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Influence of apolipoprotein E genotype on the reliability of the Friedewald formula in the estimation of low-density lipoprotein cholesterol concentrations

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Abstract

Lipoprotein data and apolipoprotein (apo) E genotype from 1302 participants, covering a wide range of total plasma cholesterol levels, were used to examine the impact of apo E genotype on the estimation of low-density lipoprotein cholesterol (LDL-C) concentrations by the Friedewald formula using high-density lipoprotein cholesterol and triglyceride (TG) concentrations as compared with the β-quantification reference procedure. The results showed that participants with apo E2/E2 genotype had significantly higher very low-density lipoprotein cholesterol (VLDL-C) concentrations and VLDL-C/TG ratio as well as lower LDL-C concentrations than participants with other apo E genotypes. Heterozygous carriers of the ε2 allele had significantly higher VLDL-C than participants with apo E3/E3 and E4/E3 genotypes. The mean absolute error and the mean percentage of bias in calculated LDL-C according to all apo E genotypes, except E2/E2 genotype, were less than 0.16 mmol/L and 4.4%, respectively. Indeed, the mean error and the mean percentage of bias associated with the LDL-C calculated by the Friedewald formula in the apo E2/E2 group were 0.93 mmol/L and 40.6%, respectively. However, participants with the apo E2/E2 genotype and a type III phenotype showed a mean error and a mean percentage of bias reaching 1.53 mmol/L and 63.5%, respectively, whereas E2/E2 participants with a non–type III phenotype had a mean error and a mean precentage of bias of 0.18 mmol/L and 11.0%, respectively. Moreover, 41.9% to 57.1% of the participants had an absolute bias higher than 5% according to the apo E genotype, except for the apo E2/E2 genotypic group where 88.6% of the participants had an absolute bias higher than 5%. Stepwise multiple linear regression analyses revealed that the apo E genotype contributed to 39.0% of the VLDL-C/TG ratio variance, whereas sex, age, and high-density lipoprotein cholesterol explained between 0.5% and 3.2% of the variance. These results indicate that the apo E genotype exerts a significant influence on the estimation of LDL-C concentrations by the Friedewald formula as compared with the β-quantification.

1. Introduction

Plasma low-density lipoprotein cholesterol (LDL-C) is an important risk factor for coronary heart disease [1,2]. Evidence from clinical trials indicates that reducing plasma cholesterol by dietary and/or pharmacological means leads to reductions in the incidence of major cardiovascular disease. The Friedewald formula [3], which estimates very low-density lipoprotein cholesterol (VLDL-C) from measured triglyceride (TG) and then uses this estimate to calculate LDL-C, is widely accepted and used because of its convenience. The formula assumes that most of the circulating TG is carried in the VLDL and that the relationship between cholesterol and TG in this fraction is constant (1:5 mg/dL). This assumption has been demonstrated to be inaccurate in some primary hyperlipidemias such as the WHO types I and III [4], as well as in certain secondary hyperlipidemias such as nephrotic syndrome and diabetes [5,6].

Variation in the apolipoprotein (apo) E genotype is a major determinant of the interindividual variation in susceptibility to coronary heart disease. In humans, 3 common alleles, designated ε4, ε3, and ε2, code for 3 major apo E isoforms in plasma, respectively, designated apo E4, apo E3, and apo E2. Apo E is a key protein in the modulation of the metabolism of the highly atherogenic apo B–containing lipoproteins [7]. As compared with the apo E3 isoform, apo E2 isoform has a markedly reduced affinity for hepatic lipoprotein receptors and is associated with hypocholesterolemia and hypertriglyceridemia, whereas the apo E4 isoform possesses the highest

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affinity for the hepatic B-E receptors and is associated with hypercholesterolemia [8]. In some instances, the apo E2 isoform leads to an impaired removal of remnants of chylomicrons and VLDL, and to the accumulation of a highly atherogenic lipoprotein fraction (β-VLDL) associated with type III dysbetalipoproteinemia.

