Context-dependent and invariant associations between lipids, lipoproteins, and apolipoproteins and apolipoprotein E genotype

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Abstract Variation in apolipoprotein (apo)E genotypes predicts variation in plasma cholesterol and apoB; however, the context-dependent associations between high density lipoprotein (HDL) cholesterol, apoA-I, triglycerides, and lipoprotein[a] (Lp[a]) and this polymorphism remain unsettled. We genotyped 5,025 women and 4,035 men sampled to represent a white general population in the age range 20 to 80+ years (mean ages 58 and 57 years for women and men, respectively). The relative frequencies of the ε22, ε32, ε42, ε33, ε43, and ε44 genotypes were 0.005, 0.127, 0.027, 0.564, 0.251, and 0.027, respectively. Variations in apoE genotype (in the order listed above) predicted stepwise increases in cholesterol and apoB in both genders (all ANOVAs: $P <$ 0.001), and stepwise decreases in HDL cholesterol and apoA-I in women (both ANOVAs: $P < 0.001$), but not in men. In both genders ε33 individuals had the lowest levels of nonfasting triglycerides, whereas the highest levels were found in individuals with ε22 and ε44 genotypes (both ANOVAs: $P < 0.001$). Finally, a stepwise increase in Lp[a] was seen in women (ANOVA: $P < 0.001$), but not in men. In women, the association between variation in nonfasting triglycerides and Lp[a], and variation in apoE genotypes was mainly seen in those with the highest alcohol consumption, similar to the consumption of most men. Variations in apoE genotype predicted 5% and 11% in women, and 2% and 6% in men, of the total variation in plasma cholesterol and apoB, respectively. Variation in levels of plasma lipoproteins is associated with variation in apoE genotypes in the population at large, with the most pronounced association in women, except for nonfasting triglycerides, for which the association is most pronounced in men. Whereas the associations between variation in plasma cholesterol and apoB and the variation in apoE genotypes seem invariant, the associations with variation in plasma HDL cholesterol, apoA-I, nonfasting triglycerides, and Lp[a] seem context dependent.—Frikke-Schmidt, R., B. G. Nordestgaard, B. Agerholm-Larsen, P. Schnohr, and A. Tybjærg-Hansen. **Context-dependent and invariant associations between lipids, lipoproteins, and apolipoproteins and apolipoprotein E genotype. *J. Lipid Res.* 2000.** 41: **1812–1822.**

Supplementary key words genes • atherosclerosis• interactions

Three different apolipoprotein (apo)E alleles (ε2, ε3, and ε4) on the long arm of chromosome 19, each allele encoding one apoE isoform (apoE-2, apoE-3, apoE-4), result in six different genotypes (ε22, ε32, ε42, ε33, ε43, and ε44) (1, 2). The apoE polymorphism explains about 20% of the variance in plasma levels of apoE (3). ApoE-2 differs from apoE-3 by a cysteine-for-arginine substitution at amino acid residue 158, whereas apoE-4 differs from apoE-3 by an arginine-for-cysteine substitution at residue 112 (4). ApoE mediates the catabolism of chylomicron and very low density lipoprotein (VLDL) remnants via a "remnant" receptor, and the binding of chylomicron remnants, VLDL, and intermediate density lipoproteins to the low density lipoprotein (LDL) receptor (4).

The apoE polymorphism is potentially one of the most important genetic predictors of plasma lipoprotein levels and thus of risk of ischemic heart disease (4–14). It is well established that variation in plasma levels of cholesterol and apoB is associated with variation in apoE genotypes, and that these associations seem to exist in most contexts, that is, appear to be invariant. In contrast, the associations between variation in plasma levels of high density lipoprotein (HDL) cholesterol, apoA-I, triglycerides, and lipoprotein[a] (Lp[a]), and variation in apoE genotypes remain unsettled, possibly because these associations may be context dependent. Most previous studies

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; apo, apolipoprotein; BMI, body mass index; BP, blood pressure; HDL, high density lipoprotein; HRT, hormonal replacement therapy; LDL, low density lipoprotein; Lp[a], lipoprotein[a]; PCR, polymerase chain reaction; VLDL, very low density lipoprotein.

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(4–9) have explored these associations in women and men combined or in men alone, and have not explored other contexts separately.

We conducted a study of 5,025 women and 4,035 men from the Danish general population, the Copenhagen City Heart Study (15), to assess whether variations in apoE genotypes predicted variation in plasma cholesterol, apoB, HDL cholesterol, apoA-I, nonfasting triglycerides, and Lp[a]. A priori we stratified our analysis by gender, but also determined whether these associations were context dependent within each gender. A particularly important aspect of our study is that we were able to explore whether apoE genotypes could predict variation in plasma nonfasting triglycerides. Because this is the natural state for most humans in the larger part of a 24-h cycle, it could be argued that this context is more important to study than that of the conventional fasting state, which occurs for only a few hours every morning. It should be emphasized, however, that conclusions about the associations between variation in nonfasting triglycerides and variation in apoE genotypes may not be comparable to conclusions based on fasting data.

MATERIALS AND METHODS

Subjects

We studied individuals who participated in the third examination of the Copenhagen City Heart Study from 1991 through 1994. This prospective cardiovascular population study includes an almost equal number of women (55%) and men stratified into 10-year age groups from 20 to 80 years and above, drawn randomly from the general population of the city of Copenhagen around Copenhagen University Hospital, Rigshospitalet, using the Copenhagen Central Population Register. A detailed description of the first (1976 through 1978) and second (1981 through 1983) examination of this study has been published previously (15). The original cohort from 1976–1978, supplemented with five hundred 20- to 24-year-olds at the second examination, and 500 individuals in each of the age groups 20 through 24, 25 through 29, 30 through 34, 35 through 39, 40 through 44, and 45 through 49 years at the third examination, were all invited to participate in the third examination at Rigshospitalet, Copenhagen. Of the 17,180 individuals invited, 10,049 participated, 9,259 gave blood, and 9,241 were genotyped. Fewer than 1% were nonwhites and 98.8% were Danish citizens, that is, for practical purposes were of Danish descent (16). All participants gave informed consent; 181 were excluded because of lipid-lowering medication. The study was approved by the Danish ethics committee for Copenhagen and Frederiksberg (No. 100.2039/91).

Questionnaire

A self-administered questionnaire was filled in before the examination, and validated by an investigator on the day of attendance. All subjects reported the weekly intake of alcoholic beverages in the form of number of beers, glasses of wine, or units of spirits [1 unit = 12 g of alcohol and corresponds to 1 beer (33 cl), 1 glass of wine, or 2 cl of spirits], and reported use of antihypertensive medication and diuretics, treatment for diabetes mellitus, smoking status, and physical activity, and women in addition reported menopausal status, and whether they used hormonal replacement therapy (HRT) (15).

