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Apolipoprotein E polymorphism in the Netherlands and its effect on plasma lipid and apolipoprotein levels

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Summary. By isoelectric focusing of delipidated sera followed by immunoblotting we studied the apolipoprotein (apo) E polymorphism in 2018 randomly selected 35-years-old males from three different areas in the Netherlands. Comparison of the APOE allele (E*2, E*3, and E*4) frequencies estimated in this study with those reported for several other population samples showed that there are marked differences between the Dutch population and the populations of Japan, New Zealand, Finland, and the United States. These differences in APOE allele frequencies appeared to be mainly due to differences in frequencies of the E*2 allele (decreased in Japan and Finland; increased in New Zealand) and the E*4 allele (increased in Finland; decreased in Japan and the United States). No difference in APOE allele frequencies was found between the Dutch population and the populations of West Germany and Scotland. Measurements of plasma cholesterol and apo B and E concentrations showed that the E*4 allele is associated with elevated plasma cholesterol and apo B levels and with decreased apo E concentrations, whereas the opposite is true for the E*2 allele. In the Dutch population, the sum of average allelic effects of the common APOE alleles on plasma cholesterol and apo B levels is 6.8% and 14.2%, respectively, of the total population mean. The total average allelic effect on plasma apo E concentrations was more pronounced (50.1%), suggesting that the APOE alleles primarily affect apo E concentrations rather than plasma cholesterol and apo B levels. This hypothesis is sustained by the observation that for plasma apo E levels the genetic variance associated with the APOE gene locus contributed about 18% to the total phenotypic variance. For plasma cholesterol and apo B this contribution was only 1.4% and 2.3% and is relatively low as compared with that reported for other population samples.

Introduction

The apolipoprotein E (apo E) present on chylomicron and very low density lipoprotein (VLDL) remnants plays a central role in the hepatic metabolism of these particles, as this apolipoprotein is recognized with high affinity by hepatic lipoprotein receptors (Sherrill et al. 1980; Weisgraber et al. 1982).

Human apo E can be separated by isoelectric focusing into three major isoforms, E2, E3, and E4, which differ in pI by a single charge unit, apo E4 being the most basic and E2, the most acidic isoform. This heterogeneity is the result of three different APOE alleles, E*4, E*3, and E*2, at one single genetic locus (Zannis and Breslow 1981; Utermann et al. 1982).

Apo E3 is the most commonly occurring, or wild-type, form. Apo E4 is derived from E3 by a Cys → Arg substitution at position 112 and is designated E4(Cys_{112} → Arg). Apo E2 is derived from E3 by an Arg → Cys substitution at position 158 and is designated E2(Arg_{158} → Cys). Up till now a number of very rare mutants of apo E have been described. Some variants are either more basic than apo E4 or more acidic than apo E2 while others have the same electric charge as E2 or E3 (Rall et al. 1982, 1983; Innerarity et al. 1984; Yamamura et al. 1984a, b; Havel et al. 1983; Ghiselli et al. 1984; Havekes et al. 1986).

Several population studies on apo E polymorphism have been reported (Utermann et al. 1979, 1984a; Menzel et al. 1983; Wardell et al. 1982; Robertson and Cumming 1985; Ehnholm et al. 1986; Utermann 1987; Eto et al. 1986b; Ordovas et al. 1987). Although the APOE gene frequencies in some European and the North American populations seem quite similar, differences in APOE allele frequencies between different populations have also been reported (Eto et al. 1986a; Ehnholm et al. 1986; Utermann 1987; Boerwinkle et al. 1987).

From these population studies it has been firmly established that the apo E polymorphism affects plasma lipid levels. The E*2 allele appeared to be associated with subnormal plasma and LDL cholesterol levels whereas the E*4 allele is associated with elevated plasma cholesterol levels (Utermann et al. 1979, 1984a; Ehnholm et al. 1986; Utermann 1987; Ordovas et al. 1987).

In this paper we report the APOE phenotype and gene frequencies together with plasma levels of cholesterol, triglyceride, apo B, and apo E in 2018 randomly selected 35-years-old male individuals from the Dutch population. From this population study we calculated the average effects of APOE allelic substitution on plasma lipid and apolipoprotein levels as well as the contribution of the genetic variance associated with the APOE gene locus to the total phenotypic variance of these lipoprotein parameters.