Data from a previous study by our laboratory [9] showed that the VLDL-C/TG ratio explained nearly 63% of the variance of the difference between calculated LDL-C and LDL-C measured by β-quantification. However, the impact of apo E genotype on the validity of the Friedewald formula to estimate LDL-C remains to be fully characterized. The aim of the present study was to examine the influence of apo E genotype on the estimation of LDL-C concentrations by the Friedewald formula as compared with the β-quantification reference procedure (sequential preparative ultracentrifugation/heparin–manganese chloride precipitation) in 1302 individuals with a wide range of total plasma cholesterol, LDL-C, and TG levels below 4.52 mmol/L.

2. Material and methods

2.1. Participants

Data from 1302 participants seen in consultation between 1991 and 2001 at the Lipid Clinic of the Laval University Medical Center in Quebec City were included in this report. Patients ranged in age from 3 to 85 years. All individuals were instructed to fast at least 12 hours before blood samples were drawn. The research protocol was approved by the Laval University Medical Center ethical review committee.

2.2. Characterization of plasma lipids and lipoproteins

Venous blood samples were obtained from an antecubital vein into Vacutainer tubes containing K_3EDTA (1 mg/mL, final concentration). Plasma was separated from blood cells by centrifugation at 3000 rpm for 10 minutes at 4°C. Plasma VLDL fraction was isolated by ultracentrifugation using a 50.3 rotor (Beckman Instruments Inc, Palo Alto, Calif) at a density of 1.006 g/mL as described by Havel et al [10]. The top fraction containing VLDL was recovered by tube slicing. Heparin and manganese chloride [11] were added to the infranatant (density of more than 1.006 g/mL and containing both LDL and high-density lipoprotein [HDL]) to precipitate apo B–containing LDL, leaving the HDL in solution. The concentration of LDL was obtained by differences from the values of cholesterol in the infranatant, measured before and after the precipitation step. The cholesterol and TG in plasma and lipoprotein fractions were quantified on an AutoAnalyzer RA-500 (Technicon Instruments Corporation, Tarrytown, NY). The mean recovery of cholesterol in the lipoprotein fractions averaged 95% and ranged from 92% to 102%. The coefficients of variation of cholesterol and TG determinations were below 2%, as previously reported [12]. The LDL-C was also calculated according to the equation described by Friedewald et al [3]: $[\text{LDL-C}_{\text{Fried}}] = [\text{total cholesterol}] - [\text{HDL} - \text{C}] - [\text{TG}]/2.2$.

2.3. Definition of the type III and non–type III phenotype

All participants with type III phenotype had to be homozygous for apo E2 and have a VLDL-C/TG ratio of 0.7 or higher. In contrast, the non–type III phenotype applied to apo E2 homozygote with a VLDL-C/TG ratio of less than 0.7.

2.4. DNA analysis

Genotyping of apo E was done by polymerase chain reaction amplification of a 244–base pair fragment of the exon 4 of the *apoE* gene with oligonucleotides F4 and F6 and digestion of polymerase chain reaction fragments with the restriction enzyme *Hha*I [13].

Table 1
Distribution of plasma triglyceride and cholesterol, VLDL-C, LDL-C, and HDL-C levels measured by β-quantification (TG levels ≤ 4.52 mmol/L)

