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Original Article

Impact of age and body size on inter-individual variation in measures of lipid metabolism: influence of gender and *apolipoprotein E* genotype

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This study was undertaken in 1695 adult subjects (870 women and 825 men) in order to further document the complexity of the influence of the *apolipoprotein (apo) E* genotypes on the mean levels and intragenotypic variability of seven measures of lipid metabolism. In addition, the statistical relationships between variability in these traits and variation in age, body mass index (BMI) and waist-to-hip ratio (WHR) were assessed. The contribution of variation in age and body size to inter-individual variation was found to be dependent on context, defined by gender and *apo E* genotype. Our findings are consistent with the reality that it is neither genes nor environments, but their interactions that are responsible for the variation in risk of cardiovascular disease.

S Lussier-Cacan[a], A Bolduc[a], M Xhignesse[b], T Niyonsenga[b], PW Connelly[c] and CF Sing[d]

[a] Institut de recherches cliniques de Montréal, Montréal, Québec, [b] CRC-CUSE, Université de Sherbrooke, Sherbrooke, Québec, [c] Department of Medicine, University of Toronto, Toronto, Ontario, Canada, [d] Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA

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Corresponding author: Dr S. Lussier-Cacan, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal (Quebec) H2W 1R7, Canada. Tel: +1 514 987 5630; fax: +1 514 987 5700; e-mail: cacans@ircm.qc.ca

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Elevated plasma lipid, lipoprotein and apolipoprotein (apo) B levels represent major risk factors for cardiovascular disease (CVD) (1, 2). Inter-individual variability in these traits is attributable to interactions of exposures to environmental factors, with biological characteristics that are influenced by genetic agents (3, 4). One of the genetic agents involved in the regulation of lipid metabolism is the gene coding for apo E. Many studies have demonstrated that the three common alleles of this gene, *ε2*, *ε3* and *ε4*, influence plasma lipoprotein levels in both health and disease (5). Individuals who carry the *ε4* allele generally have the highest, while those with the *ε2* allele have the lowest levels of low density lipoprotein (LDL) cholesterol (C).

Traditionally, analyses of the influence of genetic variability on lipid levels have been carried out on data that have been adjusted by statistical methods for variability in gender, age and measures of body size. However, studies of the *apo E* gene by Reilly et al. (6) have suggested that such adjustments may be inappropriate, as the association of lipid levels with age and body size may be heterogeneous among gender and genotype strata. Furthermore, when the penetrance function of each of the *apo E* genotypes has been estimated (7), the phenotypic variances of measures of lipid metabolism were found to be dependent on gender and genotype. This study was undertaken in adult subjects to further document the complexity of the influence of *apo E* genotypes on the mean levels and intragenotypic variability of seven measures of lipid metabolism. Moreover, the statistical relationships between variability in these traits and variation in age, body mass index (BMI) and waist-to-hip ratio (WHR) were assessed. Our analyses confirm that the means, the intragenotypic variances and the regressions of lipid, lipoprotein and apolipoprotein

traits on age and body size vary significantly among the most common *apo E* genotypes in a gender-specific manner. The heterogeneity of phenotypic variation among the *apo E* genotypes was greatest for measures of LDL metabolism, and least for the measures of high density lipoprotein (HDL) metabolism.

Materials and methods

Sample

The subjects were non-institutionalized men and women, aged between 18 and 74 years, who participated in two complementary studies in the Province of Quebec (Canada), the Heart Health Survey and the Nutrition Survey, during the autumn of 1990. They were selected from the health insurance registries, according to a survey sampling design described in the Study Report (8). The sample included approximately equal numbers of individuals in different risk categories defined by gender and age. This resulted in the over-sampling of young participants (18–34 years) to obtain sufficient numbers of 'at risk individuals' who have the highest priority for intervention strategies (8). In the original Heart Health Survey, 2354 subjects were identified and contacted, 2096 attended a clinic visit and a fasting blood sample was obtained from 2055 (1052 women and 1003 men). During an auxiliary study of plasma apolipoproteins (9), apo AI and apo B levels were determined, and apo E phenotyping was performed. At this point, data were available on a sample of 2010 subjects (1025 women and 985 men). We then excluded 196 subjects because of missing data for plasma lipoproteins, anthropometric measures, diet or other lifestyle habits. Finally, 26 subjects were excluded because they were taking lipid-lowering drugs, leaving a sample of 916 women and 872 men for the analyses reported here.

The distribution of apo E phenotypes was comparable between women and men, and corresponded to that reported in Occidental populations (5). Because of the typically small numbers of subjects presenting with E2/2, E4/2 and E4/4 phenotypes, we decided to focus our analyses on subjects with the three common phenotypes E3/2, E3/3 and E4/3. Removal of the rare phenotypes resulted in a sample of 870 women and 825 men (denoted as sample A). All analyses were carried out on sample A, and on a reduced sample of 606 women and 786 men (denoted sample B). Sample B did not include 210 women using exogenous sex hormones, and 54 women and 39 men who were taking drugs that affect lipid levels, such as certain anti-hypertensive agents and corticosteroids.

Data collection and laboratory methods

Anthropometric measurements (height, weight, waist and hip circumferences) and other personal information were obtained during the clinic visit

Table 1. Means (± SD) of concomitants by *apo E* genotype in women and men

	Sample A				Sample B			
Women	Apo E 3/2 (n = 129)	Apo E 3/3 (n = 562)	Apo E 4/3 (n = 179)	Prob[a]	Apo E 3/2 (n = 75)	Apo E 3/3 (n = 404)	Apo E 4/3 (n = 127)	Prob[a]
Age (years)	39.1 ± 17.3	39.5 ± 16.2	41.6 ± 17.0	NS	39.9 ± 16.7	40.0 ± 15.2	41.4 ± 16.1	NS
Height (m)	1.60 ± 0.08	1.61 ± 0.07	1.60 ± 0.07	NS	1.59 ± 0.08[b]	1.61 ± 0.06	1.60 ± 0.07	<0.05
Weight (kg)	60.2 ± 11.0	61.4 ± 11.5	61.4 ± 12.5	NS	60.1 ± 10.8	61.4 ± 11.5	61.7 ± 13.3	NS
BMI (kg/m^2)	23.8 ± 5.0	23.8 ± 4.6	24.2 ± 5.2	NS	23.9 ± 4.5	23.7 ± 4.4	24.1 ± 5.4	NS
WHR	0.77 ± 0.07	0.77 ± 0.07	0.77 ± 0.06	NS	0.77 ± 0.06	0.77 ± 0.07	0.77 ± 0.06	NS
Men	Apo E 3/2 (n = 121)	Apo E 3/3 (n = 542)	Apo E 4/3 (n = 162)		Apo E 3/2 (n = 116)	Apo E 3/3 (n = 515)	Apo E 4/3 (n = 155)	
Age (years)	40.6 ± 17.8	40.0 ± 16.8	41.8 ± 16.4	NS	39.3 ± 17.0	38.8 ± 16.1	41.0 ± 16.2	NS
Height (m)	1.72 ± 0.09	1.73 ± 0.07	1.72 ± 0.07	NS	1.72 ± 0.09	1.74 ± 0.07	1.72 ± 0.07	NS
Weight (kg)	74.7 ± 13.3	75.9 ± 13.0	74.4 ± 13.0	NS	74.5 ± 12.8	75.7 ± 12.8	74.3 ± 12.8	NS
BMI (kg/m^2)	25.1 ± 3.9	25.2 ± 3.9	25.0 ± 4.2	NS	25.0 ± 3.8	25.1 ± 3.9	25.0 ± 4.2	NS
WHR	0.89 ± 0.07	0.89 ± 0.07	0.89 ± 0.07	NS	0.89 ± 0.07	0.89 ± 0.07	0.89 ± 0.07	NS

