Effect of the apolipoprotein A-IV Q360H polymorphism on postprandial plasma triglyceride clearance

Karen J. Hockey,* Rachel A. Anderson,* Victoria R. Cook,* Roy R. Hantgan,† and Richard B. Weinberg[1],*

Section of Gastroenterology,* Department of Internal Medicine and Department of Biochemistry,† Wake Forest University School of Medicine, Winston-Salem, NC 27157

Abstract **Apolipoprotein (apo)A-IV is synthesized in the small intestine during fat absorption and is incorporated onto the surface of nascent chylomicrons. In circulation, apoA-IV is displaced from the chylomicron surface by high density lipoprotein-associated C and E apolipoproteins; this exchange is critical for activation of lipoprotein lipase and chylomicron remnant clearance. The variant allele A-IV-2 encodes a Q360H polymorphism that increases the lipid affinity of the apoA-IV-2 isoprotein. We hypothesized that this would impede the transfer of C and E apolipoproteins to chylomicrons, and thereby delay the clearance of postprandial triglyceride-rich lipoproteins. We therefore measured triglycerides in plasma, $S_f > 400$ chylomicrons, and very low density lipoproteins (VLDL) in 14 subjects heterozygous for the A-IV-2 allele (1/2) and 14 subjects homozygous for the common allele (1/1) who were fed a standard meal containing 50 gm fat per m^2 body surface area. All subjects had the apoE-3/3 genotype. Postprandial triglyceride concentrations in the 1/2 subjects were significantly higher between 2–5 h in plasma, chylomicrons, and VLDL, and peaked at 3 h versus 2 h for the 1/1 subjects. The area under the triglyceride time curves was greater in the 1/2 subjects (plasma, $P = 0.045$; chylomicrons, $P = 0.027$; VLDL, $P = 0.063$). A post-hoc analysis of the frequency of the apoA-IV T347S polymorphism suggested that it had an effect on triglyceride clearance antagonistic to that of the A-IV-2 allele. We conclude that individuals heterozygous for the A-IV-2 allele display delayed postprandial clearance of triglyceride-rich lipoproteins.**—Hockey, K. J., R. A. Anderson, V. R. Cook, R. R. Hantgan, and R. B. Weinberg. **Effect of the apolipoprotein A-IV Q360H polymorphism on postprandial plasma triglyceride clearance.** ***J. Lipid Res.*** **2001.** 42: **211–217.**

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The intravascular transport of dietary lipids is one of the central functions of lipoprotein metabolism (1, 2). Dietary triglycerides are hydrolyzed in the lumen of the small intestine, absorbed by the intestinal enterocytes, re-esterified, and secreted into the mesenteric lymph as chylomicrons. Following their entry into circulation, chylomicrons acquire apolipoprotein (apo)C-II, the cofactor for lipoprotein lipase, and apoE, a ligand for hepatic chylomicron remnant uptake, which are transferred to their surface from high density lipoproteins (HDL). Chylomicron triglycerides are then hydrolyzed by lipoprotein lipase in the vascular beds of the periphery. As hydrolysis proceeds, the chylomicrons shrink in size and become enriched with cholesterol esters. Finally, chylomicron remnants are removed from circulation by the hepatic chylomicron remnant receptor (3). Multiple factors have been found to affect chylomicron metabolism including age (4, 5), adiposity (6, 7), dietary fat intake (8, 9), baseline lipid levels (10), and genetic polymorphisms of lipolytic enzymes (11–13), apoB (14–16), and apoE (10, 17–21).

ApoA-IV is 46 kd plasma glycoprotein (22), which is synthesized by intestinal enterocytes of mammalian species (23) during lipid absorption (24). ApoA-IV enters circulation on the surface of nascent chylomicrons (25, 26) and, thereafter, circulates primarily as a lipid-free protein (27). Although a broad spectrum of physiologic functions has been proposed for apoA-IV, a preponderance of evidence suggests that its primary biological role is in intestinal lipid metabolism (28). A specific function of apoA-IV in chylomicron metabolism was first suggested by the observations that apoA-IV is displaced from the surface of native (25) and model (29) chylomicrons by HDL-associated C apolipoproteins. Goldberg et al. (30) subsequently observed, using a gum-stabilized triglyceride emulsion, that this exchange facilitated the activation of lipoprotein lipase by its co-factor, apoC-II.

