Effect of apolipoprotein A-IV genotype and dietary fat on cholesterol absorption in humans

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Abstract **We investigated the effect of the A-IV-2 allele, which encodes a Q360H substitution in apolipoprotein (apo) A-IV, and dietary fat on cholesterol absorption in humans. In three separate studies we compared fractional intestinal cholesterol absorption between groups of subjects heterozygous for the A-IV-2 allele (1/2) and homozygous for the common allele (1/1) receiving high cholesterol (~800 mg/day) diets with different fatty acid compositions. All subjects had the apoE 3/3 genotype. There was no difference in cholesterol absorption between the two genotype groups receiving a high saturated fat diet (33% of total energy as fat; 18% saturated, 3% polyunsaturated, 12% monounsaturated) or a low fat diet (22% of total energy as fat; 7% saturated, 7% polyunsaturated, 8% monounsaturated) diet. However, on a high polyunsaturated fat diet (32% of total energy as fat; 7% saturated, 13% polyunsaturated, 12% monounsaturated) mean fractional cholesterol absorption was 56.7% ± 1.9 in 1/1 subjects versus 47.5% ± 2.1 in 1/2 subjects ($P = 0.004$). A post hoc analysis of the effect of the apoA-IV T347S polymorphism across all diets revealed a Q360H × T347S interaction on cholesterol absorption, and suggested that the A-IV-2 allele lowers cholesterol only in subjects with the 347 T/T genotype. We conclude that a complex interaction between apoA-IV genotype and dietary fatty acid composition modulates fractional intestinal cholesterol absorption in humans.**—Weinberg, R. B., B. W. Geissinger, K. Kasala, K. J. Hockey, J. G. Terry, L. Easter, and J. R. Crouse. **Effect of apolipoprotein A-IV genotype and dietary fat on cholesterol absorption in humans.** ***J. Lipid Res.*** **2000.** 41: **2035–2041.**

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Plasma low density lipoprotein (LDL) cholesterol is a strong risk factor for atherosclerotic cardiovascular disease (1), and interventions that lower LDL levels can reduce the risk for these lethal disorders (2). Because dietary saturated fats and cholesterol raise LDL cholesterol in most people (3), current Dietary Guidelines recommend a reduction in the consumption of foods containing these lipids, such as eggs, certain meats, and dairy products (4). However, the plasma LDL response to dietary fats and cholesterol is heterogeneous (5), and some individuals can consume "atherogenic" diets with little effect on plasma lipids (6, 7). Genetic variations in the plasma apolipoproteins may be the major factors that modulate the impact of diet on the lipoprotein response (8). However, of the reported genetic variants in the human apolipoprotein gene family, only apolipoprotein E (apoE) and apoA-IV have polymorphisms that exist at frequencies >1% in the world's populations (9).

ApoA-IV is a 46-kDa plasma apolipoprotein (10) that is synthesized by the intestinal enterocytes of mammalian species (11) during lipid absorption (12). ApoA-IV enters the circulation on the surface of nascent chylomicrons (13, 14), and thereafter dissociates from the chylomicron surface (14) and circulates primarily as a lipid-free protein (15). 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 absorption (16). Approximately 15% of the U.S. population carry a common variant allele, A-IV-2, which encodes a Q360H substitution near the C terminus of apoA-IV (17) and significantly alters its biophysical properties (18).

Two studies have observed that the A-IV-2 allele attenuates the LDL response to a high dietary cholesterol intake (19, 20). Given the role of intestinal cholesterol absorption in regulating plasma LDL levels (21, 22), and the involvement of apoA-IV in intestinal lipid absorption, we hypothesized that the A-IV-2 allele might affect the efficiency of intestinal cholesterol absorption. We therefore compared fractional intestinal cholesterol absorption between

Abbreviations: ANOVA, analysis of variance; apo, apolipoprotein; BMI, body mass index; HDL, high density lipoprotein; HIPOLY, high cholesterol, high polyunsaturated fat; HISAT, high cholesterol, high saturated fat; LDL, low density lipoprotein; LOFAT, high cholesterol, low fat.

