Am. J. Hum. Genet. 54:341–360, 1994

Apolipoprotein E Polymorphism Influences Postprandial Retinyl Palmitate but Not Triglyceride Concentrations

Eric Boerwinkle,* Spencer Brown,† A. Richey Sharrett,‡ Gerardo Heiss§ and Wolfgang Patsch†

*Genetics Centers, The University of Texas Health Science Center, and †Department of Internal Medicine, Methodist Hospital and Baylor College of Medicine, Houston; ‡Epidemiology and Biometry Program, National Heart, Lung, and Blood Institute, Bethesda; and §Department of Epidemiology, The University of North Carolina, Chapel Hill

Summary

To quantify the effect of the apolipoprotein (apo) E polymorphism on the magnitude of postprandial lipemia, we have defined its role in determining the response to a single high-fat meal in a large sample of (N = 474) individuals taking part in the biethnic Atherosclerosis Risk in Communities Study. The profile of postprandial response in plasma was monitored over 8 h by triglyceride, triglyceride-rich lipoprotein (TGRL)–triglyceride, apo B-48/apo B-100 ratio, and retinyl palmitate concentrations, and the apo E polymorphism was determined by DNA amplification and digestion. The frequency of the apo E alleles and their effects on fasting lipid levels in this sample were similar to those reported elsewhere. Postprandial plasma retinyl palmitate response to a high-fat meal with vitamin A was significantly different among apo E genotypes, with delayed clearance in individuals with an ε2 allele, compared with ε3/3 and ε3/4 individuals. In the sample of 397 Caucasians, average retinyl palmitate response was 1,489 μg/dl in ε2/3 individuals, compared with 1,037 μg/dl in ε3/3 individuals and 1,108 μg/dl in ε3/4 individuals. The apo E polymorphism accounted for 7.1% of the interindividual variation in postprandial retinyl palmitate response, a contribution proportionally greater than its well-known effect on fasting LDL-cholesterol. However, despite this effect on postprandial retinyl palmitate, the profile of postprandial triglyceride response was not significantly different among apo E genotypes. The profile of postprandial response was consistent between the sample of Caucasians and a smaller sample of black subjects. While these data indicate that the removal of remnant particles from circulation is delayed in subjects with the ε2/3 genotype, there is no reported evidence that the ε2 allele predisposes to coronary artery disease (CAD). The results of this study provide not only a reliable estimate of the magnitude of the effect of the apo E polymorphism on various measurements commonly used to characterize postprandial lipemia, but also provide mechanistic insight into the effects of the apo E gene polymorphism on postprandial lipemia and CAD.

Introduction

The role of plasma triglycerides in the pathogenesis of coronary artery disease (CAD) is controversial. Numerous epidemiological studies have found that fasting plasma triglyceride concentrations are associated with CAD (Austin 1991). However, on multivariate analyses including the effects of other lipids, especially HDL-cholesterol, the association between triglycerides and CAD is not maintained. Even though HDL-cholesterol may be a better predictor of CAD, animal experiments and studies of the postprandial state in man provide evidence that the concentration of HDL-cholesterol is largely dependent on the metabolism of triglyceride-rich lipoproteins (Patsch et al. 1984; Miesenbock and Patsch 1990). It has been argued that prolonged postprandial lipemia is conducive to the development of atherosclerosis (Zilversmit 1979), and case-control studies have shown that late postprandial lipid levels are predictive of CAD, even when HDL-lipoproteins are simultaneously considered (Groot et al. 1991; Patsch et al. 1992*a;* Ryu et al. 1992).

While the magnitude and duration of postprandial lipemia is relatively constant within an individual given

Received June 23, 1993; revision received September 24, 1993.
Address for correspondence and reprints: Eric Boerwinkle, Ph.D., Genetics Center, The University of Texas Health Science Center, P.O. Box 20334, Houston, TX 77225.
© 1994 by The American Society of Human Genetics. All rights reserved.
0002-9297/94/5402-0018$02.00

uniform meal challenges, there are considerable differences in postprandial response among individuals (Patsch et al. 1984, 1987; Brown et al. 1992). A number of factors have been identified that influence postprandial response, including age (Krasinski et al. 1990), sex (Cohn et al. 1988*b*), the concentration of fasting triglycerides (Nestel 1964; Cohn et al. 1988*a;* Brown and Roberts 1991), and alcohol consumption (Wilson et al. 1970). The extent of postprandial lipemia is also influenced by genetic factors. Patients with genetically determined CETP deficiency (Inazu et al. 1990), type III hyperlipidemia (Weintraub et al. 1987), and lipoprotein lipase deficiency (Brunzell 1989; Miesenbock et al. 1993) all have exaggerated postprandial lipid responses. However, the rare frequency of these genetic conditions dictates that the underlying mutations are not responsible for a large fraction of the variability in postprandial response in the general population.

Because of its recognized importance in remnant clearance, the apolipoprotein (apo) E gene has been targeted for the study of postprandial lipid response (Mahley and Rall 1989). Apo E plays a major role in lipid metabolism through cellular uptake of lipoprotein particles by apo E–specific and apo B/E receptors on the liver and other tissues (Mahley et al. 1990). E2-containing lipoproteins have lower affinity for hepatic lipoprotein receptors, compared with lipoproteins containing E3 (Hui et al. 1984; Schneider et al. 1981; Weisgraber et al. 1982). In addition, the conversion of VLDL to LDL in the plasma of patients with familial type III hyperlipoproteinemia is impeded by the apo E2 isoform but can be restored by addition of apo E3 (Ehnholm et al. 1984), suggesting a role of apo E in the conversion of VLDL to LDL. Consistent with the function of the apo E2 isoform, several studies have shown a reduction in the clearance of postprandial lipoproteins in subjects carrying an ε2 allele (Gregg et al. 1986; Brenninkmeijer et al. 1987; Weintraub et al. 1987; Brown and Roberts 1991; Superko and Haskell 1991). In vitro, the receptor-binding affinity of E4-containing lipoproteins may be similar to those containing the common E3 isoform, but, in vivo, apo E4–containing lipoproteins are cleared more rapidly from circulation than are those containing apo E3. Dallongeville et al. (1992) and Brown and Roberts (1991) have reported that the apo E polymorphism influences fasting plasma triglyceride levels. Compared with individuals with the ε3/3 genotype, ε3/4 subjects had higher fasting triglyceride levels, which, in turn, are related to the magnitude and duration of postprandial lipemia (Cohn et al. 1988*a;* Brown and Roberts 1991). Results from studies on the role of the ε4 allele in postprandial lipid metabolism are inconsistent, showing either decreased (Brenninkmeijer et al. 1987; Weintraub et al. 1987) or increased (Brown and Roberts 1991) postprandial response.

On the basis of results of several epidemiologic studies, an association between apo E genotypes and prevalent CAD has been established (Menzel et al. 1983; Cumming and Roberts 1984; Lenzen et al. 1986). The frequency of the ε2 allele is reduced among CAD patients, while the frequency of the ε4 allele is increased. Results from these studies are commonly attributed to differences in LDL-cholesterol levels, qualifying an apparent importance of remnants and postprandial lipemia in atherogenesis. To quantitatively estimate the contribution of the apo E polymorphism to distinct metabolic aspects of postprandial lipoprotein metabolism, we have measured multiple parameters of postprandial response in a biracial sample of 474 individuals taking part in the Atherosclerosis Risk in Communities (ARIC) Study. We report here that the ε2 allele increased late postprandial retinyl palmitate concentrations in subjects consuming a high-fat test meal containing vitamin A but had no effect on late postprandial triglycerides. The ε4 allele had no significant effect on any of the postprandial variables measured, even after correction for fasting triglycerides. Furthermore, the effect of the apo E polymorphism on postprandial retinyl palmitate levels was larger than the well-established effect of this system on LDL-cholesterol. Hence, these data indicate that the effects of the common apo E polymorphism on remnant and LDL metabolism do not fully explain the observed association of this gene with CAD.

Methods

Sampling and Postprandial Protocol

Participants were selected from the ARIC study, a prospective, multicenter investigation of atherosclerosis and its sequelae in women and men 45–64 years of age. Approximately 16,000 residents were recruited into the ARIC study, 4,000 in each of four communities: Forsyth County, NC; the city of Jackson, MS; the northwestern suburbs of Minneapolis; and Washington County, MD. Each sample was a probability sample designed to be representative of residents living in that community, except for the Jackson sample, which consisted only of black residents. The general design of the ARIC study is described elsewhere (ARIC Investigators 1989). Participants were selected from the total ARIC population on the basis of the average thickness of the

carotid arteries as determined by B-mode ultrasound (Bond et al. 1991). Subjects were designated cases if the maximum carotid arterial wall thickness was greater than 2.5 mm or if there was bilateral thickening above approximately the 90th percentile of the ARIC study cohort. Controls were defined by a carotid artery far-wall thickness of less than the 75th percentile. Cases and controls were group matched for race, gender, 10-year age group, field center, and date of examination. Therefore, the conclusions presented here are not directly extendable to the general population. Analyses characterizing the relationship between the extent and duration of postprandial lipemia and case status are the subject of future publications. In this paper, we focus on the role of the apo E polymorphism in determining the postprandial profile in this sample of ARIC participants. Analyses were carried out to test whether the effect of the apo E polymorphism on postprandial response was not significantly different between cases and controls; it was not (results not shown). Exclusion criteria for participation in the postprandial study were (1) evidence of symptomatic cardiovascular or cerebrovascular disease defined by history of angina on effort; (2) physician-diagnosed heart attack, transient ischemic attack, stroke, or intermittent claudication; (3) use of lipid-altering medication such as beta blockers, thiazides, hypolipidemics, or thyroid medication; (4) use of sex hormones, including oral contraceptives in premenopausal women; (5) diabetes mellitus; (6) renal disease; (7) liver disease; (8) hypertriglyceridemia defined as a fasting triglyceride level >400 mg/dl; (9) nonstandard diets, such as a vegetarian diet; (10) lactose intolerance; or (11) history of pancreatitis.

Data were collected from a total of 474 individuals consisting of 397 Caucasians and 77 blacks, 295 males and 179 females. For technical reasons, the number of observations for triglyceride-rich lipoproteins (TGRL), the apo B-48/apo B-100 ratio in TGRL, and retinyl palmitate in blacks was less than the total number of black participants. The average (±SD) age and body-mass index of the Caucasians were 55.9 (±5.2) years and 26.4 (±4.1) kg/m^2, respectively. In blacks these values were 55.8 (±5.9) years and 27.9 (±5.4) kg/m^2, respectively. The influence of the apo E polymorphism on the magnitude and duration of the postprandial response was not significantly different between males and females (P = .67 for retinyl palmitate and P = .87 for triglycerides). Therefore, in this paper, results are presented for the combined sample of males and females.

On the day of the fat-tolerance test, study participants arrived at the field centers after fasting for 12 h, and a blood specimen was taken before administration of the test meal. For each two square meters of body surface area as determined from weight and height measurements (Lohman et al. 1988), a liquid meal was ingested containing 1,237 kcal, 32.7 g of protein, 47.8 g of carbohydrate, 300 mg of cholesterol, 105 g of fat, and 100,000 IU of vitamin A (Aquasol; Armour Pharmaceutical, Kankakee, IL). The polyunsaturated-fat/saturated-fat ratio was 0.369, and the polyunsaturated-fat/monounsaturated-fat ratio was 0.754. Participants were instructed not to take anything by mouth except water, sugarless drinks, or coffee, for a period of 8 h after ingestion of the test meal. Postprandial blood specimens were collected into EDTA-containing tubes (1.5 mg/ml of blood) at 3.5 and 8 h. Plasma was separated by centrifugation (1,500 *g* for 20 min at 4°C) and was stored in the dark at 4°C for 1–3 d. All plasma specimens were shipped on crushed ice to the Central Lipid Laboratory at Baylor College of Medicine, Houston. Lipid measurements were performed within 7 d after arrival, with the exception of retinyl palmitate, which was analyzed in batches from plasma stored under nitrogen in light-protected ampules.