Of the known genetic polymorphisms of human apoA-IV (31), the best studied is the variant allele, A-IV-2, which occurs with a frequency of 7–9% in the United States and Western Europe (32). This allele encodes a Q360H substi-

Abbreviations: apo, apolipoprotein; AUC, area under the curve; BMI, body mass index; EDTA, ethylenediaminetetraacetic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

[1] To whom correspondence should be addressed.
e-mail: weinberg@wfubmc.edu

tution (33) that significantly increases the lipid affinity (34), lecithin:cholesterol acyltransferase activation (34), and plasma residence time (35) of the apoA-IV-2 isoprotein. Recently, several studies have observed that the A-IV-2 allele affects the plasma lipoprotein response to dietary fat and cholesterol (36–39), and may also modulate the efficiency of intestinal cholesterol absorption (40).

In this study, we investigated the effect of the apoA-IV Q360H polymorphism on postprandial triglyceride clearance. We hypothesized that the increased lipid affinity of the A-IV-2 isoprotein would impede the transfer of apoC-II and apoE to chylomicrons, and thereby delay lipolysis and/or hepatic clearance of triglyceride-rich lipoproteins. We therefore compared postprandial triglyceride concentrations in plasma, $S_f > 400$ chylomicrons, and very low density lipoproteins (VLDL) between a group of subjects heterozygous for the A-IV-2 allele and a control group of subjects homozygous for the A-IV-1 allele after ingestion of a standard fat load. To control for the effect of apoE polymorphism on chylomicron metabolism (10, 17–21), all subjects selected for this study had the apoE-3/3 genotype.

METHODS

Subjects

Subjects homozygous for the common A-IV-1 allele (1/1) and heterozygous for the A-IV-2 allele (1/2) were recruited from a cohort of subjects whose apoA-IV (41) and apoE (42) genotypes were determined by polymerase chain reaction DNA restriction fragment length polymorphism analysis of buffy coat DNA. Inclusion criteria were *1*) homozygosity for the apoE-3 allele (E-3/3); *2*) total plasma cholesterol <200 mg/dl and plasma triglycerides <150 mg/dl; *3*) body mass index (BMI) within 80–120% of ideal for sex and age; *4*) no use of medications that could affect lipoprotein metabolism, with the exception of oral contraceptives; and *5*) no use of tobacco products. All subjects signed a statement of informed consent approved by the Wake Forest University School of Medicine Institutional Review Board. At the conclusion of the study, a post hoc analysis for the apoA-IV T347S polymorphism (41) was performed on buffy coat DNA that had been stored at −70°C.

Study protocol

The study was conducted in the General Clinical Research Center of Wake Forest University School of Medicine. Subjects abstained from alcohol for 24 h before the study and took nothing by mouth for 12 h before the study. The subjects' weight and height were determined, a baseline blood sample was drawn into acid-citrate-dextrose-containing tubes (Becton Dickinson, Rutherford, NJ), and an indwelling venous catheter was placed. Subjects then ingested a standard high fat meal within a 10-min period, provided as a milkshake made from heavy cream, chocolate or vanilla flavoring, and sugar. The milkshake volume for each subject was calculated to provide 50 g of fat per m^2 body surface area. Venous blood samples were then obtained hourly for eight h. During this period, the subjects were permitted to drink only water, black coffee, or black tea ad libitum.

Plasma lipids and lipoproteins

Plasma was separated by low speed centrifugation and assayed within 24 h of collection for triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol, and HDL cholesterol in the CDC standardized Lipid Laboratory at the Wake Forest University School of Medicine. Plasma for isolation of triglyceride-rich lipoproteins was stored under nitrogen at 4°C in the dark.

