review

Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis

J. Dallongeville,[1] S. Lussier-Cacan, and J. Davignon

Hyperlipidemia and Atherosclerosis Research Group, Clinical Research Institute of Montreal, and Department of Nutrition, University of Montreal, Montreal, Quebec, Canada

Abstract The relationship between apoE phenotype and plasma lipid levels was analyzed in the combined data of published studies. Accordingly, 45 population samples from 17 different countries were included in the analysis. The mean plasma values of cholesterol (CH), triglyceride (TG), and high density lipoprotein (HDL)-CH of the apoE 2/2, 3/2, 4/3, 4/4, and 4/2 groups were compared with the same parameters of the E 3/3 subset. The standardized difference between the plasma lipid concentrations of the apoE subgroups and of their respective apoE 3/3 control (Z-score), as well as their mean weighted value, were calculated for each study and in each subgroup. The analysis confirmed that subjects carrying the ε2 and ε4 alleles had, respectively, lower ($Z_{2/2} = -0.39$, $Z_{3/2} = -0.34$) and higher ($Z_{4/3} = 0.15$, $Z_{4/4} = 0.29$) plasma cholesterol values than subjects carrying the ε3/ε3 genotype. In addition, results indicated a consistent relationship between plasma TG levels and apoE phenotype among different populations. TG concentrations were significantly higher in apoE 2/2, 3/2, 4/3 and E 4/2 than in E 3/3 subsets ($Z_{2/2} = 0.42$, $Z_{3/2} = 0.14$, $Z_{4/3} = 0.13$, $Z_{4/2} = 0.19$). Further, this trend was found in samples of normolipidemic adults and children, in diabetic and obese individuals, as well as in hyperlipidemic subjects indicating an ubiquitous relationship. Concurrently, HDL-CH was significantly lower in the apoE 4/3 ($Z_{4/3} = -0.09$) than in the E 3/3 subset. In conclusion, the analysis suggests that in addition to elevated cholesterol levels, the cardiovascular risk that has been proposed for the apo E ε4 allele may, in individuals with the apoE 4/3 phenotype, be mediated by elevated plasma levels of triglycerides and low concentrations of HDL-cholesterol.—**Dallongeville, J., S. Lussier-Cacan, and J. Davignon.** Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J. Lipid Res.* 1992. **33:** 447–454.

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Apolipoprotein E (apoE) is a normal constituent of very low density lipoprotein (VLDL) and high density lipoprotein (HDL) (1). The structural gene locus of this apolipoprotein is polymorphic (2, 3). Three common alleles (ε2, ε3, and ε4) code for three apoE isoforms: E2, E3, and E4. ApoE2 and apoE4 differ from apoE3 by a single cysteine or arginine interchange at amino acids 158 and 112 of the 299 amino acid peptide chain (4).

The primary function of apoE yet known is to serve as a ligand for lipoproteins to cellular receptors (5,6). In addition, apoE, which contains a heparin-binding site (7), interacts with various proteoglycans and could be implicated in the anchoring process of lipoproteins to endothelial lipases (8, 9).

Since the pioneer work of Utermann, Pruin, and Steinmetz (10), a large number of studies have demonstrated a relationship between apoE phenotype and plasma lipid levels (11–48). This association was remarkably consistent among populations and families for plasma cholesterol and LDL-cholesterol levels (49). However, several of these studies lacked enough statistical power to establish a firm association with triglycerides and HDL-cholesterol. In our study, we have used the powerful tool of the meta-analysis to assess this particular relationship.

METHODS

Data collection

The publications included in this analysis were those that examined the relationship between apoE phenotype and plasma lipid and lipoprotein levels in male and female individuals. Articles were identified through the MedLine search facility and from the reference lists of articles that were surveyed. One study that assessed this relationship in neonates before onset of feeding (36) was not included because it represented a unique metabolic situation that was not representative of human lipoprotein physiology.

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein.

[1]To whom correspondence should be addressed at: IRCM, 110 Pine Avenue West, Montreal, Quebec, Canada, H2W 1R7.

