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N Bergeron, R J Havel

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Research Article

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Prolonged Postprandial Responses of Lipids and Apolipoproteins in Triglyceride-rich Lipoproteins of Individuals Expressing an Apolipoprotein ε4 Allele

Nathalie Bergeron and Richard J. Havel
Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, California 94143-0130

Abstract

The postprandial responses of apo B48, B100, E and lipids in triglyceride-rich lipoproteins (TRL) to a meal containing one-third of daily energy (39% fat calories) were compared in normolipidemic young men with apo E3/3 and apo E4/3 phenotypes. After the two groups consumed a diet rich in polyunsaturated fat for 15–29 d, their postabsorptive concentrations of TRL triglycerides, apo B48, and apo B100 were virtually identical, but their postprandial responses differed. In both groups, TRL apo B48 increased at 3 h but returned to postabsorptive values at 6 h only in the apo E3/3 group; in the apo E4/3 group the concentration of apo B48 at 6 h was 80% higher than postabsorptive values. TRL apo B100 also increased at 3 h in the two groups and fell to postabsorptive values at 6 h in the apo E3/3 group but remained 51% higher than postabsorptive concentrations in the apo E4/3 group; this response was closely coupled to that of TRL cholesterol and apo E. These observations suggest that clearance of intestinal and hepatogenous TRL remnants is impaired in young men with an apo E4/3 phenotype. (*J. Clin. Invest.* 1996. 97:65–72.) Key words: apo E polymorphism • chylomicrons • very low density lipoproteins • apo B48 • apo B100

Introduction

Apo E, a component of VLDL and HDL, mediates the endocytosis of triglyceride-rich lipoproteins (TRL)[1] by the LDL receptor (1) and the LDL receptor-related protein (LRP) (2). In humans, the gene locus for apo E is polymorphic (3): three common alleles (ε2, ε3, ε4) code for three major isoforms, E2, E3 and E4. Relative to apo E3, apo E2 binds poorly to hepatic lipoprotein receptors (4), leading to accumulation of chylomicron and VLDL remnants in 5% of ε2 homozygotes (5). Apo E4 binds to lipoprotein receptors in vitro with the same affinity as apo E3 (4) but the influence of apo E4 on the clearance of TRL is unclear (6–8).

Based upon measurements of retinyl esters (6–8) and triglycerides (7, 8) in TRL, apo E4 has either been shown to increase (6), delay (7), or have no effect (8) on the rate of clearance of postprandial TRL relative to apo E3. Lack of agreement about the influence of apo E4 on TRL clearance may, in part, be related to the different methods used to quantify TRL. In addition, analysis of the effect of the apo E polymorphism on TRL clearance has been complicated by differences in the postabsorptive concentration of plasma triglycerides among apo E phenotype groups (6–8).

Although it has been suggested that the clearance of VLDL remnants as well as chylomicron remnants is faster in individuals with an apo ε4 allele than in ε3 homozygotes (6, 9), the meta-analysis of Dallongeville and associates (10) in 45 population samples from 17 countries has shown that the concentration of plasma triglycerides is significantly higher in individuals with an apo E4/3 phenotype than in those with an apo E3/3 phenotype. Population studies have also shown that as compared with individuals with phenotype apo E3/3, individuals with phenotypes apo E4/3 and apo E4/4 have higher plasma and LDL cholesterol concentrations (5, 10) and increased susceptibility to coronary heart disease (5, 11–13). In a recent report of 1,950 adults participating in the Framingham offspring study, the increased prevalence of coronary heart disease associated with the ε4 allele persisted even after adjustment for conventional risk factors including LDL and HDL cholesterol (12). These results suggest that factors other than the higher concentrations of plasma and LDL cholesterol contribute to the increased risk of coronary heart disease associated with apo E4. Our current observations of the postprandial responses of apo B48 and apo B100 in TRL suggest that the clearance of intestinal and hepatogenous TRL particles postprandially is slower in young men with an apo E4/3 phenotype than in those with an apo E3/3 phenotype.

Methods

Subjects. Blood samples were obtained from 16 healthy Caucasian men, 25–40 yr of age, eight each with phenotypes apo E3/3 and apo E4/3, who were participating in a study on the influence of dietary fat saturation on postprandial lipemia. All subjects were normolipidemic (plasma cholesterol < ninety-fifth percentile; plasma triglycerides < ninetieth percentile but > tenth percentile; HDL cholesterol > tenth percentile [14]), nonsmokers with a body mass index < seventy-fifth percentile for their age group (15). None of the subjects had a history of cardiovascular disease, diabetes, thyroid, or renal disease. All study procedures were approved by the Committee of Human Research of the University of California at San Francisco, and the subjects gave their written consent to participate.