[a] Test of difference among genotype means: NS, not significant at the 0.05 level of probability.

[b] Significantly different from Apo E 3/3 at the 0.05 level of probability.

Sample A: excludes all subjects taking lipid-lowering drugs.

Sample B: excludes all subjects taking lipid-lowering drugs and other drugs potentially affecting lipids (including exogenous sex hormones).

BMI, body mass index (weight in kg/height in m^2); WHR, waist-to hip ratio (waist circumference/hip circumference in cm).

Table 2. Summary of lipid, lipoprotein and apolipoprotein phenotypic variation and contribution of age, age^2, age^3, body mass index (BMI) and waist-to-hip ratio (WHR) by *apo E* genotype in women

Traits		Sample A (n = 870)				Sample B (n = 606)			
		Apo E 3/2 (n = 129)	Apo E 3/3 (n = 562)	Apo E 4/3 (n = 179)	Prob[a]	Apo E 3/2 (n = 75)	Apo E 3/3 (n = 404)	Apo E 4/3 (n = 127)	Prob[a]
TC (mmol/l)	Mean	4.61 ± 0.94	5.13 ± 0.97	5.34 ± 1.19	<0.001	4.52 ± 0.93	5.05 ± 0.99	5.23 ± 1.22	<0.001
	Variance	0.8828	0.9456	1.4181	<0.01	0.8705	0.9780	1.4826	<0.01
	R^2 (×100)[b]	42.15***	34.69***	34.86***	NS	47.40***	41.02***	41.20***	NS
	Residual variance[c]	0.5314	0.6231	0.9505	<0.001	0.4911	0.5841	0.9078	<0.01
	Concomitants selected[d]	AGE, BMI	AGE^2, BMI	AGE^2, BMI		AGE, BMI, WHR	AGE, BMI	AGE, BMI	
LDL-C (mmol/l)	Mean	2.60 ± 0.84	3.11 ± 0.84	3.36 ± 1.03	<0.001	2.55 ± 0.79	3.07 ± 0.85	3.31 ± 1.05	<0.001
	Variance	0.7056	0.7036	1.0557	<0.01	0.6176	0.7224	1.1129	<0.01
	R^2 (×100)	36.21***	29.88***	29.57***	NS	39.85***	35.83***	39.08***	NS
	Residual variance	0.4684	0.4978	0.7650	<0.001	0.3984	0.4694	0.7060	<0.01
	Concomitants selected	AGE, BMI	AGE^2, BMI, WHR	AGE^2, BMI		AGE, BMI	AGE, BMI, WHR	AGE, BMI	
APO B (g/dl)	Mean	0.94 ± 0.26	1.10 ± 0.28	1.18 ± 0.34	<0.001	0.90 ± 0.26	1.07 ± 0.29	1.14 ± 0.35	<0.001
	Variance	0.0662	0.0794	0.1130	<0.01	0.0700	0.0828	0.1219	<0.01
	R^2 (×100)	34.28***	34.60***	37.90***	NS	45.79***	45.13***	45.88***	NS
	Residual variance	0.0453	0.0524	0.0722	<0.01	0.0407	0.0460	0.0687	<0.01
	Concomitants selected	AGE, BMI	AGE^2, BMI, WHR	AGE^2, WHR		AGE, BMI	AGE, BMI, WHR	AGE, WHR	
Log VLDL-C (mmol/l)	Mean[e]	0.52 ± 1.55	0.52 ± 1.62	0.55 ± 1.58	NS	0.49 ± 1.62	0.49 ± 1.62	0.50 ± 1.55	NS
	Variance	0.0370	0.0427	0.0413	NS	0.0434	0.0431	0.0358	NS
	R^2 (×100)[b]	18.43***	24.93***	30.74***	NS	36.97***	31.45***	33.22***	NS
	Residual variance[c]	0.0314	0.0323	0.0294	NS	0.0294	0.0299	0.0249	NS
	Concomitants selected[d]	AGE, WHR	AGE, AGE^2, AGE^3, BMI, WHR	AGE^3, BMI, WHR		AGE, WHR	AGE^2, BMI, WHR	AGE, BMI	
Log TG (mmol/l)	Mean[e]	1.15 ± 1.55	1.17 ± 1.62	1.23 ± 1.58	NS	1.07 ± 1.62	1.07 ± 1.62	1.12 ± 1.55	NS
	Variance	0.0369	0.0427	0.0413	NS	0.0434	0.0431	0.0358	NS
	R^2 (×100)	18.57***	24.88***	30.69***	NS	37.29***	31.39***	33.20***	NS
	Residual variance	0.0313	0.0324	0.0294	NS	0.0292	0.0300	0.0249	NS
	Concomitants selected	AGE, WHR	AGE, AGE^2, AGE^3, BMI, WHR	AGE^3, BMI, WHR		AGE, WHR	AGE^2, BMI, WHR	AGE, BMI	
HDL-C (mmol/l)	Mean	1.43 ± 0.34	1.42 ± 0.33	1.35 ± 0.30	≤0.05	1.42 ± 0.33	1.42 ± 0.33	1.36 ± 0.31	NS
	Variance	0.1159	0.1059	0.0908	NS	0.1089	0.1062	0.0933	NS
	R^2 (×100)	11.61 **	11.64 ***	13.57 ***	NS	15.06 *	11.88 ***	10.55 *	NS
	Residual variance	0.1066	0.0944	0.0808	NS	0.0992	0.0948	0.0869	NS
	Concomitants selected	AGE, BMI, WHR	AGE, AGE^3, BMI, WHR	AGE^2, AGE^3, WHR		WHR	AGE, AGE^3, BMI, WHR	WHR	

Table 2. (Continued)