Materials and methods

Collection of samples

Two thousand eighteen 35-year-old males were randomly selected from three different areas in the Netherlands. EDTA

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plasmas were obtained by venipuncture and stored at −20°C until the assays were performed.

Apo E phenotyping

Apo E phenotyping was performed using a recently developed rapid micromethod, which is based on isoelectric focusing of delipidated plasma samples followed by immunoblotting (Havekes et al. 1987) using a polyclonal anti-apo E antiserum as first antibodies. This method is especially suitable for large-scale screening.

Determination of plasma cholesterol, triglyceride, apo B, and apo E levels

Plasma cholesterol and triglycerides were measured enzymatically using Boehringer test-kits (cholesterol CHOD-PAP and triglyceride GPO-PAP, respectively). Apo B concentrations were measured by immunonephelometric assay (INA) as described by Rosseneu et al. (1981). Plasma apo E levels were measured by enzyme-linked immunosorbent assay (ELISA) as described by Bury et al. (1986).

Statistical analyses

Allele frequencies were estimated using the gene-counting method. Differences in APOE phenotype distribution between different population samples were determined by chi-square analysis. Differences in mean lipid and apolipoprotein levels between APOE phenotypic groups were evaluated by parametric (one-way analysis of variance) and nonparametric (Kruskal-Wallis) tests, In-pairs differences between APOE phenotypic groups were estimated using the procedure of Scheffe (parametric test) as well as the Mann-Whitney U-Wilcoxon rank sum (nonparametric) test. The average effects of the APOE alleles on the plasma cholesterol apo B and apo E concentrations and the variance of these parameters attributable to genotypic differences were estimated exactly according to the method of Sing and Davignon (1985).

Results

APOE phenotype distribution and allele frequencies

The sample of 2018 35-year-old males was randomly selected from three different geographic areas in the Netherlands. The APOE phenotype distribution and the APOE allele frequencies are presented in Table 1. The distribution of the different APOE phenotypes was in Hardy-Weinberg equilibrium ($\chi^2 = 2.82$; $P < 0.05$ at $\chi^2 < 11.0$; $df = 5$).

In Table 2 the apo E allele frequencies obtained in the present study are compared with those observed in other populations. In this table, only populations with more than 300 subjects are considered. A chi-square test of heterogeneity indicates statistically significant differences in the APOE allele frequency distribution between the different populations ($df = 16$; $\chi^2 = 149$; $P < 0.001$). Two-sample χ^2 analysis showed that the allele frequencies of the Dutch population differ highly significantly from those of the two Japanese populations and the populations of Finland, New Zealand ($P < 0.001$), and the United States ($P < 0.005$). No significant differences were found with the German populations and the

Table 1. APOE phenotype and allele frequencies in randomly selected 35-years-old males. χ^2 Hardy-Weinberg distribution is 2.82 ($df = 5$)

Phenotype	No. observed	Relative frequency (%)
E4/E4	59	2.9
E4/E3	512	25.4
E4/E2	45	2.2
E3/E3	1128	55.9
E3/E2	261	12.9
E2/E2	13	0.7
Total	2018	100
	Gene frequencies	
E*4	0.167	
E*3	0.750	
E*2	0.082	

population of Scotland. From the tables generated from the respective two-sample χ^2 analyses (tables not shown), we were able to calculate the separate contribution of the χ^2 data for each allele frequency to the total χ^2 value (Table 3).

From the data presented in Table 3, it is obvious that 50% of the differences in APOE allele frequencies, measurd as χ^2 values, between the Dutch and Japanese population from Asahikawa (Eto et al. 1986a) is due to the relatively low E*2 allele frequency in this Japanese population. However, in the other Japanese population (Utermann 1987) more than 80% of the difference between the Dutch population in apo E allele frequencies is due to a low E*4 allele frequency. From these results we calculated that the two Japanese populations differ significantly from each other regarding APOE allele frequencies ($df = 2$; $\chi^2 = 22.0$; $P < 0.001$). The APOE allele frequencies of the New Zealand population (Wardell et al. 1982) differ from that of the Dutch population mainly (78%) because of its high E*2 allele frequency. The Finnish population (Ehnholm et al. 1986) differs from the Dutch population in APOE allele frequencies both by a decreased E*2 allele and an increased E*4 allele frequency. For the American population (Ordovas et al. 1987) the E*4 allele fequency is the major contributor (72%) to the difference in APOE allele frequencies.