Protocol. As participants in a strictly controlled diet study, subjects ate all and only the food provided by the kitchen of the General

Address correspondence to Richard J. Havel, Cardiovascular Research Institute, Department of Medicine, University of California, San Francisco, CA 94143-0130.

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1. *Abbreviations used in this paper:* IDL, intermediate density lipoproteins; P/S, polyunsaturated to saturated fat ratio; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid; TRL, triglyceride-rich lipoprotein.

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Clinical Research Center for 29 days. The diet consisted of whole foods prepared daily in weighed individual portions so as to meet each subject's energy requirements, estimated by the Harris-Benedict equation (16) and a detailed physical activity questionnaire. As determined by chemical analysis of food composites prepared for each of the 3 days of the cycle menu, the diet was rich in polyunsaturated fatty acids (PUFA) (polyunsaturated to saturated fat ratio [P/S] = 1.3) and provided, as percent energy, 40% fat (11% saturated, 15% monounsaturated including 2% *trans*-fatty acids, 14% polyunsaturated), 15% protein, 45% carbohydrate, and 200 mg cholesterol/1,000 calories (Hazleton Laboratories America, Madison, WI and, for *trans*-fatty acids, Prince Edward Island Food Technology Center, Charlettetown, Canada).

Postprandial studies were conducted on days 15 and 29. Venous blood was drawn after the subjects had fasted for 12 h (0 h sample collected at 6:00 a.m.) and 3, 6, 9, and 12 h after the consumption of a challenge meal rich in PUFA (P/S = 1.3; day 15). For the second postprandial study conducted on day 29, one-half of the subjects with each apo E phenotype was given the PUFA-rich challenge meal whereas the other one half was given a saturated fatty acid (SFA)-rich meal (P/S = 0.2) to determine whether the fatty acid composition of a single challenge meal affects the postprandial response. The PUFA-rich meal and SFA-rich meal consisted of whole foods and provided one-third of daily energy (39% from fat, 15% from protein, 45% from carbohydrate, and $\sim$ 250 mg cholesterol/1,000 calories). Subjects ate the meal within 15–20 min and were instructed not to consume anything except water until the 12 h blood sample was drawn.

Separation and analysis of plasma lipoproteins. Postabsorptive and postprandial blood samples were collected into tubes containing disodium EDTA (0.05%) and benzamidine (0.03%) (17). The samples were kept on ice until plasma was separated by centrifugation (720 *g*, 4°C for 30 min); a portion was then subjected to ultracentrifugation (18) in a 50.3 rotor at d = 1.006 g/ml (93,000 *g*, 12°C for 18 h) (Beckman Instruments, Inc., Fullerton, CA) to separate TRL. Tubes were then sliced, and the TRL supernatants and infranatants were collected quantitatively. A portion of postabsorptive plasma was also subjected to ultracentrifugation at d = 1.019 g/ml under the same conditions as above to isolate a fraction containing VLDL + intermediate density lipoproteins (IDL).

The concentrations of total and free cholesterol and triglycerides in plasma were determined by enzymatic assays on a Cobas Mira analyzer (Roche Diagnostics, Nutley, NJ) (19, 20). In TRL collected 3, 6, and 9 h after the challenge meal, total cholesterol was measured with an ABA-100 chromatic analyzer (Abbott Laboratories, North Chicago, IL) because lactescence affected the linearity of the Roche-Diagnostics cholesterol assay. Lipids in HDL were measured in the supernatant collected after precipitation of apo B–containing lipoproteins by dextran sulfate and magnesium chloride (21). All lipid analyses were in duplicate and were calibrated against a pooled serum sample. Lipids in IDL were calculated as the difference between lipids in d = 1.019 g/ml supernatant and lipids in TRL. Lipid concentrations in LDL were calculated as the difference between lipids in the TRL infranatant and HDL + IDL.

Apo B100, apo B48 and apo E in TRL were quantified by densitometric scanning of apolipoprotein bands separated by electrophoresis in 3–10% SDS polyacrylamide slab gels and stained with Coomassie blue, as recently described (22). The concentration of apo E in plasma was measured by RIA (23). To determine apo E phenotypes, delipidated TRL were subjected to isoelectric focusing electrophoresis (24).

Statistical analysis. Data from one subject with an apo E4/3 phenotype, whose fasting hypertriglyceridemic state throughout the study was not detected upon screening, were excluded from the analysis. Differences in the postabsorptive concentration of plasma lipids and lipoproteins among the apo E3/3 (n = 8) and apo E4/3 (n = 7) groups were analyzed by the nonparametric Mann-Whitney U-test. Two-way ANOVA for repeated measures was used to test for the overall significance of postprandial measurements over time and between apo E phenotype groups. When the overall F statistic was significant ($P < 0.05$), comparisons within a given group were tested by the nonparametric Wilcoxon matched pairs test. Data in text, tables, and figures are expressed as mean±SD.

