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Carbohydrate intake modifies associations between *ANGPTL4* [E40K] genotype and HDL-cholesterol concentrations in White men from the Atherosclerosis Risk in Communities (ARIC) study

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Abstract

Background—Common allelic variation in the angiopoietin-like 4 gene (*ANGPTL4*[E40K]) has been associated with low triglyceride (TG) and high HDL-C.

Objective—We examined whether dietary macronutrient intake modified associations between *ANGPTL4*[E40K] variation and TG and HDL-C in White men and women from the Atherosclerosis Risk in Communities study.

Design—Diet was assessed by food frequency questionnaire. Intake of fat (total fat [TF], saturated fat [SF], monounsaturated fat [MUFA], polyunsaturated fat [PUFA], and n-3 PUFA) and carbohydrate were expressed as percentage of total energy intake. *ANGPTL4* A allele carriers ($n = 148$ in men, 200 in women) were compared to non-carriers ($n = 3667$ in men, 4496 in women). Interactions were tested separately in men and women, adjusting for study center, age, smoking, physical activity, BMI, and alcohol intake.

Results—*ANGPTL4* A allele carriers had significantly greater HDL-C and lower TG than non-carriers ($p \leq 0.001$). In all participants, carbohydrate intake was inversely associated with HDL-C and positively associated with TG, whereas TF, SF, and MUFA showed opposite associations with TG and HDL-C ($p < 0.001$). These relations were uniform between sex-specific genotype groups, with one exception. In men, but not women, the inverse association between carbohydrate and HDL-C was stronger in A allele carriers ($\beta \pm \text{S.E.} -1.80 \pm 0.54$) than non-carriers ($\beta \pm \text{S.E.} -0.54 \pm 0.11$, $p_{\text{interaction}} = 0.04$ in men and 0.69 in women; p 3-way interaction = 0.14).

Conclusions—These data suggest that *ANGPTL4* variation and relative contributions of dietary fat and carbohydrate influence TG and HDL-C concentrations. In men, *ANGPTL4* variation and dietary carbohydrate may interactively influence HDL-C.

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Conflicts of interest

The authors have no conflicts of interest to report.

Author contributions: Dr. Nettleton had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Nettleton and Boerwinkle. *Acquisition of data:* Boerwinkle. *Analysis and interpretation of data:* Nettleton and Boerwinkle. *Drafting of the manuscript:* Nettleton. Critical revision of the manuscript for important intellectual content: Nettleton, Volcik, Hoogeveen, Boerwinkle. Statistical analysis: Nettleton.

Keywords

ANGPTL4[E40K]; Dietary carbohydrate; HDL; C; Atherosclerosis Risk in Communities (ARIC) study

1. Introduction

Adipokines are proteins secreted by the adipose tissue and are involved in glucose and lipid metabolism [1]. The adipokine angiopoietin-like 4 (*ANGPTL4*) is thought to regulate fatty acid transport among tissues via inhibition of lipoprotein lipase (LPL), a key enzyme in HDL-cholesterol (HDL-C) and triglyceride (TG) metabolism [2–4]. A nonsynonymous sequence variant in the *ANGPTL4* gene (*ANGPTL4*[E40K]) results in a lysine for glutamic acid substitution and a loss of function of the *ANGPTL4* protein [5]. This variant has been associated with significantly lower triglyceride levels in three cohort studies (Dallas Heart Study, Atherosclerosis Risk in Communities [ARIC] study, Copenhagen Heart Study) [5], and significantly higher HDL-C in ARIC, the largest of these three cohorts [5].

Dietary intake, particularly the relative amount of dietary carbohydrate and fat as well as specific fatty acid classes, influence plasma triglyceride and HDL-C concentrations [6–8]. However, the association between dietary intake and triglycerides and HDL-C may be influenced by other lifestyle and genetic factors [9]. Analogously, associations between plasma lipids and polymorphisms in genes involved in the regulation of lipid transport and metabolism may vary as a function of dietary intake. Although no studies have evaluated this possibility with respect to *ANGPTL4*, it is plausible that dietary intake and *ANGPTL4* genotype interactively influence TG and HDL-C concentrations. For example, from both mechanistic and health prevention viewpoints, it would be valuable to know whether dietary composition operates similarly to influence plasma lipid concentrations in persons with genetically low TG or high HDL-C concentrations (i.e., *ANGPTL4*[E40K] variants).

Therefore, we explored the interaction between *ANGPTL4*[E40K] and dietary intake, particularly the percentage of calories from carbohydrate and total fat and specific fatty acid classes including saturated fat (SF), monounsaturated fat (MUFA), polyunsaturated fat (PUFA) and n-3 PUFA, with respect to TG and HDL-C concentrations in a well-characterized population-based sample of European Americans in ARIC study.