Traits		Sample A (n = 870)				Sample B (n = 606)			
		Apo E 3/2 (n = 129)	Apo E 3/3 (n = 562)	Apo E 4/3 (n = 179)	Prob[a]	Apo E 3/2 (n = 75)	Apo E 3/3 (n = 404)	Apo E 4/3 (n = 127)	Prob[a]
APO AI (g/dl)	Mean	1.56 ± 0.25	1.53 ± 0.24	1.49 ± 0.22	≤0.05	1.50 ± 0.21	1.50 ± 0.23	1.47 ± 0.22	NS
	Variance	0.0632	0.0576	0.0500	NS	0.0460	0.0519	0.0464	NS
	R^2 (× 100)	9.40 *	9.08 ***	11.52 ***	NS	13.62	10.73 ***	13.10 **	NS
	Residual variance	0.0596	0.0528	0.0455	NS	0.0426	0.0469	0.0420	NS
	Concomitants selected	AGE[2], BMI	AGE, BMI, WHR	AGE, WHR		AGE, WHR	AGE, BMI, WHR	AGE, WHR	

[a] Test of homogeneity among genotypes: NS, not significant at the 0.10 of probability.
[b] Percentage of sample variability on lipid traits associated with a regression model that includes all the concomitants (age, age^2, age^3, BMI, WHR); *p < 0.05; **p < 0.01; ***p < 0.001.
[c] Variance after adjustment for variation in all of the concomitants considered.
[d] Concomitants selected to be significant at the 0.15 of probability in a forward/backward stepwise regression.
[e] Antilog values.
Sample A: excludes all subjects taking lipid-lowering drugs.
Sample B: excludes all subjects taking lipid-lowering drugs and other drugs potentially affecting lipids (including exogenous sex hormones).
TC, total cholesterol; log VLDL-C, $\log_{10}$ transformed very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; log TG, $\log_{10}$ transformed total triglycerides; APO, apolipoprotein.

and the interview, as part of the Heart Health Survey. Plasma total cholesterol (TC), total triglyceride (TG), high density lipoprotein cholesterol (HDL-C), apo AI and apo B concentrations were determined from the fasting blood sample. All of these analyses were carried out at the Lipid Research Laboratory of the University of Toronto, as described by Connelly et al. (9, 10). LDL-C was calculated using the equation of Friedewald et al. (11) and very low density lipoprotein cholesterol (VLDL-C) was derived from TC after subtracting LDL-C and HDL-C. Thus, 3 women and 14 men whose triglycerides were ≥ 5.00 mmol/l were excluded from this study because of missing data for LDL-C. Apo E phenotyping was performed at the Clinical Research Institute of Montreal, according to Hill et al. (12). Six *apo E* genotypes, *ε2/2*, *ε3/2*, *ε3/3*, *ε4/3*, *ε4/2* and *ε4/4*, coded by the three polymorphic alleles, *ε2*, *ε3* and *ε4*, were inferred from the isoform phenotypes E2/2, E3/2, E3/3, E4/3, E4/2 and E4/4.

Statistical analyses

All statistical analyses were carried out separately for women and men, for the complete and the reduced samples (samples A and B, respectively) of those with the three common *apo E* genotypes (*ε3/2*, *ε3/3* and *ε4/3*), using the SAS software (SAS Institute Inc., Cary, NC, 1993, Version 6). Separate regression equations were estimated for women and men, as the distributions of lipid traits and relationships with most other traits are known to be gender specific (6, 13–15), and the natural history of coronary heart disease is different in women and men (16).

We first tested the null hypothesis of homogeneity of the means and variances of each of the concomitant, lipid, lipoprotein and apolipoprotein traits among the three genotype subgroups. Bartlett's test (17) was used to detect heterogeneity of variance. The analysis of variance and the Student–Newman–Keuls (SNK) procedure for multiple comparison (17) were used to detect heterogeneity of a trait mean among genotype subgroups when there was no statistically significant evidence of heterogeneity of trait variance. When there was evidence for heterogeneity of variance, the Kruskal–Wallis non-parametric test (17) was used to test the null hypothesis of homogeneity of the trait mean among genotypes.

Second, we estimated the contribution of the concomitants, i.e. age, age^2, age^3, body mass index (BMI, weight in kg/height in m^2) and waist-to-hip ratio (WHR, waist and hip circumferences in cm), to variability of the lipid traits for each of the three

Table 3. Summary of lipid, lipoprotein and apolipoprotein phenotypic variation and contribution of age, age^2, age^3, body mass index (BMI) and waist-to-hip ratio (WHR) by *apo E* genotype in men