Care was taken to include only one publication per survey (the one with the largest number of individuals or the most recently published paper of the same project). Studies presenting results that could not be used for the meta-analysis statistical calculations were not included in the analysis, i.e., pooled apoE phenotypes (10–12, 35) or missing standard deviation (15, 16, 29, 46).

The following information was collected from each publication: population characteristics (gender, mean and range of age, origin of sample), sampling methodology, methods of dealing with confounding variables, statistical approach, size of the sample, mean and standard deviation values of plasma cholesterol, triglycerides, and HDL-cholesterol classified by apoE phenotype. Data on LDL and VLDL-cholesterol were not available in most of the publications.

Following this procedure, 27 studies were analyzed that included 45 samples of both genders (13, 14, 17, 19–21, 24–28, 30–34, 37–45, 47, 48) (**Table 1**).

The number of subjects per sample ranged from 44 (40) to 2018 (31). The mean sample ages ranged from 21 (19) to 86 years (26). Samples were selected from 17 different countries (Austria, Benin, Canada, China, Finland, France, Germany, Hungary, Iceland, India, Japan, Malaysia, Mexico, the Netherlands, Sudan, Switzerland, the United States) and were thus multi-ethnic. In some cases, individuals were selected for cardiovascular disease (14, 17, 37), diabetes (20, 27, 33), obesity (32, 39), age (19, 26, 34, 41), or hyperlipidemia (13, 30, 47). Subjects were sampled from large populations or from close communities, from factory or institution workers, or were individuals examined in hospitals or out-patient clinics. Selection criteria had been used for lipid levels in a few studies that excluded hyperlipidemic subjects (30, 45), while in other reports, plasma lipids were adjusted for covariates (34, 38, 40, 42–45, 47).

For the purpose of our analysis, the standard deviation was calculated from the standard error of the mean whenever necessary. Cholesterol values were available in all the studies, triglyceride in all studies but two (43, 48), and HDL-cholesterol was missing in several studies (25, 27, 28, 31, 34, 40, 43, 48).

In order to evaluate the possible effect of apoE polymorphism on lipid levels, the mean plasma values of cholesterol (CH), triglyceride (TG), and high density lipoprotein (HDL)-CH of the apoE 2/2, 3/2, 4/3, 4/4, and 4/2 groups were compared with those of the E 3/3 subset. Because of the variability among studies in both the mean and standard deviation of these variables, the data were converted to a form suitable for comparison by calculating the Z-score. To estimate the overall effect of each polymorphism, the mean Z-score and the 95% confidence intervals were calculated in each subset by weighting the Z-score according to the number of subjects. Accordingly, studies with the larger number of subjects or smaller variance were more predictive of the mean Z-score.

The analysis of the data was carried out in two steps. First, the Z-score and mean weighted Z-score were calculated only for data obtained from randomly selected samples of populations. Thus, studies on diabetic (20, 27, 33) and obese (32, 39) subjects, on individuals with cardiovascular disease (14, 17, 37) or with hyperlipidemia (13, 30, 47) were excluded. Second, the analysis was conducted on the data from all selected samples.

Statistical analysis

The following methods were used for pooling and weighting different estimates of size effect (50). Differences between the less frequent apoE phenotypes and the apoE 3/3 subset were calculated in each study for cholesterol, triglycerides, and HDL-cholesterol. Values were expressed as a proportion of the pooled standard deviation (Z score):

$$Z_{i/j} = \frac{(m_{i/j} - m_{3/3})}{SD}$$

where $m_{i/j}$ represents the mean values of the variable of interest in apoE 2/2, apoE 3/2, apoE 4/3, apoE 4/4, or apoE 4/2 subgroups. SD is the pooled standard deviation for the apoE i/j and apoE 3/3 subsets and was calculated for each study as:

$$SD = \left[\frac{(n_{i/j}-1)\,SD^2_{i/j} + (n_{3/3}-1)\,SD^2_{3/3}}{(n_{i/j} + n_{3/3} - 2)}\right]^{1/2}$$

where $n_{i/j}$ and $n_{3/3}$ are the number of subjects and $SD_{i/j}$ and $SD_{3/3}$ are the standard deviations in apoE i/j and apoE 3/3 subgroups, respectively.