Isolation of triglyceride-rich lipoproteins

Three-milliliter aliquots of plasma were placed into 4.5-ml Seton poly-bottles and carefully layered with 1 ml of 0.9% NaCl, 0.05% ethylenediaminetetraacetic acid (EDTA). The tubes were spun in a Beckman L8-70M ultracentrifuge (Beckman Coulter, Fullerton, CA) in a 50.3Ti rotor at 40,000 rpm for 1 h at 10°C. The top 1-ml layer from each tube, containing $S_f > 400$ chylomicrons, was carefully removed and analyzed for triglyceride content and particle size. The remaining plasma was again carefully layered with 1 ml of 0.9% NaCl, 0.05% EDTA, and spun in a 50.3Ti rotor at 45,000 rpm for 16 h at 4°C. The top 1-ml layer from each tube, containing $S_f = 20$ to 400 chylomicron remnants and VLDL, was carefully removed and analyzed for triglyceride content.

Analysis of chylomicron particle size

The particle size distribution in the chylomicron fractions was determined by dynamic laser light scattering using a BI-2030 AT correlator, a BI-200 SM light scattering goniometer/photon counting detector (Brookhaven Instruments, Holtsville, NY), and a Spectra Physics 127 He-NE laser (Mountain View, CA) (43). Samples were diluted 1:10 or 1:20 in phosphate-buffered saline buffer and filtered into plastic cuvettes through a 0.45-μm filter immediately before analysis. For each subject, the particle size distribution was determined at the time point with the highest triglyceride concentration and at 4 h.

Data analysis

All data are presented as means ± SE. For each subject, the area under the curve (AUC) for plasma, chylomicron, and VLDL triglyceride time curves was calculated by the trapezoidal rule. The significance of differences in mean baseline parameters and mean AUC between genotype groups was determined by two-tailed unpaired Student's *t*-tests. The significance of changes in mean plasma and lipoprotein triglycerides over time within each genotype group and between genotype groups at each time point was determined by two-way repeated measures analysis of variance (ANOVA) with Tukey post hoc testing. The interaction between gender and the apoA-IV Q360H and T347S polymorphisms on AUCs was examined by two-way ANOVA with Tukey post hoc testing. The relationship between mean baseline parameters and postprandial responses was examined by Pearson product moment analysis.

RESULTS

There were no significant differences in baseline plasma lipids, age, or BMI between the two genotype groups (**Table 1**). Plasma total cholesterol, LDL, and HDL levels did not change significantly over the 8-h postprandial period in either genotype group (data not shown).

Mean triglyceride levels increased significantly following the oral fat load in plasma and in $S_f > 400$ chylomicrons in both the 1/1 and 1/2 genotype groups, and in the VLDL fraction in the 1/2 group, and returned to baseline levels by 8 h (**Figs. 1–3**). There was a significant allele × time interaction on postprandial triglyceride levels: mean triglycerides in plasma, $S_f > 400$ chylomicrons, and VLDL peaked at 3 h in the 1/2 subjects versus 2 h in the 1/1 sub-

TABLE 1. Subject baseline data

	ApoA-IV-1/1	ApoA-IV-1/2
Number of subjects	14	14
Male/female	7/7	7/7
Age[a]	26.1 ± 0.9	28.6 ± 1.4
BMI[a]	22.4 ± 0.7	25.4 ± 1.0
Total cholesterol[a]	128 ± 4	136 ± 8
Triglycerides	53 ± 6	74 ± 9
LDL cholesterol	74 ± 4	82 ± 6
HDL cholesterol	44 ± 2	40 ± 2

[a] Data are means ± SE; lipid and lipoprotein values are in mg/dl. There were no significant differences in mean baseline parameters between genotype groups.

jects, and were significantly higher in the 1/2 subjects between 2–5 h.

The integrated postprandial response, calculated as mean triglyceride AUC (**Fig. 4**), was higher in the 1/2 subjects in plasma ($P = 0.045$), chylomicrons ($P = 0.027$), and VLDL ($P = 0.063$). No significant allele × gender interactions were found for mean plasma, chylomicron, or VLDL triglyceride AUCs. A strong positive correlation was found between baseline plasma triglycerides and plasma ($r = 0.896$), chylomicron ($r = 0.909$), and VLDL ($r = 0.857$) triglyceride AUCs; positive correlations were also noted between baseline LDL and plasma ($r = 0.516$), chylomicron ($r = 0.558$), and VLDL ($r = 0.558$) triglyceride AUCs. A negative correlation was found between baseline HDL and plasma ($r = -0.471$), chylomicron ($r = -0.425$), and VLDL ($r = -0.476$) triglyceride AUCs.