2. Methods

2.1. Subjects

The ARIC study was initiated in 1987 to investigate the etiology and occurrence of atherosclerosis and atherosclerotic diseases [10]. Men and women, aged 45–64, were recruited from four U.S. communities: Forsyth County, North Carolina; Jackson, Mississippi; Northwest Minneapolis suburbs, Minnesota; Washington County, Maryland. After providing informed consent, 15,792 participants were enrolled in the study (8710 women and 7082 men). Study protocols were approved by the institutional review boards at each center. Only African Americans were recruited from the center in Jackson, Mississippi, whereas mostly Whites were recruited from the remaining three centers. The current investigation includes only White participants, thus, only recruits from North Carolina, Minnesota, and Maryland (4314 non-Whites were excluded). The current investigation also excluded participants who had a history of diabetes ($n = 1046$), who were taking cholesterol-lowering medications ($n = 386$), who were not typed for *ANGPTL4*[E40K] ($n = 973$), who fasted <8 h ($n = 234$), who regularly consumed large quantities of alcohol (>20 g/d for women and >30 g/d for men) ($n = 840$), or who provided insufficient dietary data (>10 missing items on food frequency questionnaire) or had extreme

energy intakes (kcal intake <600 or >4200 for men or <500 or >3600 for women; approximate lower and upper 1 percentiles of the energy-intake distribution of the full cohort) ($n = 219$). After these exclusions, 3815 White men and 4696 White women remained for analysis.

2.2. Triglyceride and HDL-cholesterol (HDL-C) and other CVD risk factors

The laboratory methods used to quantitate total cholesterol, LDL-C, triglycerides, and HDL-C have been described previously in detail [11]. Cholesterol and triglycerides were measured by enzymatic procedures [12,13], with reagents from Boehringer Mannheim Biochemical (analysis adapted for use in Cobas-Bio Analyzer, Roche). High density lipoprotein cholesterol was measured after dextran-magnesium precipitation of non-high density lipoproteins [14]. LDL-C was calculated with the Friedewald equation [15]. Plasma pools from the US Centers for Disease Control and Prevention (CDC) were used as internal quality controls in measurement of plasma cholesterol, triglycerides, and HDL. External control consisted of successful participation in the CDC's Lipid Standardization Program. Laboratory-prepared in-house pools were used as quality controls for HDL, HDL₃-C, apoA-I, and apoB. Coefficients of variation for total cholesterol, LDL-C, triglycerides, and HDL were 5%, 10%, 7%, and 5%, respectively.

Fasting plasma glucose was determined by a hexokinase method according to standard ARIC protocols [16]. Seated blood pressure was measured three times with a random-zero sphygmomanometer, and the last two measurements were averaged. Self-reported medication use was verified by the inspection of medication bottles. Body mass index (BMI) was calculated from measured weight (kg)/height (m)².

2.3. Genotyping

The ANGPTL4 polymorphism was measured as previously described [5] using previously isolated genomic DNA and the TaqMan assay (Applied Biosystems, Foster City, CA). The TaqMan assays were read on a 7900HT real-time CR instrument (Applied Biosystems, Foster City, CA) and genotypes were called using a semi-automated clustering routine. The common G allele encodes the glutamic acid (E) residue in the ANGPTL4 protein, while the lower frequency A allele encodes a lysine (K). Due to small numbers of A allele homozygotes (10 in our total sample), G/A heterozygotes and A/A homozygotes were combined and their values compared with those of G/G homozygotes.

2.4. Diet assessment

At the baseline examination, participants completed an interviewer-administered, semi-quantitative food frequency questionnaire (FFQ), modified from the 61-item FFQ designed and validated by Willett and co-workers [17]. Participants were asked the frequency they consumed specific foods according to predefined frequency categories, ranging from never or <1 time/mo to ≥ 6 times/d. Standard portion sizes given as a reference for intake estimation, with food models used to assist in estimation. Interviewers obtained additional information on dietary habits, including the type of fat usually used in frying and in baking (butter, margarine, vegetable oil, vegetable shortening, lard) and the brand name of the breakfast cereal usually consumed (open-ended response). Intakes of each wine, beer, liquor were ascertained on a separate interview form. Nutrient intakes were derived from the FFQ responses using the Harvard Nutrient Database. The macronutrients studied (carbohydrate, total fat, SF, MUFA, PUFA, and n-3 PUFA) were each expressed as percentage energies (calories from each macronutrient/total caloric intake).

2.5. Statistical analyses

All analyses were conducted with SAS 9.1. Because of gender differences in dietary intake and previous data suggesting the gender-specific nature of diet $\times$ gene interactions for plasma lipids in this cohort [18], and in other studies [9,19–21], all analyses were stratified by gender. Means or percentages of demographic characteristics, lifestyle factors, and cardiovascular risk factors were calculated according to *ANGPTL4*[E40K] genotype in each men and women separately.