The overall estimate of the Z score was obtained by weighting each study estimate by $W_{i/j}$ calculated as follows:

$$W_{i/j} = \frac{\dfrac{(n_{i/j}\, n_{3/3})}{(n_{i/j} + n_{3/3})}}{\Sigma_1^N \dfrac{(n_{i/j}\, n_{3/3})}{(n_{i/j} + n_{3/3})}}$$

Thus the overall estimate of effect size for each variable (mean weighted Z-score) was:

$$\bar{Z}_{i/j} = \Sigma_1^N Z_{i/j} W_{i/j}$$

The 95% confidence interval was determined as follows:

$$\bar{Z}_{i/j} \pm 1.96\left[\frac{(n_{i/j}\, n_{3/3})}{(n_{i/j} + n_{3/3})}\right]^{1/2}$$

TABLE 1. Summary of the studies used in the present analysis

Authors	Year	Country	Region	Gender	Age	N	Pop	Ref
Bouthillier	1983	CAN	Montreal	m/f	22	64		13
Menzel	1983	GER	Munster	m/f	50	439	CTL	14
	1983	GER	Munster	m/f	37	1000	CTL	
	1983	GER	Munster	m/f	54	465	CAD	
Lenzen	1986	GER	Munster	m	53	570	CAD	17
Eto	1986	JAP	Asahikawa	m/f	49	132	OLD	19
	1986	JAP	Asahikawa	m/f	21	45	YOU	
Eto	1986	JAP	Asahikawa	m	52	105	DM	20
Ehnholm	1986	FIN	Helsinki	m/f		207		21
Ordovas	1987	USA	Framingham	m	22–71	599		24
	1987	USA	Framingham	f	22–71	605		
Tsuchiya	1987	JAP	Tokyo	m	47	140	CTL	25
Davignon	1987	CAN	Montreal	m	86	118	OCT	26
	1987	CAN	Montreal	f	84	118	OCT	
James	1987	SWI	Geneva	m/f		173	CTL	27
	1987	SWI	Geneva	m/f		195	DM	
Kequin	1987	CHI	Beijing	m/f	32	95		28
Kobori	1987	JAP	Kumamoto	m/f	30–69	188	CTL	30
	1987	JAP	Kumamoto	m/f	30–69	442	HLP	
Smit	1988	NL		m	35	2018		31
Fumeron	1988	FRA	Paris	m/f		172	OBS	32
Imari	1988	JAP	Fukuoka	m/f	38	91	CTL	33
	1988	JAP	Fukuoka	m/f	67	85	DM	
Gueguen	1989	FRA	Nancy	m/f	24–67	303		34
	1989	FRA	Nancy	m/f	4–19	155	CHI	
Kuusi	1989	FIN	Helsinki	m	52	91	CAD	37
Sepehrnia	1989	BEN	Benin City	m/f	30	365		38
Eto	1989	JAP	Asahikawa	m/f	50	87	OBS	39
Eichner	1989	USA	Allegheny	f	42–50	44	COL	40
Lehtimaki	1990	FIN		m/f	9–24	1572	CHI	41
Eichner	1989	USA	Allegheny	f	42–50	458	WHI	42
Kamboh	1991	MEX	Yucatan	m/f		95		43
Hanis	1991	USA	Starr	m/f		927		44
Xhignesse	1991	CAN	Montreal	m	20–59	374		45
	1991	CAN	Montreal	f	20–59	201		
Dallongeville	1991	CAN	Montreal	m/f	44	182	HTG	47
	1991	CAN	Montreal	m/f	38	98	FH	
Hallman	1991	AUS	Tyrol	m/f		469		48
	1991	ICE		m/f		185		
	1991	HUN		m/f		202		
	1991	CHI	Singapore	m/f		190		
	1991	JAP		m/f		319		
	1991	IND	Singapore	m/f		142		
	1991	SUD	Khartoum	m/f		103		
	1991	MAL	Singapore	m/f		118		