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subjects heterozygous for the A-IV-2 allele and homozygous for the common allele, A-IV-1. Because genetic polymorphisms of apoE have been found in some studies to affect cholesterol absorption (23, 24), we studied only subjects with the apoE 3/3 genotype. Moreover, because dietary total fat and fatty acid content may also exert a strong effect on cholesterol absorption (22, 24), we measured cholesterol absorption on high cholesterol diets with three different fatty acid compositions: high saturated fat, high polyunsaturated fat, and low total fat.

MATERIALS AND METHODS

Subjects

Subjects heterozygous for the A-IV-2 allele (1/2) and homozygous for the common allele, A-IV-1 (1/1), were recruited from a cohort of subjects whose apoA-IV and apoE phenotypes were determined by isoelectrofocusing-immunoblot analysis (25, 26). The Q360H genotype was confirmed by restriction fragment length polymorphism analysis of buffy coat DNA (27); DNA from subjects with known apoA-IV 1/1, 1/2, and 2/2 genotypes was included with each analysis for quality control. All subjects selected had the apoE 3/3 genotype. Exclusion criteria were as follows: *1*) diabetes or cardiovascular, kidney, or liver disease; *2*) total cholesterol >240 mg/dl or triglycerides >200 mg/dl; *3*) body mass index >120% or <80% of ideal for sex and age; *4*) use of medications that affect lipoprotein metabolism, with the exception of oral contraceptives; and *5*) use of tobacco products. All subjects signed a statement of informed consent approved by the Wake Forest University School of Medicine Institutional Review Board.

Study design

Cholesterol absorption studies were conducted in the Wake Forest University School of Medicine General Clinical Research Center (GCRC, Winston-Salem, NC). Subjects were studied on three different diets: a high cholesterol, high saturated fat diet (HISAT); a high cholesterol, high polyunsaturated fat diet (HIPOLY); and a high cholesterol, low fat diet (LOFAT) diet. Each individual cholesterol absorption study lasted 4 weeks. During week 1, subject baseline dietary intake was determined with a modified, validated version of the National Cancer Institute Health Habits and History Questionnaire (NCI-HHHQ), a semiquantitative food frequency questionnaire (28). During weeks 2–4, the subjects consumed only food prepared by the GCRC metabolic kitchen. Fractional intestinal cholesterol absorption was measured during week 4.

The optimum design for this study would have been either to feed the three diets in parallel to a large cohort of subjects, or to feed all three diets to each subject in a sequential, random sequence. However, given the logistics of identifying and recruiting equal-sized groups of subjects with defined genotypes, this was not feasible. Thus, these studies were conducted in three waves between 1995 and 1998, and some subjects participated in more than one diet study. Three subjects participated in all three diet studies; nine subjects participated in both the HISAT and HIPOLY studies; four subjects participated in both the HIPOLY and LOFAT studies; and one subject participated in both the HISAT and LOFAT studies. In all, a total of 52 subjects participated in these studies; 4 were Asian, 2 were black, 1 was Hispanic, and 45 were white.

Experimental diets

A 5-day menu cycle was designed with low fat and nonfat foodstuffs, whole eggs, and dried egg yolks to provide 20% of total calories as protein and 800 mg of cholesterol per day. For the HISAT and HIPOLY diets, butterfat or safflower oil, respectively, were added to a selected item of prepared food served at each meal; for the LOFAT diet no additional fat was added. During the studies the subjects ate only the food provided by the metabolic kitchen, and consumed no vitamin or mineral supplements. Any deviations from the provided diet were reported to the dietitian. Subject weight was monitored daily, and the total caloric content of individual diets was adjusted, when necessary, to maintain weight within ± 1 kg. No subjects dropped out from any study.

The HISAT diet cholesterol absorption studies were conducted between May 1995 and January 1996. The HISAT diet provided 20% of total energy as protein, 45% as carbohydrate, and 33% as fat (18% of total energy as saturated fat, 3% of total energy as polyunsaturated fat, and 12% of total energy as monounsaturated fat).

The HIPOLY diet cholesterol absorption studies were conducted between April 1996 and February 1997. The HIPOLY diet provided 20% of total energy as protein, 46% as carbohydrate, and 32% as fat (7% of total energy as saturated fat, 13% of total energy as polyunsaturated fat, and 12% of total energy as monounsaturated fat).