First, to establish the main effects of dietary macronutrient intake on TG and HDL-C, general linear regression was used to evaluate the relation between percentage energy from dietary macronutrients and TG and HDL-C concentrations. In addition to the macronutrient of interest (expressed as a percentage of total energy intake), the multivariable model included total Calories/d, age (y), center (Minnesota, Maryland, North Carolina), physical activity level (Baecke score), alcohol intake (g/d), smoking status (current/former/never), cigarette years, and education level (up to and including grade school, high school without diploma, high school graduate, vocational school, college graduate, graduate school/professional school).

Second, to determine if diet–lipid associations were uniform regardless of *ANGPTL4*[E40K] genotype, similar, genotype-stratified analyses were conducted separately in men and women. Regression estimates for associations between percentage energy from individual macronutrients and TG and HDL-C for each genotype were compared within gender group and between gender groups. Interactions were formally tested by adding a cross-product term to multivariable-adjusted models if estimates materially differed between genotypes. If estimates also differed by gender, 3-way interactions (gender $\times$ diet $\times$ genotype) were also tested.

Because of non-normally distributed data, TG concentrations were analyzed on the natural log scale. Therefore, regression coefficients from models where $\ln$ TG served as the dependent variable can be interpreted as estimated percent change in TG concentration per specified unit change in the independent variable. HDL-C concentrations were normally distributed and, thus, were not transformed for analysis. In the case of HDL-C, regression coefficients can be interpreted as the estimated change in HDL-C in mg/dL per specified unit change in the independent variable. Regression coefficients are presented according to an increase of 5% of energy from the macronutrient exposure of interest.

3. Results

3.1. Participant characteristics and main effects of genotype in men and women

The minor allele (A) frequency was 0.02 in men and in women, and *ANGPTL4*[E40K] was in Hardy Weinberg equilibrium in both gender groups. Characteristics of G/G homozygotes are compared to A allele carriers (A/G heterozygotes and A/A homozygotes) separately within each gender group in Table 1. As previously demonstrated [5], A allele carriers had significantly lower TG and significantly higher HDL-C concentrations than G/G homozygotes in ARIC Whites. Insulin concentrations were significantly lower in male A allele carriers compared to male G/G homozygotes. In women, lower total and LDL-C concentrations were observed in A allele carriers versus G/G homozygotes. Other demographic, lifestyle, and CVD risk factors did not differ by genotype, with the exception of alcohol consumption and percentage of energy intake from carbohydrate in men and whole grain intake in women.

3.2. Main effects of dietary intake on plasma lipids in men and women

In both men and women, dietary macronutrient intake (percentage contribution to total energy intake) was significantly related to TG and HDL-C concentrations after multivariable

adjustment in expected directions (Table 2). Carbohydrate intake was positively associated with TG (1–3% increase per increase of 5% energy from carbohydrate) and inversely associated with HDL-C concentrations (0.6–0.7 mg/dL decrease per increase of 5% energy from carbohydrate). In contrast total fat, SF, and MUFA were inversely associated with TG concentrations (3–6% decrease in TG per increase of 5% energy from total fat, SF, or MUFA in men and 2–4% decrease in TG per increase of 5% energy from total fat, SF, or MUFA in women) and positively associated with HDL-C concentrations (0.8–2.3 mg/dL increase per increase of 5% energy from total fat, SF, and MUFA). Total PUFA intake was not associated with HDL-C in either gender group, although n-3 PUFA intake was positively associated with HDL-C (11 mg/dL greater HDL-C [women] and 18 mg/dL greater HDL-C [men] per increase of 5% energy from n-3 PUFA).

3.3. Interactions between genotype and macronutrient intake in men and women

When data were analyzed similarly within gender group, but with further stratification by *ANGPTL4*[E40K] genotype (G/G vs. G/A + A/A), in almost all cases relations between percentage energy from dietary macronutrients and TG and HDL-C concentrations were similar between genotypes within each gender. However, there was significant heterogeneity in the associations between percentage energy from carbohydrate and HDL-C between genotype groups in men (Table 3 and Fig. 1). Specifically, a stronger inverse association between percentage energy from carbohydrate and HDL-C was noted in male A allele carriers (G/A and A/A) compared with male G/G homozygotes (p for interaction = 0.04, Table 3). Although percentage of energy from carbohydrate was inversely associated with HDL-C in both genotype groups in men, the relation was steeper in A allele carriers such that at lower levels of percentage energy intake from carbohydrate, the beneficial effect of the A allele was negated (see Fig. 1). In women, a similar interaction was not observed (p for interaction in women = 0.69; p for 3-way interaction among gender, genotype, and %energy from carbohydrate = 0.15). Adjustment for use of hormone therapy in women did not alter these data (p for interaction in women = 0.67; p for 3-way interaction = 0.14). Moreover, adjustment for fasting insulin concentrations, which differed between genotype groups in men, did not attenuate the interaction observed in men (p for interaction in men = 0.01; p for 3-way interaction = 0.01).