Laboratory analyses

Total cholesterol, apoB, HDL cholesterol, triglycerides, apoA-I, glucose (Boehringer Mannheim, Mannheim, Germany), and Lp[a] total mass (Dako, Carpinteria, CA) levels were measured in nonfasting plasma (15). The polyclonal apo[a]-specific antiserum used in this assay was sensitive to apo[a] heterogeneity.

ApoE genotypes were identified by polymerase chain reaction (PCR) followed by digestion with *Hha*I as described (17), except that, because of the large sample size, we used a 5% agarose gel instead of a polyacrylamide gel. On the agarose gel we could not always detect the 48-bp band that distinguished between the ε22 and ε32 genotypes; we therefore retyped all ε22 and ε32 genotypes, using a second PCR (sense primer, 5′-ACATGGAGGAC GTGTGCGG-3′; antisense primer, 5′-ACGCGGCCCTGTTCC ACCA-3′) followed by digestion of the PCR product (250 bp) with *Hae*II (ε22 homozygotes: 2 × 187 bp; ε32 heterozygotes: 1 × 187 bp, 1 × 152 bp, 1 × 35 bp; and common bands of 32, 18, and 13 bp). The 187-bp band and 152-bp band were clearly distinguishable on an agarose gel. Seven of 48 ε22 individuals had originally been misclassified as ε32.

Other measurements

Height, weight, waist/hip ratio, and systolic and diastolic blood pressure (BP) were measured as described (15). Body mass index (BMI) was determined as weight divided by height squared (kg/m^2).

Statistical methods

Statistical analyses were performed for each gender separately, using the SPSS (Chicago, IL) program (18). A P value of <0.05 was considered significant. The distributions of plasma cholesterol, apoB, HDL cholesterol, apoA-I, nonfasting $\log_{10}$ triglycerides, and $\log_{10}$ Lp[a] appeared approximately normally distributed on inspection of bar graphs. The $\log_{10}$ transformation of Lp[a] was slightly overtransformed; however, for simplicity we still chose this transformation instead of a more unconventional transformation. When Kolmogorov-Smirnov normality tests were performed for distributions of these six traits as a whole as well as within each of the six genotypes separately, almost all 42 normality tests for each gender showed statistical evidence of non-normal distribution. Therefore, we have chosen mainly to show the results from the nonparametric analyses, except when nonparametric tests were not available: we used Levene's test for homogeneity of variance, a parametric test not dependent on the assumption of normality (18, 19), and analysis of covariance (ANCOVA) for multivariate analyses.

To test the first study hypothesis (which states that means and variances of plasma cholesterol, apoB, HDL cholesterol, apoA-I, nonfasting triglycerides, and Lp[a] do not differ as a function of apoE genotype in either gender), the Kruskal-Wallis analysis of variance (ANOVA) was used to evaluate heterogeneity of the means across genotypes. The Mann-Whitney U test was used as a post-hoc test for two-genotype comparisons. Levene's test examined homogeneity of variance. Average allele effects were calculated as described (13), and allele frequencies were estimated by the gene-counting method.

To test the second study hypothesis (which states that the apoE genotype does not interact with other lipid or nonlipid cardiovascular risk factors in the prediction of the six lipid, lipoprotein, and apolipoprotein traits), homogeneity of the association of genotype and each of the following cardiovascular risk factors on the six lipid, lipoprotein, and apolipoprotein traits were tested using bivariate interaction terms in an ANCOVA also including genotype and the risk factor in question: cholesterol, apoB, HDL cholesterol, apoA-I, nonfasting triglycerides, Lp[a], BMI, waist/hip ratio, glucose, alcohol consumption, systolic BP,

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diastolic BP, age in 10-year groups, hypertension, diuretics, diabetes mellitus, smoking, and physical activity for both women and men separately, and in addition menopausal status and use of HRT for women.

Prior to tests for interactions, all continuous covariates were evaluated with regard to normality and homogeneity of variances across genotypes. Distributions of apoB in both genders and HDL cholesterol and glucose levels in females appeared approximately normally distributed on inspection of bar graphs, whereas statistically there was evidence of heterogeneity of variance. Logarithmically transformed, the traits still appeared approximately normally distributed and the heterogeneity of variance disappeared. Distributions of nonfasting triglycerides and Lp[a] were skewed and showed evidence of heterogeneity of variance across genotypes. Logarithmically transformed, these traits appeared approximately normally distributed on inspection of bar graphs in both genders, and showed no heterogeneity of variance across genotypes, except for Lp[a] in men; when ε22 males were excluded from the analysis this heterogeneity almost disappeared (before exclusion, $P = 0.001$; after exclusion, $P = 0.039$). We have therefore chosen to ignore this heterogeneity when testing for interactions between apoE genotype and covariates in predicting levels of Lp[a] in men, which represents a limitation of that analysis. A further limitation is that alcohol intake was not normally distributed. All other continuous variables appeared normally distributed and showed no heterogeneity of variance.

All significant bivariate interactions apparent from the 216 ANCOVAs performed were further explored by dividing the interacting covariates into categories, tertiles, or quintiles, followed by tests of heterogeneity of means across the six genotypes. Bonferroni corrections for multiple comparisons (19 for women, 17 for men) after interaction tests were performed for each lipid trait in both genders (20).

The residual marginal "effect" of apoE genotype on the total variation in the six lipid, lipoprotein, and apolipoprotein traits was determined. First, the six lipid traits were adjusted by ANCOVA for age in 10-year groups, diabetes mellitus, physical activity at leisure, antihypertensive drugs, diuretics, BMI, and alcohol consumption in both women and men, and in addition for menopausal status and use of HRT in women. Second, an ANOVA on these adjusted lipid traits was used to calculate the genetic variance due to apoE (s_G^2) and the within genotype group variance (s_W^2) from sum of squares and mean square error from the ANOVA table. Finally, the proportion of the total phenotypic variability attributable to apoE genotype was estimated by $s_G^2/(s_G^2 + s_W^2)$ (21). If there was interaction between genotype and the covariate, the residual marginal effect of apoE genotype was calculated in tertiles of the interacting covariate.