Traits		Sample A (n = 825)				Sample B (n = 786)			
		Apo E 3/2 (n = 121)	Apo E 3/3 (n = 542)	Apo E 4/3 (n = 162)	Prob[a]	Apo E 3/2 (n = 116)	Apo E 3/3 (n = 515)	Apo E 4/3 (n = 155)	Prob[a]
TC (mmol/l)	Mean	4.78 ± 1.02	5.12 ± 1.02	5.30 ± 0.95	<0.001	4.80 ± 1.03	5.09 ± 1.03	5.28 ± 0.95	<0.001
	Variance	1.0489	1.0502	0.9024	NS	1.0663	1.0548	0.9045	NS
	R^2 (×100)[b]	15.48**	22.47***	29.14***	<0.001	13.71**	21.79***	28.48***	≤0.05
	Residual variance[c]	0.9251	0.8219	0.6600	NS	0.9619	0.8331	0.6686	NS
	Concomitants selected[d]	BMI	AGE, AGE^2, WHR	AGE, AGE^3		BMI	AGE, AGE^2, BMI	AGE, AGE^3	
LDL-C (mmol/l)	Mean	2.84 ± 0.89	3.22 ± 0.90	3.35 ± 0.84	<0.001	2.86 ± 0.91	3.19 ± 0.91	3.33 ± 0.84	<0.001
	Variance	0.7974	0.8182	0.7050	NS	0.8197	0.8281	0.7069	NS
	R^2 (×100)	8.30	18.98***	23.17***	<0.01	7.61	18.33***	23.23***	≤0.05
	Residual variance	0.7631	0.6691	0.5590	NS	0.7917	0.6830	0.5609	NS
	Concomitants selected	BMI	AGE, AGE^2, WHR	AGE, AGE^3		BMI	AGE, AGE^2, WHR	AGE, AGE^3	
APO B(g/dl)	Mean	1.03 ± 0.28	1.17 ± 0.31	1.25 ± 0.31	<0.001	1.03 ± 0.28	1.15 ± 0.31	1.25 ± 0.31	<0.001
	Variance	0.0758	0.0954	0.0934	NS	0.0776	0.0946	0.0949	NS
	R^2 (×100)	20.61***	28.70***	26.30***	≤0.05	19.63***	27.14***	26.37***	NS
	Residual variance	0.0628	0.0686	0.0710	NS	0.0652	0.0696	0.0722	NS
	Concomitants selected	BMI, WHR	AGE, AGE^2, BMI, WHR	AGE, AGE^3, BMI		BMI, WHR	AGE, AGE^2, BMI, WHR	AGE, AGE^3, BMI	
Log VLDL-C (mmol/l)	Mean[d]	0.65 ± 1.66	0.60 ± 1.62	0.69 ± 1.62	<0.01	0.65 ± 1.62	0.59 ± 1.62	0.69 ± 1.62	<0.01
	Variance	0.0467	0.0462	0.0434	NS	0.0454	0.0461	0.0439	NS
	R^2 (×100)[b]	31.39***	20.57***	17.22***	NS	29.09***	19.35***	17.45***	NS
	Residual variance[c]	0.0335	0.0370	0.0371	NS	0.0337	0.0375	0.0375	NS
	Concomitants selected[d]	BMI, WHR	AGE, AGE^2, BMI, WHR	AGE, AGE^3, BMI		BMI, WHR	AGE, AGE^2, BMI, WHR	AGE, AGE^3, BMI	
Log TG (mmol/l)	Mean[d]	1.41 ± 1.66	1.32 ± 1.62	1.51 ± 1.62	<0.01	1.41 ± 1.62	1.32 ± 1.62	1.51 ± 1.62	<0.01
	Variance	0.0468	0.0462	0.0433	NS	0.0454	0.0461	0.0438	NS
	R^2 (×100)	31.54***	20.54***	17.21***	NS	29.22***	19.32***	17.44***	NS
	Residual variance	0.0334	0.0370	0.0370	NS	0.0336	0.0376	0.0374	NS
	Concomitants selected	BMI, WHR	AGE, AGE^2, BMI, WHR	AGE, AGE^3, BMI		BMI, WHR	AGE, AGE^2, BMI, WHR	AGE, AGE^3, BMI	
HDL-C (mmol/l)	Mean	1.20 ± 0.25	1.22 ± 0.29	1.18 ± 0.28	NS	1.21 ± 0.25	1.22 ± 0.29	1.17 ± 0.27	NS
	Variance	0.0648	0.0853	0.0784	NS	0.0647	0.0862	0.0729	NS
	R^2 (×100)	8.29	12.20***	14.92***	NS	7.45	12.81***	15.86***	NS
	Residual variance	0.0620	0.0756	0.0688	NS	0.0626	0.0759	0.0634	NS
	Concomitants selected	BMI, WHR	AGE, AGE^2, BMI, WHR	BMI		BMI	AGE, BMI, WHR	AGE, BMI, WHR	

Table 3. (Continued)

Traits		Sample A (n = 825)				Sample B (n = 786)			
		Apo E 3/2 (n = 121)	Apo E 3/3 (n = 542)	Apo E 4/3 (n = 162)	Prob[a]	Apo E 3/2 (n = 116)	Apo E 3/3 (n = 515)	Apo E 4/3 (n = 155)	Prob[a]
APO AI (g/dl)	Mean	1.37 ± 0.20	1.38 ± 0.21	1.36 ± 0.20	NS	1.38 ± 0.20	1.38 ± 0.21	1.35 ± 0.19	NS
	Variance	0.0409	0.0437	0.0381	NS	0.0414	0.0435	0.0345	NS
	R^2 (×100)	2.24	7.44***	5.93	NS	1.86	8.32***	5.78	NS
	Residual variance	0.0417	0.0408	0.0370	NS	0.0425	0.0403	0.0336	NS
	Concomitants selected	No variable	AGE, BMI, WHR	AGE, BMI		No variable	AGE, BMI, WHR	AGE, BMI	

[a] Test of homogeneity among genotypes: NS, not significant at the 0.10 of probability.
[b] Percentage of sample variability on lipid traits associated with a regression model that includes all the concomitants (age, age^2, age^3, BMI, WHR); *p < 0.05; **p < 0.01; ***p < 0.001.
[c] Variance after adjustment for variation in all of the concomitants considered.
[d] Concomitants selected to be significant at the 0.15 of probability in a forward/backward stepwise regression.
[e] Antilog values.
Sample A: excludes all subjects taking lipid-lowering drugs.
Sample B: excludes all subjects taking lipid-lowering drugs and other drugs potentially affecting lipids (including exogenous sex hormones).
TC, total cholesterol; log VLDL-C, $\log_{10}$ transformed very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; log TG, $\log_{10}$ transformed total triglycerides; APO, apolipoprotein.

genotype subgroups, using a linear regression model (17).

Third, the null hypothesis of homogeneity among genotypes of the separate and the total contribution of concomitants to lipid, lipoprotein and apo B trait variability were tested. When statistically significant heterogeneity was detected, we proceeded to carry out pair-wise comparisons of the *ε3/2* or the *ε4/3* genotype to the *ε3/3* genotype. We also used the backward/forward stepwise regression procedure and the 0.15 criterion of selection, in order to evaluate the influence of each of the concomitants on lipid, lipoprotein and apolipoprotein traits in each of the three genotype subgroups.

Fourth, we tested the null hypothesis of homogeneity of the residual variance, after adjusting for all the concomitants within each genotype subgroup, among the three genotypes using Bartlett's test.

To reduce positive skewness, the log (base 10) transformation of the TG and VLDL-C values was used in all analyses. Unless otherwise specified, we considered the 0.05 level of probability as the criterion for significance of a test statistic.

Results

The means and SDs of concomitants for each genotype are given separately in Table 1 for women and men. Except for mean height in sample B of women, there is no evidence for statistically significant heterogeneity of the means, or the intragenotypic variances, of the concomitants among genotypes in women or men.

The means and variances of the lipid, lipoprotein and apolipoprotein traits, and the contribution of concomitants to trait variability are given in Table 2 (women) and Table 3 (men) for each of the three *apo E* genotypes. The separate contributions of age, BMI and WHR to trait variability are given for each genotype–gender stratum in Tables 4–7.

TC, LDL-C and apo B

In both samples A and B, the mean levels of TC, LDL-C and apo B in women and men are significantly different among *apo E* genotypes, a positive gradient being observed from *ε3/2* to *ε3/3* to *ε4/3*. The intragenotypic variances of the unadjusted values of these traits are significantly heterogeneous among genotypes in women but not in men, the largest variance being associated with the *ε4/3* genotype. In general, the contribution of concomi-

tants to variability in TC, LDL-C and apo B (R^2 ($\times 100$)) (Tables 2 and 3) is larger in women than in men but is similar in samples A and B for each gender. The contribution of concomitants ranges from 35 to 47% in women ($p < 0.001$) and from 8 to 30% in men ($p < 0.001$ for the *ε3/3* and *ε4/3* genotypes). The percentage of variability (R^2 ($\times$ 100)) associated with variation in concomitants is significantly heterogeneous among genotypes in men (except for *apo B* in sample B), but not in women.

