 and 3.73 ± 0.22 mmol/L and the M^+/M^+ subjects had concentrations of 5.32 ± 0.08 and 3.14 ± 0.09 mmol/L, respectively ($P < 0.05$ for both, Tukey's test). The B^-/B^- subjects had plasma total and LDL-cholesterol concentrations of 6.36 ± 0.32 and 4.09 ± 0.33 mmol/L, the B^+/B^- subjects had concentrations of 5.41 ± 0.12 and 3.25 ± 0.13 mmol/L, and the B^+/B^+ subjects had concentrations of 5.30 ± 0.10 and 3.12 ± 0.11 mmol/L, respectively ($P < 0.01$ and $P < 0.05$). In addition, the apo B signal peptide polymorphism was associated with the plasma LDL-cholesterol concentration: the *D*/*D* subjects had the highest LDL-cholesterol concentration (3.98 ± 0.32 mmol/L), the *I*/*D* subjects had intermediate values (3.29 ± 0.13 mmol/L), and the *I*/*I* subjects had the lowest values (3.09 ± 0.11 mmol/L; $P < 0.05$).

The demographic data of the 44 study subjects are shown in Table 1. The baseline plasma total cholesterol values of the study subjects were higher among those with the X^+/X^+ genotype than in those with the X^-/X^- and X^+/X^- genotypes (Table 2). No other significant differences between the genotypes were found in the baseline lipid values.

Effect of apo B polymorphisms on response to diet

The plasma total, HDL-cholesterol, LDL-cholesterol, apo B, and apo A-I concentrations decreased significantly during the low-fat diet and increased during the high-fat diet. In addition, the plasma triacylglycerol concentrations increased significantly during the low-fat diet and decreased during the high-fat diet (Table 2).

The *Xba*I polymorphism significantly affected the diet-induced responses of plasma total and LDL-cholesterol concentrations when all the dietary periods (baseline, low fat, high fat, and switch back) were analyzed together ($P < 0.05$, repeated-measures ANOVA). The subjects with the X^-/X^- genotype had the greatest increase in their plasma total and LDL cholesterol during the high-fat diet and the greatest decrease during the low-fat diet (Table 2 and **Figure 1**). The effects of the *Eco*RI, *Bsp* 1261I, *Msp*I, and signal peptide polymorphisms on the response to diet were not significant when all the diet periods were analyzed together. The percentage increase in plasma total cholesterol during the high-fat diet was significantly greater in the subjects with the M^+/M^+ genotype than in those with the M^+/M^- genotype (29 ± 2% compared with 16 ± 5%; P = 0.02) and greater in the R^-/R^- subjects (35 ± 6%) than in the R^+/R^- (28 ± 3%) and R^+/R^+ subjects (26 ± 2%; P = 0.21). The percentage changes in plasma LDL-cholesterol concentrations in the different genotype groups are shown in Figure 1.

Meta-analysis

The dietary intervention studies included in the meta-analysis are listed in **Table 3**. The data from the study by Friedlander et al (35) were divided into 2 separate data sets (a and b), because there were data from 2 separate dietary trials. All the studies had been conducted on healthy subjects, and the average duration of the

TABLE 3
Baseline characteristics and respective percentage changes in plasma LDL cholesterol concentration in relevant dietary intervention studies included in the meta-analysis[1]

Reference	Mean age	Mean BMI	Plasma total cholesterol	Plasma LDL cholesterol	Change in plasma LDL[2]
	y	*kg/m²*	*mmol/L*	*mmol/L*	*%*
(6) (*n* = 22 F, 22 M whites)	38	23	5.40 ± 0.25[3]	3.30 ± 0.05	39
(7, 27–30) (*n* = 52 F, 55 M whites)	43	26	6.49 ± 0.20	4.51 ± 0.17	35
(33) (*n* = 22 F, 15 M whites)	33	26	4.60 ± 0.82	2.87 ± 0.66	13
(35a)[4] (*n* = 23 M whites)	21	21	3.96 ± 0.52	2.40 ± 0.48	15
(35b)[4] (*n* = 40 M whites)	21	21	3.72 ± 0.51	2.22 ± 0.42	13
(20) (*n* = 29 M whites)	54	26	5.97 ± 1.22	3.24 ± 1.10	22
(37) (*n* = 32 F, 23 M whites)	52	26	6.20 ± 0.10	4.04 ± 0.10	15
(36) (*n* = 43 F, 44 M whites)	51	27	5.52 ± 0.82	3.34 ± 0.69	12
(32) (*n* = 30 F, 21 M whites)	50	NA	6.18 ± 1.05	3.90 ± 0.90	3
(34) (*n* = 29 F, 20 M whites)	49	25	5.75 ± 0.95	4.00 ± 0.95	7
(31) (*n* = 71 M, mixed)[5]	35	NA	4.24 ± 0.70	2.50 ± 0.60	20

[1] Studies are listed in the order of decreasing number of apolipoprotein B polymorphisms reported. NA, not applicable.
[2] The percentage change in plasma LDL-cholesterol concentration is referenced to dietary changes described in Table 4.
[3] $\bar{x}$ ± SEM.
[4] Reference 35 describes 2 different dietary intervention studies.
[5] Study population consisted of white, Mexican American, and Asian subjects.