Effect of allele substitution at the APOE gene locus on plasma lipid and apolipoprotein levels

To evaluate whether the allelic variation at the APOE locus significantly affects the serum lipid and apolipoprotein levels, plasma cholesterol, triglycerides, and apo B and E were assayed. Table 4 presents the mean plasma cholesterol, triglyceride, apo B, and apo E levels in the different APOE phenotype groups. We used the one-way analysis of variance for testing the equality of the mean values among APOE phenotypes. As we could not find homogeneity of the variance among phenotypes and within phenotypes, we also estimated levels of significance using the nonparametric test of Kruskal-Wallis. Both statistical analyses showed that plasma cholesterol, apo B, and apo E levels differ among APOE phenotype groups. The triglyceride level was not affected by the APOE phenotype. Using the procedure of Scheffe (parametric test) as well as the Mann-Whitney U-Wilcoxon rank sum test (non-

Table 2. APOE gene frequencies in several random population samples. χ^2 values at $df = 2$ and P values of 0.01 and 0.001 are 9.21 and 13.95, respectively

Population sample	No. of subjects	APOE allele frequency			Hardy-Weinberg distribution		Difference from the Dutch population		Reference
		E*2	E*3	E*4	χ^2 ($df = 5$)	P	χ^2 ($df = 2$)	P	
The Netherlands	2018	0.082	0.751	0.167	2.83	N.S.	–	–	This study
Scotland	400	0.083	0.770	0.145	3.70	N.S.	2.31	N.S.	Cumming and Robertson (1984)
FRG (Münster)	1000	0.078	0.783	0.139	7.15	N.S.	8.71	N.S.	Menzel et al. (1983)
FRG (Marburg)	1031	0.077	0.773	0.150	7.24	N.S.	3.74	N.S.	Utermann et al. (1984)
USA	1204	0.075	0.786	0.135	15.30	<0.01	13.34	<0.005	Ordovas et al. (1987)
Finland	615	0.041	0.733	0.227	7.09	N.S.	41.02	<0.001	Ehnholm et al. (1986)
New Zealand	426	0.119	0.739	0.141	14.28	<0.05	13.96	<0.001	Wardell et al. (1982)
Japan (Asahikawa)	576	0.037	0.846	0.117	2.70	N.S.	50.05	<0.001	Eto et al. (1986a)
Japan	319	0.081	0.849	0.067	3.51	N.S.	37.41	<0.001	Utermann (1987)

Table 3. Relative contribution of the different APOE alleles to the total χ^2 value as estimated for the difference in allele frequencies between the Dutch and other populations. ↑, ↓, The contribution to the total χ^2 is due to an increased or decreased allele frequency, respectively

Apo E allele	Population (reference)				
	Japan[a]		New Zealand (Wardell et al. 1982)	Finland (Ehnholm et al. 1986)	USA (Ordovas et al. 1987)
	Eto et al. (1986)	Uterman (1987)			
E*2	0.50 ↓	0.01 ↓	0.78 ↑	0.54 ↓	0.06 ↓
E*3	0.21 ↑	0.17 ↑	0.01 ↓	0.01 ↓	0.22 ↑
E*4	0.29 ↓	0.82 ↓	0.21 ↓	0.45 ↑	0.72 ↓

[a] Two-sample χ^2 analysis showed a significant difference in APOE allele frequencies between both Japanese population samples ($df = 2$; $\chi^2 = 22.0$; $P < 0.001$)

Table 4. Mean plasma cholesterol, triglyceride, apo B, and apo E levels (in mg/dl) among different APOE phenotypes