	Age (y)	Plasma TG (mmol/L)	Plasma cholesterol (mmol/L)	VLDL-C (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	VLDL-C/TG ratio
Quantiles							
100.0% (Maximum)	85.1	4.52	16.94	5.23	15.51	2.55	1.31
99.5%	75.2	4.50	10.89	4.04	8.97	2.00	1.12
97.5%	69.8	4.37	9.35	2.64	7.20	1.66	0.83
90.0%	63.0	3.92	7.82	1.82	5.61	1.38	0.54
75.0%	56.2	3.27	6.82	1.38	4.60	1.15	0.46
50.0% (Median)	48.3	2.35	5.77	0.94	3.68	0.94	0.39
25.0%	38.0	1.56	4.84	0.58	2.89	0.81	0.34
10.0%	24.6	1.09	4.13	0.35	2.28	0.69	0.29
2.5%	10.3	0.71	3.28	0.15	1.63	0.52	0.20
0.5%	6.4	0.51	2.52	0.07	0.99	0.30	0.09
0.0% (Minimum)	3.8	0.36	1.78	0.03	0.58	0.08	0.06
Geometric mean	46.2	2.43	5.90	1.05	3.86	0.99	0.41
SD	14.6	1.05	1.52	0.66	1.42	0.29	0.14
n	1302	1302	1302	1302	1302	1302	1302

$\text{LDL-C}_{\text{Ultra}}$ indicates LDL-C determined by β-quantification. 59.4% (n = 773) and 40.6% (n = 529) of the participants were men and women, respectively.

Table 2
Distribution of plasma triglyceride and cholesterol, VLDL-C, LDL-C, and HDL-C levels measured by β-quantification and VLDL-C/TG ratio according to apo E genotype (TG levels $\leq$ 4.52 mmol/L)

Genotype	n (%)	Plasma TG (mmol/L)	Plasma cholesterol (mmol/L)	VLDL-C (mmol/L)	LDL-C_{Ultra} (mmol/L)	HDL-C (mmol/L)	VLDL-C/TG ratio
E4/E4	35 (2.7)	2.71 ± 1.12	5.94 ± 1.09	1.06 ± 0.53	3.93 ± 1.08	0.94 ± 0.29	0.38 ± 0.10
E4/E3	280 (21.5)	2.37 ± 1.05	6.04 ± 1.47	0.94 ± 0.53	4.10 ± 1.36	0.99 ± 0.30	0.38 ± 0.10
E3/E3	689 (52.9)	2.36 ± 1.03	5.95 ± 1.55	0.92 ± 0.49	4.03 ± 1.42	0.99 ± 0.29	0.38 ± 0.09
E4/E2	49 (3.8)	2.61 ± 1.24	5.86 ± 1.80	1.21 ± 0.72[b,c]	3.72 ± 1.76	0.92 ± 0.26	0.43 ± 0.13[c]
E3/E2	170 (13.0)	2.62 ± 1.02	5.62 ± 1.37	1.19 ± 0.61[b,c]	3.43 ± 1.17[b,c]	0.99 ± 0.28	0.44 ± 0.11[a,b,c]
E2/E2	79 (6.1)	2.61 ± 0.98	5.64 ± 1.59	2.08 ± 1.22[a,b,c,d,e]	2.47 ± 1.00[a,b,c,d,e]	1.05 ± 0.22	0.75 ± 0.24[a,b,c,d,e]
E2/E2 non–type III	35 (2.7)	2.00 ± 0.79	4.61 ± 1.37	1.07 ± 0.52	2.40 ± 1.30	1.12 ± 0.25	0.53 ± 0.11
E2/E2 type III	44 (3.4)	3.10 ± 0.84[f]	6.46 ± 1.24[f]	2.89 ± 0.99[f]	2.53 ± 0.68	0.99 ± 0.19[f]	0.92 ± 0.15[f]

Mean ± SD.
[a] $P < .05$ vs E4/E4.
[b] $P < .05$ vs E4/E3.
[c] $P < .05$ vs E3/E3.
[d] $P < .05$ vs E4/E2.
[e] $P < .05$ vs E3/E2.
[f] $P < .05$ E2/E2 type III (VLDL-C/TG ratio $\geq$0.7) vs E2/E2 non–type III (VLDL-C/TG ratio < 0.7).

2.5. Statistical analysis

Plasma TGs were log-transformed to normalize their distribution. Continuous variables were analyzed using analysis of variance and Tukey tests. Pearson correlation coefficients were determined to assess the significance of associations. Stepwise multiple linear regression analyses were used to interpret the relationship of these associations. All analyses were performed using JMP Statistical Software (version 5.1, SAS Institute, Cary, NC).