The LOFAT diet cholesterol absorption studies were conducted between July 1997 and March 1998. The LOFAT diet provided 20% of total energy as protein, 55% as carbohydrate, and 22% as fat (7% of total energy as saturated fat, 7% of total energy as polyunsaturated fat, and 8% of total energy as monounsaturated fat).

Plasma lipids and lipoproteins

In each study blood samples for plasma lipid and lipoprotein analyses were obtained on two consecutive days at the beginning of week 2 and again at the end of week 4. Blood was collected before breakfast after a 12-h fast into ethylenediamintetraacetic acid tubes. Plasma was separated and assayed within 24 h for triglycerides, total cholesterol, LDL cholesterol, and high density lipoprotein (HDL) cholesterol in the Centers for Disease Control-standardized Lipid Laboratory of the Wake Forest University School of Medicine (26). The lipid and lipoprotein data for each 2-day period were averaged to yield individual baseline and postdiet values.

Fractional cholesterol absorption

Fractional intestinal cholesterol absorption was measured by a continuous feeding isotope ratio method (29), with a technician blinded to subject genotype. [1,2-α-^{3}H]cholesterol (45.6 mCi/mmol) and β-[4-^{14}C]sitosterol (56 mCi/mmol) were purchased from Amersham (Arlington Heights, IL); radiochemical purity was confirmed by HPLC. During week 4 on each diet, subjects ingested 0.38 μCi of [^{3}H]cholesterol and 0.11 μCi of β-[^{14}C]sitosterol with their morning meal for 5 consecutive days. Stools were collected for 4 days after the first day of isotope ingestion. A 1-g sample from each fecal collection was saponified with NaOH in 90% ethanol and extracted with hexane. Extracts were evaporated to dryness, transferred to ash-free paper cones, and the cones were combusted in a Packard (Downers Grove, IL) 306 Tri-Carb oxidizer that separates and traps $^{14}CO_2$ and $^{3}H_2O$ (30). ^{3}H and ^{14}C were quantitated by scintillation counting. Samples were analyzed in duplicate for the final 3 days of feces collection and the six isotope values for each subject were averaged. Fractional cholesterol absorption was computed as the isotope ratio in the feces divided by the isotope ratio in the diet. The mean coefficient of variation for the entire study was 8.8% (n = 72 measurements).

Statistical analysis

Descriptive data are presented as means ± SEM. For the purpose of statistical analysis, the three diets were treated as three independent studies. The significance of differences in entry parameters, fractional cholesterol absorption, and baseline→diet change in dietary intake between the 1/1 and 1/2 genotype groups on each diet was determined by two-tailed unpaired Student's *t*-test. The significance of changes in plasma lipoprotein levels between weeks 2 to 4 within genotype groups on each diet was determined by two-tailed paired Student's *t*-test. The interaction between gender, diet, and the apoA-IV Q360H and T347S polymorphisms on cholesterol absorption across all three studies was determined by analysis of variance by Tukey post hoc testing. The relationship between intestinal cholesterol absorption and pre-post changes in lipoprotein levels was examined by Pearson product moment analysis.

RESULTS

In each of the three separate diet studies, there were no significant differences between the 1/1 and 1/2 groups in mean age, body mass index (BMI), or plasma lipids at entry (**Table 1**). In each of the three diet studies, there were no significant differences between the 1/1 and 1/2 groups in baseline intake of cholesterol, or in the percentage of total calories derived from protein, carbohydrate, total fat, or saturated, polyunsaturated, or monounsaturated fatty acids on an ad libitum diet. Likewise, in each of the three diet studies there was no significant difference between the 1/1 and 1/2 groups in the baseline ad libitum→ experimental diet change in the intake of any dietary component.