Pair-wise tests (*ε3/2* vs *ε3/3* and *ε3/3* vs *ε4/3*) show that the heterogeneity of the contribution of the concomitants among genotypes in men is a result of significantly lower R^2 values in those with the *ε3/2* genotype (tests not shown). This observation is primarily a consequence of the heterogeneity in the contribution of age in men, but not in women (see Table 6). Fig. 1 illustrates graphically the dependency of the association between LDL-C levels and age on gender and genotype, using data from sample A. Whereas the differences among genotype means are similar across the age range for women, in men differences among genotypes are small in the second and third decades, becoming greater after 50 years of age. Analyses of the impact of age variation on LDL-C variability show a significant influence in those with the *ε3/3* and *ε4/3* genotypes but not in those with the *ε3/2* genotype (Table 6).

The stepwise regression procedure, using the selection criterion of 0.15 probability for inclusion, suggests that the influence of variation in the three concomitants on variation in lipid traits is variable among genotypes. In women, where the contribution of all the concomitants is not significantly different among *apo E* genotypes, age and BMI are generally selected, but not WHR, in all three genotype subgroups for TC, LDL-C and apo B in samples A and B. In men, where the contribution of all the concomitants to variation in TC and LDL-C was found to be heterogeneous among *apo E* genotypes, only BMI is selected in those with the *ε3/2* genotype and only age, in those with the *ε4/3* genotype. For apo B, age is still not selected in the *ε3/2* genotype subgroup but age and BMI are selected in the *ε3/3* and *ε4/3* subgroups. The heterogeneity of the influence of WHR on TC, LDL-C and apo B among genotypes in women, and of age on TC, LDL-C and apo B among genotypes in men, revealed by the stepwise regression analyses, is generally confirmed by the test of homogeneity of the contribution of each concomitant (results in Tables 4–7).

After adjustment for age, BMI and WHR, the residual intragenotypic variance remains heterogeneous among genotypes in women but not in men. This was the case for both samples A and B.

VLDL-C, TG, HDL-C and apo AI

In both samples A and B, the means of log VLDL-C and log TG are significantly different among the three *apo E* genotypes in men, but not in women. The means are lower in those with the *ε3/3* genotype than in those with the *ε3/2* and *ε4/3* genotypes in both samples. In both women and men, there is no statistically significant evidence for heterogeneity among genotypes for the variance of log VLDL-C or log TG, or the association of variation of each of these traits with variation in concomitants. However, as observed for TC, LDL-C and apo B, the stepwise regression analyses did not select age as a predictor of VLDL-C and TG in the subgroup of men with the *ε3/2* genotype.

Except for differences among the genotype means in sample A, the means and intragenotypic variances of HDL-C and apo AI and their relationships with concomitants were not significantly heterogeneous among *apo E* genotypes in women. In men, there is no statistically significant evidence for heterogeneity among genotypes for the HDL-C and apo AI means or variances, or for their relationships with concomitants.

After adjustment for all the concomitants, the residual variance for log VLDL-C, log TG, HDL-C and apo AI is not significantly heterogeneous among genotypes in women or men in either sample A or B.

Discussion

Our goal in this study was to evaluate the role of context defined by the common *apo E* genotypes and gender in determining the frequency distribution of inter-individual variation in traits that are measures of lipid metabolism. We carried this out by testing the null hypothesis that the 'norm of reaction' (i.e. 'adaptive response' of a genotype to variations in other genetic and environmental agents that influence trait variation (18)) does not vary among the common *apo E* genotypes separately by gender. Few studies of adults have investigated the dependency of the associations between plasma lipid, lipoprotein and apolipoprotein traits and age and body size on the context defined by gender and *apo E* genotype (for example (6, 14)). Inferences from our study are generally consistent with these studies, further documenting the complexity of the etiological relationships that might be expected between inter-individual variation in measures of lipid metabolism and genetic varia-

Table 4. Regression of lipid, lipoprotein and apolipoprotein traits on age[a], BMI or WHR one at a time in women for each genotype (sample A)

		APO E 3/2 (n = 129) R^2 (×100) (Prob[b])	APO E 3/3 (n = 562) R^2 (×100) (Prob[b])	APO E 4/3 (n = 179) R^2 (×100) (Prob[b])	Prob[c]
TC	AGE	39.58 (0.0001)	34.02 (0.0001)	33.57 (0.0001)	NS
	BMI	9.89 (0.0003)	6.58 (0.0001)	13.72 (0.0001)	NS
	WHR	8.21 (0.0010)	7.81 (0.0001)	11.10 (0.0001)	NS
LDL-C	AGE	33.22 (0.0001)	28.57 (0.0001)	28.03 (0.0001)	NS
	BMI	10.71 (0.0002)	6.65 (0.0001)	12.20 (0.0001)	NS
	WHR	7.92 (0.0012)	8.69 (0.0001)	10.92 (0.0001)	NS
APO B	AGE	28.58 (0.0001)	30.70 (0.0001)	34.30 (0.0001)	NS
	BMI	14.54 (0.0001)	10.28 (0.0001)	14.83 (0.0001)	NS
	WHR	13.53 (0.0001)	13.45 (0.0001)	18.84 (0.0001)	NS
Log VLDL-C	AGE	10.49 (0.0030)	17.24 (0.0001)	24.96 (0.0001)	NS
	BMI	8.33 (0.0009)	12.64 (0.0001)	15.34 (0.0001)	NS
	WHR	13.85 (0.0001)	11.77 (0.0001)	17.60 (0.0001)	NS
Log TG	AGE	10.63 (0.0028)	17.19 (0.0001)	24.89 (0.0001)	NS
	BMI	8.39 (0.0009)	12.66 (0.0001)	15.30 (0.0001)	NS
	WHR	13.91 (0.0001)	11.74 (0.0001)	17.62 (0.0001)	NS
HDL-C	AGE	1.84 (0.5078)	0.92 (0.1604)	3.96 (0.0688)	NS
	BMI	5.44 (0.0078)	5.53 (0.0001)	2.05 (0.0559)	NS
	WHR	5.44 (0.0078)	6.46 (0.0001)	7.28 (0.0003)	NS
APO AI	AGE	4.07 (0.1566)	3.63 (0.0001)	6.15 (0.0110)	NS
	BMI	2.26 (0.0893)	0.92 (0.0232)	0.07 (0.7341)	NS
	WHR	1.27 (0.2032)	1.94 (0.0009)	1.67 (0.0849)	NS

[a] Includes age, age^2, age^3.
[b] P-value of the regression model.
[c] Test of homogeneity among genotypes.
NS, not significant at the 0.10 level of probability.
TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; APO, apolipoprotein; log VLDL-C, $\log_{10}$ transformed very low density lipoprotein cholesterol; log TG, $\log_{10}$ transformed total triglycerides; HDL-C, high density lipoprotein cholesterol.

tion. We review here the implications of our findings for understanding genotype–phenotype relationships in the population at large.