Results

Subject characteristics at baseline. The average age, body mass index, and maintenance energy requirements were similar among individuals with an apo E3/3 and E4/3 phenotype (Table I). Based upon a 24-h dietary recall, both groups reported consuming similar habitual diets providing $\sim$ 30–32% of energy from fat (11–13% saturated, 11–12% monounsaturated, and 8% polyunsaturated fatty acids) and 105–120 mg cholesterol/1,000 kcal.

Postabsorptive lipoprotein lipids at baseline and after consuming a diet rich in PUFA. Before starting the PUFA-rich diet, subjects with phenotype apo E4/3 had significantly higher postabsorptive concentrations of plasma and TRL triglycerides than those with phenotype apo E3/3. Feeding a diet rich in PUFA to individuals with an apo E4/3 phenotype significantly reduced the postabsorptive concentrations of triglycerides in plasma and TRL by 34 and 45%, respectively, on days 15 and 29 (Table II). The concentration of IDL triglycerides in the apo E4/3 group was reduced by 66% on day 29. The PUFA-rich diet also significantly reduced plasma triglycerides in the apo E3/3 group (−21%). The magnitude of the PUFA-induced reduction in triglycerides was significantly greater in subjects with phenotype apo E4/3 than in those with phenotype apo E3/3 (P = 0.02) so that on days 15 and 29, plasma and lipoprotein triglycerides were remarkably similar in the two groups.

Table I. Anthropometric Characteristics and Postabsorptive Plasma Lipid and Lipoprotein Concentrations of Subjects on Day 1

Characteristics	E3/3 phenotype (n = 8)	E4/3 phenotype (n = 7)
Age (yr)	30.8±4.20	32.0±3.60
BMI (kg/m^2)	24.1±1.00	23.4±1.80
Weight (kg)	74.9±4.8	76.6±7.3
Maintenance energy Requirements (kcal)	3114±217	3170±217
Cholesterol (mg/dl)		
plasma	176.4±20.1	171.9±12.2
VLDL	9.10±3.72	10.7±3.48
IDL	3.45±4.28	3.26±1.90
LDL	114.8±28.2	106.9±8.12
HDL	45.3±11.8	47.1±6.90
Triglycerides (mgdl)		
plasma	84.9±19.1	108.0±17.6*
VLDL	46.5±12.8	70.4±14.2*
IDL	5.78±6.16	7.03±3.09
LDL	15.9±8.82	12.4±8.79
HDL	12.2±1.65	11.2±2.70

Values are mean±SD. *Significantly different from the apo E3/3 group by the Mann-Whitney U-test ($P < 0.05$). BMI, body mass index.

Table II. Postabsorptive Plasma Lipid and Lipoprotein Concentrations (mg/dl) in Subjects Fed a PUFA-rich Diet

	Plasma		TRL		IDL		LDL		HDL	
Apo E phenotype	cholesterol	triglycerides	cholesterol	triglycerides	cholesterol	triglycerides	cholesterol	triglycerides	cholesterol	triglycerides
E3/3 (n = 8)										
Day 15	163.8±18.6	66.5 ± 15.0*	6.89 ± 10.8*	37.2 ± 12.4	3.51 ± 1.94	3.09 ± 3.16	106.8 ± 21.8	13.2 ± 4.30	44.99 ± 8.15	11.56 ± 2.81
Day 29	164.9 ± 15.3*	66.3 ± 19.7*	7.20 ± 3.90	35.3 ± 15.9	3.86 ± 1.99	3.36 ± 2.59	106.4 ± 24.8	12.7 ± 5.30	45.91 ± 9.79	12.48 ± 3.18
E4/3 (n = 7)										
Day 15	168.0 ± 20.7	71.4 ± 13.2*	7.44 ± 2.96*	39.5 ± 10.4*	2.82 ± 1.45	3.68 ± 1.89	107.3 ± 14.8	14.5 ± 6.77	48.57 ± 8.61	12.69 ± 3.99
Day 29	162.3 ± 24.2	71.3 ± 10.8*	6.22 ± 1.43*	37.9 ± 8.27*	3.98 ± 1.55	2.34 ± 2.01*	99.2 ± 16.1	17.0 ± 5.74	48.99 ± 9.21	11.22 ± 3.04

Vales are mean±SD. *Significantly different from day 1 by the Wilcoxon matched pairs test (P<0.05).

The postabsorptive concentrations of plasma and lipoprotein cholesterol in subjects with apo E3/3 and E4/3 phenotypes were similar on day 1 (Table I). The PUFA-rich diet reduced plasma cholesterol significantly only in the apo E3/3 group (−7%). On days 15 and 29 of the PUFA-rich diet, the postabsorptive concentrations of plasma lipoprotein lipids were closely similar in the two groups (Table II). In contrast to an earlier report (25), the ratio of free to total cholesterol in HDL (apo E3/3: 0.20; apo E4/3: 0.19) as well as in other lipoprotein fractions was similar in the two apo E phenotype groups.