Dynamic light scattering analysis of postprandial $S_f > 400$ chylomicrons revealed a multimodal particle distribution with three distinct peaks of particle diameters with size ranges of 180–300 nm, 28–60 nm, and 3–8 nm (**Table 2**). The large- and medium-sized distributions probably correspond to populations of large and small chylomicrons, as previously noted for $S_f > 400$ chylomicrons collected from human thoracic duct lymph (44). The smallest peak may represent discoidal particles formed from surface material shed by the particles during processing. There were no significant differences in the particle size distributions between the two allele groups.

After we began this study, Ostos et al. (45) reported that the apoA-IV T347S polymorphism, which has worldwide gene frequency of 16–21% (41, 46), decreases the magnitude of postprandial lipidemia. We therefore conducted a post hoc analysis of the effect of the T347S polymorphism

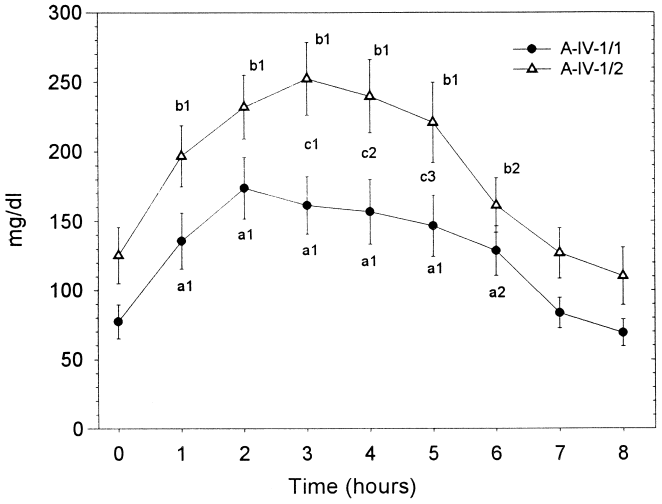

Fig. 2. Chylomicron triglycerides in subjects with the A-IV-1/1 and A-IV-1/2 genotypes. Time points for the 1/1 subjects significantly different from time 0: a1, $P < 0.001$; a2, $P = 0.005$. Time points for the 1/2 subjects significantly different from time 0: b1, $P < 0.001$; b2, $P = 0.005$. Time points significantly different 1/1 versus 1/2 subjects: c1, $P = 0.011$; c2, $P = 0.026$; c3, $P = 0.052$. Values are means ± SE (n = 14).

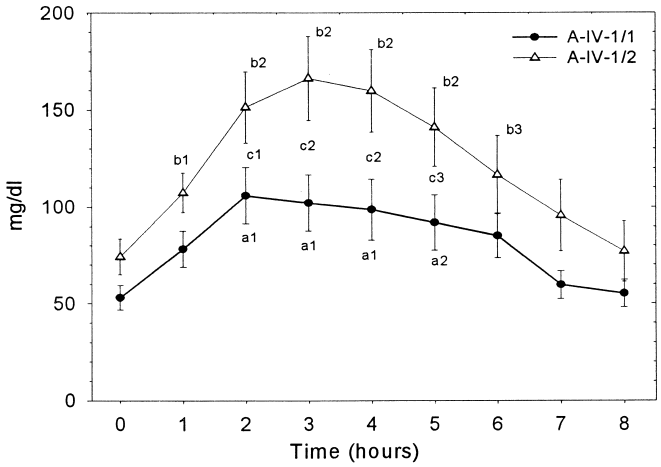

Fig. 1. Total plasma triglycerides in subjects with the A-IV-1/1 and A-IV-1/2 genotypes, measured after ingestion of 50 grams of fat per m^2 body surface area. Time points for the 1/1 subjects significantly different from time 0: a1, $P < 0.001$; a2, $P = 0.007$. Time points for the 1/2 subjects significantly different from time 0: b1, $P = 0.042$; b2, $P < 0.001$; b3, $P = 0.002$. Time points significantly different 1/1 versus 1/2 subjects: c1, $P = 0.039$; c2, $P = 0.007$; c3, $P = 0.027$. Values are means ± SE (n = 14).