RESULTS

The relative apoE genotype and allele frequencies in these white Danish individuals sampled from the general population were as follows: ε22, 0.005; ε32, 0.127; ε42, 0.027; ε33, 0.564; ε43, 0.251; and ε44, 0.027; also ε2, 0.082; ε3, 0.753; and ε4, 0.165. Genotype frequencies were similar for women and men (χ^2: $P = 0.4$), and did not differ significantly from those predicted by the Hardy-Weinberg equilibrium (χ^2: $0.2 < P < 0.3$). However, as shown in previous studies (22–25) the frequency of the ε4 allele decreased significantly with age from 0.156 in the 20- to 30-year-olds to 0.137 in individuals above 80 years of age ($P = 0.05$). **Table 1** shows basic characteristics of the 5,025 women and 4,035 men sampled from the general population.

Means and variances of lipids, lipoproteins, and apolipoproteins as a function of apoE genotype

There was a stepwise increase as a function of genotype (ε22, to ε32, to ε42, to ε33, to ε43, to ε44) and alleles (ε2, to ε3, to ε4) in cholesterol and apoB, in both

TABLE 1. Characteristics of individuals

Characteristic	Women	Men	*P* Value[a]		
			Means	Variance	Frequency
No. of individuals	5,025	4,035			
Age (years)[b]	58 ± 0.2	57 ± 0.2	<0.001	0.89	
Cholesterol (mmol/l)	6.3 ± 0.02	6.0 ± 0.02	<0.001	<0.001	
ApoB (mg/dl)	86 ± 0.3	86 ± 0.4	0.23	<0.001	
HDL cholesterol (mmol/l)	1.7 ± 0.01	1.4 ± 0.01	<0.001	<0.001	
ApoA-I (mg/dl)	151 ± 0.4	130 ± 0.4	<0.001	<0.001	
Triglycerides (mmol/l)[c]	1.7 ± 0.02	2.1 ± 0.03	<0.001	<0.001	
Lp[a] (mg/dl)	32 ± 6	29 ± 6	<0.001	<0.001	
BMI (kg/m^2)	25 ± 0.1	26 ± 0.1	<0.001	<0.001	
Hypertension (%)	19	21			0.05
Diabetes mellitus (%)	2	4			<0.001
Smokers (%)	46	52			<0.001
Ex-smokers (%)	23	30			
Nonsmokers (%)	31	18			
Alcohol consumption (units/week)[d]	6	14	<0.001	<0.001	
Premenopausal (%)	29	—			—
Postmenopausal (%)	71	—			—
−HRT (%)	21	—			—

Values are means ± SEM or frequencies.
[a] Mann Whitney U test, Levene's test, and χ^2 tests.
[b] Age was not normally distributed in the population, which represents a limitation of the given mean values.
[c] Nonfasting.
[d] One unit = 12 g of alcohol = 1 beer = 1 glass of wine.

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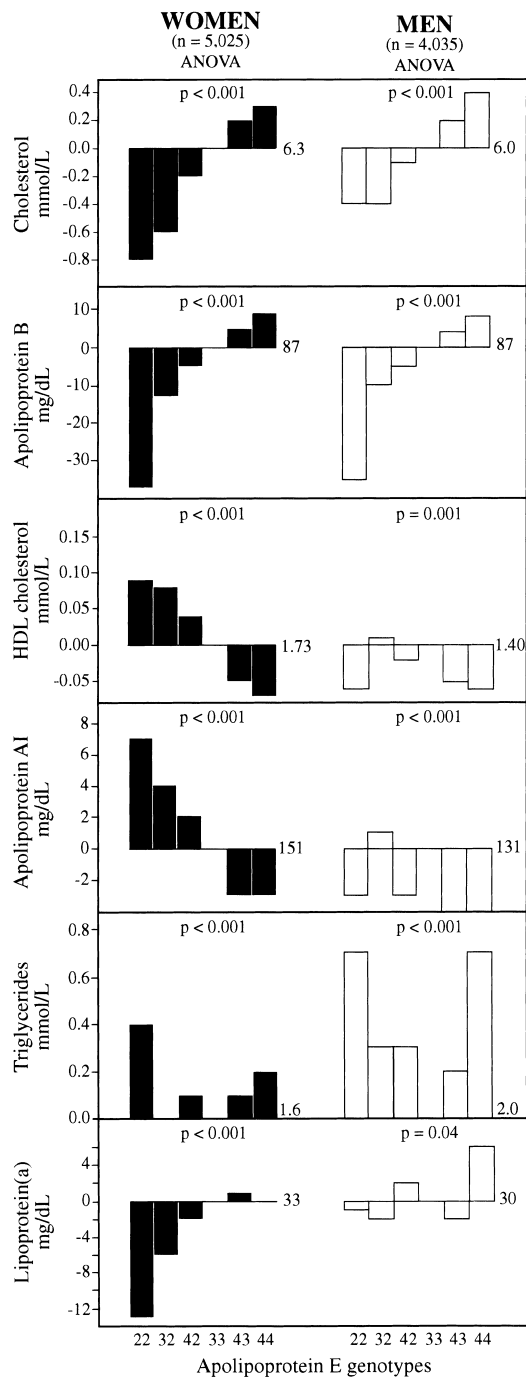

Fig. 1. Lipids, lipoproteins, and apolipoproteins as a function of apoE genotype in women and men sampled from the general population; triglycerides were nonfasting. Values represent changes in absolute mean values relative to the ε33 genotype; the absolute level in ε33 individuals is shown on the right side of the columns. Traits were adjusted for age in 10-year age groups by ANCOVA. Age did not interact with apoE genotype in predicting the six lipid traits, except for an interaction on apoA-I at the 0.03 level: on stratified data this looked like a chance event, and was therefore disregarded. Variation in apoE genotypes interacted with levels of glucose in the prediction of HDL cholesterol, and apoA-I in women, and with HDL cholesterol, apoA-I, and nonfasting triglycerides (Tables 3 and 5, and Fig. 3), which indicates that the overall pattern shown here differs in the different glucose strata. Furthermore, variation in apoE genotypes interacted with alcohol consumption in the prediction of nonfasting triglycerides and Lp[a] in women (Tables 3 and 5, and Fig. 4), indicating that the overall pattern shown here differs according to alcohol consumption in women.

women and men (all ANOVAs: $P < 0.001$) (**Figs. 1** and **2**); for cholesterol this pattern was confirmed in the same individuals on levels measured 10 and 15 years earlier (15) (all ANOVAs: $P < 0.001$; data not shown). On post-hoc Mann-Whitney U tests, most two-genotype comparisons (**Table 2**) of mean values (Fig. 1) were statistically significant for both traits in either gender. The absolute increase from ε22 to ε44 of age-adjusted