Both apoA-IV genotype and dietary fat composition affected fractional intestinal cholesterol absorption (**Fig. 1**). In the subjects consuming the HISAT diet, mean cholesterol absorption was 52.3 ± 1.6 for the 1/1 group versus 50.6 ± 2.1 for the 1/2 group ($P = 0.514$). In the subjects consuming the HIPOLY diet, mean cholesterol absorption was 56.7 ± 1.9 for the 1/1 group versus 47.5 ± 2.1 for the 1/2 group ($P = 0.004$). In the subjects consuming the LOFAT diet, mean cholesterol absorption was 48.9 ± 2.7 for the 1/1 group versus 46.9 ± 2.0 for the 1/2 group ($P = 0.580$).

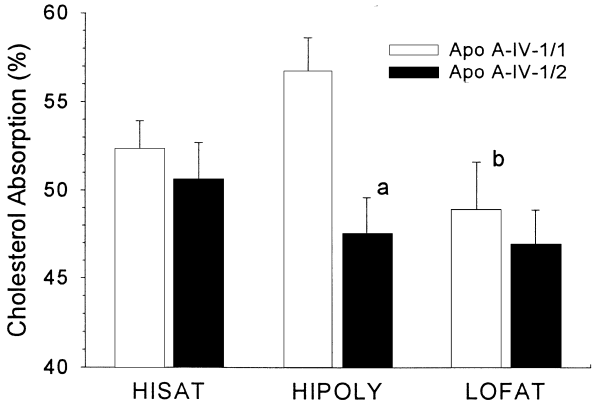

Fig. 1. Mean fractional intestinal cholesterol absorption in subjects with the A-IV-1/1 and A-IV-1/2 genotypes, measured on three different high cholesterol diets: HISAT, high saturated fat; HIPOLY, high polyunsaturated fat; LOFAT, low total fat. [a] $P = 0.004$, A-IV-1/1 HIPOLY versus A-IV-1/2 HIPOLY; [b] $P = 0.04$, A-IV-1/1 LOFAT versus A-IV-1/1 HIPOLY.

Although the design of this study was such that most subjects were studied on only one diet, a sufficient number of subjects were studied on both the HISAT and HIPOLY diets to enable a comparison of the diet response within subjects. Among the 1/1 subjects who were studied on both the HISAT and HIPOLY diets (n = 5) mean cholesterol absorption was 51.9 ± 3.1 on the HISAT diet versus 56.1 ± 2.4 on the HIPOLY diet. In the 1/2 subjects who were studied on both the HISAT and HIPOLY diets (n = 4), mean cholesterol absorption was 52.9 ± 3.6 on the HISAT diet versus 46.0 ± 2.8 on the HIPOLY diet. The HISAT→HIPOLY difference in cholesterol absorption between the 1/1 and 1/2 groups, 4.3 ± 3.1 versus −6.9 ± 3.1, was significantly different at $P = 0.039$.

When analyzed by analysis of variance (ANOVA) across all diets, apoA-IV genotype had an independent effect on cholesterol absorption, 52.6 ± 1.2 for 1/1 subjects versus 48.3 ± 1.3 for 1/2 subjects ($P = 0.014$). However, across genotype, diet had an independent effect on cholesterol absorption in the 1/1 subjects ($P = 0.040$), but not in the 1/2 subjects ($P = 0.409$). Two-way ANOVA of pooled data from all three studies found no gender or gender × allele

TABLE 1. Subject baseline data

	Saturated Fat Diet		Polyunsaturated Fat Diet		Low Fat Diet	
	1/1	1/2	1/1	1/2	1/1	1/2
N	14	12	13	11	12	10
M/F	9/5	6/6	7/6	6/5	6/6	6/4
Age	24.9 ± 0.9	27.8 ± 1.9	25.2 ± 1.0	28.5 ± 2.0	28.7 ± 1.9	32.8 ± 2.9
BMI	21.7 ± 0.6	23.0 ± 0.6	23.2 ± 1.3	24.4 ± 0.9	23.4 ± 1.1	26.3 ± 1.0
TCHOL	169 ± 7	173 ±10	167 ± 9	161 ± 8	159 ± 8	166 ± 10
TRIG	101 ± 11	110 ± 14	100 ± 18	88 ± 14	75 ± 9	84 ± 12
LDL	99 ± 7	105 ± 8	101 ± 7	97 ± 5	91 ± 6	104 ± 10
HDL	50 ± 2	47 ± 2	46 ± 2	48 ± 4	53 ± 4	52 ± 3

Lipid and lipoprotein values are mg/dl, means ± SEM. For each diet there were no significant differences in mean baseline parameters between genotype groups.