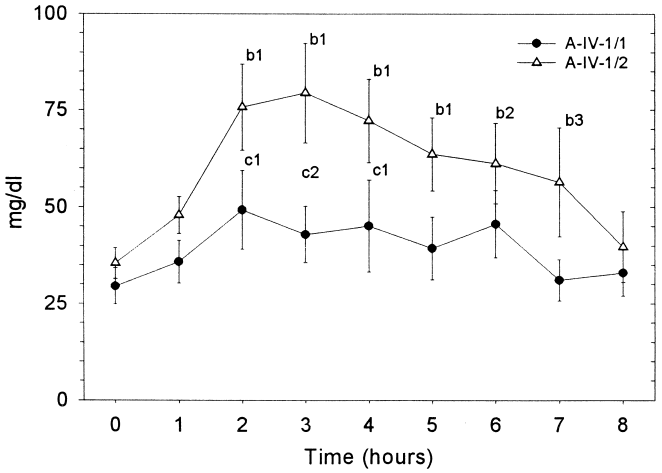

Fig. 3. VLDL triglycerides in subjects with the A-IV-1/1 and A-IV-1/2 genotypes. Time points for the 1/2 subjects significantly different from time 0: b1, $P < 0.001$; b2, $P = 0.003$; b3, $P = 0.045$. Time points significantly different 1/1 versus 1/2 subjects: c1, $P = 0.045$; c2, $P = 0.007$. Values are means ± SE (n = 14).

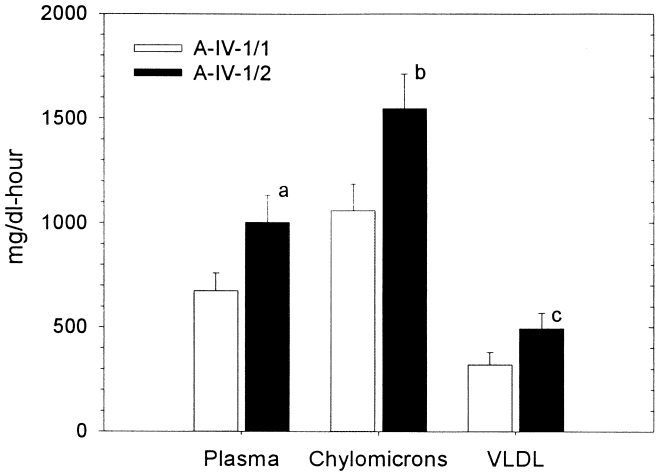

Fig. 4. Areas under the triglyceride versus time curves in subjects with the A-IV-1/1 and A-IV-1/2 genotypes. Values for 1/1 versus 1/2 subjects significantly different: [a] $P = 0.045$; [b] $P = 0.027$; [c] $P = 0.063$. Values are means ± SE.

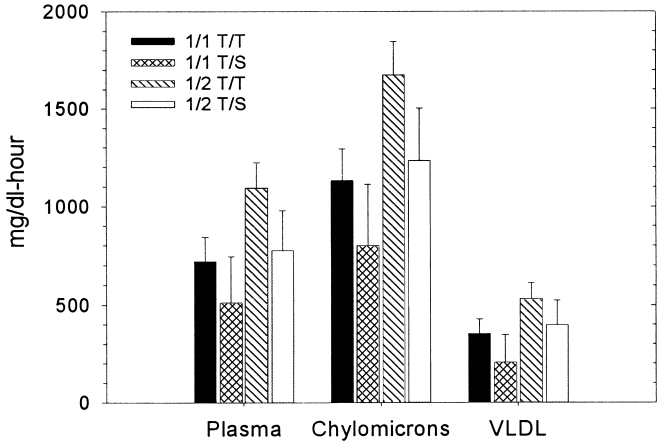

Fig. 5. Areas under the triglyceride versus time curves in subjects with the A-IV-1/1 and A-IV-1/2 genotypes by 347 T/S genotype. There were no significant differences among the values for the four haplotypes. Values are means ± SE.

on the outcome variables. DNA from 27 of the 28 subjects was available for T347S genotyping. In the 1/1 group, one subject had the 347S/S genotype and two subjects had the 347T/S genotype; in the 1/2 group, four subjects had the 347T/S genotype. Although in both 1/1 and 1/2 groups, the mean AUC for plasma, chylomicron, and VLDL triglycerides was lower in subjects carrying a 347S allele (**Fig. 5**), the differences among the means did not reach significance.