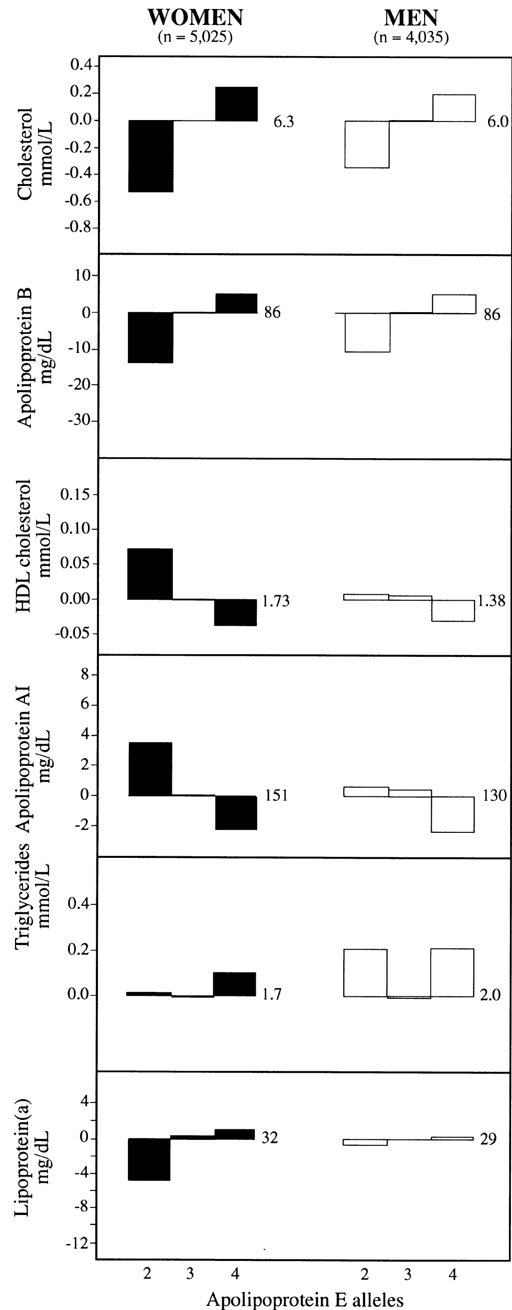

Fig. 2. Average allele effects for lipids, lipoproteins, and apolipoproteins as a function of apoE alleles; the population mean level is shown on the right side of the columns. Traits were adjusted for age in 10-year age groups by ANCOVA prior to average allele effect calculations. The overall pattern for HDL cholesterol, apoA-I, nonfasting triglycerides, and Lp[a] as a function of apoE alleles could differ in strata of glucose levels and alcohol consumption due to interactions between these covariates and apoE genotypes in the prediction of the above-mentioned lipid traits.

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TABLE 2. Post-hoc Mann-Whitney U tests

	Women						Men				
	ε22	ε32	ε42	ε33	ε43		ε22	ε32	ε42	ε33	ε43
Cholesterol						Cholesterol					
ε32	0.02					ε32	0.42				
ε42	0.001	0.000				ε42	0.14	0.04			
ε33	0.000	0.000	0.09			ε33	0.03	0.000	0.09		
ε43	0.000	0.000	0.000	0.000		ε43	0.01	0.000	0.003	0.000	
ε44	0.000	0.000	0.000	0.001	0.43	ε44	0.006	0.000	0.001	0.002	0.07
ApoB						ApoB					
ε32	0.000					ε32	0.000				
ε42	0.000	0.000				ε42	0.000	0.008			
ε33	0.000	0.000	0.009			ε33	0.000	0.000	0.009		
ε43	0.000	0.000	0.000	0.000		ε43	0.000	0.000	0.000	0.000	
ε44	0.000	0.000	0.000	0.000	0.19	ε44	0.000	0.000	0.000	0.000	0.03
HDL cholesterol						HDL cholesterol					
ε32	0.60					ε32	0.60				
ε42	0.90	0.27				ε42	0.94	0.44			
ε33	0.94	0.001	0.70			ε33	0.72	0.36	0.66		
ε43	0.51	0.000	0.12	0.000		ε43	0.79	0.001	0.37	0.000	
ε44	0.33	0.000	0.07	0.02	0.34	ε44	0.60	0.03	0.22	0.04	0.48
ApoA-I						ApoA-I					
ε32	0.87					ε32	0.38				
ε42	0.72	0.27				ε42	0.89	0.16			
ε33	0.62	0.001	0.72			ε33	0.65	0.10	0.47		
ε43	0.31	0.000	0.13	0.000		ε43	0.77	0.000	0.47	0.000	
ε44	0.33	0.002	0.17	0.09	0.74	ε44	0.69	0.01	0.34	0.04	0.52
Nonfasting triglycerides						Nonfasting triglycerides					
ε32	0.003					ε32	0.05				
ε42	0.03	0.08				ε42	0.17	0.21			
ε33	0.002	0.47	0.03			ε33	0.008	0.002	0.004		
ε43	0.01	0.23	0.27	0.01		ε43	0.03	0.29	0.07	0.02	
ε44	0.11	0.02	0.58	0.06	0.10	ε44	0.35	0.08	0.62	0.001	0.02
Lp[a]						Lp[a]					
ε32	0.04					ε32	0.93				
ε42	0.03	0.44				ε42	0.70	0.45			
ε33	0.004	0.000	0.26			ε33	0.57	0.007	0.61		
ε43	0.003	0.000	0.24	0.80		ε43	0.82	0.42	0.72	0.02	
ε44	0.02	0.51	0.98	0.18	0.17	ε44	0.44	0.13	0.51	0.65	0.23

P Values are shown; 0.000 represents $P < 0.001$. Post-hoc tests for mean values in Fig. 1.

levels of cholesterol and apoB was 1.1 mmol/l and 46 mg/dl in women, and 0.8 mmol/l and 43 mg/dl in men, respectively.

A significant stepwise decrease was observed for HDL cholesterol and apoA-I as a function of apoE genotype in women ($P < 0.001$ for both traits), but not in men (Figs. 1 and 2); for HDL cholesterol this pattern was confirmed on levels measured 10 years earlier in the same individuals (15) (women, $P < 0.001$; men, $P = 0.17$; data not shown). For apoE alleles from ε2 to ε3 to ε4 there were stepwise decreases in HDL cholesterol and apoA-I in women, with a weak similar trend in men (Fig. 2). On post-hoc Mann-Whitney U tests, ε32 versus ε33, ε32 versus ε43, ε32 versus ε44, and ε33 versus ε43 differed for both HDL cholesterol and apoA-I in women (Table 2, Fig. 1). The absolute decrease from ε22 to ε44 of age-adjusted levels of HDL cholesterol and apoA-I in women was −0.16 mmol/l and −10 mg/dl, respectively (Fig. 1).