Laboratory Measures

Cholesterol and triglyceride concentrations were measured enzymatically on a Cobas-Fara centrifugal analyzer (Roche Diagnostics, Montclair, NJ) with the respective enzymatic kits (catalog nos. 236691 and 701912; Boehringer-Mannheim Diagnostics, Indianapolis). HDL-cholesterol was determined by measuring cholesterol in the supernatant after precipitation with $MgCl_2$ and dextran sulfate (Warnick et al. 1982; Patsch et al. 1989). Plasma apo B and A-I levels were measured by radioimmunoassay according to the methods of Schonfeld et al. (1974) and Brown et al. (1988), respectively.

TGRL were isolated from 5 ml of plasma from the 0-, 3.5-, and 8-h blood specimens by ultracentrifugation (Lipid Research Clinics Program 1982). Samples were spun for 18 h at 10°C at 105,000 *g* in a Beckman 50.3 rotor. The top fraction was obtained by tube slicing, and triglycerides were determined by enzymatic methods as described above. Cholesterol and apo B were measured in the infranate. LDL-cholesterol was calculated by subtraction of total HDL-cholesterol. To determine the apo B-48/apo B-100 ratio in TGRL, aliquots of the top fraction were precipitated with 10% trichloroacetic acid and 0.03% desoxycholate. Precipitates were dissolved in sample buffer containing

0.025% bromophenol blue and were subjected to SDS gel electrophoresis in a Bio-Rad Mini-Protean II cell (Bio-Rad Laboratories, Hercules, CA) using a 3% stacking gel and a 5% separating gel according to the method of Laemmli (1970). Electrophoresis was performed for 90 min at 100 V. Gels were stained with 0.25% Coomassie blue G-250 for 1 h and destained in 40% methanol and 10% acetic acid (v%) for 20 h. Relative abundance of apo B-48 and apo B-100 (the apo B-48/apo B-100 ratio) was determined by scanning of the destained gels by using a laser densitometer equipped with an automated integration module (LKB Ultrascan XL; Pharmacia LKB Biotechnology, Piscataway, NJ).

Retinyl palmitate levels in plasma were determined by high-pressure liquid chromatography (HPLC) (Waters 501 HPLC pump, Waters 486 tunable absorbance detector, Waters 712 sample processor, and Waters 743 data module; Waters Instruments, Milford, MA). The assay was carried out in subdued light with HPLC-grade solvents. Retinol from 0.5 ml of plasma denatured with an equal volume of absolute ethanol was extracted by the addition of 7.5 ml of hexane. Six and one half milliliters of the hexane phase were removed and evaporated to dryness under a gentle stream of N_2 at room temperature. The resulting residues were dissolved in 200 μl of methanol/chloroform (4/1 [v/v]) and were passed through a 0.45-μm HV filter (Millipore, Milford, MA), and 50-μl aliquots were injected into a 3.9 × 150-mm Nova-Pack C 18 4-μm HPLC column (Waters, Milford, MA). Acetonitrile/methanol/methylene chloride (70/15/15 [v/v/v]) was used as the mobile phase at a flow rate of 1.8 ml/min (Amadee-Manesme et al. 1984). The effluent was monitored at 325 nm, and the peak of retinyl palmitate was identified by comparison with the retention time of a purified standard (Sigma Chemical, St. Louis). The amount of retinyl palmitate in plasma was quantified by regression analysis using integrated areas of unknowns and standard dilutions. The correlation between concentration and integrated areas in five dilutions of standard unknowns was typically >.98.

Apo E genotypes were determined by restriction isotyping (Hixson and Vernier 1990). Genomic DNA was extracted from frozen buffy coats, amplified by the PCR (Saiki et al. 1988) using the primers reported by Emi et al. (1988), and was restricted with *Hha*I. The apo E genotypes were typed directly from the ethidium bromide–stained 12% polyacrylamide gels.

Statistical Analyses

Allele frequencies were estimated by the method of gene counting. Agreement with Hardy-Weinberg equilibrium expectations was tested using a χ^2 goodness-of-fit test. Differences in allele frequencies among Caucasians and blacks were tested using a 2 × 3 contingency χ^2 (Snedecor and Cochran 1980).

Prior to analysis of the effects of the apo E gene on fasting lipid, lipoprotein, apo, and retinyl palmitate levels, these measures were adjusted, by multiple linear regression (Neter et al. 1990), for the concomitant effects of sex, age, body-mass index, disease status, cigarette smoking, and alcohol consumption. Retinyl palmitate was also adjusted for the influence of fasting triglyceride levels. Both the analysis of variance (Neter et al. 1990) and a more robust nonparametric test, the Kruskal-Wallis test (Conover 1980; Daniel 1990), were used to investigate the effects of the apo E gene on the adjusted fasting measures of lipid metabolism. Normalizing transformations such as logarithms and square root were avoided because they lack intuitive appeal and result in loss of the original scale.

The effect of the apo E polymorphism on the postprandial profile of lipid, lipoprotein, apo, and retinyl palmitate levels was investigated using either a repeated-measures analysis-of-variance model (Neter et al. 1990) or profile analysis (Morrison 1976). Specifically, the hypothesis of primary interest was the *F*-test of interaction between the apo E polymorphism and time, which tests whether the response to the high fat load was the same among apo E types. Postprandial "response" was defined as the difference between levels 8 h postprandially and fasting, a measure that was found to be a highly informative predictor of CAD in a cross-sectional study (Patsch et al. 1992*a*). The effect of the apo E polymorphism on this measure of response was tested using the analysis-of-variance (Neter et al. 1990) and the Kruskal-Wallis nonparametric test (Conover 1980; Daniel 1990). The proportion of interindividual variation, in this response, attributable to the apo E polymorphism was calculated using the R^2 from the analysis of variance.

Results

The observed frequencies of each of the apo E genotypes are shown, for Caucasians and blacks, respectively, in the first row of table 1 and the first row of table 2. As expected on the basis of the results of numerous other studies (Hallman et al. 1991; Gerdes et al. 1992), the most common group in both races was the ε3/3 genotype, followed by the ε3/4 and ε2/3 genotypes. In the sample of Caucasians there were only two ε2/2 individuals. In the sample of blacks no individuals

Table 1

Fasting Plasma Lipid, Lipoprotein, and Apo Levels in the Sample of Caucasians

Variable	Mean (SD)								
	ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	Overall	P^{a}	P^{b}
No. of subjects	2	44	10	260	73	8	397		
Total cholesterol	194.3 (40.6)	196.3 (39.9)	187.9 (36.6)	202.9 (34.9)	207.1 (31.4)	196.5 (22.4)	202.4 (34.7)	.35 (.28)	.27 (.15)
LDL-cholesterol	118.9 (33.8)	117.7 (31.7)	115.6 (26.3)	136.2 (34.1)	136.6 (28.5)	128.0 (26.7)	133.5 (33.0)	.01 (.01)	.005 (.003)
Apo B	101.4 (38.8)	93.5 (27.8)	99.6 (34.4)	103.3 (31.9)	102.5 (26.0)	101.2 (29.0)	101.9 (30.5)	.60 (.48)	.17 (.11)
Apo B in LDL	80.7 (22.8)	74.4 (18.2)	86.0 (30.3)	85.2 (29.3)	92.2 (26.2)	91.2 (25.7)	85.5 (27.9)	.07 (.04)	.008 (.004)
Apo B-48/ apo B-100	.059	.034 (.023)	.028 (.027)	.031 (.021)	.033 (.020)	.046 (.037)	.032 (.022)	.32 (.47)	.75 (.72)
Triglycerides	165.7 (58.0)	135.8 (75.4)	122.7 (75.4)	122.6 (57.8)	141.9 (111.6)	119.7 (42.2)	127.8 (72.9)	.40 (.70)	.11 (.63)
Triglycerides in VLDL	107.2 (70.2)	75.5 (54.5)	74.5 (66.8)	77.4 (60.7)	97.4 (113.1)	69.4 (30.2)	80.8 (72.5)	.45 (.89)	.13 (.81)
HDL-cholesterol	41.7 (6.52)	44.3 (14.5)	49.2 (10.9)	43.9 (11.9)	44.4 (8.60)	46.7 (13.3)	44.2 (11.6)	.78 (.51)	.94 (.51)
Apo AI	132.8 (38.7)	125.0 (27.2)	127.2 (21.2)	120.0 (26.6)	124.6 (21.3)	134.4 (23.4)	121.9 (25.7)	.37 (.43)	.28 (.34)

[a] Probability that average levels are not significantly different among the six apo E genotypes. The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).

[b] Probability that average levels are not significantly different among the three common apo E genotypes (ε2/3, ε3/3, and ε3/4). The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).

were observed with the ε2/2 or ε4/4 genotype, and only one person was observed with the ε2/4 genotype. Because the depth of inference based on these rare classes is limited, the analyses presented below were carried out for all apo E genotypes and then were repeated considering only the three common genotypes —ε2/3s, ε3/3s, and ε3/4s. The estimated frequencies of the ε2, ε3, and ε4 alleles in the sample of Caucasians

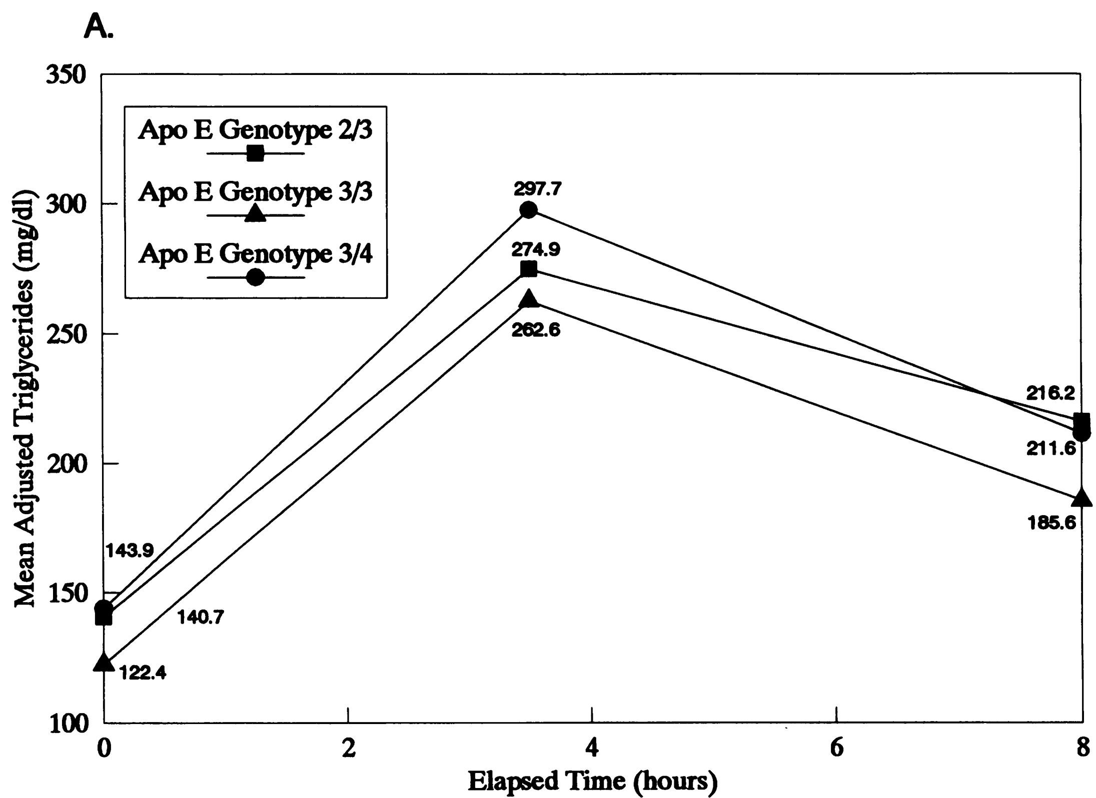
Table 2

Fasting Plasma Lipid, Lipoprotein, and Apo Levels in the Sample of Blacks

Variable	Mean (SD)						
	ε2/3	ε2/4	ε3/3	ε3/4	Overall	P^{a}	P^{b}
No. of subjects	13	1	47	16	77		
Total cholesterol	196.8 (50.0)	170.6	215.4 (33.1)	198.0 (42.5)	208.0 (38.9)	.19 (.10)	.15 (.09)
LDL-cholesterol	109.7 (51.8)	119.5	136.5 (32.2)	130.0 (38.0)	130.9 (36.5)	.44 (.73)	.27 (.58)
Apo B	91.7 (36.7)	80.9	97.8 (21.8)	115.8 (38.6)	100.5 (29.5)	.19 (.50)	.12 (.40)
Apo B in LDL	109.7 (51.8)	119.5	136.5 (32.2)	130.0 (38.0)	130.9 (36.5)	.44 (.65)	.91 (.91)
Apo B-48/ apo B-100	.037 (.029)	.054	.037 (.030)	.029 (.018)	.035 (.027)	.78 (.62)	.73 (.88)
Triglycerides	124.6 (56.5)	83.6	96.8 (40.0)	108.7 (57.0)	103.8 (47.2)	.28 (.36)	.16 (.23)
Triglycerides in VLDL	88.3 (64.4)	58.4	54.3 (33.2)	71.5 (65.5)	63.3 (47.8)	.35 (.45)	.20 (.27)
HDL-cholesterol	44.4 (13.0)	37.1	54.9 (16.9)	45.6 (16.6)	51.0 (16.7)	.08 (.04)	.05 (.03)
Apo AI	124.0 (18.5)	131.6	134.5 (31.5)	132.9 (43.9)	132.5 (33.1)	.89 (.64)	.74 (.47)

[a] Probability that average levels are not significantly different among the four apo E genotypes. The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).