DISCUSSION

The magnitude of the postprandial rise in plasma triglycerides is determined by a dynamic balance between the synthesis and clearance of triglyceride-rich lipoproteins. A number of dietary, metabolic, and genetic factors are known to affect these processes. Alcohol (47), age (4, 5), gender (48), adiposity (6, 7), dietary fat intake (8, 9), hormonal status (49, 50), and baseline lipid levels (10), have all been shown to affect the postprandial plasma triglyceride response. Allelic variations in the apoE gene have long been known for their potent effect on postprandial lipid metabolism (10, 17–21), but recently, genetic polymorphisms in the genes for lipoprotein lipase (11–13), apoB (14–16), and intestinal fatty acid binding protein 2 (51) have also been found to affect the postprandial lipoprotein response.

Following a standard fat meal, subjects carrying an A-IV-2 allele displayed higher triglyceride levels during peak lipemia, a delayed triglyceride peak, and increased chylomicron and VLDL AUCs. These observations suggest that the A-IV-2 allele delays the postprandial clearance of triglyceride-rich lipoproteins and may play a role in the regulation of postprandial lipid metabolism. Candidate mechanisms by which the A-IV-2 allele could modulate postprandial lipidemia include an effect on the rate and/or efficiency of chylomicron synthesis, peripheral lipolysis, or hepatic remnant uptake. The observation that triglyceride-rich lipoprotein levels peaked later in the 1/2 subjects suggests the possibility that chylomicron synthesis or secretion could be delayed in these subjects. In this regard, intracerebral infusion of apoA-IV in rats slows gastric emptying (52), and thus delays secretion of chylomicrons into plasma. Nonetheless, it is not known whether neural effects of apoA-IV affect gastric emptying in humans or whether apoA-IV polymorphisms could modulate its effect on gastrointestinal function. We have shown that the A-IV-2 allele reduces the efficiency of cholesterol absorption in subjects consuming a polyunsaturated fat diet (40), which suggests that apoA-IV may play a role in modulating chylomicron assembly. However, intestinal lipid absorption is grossly normal in apoA-IV knock-out mice (53); hence, the function of apoA-IV in chylomicron synthesis must be an accessory one.

TABLE 2. Chylomicron particle diameter

	Sampled at Maximum Value			Sampled at T = 4 h		
Subjects	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
ApoA-IV-1/1 (n= 14)	247.3 ± 8.6[a]	40.5 ± 2.3	5.5 ± 0.3	238.2 ± 7.9	34.9 ± 1.3	6.7 ± 1.0
ApoA-IV-1/2 (n = 12)	232.9 ± 7.6	37.6 ± 2.3	4.8 ± 0.5	236.8 ± 10.1	37.5 ± 1.1	5.1 ± 0.5

[a] Data are means ± SE; particle diameters are in nm. There were no significant differences in particle size between genotype groups.

Following entry into circulation, chylomicron triglycerides are hydrolyzed by lipoprotein lipase in the vascular beds of the periphery (1, 2). Metabolic and genetic factors that modulate the activity or plasma levels of lipoprotein lipase or its co-factor, apoC-II, have a profound impact on the magnitude of postprandial lipidemia (1, 2). Before chylomicron hydrolysis can commence, apoA-IV on the surface of nascent chylomicrons must be displaced by apoC-II, which is transferred from its interprandial reservoir on plasma HDL. Because the apoA-IV-2 isoprotein has increased affinity for lipoproteins (34), it could inhibit adsorption of apoC-II, thereby slowing the rate of peripheral lipolysis. If this were true, we would expect that at any given time, circulating chylomicrons would be larger in the 1/2 subjects. However, we found no difference in the chylomicron particle size distribution between the two genotype groups, which suggests that delayed hepatic uptake, rather than delayed lipolysis, could be the basis for the A-IV-2 allele effect.