Year: year of publication. Country of sample origin: AUS, Austria; BEN, Benin; CAN, Canada; CHI, China; FIN, Finland; FRA, France; GER, Germany; HUN, Hungary; ICE, Iceland; IND, India; JAP, Japan; MAL, Malaysia; MEX, Mexico; NL, The Netherlands; SUD, Sudan; SWI, Switzerland; USA, United States. Gender: m, male; f, female. Age: mean age and range of age. Pop: sample type (CTL, control group; CAD, group selected for cardiovascular disease; individuals selected for age: OLD, age between 35 and 64 years, YOU, young age between 18 and 25, OCT, octogenarians; DM, diabetes mellitus; OBS, obesity; CHI, children; Sample selected for race: COL, black, WHI, white; Sample selected for hyperlipidemia (HLP): HTG, hypertriglyceridemia, FH, familial hypercholesterolemia.

RESULTS

The results presented in the following section summarize the analysis conducted on the total set of samples including those selected on morbidity criteria (obesity, diabetes, cardiovascular disease, and hyperlipidemia). These results and the conclusions are identical to those that were obtained when the analysis was limited to the samples of representative healthy populations.

A total number of 14,799 individuals were screened for cholesterol (0.6% apoE 2/2, 10.7% apoE 3/2, 62.4% apoE 3/3, 22.3% apoE 4/3, 2.3% apoE 4/4, and 1.6% apoE 4/2); 12,880 individuals were screened for triglycerides (0.5% apoE 2/2, 10.6% apoE 3/2, 62.0% apoE 3/3, 22.8% apoE 4/3, 2.3% apoE 4/4, and 1.6% apoE 4/2), and 9,751 individuals for HDL-cholesterol (0.3% apoE 2/2, 9.7% apoE 3/2, 63% apoE 3/3, 23% apoE 4/3, 2.5% apoE 4/4, and 1.5% apoE 4/2). As expected, percentages were much smaller in the E 2/2, E 4/2, and E 4/4 subsets. This was partially due to the naturally low frequency of these phenotypes but also to the fact that these subgroups were not reported in several studies. There-

fore, the reliability of the mean Z-score estimate is greater in apoE 3/2 and apoE 4/3 than in the apoE 2/2, 4/2, and 4/4 subsets.

The relationship between apoE phenotype and cholesterol levels is shown in **Fig. 1**. Results are illustrated in the form of Z-score. Compared to their respective apoE 3/3 controls, mean cholesterol concentrations were lower in all studies but one (1/19) for the apoE 2/2 subgroups and, in 38 of 44 samples, for the apoE 3/2 subgroups. Conversely, mean cholesterol concentrations were higher in 25 of 29 samples in the apoE 4/4 subgroup and, in 34 of 45 studies, in the apoE 4/3 subgroup. Results from the apoE 4/2 subgroup were more dispersed below and above the value of the apoE 3/3 subset. Consequently, mean Z scores for cholesterol levels were significantly lower and higher in the subjects carrying the ε2 and ε4 alleles, respectively, compared to homozygous ε3/ε3 (**Table 2**).

Fig. 2 presents the relationship between apoE phenotype and triglyceride levels. Compared to the apoE 3/3 subsets, mean triglyceride concentrations

TABLE 2. Mean Z-scores and 95% confidence intervals of the apoE 2/2, 3/2, 4/3, 4/4, and 4/2 groups relative to the apoE 3/3 group for cholesterol, triglycerides, and HDL-cholesterol

Apo E Phenotype	Mean Z score	95% Confidence Intervals	
Cholesterol			
2/2	−0.39	−0.61	−0.17
3/2	−0.34	−0.39	−0.29
4/3	0.15	0.11	0.19
4/4	0.29	0.18	0.40
4/2	−0.14	−0.27	−0.01
Triglycerides			
2/2	0.42	0.17	0.67
3/2	0.14	0.08	0.20
4/3	0.13	0.08	0.17
4/4	0.03	−0.09	0.15
4/2	0.19	0.05	0.33
HDL-cholesterol			
2/2	−0.03	−0.34	0.27
3/2	−0.01	−0.08	0.06
4/3	−0.09	−0.14	−0.04
4/4	0.09	−0.05	0.22
4/2	0.02	−0.15	0.19