Effect of apo E phenotype on the postprandial lipid and apolipoprotein responses in TRL. The average postabsorptive concentrations of TRL apo B48 in individuals with an apo E3/3 and E4/3 phenotype were almost identical (0.15 and 0.16 mg/dl, respectively) (Fig. 1). In contrast, the postprandial changes in apo B48 differed significantly. In those with phenotype apo E3/3, TRL apo B48 increased more than three-fold 3 h after the meal and returned to near postabsorptive values at 6 h. In individuals with an ϵ4 allele, the concentration of apo B48 also increased more than three-fold at 3 h, but remained significantly higher than postabsorptive values 6 h after the meal.

The average concentration of TRL apo B100 in the postabsorptive state was also almost identical in individuals with an apo E3/3 and E4/3 phenotype (3.19 and 3.14 mg/dl, respectively). Again, the postprandial response differed significantly in the two groups (Fig. 1). In the apo E3/3 group, the concentration of TRL apo B100 increased 1.5-fold at 3 h and fell to near baseline values 6 h after the meal. In contrast, whereas TRL apo B100 also increased 1.6-fold at 3 h in the apo E4/3 group, it failed to return to postabsorptive concentrations at 6 h, reflecting the accumulation of hepatogenous TRL.

The postabsorptive concentration of triglycerides in TRL was, on average, remarkably similar in individuals with apo E3/3 and E4/3 phenotypes (37.2 and 39.5 mg/dl). The increment in TRL triglycerides that follows the consumption of such a meal has been shown to be predominantly, but not exclusively, in apo B48-particles (26). In the current study the postprandial pattern of TRL-triglycerides was closely coupled to that of apo B48 (Fig. 1). TRL triglyceride concentration increased three-fold 3 h after the meal in both groups. After 6 h, TRL triglyceride concentrations fell to 10 and 25 mg/dl above postabsorptive values in the apo E3/3 and E4/3 groups, respectively (group difference not significant).

The postabsorptive concentration of TRL cholesterol was also similar in the apo E3/3 and E4/3 groups (6.79 and 7.44 mg/dl, respectively). The concentration of TRL apo E was slightly, but not significantly, higher in individuals with phenotype apo E4/3 than in those with phenotype apo E3/3 (0.68 and 0.55 mg/

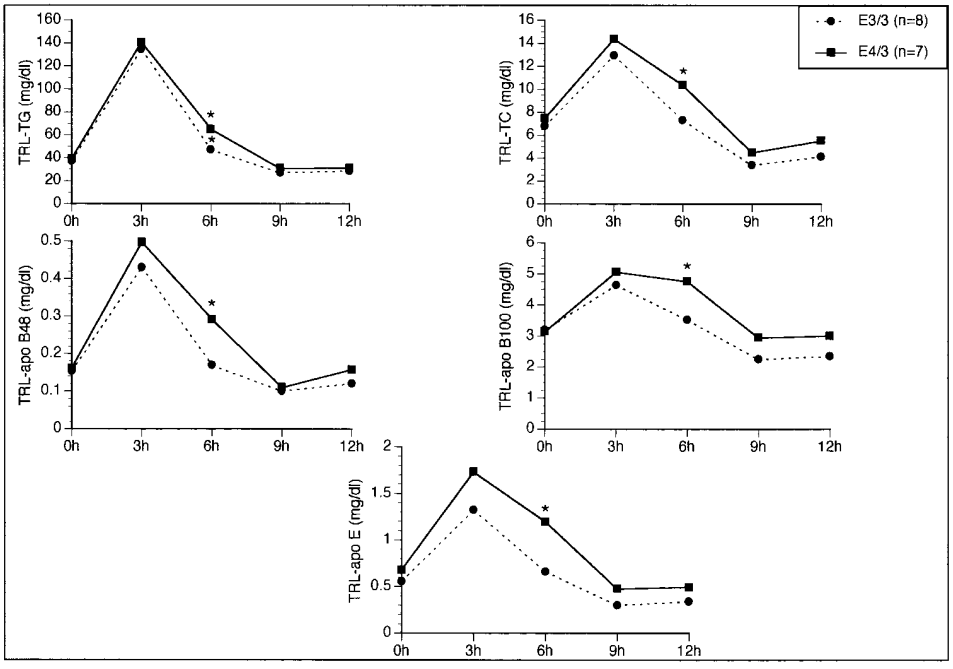

Figure 1. Mean postprandial changes in TRL triglycerides (*TG*), apo B48, cholesterol (*TC*), apo B100, and apo E in individuals with apo E3/3 and apo E4/3 phenotypes fed a PUFA-rich diet and challenge meal (day 15). All components of TRL at 3 h were significantly higher than 0 h values ($P < 0.01$). * Significantly different from 0 h values by the Wilcoxon matched pairs test ($P < 0.05$).