[b] Probability that average levels are not significantly different among the three common apo E genotypes (ε2/3, ε3/3, and ε3/4). The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).



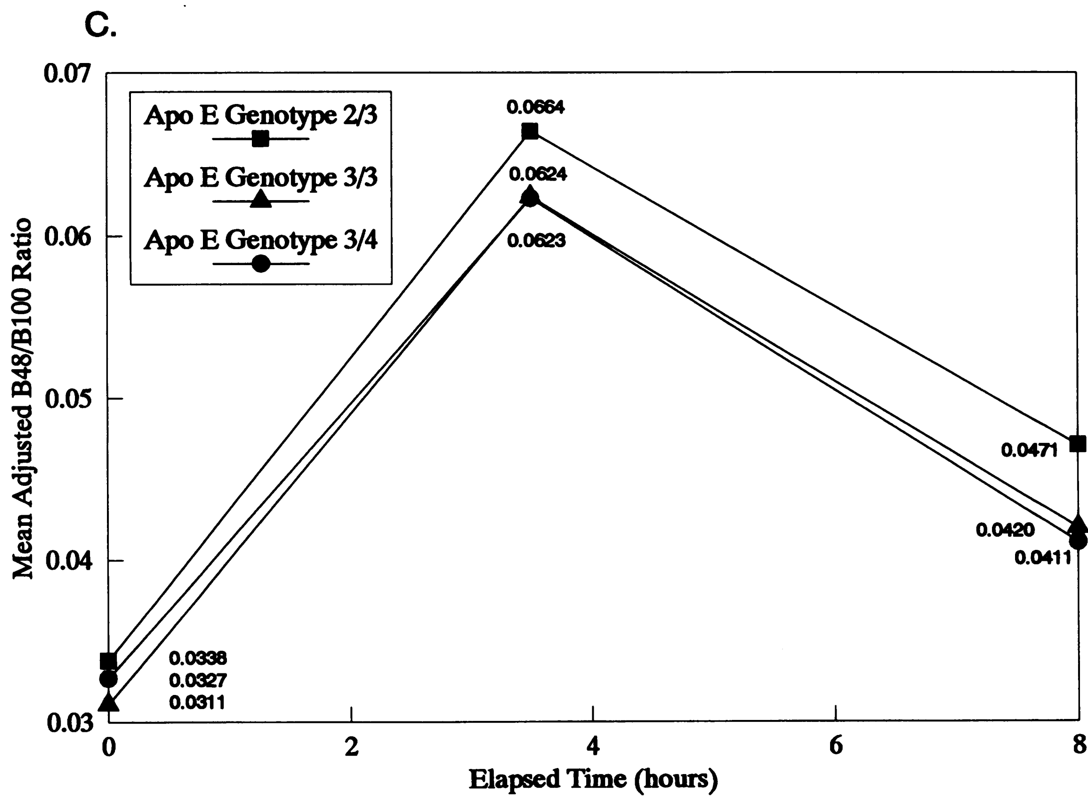
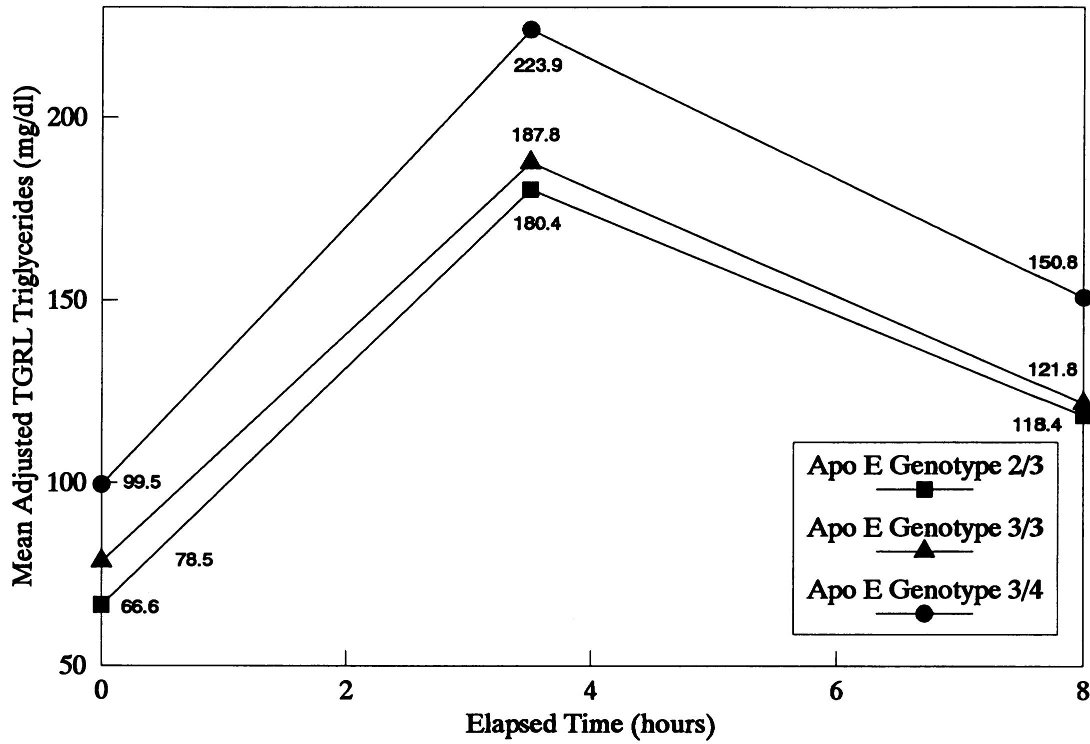


Figure 1 Profile of postprandial triglyceride (*A*), TGRL-triglyceride (*B*), apo B-48/apo B-100 ratio (*C*), and retinyl palmitate (*D*) levels, for each of the common apo E genotypes in 397 Caucasian subjects from the ARIC study. *E–H*, Profile of postprandial triglyceride, TGRL-triglyceride, apo B-48/apo B-100 ratio, and retinyl palmitate levels, respectively, in the sample of 77 black subjects.



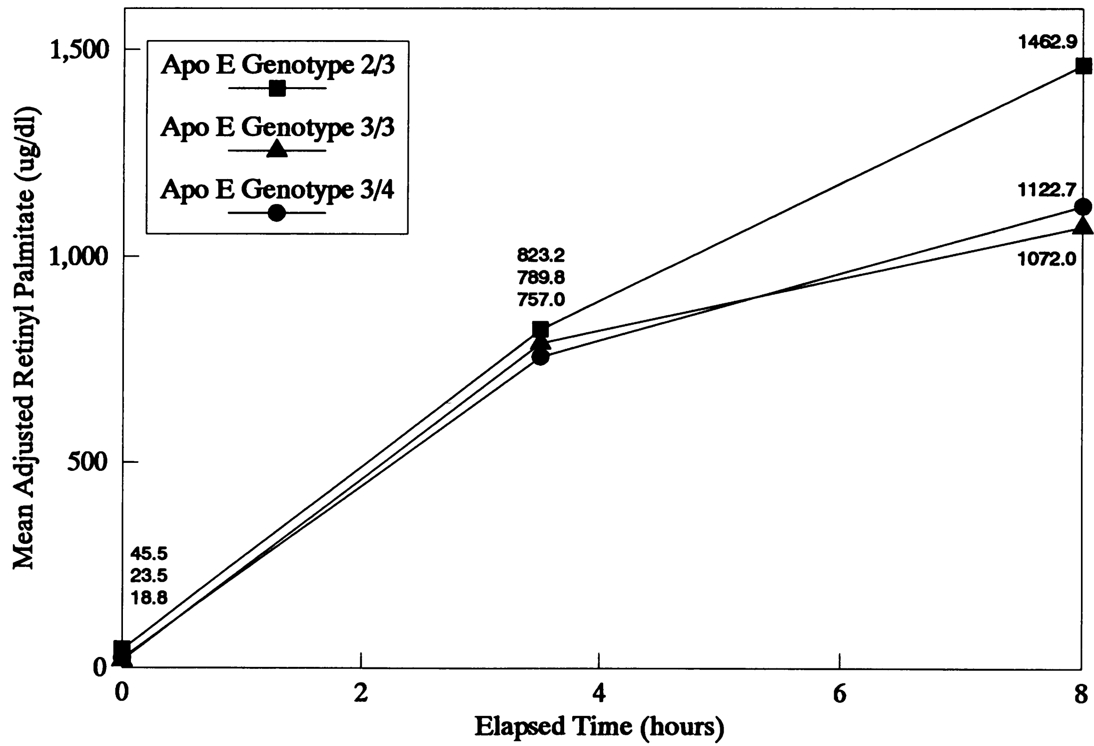
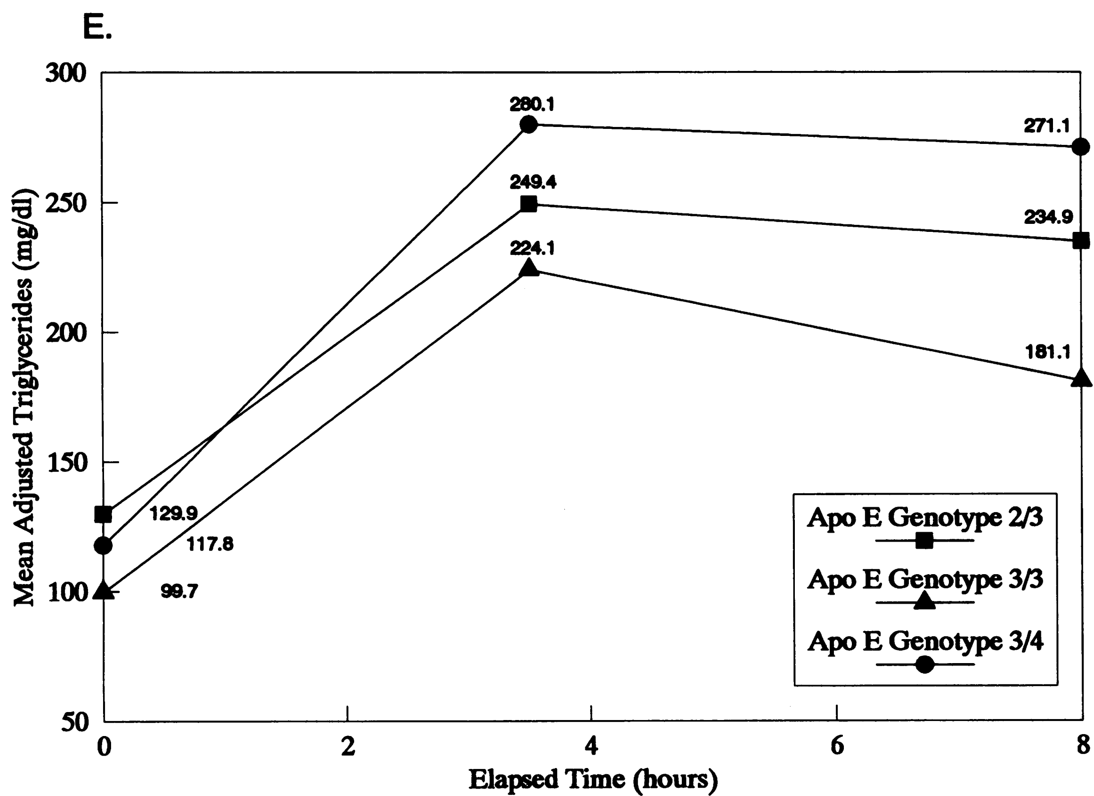