dietary intervention was 6 wk (from 3 to 12 wk). The distribution of apo E phenotypes in the present study and that of Gylling et al (20) differed from that of the other studies, because these studies had been designed to include equal numbers of subjects with apo E3 (*E3/3*) and apo E4 (*E3/4* or *E4/4*) phenotypes. Most of the studies had been designed to alter serum lipids, with several dietary factors being modified simultaneously, ie, the intake of total fat, saturated fatty acids, polyunsaturated fatty acids, or a combination of these, and the intake of dietary cholesterol (6, 7, 20, 27–30, 32–37), whereas one study was designed to alter serum lipids with one single dietary modification (31) (**Table 4**). Because all of the dietary modifications resulted in altered plasma total and LDL-cholesterol and apo B concentrations, and because it has been shown that persons responding to dietary cholesterol also tend to be more sensitive to changes in the quality of dietary fat (53), the different dietary studies were not considered to be distinct from each other, and thus suitable for meta-analysis. The X^+ allele was significantly less common in one study (33) and the R^- allele more frequent in one study (34) than in the other studies (**Table 5**). The main results of the meta-analysis are presented in **Table 6**. The comparison of the diet-induced changes (*z* scores) in plasma LDL-cholesterol concentrations between genotype groups +/− and +/+ are presented in **Figure 2**.

Effect of apo B Xba*I polymorphism on response to diet*

The average diet-induced alterations in the plasma total cholesterol, HDL-cholesterol, and triacylglycerol concentrations did not differ between the subjects with the genotypes X^+/X^+, X^+/X^-, or X^-/X^-. The heterozygous presence of the *Xba*I restriction site was associated with a greater increase in the plasma LDL-cholesterol and apo A-I concentrations than was the homozygous presence of the restriction site (Figure 2 and Table 6). The

TABLE 4
Comparison of dietary variables in the relevant intervention studies included in the meta-analysis[1]

Reference	Dietary fat		Dietary cholesterol		P-S ratio		Duration	Design
	LF	HF	LF	HF	LF	HF		
	% of energy		*mg/d*				*wk*	
(6)	24	36	240	420	1.0	0.2	4	Sequential
(7, 27–30)	24	39	300	540	1.0	0.1	6	Sequential
(33)	35	35	150	600	1.1	0.6	5	Crossover
(35a)[2]	33	40	300	NA	1.4	1.3	12	Sequential
(35b)[2]	33	40	300	NA	1.5	1.3	8	Sequential
(20)	24	38	208	574	0.6	0.3	6	Sequential
(37)	36	38	192	280	1.2	0.2	6	Crossover
(36)	24	34	279	337	0.6	0.4	8	Sequential
(32)	25	30	180	650	1.4	NA	4	Sequential
(34)	19	35	180	750	0.4	NA	3	Crossover
(31)	38	41	310	1430	0.4	NA	3	Sequential

[1] Studies are listed in the order of decreasing number of polymorphisms reported. P-S ratio, ratio of dietary polyunsaturated to saturated fatty acids; LF, low fat (plasma cholesterol–lowering diet); HF, high fat (plasma cholesterol–increasing diet); NA, not applicable.
[2] Reference 35 describes 2 different dietary intervention studies.

TABLE 5
Allele frequencies of the rare alleles of apolipoprotein B restriction-fragment-length polymorphisms in relevant dietary intervention studies[1]

Reference	*Xba*I (X^+)	*Eco*RI (R^-)	*Msp*I (M^-)	SP (*D*)	*Bsp*I (B^-)
			%		
(6)	39	24	6	25	26
(7, 27–30)	39	15	6	22	27
(33)	23[2]	16	4	23	NA
(35a)[3]	39	11	7	28	NA
(35b)[3]	36	11	6	15	NA
(20)	45	17[4]	12[4]	NA	NA
(37)	48	NA	NA	24	NA
(36)	38	NA	NA	30	NA
(32)	NA	15	NA	NA	NA
(34)	NA	29[2]	NA	NA	NA
(31)	NA	NA	NA	32	NA

[1] Studies are listed in the order of decreasing number of polymorphisms reported. NA, not applicable. The apo B genotype groups are as follows: X^+, presence of the *Xba*I restriction site; R^-, absence of the *Eco*RI restriction site; M^-, absence of the *Msp*I restriction site; *D*, deletion of the signal peptide; B^-, absence of the *Bsp*I restriction site.

[2] Significantly different allele frequency than reported in other studies, $P < 0.05$.

[3] Reference 35 describes 2 different dietary intervention studies.

[4] The allele frequencies of the + and − alleles were reported previously in an opposite order.

plasma apo B concentration increased more in the X^+/X^+ subjects than in the X^-/X^- subjects (Table 6).

Effect of apo B Eco*RI polymorphism on response to diet*

The distribution of EcoRI polymorphisms in different dietary studies was skewed, and most studies included only one female or one male R^-/R^- subject. The overall effect analysis showed that the increase in plasma total cholesterol, LDL-cholesterol, and apo B concentrations was significantly greater in R^-/R^- subjects than in R^+/R^+ subjects (Table 6).