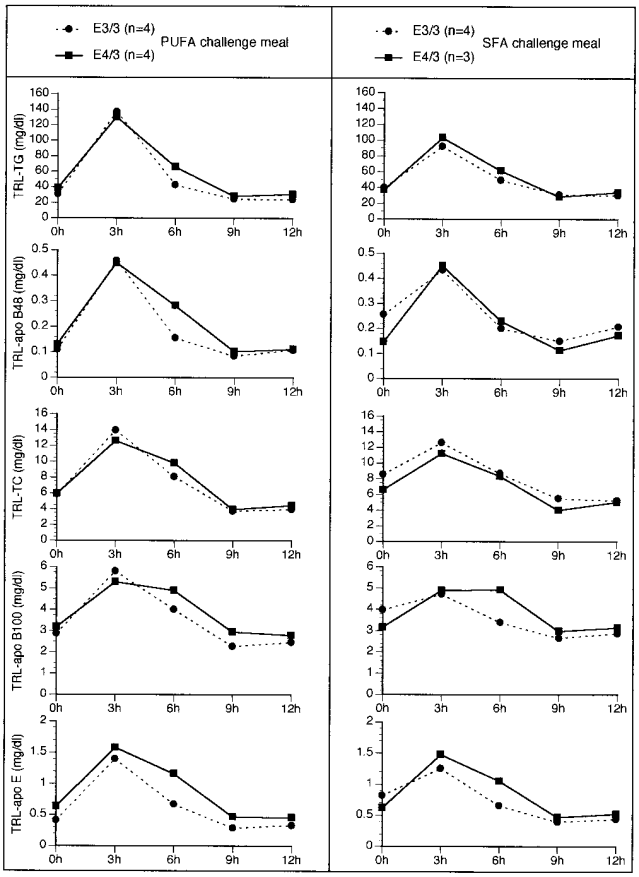

Figure 2. Mean postprandial changes in TRL triglycerides (*TG*), apo B48, cholesterol (*TC*), apo B100, and apo E in individuals with apo E3/3 and apo E4/3 phenotypes fed a PUFA-rich diet and given a PUFA-rich or SFA-rich challenge meal (day 29).

dl, respectively; $P = 0.25$). Whereas postprandial triglycerides associate predominantly with apo B48 particles, cholesterol and apo E have been shown to associate predominantly with apo B100 particles postprandially (26). In our study, we found the postprandial responses of TRL cholesterol and apo E to be closely coupled to that of apo B100, increasing 2–2.5-fold at 3 h but returning to baseline at 6 h only in the apo E3/3 group. In contrast, in the apo E4/3 group TRL cholesterol and apo E concentrations were still elevated at 6 h postprandially and, as for all other components of TRL, fell below postabsorptive concentrations 9 h after the meal.

The distinct postprandial responses of individuals with an apo E3/3 and E4/3 phenotype were confirmed by the second challenge meals given on day 29 when one-half of each group was given the PUFA-rich or the SFA-rich challenge meal (Fig. 2). In subjects given the PUFA-rich meal (*left*), the postprandial responses of all components of TRL duplicated the responses observed on day 15. In subjects given the SFA-rich meal (*right*), the postabsorptive concentrations of all components of TRL except triglycerides were slightly, but not significantly, higher in the apo E3/3 group. The postprandial response to the SFA-rich meal mimicked that of the PUFA-rich meal, in that all components of TRL fell to near baseline at 6 h in individuals with phenotype apo E3/3 but not in those with phenotype apo E4/3. Although the 3-h increment in TRL components tended to be higher after the PUFA-rich meal than after the SFA-rich meal, this did not affect the pattern of return to postabsorptive values in either apo E phenotype group. Consequently, the SFA- and PUFA-meal data for a given apo E phenotype were pooled and analyzed together to increase statistical power. Again, we found that the concentrations of TRL apo B48, apo B100, triglycerides, cholesterol and apo E at 6 h were all significantly higher than postabsorptive values only in individuals with an apo E4/3 phenotype ($P \leq 0.02$).

Effect of apo E phenotype on the distribution of apo E in plasma and TRL. In agreement with previous observations (9), the concentration of apo E in postabsorptive plasma tended to be lower in individuals with an apo E4/3 phenotype than in those with an apo E3/3 phenotype ($P = 0.20$) (Table III). Plasma apo E did not change postprandially in either group. Because the postabsorptive concentration of apo E was lower in plasma but higher in TRL of individuals with an apo E4/3 phenotype, the percentage of apo E in postabsorptive TRL tended to be higher in these individuals than in those with an apo E3/3 phenotype. 6, 9, and 12 h after the meal, the percentage of apo E in TRL was significantly higher in the apo E4/3 group than in the apo E3/3 group.