Abbreviations: N, number of subjects; M/F, number of male and female subjects; BMI, body mass index; TCHOL, total plasma cholesterol; TRIG, plasma triglycerides; LDL, plasma low density lipoprotein cholesterol; HDL, plasma high density lipoprotein cholesterol.

TABLE 2. Two-way ANOVA analysis of all cholesterol absorption data for subjects genotyped for both apoA-IV Q360H and T347S polymorphisms

	Gender	Diet	Q360H
T347S	0.201[a]	0.320	4.891
	0.655[b]	0.727	0.031
Q360H	0.173	2.873	
	0.679	0.064	
Diet	0.240		
	0.788		

[a] F value.
[b] *P* value.

effects on cholesterol absorption. There was no correlation in either genotype group between fractional cholesterol absorption and the baseline, postdiet, or baseline→diet changes in plasma lipids and lipoproteins.

As we were concluding these studies, Jansen et al. (31) reported that another common apoA-IV polymorphism, T347S, increases the plasma LDL response to a high cholesterol diet, suggesting that it, too, might affect fractional cholesterol absorption. We therefore performed T347S genotyping on stored buffy coat DNA from the participants in these studies; DNA from 46 of the 52 subjects was available for analysis. ANOVA of data pooled from all three diet studies revealed a Q360H × T347S polymorphism interaction on cholesterol absorption (**Table 2**). In subjects with the 347T/T genotype, cholesterol absorption was significantly lower in subjects carrying the A-IV-2 allele (**Fig. 2**); however, there was no difference in cholesterol absorption between the A-IV-1/1 and 1/2 groups in subjects with the 347 T/S or S/S genotype.

Although these studies were not principally designed to examine the effect of genotype on the lipid response to dietary modification, we nonetheless measured plasma lipids and lipoproteins at the beginning and end of dietary modification. On the HISAT diet, total cholesterol, LDL, and HDL increased and triglycerides decreased significantly in both genotype groups. On the HIPOLY diet, only triglycerides decreased significantly in both groups, and on the LOFAT diet, there were no significant changes in any lipid parameter in either group. Nonetheless, there was no significant difference between genotypes in the baseline→experimental diet change in LDL or HDL on the three diets (**Figs. 3** and **4**).

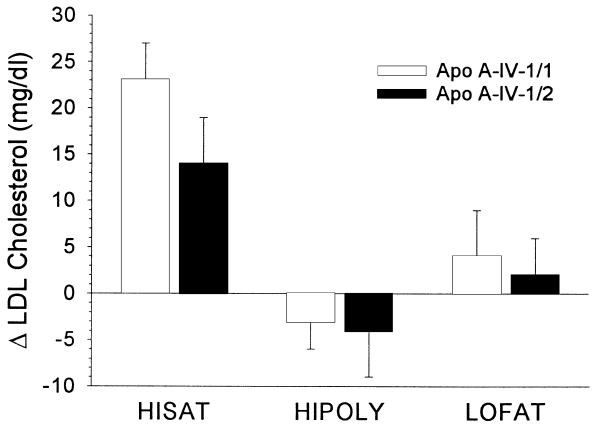

Fig. 3. Mean change in plasma LDL cholesterol in subjects with the A-IV-1/1 and A-IV-1/2 genotypes between baseline and after 3 weeks on different high cholesterol diets: SAT, high saturated fat; POLY, high polyunsaturated fat; LOW, low total fat. There were no significant differences in the mean changes in LDL between the A-IV-1/1 and A-IV-1/2 groups on any diet.