As hydrolysis proceeds, chylomicrons shrink and become enriched with cholesterol esters (3). When they have shrunk to remnant particles of ~1,000 Å, they can pass into the space of Disse, gain access to the surface of sinusoidal hepatocytes, and be removed from circulation by hepatic remnant receptors (3). ApoE serves as the receptor ligand in this process and, hence, before remnants can be cleared from circulation, they must acquire apoE on their surface. As with apoC-II, apoE may be adsorbed to the chylomicron surface in exchange with apoA-IV (25, 54). Thus, increased lipid affinity of the apoA-IV-2 isoprotein could impede adsorption of apoE, and thereby delay hepatic chylomicron remnant uptake. The observations that the VLDL fraction, which included chylomicron remnants, increased over baseline levels only in the 1/2 subjects and was significantly higher in the 1/2 subjects than in the 1/1 subjects at 2–4 h support this possibility. If so, this could explain why individuals carrying the A-IV-2 allele display an attenuated rise in plasma LDL to dietary cholesterol (36, 37). As cholesterol-enriched chylomicron remnants reach the liver, they downregulate hepatic LDL receptors, which in turn, raises plasma LDL levels. However, if the A-IV-2 allele retarded chylomicron remnant clearance, hepatic LDL receptors would be less affected and the increase in plasma LDL would be blunted. The apoE-2 allele is thought to lower plasma LDL levels by such a mechanism (55).

The apoA-IV T347S polymorphism appears to have effects opposite to those of the H360 allele in modulating the lipid response to dietary fat. Subjects carrying the S347 allele display greater rises in LDL and apoB in response to a high fat diet (56) and display decreased postprandial lipidemia compared to subjects homozygous for the T347 allele (45). Although our analysis of the impact of the S347 genotype was retrospective and, consequently, the haplotype subgroups were not well balanced, the trends in our data nonetheless support the findings of Ostos et al. (45), and suggest that an antagonistic interaction between the apoA-IV Q360H and T347S polymorphisms may modulate the ultimate magnitude of the postprandial lipid response. Moreover, although the biophysical properties of the apoA-IV 347S isoprotein have not been examined to date, these observations predict that it will be found to have lower lipid affinity.

Abnormal or delayed postprandial chylomicron clearance has been implicated in the pathogenesis of atherosclerosis (57, 58). Although the A-IV-2 allele does not appear to be an independent risk factor for atherosclerotic cardiovascular disease (59, 60), it might contribute to the risk of atherosclerosis in states where there is a metabolic predisposition to hypertriglyceridemia. In a study of cardiovascular events in patients with non-insulin-dependent diabetes mellitus, the A-IV-2 allele was associated with a 5- to 8-fold increased risk for myocardial infarction in obese diabetics (who had elevated plasma triglyceride levels) compared with groups of subjects with normal glucose tolerance or diabetics with a normal BMI (61). Similarly, the A-IV-2 allele might play a permissive role in other hypertriglyceridemic disorders. For example, the apoE-2/2 genotype is a necessary, but not sufficient, condition for the genesis of type III hyperlipoproteinemia; only a small subset of individuals homozygous for the E-2 allele display the type III phenotype (62). Recently an increased frequency of the N291S polymorphism in the lipoprotein lipase gene (63) and an *Sst* I polymorphism in the apoC-III gene (64) have been noted in hyperlipidemic E-2/2 homozygotes. It would be of interest to determine whether the A-IV-2 allele is a factor that contributes to the development of dysliproteinemia in E-2/2 homozygotes.

In summary, our data suggest that the A-IV-2 allele delays postprandial clearance of triglyceride-rich lipoproteins. Furthermore, our data suggest that there may be an antagonistic interaction between the H360 and S347 alleles that determines the ultimate impact of apoA-IV polymorphisms on the postprandial lipid response. Finally, we speculate that the A-IV-2 allele could increase the risk of atherosclerosis in states where there is a metabolic predisposition to hypertriglyceridemia. Further studies will be needed to elucidate the impact of apoA-IV alleles and their interaction with other apoprotein polymorphisms on postprandial lipoprotein metabolism.

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