Discussion

Population studies have shown that the apo E polymorphism has a substantial effect on plasma lipids and lipoproteins (5, 10). In our group of normolipidemic young men, the postabsorptive concentrations of LDL cholesterol on day 1 were sim-

Table III. Total Apo E in Plasma and Percent Apo E in TRL in the Postabsorptive and Postprandial States in Individuals with an Apo E3/3 and Apo E4/3 Phenotype

	Apo E3/3 group ($n = 8$)		Apo E4/3 group ($n = 7$)	
Hours after the meal	Plasma apo E	TRL apo E	Plasma apo E	TRL apo E
	mg/dl	*% of plasma apo E*	*mg/dl*	*% of plasma apo E*
0	4.33±0.88	13.0±3.93	3.90±1.04	17.6±6.87
3	4.25±0.78	30.6±10.1	4.43±1.44	38.2±8.44
6	4.08±0.82	16.5±7.87	4.01±1.26	29.7±10.4*
9	4.14±0.72	7.30±2.87	3.81±0.94	13.1±2.99‡
12	4.43±0.75	7.72±3.16	4.33±1.17	11.5±2.77*

Values are mean±SD (day 15). *Significantly different from the apo E3/3 group by the Mann-Whitney U-test ($P < 0.03$); ‡Significantly different from the apo E3/3 group by the Mann-Whitney U-test ($P = 0.004$).

ilar in both apo E phenotype groups, but plasma and TRL triglycerides were, respectively, 27 and 51% higher in men with an apo E4/3 phenotype than in those with an E3/3 phenotype. These findings are consistent with the meta-analysis of Dallongeville and associates (10), who found significantly higher plasma triglyceride concentrations in individuals with an apo E4/3 phenotype than in those with an apo E3/3 phenotype, but could also reflect differences in habitual dietary habits, not detected in the 24-h recall. It has been suggested that the magnitude of the population effect of the ϵ4 allele on LDL-cholesterol concentrations is directly related to dietary cholesterol and fat consumption (27, 28). In the populations surveyed by Dallongeville et al. (10), such a relationship for plasma triglycerides is unclear, although the effect appears to be small in Japan (29–31). By contrast, among obese individuals (who presumably consume more fat) having an ϵ4 allele has been shown to increase the risk of hypertriglyceridemia (32).

In agreement with previous data (33, 34), feeding a diet rich in PUFA for 2 wk reduced the postabsorptive concentrations of plasma and TRL triglycerides; this effect was more prominent in the apo E4/3 group, but also occurred in the apo E3/3 group so that on days 15 and 29 the postabsorptive concentrations of plasma and TRL triglycerides, as well as TRL apo B48, apo B100, cholesterol, and apo E, were virtually identical in the two groups. That we found a more pronounced PUFA-induced lowering of triglycerides in individuals with an apoE4/3 phenotype than in those with phenotype E3/3 is of interest as others have shown that lowering the amount of total fat (from 39 to 26% of energy), saturated fat (from 15 to 8% of energy), and cholesterol (from 435 to 200 mg/d) in the diet reduces LDL cholesterol more in individuals with an apo E4/3 phenotype than in those with an apo E3/3 phenotype (35).

Although the postabsorptive concentration of apo B48 was similar in the apo E3/3 and E4/3 groups, the postprandial response of this structural component of chylomicrons was prolonged in individuals with an apo E4/3 phenotype. The postabsorptive concentration of TRL apo B48 increased and peaked 3 h after the meal in both groups but returned to postabsorptive values at 6 h only in individuals with an apo E3/3 phenotype. In contrast, in individuals with an apo E4/3 phenotype, the concentrations of TRL apo B48 and TRL triglycerides at 6 h were, respectively, 80 and 65% higher than postabsorptive values. The prolonged accumulation of apo B48 in the apo E4/3 group was highly reproducible and did not depend on the fatty acid composition of the challenge meal. Because the rate of return of TRL apo B48 to postabsorptive concentrations is mainly influenced by the clearance of apo B48 particles by receptor-mediated processes (36), the current findings suggest that the clearance of chylomicron remnants is delayed in individuals with an apo E4/3 phenotype consuming a PUFA-rich diet.