Trait	Pooled	Phenotype						Significance	
		E2/E2	E3/E2	E4/E2	E3/E3	E4/E3	E4/E4	P^a	P^b
Cholesterol	215.5	209.0	202.4	207.4	216.8	219.0	225.2	<0.001	<0.001
	(41.7)[c]	(65.0)	(48.8)	(41.0)	(39.5)	(40.7)	(40.7)		
Triglyceride	154.2	155.8	158.4	168.7	149.7	161.1	152.0	0.186	0.580
	(90.9)	(71.8)	(98.2)	(110.6)	(85.0)	(98.9)	(75.1)		
Apo B	117.4	80.0	107.8	111.9	117.3	122.9	127.0	<0.001	<0.001
	(37.4)	(26.5)	(42.5)	(35.7)	(35.2)	(38.6)	(34.6)		
Apo E	5.6	13.8	7.3	6.7	5.5	5.0	4.4	<0.001	<0.001
	(2.4)	(3.8)	(2.9)	(2.1)	(2.0)	(2.0)	(2.0)		

[a] Level of significance estimated by one-way analysis of variance
[b] Level of significance estimated by the nonparametric test of Kruskal-Wallis
[c] Values in parentheses represent standard deviations

parametric test), we estimated the significance of the differences in mean lipid and apolipoprotein levels between the phenotypic groups in pairs (Table 5). Again, gross differences between the parametric and nonparametric test were not found. From the results presented in Table 5 it is obvious that there are statistically significant differences in mean levels of plasma cholesterol, apo B, and apo E among several APOE phenotype groups. Compared with the most common E*3 allele, the E*4 allele leads to elevated plasma cholesterol levels, whereas the E*2 allele is associated with a decreased plasma cholesterol concentration. The mean plasma cholesterol level of the E2/E2 homozygotes did not differ significantly from that of the other phenotypes, as 3 of the 13 E2/E2 homozygotes were hyperlipidemic.

For plasma apo B levels the statistically significant differences between the APOE phenotypes was more pronounced than for plasma cholesterol. The effect of allelic substitution at the APOE gene locus on apo E levels is the opposite to that on plasma cholesterol and apo B. The E*4 allele leads to decreased apo E levels, whereas the E*2 allele is strongly associated with an increased apo E concentration. When the mean apo B and apo E levels calculated for each phenotype group

Table 5. In pairs statistical analyses[a] of the differences in mean cholesterol, triglyceride, apo B, and apo E levels between the different APOE phenotype groups

Different from	Cholesterol						Apo B						Apo E					
	2/2	3/2	4/2	3/3	4/3	4/4	2/2	3/2	4/2	3/3	4/3	4/4	2/2	3/2	4/2	3/3	4/3	4/4
	Mean (mg/dl)						Mean (mg/dl)						Mean (mg/dl)					
	209.0	202.4	216.7	207.4	219.0	225.2	80.0	107.8	117.3	111.9	122.9	127.0	13.8	7.26	6.68	5.45	5.00	4.37
2/2								0	0	*0	*0	*0		*0	*0	*0	*0	*0
3/2				0*	0*	0*				*0	*0	*0				*0	*0	*0
4/2																*0	*0	*0
3/3																	*0	*0
4/3																		
4/4																		

[a] In pairs statistical analysis was performed by a parametric test (procedure Scheffe, $P<0.05$;*) and a nonparametric test (Mann-Whitney U-Wilcoxon Rank sum; $P<0.01$;0)

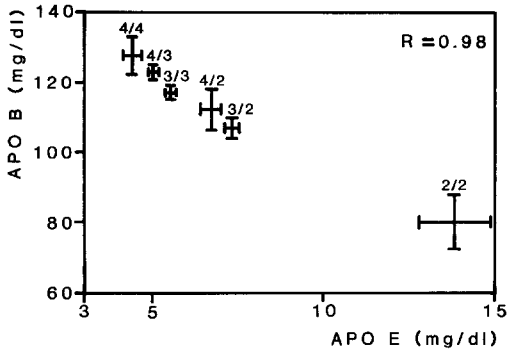

Fig. 1. Inverse relationship between mean apo B and apo E levels calculated in each phenotype group. *Vertical* and *horizontal bars* represent ± SEM for apo B and apo E, respectively

Table 6. Average effects (in mg/dl) of the common APOE alleles on plasma cholesterol, apo B, and apo E levels

Apo E allele	Average effect[a]					
	Cholesterol		Apo B		Apo E	
E*2	−9.7	(−4.5)	−11.4	(−9.7)	2.1	(37.6)
E*3	0.6	(0.3)	0.0	(0.0)	−0.1	(−1.8)
E*4	4.3	(2.0)	5.3	(4.5)	−0.6	(−10.7)

[a] Values in parentheses represent the average effects expressed as percentages of the respective population means

Table 7. Relative contribution of the genetic variance associated with the APOE locus to the total phenotypic variance

Lipoprotein parameter	Total genetic variance associated with the APOE locus (% of total phenotypic variance)	Total phenotypic variance $(mg/dl)^2$
Cholesterol	1.4	1739
Apo B	2.3	1399
Apo E	18.1	5.7

were considered separately, a strong inverse relationship was observed between apo B and apo E (Fig. 1).