In both genders ε22 and ε44 individuals had the highest nonfasting triglyceride levels whereas ε33 individuals had the lowest levels (Fig. 1, Table 2). This nonfasting triglyceride pattern was confirmed in the same individuals for values measured 15 years earlier (15) (women, $P = 0.02$; men, $P = 0.001$; data not shown). Most heterozygotes appeared to have intermediate levels of plasma triglycerides (Fig. 1). Accordingly, both the ε2 and ε4 alleles predicted higher levels of nonfasting triglycerides than the ε3 allele (Fig. 2). The absolute increases in plasma triglycerides from ε33 to ε22 in women and men were +0.4 and +0.7 mmol/l, and from ε33 to ε44 +0.2 and +0.7 mmol/l, respectively (Fig. 1).

Levels of Lp[a] increased from ε22 to ε33 in women, but not in men (Fig. 1). On post-hoc Mann-Whitney U tests, ε22 versus ε32, ε22 versus ε42, ε22 versus ε33, ε22 versus ε43, ε32 versus ε33, and ε32 versus ε43 differed significantly in women (Table 2, Fig. 1). Accordingly, the ε2 allele predicted lower Lp[a] levels than the two other alleles in women, but not in men (Fig. 2). The absolute increase in Lp[a] levels from ε22 to ε33 in women was 13 mg/dl (Fig. 1).

There was evidence of heterogeneity of variance between apoE genotypes for cholesterol, not present in HDL or Lp[a] in both genders, and for $\log_{10}$ Lp[a] in men (data not shown): this appeared to be explained by

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a larger variance for ε22 individuals than for individuals with other genotypes. A likely explanation is that some but not all ε22 individuals develop type III hyperlipoproteinemia (1). The heterogeneity of variance seen for apoB in both genders across genotypes and for HDL cholesterol in women mainly reflected that the variance increased with increasing mean levels of apoB and HDL cholesterol.

Interaction of apoE genotype with other cardiovascular risk factors in the prediction of lipid, lipoprotein, and apolipoprotein traits

Of 216 interactions tested (**Table 3**), 25 were significant ($P < 0.05$): in 16 interactions, the association between variation in the trait examined and variation in apoE genotypes showed irregular patterns rather than monotonic and consistent associations in different strata of the interacting covariate, and thus suggested chance observations, whereas the 9 interactions described below appeared with some consistency between related traits, and/or appeared biologically plausible, that is, were consistent with knowledge that the interacting covariate and the dependent lipid trait might be associated, and that the same or a similar association was found with related traits.

Three of four interactions involving apoE genotype and cholesterol predicting variation in apoB, or apoE genotype and apoB predicting variation in cholesterol, were highly significant ($P < 0.001$). The cause of these interactions may be that individuals with the ε22 genotype (28 women and 20 men) were not equally distributed in the apoB quintiles (data not shown): there were 22 women in the first, 5 in the second, and 1 woman in the third quintile;

TABLE 3. Bivariate interactions between apolipoprotein E genotype and lipid and nonlipid cardiovascular risk factors in predicting six lipid traits

	Cholesterol	ApoB[a]	HDL cholesterol[a]	ApoA-I	Triglycerides[a,b]	Lp[a][a]
Women						
Cholesterol	—	0.000[c]	0.73	0.08	0.41	0.30
ApoB[a]	0.000[c]	—	0.20	0.08	0.61	0.26
HDL cholesterol[a]	0.67	0.03	—	0.02	0.28	0.15
ApoA-I	0.23	0.13	0.52	—	0.20	0.13
Triglycerides[a,b]	0.25	0.008	0.20	0.13	—	0.11
Lp[a][a]	0.36	0.27	0.08	0.78	0.86	—
BMI	0.69	0.60	0.60	0.27	0.64	0.04
Waist/hip	0.18	0.32	0.42	0.33	0.98	0.19
Glucose[a]	0.05	0.36	0.006	0.007	0.32	0.76
Alcohol consumption	0.63	0.05	0.23	0.53	0.03	0.02
Systolic BP	0.25	0.05	0.02	0.09	0.35	0.64
Diastolic BP	0.37	0.89	0.88	0.96	0.63	0.37
Age	0.44	0.13	0.21	0.03	0.75	0.08
Hypertension	0.24	0.67	0.73	0.71	0.67	0.44
Diuretics	0.23	0.57	0.67	0.63	0.27	0.11
Diabetes mellitus	0.44	0.72	0.94	0.84	0.19	0.11
Smoking	0.38	0.46	0.79	0.83	0.97	0.008
Physical activity	0.03	0.009	0.44	0.39	0.45	0.27
Menopause	0.55	0.68	0.38	0.95	0.43	0.11
HRT	0.62	0.06	0.15	0.09	0.59	0.37
Men						
Cholesterol	—	0.000[c]	0.19	0.04	0.74	0.008
ApoB[a]	0.22	—	0.10	0.14	0.57	0.22
HDL cholesterol	0.07	0.14	—	0.48	0.06	0.07
ApoA-I	0.12	0.13	0.58	—	0.18	0.21
Triglycerides[a,b]	0.008	0.03	0.64	0.61	—	0.002[c]
Lp[a][a]	0.73	0.27	0.39	0.08	0.79	—
BMI	0.02	0.08	0.59	0.90	0.41	0.83
Waist/hip	0.41	0.34	0.52	0.25	0.85	0.39
Glucose	0.44	0.35	0.09	0.04	0.002[c]	0.41
Alcohol consumption	0.001[c]	0.52	0.93	0.83	0.39	0.28
Systolic BP	0.27	0.27	0.10	0.03	0.50	0.47
Diastolic BP	0.18	0.32	0.05	0.02	0.37	0.71
Age	0.11	0.21	0.99	0.73	0.39	0.05
Hypertension	0.25	0.33	0.46	0.45	0.75	0.67
Diuretics	0.63	0.64	0.30	0.25	0.59	0.08
Diabetes mellitus	0.06	0.37	0.05	0.14	0.19	0.35
Smoking	0.45	0.39	0.82	0.53	0.51	0.72
Physical activity	0.21	0.33	0.96	0.62	1.00	0.88

All covariates in the vertical column were tested one by one in an interaction term with apoE genotype in an analysis of covariance in predicting the six lipid traits (horizontal row). *P* Values are shown; 0.000 represents $P < 0.001$.
BP, blood pressure; Age, age in 10 year groups.
[a] Logarithmically transformed prior to statistical analysis to approach normal distribution, and/or to obtain equal variances (HDL cholesterol and glucose levels only in women).
[b] Nonfasting.
[c] $P < 0.05$ after correction for multiple comparison by the Bonferroni method.