In young men given 0.73 g fat/kg body wt (P/S = 0.4), Brown and Roberts (7) also reported delayed peaks and significantly greater 8-h postprandial response curves for plasma triglycerides and retinyl esters in $S_f > 1{,}000$ "chylomicrons," and a tendency towards a later peak in retinyl esters in $S_f < 1{,}000$ "remnant" particles in 5 individuals with apo E4/3 and E4/4 phenotypes than in 14 with an apo E3/3 phenotype. After adjusting for higher postabsorptive plasma triglycerides in the apo E4 group by expressing data as a percent change from postabsorptive values, the investigators found that the triglyceride response was reversed and lowest in individuals with apo E4/3 and E4/4 phenotypes. The validity of such an adjustment, which is based upon the assumption that the relationship between the postabsorptive and postprandial response in plasma triglycerides is linear (37–39), is uncertain because adjustments were also made to account for differences in triglyceride concentrations produced by olive and fish oil supplementation of their diets. Whether the increased postprandial response and delayed peak in retinyl esters in $S_f > 1{,}000$ chylomicrons or $S_f < 1{,}000$ remnant particles was related only to the higher postabsorptive plasma triglycerides in their apo E4 group is also unclear as the linearity of the relationship between these variables is not well established (6, 40).

Our findings for TRL apo B48 differ markedly from those of Weintraub and associates (6) who gave a meal containing 50 g fat/m^2 body surface and reported a significantly lower 12-h area under the retinyl ester curve in $S_f < 1{,}000$ remnants in subjects with apo E4/3 and E4/4 phenotypes than in those with an apo E3/3 phenotype. They concluded that apo E4 accelerates the clearance of chylomicron remnants. The basis for this discrepancy with our findings is uncertain but may, in part, be related to differences in the methods used to quantify chylomicron particles. Whereas apo B48, as measured in our study, is a structural component specific to chylomicrons, retinyl esters, as measured in the study of Weintraub et al., associate with lipoproteins other than chylomicrons particularly during later stages of postprandial lipemia (41, 42). In addition, in the study of Weintraub et al. (6), differences in the postabsorptive concentrations of plasma triglycerides (18% lower in individuals with phenotypes apo E4/3 and E4/4 than in those with phenotype apo E3/3) may also have influenced the results. Had triglyceride concentrations been taken into account, the difference between the apo E4/3, E4/4 group and the apo E3/3 group in postprandial retinyl palmitate responses in $S_f < 1{,}000$ remnant particles may have been smaller. In the current study the postabsorptive concentrations of plasma triglycerides and all TRL components were remarkably similar, and only the postprandial responses differed among apo E phenotype groups.

Our findings also differ from those of Boerwinkle and associates (8) who found no difference in postprandial responses in older groups of apo E4/3 and apo E3/3 Caucasian men and women on uncontrolled diets. They evaluated the postprandial response by measuring the concentrations of plasma triglycerides and retinyl palmitate, as well as TRL triglycerides and TRL apo B48/apo B100 dye absorbance ratios 0, 4, and 8 h after a meal containing 105 g fat (P/S = 0.4). The magnitude and duration of the postprandial plasma triglyceride response is a function of the amount of fat consumed (43, 44). Lack of agreement with our findings could therefore be related, in part, to the large fat challenge used by Boerwinkle and associates. Perhaps more critically, their use of the dye absorbance ratio of apo B48/apo B100 to estimate remnant concentrations may be inappropriate. For example, we found that the percent increments in TRL apo B48 and apo B100 were similar 6 h after the meal, so that the apo B48/apo B100 ratio did not differ significantly from the postabsorptive value in either of our apo E phenotype groups (postabsorptive ratio 0.049 (apo E3/3), 0.048 (apo E4/3); 6h ratio 0.048 (apo E3/3), 0.061 (apo E4/3).

In addition to affecting the concentration of chylomicron remnant particles postprandially, the presence of an ϵ4 allele was also associated with a prolonged postprandial response of apo B100, the structural component of VLDL, as well as cho-

lesterol and apo E in TRL. Although the postabsorptive concentrations and the 3-h postprandial responses of apo B100, cholesterol, and apo E were similar in both groups, these components of TRL returned to postabsorptive values 6 h after the meal only in the apo E3/3 group. In contrast, in the group with an apo E4/3 phenotype the concentrations of apo B100, cholesterol, and apo E at 6 h were respectively 51, 39, and 77% higher than postabsorptive values, evidently reflecting a continued accumulation of hepatogenous TRL. This prolonged postprandial response was consistent and occurred whether subjects with an apo E4/3 phenotype were given a PUFA-rich or SFA-rich challenge meal. To our knowledge, the postprandial response of apo B100–containing TRL in individuals with an ϵ4 allele has not been reported previously. It is noteworthy, however, that "double prebetalipoproteinemia," which is characterized by the accumulation of remnant-like VLDL in postabsorptive plasma (24), has been found to be significantly more prevalent in normolipidemic Finns and hyperlipidemic Italians with apo E4/4 and E4/3 phenotypes than in those with an apo E3/3 phenotype (45). The combined data from the two populations showed that double prebetalipoproteinemia occurred in 34 out of 56 individuals (61%) with phenotypes E4/3 and E4/4, but only in 31 out of 117 individuals (27%) with phenotype E3/3.