DISCUSSION

Although there is a considerable body of data in mice supporting a powerful effect of genotype on the efficiency of intestinal cholesterol absorption (32–34), to date only

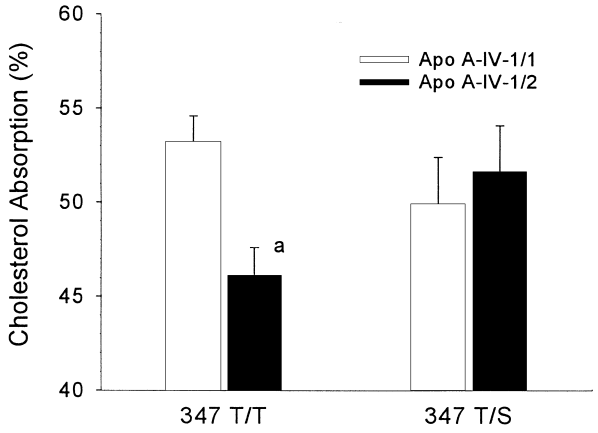

Fig. 2. Mean fractional intestinal cholesterol absorption in subjects genotyped for both A-IV-1/1 and A-IV-1/2, and the T347S polymorphism. Cholesterol absorption data from the three diet studies were analyzed by two-way ANOVA. 347 T/T, subjects homozygous for the T347 allele; 347 T/S, subjects heterozygous or homozygous for the S347 allele. [a] $P = 0.002$, A-IV-1/1 versus A-IV-1/2.

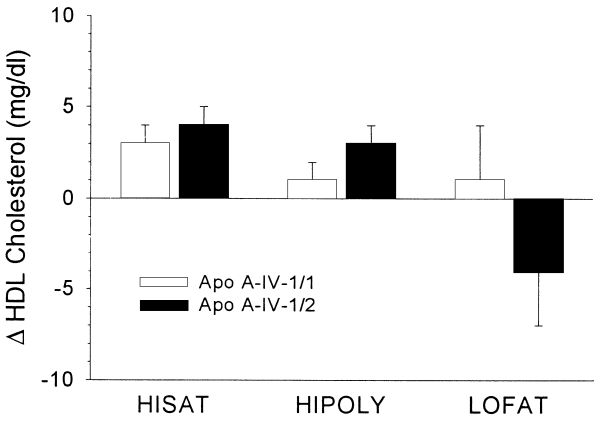

Fig. 4. Mean change in plasma HDL cholesterol in subjects with the A-IV-1/1 and A-IV-1/2 genotypes between baseline and after 3 weeks on different high cholesterol diets: SAT, high saturated fat; POLY, high polyunsaturated fat; LOW, low total fat. There were no significant differences in the mean changes in HDL between the A-IV-1/1 and A-IV-1/2 groups on any diet.

a few studies have examined the impact of genetic polymorphisms on cholesterol absorption in humans. In two studies in which the subjects consumed ad libitum diets, fractional cholesterol absorption was found to be lowest in subjects carrying an apoE2 allele, intermediate in apoE3/3 subjects, and highest in subjects with E3/4 or E4/4 phenotypes (22, 23). However, on defined low or high cholesterol diets, the differences in absorption among apoE genotype groups were not significant (24). Nonetheless, to eliminate any possible confounding effects of apoE polymorphisms on cholesterol absorption, in this investigation we studied only subjects with the apoE3/3 genotype.

Our data establish that subjects heterozygous for the A-IV-2 allele have lower fractional cholesterol absorption than A-IV-1/1 homozygous subjects on a high cholesterol, high polyunsaturated fat diet. Moreover, we also observed that cholesterol absorption in the A-IV-1/1 subjects increased on a HIPOLY diet, as previously noted in animal studies (35–38), whereas in the A-IV-1/2 subjects dietary fatty acid content had little impact on cholesterol absorption. Finally, we observed that another apoA-IV polymorphism, T347S, may determine the ultimate impact of the A-IV-2 allele on cholesterol absorption. Together, these data suggest that a complex apoA-IV genotype-dietary fatty acid interaction may modulate the efficiency of cholesterol absorption in humans.