3. Results

3.1. Participants

Data from 1302 participants are presented in this study; 59.4% of these were men with a mean age of 47.4 ± 16.1 years, and 40.6% were women with a mean age of 45.3 ± 13.4 years. Table 1 contains the distribution of plasma TG levels as well as plasma cholesterol, VLDL-C, LDL-C, and HDL-C concentrations measured by β-quantification. All participants included in this study had plasma TG levels below 4.52 mmol/L. They had a mean plasma cholesterol, VLDL-C, LDL-C, and HDL-C concentrations and VLDL-C/TG ratio of 5.90 ± 1.52, 1.05 ± 0.66, 3.86 ± 1.42, and 0.99 ± 0.29 mmol/L and 0.41 ± 0.14, respectively.

3.2. Lipid-lipoprotein profile according to apo E genotype

Table 2 shows the distribution of plasma TG and cholesterol in the different lipoprotein fractions according to the apo E genotype. The apo E3/E3 (52.9%) and E4/E3 (21.5%) genotypes represent nearly 75% of the apo E genotypes found in the participants. The apo E2/E2 genotype was found in 6.1% of the participants, and 55.7% of them were characterized by a type III phenotype with a VLDL-C/TG

Table 3
Determination of LDL-C using the Friedewald formula and β-quantification according to apo E genotype with TG levels $\leq$ 4.52 mmol/L

Genotype	n	Mean LDL-C_{Ultra} (mmol/L)	Mean LDL-C_{Fried} (mmol/L)	ΔLDL-C (mmol/L)	Bias (%)	R	P
E4/E4	35	3.93 ± 1.08	3.77 ± 1.07	−0.16 ± 0.25	−4.1 ± 7.1	0.97	<.0001
E4/E3	280	4.10 ± 1.36	3.97 ± 1.40	−0.13 ± 0.23	−3.6 ± 6.7	0.99	<.0001
E3/E3	689	4.03 ± 1.42	3.88 ± 1.45	−0.15 ± 0.19	−4.4 ± 6.7	0.99	<.0001
E4/E2	49	3.72 ± 1.76	3.75 ± 1.76	0.03 ± 0.29[a,b,c]	0.7 ± 8.7[c]	0.99	<.0001
E3/E2	170	3.43 ± 1.17	3.44 ± 1.22	0.01 ± 0.31[a,b,c]	−0.07 ± 10.93[b,c]	0.97	<.0001
E2/E2	79	2.47 ± 1.00	3.41 ± 1.35	0.93 ± 0.89[a,b,c,d,e]	40.2 ± 36.9[a,b,c,d,e]	0.75	<.0001
E2/E2 non–type III	35	2.40 ± 1.30	2.58 ± 1.25	0.18 ± 0.27	11.0 ± 16.6	0.98	<.0001
E2/E2 type III	44	2.53 ± 0.68	4.06 ± 1.04	1.53 ± 0.74[f]	63.5 ± 31.6[f]	0.70	<.0001
Total	1302	3.86 ± 1.42	3.81 ± 1.42	−0.05 ± 0.40	−0.7 ± 15.7	0.96	<.0001

Mean ± SD. ΔLDL-C: mean LDL-C_{Fried} − mean LDL-C_{Ultra}; *Bias*: LDL-C_{Fried} − LDL-C_{Ultra}/LDL-C_{Ultra} × 100. LDL-C_{Fried} indicates calculated LDL-C; R, correlation coefficient.
[a] $P < .05$ vs E4/E4.
[b] $P < .05$ vs E4/E3.
[c] $P < .05$ vs E3/E3.
[d] $P < .05$ vs E4/E2.
[e] $P < .05$ vs E3/E2.
[f] $P < .05$ E2/E2 type III (VLDL-C/TG ratio $\geq$0.7) vs E2/E2 non–type III (VLDL-C/TG ratio < 0.7).