It is commonly believed that the increased concentration of LDL cholesterol found in most populations of the world among those expressing an ϵ4 allele (5, 28) results from an increased rate of clearance of chylomicron and VLDL remnants and consequent down-regulation of the hepatic LDL receptor (5, 6, 9). Our data and those of Pagnan et al. (45) suggest that this view may need to be reevaluated. As reported by others (9, 46) and confirmed here, the concentration of apo E in blood plasma tends to be lower in subjects with an ϵ4 allele than in ϵ3 homozygotes, and a larger fraction of apo E is found in VLDL and less in HDL from subjects with an ϵ4 allele. Furthermore, in subjects with an apo E4/3 phenotype, VLDL and HDL isolated by ultracentrifugation or gel permeation chromatography are enriched in apo E4 and apo E3, respectively (46). Weisgraber (47) has shown that the preferential association of apo E4 with VLDL is a function of the positive charge associated with arginine at amino acid residue 112 of apo E, and also of dimerization via disulfide bridging of apo E3, but not apo E4 with apo AII in HDL (48). Gregg et al. (9) have shown that radioiodinated apo E4 associates preferentially with VLDL and is removed from the blood more rapidly than apo E3. Among lipoprotein fractions, VLDL are depleted more rapidly of the labeled apo E than are other fractions , including HDL. Added apo E exchanges readily among lipoprotein fractions (49), so that removal of apo E from the plasma compartment will be influenced by the steady state fraction of apo E associated with lipoproteins of differing residence times. Preferential association with VLDL would therefore be expected to lead to a shorter residence time in the plasma. The shorter residence time of apo E4 in plasma cannot, therefore, be taken as evidence for more rapid clearance of VLDL remnants in persons with phenotypes apo E4/3 or E4/4.

More rapid clearance of chylomicron remnants in persons with one or more ϵ4 alleles has been postulated to lead to removal of remnants from the blood at an earlier stage of delipidation by lipoprotein lipase (8) so that the liver would be exposed to a larger fraction of dietary fat, leading to down-regulation of LDL receptors. In discoidal complexes with phosphatidylcholine, apo E4 and apo E3 have indistinguishable affinity for the LDL receptor (4), but the affinity of spherical TRL bearing these forms of apo E is unknown. What is known is that the binding affinity of VLDL particles containing apo E for the LDL receptor is highly dependent upon the conformation of apo E (50–53). Thus, VLDL from normal individuals bind poorly to LDL receptors (54), whereas large VLDL from hypertriglyceridemic individuals tend to bind with high affinity (50). High affinity has been associated with susceptibility of a specific site in apo E to thrombin (51, 52). Multiple molecules of apo E on VLDL particles do not necessarily confer high affinity for LDL receptors (55). Among our subjects, the average concentration of apo E in TRL was actually higher in those with phenotype apo E4/3 than in those with phenotype apo E3/3 both postabsorptively and postprandially. Because apo E dissociates from lipoproteins during ultracentrifugation (23), the actual concentrations in TRL in vivo may be somewhat higher, but our data indicate no deficiency in apo E in TRL of our subjects with an ϵ4 allele. We speculate that the apo E on chylomicron remnants of persons with an apo E4/3 phenotype may be less accessible to hepatic lipoprotein receptors than apo E from persons with an apoE3/3 phenotype. The same may apply to VLDL remnants which also appear to accumulate in persons with an apo E4/3 phenotype (45). Studies in homozygous Watanabe heritable hyperlipidemic rabbits have shown that reduced hepatic uptake of VLDL remnants is associated with increased conversion of the remnants to LDL, accounting for a substantial fraction of the increased concentration of LDL in these animals expressing an abnormal LDL receptor (56). We thus suggest that a prolonged residence time of VLDL remnants in persons carrying an ϵ4 allele raises LDL concentration by increasing the fraction of VLDL converted to LDL.

In summary, we have found that the postprandial increases in TRL apo B48 and apo B100 are prolonged in persons with an apo E4/3 phenotype as compared with those with the common apo E3/3 phenotype. This prolonged response, which is independent of the fatty acid composition of a challenge meal, may contribute to the tendency of individuals with an apo E4/3 phenotype to have increased concentrations of VLDL-triglycerides as well as an increased concentration of LDL-cholesterol. All of the lipoproteins that appear to accumulate to a greater extent in persons with an apo E4/3 phenotype (chylomicron remnants, VLDL remnants, and LDL) are thought to be atherogenic (36) and may account for the increased risk of atherosclerotic vascular disease in the $\sim$ 25% of individuals who carry one or more ϵ4 alleles. Our observations need to be confirmed in larger and more diverse population samples. Investigations are also needed to determine the influence of the quality and quantity of dietary fat upon the postabsorptive and postprandial concentrations of lipoproteins among those with phenotypes apo E3/3, 4/3, and 4/4.

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