Figure 1 (continued)



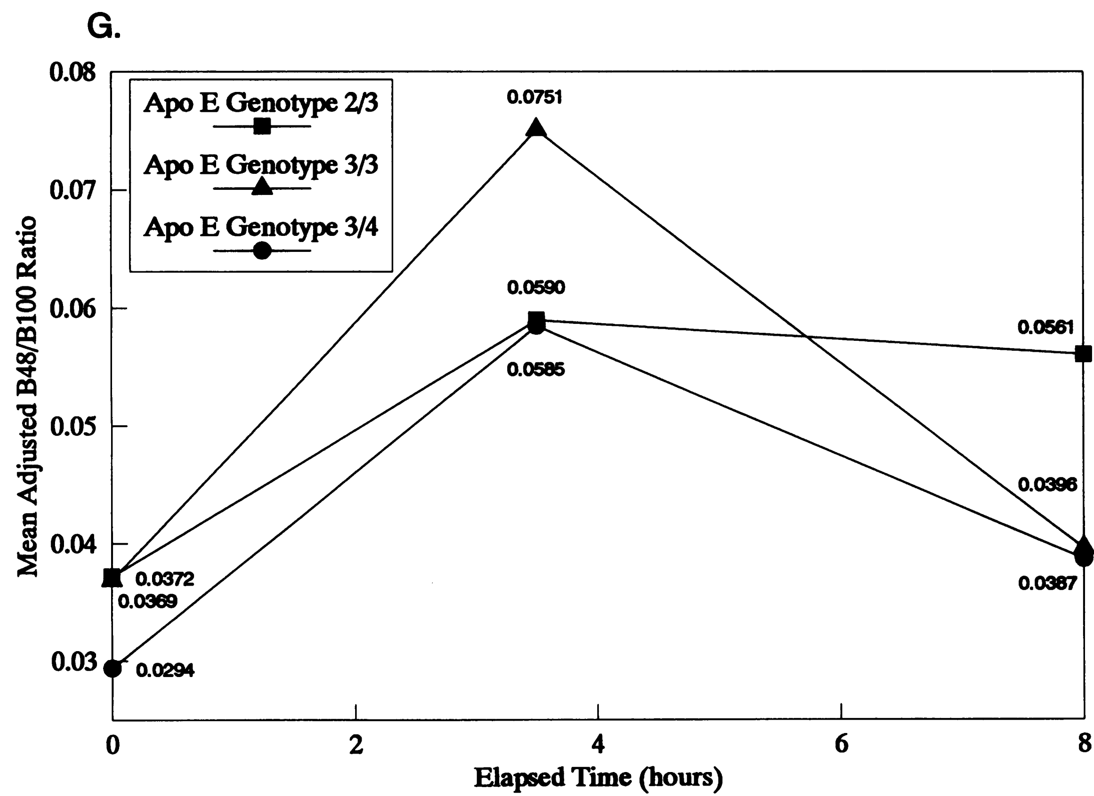
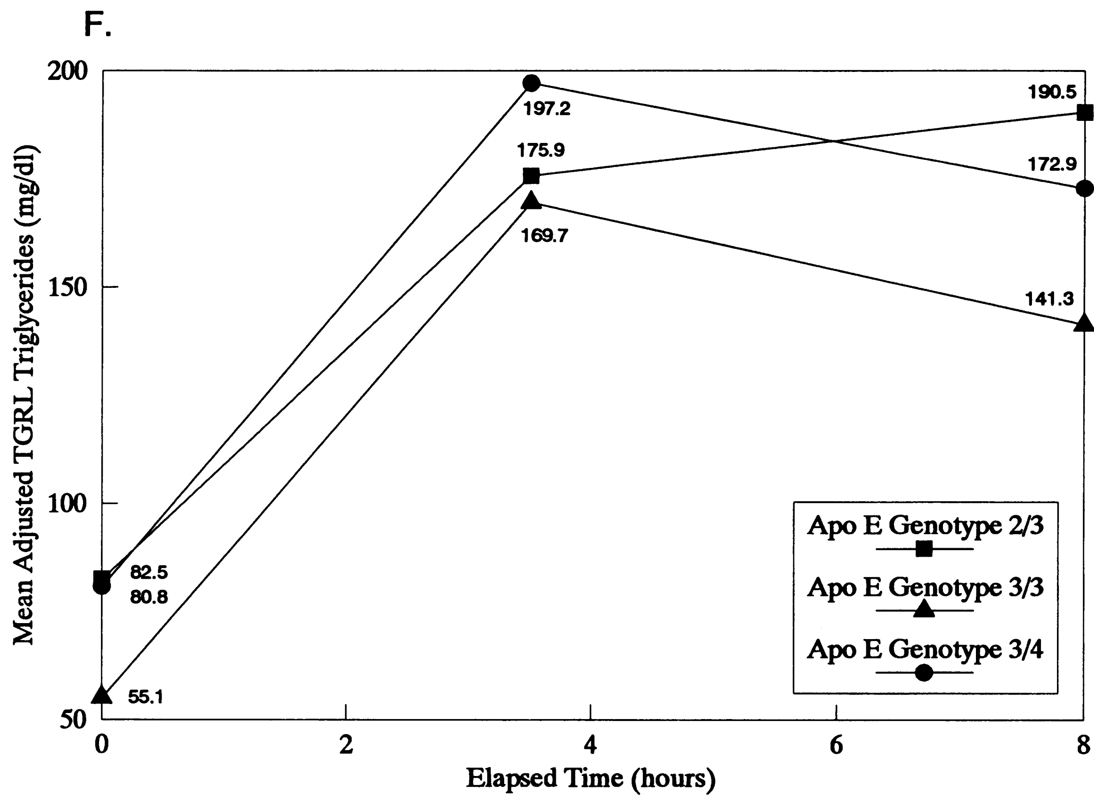


Figure 1 (continued)



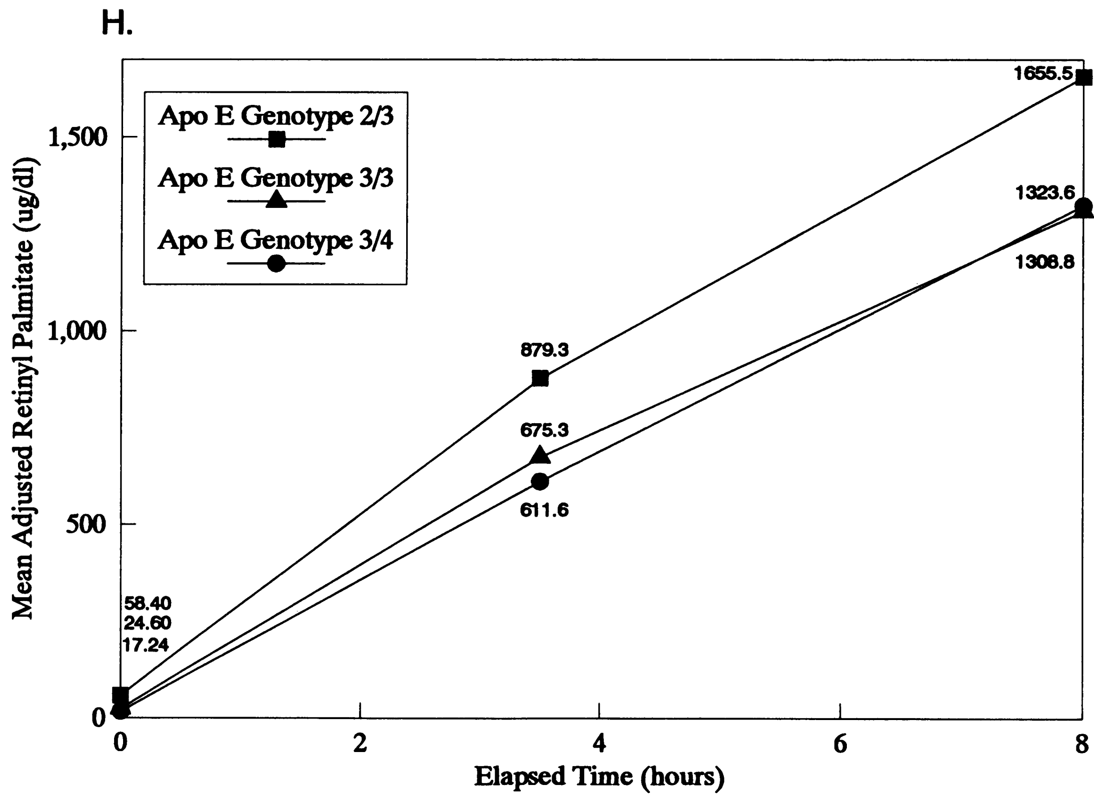
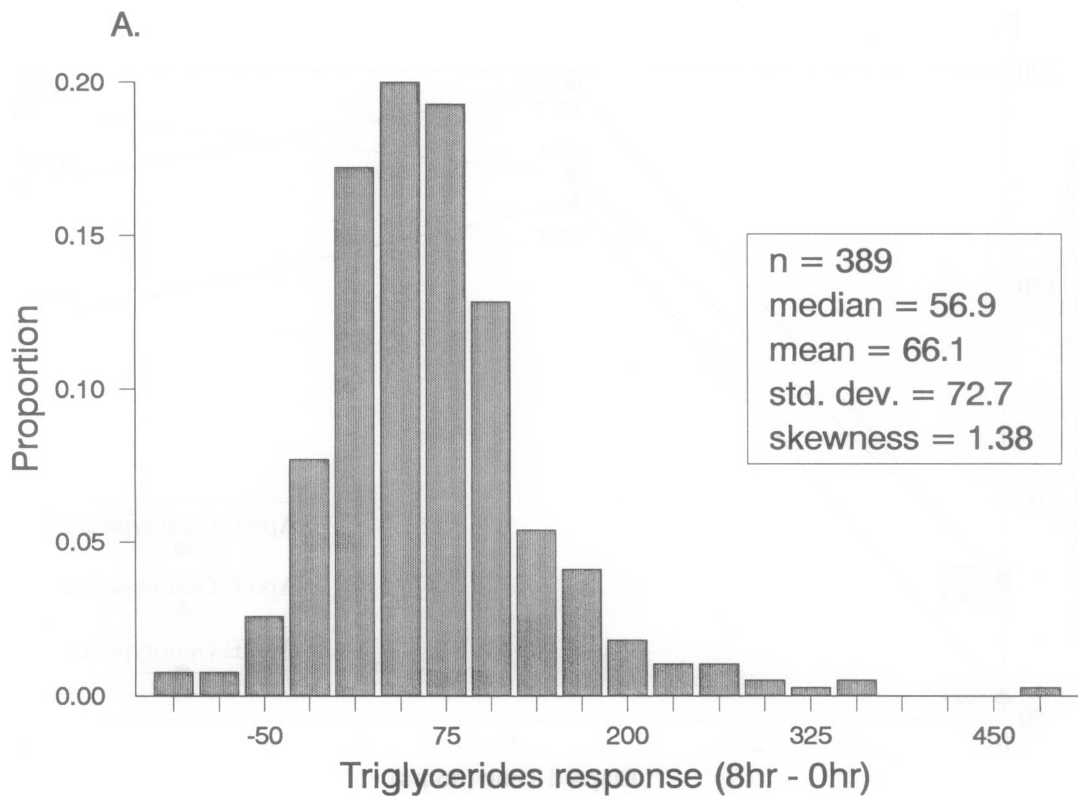