In speculating on mechanisms by which apoA-IV polymorphisms could modulate cholesterol absorption, it is relevant that disruption of chylomicron assembly totally abolishes cholesterol absorption (39). In the first stage of chylomicron assembly, apoB-48 is cotranslationally lipidated by microsomal triglyceride transfer protein to form HDL-sized particles (40). These particles, which already have apoA-IV on their surface (41), then acquire additional triglyceride and expand to diameters of up to 10,000 Å. As the nascent particles expand, without a mechanism to maintain the packing density of surface lipids, free cholesterol influx from intracellular membranes could increase particle cholesterol content. Indeed, during fat absorption 27% of newly synthesized enterocyte free cholesterol is packaged into chylomicrons and appears in lymph (42). The C-terminal half of apoB-100 contains two α-helical domains that can adapt their surface conformation in response to changes in particle size, and fulfill this role in hepatic VLDL assembly, but intestinal apoB-48 lacks these domains (43). Thus the evolutionary appearance of intestinal apoB editing (44) may have created a need for an auxiliary apolipoprotein to control molecular packing on the chylomicron surface.

The biological and biophysical properties of apoA-IV may be optimally suited for this role. Intestinal apoA-IV synthesis is specifically stimulated by absorption of long-chain fatty acids (45, 46) but not short-chain fatty acids (47) (which do not form chylomicrons), and is blocked by Pluronic L-81, a surfactant that selectively disrupts chylomicron synthesis (48). This suggests that apoA-IV plays a specific role in the process of chylomicron assembly. Moreover, human apoA-IV displays unique conformational flexibility and elasticity at lipid/water interfaces (49), properties that are critical in stabilizing oil/water emulsion particles (50). Thus, by controlling molecular area at the expanding chylomicron surface during core lipidation, apoA-IV could modulate the influx of free cholesterol to the particle surface, thereby regulating particle cholesterol composition, and, ultimately, the efficiency of cholesterol absorption.

Such a mechanism could explain why the A-IV-2 allele had an impact on cholesterol absorption only on the HIPOLY diet. Polyunsaturated phospholipids have an expanded interfacial conformation and a lower lateral packing density (51, 52), properties that increase the rate of interfacial free cholesterol exchange (51, 53). Hence, increased transfer of intracellular free cholesterol to polyunsaturated phospholipid-enriched chylomicrons may have increased cholesterol absorption in the 1/1 subjects on the HIPOLY diet. However, the apoA-IV-2 isoprotein has higher surface activity than apoA-IV-1 (18), so increased binding of apoA-IV-2 to expanding chylomicrons may have restricted free cholesterol influx (54), thus constraining cholesterol absorption in the 1/2 subjects. Although its biophysical properties have not been studied to date, this hypothesis predicts that the 347S isoprotein will be found to have lower lipid affinity.

The lack of an effect of apoA-IV genotype on the LDL response to the fat-modified diets, despite an almost 3-fold increase in cholesterol intake, warrants comment. In a previous study, when cholesterol intake was increased 5-fold with no change in baseline fat intake (~30% of total calories), the A-IV-2 allele attenuated an increase in LDL cholesterol (19). Conversely, when cholesterol intake was decreased 50% with a modest reduction in total fat intake, subjects carrying the A-IV-2 allele had a smaller decrease in LDL (20). However, in another study in which cholesterol intake was held constant and saturated and monounsaturated fat intake was varied, no impact of the A-IV-2 allele on LDL levels was observed (55), although subjects carrying the A-IV-2 allele had greater changes in HDL levels. Similarly, in studies in which saturated fat and cholesterol were decreased simultaneously, no effect on LDL was noted (56, 57). These observations suggest that dietary fatty acids can efface the impact of the A-IV-2 allele on the LDL response to changes in cholesterol intake alone, perhaps because fatty acids are a much more potent factor in regulating hepatic LDL receptors than dietary cholesterol (5, 58). This could also explain why we found no correlation between fractional cholesterol absorption and the baseline→diet changes in plasma lipoproteins.

In summary, we observed that the A-IV-2 allele constrains cholesterol absorption in the setting of a high polyunsaturated fat diet. This constitutes the first demonstration of an apolipoprotein allele-dietary fatty acid interaction modulating human intestinal cholesterol absorption. Nonetheless, the A-IV-2 allele had no effect on the lipoprotein response to fat-modified, high cholesterol diets. Thus, the impact of apoA-IV genotype on the lipoprotein response to diet may ultimately be determined by a complex interaction between a relatively weak genotype effect on cholesterol absorption and a much more potent effect of dietary fatty acids on hepatic cholesterol metabolism.

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