ratio of 0.70 or higher. No significant difference was observed for plasma TG, plasma cholesterol, and HDL-C between the apo E genotypes. Participants with apo E2/E2 genotype had significantly higher VLDL-C concentrations and VLDL-C/TG ratio as well as lower LDL-C concentrations than participants with the other genotypes. Heterozygous carriers of the ε2 allele had significantly higher VLDL-C than participants with apo E3/E3 and E4/E3 genotypes. Participants with apo E3/E2 genotype had lower LDL-C concentrations than participants with apo E3/E3 and E4/E3 genotypes. Carriers of the ε2 allele had also higher VLDL-C/TG ratio than participants with the apo E3/E3 genotype. Finally, carriers of the apo E2/E2 genotype associated with a type III phenotype had significantly higher plasma TG, plasma cholesterol, and VLDL-C concentrations as well as higher VLDL-C/TG ratio and lower HDL-C concentrations than non–type III participants.

3.3. Impact of apo E genotype on the accuracy of the Friedewald equation

We then investigated the influence of the apo E genotype on the accuracy of the Friedewald formula (Table 3) in patients with plasma TG levels 4.52 mmol/L or lower. We found that the mean absolute error and the mean percentage of bias in calculated LDL-C according to any apo E genotype, except E2/E2 genotype, were less than 0.16 mmol/L and 4.4%, respectively. Indeed, the mean error and the mean percentage of bias associated with the LDL-C calculated by the Friedewald formula in the apo E2/E2 group were 0.93 mmol/L and 40.2%, respectively. However, in participants with the apo E2/E2 genotype associated with a type III phenotype, the mean error and the mean percentage of bias reached 1.53 mmol/L and 63.5%, respectively. Apo E2/E2 carriers with a non–type III phenotype had a mean error and a mean precentage of bias of 0.18 mmol/L and 11.0%, respectively. Finally, the difference between calculated and measured LDL-C was greater in carriers of an ε2 allele than in noncarriers, indicating that the Friedewald formula overestimates plasma LDL-C concentrations.

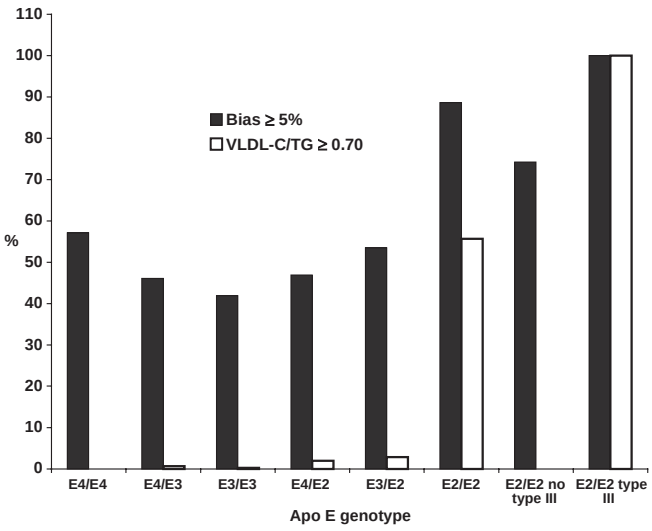

Fig. 1. Proportion of participants with a bias ≥5% associated to the calculation of LDL-C concentrations by the Friedewald formula as compared with the β-quantification and the proportion of participants showing a VLDL-C/TG ratio ≥0.70 according to the apo E genotype. All participants had plasma TG levels ≤4.52 mmol/L. (E4/E4, n = 35; E4/E3, n = 280; E3/E3, n = 689; E4/E2, n = 49; E3/E2, n = 170; E2/E2, n = 79; E2/E2 non–type III, n = 35; E2/E2 type III, n = 44).