Effect of apo B Msp*I polymorphism on response to diet*

The *Msp*I polymorphism was analyzed in 5 dietary trials (6, 20, 29, 33, 35) (Table 4), and the distribution of this polymorphism was also skewed, M^-/M^- homozygotes being very rare. The M^+/M^+ genotype was associated with a greater alteration in plasma LDL-cholesterol, plasma triacylglycerol, and apo A-I concentrations than the M^+/M^- genotype. There was no significant effect of the apo B *Msp*I polymorphism on the plasma total cholesterol, HDL- cholesterol, or apo B response (Table 6).

Effect of the apo B signal peptide polymorphism on response to diet

The apo B signal peptide insertion-deletion polymorphism was determined in 7 dietary trials (6, 28, 31, 33, 35–37); meta-analysis revealed no significant effect of this polymorphism on the plasma total, LDL-cholesterol, HDL-cholesterol, triacylglycerol, or apo A-I response. Plasma apo B concentration increased more with high-fat diet in *I*/*D* subjects than in *I*/*I* subjects (Table 6).

Effect of apo B Bsp *1261I polymorphism on response to diet*

The *Bsp* 1261I polymorphism was determined in only 2 of the dietary studies available, both of them conducted in Finland (6, 29). The distributions of the allele frequencies did not differ significantly between these 2 studies. The apo B *Bsp* 1261I polymorphism had a significant effect on the plasma total and LDL-cholesterol, triacylglycerol, and apo A-I responses: the B^-/B^- genotype was associated with a greater increase with diet in the plasma total cholesterol, LDL-cholesterol, and apo A-I concentrations and a significantly smaller alteration in the plasma triacylglycerol concentration than the B^+/B^- genotype (Table 6).

DISCUSSION

Apo B mutations might affect the plasma lipid responses or the plasma apo B concentrations during dietary modifications by altering apo B secretion, structural stability, affinity for the LDL receptor, or interactions of apo B–containing lipoproteins with other lipoproteins, cells, or enzymes (eg, hepatic or lipoprotein lipase). The physiologic role of the apo B *Xba*I polymorphism in codon 2488 in exon 26 is still unclear. The polymorphism alters plasma lipid concentrations (23–26, 54, 55) and LDL catabolism (56–58) even though it does not alter the amino acid sequence. In our dietary intervention study, the genotype distribution of the subjects did not differ from that of other cohorts in Finland (20, 27, 36). In accordance with some previous studies (23–26, 55), the X^+ allele was associated with higher basal plasma total and LDL-cholesterol concentrations, although this association was not observed in all studies (59–61). The greatest diet-induced response in plasma total and LDL cholesterol was related to the dose of the X^- allele, which agrees with the results of some previous studies (35, 36). The X^-/X^- genotype has also been associated with a greater postprandial response than the X^+/X^+ genotype (62), although an opposite result was published for a Finnish population (27). Because the previous studies have given inconclusive or contradictory results, a meta-analysis could be expected to clarify the role of the apo B *Xba*I polymorphism as a modifier of diet-induced responsiveness. The meta-analysis did not support the results of our own dietary trial, however. Actually, the comparison of effect sizes between all genotype groups did not reveal any systematic effect of either *Xba*I allele; it can thus be concluded that the data available now do not strengthen the role of the apo B *Xba*I polymorphism in diet-induced plasma lipid changes.

The discrepancy between our results about the *Xba*I polymorphism and the meta-analysis may be due to several reasons. First, our finding could be due to chance alone (type 1 statistical error). In that case, the meta-analysis would increase the statistical power. Second, the comparability of the separate studies pooled in the meta-analysis may have varied according to the selection of study subjects, the dietary interventions carried out, or the compliance of the subjects, and the combination of data may hence have masked the true effect. However, all the studies included are considered reliable and, furthermore, it has been shown that the diet-induced responsiveness of plasma lipids does not differ between modifications of dietary cholesterol content or the quality of fat (53). Our study differed in some respects from the others. Here, the number of subjects with apo E3 and apo E4 phenotypes did not differ between the apo B *Xba*I genotype groups, even though the apo E phenotype itself did not explain the responsiveness to diet (6). In addition to the apo E phenotype, there may have been some other, so far unknown, confounding genetic factors. As long as the possible functionally effective mutation linked with the *Xba*I polymorphism is unknown, the role of the apo B *Xba*I polymorphism will remain unclear.