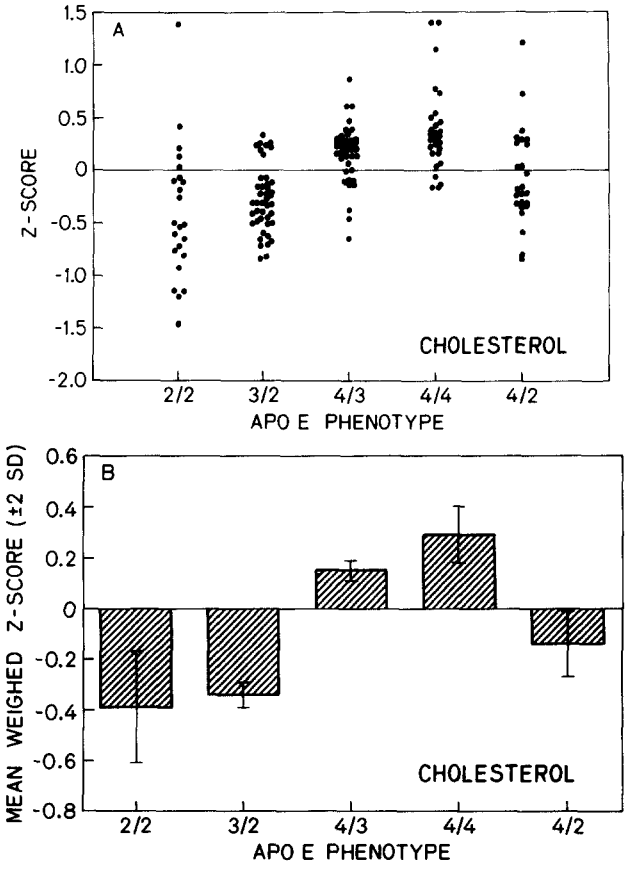

Fig. 1. Standardized difference between mean cholesterol values of individual apoE phenotype subgroups and the corresponding mean value of the apoE 3/3 subgroup (Z-score) for each study (panel A) and the mean weighted Z-scores ± 2 SD representing the 95% confidence intervals (panel B) for the same variable plotted by apoE phenotype.

were higher in 9 of 14 studies in the apoE 2/2 subgroups, in 30 of 36 studies in the apoE 3/2, in 30 of 36 in the apoE 4/3, and in 15 of 20 in the apoE 4/2 subsets. Triglyceride levels were either higher or lower in the apoE 4/4 than in the apoE 3/3 subset. The mean weighted Z score was significantly higher in subjects carrying the ε3/ε2, ε4/ε3 and ε4/ε2 genotypes than in subjects with the ε3/ε3 genotype (Table 2).

The relationship between apoE phenotype and HDL-cholesterol levels is illustrated in **Fig. 3**. There was no clear association between HDL-cholesterol concentrations and apoE phenotype in the apoE 2/2, E 3/2, and E 4/2 subsets. However, mean HDL-CH levels were found to be lower in 17 of 28 samples in apoE 4/3 and higher in 14 of 17 in the apoE 4/4 subgroups than their respective apoE 3/3 controls. Accordingly, only HDL-cholesterol of the apoE 4/3 subset was significantly lower than that of the apoE 3/3 group (Table 2).

DISCUSSION

Several studies have demonstrated an association between the apoE phenotype and lipid levels. However, among these, a significant and consistent relationship between the apoE phenotype and the plasma triglyceride and HDL-cholesterol levels was not demonstrated. Our work was specifically designed to assess this particular point.