Figure 1 (continued)



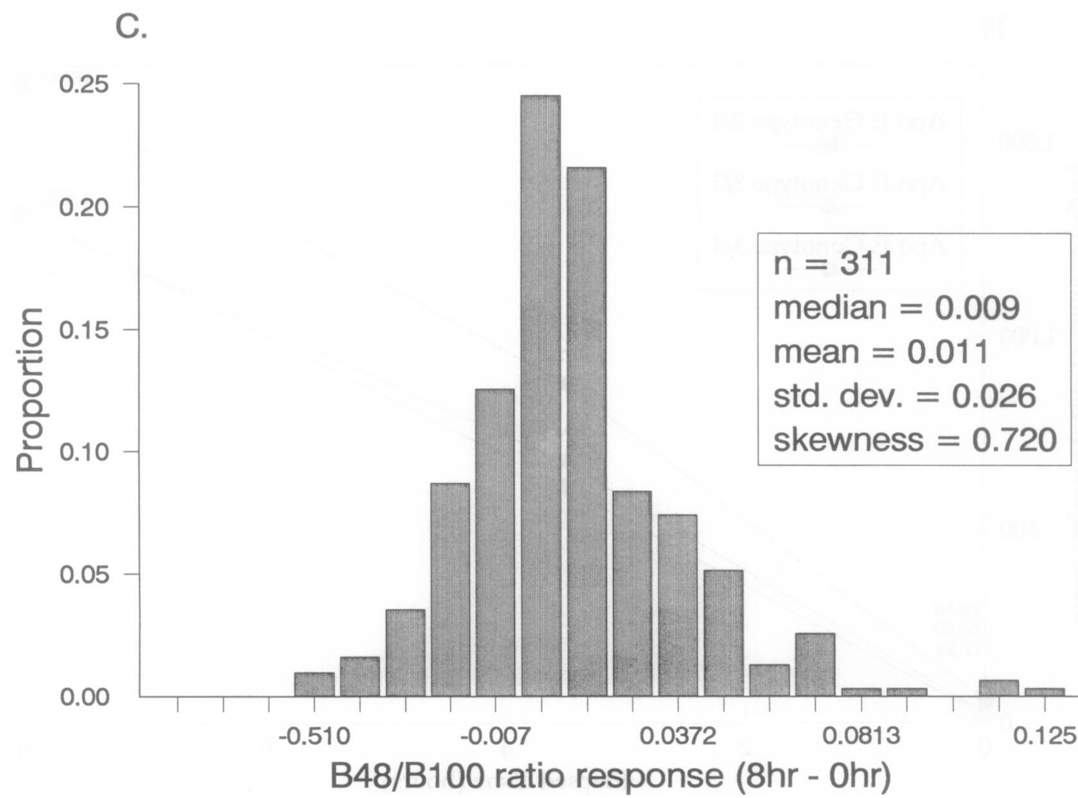
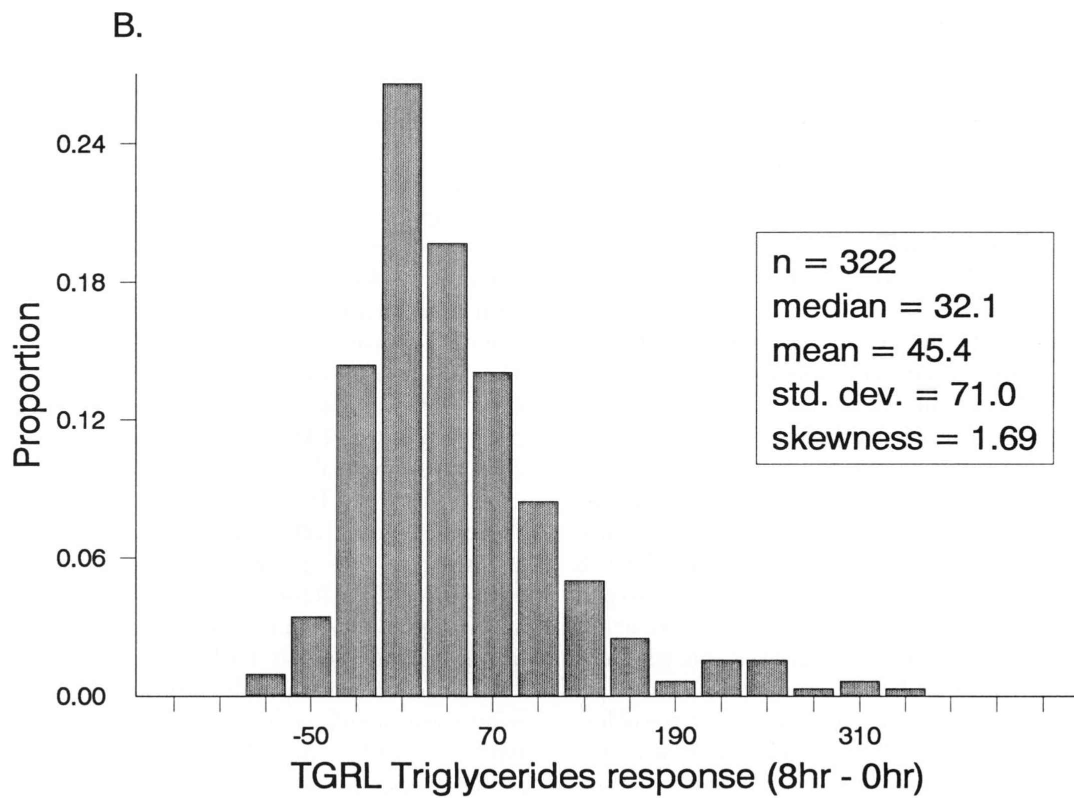


Figure 2 Distribution of postprandial response as measured by the difference between 8 h and fasting, for triglyceride (*A*), TGRL-triglyceride (*B*), apo B-48/apo B-100 ratio (*C*), and retinyl palmitate (*D*) levels in 397 Caucasian subjects from the ARIC study.



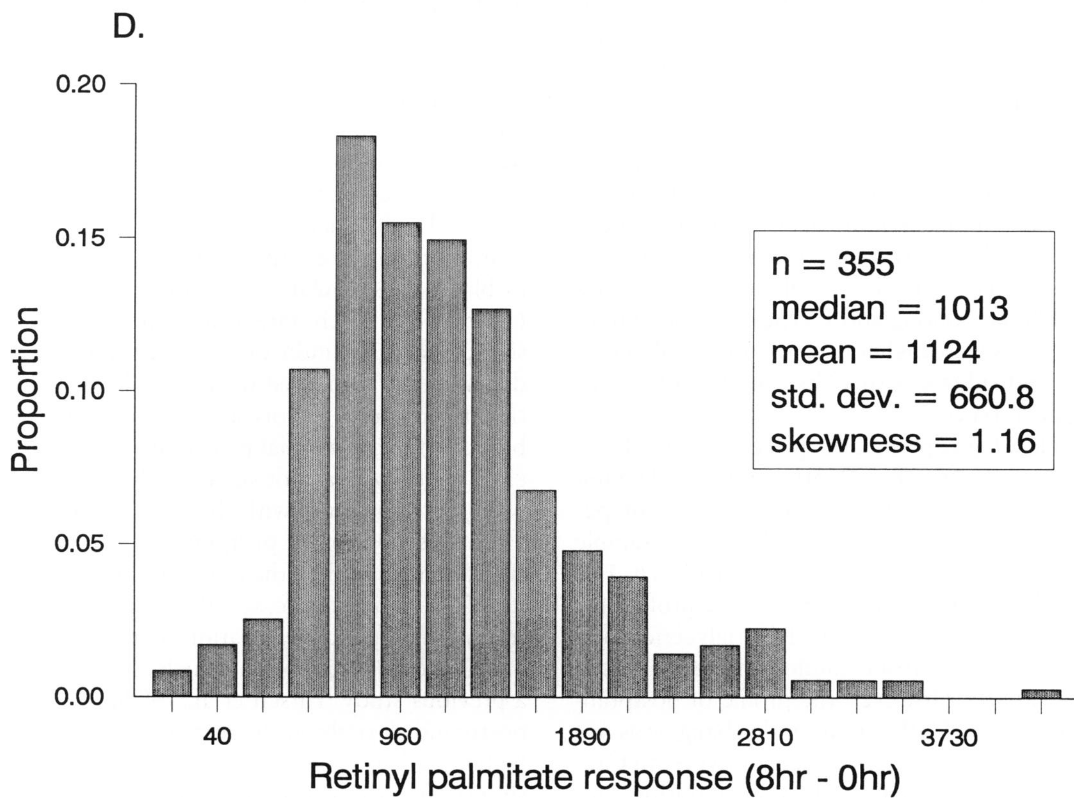


Figure 2 (continued)

were .073, .802, and .125, respectively. In the sample of blacks, these values were .091, .799, and .110, respectively. The estimated allele frequencies were not significantly different between Caucasians and blacks ($\chi^2 = 0.76$, $P = .68$). In addition, the observed genotype frequencies shown in tables 1 and 2 are in agreement with Hardy-Weinberg expectations, in both racial groups.

The effect of the apo E polymorphism on fasting plasma lipid, lipoprotein, and apo levels is shown in tables 1 and 2, for Caucasians and for blacks, respectively. When the test of interaction from a two-way analysis of variance was used, the effects of the apo E polymorphism on these measures were not significantly different between Caucasians and blacks. In Caucasians, average LDL-cholesterol and LDL–apo B levels were consistently different among apo E genotypes. Both LDL-cholesterol and LDL-apo B levels were significantly lower in individuals with at least one ε2 allele, compared with those with the common ε3/3 genotype. LDL-apo B levels were higher, and LDL-cholesterol levels were only slightly higher, in individuals with the ε3/4 genotype, compared with those with the common ε3/3 genotype. Of particular relevance to the analysis of postprandial response presented below, individuals with the ε3/4 genotype had higher, but not significantly higher, plasma triglyceride and TGRL-triglyceride levels than did individuals with either of the other two common apo E genotypes. As in the sample of Caucasians, average LDL-cholesterol and LDL–apo B levels were lower in black individuals with an ε2 allele, but these effects did not reach statistical significance. In this sample of black subjects, HDL-cholesterol levels were also different among apo E types. Average HDL-cholesterol levels were higher in ε3/3 individuals, compared with individuals with either of the other two common apo E types.

The profile of postprandial triglyceride, TGRL-triglyceride, apo B-48/apo B-100 ratio, and retinyl palmitate response for each of the common apo E genotypes is shown in figure 1*A*–1*D*, respectively, for the sample of Caucasians. These data are plotted in figure 1*E*–1*H*, for the sample of blacks. The characteristic profiles for postprandial triglycerides and TGRL-triglycerides are significantly different from random in both racial groups ($P < .0001$). However, the profile of postprandial triglyceride and TGRL-triglyceride change was not different among the common apo E genotypes. In Caucasians, postprandial triglyceride concentrations increased from 128.6 (±75.2) mg/dl at fasting to 270.8 (±130.5) mg/dl at 3.5 h, followed by a decrease to 193.7 (±137.1) mg/dl at 8 h. Average TGRL-triglyceride concentrations were 81.2 (±72.8) mg/dl at fasting, 194.9 (±114.0) mg/dl at 3.5 h, and 126.9 (±116.9) mg/dl at 8 h.

The apo B-48/apo B-100 ratio determined here is a measure of the relative contribution of intestinally derived apo B-48–based lipoproteins, such as chylomicrons and chylomicron remnants, to apo B-100–containing lipoproteins primarily VLDL, from the liver. The pattern of postprandial response for the apo B-48/apo B-100 ratio (fig. 1*C*) is similar to those observed for triglycerides (fig. 1*A*) and TGRL-triglycerides (fig. 1*B*). This pattern was also not significantly different among apo E genotypes. In Caucasians, the mean value of the apo B-48/apo B-100 ratio increased from .032 (±.022) at fasting to .063 (±.033) at 3.5 h, followed by a decrease to .043 (±.028) at 8 h. In this sample of blacks, average apo B-48/apo B-100 ratios were .036 (±.027) at fasting, .068 (±.062) at 3.5 h, and .042 (±.028) at 8 h.