TABLE 6
Overall scaled effect sizes (*z* scores) and 95% CIs of apolipoprotein (apo) B genotypes on diet-induced responsiveness of plasma lipids and lipoproteins[1]

	Total cholesterol	LDL cholesterol	HDL cholesterol	Triacylglycerols	Apo B[2]	Apo A-I[2]
*Xba*I	418[3]	418	418	418	333	304
X^-/X^- (n = 160) × X^+/X^- (n = 190)	0.08 (−0.13, 0.29)[4]	0.21 (−0.01, 0.43)	0.22 (−0.01, 0.45)	0.05 (−0.16, 0.26)	0.22 (−0.02, 0.46)	0.38 (0.11, 0.65)[5]
X^+/X^- × X^+/X^+ (n = 68)	−0.01 (−0.30, 0.28)	−0.38 (−0.67, −0.09)[5]	−0.05 (−0.35, 0.25)	−0.03 (−0.31, 0.25)	0.09 (−0.23, 0.41)	−0.40 (−0.76, −0.04)[6]
X^-/X^- × X^+/X^+	0.17 (−0.13, 0.47)	−0.02 (−0.32, 0.28)	0.13 (−0.18, 0.44)	0.08 (−0.21, 0.37)	0.38 (0.04, 0.72)[6]	−0.06 (−0.43, 0.31)
*Eco*RI	380[3]	380	380	380	221	192
R^-/R^- (n = 10) × R^+/R^- (n = 110)	−0.85 (−2.13, 0.43)	−1.07 (−2.38, 0.24)	0.52 (−0.73, 1.77)	−0.68 (−1.94, 0.58)	−2.54 (−4.16, −0.92)[5]	−0.57 (−1.65, 0.51)
R^+/R^- × R^+/R^+ (n = 260)	−0.07 (−0.30, 0.16)	−0.08 (−0.31, 0.15)	0.10 (−0.14, 0.34)	0.10 (−0.10, 0.30)	0.00 (−0.30, 0.30)	0.05 (−0.28, 0.38)
R^-/R^- × R^+/R^+	−1.27 (−2.50, −0.04)[6]	−1.51 (−2.78, −0.24)[6]	0.00 (−1.14, 1.14)	0.18 (−0.97, 1.33)	−1.51 (−2.78, −0.24)[6]	−0.39 (−1.39, 0.61)
*Msp*I	276[3]	276	276	276	214	185
M^+/M^- (n = 33) × M^+/M^+ (n = 243)	0.32 (−0.05, 0.69)	0.37 (0.03, 0.71)[6]	0.05 (−0.32, 0.42)	0.39 (0.02, 0.76)[6]	0.06 (−0.36, 0.48)	0.91 (0.43, 1.39)[7]
Signal peptide	446[3]	446	446	446	383	383
D/D (n = 35) × I/D (n = 160)	0.13 (−0.29, 0.55)	0.39 (−0.03, 0.81)	0.01 (−0.41, 0.43)	0.19 (−0.20, 0.58)	−0.13 (−0.60, 0.34)	0.14 (−0.34, 0.62)
I/D × I/I (n = 251)	−0.08 (−0.28, 0.12)	−0.16 (−0.36, 0.04)	−0.07 (−0.27, 0.13)	0.02 (−0.17, 0.21)	−0.32 (−0.55, −0.09)[5]	−0.06 (−0.29, 0.17)
D/D × I/I	−0.04 (−0.44, 0.36)	0.21 (−0.20, 0.62)	0.10 (−0.30, 0.50)	−0.66 (−0.43, 0.31)	−0.45 (−0.92, 0.02)	0.18 (−0.29, 0.65)
*Bsp*I	151[3]	151	151	151	151	151
B^-/B^- (n = 8) × B^+/B^- (n = 64)	−0.65 (−1.21, −0.09)[6]	−0.80 (−1.37, −0.23)[5]	−0.34 (−0.89, 0.21)	0.98 (0.40, 1.56)[7]	−0.15 (−0.70, 0.40)	−0.66 (−1.22, −0.10)[6]
B^+/B^- × B^+/B^+ (n = 79)	0.24 (−0.11, 0.59)	0.18 (−0.17, 0.53)	−0.12 (−0.45, 0.21)	0.39 (0.06, 0.72)[6]	0.02 (−0.31, 0.35)	0.07 (−0.26, 0.40)
B^-/B^- × B^+/B^+	−0.70 (−1.61, 0.21)	−0.77 (−1.69, 0.15)	−0.16 (−1.06, 0.74)	0.74 (−0.17, 1.65)	−0.09 (−0.98, 0.80)	−0.85 (−1.77, 0.07)

[1] The apo B genotype groups are as follows: X^- and X^+, absence and presence of the *Xba*I restriction site; R^- and R^+, absence and presence of the *Eco*RI restriction site; M^- and M^+, absence and presence of the *Msp*I restriction site; *I* and *D*, insertion and deletion of the signal peptide; B^- and B^+, absence and presence of the *Bsp*I restriction site.
[2] Not determined in every dietary trial.
[3] *n*.
[4] *z* score; 95% CI in parentheses.
[5–7] Significance in meta-analysis: [5] $P < 0.01$, [6] $P < 0.05$, [7] $P < 0.001$.