In agreement with previous studies (6, 14, 19–22), we found that, on average, those individuals with the *ε4* allele had higher, and those individuals with the *ε2* allele had lower TC, LDL-C and apo B values, regardless of gender or whether the sample included those taking drugs or hormones. Less consistent among studies is the influence of genotype on the mean values for TG, VLDL-C, HDL-C and apo AI (23). Compared with the *ε3/3* genotype, we found that the means of TG and VLDL-C were significantly higher in men (but not women) with the *ε4/3* genotype, regardless of whether the subjects were taking drugs potentially affecting lipid levels. In contrast, the average HDL-C and apo AI levels were found to be significantly lower in women (but not men) with the *ε4/3* genotype, but only in the sample that included individuals taking drugs or exogenous hormones. We reported similar results for VLDL-C and TG in healthy subjects from a previous study by our group (19). These findings support the argument that influences of a particular genetic variation (*apo E* is our example) on the average level of a trait may be invariant with regard to effects indexed by gender (TC, LDL-C and apo B are examples for the *apo E* gene), or be dependent on effects indexed by gender (TG is an example for the *apo E* gene). Our study further illustrates that variation in a gene may influence variation in different measures of a physiological subsystem in different ways. The gender dependency of the heterogeneity of the pleiotropic effects of the *apo E* gene on measures of lipid metabolism is likely a consequence of gender-specific interactions of different agents on the phenotypic values of different traits.

Our finding that the relationship between variation in measures of lipid metabolism and variation in age, BMI and WHR is dependent on context defined by gender and *apo E* genotype is further documentation of the complexity of the biological relationships that link genotypic variation with phenotypic variation (4). The observed gender differences in the heterogeneity among genotypes in the association of TC, LDL-C and apo B levels

with age and measures of body size is consistent with the *apo E* genotype by gender and body-size interaction effects first reported by Reilly et al. (6). These studies bring to attention the reality that, in general, the utility of non-genetic risk factor information for predicting and understanding risk of diseases like CVD that have a complex multi-factorial etiology will be genotype dependent.

The traditional strategy for genetic studies has been to adjust the entire sample for variation in concomitants and then to proceed with the estimation of genetic effects. The context-dependency of *apo E* effects emphasizes that such a procedure can result in misrepresentation of the influence of genetic variation on trait variability. When gene by environment interaction plays a large role in the biology of a risk factor, estimation of genetic effects using pooled male and female data that have been adjusted for concomitant variation can understate the role of a gene in determining trait variation. This would result in the minimization of the utility of genetic variation for predicting risk of CVD. Equally important is the futility of searching for consistency of genetic effects among studies that differ in their gender composition, concomitants considered in data adjustment prior to genetic analyses, and the mix of interacting non-genetic agents (24).

Bradshaw (25), Murphy (26), Bishop et al. (27), Berg (28) and Sing et al. (4) are among those who have suggested that genetic variability may influence intragenotypic phenotypic variance among individuals. Few studies have estimated the influence of genotypic variation on intragenotypic phenotypic variance of human quantitative CVD risk factors. Reilly et al. presented the first studies of *apo E* genotype-specific phenotypic variances of measures of lipid metabolism in 1991 (14). Their analyses of published data suggested that the effects of *apo E* genotypic variation on the intragenotypic phenotypic variability that they observed might be generalized to other populations. Our study further documents that the *apo E* gene is an example of a 'variability' gene, as well as a 'level' gene. Variability in the average level of a risk factor among genotypes is but one measure of risk associated with genetic information. Knowledge about heterogeneity of the intragenotypic

Table 5. Regression of lipid, lipoprotein and apolipoprotein traits on age[a], BMI or WHR one at a time in women for each genotype (sample B)

		APO E 3/2 (n = 75) R^2 (×100) (Prob[b])	APO E 3/3 (n = 404) R^2 (×100) (Prob[b])	APO E 4/3 (n = 127) R^2 (×100) (Prob[b])	Prob[c]
TC	AGE	42.55 (0.0001)	40.31 (0.0001)	39.22 (0.0001)	NS
	BMI	6.21 (0.0311)	5.86 (0.0001)	15.53 (0.0001)	NS
	WHR	2.36 (0.1883)	8.41 (0.0001)	10.85 (0.0002)	NS
LDL-C	AGE	34.51 (0.0001)	34.30 (0.0001)	37.07 (0.0001)	NS
	BMI	6.96 (0.0221)	6.80 (0.0001)	13.85 (0.0001)	NS
	WHR	2.20 (0.2043)	9.09 (0.0001)	13.82 (0.0001)	NS
APO B	AGE	40.12 (0.0001)	40.75 (0.0001)	42.36 (0.0001)	NS
	BMI	9.64 (0.0067)	10.94 (0.0001)	14.88 (0.0001)	NS
	WHR	8.82 (0.0097)	15.92 (0.0001)	20.51 (0.0001)	NS
Log VLDL-C	AGE	26.67 (0.0001)	23.72 (0.0001)	27.66 (0.0001)	NS
	BMI	7.33 (0.0188)	12.53 (0.0001)	17.88 (0.0001)	NS
	WHR	21.23 (0.0001)	14.75 (0.0001)	13.55 (0.0001)	NS
Log TG	AGE	26.88 (0.0001)	23.66 (0.0001)	27.60 (0.0001)	NS
	BMI	7.47 (0.0177)	12.54 (0.0001)	17.86 (0.0001)	NS
	WHR	21.39 (0.0001)	14.71 (0.0001)	13.62 (0.0001)	NS
HDL-C	AGE	1.97 (0.7000)	0.60 (0.4918)	1.00 (0.7429)	NS
	BMI	3.14 (0.1282)	7.23 (0.0001)	1.29 (0.2042)	NS
	WHR	12.20 (0.0021)	6.92 (0.0001)	8.69 (0.0008)	NS
APO AI	AGE	9.37 (0.0707)	4.76 (0.0002)	3.69 (0.2000)	NS
	BMI	0.31 (0.6342)	1.45 (0.0155)	0.10 (0.7248)	NS
	WHR	1.68 (0.2674)	1.53 (0.0128)	3.31 (0.0406)	NS

[a] Includes age, age^2, age^3.

[b] P-value of the regression model.

[c] Test of homogeneity among genotypes.

NS, not significant at the 0.10 level of probability.

TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; APO, apolipoprotein; log VLDL-C, $\log_{10}$ transformed very low density lipoprotein cholesterol; log TG, $\log_{10}$ transformed total triglycerides; HDL-C, high density lipoprotein cholesterol.

