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Apolipoprotein E polymorphism in men and women from a Spanish population: allele frequencies and influence on plasma lipids and apolipoproteins

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Abstract

The apolipoprotein (apo) E phenotype and its influence on plasma lipid and apolipoprotein levels were determined in men and women from a working population of Madrid, Spain. The relative frequencies of alleles ε2, ε3 and ε4 for the study population ($n = 614$) were 0.080, 0.842 and 0.078, respectively. In men, apo E polymorphism was associated with variations in plasma triglyceride and very low-density lipoprotein (VLDL) lipid levels. It was associated with the proportion of apo C-II in VLDL, and explained 5.5% of the variability in the latter parameter. In women apo E polymorphism was associated with the concentrations of plasma cholesterol and low-density lipoprotein (LDL) and high-density lipoprotein (HDL) related variables. The allelic effects were examined taking allele ε3 homozygosity as reference. In men, allele ε2 significantly increased VLDL triglyceride and VLDL cholesterol concentrations, and this was accompanied by an increase of the apo C-II content in these particles. Allele ε4 did not show any significant influence on men's lipoproteins. In women, allele ε2 lowered LDL cholesterol and apo B levels, while allele ε4 increased LDL cholesterol and decreased the concentrations of HDL cholesterol, HDL phospholipid and apo A-I. These effects were essentially maintained after excluding postmenopausal women and oral contraceptive users from the analysis. In conclusion: (1) the population of Madrid, similar to other Mediterranean populations, exhibits an underexpression of apo E4 compared to the average prevalence in Caucasians, (2) gender interacts with the effects of apo E polymorphism: in women, it influenced LDL and HDL levels, whereas in men it preferentially affected VLDL, and (3) allele ε2 decreased LDL levels in women, while it increased both VLDL lipid levels and apo C-II content in men, but, in contrast to allele ε4, it did not show an impact on HDL in either sex.

Keywords: Apolipoprotein E polymorphism; Lipoproteins; Cholesterol; Triglyceride; Apolipoproteins; Gender

1. Introduction

Apolipoprotein (apo) E, as a common constituent of chylomicrons, very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL), plays a key role in the metabolism of plasma lipoproteins. In humans, apo E is coded by three major codominant alleles (ε2, ε3 and ε4) for a single gene locus, whose products (apo E2, apo E3 and apo E4, respectively) give rise to six different phenotypes [1,2].

By influencing plasma cholesterol concentrations, apo E polymorphism is recognized as one of the most important genetic determinants for coronary disease in the general population [3,4]. Compared to apo E3, the E4 isoform is associated with higher levels of plasma and low-density lipoprotein (LDL) cholesterol and apo

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B [3,4]. Moreover, apo E4 may decrease HDL cholesterol and raise triglyceride levels [5]. Consistent with these effects, apo E4 is associated with a greater risk of coronary disease [6]. It is significant that this relationship has been reported to persist after adjustment for traditional risk factors, including LDL and HDL cholesterol [7]. On the other hand, the E2 isoform has opposite effects on cholesterol levels to those of apo E4 [3,4]. Despite this, according to a recent meta-analysis, a cardioprotective role of apo E2 is not certain [6], probably because of its tendency to increase triglyceride levels [5]. This is primarily attributed to the lower affinity of apo E2 for the LDL receptor than apo E3 and apo E4, which results in a delayed clearance of apo E2-bearing remnant particles [1,2]. An impaired lipolytic conversion of VLDL into LDL may also be involved [8–10]. The accumulation of remnant particles is most pronounced when ε2 is found in homozygosity, which, in combination with certain additional disorders, may lead to type III hyperlipoproteinemia, a pathology associated with increased peripheral and coronary atherosclerosis [11]. In keeping with this, there is a growing body of evidence for a significant role of triglyceride-rich (TGR) lipoproteins in the development of atherosclerosis [12,13].

Apart from apo E, the C apolipoproteins are important determinants of the metabolic fate of TGR particles. Apo C-II is the physiological activator of lipoprotein lipase (LPL), the key enzyme for plasma triglyceride hydrolysis, and apo C-III has been suggested to be an inhibitor of LPL [14,15]. On the other hand, the different apo C peptides have been shown to interfere with the recognition of apo E by the LDL receptor [16,17] and the LDL receptor-related protein (LRP) [18,19]. Therefore, the interplay between the different apolipoproteins contained in VLDL may be relevant for the catabolic processing of these particles in vivo. In fact, the relative contents of apo C-II and apo C-III correlate with the fractional catabolic rate (FCR) of VLDL triglyceride [20]. Since apo E polymorphism directly affects plasma VLDL catabolism, it would be interesting to know whether this is accompanied by changes in the VLDL apo C's composition.