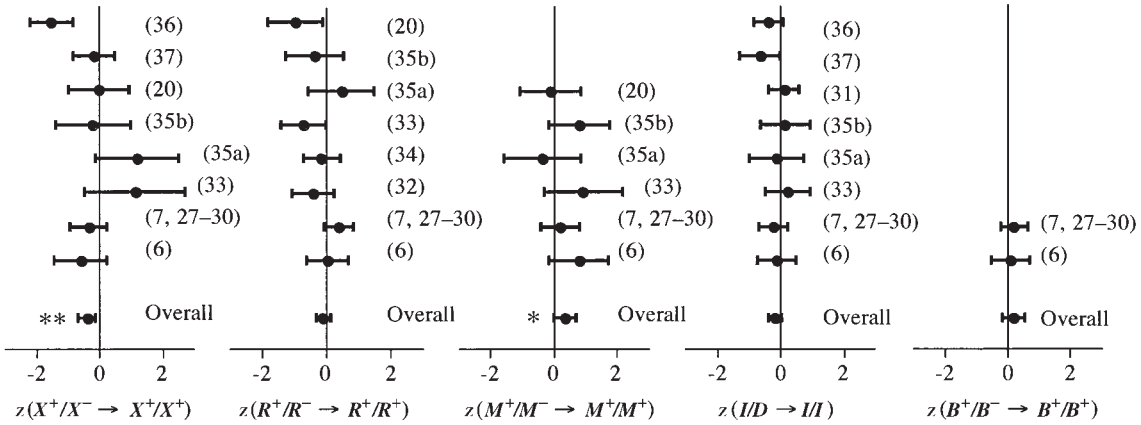

FIGURE 2. Comparison of diet-induced changes (*z* scores) in plasma LDL-cholesterol concentrations between 2 genotype groups (+/− and +/+). The apolipoprotein B genotypes are explained in more detail in Methods. The horizontal bars represent 95% CIs. Overall: meta-analysis of the mean weighted *z* scores over separate studies ($^{*}P < 0.05$, $^{**}P < 0.01$). The numbers in parentheses correspond to the reference number of the relevant dietary intervention study.

The apo B *Eco*RI polymorphism in exon 29 changes the amino acid sequence but its functional role is unclear. The R^- allele has been associated with high plasma total cholesterol concentrations and coronary heart disease in some studies (59–61, 63), but not in all (55, 64). The *Eco*RI polymorphism has been advocated as a "variability" gene affecting the plasma apo B concentration (65, 66). Moreover, the polymorphism is restrictive, ie, in identical twins it reduces the variability in the serum cholesterol concentration between co-twins (67). Because identical twins share the same genetic code, the reason for the difference in the cholesterol concentration between co-twins must lie in environmental factors such as diet.

In our dietary intervention study, plasma LDL-cholesterol concentrations increased during the high-fat diet by as much as 59 ± 10% in R^-/R^- subjects, whereas smaller responses were seen in R^+/R^+ (26 ± 2%) and R^+/R^- (39 ± 6%) subjects. In the present meta-analysis, the *Eco*RI polymorphism affected the response of the plasma total cholesterol, LDL-cholesterol, and apo B concentrations to diet. When switched from the low-fat to the high-fat diet, the R^-/R^- subjects had the greatest increase in plasma lipids. To confirm this result, a prospective study of a large number of R^-/R^- individuals should be carried out.

The apo B *Msp*I restriction site in codon 3611 is located in the same exon as the *Xba*I restriction site. The exact role of this variation in the apo B gene, which causes an amino acid substitution (arginine to glutamine), is unclear. Interestingly, in the screening population of the present study, the rare allele (M^-) was associated with a higher basal cholesterol concentration, whereas in our dietary intervention study, the common allele (M^+) was associated with a greater response in the plasma LDL-cholesterol, triacylglycerol, and apo A-I concentrations. This finding agrees with that of Series et al (68), who showed that the M^- allele is associated with diet-resistant hypercholesterolemia.

The apo B signal peptide insertion-deletion polymorphism alters the N-terminal signal sequence and may alter the apo B secretion and lipoprotein particle responses in the postprandial state (69, 70). The plasma lipid concentrations in subjects with the deletion allele may also be less responsive to an increased amount of dietary fat (69, 71). In the present meta-analysis, no significant effect was associated with either the insertion or the deletion allele in the plasma total, LDL-, or HDL-cholesterol response, but the apo B response was associated with the *D* allele. The role of the signal peptide polymorphism may be more important in the postprandial plasma lipid response than in the general dietary one.

The apo B *Bsp*I polymorphism in codon 71 causes a change in the amino acid sequence. Because this polymorphism has been determined in only 2 dietary intervention trials (6, 27), its role in dietary responsiveness remains unconfirmed.

The response in our own dietary trial was very good, the average percentage increase in plasma LDL cholesterol being 39% with the high-fat diet. The remarkable plasma lipid response in these free-living subjects was possible partly because all the food consumed was supplied by the hospital kitchen and because compliance was very good. As shown before, the apo E4 phenotype was not associated with dietary responsiveness in our trial (6) or in many others (7–14), although some reports showed greater responsiveness in subjects with the apo *E4* allele (15–21). Because our study population had more subjects with the apo E4 phenotype than did the other studies included in the meta-analysis, the apo E phenotype may have been a confounding factor in the meta-analysis.

In conclusion, a dietary intervention in 44 healthy subjects showed an association between the apo B *Xba*I, *Eco*RI, and *Msp*I RFLP-determined genotypes and the diet-induced plasma lipid response. The results of the meta-analysis supported our finding of the association between the apo B *Eco*RI and *Msp*I genotypes and responsiveness to diet. However, the determination of the apo B DNA polymorphisms does not now add much clinical value to dietary counseling. The heterogeneity of the study populations with respect to other possible genetic variations affecting plasma lipids, eg, the apo E polymorphism, may be a major confounder and should be taken into consideration in future studies.