In Caucasians, the profile of postprandial retinyl palmitate was significantly different among apo E genotypes ($F = 4.08$; 4 and 654 df; $P = .003$); however, the profile of response was very similar between fasting and 3.5 h but diverged between 3.5 h and 8 h. Late postprandial retinyl palmitate levels were significantly higher in individuals with the ε2/3 genotype, compared with individuals with either of the other apo E genotypes. For example, average late postprandial retinyl palmitate levels were 1,462.9 (±736.9) μg/dl in ε2/3 individuals, compared with 1,072.0 (±604.2) μg/dl in the ε3/3 individuals. The influence of the apo E polymorphism on the postprandial retinyl palmitate profile in blacks was similar to that observed in Caucasians, but it did not reach statistical significance. As in Caucasians, black ε2/3 individuals showed a larger postprandial increase, compared with individuals with either of the other apo E genotypes. In both Caucasians and blacks, the postprandial profile of individuals with the ε3/4 genotype was not significantly different from the profile in individuals with the ε3/3 genotype.

The distribution of postprandial response in Caucasians, as measured by the difference in plasma triglyceride, TGRL-triglyceride, apo B-48/apo B-100 ratio, and retinyl palmitate concentrations between 8 h and baseline, is shown in figure 2, in panels *A*–*D*, respectively. In a previous study (Patsch et al. 1992*a*) this measure of postprandial triglyceride response was shown to be highly predictive of CAD. The average and median responses for triglycerides were 66.1 and 56.9 mg/dl, respectively. As a measure of general spread of the data, the SD of triglyceride response was 72.7 mg/dl, and the

Table 3

Average Postprandial Response of Plasma Triglyceride and Retinyl Palmitate Levels in Caucasians

VARIABLE	MEAN (SD)								
	ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	Overall	P[a]	P[b]
No. of subjects	2	44	10	260	73	8	397		
Triglycerides	92.0 (10.1)	73.6 (73.3)	89.1 (58.8)	66.0 (74.1)	62.1 (71.9)	28.9 (46.2)	66.1 (72.7)	.55 (.25)	.72 (.65)
Triglycerides in VLDL	43.8	44.0 (65.8)	66.8 (31.4)	43.8 (73.8)	52.6 (67.1)	6.7 (75.5)	45.4 (71.0)	.60 (.32)	.69 (.54)
Apo B-48/apo B-100	.0023	.0083 (.023)	.027 (.023)	.012 (.028)	.0072 (.019)	.011 (.016)	.011 (.026)	.38 (.27)	.44 (.34)
Retinyl palmitate	2,064 (34)	1,489 (701)	1,598 (499)	1,037 (595)	1,108 (75)	1,283 (869)	1,124 (661)	.0001 (.0001)	.0004 (.0005)

[a] Probability that average levels are not significantly different among the six apo E genotypes. The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).

[b] Probability that average levels are not significantly different among the three common apo E genotypes (ε2/3, ε3/3, and ε3/4). The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).

distribution was positively skewed (γ_1 = .886). Most individuals showed a moderate increase in triglycerides; half the individuals in this sample had a response between 22.0 and 94.7 mg/dl. However, some individuals had a very high response (10% were >153 mg/dl), and some individuals even had a negative response when measured by this simple difference. For TGRL-triglycerides, the average and median responses were 45.4 and 32.1 mg/dl, respectively, with an SD of 71.0 mg/dl. For the apo B-48/apo B-100 ratio, the average and median responses were .011 and .0088, respectively, with an SD of .026. For retinyl palmitate, the average and median responses were 1,123.8 and 1,012.8 μg/dl, respectively, with an SD of 660.8 μg/dl. The 25th and 75th percentiles of retinyl palmitate response were 662.0 and 1,416.7 μg/dl, respectively. However, there is a substantial group of people (10%) with a postprandial retinyl palmitate response of >2,000 μg/dl.

The effect of the apo E polymorphism on the postprandial response of triglycerides, TGRL-triglycerides, apo B-48/apo B-100 ratio, and retinyl palmitate are presented in tables 3 and 4. In Caucasians (table 3), average triglyceride response is not significantly different among apo E genotypes. When only the common apo E genotypes are considered, average triglyceride response was somewhat higher in ε2/3 individuals and lower in ε3/4 individuals, compared with that in ε3/3 individuals. In blacks (table 4), average triglyceride response was higher in ε3/4 individuals, compared with individuals with either of the other two common apo E types—an effect opposite to that observed in Caucasians. However, these differences did not reach statistical significance in either race. As was seen for total triglycerides, average TGRL-triglycerides were not significantly different among apo E genotypes in either Caucasians or blacks, and no clear pattern emerged for the effect of the apo E polymorphism on this trait. Therefore, we conclude that there is no significant evidence that the common apo E polymorphism affects postprandial total triglyceride or TGRL-triglyceride response in this sample. Similarly, the data shown in tables 3 and 4 suggest that there is no significant effect of the apo E polymorphism on the apo B-48/apo B-100 ratio.

In contrast to triglycerides and the apo B-48/apo B-100 ratio, the postprandial response of retinyl palmitate was significantly different among apo E genotypes. In Caucasians, average retinyl palmitate response was 1,489.4 μg/dl in ε2/3 individuals, compared with 1,036.7 μg/dl in ε3/3 individuals. Even though the number of ε2/2 individuals is small, their average postprandial response is even higher than that of the ε2/3 individuals, potentially indicating an additive effect of the ε2 allele on postprandial response. Postprandial response of retinyl palmitate concentrations was very similar between ε3/3 and ε3/4 subjects. In blacks, the direction and magnitude of the effect of the apo E polymorphism on retinyl palmitate response was the same as that observed in Caucasians, but it did not reach statistical significance. Average retinyl palmitate response was 1,619.7 μg/dl in ε2/3 individuals, compared with 1,284.3 μg/dl in ε3/3 individuals. When a two-way analysis of variance (Neter et al. 1990) was used on the pooled sample of Caucasians and blacks, the effect of the apo E polymorphism on postprandial retinyl palmitate response was statistically significant,

Table 4

Average Postprandial Response of Plasma Triglyceride and Retinyl Palmitate Levels in Blacks

Variable	Mean (SD)						
	ε2/3	ε2/4	ε3/3	ε3/4	Overall	P^a	P^b
No. of subjects	13	1	47	16	77		
Triglycerides	92.1 (88.9)	−13.0	96.2 (95.0)	151.5 (186.7)	105.1 (118.6)	.30 (.26)	.26 (.43)
Triglycerides in VLDL	95.3 (72.6)	.76	79.1 (114.3)	119.5 (98.5)	89.3 (103.8)	.62 (.17)	.59 (.23)
Apo B-48/ apo B-100	.012 (.016)	.0027	.0021 (.035)	.016 (.018)	.0075 (.029)	.53 (.38)	.34 (.23)
Retinyl palmitate	1,619.7 (786.3)	459.7	1,284.3 (590.9)	1,356.3 (661.8)	1,334.5 (642.3)	.34 (.33)	.47 (.54)

[a] Probability that average levels are not significantly different among the four apo E genotypes. The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).

[b] Probability that average levels are not significantly different among the three common apo E genotypes (ε2/3, ε3/3, and ε3/4). The value given in parentheses is this probability based on a nonparametric Kruskal-Wallis test (see Methods).

but the interaction between the apo E polymorphism and race was not significant (P = .92). We conclude from these data that this gene polymorphism does have a significant influence on the postprandial retinyl palmitate response, but this effect is not different between Caucasians and blacks.

We next calculated the contribution of differences in apo E genotypes to the interindividual variation in postprandial response. In both Caucasians and blacks, 7.13% of the interindividual variation in postprandial retinyl palmitate response was attributable to the apo E polymorphism. As expected, the contribution of the apo E polymorphism to the other three response variables was less than that for retinyl palmitate. Among both Caucasians and blacks, the contribution of the apo E polymorphism to the interindividual variation in response was <2% for total triglycerides, TGRL-triglycerides, and the apo B-48/apo B-100 ratio. For comparative purposes, 4.1% of the variance in fasting LDL-cholesterol concentrations in this sample of Caucasians was attributable to the apo E polymorphism; this value is similar to that reported elsewhere (Boerwinkle and Utermann 1988) in other populations. Therefore, the proportional contribution of apo E to the interindividual variance in postprandial response as measured by retinyl palmitate is greater than that for fasting LDL-cholesterol.

Discussion

Interindividual variability in postprandial lipid transport after a standard meal exceeds that observed in the fasting state (Patsch et al. 1984, 1987; Brown et al. 1992) and is influenced by numerous environmental and genetic factors affecting the synthesis and catabolism of TGRL originating from the liver and intestine. To quantify the effect of the apo E polymorphism on the magnitude of the postprandial lipemia, we have investigated its effect on postprandial triglyceride, TGRL-triglyceride, apo B-48/apo B-100 ratio, and retinyl palmitate profiles in a large sample from the ARIC study. The frequency of the apo E alleles and the effects of the apo E polymorphism on fasting lipid levels in this sample were similar to those reported in other studies (Hallman et al. 1991; Gerdes et al. 1992). The profiles of postprandial triglyceride, TGRL-triglyceride, and apo B-48/apo B-100 ratio were not significantly different among apo E genotypes. Average levels of each of these three variables increased during the first 3.5 h postprandially but decreased between 3.5 and 8 h. Average retinyl palmitate levels increased throughout the 8-h time period. The postprandial profile and response of plasma retinyl palmitate levels were significantly different among apo E genotypes. Individuals with an ε2 allele had significantly higher postprandial retinyl palmitate response, compared with ε3/3 and ε3/4 subjects. The apo E polymorphism accounted for 7.1% of the interindividual variation in 8-h postprandial retinyl palmitate response, a value greater than that observed for fasting LDL-cholesterol.

Because of the large sample size and outpatient design of this study, only two postprandial blood specimens were collected, at 3.5 and 8 h. The rationale for selecting these two time points was to obtain basic in-

formation about the shape of the postprandial curve for select analytes and to include a late postprandial sample, shown previously to discriminate patients with angiographically verified CAD from control subjects without CAD (Patsch et al. 1992*a*). The analyses included total plasma triglycerides as well as triglycerides from the $d < 1.006$-g/ml fraction, as measures of general postprandial lipemia. They also included retinyl palmitate concentrations in plasma, as a measure more specific to intestinally derived lipoproteins (Hazzard and Bierman 1976; Berr and Kern 1984). Indeed, postprandial retinyl palmitate concentrations are thought to provide a better measure of chylomicron remnant clearance than do postprandial triglyceride concentrations (Brenninkmeijer et al. 1987; Weintraub et al. 1987). To obtain an additional estimate of the contribution of intestinally derived lipoproteins, the apo B-48/apo B-100 ratio in $d < 1.006$-g/ml lipoproteins was determined. The validity of this ratio is, however, not absolute, since the intestine may also synthesize and secrete a variable fraction of apo B-100 (Hoeg et al. 1990).

Numerous reports have indicated that the apo E polymorphism influences plasma lipid levels. Hallman et al. (1991) have studied the frequency and effects of the apo E polymorphisms among nine ethnically and geographically diverse populations. They concluded that, although the frequencies of the apo E alleles are heterogeneous among populations, the effects of this gene are relatively consistent: the average effect of the ε2 allele is to lower plasma cholesterol levels, and the average effect of the ε4 allele is to raise plasma cholesterol levels. Similar results have been obtained in studies of U.S. blacks (Eichner et al. 1989). Even though the relative frequency of the ε4 allele is higher in blacks compared with Caucasians, the same effects of the apo E polymorphism on plasma cholesterol levels are apparent (Eichner et al. 1989). These alterations in total cholesterol levels are primarily due to the effects of this gene on LDL-cholesterol specifically (Boerwinkle and Utermann 1988). The apo E polymorphism may also influence plasma triglyceride concentrations (Dallongeville et al. 1992). In particular, individuals with the ε3/4 genotype are usually reported to have elevated triglycerides, compared with ε3/3 subjects. In our sample, average fasting and postprandial triglyceride concentrations in both Caucasians and blacks tended to be higher in both the ε2/3s and the ε3/4s, compared with those in individuals with the common ε3/3 genotype, though this relationship was not statistically significant or consistent. In our sample of blacks, marginally significant differences in HDL-cholesterol among apo E types were observed, with the ε3/3s having higher HDL-cholesterol, compared with the ε2/3s and ε3/4s. It is interesting that Srinivasan et al. (1989) also reported that serum apo E concentrations, which are greatly affected by the apo E polymorphism (Boerwinkle and Utermann 1988), are related to HDL-cholesterol levels in blacks. The exact mechanism of these observations is unknown but may result from the ability of the apo E isoforms to complex with apo AII (Innerarity et al. 1978). Alternatively, it may reflect differences in plasma triglycerides among apo E genotypes (Dallongeville et al. 1992) and the inverse relationship between triglycerides and HDL-cholesterol (Patsch et al. 1992*b*).