The following two important features should be considered in relation to the impact of apo E polymorphism on lipid levels in a given population. First, the magnitude of the effects of apo E polymorphism on lipid and apolipoprotein traits differs considerably among populations; this has led to the notion that factors like ethnic origin, lifestyle and diet interact with those effects [21]. Similarly, evidence for an interaction of gender with the influences of apo E polymorphism has been obtained, but, again, the expression of this phenomenon is not uniform from one human group to another [21]. Second, there is a wide variation in the distribution of the apo E alleles across populations around the world [3,4]. In Europe, the frequency of allele ε4 is distributed along a decreasing north-south gradient, which parallels the gradient of ischaemic heart disease (IHD) mortality rates [4,22]. Interestingly, the decline of allele ε4 frequency with latitude has been made evident even within specific countries such as France [23] and mainland Italy [24]. However, the lowest prevalence of allele ε4 (0.052) registered in the latter country, and the lowest reported so far for a Caucasian population, was encountered in Sardinia [24], a Mediterranean island found to the west of mainland Italy.

The underexpression of apo E4 has been suggested as a contributing factor to the low incidence of IHD exhibited by Mediterranean countries [25,26]. Accordingly, Spain enjoys one of the lowest IHD mortality rates among European populations [27]. Nevertheless, a recent report has revealed a considerable variability in these rates across the Spanish geography [28]. Some differences in dietary habits that may contribute to this variability have been described [29]. However, data on the prevalence of the apo E isoforms as well as their impact on lipid levels in the different Spanish regions are still scarce.

In the present study the frequency of the three most common apo E isoforms was measured in a population from Madrid, located in the center of mainland Spain and pertaining to a region that enjoys one of the lowest mortality rates by IHD in that country [28]. On the other hand, we determined the influence of apo E polymorphism on lipoprotein levels and the apolipoprotein composition of VLDL in males and females among that population.

2. Methods

2.1. Study population

Subjects were recruited from the working population of the Hospital Ramón y Cajal, Madrid. Volunteers were randomly selected, the participation rate being 82%. The male-to-female ratio of the subjects that entered the study (221 men and 393 women) is representative of the working population in such a center (men 40.3%, women 59.7%). The average age for males was 42.5 ± 0.7 years (mean $\pm$ S.E., range 24–67) and for females 41.0 ± 0.5 (range 24–72). An appropriate questionnaire was provided to the subjects in order to record their smoking habits, menopausal status, oral contraceptive use and medical history. Dietitians assessed alcohol consumption and dietary habits through a week-based food record. Body weight and height were measured and the body mass index (BMI) calculated. Subjects taking hypolipidemic medication were not included in the study.

2.2. Laboratory analysis

Venous blood was drawn from fasting, sitting subjects between 08:30 and 09:30 h, and 1 mg Na_2-EDTA/ml of blood was immediately added. After centrifugation, the plasma was separated and part of it used to isolate VLDL ($d < 1.006$ kg/l) by ultracentrifugation. HDL lipids were measured after precipitation of apo B-containing lipoproteins with phosphotungstic acid and Mg. LDL lipids were determined by subtracting VLDL and HDL lipids from plasma values. Cholesterol, triglyceride and choline-containing phospholipids were measured enzymatically (Menarini, Firenze, Italy) with a Technicon Autoanalyzer. Plasma apo B and apo A-I concentrations were quantified by immunonephelometry (Array System, Beckman Instruments). Lipoprotein (a) (Lp(a)) was assayed by ELISA (TintElize Lp(a), Biopool, Umëa, Sweden).

To analyze the apolipoprotein content of VLDL, the apo E, apo C-II and apo C-III isoforms were quantified by isoelectric focusing of delipidated VLDL and scanning the gel as previously described [30]. Apo E phenotyping was performed according to the criteria of Ordovás et al. [31]. As a control, an aliquot of a frozen VLDL stock solution from a pool of normolipidemic sera was run in all the gels. Blind apo E phenotyping from repeated blood samples drawn from some of the subjects resulted in the same assigned phenotype. To further test the reliability of our phenotyping procedure, after completing the collection of all the samples, 30 of the participants comprising all the major apo E phenotypes were randomly selected and called for a new blood sample. Subsequently, DNA of the cells was extracted and apo E genotyping was performed at the laboratory of Dr Fernando Valdivieso (Department of Molecular Biology, Universidad Autónoma, Madrid) [32]. In all cases the resulting genotypes confirmed previously assigned phenotypes.