First, by combining the results of individual studies, we have confirmed previous observations that subjects

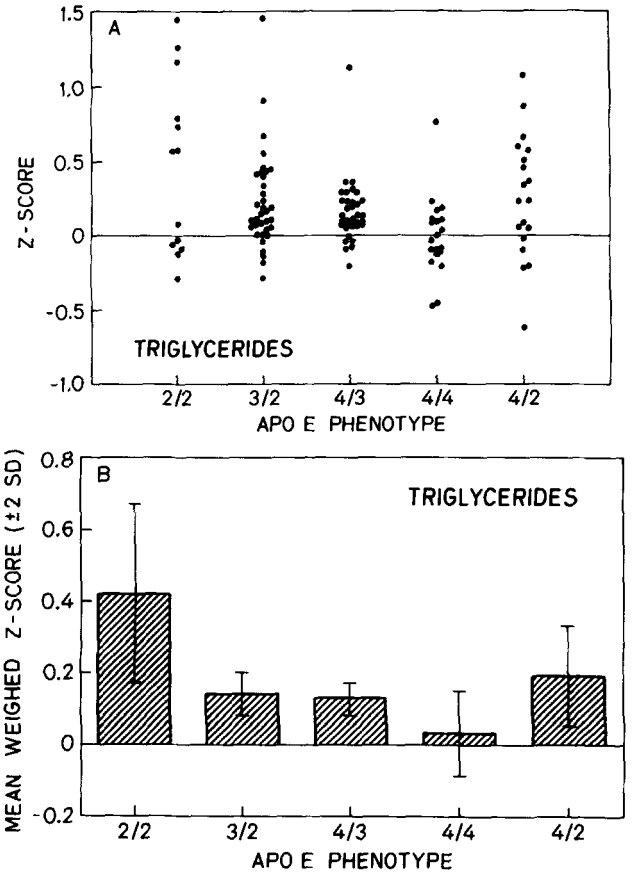

Fig. 2. Z-score values of each study analyzed in the meta-analysis (panel A) and mean weighted Z-score ± 2 SD representing the 95% confidence intervals (panel B) plotted by apoE phenotype for the variable triglyceride.

carrying the ε2 and ε4 alleles have, respectively, lower and higher levels of plasma cholesterol than individuals with the ε3/ε3 genotype. The relationship was found to be similar among the different samples indicating that the role of the apoE polymorphism in determining individual differences in plasma cholesterol is homogenous among different ethnic groups or metabolic situations. However, the amplitude of the effect of the various apoE alleles could differ among populations (48, 51).

Plasma triglyceride levels vary widely among and within individuals (52). This variability could mask a clear effect of apoE phenotype on triglyceride levels. Pooling the data from the diverse studies allowed us to improve the statistical power of the analysis. This clearly showed a consistent relationship between apoE phenotype and plasma triglyceride concentrations. Triglycerides were higher in subjects carrying the ε2 allele and subjects with the ε4/ε3 genotype than in individuals with the ε3/ε3 genotype. Not unexpectedly, more scatter was found for triglyceride than for cholesterol standardized scores. Despite this variability, the relationship was observed in populations of different origins and in selected samples such as children (34, 41), obese individuals (32, 39), diabetic (20, 27, 33) and hyperlipidemic subjects (13, 30, 47), thus demonstrating an ubiquitous relationship.

The higher levels of triglycerides observed in subjects carrying the ε2 allele can be explained by their slower plasma clearance of chylomicron and VLDL remnants (53–55). In addition, a defect in the lipolysis of VLDL from subjects with the apoE 2/2 phenotype has been observed (56, 57) suggesting that an alteration in the lipolytic process may also contribute to plasma triglyceride accumulation in subjects carrying the ε2 allele.

One of the most striking findings from our work was the positive, homogeneous, and significant relationship between the apoE 4/3 phenotype and triglyceride levels, suggesting a delayed catabolism relative to production in this subset. In support of our results, previous reports have indicated that the ε4 allele may be implicated in the pathogenesis of Type V hyperlipoproteinemia (58–60). Further, another study has