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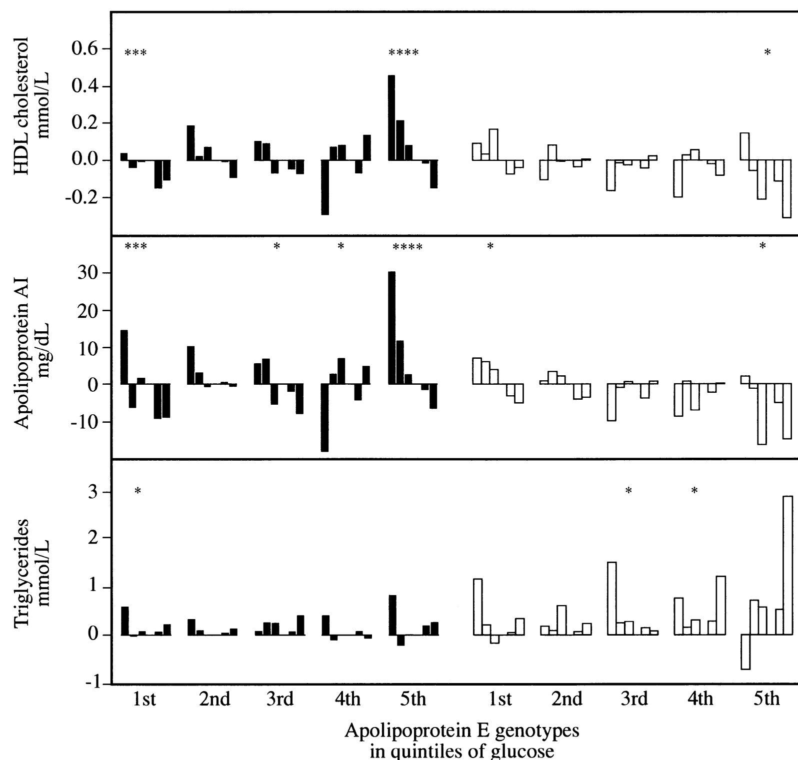

Fig. 3. Changes in absolute mean values of HDL cholesterol, apoA-I, and nonfasting triglycerides as a function of apoE genotype relative to the ε33 genotype (left to right: ε22, ε32, ε42, ε33, ε43, ε44) in women and men by glucose quintiles. Kruskal-Wallis analysis of variance: * $P < 0.05$, *** $P < 0.005$, **** $P < 0.001$.

for men there were 18 in the first, 1 in the second, and 1 in the fifth quintile. The stepwise increase in apoB by apoE genotypes remained significant in all cholesterol quintiles; however, the stepwise increase in cholesterol disappeared when data were stratified in apoB quintiles (data not shown).

Four significant interactions (and one borderline) between apoE genotype and glucose in predicting variation in HDL cholesterol and apoA-I in both genders and nonfasting triglycerides in men, were present: in women, the stepwise decrease in HDL cholesterol and apoA-I as a function of genotype was present only in the highest glucose quintile, and this was most likely the cause of the interaction, whereas for the remaining three interactions the cause was not evident (**Fig. 3**).

Alcohol consumption interacted with apoE genotype in women in predicting variation in nonfasting triglycerides and Lp[a]: variations in apoE genotypes predicted variation in nonfasting triglycerides and Lp[a] mainly in the highest tertile of alcohol consumption (**Fig. 4**). Although

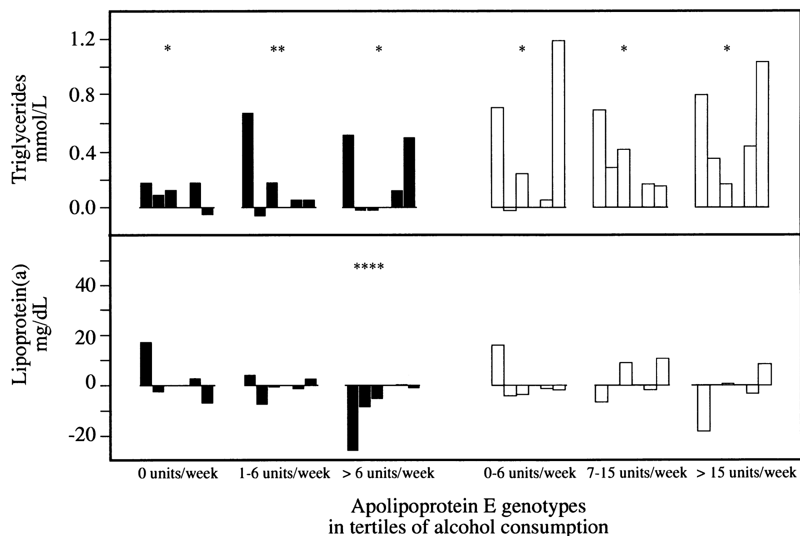

Fig. 4. Changes in absolute mean values of nonfasting triglycerides and Lp[a] as a function of apoE genotype relative to the ε33 genotype (left to right: ε22, ε32, ε42,ε33, ε43, ε44) in women and men by tertiles of alcohol consumption. Kruskal-Wallis analysis of variance: * $P < 0.05$, ** $P < 0.01$, **** $P < 0.001$.

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TABLE 4. Contribution of apoE genotype to variability

Variable	Women				Men			
	s_W^2	s_G^2	$s_G^2 / s_G^2 + s_W^2$	P	s_W^2	s_G^2	$s_G^2 / s_G^2 + s_W^2$	P
Cholesterol	1.26	0.059	0.045	<0.001	1.28	0.030	0.023	<0.001
ApoB	0.0099	0.0012	0.107	<0.001	0.0107	0.00073	0.064	<0.001
HDL cholesterol	[a]	[a]	[a]		[a]	[a]	[a]	
ApoA-I	[a]	[a]	[a]		[a]	[a]	[a]	
Triglycerides[b]	[c]	[c]	[c]		[a]	[a]	[a]	
Lp[a]	[c]	[c]	[c]		0.602	0.0023	0.004	<0.005

[a] Indicates significant interactions between apoE genotype and glucose levels (see Table 5).
[b] Nonfasting.
[c] Indicates significant interactions between apoE genotype and alcohol consumption (see Table 5). For calculations see statistical methods.

these interactions were not found in men, the stratified results are still shown for men for comparison.