2.3. Statistical analysis

Allele frequencies were determined by the gene-counting method. Frequency distributions of the phenotypes were analyzed by the χ^2 goodness of fit test.

To examine the association between the apo E phenotype and lipid and apolipoprotein traits the subjects were divided into three groups: the E2 group (E2/2 and E3/2 subjects), the E3 group (E3/3 subjects) and the E4 group (E4/4 and E4/3 subjects). The subjects with apo E4/2 phenotype were not included in this analysis. The analyses were performed separately for men and women. Significant covariates ($P < 0.05$) for each dependent variable were identified using stepwise regression to select backwards the most parsimonious set of covariates. The possible covariates considered were the linear effects of age, BMI, smoking, alcohol consumption and the polyunsaturated to saturated fatty acid intake ratio in men, and the same variables together with menopausal status were considered in women. Each dependent variable was then adjusted for its respective set of covariates. Analysis of variance (ANOVA) was used to compare lipid and apolipoprotein levels between phenotypes. When statistically significant differences arose ($P < 0.05$), the differences between each pair of groups were assessed by the Newman–Keuls test.

The independent effects of alleles ε2 and ε4 on each lipoprotein parameter taking ε3 homozygosity as the reference were estimated by stepwise regression analysis. For this purpose, two dummy variables coded respectively with the number (0, 1, 2) of alleles ε2 and ε4 in each phenotype were used. The regression coefficients of the alleles were adjusted for their corresponding confounders. For this, the above mentioned variables were also included in the initial model to select backwards those variables that either changed the coefficients of alleles ε2 or ε4 by more than 10% or whose inclusion in the model conferred or removed statistical significance ($P < 0.05$) to the allelic effects. The goodness of the fit of the final model was tested by an F ratio. The proportion of the variance of each lipoprotein trait attributable to apo E polymorphism (R^2) was calculated as the ratio of the sum of squares due to the polymorphism to the covariate-adjusted total sum of squares (total sum of squares minus the covariate sum of squares). A partial F was used to test the statistical significance of the resulting R^2 value.

Before the statistical analyses, plasma and VLDL triglyceride, VLDL cholesterol and Lp(a) concentrations were log transformed given their skewed distribution. Statistical analyses were performed using the Statgraphics software, version 5 (Statistical Graphics).

3. Results

The distribution of the observed frequencies of the different apo E phenotypes (Table 1) was in Hardy-Weimberg equilibrium in the total population ($\chi^2 = 1.19$, $P = 0.76$, 3 df), in men ($\chi^2 = 0.24$, $P = 0.89$, 2 df) and in women ($\chi^2 = 0.92$, $P = 0.82$, 3 df). There was no statistical difference in the distribution between either sex ($\chi^2 = 0.38$, $P = 0.83$, 2 df). The resulting allele frequencies are also shown in Table 1. Allele ε3 presented a relative frequency of 84% in the whole population, whereas alleles ε2 and ε4 showed almost equal frequency (8%).

To analyze the association of the apo E phenotype with lipid and apolipoprotein levels in whole plasma and lipoprotein fractions, subjects were grouped as apo E2 carriers (E2/2 and E3/2 subjects), apo E3 homozygotes and apo E4 carriers (E4/4 and E4/3 sub-

jects). The mean values after adjustment for confounders in men and women are shown in Tables 2 and 3, respectively. In men, the variations in plasma and VLDL triglyceride and VLDL cholesterol levels were significantly associated with the apo E phenotype, being higher in the apo E2 carriers than in the apo E3/3 subjects. LDL cholesterol showed a tendency to decrease in the E2 group, but this variable was not significantly associated with apo E polymorphism (Table 2).

The influence of apo E polymorphism on the apolipoprotein composition of VLDL was also studied. In men, a highly significant association between apo E polymorphism and the apo C-II content of these particles was found, the content rising in the E2 group compared to the E3 and E4 groups (Table 2). This phenomenon was attributable to the non-sialilated form of such a peptide, i.e. apo $C\text{-}II_0$ (results not shown), which averaged 77% of total VLDL-apo C-II in men. On the other hand, apo E phenotype had no influence on the apo E or apo C-III contents (Table 2), nor on the distribution of the different non-sialilated and sialilated forms of apo C-III (results not shown).