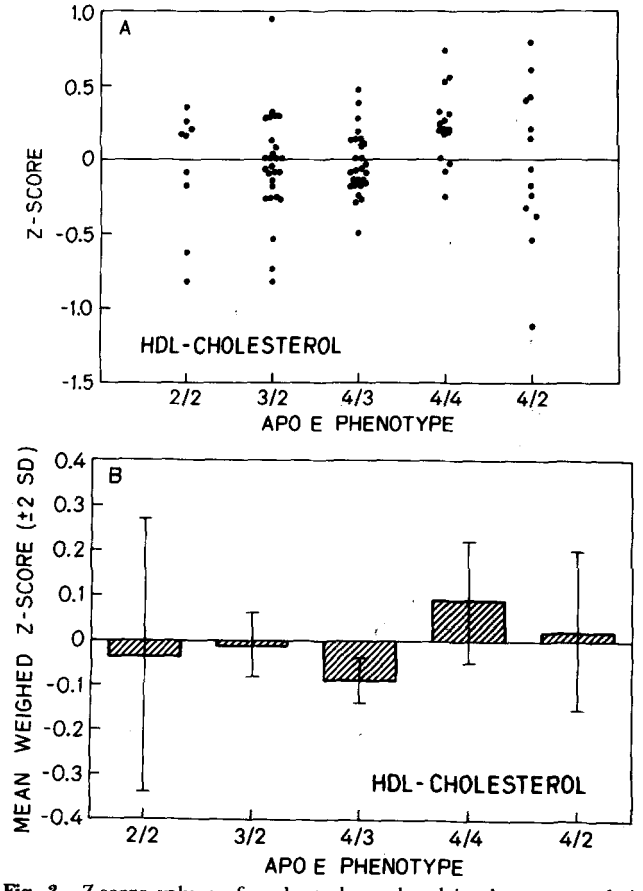

Fig. 3. Z-score values of each study analyzed in the meta-analysis (panel A) and mean weighted Z-score ± 2 SD representing the 95% confidence intervals (panel B) plotted by apoE phenotype for the variable HDL-cholesterol.

shown that subjects who are heterozygous for an apoC-II gene defect and who carry the apoE ε4/ε3 genotype have significantly higher triglyceride levels than their affected and nonaffected relatives having apoE 3/3 or 3/2 phenotypes (61). Based on these observations, it is tempting to hypothesize that apoE4, which is preferentially associated with VLDL (62–64), interferes with plasma lipase activities and/or with the triglyceride removal system resulting in delayed lipolysis and/or clearance of plasma triglycerides. This hypothesis gains support through in vitro experiments demonstrating that apoE modulates lipoprotein lipase and hepatic lipase activities (65–71).

An intriguing observation is that of subjects carrying the ε4/ε4 genotype who have triglyceride levels similar to those of ε3/ε3 genotype carriers. As mentioned above, there were fewer individuals in the apoE 4/4 compared to the apoE 4/3 and apoE 3/2 subsets. Therefore it is possible that an effect of the allele ε4 is not revealed in this subgroup due to the smaller number of individuals. An alternative hypothesis is that a mild putative defect in lipolysis observed in ε4/ε3 is compensated by a faster clearance of VLDL remnants in subjects carrying the ε4/ε4 genotype (54, 55). This would result in significantly lower triglyceride levels in this subset compared to that bearing the ε4/ε3 genotype.

Plasma HDL-cholesterol concentrations depend on the combination of HDL apolipoprotein production, VLDL lipolysis, and plasma lipid transfer protein activity. As a consequence, VLDL and HDL metabolism are intimately related. In this meta-analysis we showed conclusively that HDL-cholesterol levels were significantly lower in apoE 4/3 subjects than in those with the E 3/3 phenotype, consistent with a possible alteration of triglyceride metabolism in apoE 4/3 subjects.

In summary, we performed a meta-analysis on the relationship between plasma lipid levels and apoE phenotype. This provided new results not otherwise obtainable using the classical statistical approaches. Of particular interest was the demonstration of a consistent relationship between the apoE phenotype and triglyceride levels. The ε4 allele has been proposed as a risk factor for cardiovascular disease (49). Higher cholesterol levels were thought to mediate this risk. Our observations also indicate that subjects carrying the ε4/ε3 genotype, in addition to elevated plasma cholesterol concentrations, have higher triglyceride and lower HDL-cholesterol levels than subjects carrying the ε3/ε3 genotype. Indeed, this may contribute to the higher cardiovascular risk in this particular subset of individuals.

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