We calculated the average effects of the three APOE alleles on the lipid and apolipoprotein levels (Table 6) according to the formula of Sing and Davignon (1985). The average effect of the E*2 allele is to reduce plasma cholesterol (−9.7 mg/dl) and apo B (−11.4 mg/dl) and to raise apo E levels (+2.1 mg/dl), whereas the E*4 allele induces an opposite effect (+4.3, +5.3, and −0.6 mg/dl, respectively). In comparison with the average effects of E*2 and E*4 alleles, the E*3 allele does not seem to influence these lipoprotein parameters. Table 6 demonstrates that the relative effect of the allelic substitution at the APOE locus on plasma apo B is more pronounced than the relative effect on total plasma cholesterol levels, whereas the effect on plasma apo E levels is most dramatic and the opposite to that on plasma cholesterol and apo B.

Relative contribution of the genetic variance associated with the APOE locus to the total phenotype variance

Table 7 presents the estimates of the relative contribution of the APOE gene to the total phenotypic variation of the measured lipoprotein parameters. The total genetic variance associated with the APOE locus contributed more than 18% to the total population variability in apo E levels. The effects of the genetic variation associated with the APOE gene on the variability of total cholesterol and plasma apo B levels are much less pronounced (1.4% and 2.3%, respectively).

Discussion

In this study we present the APOE phenotype distribution and allele frequencies for the Dutch population. The blood samples were randomly selected, from 35-year-old males living in three different geographical areas (Amsterdam, Leiden, and Doetinchem). The Amsterdam and Leiden samples represent urban populations, while the Doetinchem sample represents a more or less rural community.

Statistically significant differences in APOE phenotype distribution among the three areas were not found, indicating that the combined population sample (2018 individuals) is rep-

resentative for the whole Dutch population. This close similarity in phenotpye distribution between the three areas was not surprising since a genetic drift maintained by national-geographic or social-cultural isolation is highly unlikely in the densly populated Netherlands.

Comparison of the APOE allele frequencies estimated in this study with those reported for other population samples (Table 2) showed that there are marked differences between the Dutch population and that of Japan, New Zealand, Finland and the United States. As presented in Table 3, these differences are mainly due to differences in frequencies of the E*2 alleles (decreased in Japan and Finland; increased in New Zealand) and the E*4 allele (increased in Finland; decreased in Japan and the United States), whereas the frequencies of the E*3 allele appear to be rather similar for all population samples considered.

The differences in APOE allele frequencies among the Dutch, the Finnish, and the Japanese populations may be due to differences in ethnic background and geographical isolation and are similar to the differences between the German and Finnish populations described by Ehnholm et al. (1986) and the differences between the Caucasian and Japanese populations reported by Eto et al. (1986a). The statistically significant differences observed in APOE allele frequency among the Dutch population, a community in New Zealand (Wardell et al. 1982), and a U.S. population (Ordovas et al. 1987) (Table 2) might be due to a combination of population admixture and genetic drift.

It should, however, be noted that for both the New Zealand and U.S. population samples the observed APOE phenotype distributions differ significantly from the expected Hardy-Weinberg distributions (Table 2). We calculated that more than 90% of these differences can be attributed to differences between the observed and expected numbers of phenotypes exhibiting the E*4 allele (estimated data not presented). In particular, for the U.S. population this deviation from the expected Hardy-Weinberg distribution contributes to the observed difference in APOE allele frequency compared with that of the Dutch population sample (see also Table 3).

Boerwinkle et al. (1987) also observed statistically significant differences in APOE allele frequencies among different ethnically and/or geographically distinct populations. In contrast to the present study, they also considered relatively small population samples in this respect.