Lipid and apolipoprotein values corresponding to women are shown in Table 3. The effect of apo E polymorphism in these subjects is clearly distinguishable from that found in men. In women, plasma cholesterol, LDL cholesterol and apo B levels were significantly decreased in the presence of apo E2. Apo E4 showed an opposite effect on these variables, although only LDL cholesterol was statistically higher in the E4 than in the E2 and E3 groups. Besides, apo E4 carriers had significantly lower HDL cholesterol and HDL phospholipid concentrations. Apo A-I levels were also significantly associated with apo E phenotype, being lower in the E4 subjects, although no statistically significant differences between each pair of groups appeared. In parallel to the lack of effect of apo E alleles on VLDL lipids in women, there was no association between apo E polymorphism and the apolipoprotein composition of such particles. On the other hand, and as has been observed in men, in women this polymorphism was not associated with variations in Lp(a) concentrations (Tables 2 and 3).

In order to estimate the independent quantitative effects of alleles ε2 and ε4 as compared to ε3 homozygosity on lipoprotein parameters, stepwise variable selection analysis was performed. For this, a model was used in which an additive and codominant effect of alleles ε2 and ε4 was specified. Tables 4 and 5 show the results obtained after adjustment for the indicated confounding variables for the allelic effects. As shown in Table 4, in men allele ε2 significantly increased plasma and VLDL triglyceride and VLDL cholesterol concentrations. Moreover, allele ε2 increased the apo C-II content of VLDL by 4%. With regard to allele ε4, it did not show any significant effect on men's lipoproteins (Table 4).

Apo E polymorphism explained 4.6% ($P < 0{,}01$), 6.0% ($P < 0.01$) and 5.9% ($P < 0.01$) of the variability of plasma triglyceride, VLDL triglyceride and VLDL cholesterol levels, respectively. It also accounted for 5.5% ($P < 0.01$) of that of the apo C-II content of VLDL.

The possibility exists that the raising effect of allele ε2 on the proportion of apo C-II in VLDL was dependent on the parallel allelic effect on VLDL lipid levels. To resolve this query, a multiple regression analysis including alleles ε2 and ε4 and the concentration of VLDL triglyceride as independent variables was performed. According to this model, VLDL triglyceride showed a highly significant association with the apo C-II content (regression coefficient $(\beta) = 6.40 \pm 1.41$, $P < 0.001$). The raising effect of allele ε2 on the latter

Table 1
Apo E phenotype and allele frequencies

	Total ($n = 614$)		Men ($n = 221$)		Women ($n = 393$)	
	n	%	*n*	%	*n*	%
Phenotype						
E2/2	2	0.3	0	0	2	0.5
E3/2	92	15.0	32	14.5	60	15.3
E3/3	431	70.2	159	71.9	272	69.2
E4/3	80	13.0	27	12.2	53	13.5
E4/4	7	1.1	2	0.9	5	1.3
E4/2	2	0.3	1	0.5	1	0.3
		Frequency		Frequency		Frequency
Allele						
ε2		0.080		0.075		0.083
ε3		0.842		0.853		0.836
ε4		0.078		0.072		0.081

Table 2
Lipid and apolipoprotein levels (mg/dl) and VLDL apolipoprotein content (%) as a function of the apo E phenotype in men[a]

	Apo E phenotype			ANOVA
	E2 ($n=32$)	E3 ($n=159$)	E4 ($n=29$)	
Plasma cholesterol	219.7 ± 7.5	219.7 ± 3.4	219.2 ± 8.1	NS
Plasma triglyceride	164.6 ± 12.6[b]	133.5 ± 5.7[c]	142.4 ± 13.6[b,c]	$P<0.05$
VLDL cholesterol	21.3 ± 2.1[b]	14.3 ± 0.9[c]	14.5 ± 2.2[c]	$P<0.01$
VLDL triglyceride	115.4 ± 10.9[b]	88.6 ± 5.0[c]	94.4 ± 11.8[b,c]	$P<0.01$
LDL cholesterol	154.3 ± 7.0	160.6 ± 3.2	160.8 ± 7.5	NS
HDL cholesterol	44.1 ± 1.8	44.7 ± 0.8	44.2 ± 1.9	NS
HDL phospholipid	104.9 ± 3.4	101.3 ± 1.6	102.4 ± 3.7	NS
Apo A-I	122.6 ± 4.1	125.1 ± 1.5	129.8 ± 3.1	NS
Apo B	105.0 ± 13.7	101.4 ± 3.7	99.1 ± 10.3	NS
Lp(a)	20.2 ± 3.3	16.9 ± 1.6	22.4 ± 3.6	NS
VLDL-Apo C-II (%)	17.0 ± 1.1[b]	12.5 ± 0.5[c]	13.5 ± 1.2[c]	$P<0.001$
VLDL-Apo C-III (%)	67.4 ± 2.0	69.4 ± 0.9	68.6 ± 2.2	NS
VLDL-Apo E (%)	16.0 ± 2.3	19.5 ± 1.0	20.0 ± 2.5	NS