The apo E isoforms differ by two cysteine to arginine substitutions, at positions 112 and 158 in the apo E protein (Weisgraber et al. 1981). Substitution of an arginine by a cysteine at position 158 reduces the receptor-binding affinity of the E2 isoform (Weisgraber et al. 1982; Hui et al. 1984). Reduced binding affinity of the E2 isoform for hepatic lipoprotein receptors and subsequent up-regulation of LDL-receptor density have been proposed as the most likely mechanism for the effect of this gene on plasma LDL-cholesterol and total cholesterol concentrations (Weintraub et al. 1987; Boerwinkle and Utermann 1988). It is also the likely mechanism for the increased late postprandial retinyl palmitate concentrations in ε2/3 subjects observed in the present study.

Previous studies on apo E and postprandial lipemia have considered much smaller samples than are available in the ARIC study (Gregg et al. 1986; Brenninkmeijer et al. 1987; Weintraub et al. 1987; Brown and Roberts 1991; Superko and Haskell 1991). In their seminal paper, Weintraub et al. (1987) first reported that clearance of dietary fats were different among apo E types, with ε2 individuals having delayed clearance and with ε4 individuals having faster clearance, as ascertained by both retinyl palmitate concentrations in plasma and the nonchylomicron fraction. The effect of the ε4 allele was consistent with in vivo studies showing that radioactively labeled apo E4 is cleared faster than apo E3 (Gregg et al. 1986). A more rapid transfer of apo E4 to chylomicron remnants was thought to account for its faster clearance. While these studies have provided mechanistic insights, they may not be representative of the effect of the ε4 allele on postprandial lipemia in the population at large.

The mechanism whereby the ε4 allele increases fasting plasma triglycerides is not known. It is conceivable that enhanced uptake of intestinal TGRL that results

from a more rapid association of apo E4 with chylomicrons (Innerarity et al. 1978; Gregg et al. 1986; Weintraub et al. 1987) influences plasma triglycerides. In hepatocyte preparations, uptake of chylomicron remnants is associated with increased VLDL production (Craig et al. 1988). Increased association of intestinal TGRL with apo E4 may render these particles amenable for endocytosis at an earlier stage of delipidation, thereby increasing hepatic fatty-acid concentrations, which is known to drive hepatic VLDL-triglyceride secretion. The fact that the ε4 allele is associated with hypercholesterolemia (Utermann et al. 1984) as well as with type V hyperlipemia (Ghiselli et al. 1982) is consistent with the notion that a common mechanism (i.e., uptake of triglyceride-rich remnants) may underlie these observations. Dependent on other genetic and environmental factors regulating VLDL synthesis and catabolism, including its conversion to LDL, two different phenotypes may be expressed in ε4-carrying subjects. Association of the ε4 allele with hypertriglyceridemia in obesity (Fumeron et al. 1988; Gueguen et al. 1989) suggests that the ε4 allele is conducive to enhanced hepatic lipoprotein production that is characteristic of obesity in general (Kesaniemi and Grundy 1983; National Institute of Health Consensus Development Panel on the Health Implications of Obesity 1985). Hence, impaired metabolism of TGRL may contribute to the association of the ε4 allele with CAD, but the relative importance of increased LDL-cholesterol as opposed to increased triglyceride levels awaits further study.

Our data confirm the results of others showing increased retinyl palmitate response in ε2/3 subjects. Unlike retinyl palmitate concentrations, postprandial triglyceride levels did not differ among apo E genotypes. The size of our sample provides confidence in the dissociation of postprandial triglyceride and retinyl palmitate levels, which is consistent with defective clearance of fully delipidated particles. Defective clearance of intestinal lipoproteins would be expected to increase the relative proportion of apo B-48 in the $d < 1.006$-g/ml lipoprotein fraction. However, retinyl palmitate levels in ε2/3 subjects exceeded those in ε3/3 subjects by <50%. Since apo B-48 contributed only 4% of the total apo B in the TGRL fraction of ε3/3 subjects at the 8-h time point and since quantification of apo B subspecies is associated with a larger methodological error than are other measurements, apo E–specific differences in the relative proportion of apo B subspecies may have remained undetected had they occurred. Alternatively, a very small amount of retinyl palmitate could have been transferred among lipoprotein particles (Berr 1992), slightly overestimating the contribution of intestinal lipoproteins to the remnant pool in plasma.

Using labeled cholesterol and betasitosterol feeding, Kesaniemi et al. (1987) showed that the ε2 allele is associated with lower intestinal cholesterol absorption than are the ε3 and ε4 alleles. However, as reported here, ε2-containing individuals show increased postprandial response as measured by retinyl palmitate. Should such apo E–specific differences in absorption also apply to retinol, this result would still be valid; but it may have actually underestimated the effect of the ε2 allele on remnant clearance.

At least five lines of evidence implicate postprandial lipoprotein metabolism in the genesis of CAD. First, receptor-mediated uptake of triglyceride-rich remnant particles by monocyte-derived macrophages promotes the formation of lipid-laden foam cells in the vessel wall (Brown and Goldstein 1983; Gianturco et al. 1988; Huff et al. 1991). Similarly, uptake of isolated chylomicron remnants by cultured human arterial smooth-muscle cells results in the accumulation of intracellular cholesterol (Floren et al. 1981). Second, chylomicron and VLDL remnants make a substantial contribution to the lipid content of the vessel wall in cholesterol-fed rabbits (Stender and Zilversmit 1982; Daugherty et al. 1985). Third, the level of HDL-cholesterol, an established risk factor for CAD, is dependent on both the metabolism of TGRL particles and the extent of postprandial lipemia (Nikkila et al. 1987; Patsch et al. 1987; Tall 1990). This metabolic interrelationship is mediated largely by the action of cholesteryl ester transfer protein (Albers et al. 1984; Tall 1986). Fourth, patients with familial dysbetalipoproteinemia are at high risk of CAD and show a buildup of TGRL remnants of both intestinal and hepatic origin (Fainaru et al. 1982). These remnant particles have a propensity for uptake by macrophages, which are subsequently transformed into foam cells (Mahley and Rall 1989). A similar type of hyperlipidemia can be induced in various animal species by an atherogenic diet, and the severity of atherosclerosis induced correlates with the remnant concentration attained in circulation (Stender and Zilversmit 1982; Daugherty et al. 1985). Fifth, Groot et al. (1991) and Patsch et al. (1992*a*) found in cross-sectional case-control studies that increased postprandial triglycerides, alone or in combination with increased retinyl palmitate concentrations, are associated with angiographically verified CAD. In addition, Ryu et al. (1992) reported an association between the magnitude of postprandial response and carotid artery wall thickness.

While the data of the present study and of other studies indicate that the terminal step of the remnant pathway, i.e., their removal from circulation, is delayed in subjects with the ε2/3 genotype, there is no evidence that the ε2 allele predisposes to CAD. In contrast, a moderately protective role of this allele has been suggested in some studies (Hixson et al. 1991). Thus, other factors, such as enhanced VLDL production by the liver or impaired lipolysis of TGRL, irrespective of their origin, must be implicated in the atherogenesis of remnant particles. Such a hypothesis is in keeping with both the pathophysiology of type III hyperlipoproteinemia (Mahley and Rall 1989) and recent studies showing that increased postprandial triglyceride levels are predictive of CAD (Groot et al. 1991; Patsch et al. 1992*a;* Ryu et al. 1992). In both diseases, impaired metabolism of TGRL results in rerouting of cholesteryl esters from HDL into TGRL. The amount of cholesteryl esters entering the remnant pathway in these diseases is greatly increased, and it is conceivable that their atherogenic potential is enhanced. The atherogenic potential of these cholesteryl ester–enriched remnants is further enhanced in type III hyperlipoproteinemia, because of their prolonged residence time in circulation, which, in turn, is due to the receptor-binding deficiency of the apo E2 isoform (Mahley and Rall 1989). In contrast, the ε2 allele alone is associated with increased postprandial retinyl palmitate, but not with triglyceride levels. In fact, the ε2 allele, because of its defective interaction with hepatic receptors (Hui et al. 1984; Schneider et al. 1981; and Weisgraber et al. 1982), may be associated with reduced hepatic triglyceride production, for reasons described above. Thus, our studies not only provide a reliable estimate of the magnitude of the effect of the apo E polymorphism on various measurements commonly used to characterize postprandial lipemia, but also provide mechanistic insight into the effects of the apo E gene polymorphism on postprandial lipemia and CAD.

Acknowledgments

This work was supported by grants HL-40613 and HL-27341 from the National Institutes of Health and by contracts N01-HC55015, N01-HC55016, N01-HC55018, N01-HC55019, N01-HC55020, N01-HC55021, and N01-HC55022 with the National Heart, Lung and Blood Institute. E.B. is an Established Investigator of the American Heart Association and the recipient of Research Career Development Award HL-02453 from the National Institutes of Health. We would also like to acknowledge the assistance of the ARIC investigators: Jeannette Bensen, Catherine Paton, Amy Haire, and Delilah Posey from the Forsyth County field center; Bobbie Alliston, Faye Blackburn, Catherine Britt, and Barbara Davis from the Jackson field center; John O'Brien, Linda Goldman, Barbara Kuehl, and Anne Murrill from the Minneapolis field center; Carol Christman, Sonny Harrell, Joel Hill, and Joan Nelling from the Washington County field center; Valerie Stinson, Pam Pfile, Hoang Pham, and Teri Trevino from the Central Hemostasis Laboratory; and John Crouch, Debbie Rubin-Williams, Cynthia Stahoviak, and Yi-Hsin Yang from the Coordinating Center.