Several studies (Utermann et al. 1979, 1984a; Sing and Davignon 1985; Ehnholm et al. 1986; Eto et al. 1986b; Utermann 1987) have shown a association of the E*4 allele with elevated plasma cholesterol and apo B levels, whereas the E*2 allele appeared to be associated with decreased levels of plasma cholesterol and apo B. Reciprocally the E*4 allele is associated with a reduced plasma apo E level, whereas the E*2 allele leads to a highly significant increase in plasma apo E concentration. This effect of allelic substitution at the APOE locus on plasma cholesterol, apo B, and apo E levels has been confirmed in the present study of the Dutch population. The mechanisms underlying these associations are at present assumed to be the result of (I) a more efficient catabolism of chylomicron and VLDL remnants by the liver in individuals with the E*4 allele and (II) a less efficient catabolism of these lipoprotein particles in subjects exhibiting the E*2 allele due to a defect in binding of apo E2 to hepatic lipoprotein receptors. An enhanced uptake by the liver of chylomicron and VLDL remnants will supply the liver with extra cholesterol, thereby reducing the hepatic LDL receptor activity and thus elevating plasma LDL levels. Reciprocally, a diminished uptake of lipoprotein remnants will lead to an enhanced hepatic LDL receptor activity and eventually to a lower plasma LDL concentration. A more detailed description of this suggested mechanism was presented by Utermann (1985, 1987), who suggested that the APOE gene primarily affects apo E concentrations and thus that the metabolism of apo E-containing lipoproteins thereby regulates the LDL cholesterol and apo B concentrations in plasma. This hypothesis is clearly sustained by our data (Tables 6, 7) and the data published by Eto et al. (1986b) and Boerwinkle and Utermann (1988).

In the Dutch population, the contribtuion of the genetic variance associated with the APOE locus to the total phenotypic variance of plasma cholesterol and apo B levels (Table 7) is low compared with the results of Sing and Davignon (1985) and Boerwinkle and Utermann (1988). This difference can be ascribed almost exclusively to the pronounced total phenotypic variance of these parameters in the Dutch population compared with the Canadian and German population samples. The relatively low total phenotypic variance of the Canadian population sample is most probably due to a truncation of this population by selecting subjects whose plasma cholesterol and triglyceride had been normal on a previous visit. Such a preselection of subjects was not made for our population study. Truncation of the Dutch population sample (35-year-old men) afterwards, by excluding subjects with cholesterol and apo B and apo E values outside the range of the respective mean ± 2 SD, slightly reduced the total phenotypic variance but did not result in a marked increase in the relative contribution of the genetic variance to the total phenotypic variance of plasma cholesterol and apo B.

Consequently, we concluded that the total genetic variance of cholesterol and apo B associated with APOE locus is almost negligible in the Netherlands as compared with the Canadian and German population samples. The reason for these interpopulation differences in the contribution of genetic variance associated with the APOE locus to the total phenotypic variance is at present subject to speculation.

Irrespective of the convincing data concerning the effect of allelic substitutions at the APOE locus on plasma cholesterol, apo B, and apo E levels, a simple relationship between apo E phenotype and atherosclerotic risk has not yet been established. In some reports a lower frequency of the E*4 allele was found in patients with myocardial infarction (Utermann et al. 1984b), whereas an increased frequency was also reported (Menzel et al. 1983; Cumming and Robertson 1984). The elevated plasma (LDL) cholesterol levels in individuals with the E*4 allele is due to a more efficient catabolism of chylomicrons and VLDL in these subjects (Gregg et al. 1986), thereby preventing the accumulation of atherogenic chylomicron and VLDL remnants. If the LDL concentrations are only moderately elevated, E*4-bearing individuals will be at lower risk. In individuals with the E*2 allele, the LDL cholesterol levels are low due to an impaired VLDL and chylomicron remnant catabolism. These individuals are at lower risk as long as the levels of the atherogenic remnant particles remain below the level at which atherosclerotic risk increases. This relationship between the efficiency of chylomicron and VLDL remnant catabolism on the one hand and the level of LDL cholesterol on the other hand might be responsible for the lack of a general relationship between APOE phenotype and atherosclerotic risk notwithstanding the, for some populations, firm con-

tribution of the polymorphic APOE gene locus to the plasma cholesterol, apo B, and apo E levels.

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