[a] Apo E4/2 subjects were excluded from the analysis. Values (mean ± S.E.) are after adjustment for their respective set of covariates (see Section 2). When statistically significant differences were found by ANOVA, comparisons between each pair of groups were performed by the Newman–Keuls test. Values not sharing any superscript are significantly different ($P<0.05$) by this test. NS, not significant by ANOVA.

parameter after adjusting for VLDL triglyceride was lowered from 4 to 3%, but it remained significant (Table 4). This suggests that the influence of allele ε2 on the proportion of apo C-II in VLDL was principally independent of the levels of this lipoprotein fraction. To further analyze the relationship between the apo C-II content and VLDL lipid levels, the effect of including apo C-II as an independent variable on VLDL triglyceride and VLDL cholesterol was studied. As expected, the apo C-II content showed a high correlation with the concentrations of VLDL triglyceride ($\beta = 0.014 \pm 0.003$, $P < 0.001$) and VLDL cholesterol ($\beta = 0.014 \pm 0.004$, $P < 0.001$). The adjustment for apo C-II decreased the magnitude of the effect of allele ε2 on both VLDL triglyceride and VLDL cholesterol (Table 4).

With regard to women (Table 5), allele ε2 decreased plasma and LDL cholesterol by 16 and 19 mg/dl, respectively, and apo B by 11 mg/dl as compared to ε3 homozygosity. Allele ε4 increased LDL cholesterol by 10 mg/dl and reduced HDL cholesterol, HDL phospholipid and apo A-I concentrations by 3, 6 and 3 mg/dl, respectively. On the other hand, associations between apo E polymorphism and the concentrations of VLDL cholesterol and VLDL triglyceride were observed, but they disappeared after adjusting for BMI, indicating that the influences of the apo E alleles on those traits were spurious and dependent on the effect of BMI (Table 5). The exclusion of postmenopausal women from this analysis revealed a significant lowering effect of allele ε2 on plasma cholesterol ($\beta = -18.46 \pm 5.49$, $P < 0.001$) and LDL cholesterol ($\beta = -19.90 \pm 5.25$, $P < 0.001$). The effect of allele ε4 on premenopausal women was to decrease HDL phospholipid ($\beta = -5.90 \pm 2.95$, $P < 0.05$). After excluding oral contraceptive users from the premenopausal group, the effects of allele ε2 ($\beta = -17.79 \pm 5.84$, $P < 0.001$, for plasma cholesterol, and $\beta = -19.32 \pm 5.55$, $P < 0.001$, for LDL cholesterol) and allele ε4 ($\beta = -3.76 \pm 1.66$, $P < 0.05$, for HDL cholesterol) were essentially maintained.

The most pronounced contribution of apo E polymorphism to the variability of the lipoprotein traits in women was on that of LDL cholesterol, explaining 6.2% ($P < 0.001$) of its variance, followed by 3.8% of those of plasma cholesterol and apo B levels ($P < 0.001$ and $P < 0.05$, respectively). Also significant were the contributions of apo E polymorphism to the variance of HDL cholesterol (2.4%, $P < 0.05$), HDL phospholipid (2.7%, $P < 0.01$) and apo A-I (3.0%, $P < 0.05$).

4. Discussion

In the present report, the frequency of the common apo E isoforms and their influence on plasma lipoproteins in men and women from a working population of Madrid, Spain, were determined. The results show that the relative frequency of apo E4 in this population (7.8%) is within the range of that reported for other Mediterranean countries [24,25,33] and about half the average frequency in Caucasians [3,4]. However, the relative frequency of apo E2 (8%) coincides with the average in Caucasian populations [3].

The relative frequencies of the apo E isoforms in our study population are similar to those reported for a population from Hospitalet [34], in northeastern Spain, and a population from Tenerife [26], in the Canary Islands, an Atlantic Spanish archipelago off the northwest coast of Africa. These observations suggest that in Spain the common apo E alleles are homogeneously distributed.

Present findings also show that the contribution of apo E polymorphism in explaining the variance in lipoprotein levels is clearly different in men and women. In the latter, this polymorphism was associated with the variability in the concentrations of LDL and HDL lipids and apolipoproteins, whereas in males it was associated with the variability of VLDL lipid levels. Some other studies also have found a greater effect of the apo E phenotype on LDL cholesterol and apo B concentrations in women than in men [35–37], but others found the opposite [38]. Differences in ethnic origin and/or lifestyle factors may underlie the different responses of both sexes from one population to another, as shown by Kamboh et al. in two different ethnic groups from the San Luis Valley, CO [37]. On the other hand, apo E polymorphism was not associated with variations in Lp(a) levels in men or in women, which is in concordance with previous reports [26,36,39].