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REFERENCES

1. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Ann Intern Med 1971;74:1–12.
2. Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361,662 men. Lancet 1986;2:933–6.
3. Solberg LA, Strong JP. Risk factors and atherosclerotic lesions. A review of autopsy studies. Arteriosclerosis 1983;3:187–98.
4. Katan MB, van Gastel AC, de Rover CM, van Montfort MA, Knuiman JT. Differences in individual responsiveness of serum cholesterol to fat-modified diets in man. Eur J Clin Invest 1988;18: 644–7.
5. Kesäniemi YA, Savolainen MJ, Uusitupa M, Sarkkinen E, Rantala M, Kervinen K. Apolipoprotein E phenotypes and response to dietary fat and cholesterol. In: Bray GA, Ryan DH, eds. Nutrition, genetics, and heart disease. Baton Rouge, LA: Lousiana State University Press, 1996:379–87.
6. Savolainen MJ, Rantala M, Kervinen K, et al. Magnitude of dietary effects on plasma cholesterol concentration: role of sex and apolipoprotein E phenotype. Atherosclerosis 1991;86:145–52.
7. Xu CF, Boerwinkle E, Tikkanen MJ, Huttunen JK, Humphries SE, Talmud PJ. Genetic variation at the apolipoprotein gene loci contribute to response of plasma lipids to dietary change. Genet Epidemiol 1990;7:261–75.
8. Cobb MM, Teitlebaum H, Risch N, Jekel J, Ostfeld A. Influence of dietary fat, apolipoprotein E phenotype, and sex on plasma lipoprotein levels. Circulation 1992;86:849–57.
9. Sarkkinen ES, Uusitupa MI, Pietinen P, et al. Long-term effects of three fat-modified diets in hypercholesterolemic subjects. Atherosclerosis 1994;105:9–23.
10. Zambon D, Ros E, Casals E, Sanllehy C, Bertomeu A, Campero I. Effect of apolipoprotein E polymorphism on the serum lipid response to a hypolipidemic diet rich in monounsaturated fatty acids in patients with hypercholesterolemia and combined hyperlipidemia. Am J Clin Nutr 1995;61:141–8.
11. Martin LJ, Connelly PW, Nancoo D, et al. Cholesteryl ester transfer protein and high density lipoprotein responses to cholesterol feeding in men: relationship to apolipoprotein E genotype. J Lipid Res 1993;34:437–46.
12. Jones PJ, Main BF, Frohlich JJ. Response of cholesterol synthesis to cholesterol feeding in men with different apolipoprotein E genotypes. Metabolism 1993;42:1065–71.
13. Glatz JF, Demacker PNM, Turner PR, Katan MB. Response of serum cholesterol to dietary cholesterol in relation to apolipoprotein E phenotype. Nutr Metab Cardiovasc Dis 1991;1:13–7.
14. Marshall JA, Kamboh MI, Bessesen DH, Hoag S, Hamman RF, Ferrell RE. Associations between dietary factors and serum lipids by apolipoprotein E polymorphism. Am J Clin Nutr 1996;63: 87–95.
15. Lehtimäki T, Moilanen T, Solakivi T, Laippala P, Ehnholm C. Cholesterol-rich diet induced changes in plasma lipids in relation to apolipoprotein E phenotype in healthy students. Ann Med 1992; 24:61–6.
16. Gylling H, Miettinen TA. Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men with different apoE phenotypes. J Lipid Res 1992;33:1361–71.
17. Mänttäri M, Koskinen P, Ehnholm C, Huttunen JK, Manninen V. Apolipoprotein E polymorphism influences the serum cholesterol response to dietary intervention. Metabolism 1991;40:217–21.
18. Tikkanen MJ, Huttunen JK, Ehnholm C, Pietinen P. Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high-fat diet. Arteriosclerosis 1990;10:285–8.
19. Lopez-Miranda J, Ordovas JM, Mata P, et al. Effect of apolipoprotein E phenotype on diet-induced lowering of plasma low density lipoprotein cholesterol. J Lipid Res 1994;35:1965–75.