Table 6. Regression of lipid, lipoprotein and apolipoprotein traits on age[a], BMI or WHR one at a time in men for each genotype (sample A)

		APO E 3/2 (n = 121) R^2 (×100) (Prob[b])	APO E 3/3 (n = 542) R^2 (×100) (Prob[b])	APO E 4/3 (n = 162) R^2 (×100) (Prob[b])	Prob[c]
TC	AGE	6.86 (0.0393)	21.27 (0.0001)	27.62 (0.0001)	<0.01
	BMI	9.57 (0.0006)	4.81 (0.0001)	4.03 (0.0105)	NS
	WHR	3.76 (0.0332)	8.06 (0.0001)	4.19 (0.0090)	NS
LDL-C	AGE	3.79 (0.2087)	17.71 (0.0001)	21.98 (0.0001)	<0.01
	BMI	4.74 (0.0165)	3.93 (0.0001)	3.41 (0.0187)	NS
	WHR	1.32 (0.2101)	7.63 (0.0001)	3.63 (0.0152)	NS
APO B	AGE	9.22 (0.0099)	23.57 (0.0001)	23.18 (0.0001)	<0.05
	BMI	14.01 (0.0001)	9.67 (0.0001)	8.92 (0.0001)	NS
	WHR	9.86 (0.0005)	15.51 (0.0001)	10.32 (0.0001)	NS
Log VLDL-C	AGE	10.79 (0.0038)	9.18 (0.0001)	8.79 (0.0022)	NS
	BMI	22.14 (0.0001)	14.90 (0.0001)	12.95 (0.0001)	NS
	WHR	17.22 (0.0001)	13.02 (0.0001)	8.65 (0.0001)	NS
Log TG	AGE	10.86 (0.0037)	9.18 (0.0001)	8.78 (0.0022)	NS
	BMI	22.26 (0.0001)	14.87 (0.0001)	12.93 (0.0001)	NS
	WHR	17.26 (0.0001)	12.99 (0.0001)	8.61 (0.0002)	NS
HDL-C	AGE	1.70 (0.5705)	0.09 (0.9275)	1.25 (0.5734)	NS
	BMI	6.03 (0.0066)	8.74 (0.0001)	11.10 (0.0001)	NS
	WHR	5.43 (0.0101)	7.49 (0.0001)	6.31 (0.0013)	NS
APO AI	AGE	0.66 (0.8539)	0.61 (0.3459)	1.81 (0.4086)	NS
	BMI	1.01 (0.2739)	3.60 (0.0001)	2.21 (0.0592)	NS
	WHR	0.91 (0.2974)	2.88 (0.0001)	0.83 (0.2497)	NS

[a] Includes age, age^2, age^3.
[b] P-value of the regression model.
[c] Test of homogeneity among genotypes.
NS, not significant at the 0.10 level of probability.
TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; APO, apolipoprotein; log VLDL-C, $\log_{10}$ transformed very low density lipoprotein cholesterol; log TG, $\log_{10}$ transformed total triglycerides; HDL-C, high density lipoprotein cholesterol.

phenotypic variance of a risk factor provides additional information about the fraction of individuals with a particular genotype who exceed a particular level of risk. The *ε4/3* genotype not only predicts a higher average level of TC, LDL-C and apo B than the *ε3/3* genotype, it also predicts that a larger fraction of individuals will deviate a given distance from the average. Although apparently not the case with the *apo E* gene, it is feasible that genotypic variability could influence intragenotypic phenotypic variability, but not the average genotype values of a risk factor (28).

In this study, we found more differences in the intragenotypic variance of measures of lipid metabolism among genotypes in women than in men. This is in accordance with the results of Reilly et al. who also tested the hypothesis of homogeneity of the individual contribution of four concomitants to the variability of measures of lipid metabolism among genotypes (6). Compared with our study, they found more heterogeneity among genotypes in the contribution of concomitants to intragenotypic phenotypic variability. However, age had a greater influence than weight in men and the contrary was found in women, which is comparable with the results of our study.

The rationale for conducting genetic studies most often dictates the strategy for estimating the role of the genetic variation contribution to variation in risk of disease. Some would argue that genetic variation has utility only as it supplements information on traditional non-genetic risk factors. This point of view assumes that the actions of genes are biologically independent of causative agents that are indexed by variation in the non-genetic risk factors. The evaluation of the influence of the *apo E* polymorphism on measures of lipid metabolism has relied abundantly on this assumption. The consistent finding from the application of this strategy, across a wide range of samples drawn from populations differing in ethnic background and geographic location, has been that individuals who carry the *ε4* allele have the highest, while those with the *ε2* allele have the lowest levels of plasma cholesterol. This result provides a strong argument for the merit of estimating the effects of genetic variation after considering all other risk factors.

Does the experience with the *apo E* gene justify pursuing the traditional statistical method of estimating genetic effects using data that have been adjusted for other predictors of trait variation? We believe there are three reasons why this logic may be inappropriate. First, our *apo E* study, and the previous work of Reilly et al. (6), clearly demonstrate that the impact of genetic variation can be dependent on the influence of agents that are indexed by gender, age and body size. The *apo E* gene has context-dependent effects as well as invariant effects. Second, the gene has pleiotropic effects on many measures of lipid metabolism and other intermediate biological and physiological processes involved in determining health (29). Previous work (6, 14) has clearly established that the *apo E* gene has different effects on different measures of lipid metabolism. Different combinations of concomitants make different contributions to predicting variation in different *apo E* genotypes. The genotype effects on some but not on all traits depend on age and gender. One should expect that the effects of variation in a particular gene would have invariant effects on the variation of some traits and context-dependent effects on the variation in other traits. Third, the biological reality is that neither genes nor environments, but their interactions, are the causation of phenotypic variability (3, 18).

The observed heterogeneity of the associations of measures of lipid metabolism with concomitants and that of the intragenotypic phenotypic variance among genotypes is a statistical reflection of the interaction of unmeasured genetic and environmental agents with effects of the *apo E* genotypes. Insights about the interactive biology of causation are not possible from studies that take the traditional approach to analyses that seek the invariant effects of genotypic variation. We believe that it is far better to carry out studies of the impact of genetic variation that seek to document the complexity of the biological reality and test the assumptions of independence of genetic effects than to focus solely on estimating invariant genotypic effects.