It was found in the present study that allele ε2 had a greater impact on the concentrations of apo B-containing lipoproteins than allele ε4, but, most interestingly, the effects of allele ε2 were preferentially exerted on different fractions in men and women. In the latter, allele ε2 lowered LDL levels, whereas in men it increased VLDL lipids. A higher effect of allele ε2 on plasma triglyceride levels together with a lower effect on LDL cholesterol in men as compared to women was found previously in the Turkish Heart Study [40]. Regarding the HDL fraction, allele ε4 decreased the concentrations of HDL lipids and apo A-I in women, whereas allele ε2 did not influence these traits, which is in accordance with previous results by others [23,25]. It was noteworthy that in our study population allele ε4 had no significant effects on any lipoprotein parameter in men.

The possibility that the different number of males and females studied here influenced the finding of a different impact of apo E polymorphism in both groups of subjects, is unlikely. The participants were randomly selected among all the individuals working in the center in which the study was carried out, the proportion of subjects of both sexes being representative of the proportion existing in such a population. These facts, together with the high participation rate, make selection bias unlikely. Therefore, although the effect of the apo E phenotype on men's LDL could have been underestimated, the main features arising from the analysis of the different lipoproteins in men and women, i.e. an interaction of the effect of apo E polymorphism with gender, are consistent.

Gender-specific factors, such as hormonal status, could be responsible for the different effects observed in women as compared to men. However, these differences cannot be attributed to either menopause or oral contraceptive use, since the allelic effects on lipoproteins remained essentially the same after excluding these groups from the analysis. Consistently, some previous studies also found an association of apo E polymorphism with LDL cholesterol levels in premenopausal women [37,38].

The present results for men differ from those reported for men in the above mentioned Canary population, in which the effects of allele ε2 were to decrease LDL levels while those of allele ε4 were to increase them [26]. This suggests that, despite the similar cultural and genetic backgrounds of both Spanish populations, there exist some environmental differences, including lifestyle, and/or genetic differences, modulating the effect of apo E polymorphism on lipoprotein

Table 3
Lipid and apolipoprotein levels (mg/dl) and VLDL apolipoprotein content (%) as a function of the apo E phenotype in women[a]

	Apo E phenotype			ANOVA
	E2 ($n=62$)	E3 ($n=272$)	E4 ($n=58$)	
Plasma cholesterol	188.8 ± 4.6^{b}	204.2 ± 2.2^{c}	212.5 ± 4.7^{c}	$P<0.01$
Plasma triglyceride	86.3 ± 4.4	82.7 ± 2.1	84.2 ± 4.5	NS
VLDL cholesterol	7.5 ± 0.8	6.4 ± 0.4	7.2 ± 0.8	NS
VLDL triglyceride	44.6 ± 3.6	41.2 ± 1.7	41.8 ± 3.6	NS
LDL cholesterol	125.3 ± 4.4^{b}	142.9 ± 2.1^{c}	154.0 ± 4.5^{d}	$P<0.001$
HDL cholesterol	56.2 ± 1.4^{b}	55.2 ± 0.7^{b}	51.0 ± 1.4^{c}	$P<0.05$
HDL phospholipid	117.4 ± 2.7^{b}	113.3 ± 1.3^{b}	105.4 ± 2.7^{c}	$P<0.01$
Apo A-I	125.7 ± 1.7	128.7 ± 0.8	124.4 ± 1.7	$P<0.05$
Apo B	84.9 ± 4.0^{b}	95.1 ± 1.9^{c}	100.0 ± 3.7^{c}	$P<0.05$
Lp(a)	16.6 ± 2.6	21.7 ± 1.3	18.2 ± 2.7	NS
VLDL-Apo C-II (%)	11.5 ± 0.8	10.7 ± 0.4	10.9 ± 0.9	NS
VLDL-Apo C-III (%)	71.4 ± 1.7	71.7 ± 0.8	72.8 ± 1.7	NS
VLDL-Apo E (%)	18.2 ± 1.8	19.9 ± 0.9	19.2 ± 1.9	NS

[a] Apo E4/2 subjects were excluded from the analysis. Values (mean ± S.E.) are after adjustment for their respective set of covariates (see Section 2). When statistically significant differences were found by ANOVA, comparisons between each pair of groups were performed by the Newman–Keuls test. Values not sharing any superscript are significantly different ($P<0.05$) by this test. NS, not significant by ANOVA.