20. Gylling H, Kontula K, Koivisto UM, Miettinen HE, Miettinen TA. Polymorphisms of the genes encoding apoproteins A-I, B, C-III, and E and LDL receptor, and cholesterol and LDL metabolism during increased cholesterol intake. Common alleles of the apoprotein E gene show the greatest regulatory impact. Arterioscler Thromb Vasc Biol 1997;17:38–44.
21. Miettinen TA, Gylling H, Vanhanen H, Ollus A. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. Arterioscler Thromb 1992;12:1044–52.
22. Glatz JF, Turner PR, Katan MB, Stalenhoef AF, Lewis B. Hypo- and hyperresponse of serum cholesterol level and low density lipoprotein production and degradation to dietary cholesterol in man. Ann N Y Acad Sci 1993;676:163–79.
23. Berg K. DNA polymorphism at the apolipoprotein B locus is associated with lipoprotein level. Clin Genet 1986;30:515–20.
24. Law A, Wallis SC, Powell LM, et al. Common DNA polymorphism within coding sequence of apolipoprotein B gene associated with altered lipid levels. Lancet 1986;1:1301–3.
25. Talmud PJ, Barni N, Kessling AM, et al. Apolipoprotein B gene variants are involved in the determination of serum cholesterol levels: a study in normo- and hyperlipidaemic individuals. Atherosclerosis 1987;67:81–9.
26. Aalto-Setälä K, Gylling H, Helve E, et al. Genetic polymorphism of the apolipoprotein B gene locus influences serum LDL cholesterol level in familial hypercholesterolemia. Hum Genet 1989;82: 305–7.
27. Tikkanen MJ, Xu CF, Hämäläinen T, et al. *Xba*I polymorphism of the apolipoprotein B gene influences plasma lipid response to diet intervention. Clin Genet 1990;37:327–34.
28. Xu CF, Tikkanen MJ, Huttunen JK, et al. Apolipoprotein B signal peptide insertion/deletion polymorphism is associated with Ag epitopes and involved in the determination of serum triglyceride levels. J Lipid Res 1990;31:1255–61.
29. Talmud PJ, Boerwinkle E, Xu CF, et al. Dietary intake and gene variation influence the response of plasma lipids to dietary intervention. Genet Epidemiol 1992;9:249–60.
30. Tikkanen MJ, Heliö T. Genetic variants of apolipoprotein B: relation to serum lipid levels and coronary artery disease among the Finns. Ann Med 1992;24:357–61.
31. Boerwinkle E, Brown SA, Rohrbach K, Gotto AM Jr, Patsch W. Role of apolipoprotein E and B gene variation in determining response of lipid, lipoprotein, and apolipoprotein levels to increased dietary cholesterol. Am J Hum Genet 1991;49:1145–54.
32. Abbey M, Belling B, Clifton P, Nestel PJ. Apolipoprotein B gene polymorphism associates with plasma cholesterol changes induced by dietary fat and cholesterol. Nutr Metab Cardiovasc Dis 1991; 1:10–2.
33. Friedlander Y, Kaufmann NA, Cedar H, Kark JD. *Xba*I polymorphism of the apolipoprotein B gene and plasma lipid and lipoprotein response to dietary fat and cholesterol: a clinical trial. Clin Genet 1993;43:223–31.
34. Abbey M, Hirata F, Chen GZ, et al. Restriction fragment length polymorphism of the apolipoprotein B gene and response to dietary fat and cholesterol. Can J Cardiol 1995;11(suppl):79G–85G.
35. Friedlander Y, Berry EM, Eisenberg S, Stein Y, Leitersdorf E. Plasma lipids and lipoproteins response to a dietary challenge: analysis of four candidate genes. Clin Genet 1995;47:1–12.
36. Pajukanta PE, Valsta LM, Aro A, Pietinen P, Heliö T, Tikkanen MJ. The effects of the apolipoprotein B signal peptide (ins/del) and *Xba*I polymorphisms on plasma lipid responses to dietary change. Atherosclerosis 1996;122:1–10.
37. Humphries SE, Talmud PJ, Cox C, Sutherland W, Mann J. Genetic factors affecting the consistency and magnitude of changes in plasma cholesterol in response to dietary challenge. Q J Med 1996;89:671–80.
38. MacCluer JW, Kammerer CM. Dissecting the genetic contribution to coronary heart disease. Am J Hum Genet 1991;49:1139–44 (editorial).