Table 4
Multivariate regression analysis showing independent contributions of sex, age, HDL-C, and apo E genotype on the variance of the VLDL-C/TG ratio

Independent variables	Partial ($R^2 \times 100$)	P
Sex	0.5	.0005
Age	0.6	.0002
HDL-C	3.2	<.0001
Apo E	39.0	<.0001

The multivariate model included sex, age, HDL-C, and apo E genotype.

We next examined the proportion of participants exhibiting an absolute percentage of bias higher than 5% and the proportion of participants showing a type III phenotype (VLDL-C/TG ratio ≥0.70) according to the apo E genotype (Fig. 1). We observed that 41.9% to 57.1% of the participants had an absolute bias higher than 5% according to the apo E genotype, except for the apo E2/E2 genotype where 88.6% of the participants had an absolute bias higher than 5%. Moreover, all participants characterized by the type III phenotype had an absolute bias higher than 5%, whereas 74.3% of the participants without the type III phenotype showed the same characteristic. Finally, 81% (44/54) of the participants having a VLDL-C/TG ratio 0.70 or higher were carriers of the apo E2/E2 genotype, and 92.5% (50/54) were carriers of an ε2 allele.

3.4. Impact of the apo E genotype on the VLDL-C/TG ratio

Stepwise multiple linear regression analysis was performed to assess the independent contribution of the apo E genotype to the variance of VLDL-C/TG ratio including sex, age, and HDL-C in the model. As shown in the Table 4, the apo E genotype represented 39.0% of the variance of the VLDL-C/TG ratio, whereas sex, age, and HDL-C contributed between 0.5% and 3.2% to the variance of the ratio.

4. Discussion

The accurate determination of plasma LDL-C concentration, which is a major risk factor for coronary heart disease, plays an increasingly important role in the assessment of cardiovascular risk and clinical management of individual patients [2,14]. Currently, most clinical laboratories use the Friedewald equation to calculate the LDL-C levels, because the reference method (β-quantification by ultracentrifugation) is not suitable for routine use because of its elevated cost and technician time requirement. The Friedewald formula is based on the assumption that the mass ratio of serum TGs to VLDL-C is 5:1 (for mg/dL) (equivalent to a

molar ratio of 2.2:1). However, this assumption does not hold in specific conditions in which the ratio of VLDL-C to plasma TG is altered such as in the fed state, in marked hypertriglyceridemia, and in type III hyperlipidemia [4]. The Friedewald formula has been also shown to introduce significant bias in secondary hyperlipidemia that is caused by liver disease [15], diabetes mellitus [16,17], and nephrotic syndrome [5,18]. Furthermore, the apo E genotype is well known to have a significant impact on the lipid-lipoprotein profile, mainly by modulating the highly atherogenic apo B–containing lipoprotein metabolism [7].

In the present study, we investigated the influence of the apo E genotype on the bias associated with the Friedewald formula in the estimation of LDL-C concentrations as compared with the β-quantification as the reference method in 1302 individuals with a wide range of total plasma cholesterol, LDL-C, and TG levels and apo E genotype. Moreover, we wanted to investigate the independent effect of the apo E genotype on the VLDL-C/TG ratio, as we have shown previously [9], to be the most important independent predictor of the bias associated with the Friedewald formula.

The apo E genotype has a major influence on plasma lipid-lipoprotein concentrations which can be explained by differences in the metabolic behavior of the 3 major apo E isoforms (Table 2). The present study confirms that individuals carrying the ε2 allele had significantly higher VLDL-C levels and higher VLDL-C/TG ratio as well as lower LDL-C concentrations than participants with the apo E4/E3 and E3/E3 genotype [19]. The E2 isoform showed a lower affinity to the B-E receptors, and this affinity level is one of the factors that determines the number of IDL particles that will be transformed into LDL [8], resulting in an accumulation of chylomicron and VLDL remnants that is associated with an increase in triglyceridemia and VLDL-C, thus an increase in the VLDL-C/TG ratio. In the apo E2 homozygotes, frequently found in type III patients (90%), this metabolic disorder is amplified leading to a significantly higher VLDL-C/TG ratio as compared with apo E2 heterozygotes and other apo E genotypes.