Table 4
Estimates of the allelic effects on lipid and apolipoprotein levels (mg/dl) and VLDL apolipoprotein content (%) in men[a]

Dependent variable	Coefficient ± S.E.		Adjusted for	*F*-Ratio	ANOVA
	Allele ε2	Allele ε4			
Plasma cholesterol	−4.69 ± 8.37	−4.40 ± 8.01		0.27	NS
Plasma triglyceride (log)	0.11 ± 0.04**	0.05 ± 0.03	BMI, alcohol	13.01	$P<0.001$
VLDL cholesterol (log)	0.21 ± 0.06***	0.07 ± 0.06	BMI, alcohol	12.00	$P<0.001$
	0.18 ± 0.06**	0.07 ± 0.06	BMI, alcohol, apo C-II (%)[b]	13.24	$P<0.001$
VLDL triglyceride (log)	0.17 ± 0.05***	0.07 ± 0.05	BMI, alcohol	15.11	$P<0.001$
	0.12 ± 0.05*	0.06 ± 0.05	BMI, alcohol, apo C-II (%)[b]	17.19	$P<0.001$
LDL cholesterol	−10.90 ± 7.76	−3.86 ± 7.43		1.04	NS
HDL cholesterol	−0.01 ± 2.06	−0.19 ± 2.09		<0.01	NS
HDL phospholipid	3.45 ± 3.91	0.35 ± 3.79		0.39	NS
Apo A-I	−2.91 ± 4.44	3.08 ± 3.18		0.73	NS
Apo B	5.65 ± 12.69	−2.13 ± 9.08		0.20	NS
Lp(a) (log)	0.08 ± 0.10	<0.01 ± 0.10		0.30	NS
VLDL-Apo C-II (%)	3.92 ± 1.13***	0.60 ± 1.11		5.97	$P<0.01$
	3.02 ± 1.10**	0.20 ± 1.07	VLDL triglyceride (log)[b]	11.24	$P<0.001$
VLDL-Apo C-III (%)	−2.50 ± 2.23	−1.88 ± 2.18		0.90	NS
VLDL-Apo E (%)	−2.33 ± 2.43	1.48 ± 2.38		0.73	NS

[a] Stepwise regression analysis was performed. The coefficients for alleles ε2 and ε4 were adjusted for the indicated confounders, which were selected backwards as described in Section 2. NS, not significant by ANOVA.
[b] Apo C-II and VLDL triglyceride (log) were forced into the model.
* $P<0.05$,
** $P<0.01$,
*** $P<0.001$ for the t-value of the coefficients.

levels. Some significant differences in dietary habits between the populations of the region of Madrid and the Canary Islands have been identified [29]. Interestingly, the IHD mortality rate in this archipelago is the highest, whereas in the region of Madrid it is among the lowest in Spain [28]. The relative contributions of apo E polymorphism and modifiable factors in determining the variability in the IHD mortality rate between the different regions of Spain, a country enjoying a low average incidence of IHD in association with apo E4 underexpression, deserves further investigation.

The elevation of plasma triglyceride concentration by allele ε2, as occurred in males, is consistent with the defective catabolism of apo E2-containing TGR lipoproteins [9,10]. The average VLDL levels in men were about twice as high as in women (Tables 2 and 3), suggesting underlying differences in VLDL metabolism between both sexes. In this scenario the efficient catabolism of VLDL particles in men carrying allele ε2 could be more compromised than in their women counterparts. As a result, allele ε2 may predispose men to hypertriglyceridemia to a higher extent than women. This is consistent with the fact that type III hyperlipoproteinemia, which is typically associated with ε2 homozygosity and is characterized by the accumulation of remnant particles, or β-VLDL, is much more prevalent in men than in women, and it tends to occur earlier in men [11]. Similarly, within hyperlipidemic subjects, double pre-β lipoproteinemia, which is a milder form of accumulation of VLDL remnants, has been shown to be more prevalent, on the one hand, in subjects with a single dose of allele ε2, and, on the other, in men rather than in women [41].

The parallel increase in both VLDL triglyceride and VLDL cholesterol levels in men possessing allele ε2 suggests an increase in the amount of circulating VLDL particles rather than a change in their lipid content. However, VLDL from apo E2 men contained, in per cent terms, approximately one third more apo C-II than VLDL from apo E3/3 subjects (Table 2). Kaprio et al., studying a Caucasian population from Minnesota, described that allele ε2 was associated with increased total plasma apo C-II concentration, whereas no association was found with plasma apo C-III levels [35]. Present findings suggest that the elevation in plasma apo C-II concentration can lie in an enrichment of VLDL particles in this apolipoprotein.