In summary, studies of the *apo E* gene document that it is unrealistic to believe that variation in a particular gene will have invariant effects on all

Table 7. Regression of lipid, lipoprotein and apolipoprotein traits on age[a], BMI or WHR one at a time in men for each genotype (sample B)

		APO E 3/2 (n = 116) R^2 (×100) (Prob[b])	APO E 3/3 (n = 515) R^2 (×100) (Prob[b])	APO E 4/3 (n = 155) R^2 (×100) (Prob[b])	Prob[c]
TC	AGE	6.53 (0.0550)	20.52 (0.0001)	27.12 (0.0001)	<0.05
	BMI	8.60 (0.0014)	4.85 (0.0001)	3.80 (0.0150)	NS
	WHR	3.66 (0.0396)	7.56 (0.0001)	3.75 (0.0158)	NS
LDL-C	AGE	3.58 (0.2512)	16.96 (0.0001)	22.39 (0.0001)	<0.05
	BMI	4.72 (0.0192)	4.17 (0.0001)	2.99 (0.0315)	NS
	WHR	1.39 (0.2072)	7.17 (0.0001)	3.48 (0.0201)	NS
APO B	AGE	9.71 (0.0094)	21.59 (0.0001)	23.62 (0.0001)	NS
	BMI	13.02 (0.0001)	9.44 (0.0001)	8.51 (0.0002)	NS
	WHR	9.65 (0.0007)	15.23 (0.0001)	10.29 (0.0001)	NS
Log VLDL-C	AGE	11.09 (0.0042)	7.84 (0.0001)	8.61 (0.0034)	NS
	BMI	18.57 (0.0001)	13.99 (0.0001)	13.56 (0.0001)	NS
	WHR	16.24 (0.0001)	13.04 (0.0001)	8.88 (0.0002)	NS
Log TG	AGE	11.19 (0.0039)	7.85 (0.0001)	8.60 (0.0035)	NS
	BMI	18.68 (0.0001)	13.96 (0.0001)	13.55 (0.0001)	NS
	WHR	16.30 (0.0001)	13.00 (0.0001)	8.84 (0.0002)	NS
HDL-C	AGE	0.97 (0.7783)	0.07 (0.9464)	0.60 (0.8213)	NS
	BMI	4.72 (0.0192)	8.54 (0.0001)	11.93 (0.0001)	NS
	WHR	4.57 (0.0212)	7.91 (0.0001)	8.76 (0.0002)	NS
APO AI	AGE	0.30 (0.9534)	0.79 (0.2564)	1.15 (0.6270)	NS
	BMI	0.53 (0.4363)	3.71 (0.0001)	2.19 (0.0663)	NS
	WHR	0.59 (0.4109)	3.52 (0.0001)	1.59 (0.1180)	NS

[a] Includes age, age^2, age^3.
[b] P-value of the regression model.
[c] Test of homogeneity among genotypes.
NS, not significant at the 0.10 level of probability.
TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; APO, apolipoprotein; log VLDL-C, $\log_{10}$ transformed very low density lipoprotein cholesterol; log TG, $\log_{10}$ transformed total triglycerides; HDL-C, high density lipoprotein cholesterol.

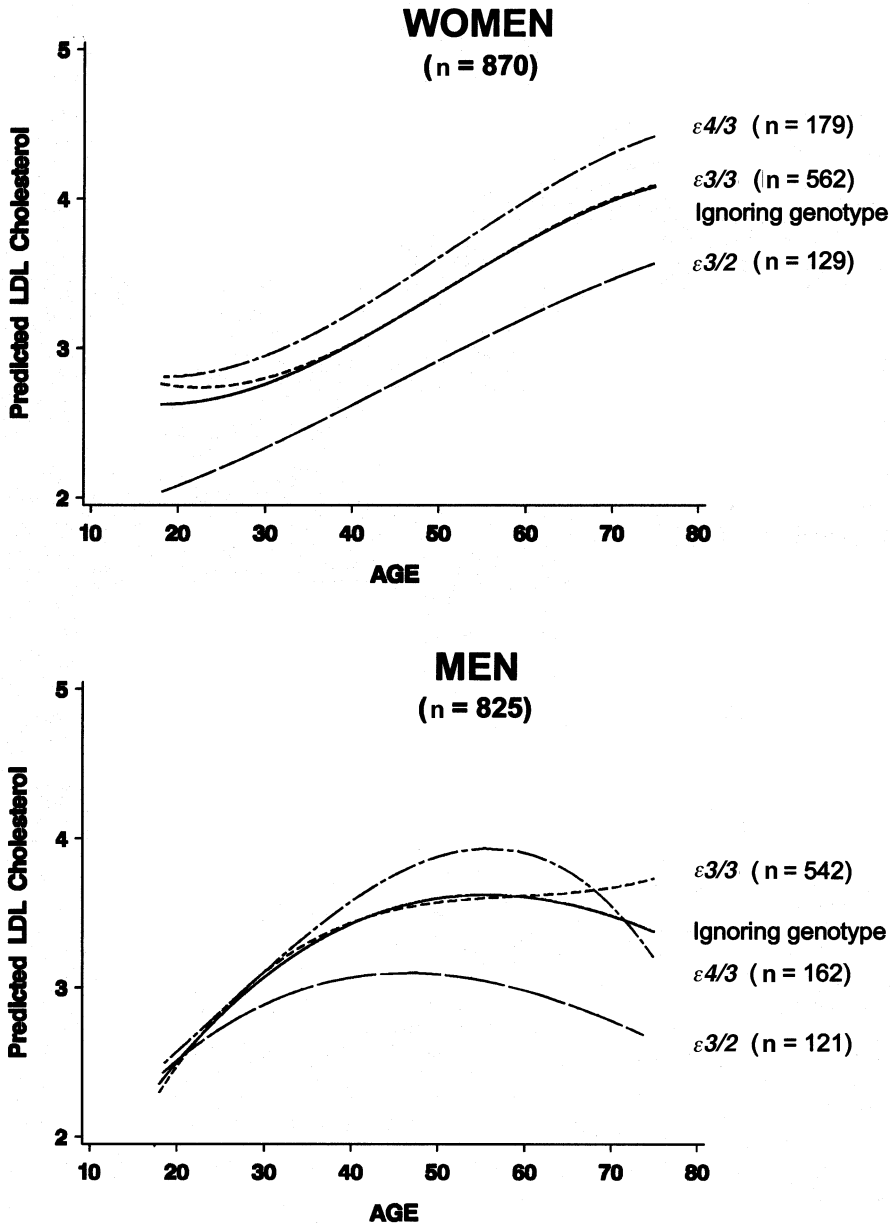

Fig. 1. Regression curves showing the relationship between age and predicted LDL-C levels in women (upper panel) and men (lower panel). The age-dependent LDL-C curves in women show a parallel increase for all 3 genotypes, the *ε3/2* sub-group curve being at lower levels at all ages. In men, genotype differences are small in the second and third decades becoming greater after the age of 50. A significant impact of age on LDL-C is noted in those with the *ε3/3* and *ε4/3* genotypes, but not in those with the *ε3/2* genotype.

traits that it influences. An analytical strategy that seeks to illuminate these context-dependent effects will minimize the risk of failing to obtain knowledge concerning the full utility of genetic variation. Our study also suggests that the utility of the traditional non-genetic risk factors for predicting risk of CVD can depend on the context defined by genotype. This finding is consistent with the reality that neither genes nor environments, but their interactions, are the causes of variation in risk of cardiovascular disease.

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