Contribution of apoE genotype to variability in lipids, lipoproteins, and apolipoproteins

In women, variation in apoE genotypes predicted 4.5 and 10.7% of the variation in cholesterol and apoB after adjustment for age in 10-year groups, diabetes mellitus, physical activity at leisure, antihypertensive drugs, diuretics, BMI, alcohol consumption, menopausal status, and use of HRT (**Table 4**). Because of significant interactions between apoE genotype and alcohol consumption in predicting variation in nonfasting triglycerides, and Lp[a] in women, and between apoE genotype and glucose levels in predicting variation in HDL cholesterol and apoA-I in women and HDL cholesterol, apoA-I, and nonfasting triglycerides in men, the marginal genotype "effects" on these lipid traits were estimated in tertiles or quintiles of the interacting covariates (**Table 5**). In men, variation in apoE genotypes predicted 2.3, 6.4, and 0.4% of the variation in cholesterol, apoB, and Lp[a], respectively (Table 4).

For comparison, age in 10-year groups alone predicted 23.5, 20.2, 0.2, 2.7, 11.2, and 0.8% in women and 7.3, 7.6, 0.8, 2.4, 2.6, and 0.1% in men of the total variation in cholesterol, apoB, HDL cholesterol, apoA-I, nonfasting triglycerides, and Lp[a], respectively (data not shown).

DISCUSSION

The main novel observations in the present study are as follows: General: *i*) comprehensive two-genotype comparisons of all possible apoE genotype combinations for six lipid, lipoprotein, and apolipoprotein traits in both genders (Fig. 1 and Table 2); *ii*) comprehensive tests of interaction between apoE genotype and 20 other covariates in this relatively large sample (Table 3). Specific: *iii*) the contribution of apoE genotype to the total variance in cholesterol and apoB is considerably greater in women than in

TABLE 5. Contribution of apoE genotype to variability in strata of interacting covariates

Covariates	HDL cholesterol		ApoA-I		Triglycerides[a]		Lp[a]	
	$s_G^2 / s_G^2 + s_W^2$	P	$s_G^2 / s_G^2 + s_W^2$	P	$s_G^2 / s_G^2 + s_W^2$	P	$s_G^2 / s_G^2 + s_W^2$	P
Alcohol women								
No alcohol	[c]		[c]		0.008	<0.001	0.004	<0.001
1–6 units[b]/week	[c]		[c]		0.013	<0.001	0.004	<0.001
>6 units[b]/week	[c]		[c]		0.009	<0.001	0.015	<0.001
Glucose women								
<4.8 mmol/l	0.026	0.001	0.022	<0.005	[d]		[d]	
4.8–5.2 mmol/l	0.008	0.62	0.003	0.74	[d]		[d]	
5.2–5.5 mmol/l	0.008	0.10	0.015	0.02	[d]		[d]	
5.5–6.1 mmol/l	0.010	0.11	0.011	0.02	[d]		[d]	
>6.1 mmol/l	0.026	<0.001	0.027	<0.001	[d]		[d]	
Glucose men								
<4.9 mmol/l	0.004	0.75	0.007	0.26	0.015	0.08	[e]	
4.9–5.3 mmol/l	0.011	0.54	0.014	0.05	0.011	0.16	[e]	
5.3–5.8 mmol/l	0.004	0.15	0.007	0.07	0.013	0.03	[e]	
5.8–6.5 mmol/l	0.003	0.40	0.003	0.51	0.028	<0.001	[e]	
>6.5 mmol/l	0.021	<0.005	0.027	<0.005	0.014	0.06	[e]	

For calculations see Statistical Methods.
[a] Nonfasting.
[b] 1 unit = 12 g of pure alcohol, which corresponds to 1 beer (33 cl), 1 glass of wine, or 2 cl of spirits.
[c] Not calculated as apoE genotype did not interact with alcohol consumption in the prediction of HDL cholesterol and apoA-I.
[d] Not calculated as apoE genotype did not interact with glucose levels in the prediction of triglycerides and Lp[a].
[e] See Table 4.

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men; *iv*) ε42 individuals have cholesterol and apoB levels between ε32 and 33 individuals; *v*) variations in apoE genotypes predict variation in plasma HDL cholesterol, apoA-I, and Lp[a] in a gender-specific manner; *vi*) in women, variations in apoE genotypes predict variation in nonfasting triglycerides and Lp[a] dependent on alcohol consumption, and variation in HDL cholesterol and apoA-I dependent on glucose levels. In men, variations in apoE genotypes predict variation in HDL cholesterol, apoA-I, and nonfasting triglycerides dependent on glucose levels.

The demonstrated cholesterol and apoB lowering associated with the ε2 allele, and the cholesterol and apoB increase associated with the ε4 allele, are well established (4, 6–10); however, the reported order of the six genotypes associated with stepwise increases of mean levels of cholesterol and apoB varies somewhat (4, 13). We and others (14) found that apoB increases from ε22 to ε32 to ε42 to ε33 to ε43 to ε44 and that cholesterol increases from ε22 ≈ ε32 to ε42 to ε33 to ε43 to ε44 in both genders.

Another novel finding is that the stepwise increase in cholesterol associated with the six apoE genotypes disappeared in quintiles of apoB, whereas in contrast quintiles of cholesterol did not abolish the stepwise increase in apoB. This suggests that apoB is the factor primarily associated with apoE genotype. These and previous studies (4, 26) support the hypothesis that the affinity of the apoE isoforms to the LDL receptor are inversely correlated with the ability of the LDL receptor to remove apoB-containing lipoproteins from plasma, and thereby positively correlated with plasma cholesterol levels.

It has previously been demonstrated that the associations between variation in lipid and lipoprotein levels with variation in apoE genotypes are context dependent, with many different environmental and biological factors as proposed interactors (27). Conflicting results concerning the association between variation in levels of HDL cholesterol and apoA-I with variation in apoE genotypes have been reported (4); however, Reilly et al. (10–12) demonstrated that these associations were influenced by gender, which could explain the controversy. In agreement with this, we demonstrated a stepwise decrease in HDL cholesterol and apoA-I levels from ε22 to ε32 to ε42 to ε33 to ε43 to ε44 in women, but not in men. Kaprio et al. (3) estimated that the ε4 allele predicted an increase in triglycerides in women, and a decrease in HDL cholesterol in men. We find, however, that the ε4 allele predicted an increase in nonfasting triglycerides and a decrease in HDL cholesterol in both women and men. Furthermore, it has been suggested that variation in HDL cholesterol associated with variation in apoE genotypes in women is dependent on the use of HRT (3, 28) in agreement with the present findings of statistically significant borderline interactions (HDL cholesterol, $P = 0.15$; apoA-I, $P = 0.09$) between apoE genotype and HRT in postmenopausal women on levels of HDL-cholesterol and apoA-I. The stepwise decreasing pattern in HDL cholesterol and apoA-I from ε22 through on to ε44 was present in premenopausal (n = 1,437) and untreated postmenopausal women (n = 2,859), but not in postmenopausal women treated with HRT (n = 683), where neither HDL cholesterol nor apoA-I showed a decreasing pattern in mean levels from ε22 to ε44 (Kruskal-Wallis ANOVA $P = 0.26$, and $P = 0.27$; data not shown). The variation in cholesterol and apoB associated with variation in apoE genotypes was not abolished by HRT; the stepwise increase from ε22 through on to ε44 was present in all three female groups.