Such enrichment in apo C-II could be a consequence of the longer residence time of VLDL in subjects possessing allele ε2 [9,10]. The question arising now is the possible consequences of this increase in the relative proportion of apo C-II on the metabolism of VLDL. The possibility that an increase in apo C-II relative to apo C-III could stimulate LPL-catalyzed hydrolysis of VLDL triglyceride, thus counteracting the slower clearance of apo E2-containing VLDL particles, seems unlikely. Except in patients homozygous for apo C-II deficiency, the amount of this peptide contained in VLDL is normally much greater than that required to maximally activate LPL [42,43]. According to Le et al.,

Table 5
Estimates of the allelic effects on lipid and apolipoprotein levels (mg/dl) and VLDL apolipoprotein content (%) in women[a]

Dependent variable	Coefficient ± S.E.		Adjusted for	F-Ratio	ANOVA
	Allele ε2	Allele ε4			
Plasma cholesterol	−15.73 ± 4.90**	7.19 ± 4.62	Age	32.18	$P<0.001$
Plasma triglyceride (log)	0.04 ± 0.02	0.01 ± 0.02		1.67	NS
VLDL cholesterol (log)	0.08 ± 0.04	0.05 ± 0.04	BMI	8.60	$P<0.001$
VLDL triglyceride (log)	0.06 ± 0.04	0.02 ± 0.03	BMI	10.63	$P<0.001$
LDL cholesterol	−18.63 ± 4.65***	9.95 ± 4.93*	Age	29.41	$P<0.001$
HDL cholesterol	0.91 ± 1.54	−3.30 ± 1.46*		3.03	$P<0.05$
HDL phospholipid	4.18 ± 2.87	−5.80 ± 2.72*		3.94	$P<0.05$
Apo A-I	−2.93 ± 1.91	−3.33 ± 1.62*	Age	6.05	$P<0.001$
Apo B	−10.92 ± 4.46*	4.80 ± 3.68	Age	9.26	$P<0.001$
Lp(a) (log)	−0.08 ± 0.08	−0.15 ± 0.07		2.44	NS
VLDL-Apo C-II (%)	1.15 ± 0.88	0.73 ± 0.86		1.05	NS
VLDL-Apo C-III (%)	−0.41 ± 1.72	0.54 ± 1.68		0.09	NS
VLDL-Apo E (%)	−1.46 ± 1.82	−1.03 ± 1.83		0.41	NS

[a] Stepwise regression analysis was performed. The coefficients for alleles ε2 and ε4 were adjusted for the indicated confounders, which were selected backwards as described in Section 2. NS, not significant by ANOVA.
* $P<0.05$,
** $P<0.01$,
*** $P<0.001$ for the t-value of the coefficients.

multivariate analysis predicted that VLDL triglyceride FCR is inversely related to the VLDL apo C-II/B ratio [20]. Thus, the enrichment of VLDL in apo C-II may preclude the efficient clearance through the hepatic receptors, since this apolipoprotein can oppose the recognition of apo E by both the LDL receptor [17] and LRP [19], and therefore inhibit the hepatic uptake of the particle [44,45]. Consistent with this view, present results have shown that the magnitude of the raising effect of allele ε2 on men's VLDL triglyceride decreased after adjustment for the VLDL-apo C-II content, pointing to the possibility that the latter parameter contributes to the high VLDL levels in apo E2 men.

To summarize, in the population of Madrid, which is the object of this study, the frequency of apo E4 is lower than in most Caucasian populations but within the range of those reported for Mediterranean countries. On the other hand, apo E polymorphism exhibited a differential impact in men and women, since in women it influenced LDL and HDL levels, whereas in men it had a preferential effect on VLDL. In both genders, allele ε2 exerted its effects on apo B-containing lipoproteins and, in contrast to allele ε4, did not have an impact on HDL levels. However, while in women allele ε2 lowered LDL levels, in men its effects were to increase both VLDL lipid levels and apo C-II content. Together with the findings from previous studies, these data suggest that lifestyle, diet and/or genetic factors modulate the impact of apo E polymorphism on lipoproteins and the eventual risk for IHD in the different Spanish regions. It is hypothesized that the repercussion of this polymorphism on the risk for IHD in the population of Madrid is exerted mainly through cholesterol-rich lipoproteins in women and through TGR lipoproteins in men. Additional studies should be undertaken to test this hypothesis.

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