References

Albers JJ, Tollefson JH, Chen C-H, Steinmetz A (1984) Isolation and characterization of human plasma lipid transfer proteins. Arteriosclerosis 4:49–58

Amadee-Manesme O, Furr HC, Oson JA (1984) The correlation between liver vitamin A concentrations in micro-(needle biopsy) and macrosamples of human liver specimens obtained at autopsy. Am J Clin Nutr 39:315–319

ARIC Investigators (1989) The Atherosclerosis Risk in Communities (ARIC) study: design and objectives. Am J Epidemiol 129:687–702

Austin ME (1991) Plasma triglyceride and coronary heart disease. Arterioscler Thromb 11:2–14

Berr F (1992) Characterization of chylomicron remnant clearance by retinyl palmitate label in normal humans. J Lipid Res 33:915–930

Berr F, Kern F Jr (1984) Plasma clearance of chylomicrons labeled with retinyl palmitate in healthy human subjects. J Lipid Res 25:805–812

Boerwinkle E, Utermann G (1988) Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B, and cholesterol metabolism. Am J Hum Genet 42:104–112

Bond MG, Barnes RW, Riley WA, Wilmoth SK, Chambless LE, Howard G, Owens B (1991) High resolution B-mode ultrasound methods in the Atherosclerosis Risk in Communities (ARIC) Study. J Neuroimaging 1:68–71

Brenninkmeijer BJ, Stuyt PMJ, Demacker PNM, Stalenhoef AFH, van't Laar A (1987) Catabolism of chylomicron remnants in normolipidemic subjects in relation to the apoprotein E phenotype. J Lipid Res 28:361–370

Brown AJ, Roberts DCK (1991) The effect of fasting triacylglyceride concentration and apolipoprotein E polymorphism on postprandial lipemia. Arterioscler Thromb 11:1737–1744

Brown MS, Goldstein JL (1983) Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in Atherosclerosis. Annu Rev Biochem 52:223–261

Brown SA, Chambless LE, Sharrett AR, Gotto AM Jr, Patsch W (1992) Postprandial lipemia: Reliability in an epidemiological field study. Am J Epidemiol 136:538–545

Brown SA, Rhodes CE, Dunn K, Gotto AM Jr, Patsch W

(1988) Effect of blood collection and processing on radioimmunoassay results for apolipoprotein A-I in plasma. Clin Chem 34:920–924

Brunzell JD (1989) Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. In: Scriver C, Beaudet A, Sly W, Valle E (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 1165–1180

Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ (1988*a*) Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. J Lipid Res 29:925–936

——— (1988*b*) Postprandial plasma lipoprotein changes in human subjects of different ages. J Lipid Res 29:469–479

Conover WJ (1980) Practical nonparametric statistics. John Wiley & Sons, New York

Craig WY, Nutik T, Cooper AD (1988) Regulation of apoprotein synthesis and secretion in the human hepatoma Hep G2: the effect of exogenous lipoprotein. J Biol Chem 263:13880–13890

Cumming AM, Roberts FW (1984) Polymorphism at the apoprotein-E locus in relation to risk for coronary disease. Clin Genet 25:310–313

Dallongeville J, Lussier-Cacan S, Davignon J (1992) Modulation of plasma triglyceride levels by apo E phenotype: a meta-analysis. J Lipid Res 33:447–454

Daniel WW (1990) Applied nonparametric statistics. PWS-Kent, Boston

Daugherty A, Lange LG, Sobel BE, Schonfeld G (1985) Aortic accumulation and plasma clearance of β-VLDL and HDL: effects of diet-induced hypercholesterolemia in rabbits. J Lipid Res 26:955–963

Ehnholm C, Mahley RW, Chappell DA, Weisgraber KH, Ludwig E, Witztum JL (1984) Role of apolipoprotein E in lipolytic conversion of β-very low density lipoproteins to low density lipoprotein·in type III hyperlipoproteinemia. Proc Natl Acad Sci USA 81:5566–5570

Eichner JE, Kuller LH, Ferrell RE, Kamboh MI (1989) Phenotypic effects of apolipoprotein structural variation on lipid profiles. IV. Apolipoprotein polymorphisms in a small group of Black women from the Healthy Women Study. Genet Epidemiol 6:681–689

Emi M, Wu LL, Robertson MA, Myers RL, Hegele RA, Williams RR, White R, et al (1988) Genotyping and sequence analysis of apolipoprotein E isoforms. Genomics 3:373–379

Fainaru M, Mahley RW, Hamilton RL, Innerarity TL (1982) Structural and metabolic heterogeneity of beta-very low density lipoproteins from cholesterol-fed dogs and from humans with type III hyperlipoproteinemia. J Lipid Res 23:701–714

Floren C-H, Albers JJ, Bierman EL (1981) Uptake of chylomicron remnants causes cholesterol accumulation in cultured human arterial smooth muscle cells. Biochim Biophys Acta 663:336–349

Fumeron F, Rigaud D, Bertiere MC, Bardon S, DeLy C, Apfelbaum M (1988) Association of apolipoprotein E4 isoform with hypertriglyceridemia. Clin Genet 34:1–7

Gerdes LU, Klausen IC, Sihm I, Faergeman O (1992) Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. Genet Epidemiol 9:155–167

Ghiselli G, Schaefer EJ, Zech LA, Gregg RE, Brewer HB Jr (1982) Increased prevalence of apolipoprotein E4 in type V hyperlipoproteinemia. J Clin Invest 70:474–477

Gianturco SH, Lin AH, Hwang SL, Young J, Brown SA, Via DP, Bradley WA (1988) Distinct murine macrophage receptor pathway for human triglyceride-rich lipoproteins. J Clin Invest 82:1633–1643

Gregg RE, Zech LA, Schaefer EJ, Stark D, Wilson D, Brewer HB Jr (1986) Abnormal in vivo metabolism of apolipoprotein E4 in humans. J Clin Invest 78:815–821

Groot PHE, van Stiphuit WAHJ, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, et al (1991) Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb 11:653–662

Gueguen R, Visvikis S, Steinmetz J, Siest G, Boerwinkle E (1989) An analysis of genotype effects and their interactions by using the apolipoprotein E polymorphism and longitudinal data. Am J Hum Genet 45:793–802

Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Császár A, Utermann G (1991) The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 49:338–349

Hazzard WR, Bierman EL (1976) Delayed clearance of chylomicron remnants following vitamin-A-containing oral fat loads in broad-beta disease (type III hyperlipoproteinemia). Metabolism 25:777–801

Hixson JE, PDAY Research Group (1991) Apolipoprotein E polymorphisms affect atherosclerosis in young males. Arterioscler Thromb 11:1237–1244

Hixson JE, Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 31:545–548

Hoeg JM, Sviridov DD, Tennyson GE, Demosky SJ Jr, Meng MS, Bojanovski D, Safonova IG, et al (1990) Both apolipoproteins B-48 and B-100 are synthesized and secreted by the human intestine. J Lipid Res 31:1761–1769

Huff MW, Evans AJ, Sawyez CG, Wolfe BM, Nestel PH (1991) Comparison of very low density lipoprotein from subjects with type III, IV, and V hyperlipoproteinemias. Arterioscler Thromb 11:221–233

Hui DY, Innerarity TL, Mahyley RW (1984) Defective hepatic lipoprotein receptor binding of β-very low density lipoproteins from type III hyperlipoproteinemic patients. J Biol Chem 259:860–869

Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhama Y, et al (1990) Increased high-density lipoprotein levels caused by a common cholesteryl-ester

transfer protein gene mutation. N Engl J Med 323:1234–1238

Innerarity TL, Mahley RW, Weisgraber KH, Bersot TP (1978) Apoprotein (E-A-II) complex of human plasma lipoproteins. II. Receptor binding activity of a high density lipoprotein subfraction modulated by the apo (E-AII) complex. J Biol Chem 253:6289–6295

Kesaniemi YA, Ehnholm C, Miettinen TA (1987) Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. J Clin Invest 80:578–581

Kesaniemi YA, Grundy S (1983) Increased low density lipoprotein production associated with obesity. Arteriosclerosis 3:170–177

Krasinski SD, Cohn JS, Schaefer EJ, Russell RM (1990) Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. J Clin Invest 85:883–892

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lenzen HJ, Assman G, Buchwalsky R, Schulte HS (1986) Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. Clin Chem 32:778–781

Lipid Research Clinics Program (1982) Manual of operations: lipid and lipoprotein analyses. US Dept of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda

Lohman TG, Roche AF, Martorell R (1988) Anthropometric standardization reference manual. Human Kinetic, Champaign, IL

Mahley RW, Innerarity TL, Rall SC Jr, Weisgraber KH, Taylor JM (1990) Apolipoprotein E: genetic variants provide insights into its structure and function. Curr Opin Lipidol 1:87–95

Mahley RW, Rall SC (1989) Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver C, Beaudet A, Sly W, Valle E (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 1195–1214

Menzel HJ, Kladetzky RG, Assman G (1983) Apolipoprotein E polymorphism and coronary disease. Arteriosclerosis 3:310–315

Miesenbock G, Holzl B, Foger B, Brandstatter E, Paulweber B, Sandhofer F, Patsch JR (1993) Heterozygous lipoprotein deficiency due to missense mutation as the cause of impaired triglyceride tolerance with multiple lipoprotein abnormalities. J Clin Invest 91:448–455

Miesenbock G, Patsch JR (1990) Relationship of triglyceride and high-density lipoprotein metabolism. Atheroscler Rev 21:119–127

Morrison DF (1976) Multivariate statistical methods. McGraw-Hill, New York

National Institute of Health Consensus Development Panel on the Health Implications of Obesity (1985) Health implications of obesity. Ann Intern Med 103:1073–1077

Nestel PJ (1964) Relationship between plasma triglycerides and removal of chylomicrons. J Clin Invest 43:943–949

Neter J, Wasserman W, Kutner MH (1990) Applied linear statistical models. RD Irwin, Homewood, IL

Nikkila EA, Taskinen M-R, Sane T (1987) Plasma high-density lipoprotein concentration and subfraction distribution in relation to triglyceride metabolism. Am Heart J 113:543–548

Patsch JR, Miesenbock G, Hopferwieser T, Muhlberger, Knapp E, Dunn JK, Gotto AM Jr, et al (1992*a*) Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 12:1336–1345

Patsch JR, Prasad S, Gotto AM Jr, Bengtsson-Olivecrona G (1984) Postprandial lipemia: a key for the conversion of high-density lipoprotein$_2$ into high-density lipoprotein$_3$ by hepatic lipase. J Clin Invest 74:2017–2023

Patsch JR, Prasad S, Gotto AM Jr, Patsch W (1987) High density lipoprotein$_2$: relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest 80:341–347

Patsch W, Brown SA, Morrisett JD, Gotto AM Jr, Patsch J (1989) A dual-precipitation method evaluated for measurement of cholesterol in high density lipoprotein subfractions HDL_2 and HDL_3 in human plasma. Clin Chem 35:265–270

Patsch W, Sharrett AR, Sorlie DP, Davis CE, Brown SA, ARIC Investigators (1992*b*) The relation of high density lipoprotein cholesterol and its subfractions to apolipoprotein A-I and fasting triglycerides: the role of environmental factors. Am J Epidemiol 136:546–557

Ryu JE, Howard G, Craven TE, Bond MG, Hagaman AP, Crouse JR (1992) Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. Stroke 23:823–828

Saiki RK, Gelfand DH, Stoffel S, Scharf S, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed amplification of DNA with thermostable DNA polymerase. Science 239:487–491

Schneider WJ, Kovanen PT, Brown MS, Goldstein JL, Utermann G, Weber W, Havel RJ, et al (1981) Familial dysbetalipoproteinemia: abnormal binding of mutatant apoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenal of rats, rabbits and cows. J Clin Invest 68:1075–1085

Schonfeld G, Lees RS, George PK, Pfleger B (1974) Assay of total plasma apolipoprotein B concentration in human subjects. J Clin Invest 53:1458–1467

Snedecor GW, Cochran WG (1980) Statistical methods. Iowa State University Press, Ames

Srinivasan SR, Wattigney W, Webber LS, Berenson GS (1989) Serum apoliprotein E in children and adolescents: The Bogalusa Heart Study. Metabolism 38:1173–1178

Stender S, Zilversmit DB (1982) Comparison of cholesteryl ester transfer from chylomicrons and other plasma lipoproteins to aorta intima-media of cholesterol-fed rabbit. Arteriosclerosis 2:493–499

Superko HR, Haskell WL (1991) The effect of apolipoprotein E isoform difference on postprandial lipoprotein in patients matched for triglycerides, LDL-cholesterol, and HDL-cholesterol. Artery 18:315–325

Tall AR (1986) Plasma lipid transfer proteins. J Lipid Res 27:361–367

——— (1990) Plasma high density lipoproteins: metabolism and relationship to atherogenesis. Atheroscler Rev 21:119–127

Utermann G, Kindermann I, Kaffarnik H, Steinmetz A (1984) Apoliprotein E polymorphism and hyperlipidemia. Hum Genet 65:232–236

Warnick GR, Benderson JM, Albers JJ (1982) Dextran sulfate-Mg^{2+} precipitation procedure for quantification of high-density-lipoprotein cholesterol. Clin Chem 28:1379–1388

Weintraub MS, Eisenberg S, Breslow JL (1987) Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 80:1471–1477

Weisgraber KH, Innerarity TL, Mahley RW (1982) Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cystein-arginine interchange at a single site. J Biol Chem 257:2518–2521

Weisgraber KH, Rall SC, Mahley RW (1981) Human E apoprotein heterogeneity: cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. J Biol Chem 256:9077–9083

Wilson DE, Schreibman PH, Brewster AC, Arky RA (1970) The enhancement of alimentary lipemia by ethanol in man. J Lab Clin Med 75:264–274

Zilversmit DB (1979) Atherogenesis: a postprandial phenomenon. Circulation 60:473–485