39. Clarke MJ, Stewart LA. Obtaining data from randomised controlled trials: how much do we need for reliable and informative meta-analyses? In: Chalmers I, Altman DG, eds. Systematic reviews. London: BMJ Publishing Group, 1995:17–36.
40. Rantala M, Savolainen MJ, Kervinen K, Kesäniemi YA. Apolipoprotein E phenotype and diet-induced alteration in blood pressure. Am J Clin Nutr 1997;65:543–50.
41. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
42. Ukkola O, Savolainen MJ, Salmela PI, von Dickhoff K, Kesäniemi YA. Apolipoprotein B gene DNA polymorphisms are associated with macro- and microangiopathy in non-insulin-dependent diabetes mellitus. Clin Genet 1993;44:177–84.
43. Huang LS, de Graaf J, Breslow JL. ApoB gene *Msp*I RFLP in exon 26 changes amino acid 3611 from Arg to Gln. J Lipid Res 1988;29:63–7.
44. Hallman DM, Visvikis S, Steinmetz J, Boerwinkle E. The effect of variation in the apolipoprotein B gene on plasmid lipid and apolipoprotein B levels. I. A likelihood-based approach to cladistic analysis. Ann Hum Genet 1994;58:35–64.
45. Visvikis S, Chan L, Siest G, Drouin P, Boerwinkle E. An insertion deletion polymorphism in the signal peptide of the human apolipoprotein B gene. Hum Genet 1990;84:373–5.
46. Henkel E, Stolz M. A newly drafted colour test for the determination of triglycerides convenient for manual and mechanized analysis. (Glycerolphosphate-oxidase-PAP method.) Frezenius Z Anal Chem 1982;311:451.
47. Allain C, Poon L, Chan C, Richmond W, Fu P. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470.
48. Lipid Research Clinics. Lipid and lipoprotein analysis. Manual of laboratory operations 1974. Washington, DC: US Department of Health, Education and Welfare, 1975. [US DHEW (NIH) publication 1:75-82.]
49. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18: 499–502.
50. Siervogel RM, Morrison JA, Kelly K, Mellies M, Gartside P, Glueck CJ. Familial hyper-alpha-lipoproteinemia in 26 kindreds. Clin Genet 1980;17:13–25.
51. National Center for Biotechnology Information at the National Library of Medicine. World Wide Web: http://www.ncbi.nlm.gov/PubMed/ (accessed December 23, 1999).
52. Hedges LV, Olkin I. Statistical methods for meta-analysis. Montreal: Academic Press, Inc, 1985.
53. Katan MB, Berns MA, Glatz JF, Knuiman JT, Nobels A, de Vries JH. Congruence of individual responsiveness to dietary cholesterol and to saturated fat in humans. J Lipid Res 1988;29:883–92.
54. Aalto-Setälä K, Kontula K, Mänttäri M, et al. DNA polymorphisms of apolipoprotein B and AI/CIII genes and response to gemfibrozil treatment. Clin Pharmacol Ther 1991;50:208–14.
55. Aalto-Setälä K, Tikkanen MJ, Taskinen MR, Nieminen M, Holmberg P, Kontula K. *Xba*I and c/g polymorphisms of the apolipoprotein B gene locus are associated with serum cholesterol and LDL-cholesterol levels in Finland. Atherosclerosis 1988;74:47–54.
56. Series J, Cameron I, Caslake M, Gaffney D, Packard CJ, Shepherd J. The *Xba*1 polymorphism of the apolipoprotein B gene influences the degradation of low density lipoprotein in vitro. Biochim Biophys Acta 1989;1003:183–8.
57. Houlston RS, Turner PR, Revill J, Lewis B, Humphries SE. The fractional catabolic rate of low density lipoprotein in normal individuals is influenced by variation in the apolipoprotein B gene: a preliminary study. Atherosclerosis 1988;71:81–5.
58. Demant T, Houlston RS, Caslake MJ, et al. Catabolic rate of low density lipoprotein is influenced by variation in the apolipoprotein B gene. J Clin Invest 1988;82:797–802.
59. Monsalve MV, Young R, Jobsis J, et al. DNA polymorphisms of the gene for apolipoprotein B in patients with peripheral arterial disease. Atherosclerosis 1988;70:123–9.
60. Hegele RA, Huang LS, Herbert PN, et al. Apolipoprotein B-gene DNA polymorphisms associated with myocardial infarction. N Engl J Med 1986;315:1509–15.
61. Genest JJ Jr, Ordovas JM, McNamara JR, et al. DNA polymorphisms of the apolipoprotein B gene in patients with premature coronary artery disease. Atherosclerosis 1990;82:7–17.
62. Lopez-Miranda J, Ordovas JM, Ostos MA, et al. Dietary fat clearance in normal subjects is modulated by genetic variation at the apolipoprotein B gene locus. Arterioscler Thromb Vasc Biol 1997;17:1765–73.
63. Myant NB, Gallagher J, Barbir M, Thompson GR, Wile D, Humphries SE. Restriction fragment length polymorphisms in the apo B gene in relation to coronary artery disease. Atherosclerosis 1989;77:193–201.
64. Ferns GA, Robinson D, Galton DJ. DNA haplotypes of the human apoprotein B gene in coronary atherosclerosis. Hum Genet 1988; 81:76–80.
65. Berg K. Role of genetic factors in atherosclerotic disease. Am J Clin Nutr 1989;49:1025–9.
66. Berg K, Powell LM, Wallis SC, Pease R, Knott TJ, Scott J. Genetic linkage between the antigenic group (Ag) variation and the apolipoprotein B gene: assignment of the Ag locus. Proc Natl Acad Sci U S A 1986;83:7367–70.
67. Berg K. Twin studies of coronary heart disease and its risk factors. Acta Genet Med Gemellol (Roma) 1987;36:439–53.
68. Series JJ, Gaffney D, Packard CJ, Shepherd J. Frequency of the *Xba*I, *Eco*RI, *Pvu*II and *Msp*I polymorphisms of the apolipoprotein B gene in relation to hypercholesterolaemia in the general population. Clin Chim Acta 1993;215:89–98.
69. Regis-Bailly A, Fournier B, Steinmetz J, Gueguen R, Siest G, Visvikis S. Apo B signal peptide insertion/deletion polymorphism is involved in postprandial lipoparticles' responses. Atherosclerosis 1995;118:23–34.
70. Peacock RE, Karpe F, Talmud PJ, Hamsten A, Humphries SE. Common variation in the gene for apolipoprotein B modulates postprandial lipoprotein metabolism: a hypothesis generating study. Atherosclerosis 1995;116:135–45.
71. Regis-Bailly A, Visvikis S, Steinmetz J, Fournier B, Gueguen R, Siest G. Effects of apo B and apo E gene polymorphisms on lipid and apolipoprotein concentrations after a test meal. Clin Chim Acta 1996;253:127–43.