The apo E genotype had also an important impact on the bias associated with the Friedewald formula as compared with the β-quantification. Indeed, this study reported that the mean bias associated with the Friedewald formula was below 5% in all apo E genotypes, except in participants with the apo E2/E2 genotype, where the mean bias was 40.2%. When we separated the apo E2/E2 group in subjects with the type III phenotype versus the non–type III phenotype, both groups were characterized by higher bias than other genotypes (63.5% and 11.0%, respectively). These results suggest that the presence of the apo E2 homozygosity is an important predictor of the bias associated with the Friedewald formula.

On the other hand, this study showed that in participants carrying an apo ε2 allele, the Friedewald formula had a tendency to overestimate the LDL-C concentrations, whereas the opposite effect was observed in noncarriers of an apo ε2 allele. This finding is related to the difference in VLDL-C/TG ratio observed in the various apo E genotypic groups because the apo ε2 allele carriers had a higher VLDL-C/TG ratio than noncarriers. In this regard, a previous study from our laboratory [9] showed a positive correlation between the difference between LDL-C concentrations calculated by the Friedewald formula and LDL-C concentrations measured by ultracentrifugation and the VLDL-C/TG ratio. This correlation indicated that the Friedewald formula underestimated LDL-C concentrations when the VLDL-C/TG ratio was low, as found in hypertriglyceridemic patients, and overestimated LDL-C concentrations in patients with high VLDL-C/TG ratio (type III hyperlipidemia). Moreover, this study also showed that the VLDL-C/TG ratio was found to be the most important independent predictor of the bias associated with the Friedewald formula, confirming that the relative proportion of cholesterol and TG in VLDL particles has a major impact on the accuracy of the Friedewald formula. The present study extends these previous results and indicates that the apo E polymorphism influences significantly the plasma lipid-lipoprotein profile as well as the VLDL-C/TG ratio and has a major impact on the assessment of VLDL composition used in the Friedewald formula.

To identify the predictors of VLDL-C/TG ratio, we performed a stepwise multiple linear regression analysis in which sex, age, HDL-C, and apo E genotype were included in the model. This procedure allows to sort out the relative contribution of all independent variables on the variance of the VLDL-C/TG ratio after adjustment for all independent variables included in the model. As expected, the apo E genotype showed the highest independent contribution (39.0%, $P < .0001$) to the variance of the VLDL-C/TG ratio, and this contribution could be explained by the fact that the apo E genotype influences directly the composition of the VLDL particles [7] and affects their TG/cholesterol ratio. The other covariates included in the model had only minor impact and explained between 0.5% and 3.2% of the variance of VLDL-C/TG ratio.

Potential limitations include the retrospective nature of the study, the lack of data on lifestyle, and the possibility of selection bias given that patients were referred to a lipid clinic. It would have been interesting to include anthropometric variables and lifestyle parameters as well as data on lipid-lowering drugs in the regression linear model, but these data were not available. It should be emphasized, however, that the inclusion of these new parameters in the model is not expected to decrease the importance of the apo E polymorphism as an independent predictor of the variance of VLDL-C/TG ratio, but rather to increase the proportion of the variance explained by the model.

In summary, our results suggest that the apo E genotype is an independent predictor of the bias associated with the Friedewald formula as compared with the β-quantification, mainly by affecting the VLDL-C/TG ratio. Hence, Friedewald formula must be used with caution in patients with an altered VLDL-C/TG ratio due to their apo E genotype, such as in patients with dysbetalipoproteinemia associated with

apo E2 homozygosity and in carriers of an $\varepsilon 2$ allele. Finally, clinicians should perform ultracentrifugation to assess lipid levels when type III hyperlipidemia is suspected based on lipid profile (elevated plasma TG levels usually above 4.52 mmol/L) or physical signs such as palmar xanthomas.

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