Why the variation in HDL cholesterol, apoA-I, and nonfasting triglycerides associated with variation in apoE genotypes is most obvious in the quintile of highest plasma glucose is not completely clear. However, it is well known that patients with elevated glucose and insulin resistance, and thus non-insulin-dependent diabetes mellitus, have hypertriglyceridemia as well as low HDL levels due to abnormalities in both production and clearance of VLDL triglycerides and HDL cholesterol/apoA-I (29); HDL particles containing both cholesterol and apoA-I are produced as excess surface material when triglycerides in VLDL are hydrolyzed. In accordance with this in the present study, levels of nonfasting triglycerides increased and levels of HDL cholesterol and apoA-I decreased as a function of increasing glucose levels in both genders (data not shown). It therefore could be speculated that the rather discrete variation in HDL cholesterol and apoA-I associated with variation in apoE genotypes is more pronounced in the highest quintile of glucose because this is a substrate-rich environment with plenty of triglycerides, and thus a great potential for production of HDL particles. In such a context, variations in apoE genotypes may in particular predict variation in HDL cholesterol and nonfasting triglyceride levels.

It is interesting that our results on variation in apoE genotypes as predictors of variation in nonfasting triglycerides are in agreement with previous results in studies of fasting triglycerides (6, 7, 9): individuals in the present study with the ε2 or ε4 allele when compared with individuals with the most common genotype (ε33, "wild-type") had the highest nonfasting triglyceride levels. However, in contrast to the present findings some studies did not find an association between the ε44 genotype and higher triglyceride levels (30, 31). In the present study alcohol consumption interacted with apoE genotype on nonfasting plasma triglycerides in women, but not in men. In women, this could be explained by an association between the ε4 allele and raised nonfasting triglyceride levels mainly in the 30% of women with a weekly alcohol intake above 6 units. In men, the mean weekly consumption of alcohol was almost 2½ times higher than in women (14 units) and 60% of men drank more than 6 units of alcohol per week, possibly explaining the lack of a statistically significant apoE-alcohol interaction in men. This may explain why levels of nonfasting triglycerides associated with ε4 were higher in men than in women in the Copenhagen City Heart Study and why high triglyceride levels were not associated with the ε44 genotype in a large Turkish study (31), where members of the largest part of the population are Muslims with restricted alcohol intake.

Our results on triglycerides in the nonfasting state may differ from those in the fasting state; however, studies of

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both fasting (6, 7, 9) and nonfasting triglycerides (30) have demonstrated that high triglyceride levels are associated with the ε4 allele, in accordance with findings in the present study on nonfasting triglycerides. In spite of this, dietary status may still have some impact on the association between, particularly, the ε44 genotype and triglyceride levels, as ε44 individuals in the Turkish Heart Study (31) had fasting triglyceride levels similar to those of ε33 individuals. Therefore, the fasting and nonfasting state may represent two different contexts with respect to apoE genotype and effect on triglycerides. Although it is conventional to measure triglycerides in the fasting state to minimize variability due to fat intake, it could be argued that studies of the nonfasting state may be more relevant as most humans spend more hours in the nonfasting state (up to 8 h after the last meal) than in the fasting state (more than 8 h after the last meal). It should be emphasized, however, that conclusions about genotypes as predictors of nonfasting variation may not be comparable to conclusions based on fasting data.

The association between variation in Lp[a] levels and variation in apoE genotypes is not quite clear (7, 32–34). One study suggests that variation in levels of Lp[a] is not directly associated with variation in apoE phenotypes: variations in apoE phenotypes predict variations in lipids and lipoproteins, which in turn seem to influence levels of Lp[a] (32). In support of this we found, at least in men, that apoE genotype interacted with levels of cholesterol and triglycerides in the prediction of Lp[a]. In women, apoE genotype interacted with alcohol consumption in predicting Lp[a], due to an effect in those who drank most. Potentially, the interaction between apoE genotype and alcohol consumption in predicting triglycerides could also explain the interaction between apoE genotype and alcohol consumption in predicting Lp[a] levels, as apo[a] associates with triglyceride-rich lipoproteins at least in the postprandial state (34). In the present study the association between variation in levels of Lp[a] and variation in apoE genotypes was strongest in women, whereas an overall association did not seem to exist in men. Another study found no overall association between variation in levels of Lp[a] and variation in apoE phenotypes in 466 white men, but suggested that the association with variation in apoE phenotypes was dependent on apo[a] isoforms (33). We suggest that variation in levels of Lp[a] associated with variation in apoE genotypes is gender specific and context dependent.

Potential limitations of the present study need to be discussed. Chance findings are always a crucial issue when multiple statistical tests are performed. We chose to perform 216 interaction tests between apoE genotype and several covariates in the prediction of the six lipid traits, because we did not want to miss any interaction that potentially could explain an important context-dependent effect. We did not trust solely in the statistical P value, but stratified all significant and borderline significant interactions in strata of the interacting covariate. On inspection of these bar graphs, we evaluated if the interaction was biologically plausible, that is, was consistent with knowledge that the interacting covariate and the dependent lipid trait might be associated, and that the same or similar associations were found with related traits, or if it showed irregular patterns most likely due to chance findings. Furthermore, misclassification of genotypes is unlikely as all photographs of gels were scrutinized by two different researchers unaware of the associations between variation in lipids, lipoproteins, and apolipoproteins and variation in apoE genotypes. In addition, the genotype distribution in the general population was similar to that predicted by the Hardy-Weinberg equilibrium, supporting that no major misclassification took place. We cannot, however, totally exclude misclassification of a few apoE genotypes, as we genotyped more than 9,000 individuals.

In conclusion, the present results suggest that in the population at large variation in plasma lipoprotein levels is associated with variation in apoE genotypes, especially in women. Whereas the associations between variation in levels of cholesterol and apoB and variation in apoE genotypes seem invariant between studies, the associations with variation in levels of HDL cholesterol, apoA-I, nonfasting triglycerides, and Lp[a] seem from our studies to be context dependent. Our data suggest that associations between cardiovascular disease and variation in apoE genotypes will most likely differ substantially by both genotype and gender due to variation in levels of lipids, lipoproteins, and apolipoproteins.

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