The nature of the interaction was similar if we modeled carbohydrate intake as g/d rather than as a percentage of total energy (-0.4 ± 0.1 mg/dL change in HDL-C per +20-g change in carbohydrate intake in male G/G and -1.2 ± 0.4 mg/dL change in HDL-C per +20-g change in carbohydrate intake in male A/G + A/A), although the test for interaction was not formally significant (p for interaction = 0.13). Results were not materially affected if we adjusted models for percentage energy from protein or percentage energy from total fat (data not shown). However, because percentage energy from carbohydrate, fat, and protein were all highly correlated in this dataset (Pearson correlation coefficient = |0.6–0.8|), simultaneous adjustment for any two variables leaves little room for independent variation in these nutrients. Adjustment for two measures of carbohydrate quality (dietary fiber and glycemic load each separately) also had no impact on our results (data not shown).

4. Discussion

We evaluated interactions between dietary macronutrient intake and *ANGPTL4*[E40K] genotype for HDL-C and TG in 8511 White men and women from the ARIC study. For most macronutrients, the associations with TG and HDL-C were uniform across *ANGPTL4*[E40K] genotype groups in both men and women. However, we did note a significant interaction between percentage of energy from carbohydrate and *ANGPTL4*[E40K] genotype for HDL-C. In men only, percentage energy intake from carbohydrate showed a stronger inverse

association with HDL-C in A allele carriers than in G allele homozygotes, essentially negating the beneficial effect of the *ANGPTL4*[E40K] mutation on HDL-C when percentage intake from carbohydrate was low.

It is well established that greater energy contribution from carbohydrate in the diet will lower HDL-C concentrations [7,22]. However, research also indicates that the quality of carbohydrate may be equally important in determining the sum effect on HDL-C [23,24]. Similarly, other dietary factors (nutrients and foods included in a single dietary pattern) may further influence associations with HDL-C ascribed to carbohydrate intake. In our study, dietary intake of several food groups significantly differed between men and women, including refined grain intake which was significantly greater in men (18 weekly servings in men vs. 16 in women, data not shown). Thus, it is possible that the significant interaction between percentage energy from carbohydrate and *ANGPTL4*[E40K] genotype that we observed in men, but not in women, was partly due to gender differences in the foods comprised by carbohydrate as well as gender differences in overall dietary patterns (additional confounding dietary factors). We attempted to address this issue by adjusting for measures of carbohydrate quality (fiber and glycemic index), but we found neither attenuation in the interaction nor change in genotype-specific regression coefficients. Hormonal differences between men and women may also have played a role, although adjustment for use of hormone therapy did not change our results.

The A for G allele substitution in *ANGPTL4* results in a substitution of lysine for glutamic acid, a loss of function of *ANGPTL4*, and a reversal of LPL inhibition [2–4]. Greater LPL activity is associated with greater TG clearance and greater HDL-C concentrations. Consistent with this, the E40K *ANGPTL4* mutation is associated with lower TG and higher HDL-C concentrations (shown here and demonstrated previously [5]). In contrast, high carbohydrate intake is thought to decrease HDL-C concentrations by decreasing hepatic fatty acid oxidation and consequently increasing hepatic production of very low-density, triglyceride-rich LDL-C (VLDL-C) particles [25]. The question of why would this effect would be magnified in E40K variants, as we observed in the current study, is uncertain and is inconsistent with the effect of the *ANGPTL4* mutation on LPL activity (increased) and the subsequent effect of LPL activity on HDL-C concentrations (increased). However, it is important to acknowledge that these relationships relate to the postprandial state. Meal composition and timing (variables not collected in the current study) may affect these relationships. Furthermore, our samples were collected after a ≥ 8 -h overnight fast, and our study did not measure LPL activity or mass.

Because carbohydrate intake is only a part of a larger landscape of dietary intake, we did attempt to account for other dietary factors by adjusting for intake of other macronutrients and measures of carbohydrate quality. Although these adjustments had little impact on our results, adjustment is likely incomplete due to residual confounding by imprecisely measured or unmeasured dietary factors. Similarly, residual confounding by other lifestyle factors is also possible, although we tried to account for differences in alcohol intake, education, smoking habits, and physical activity.

It is possible that the absence of interaction between other dietary macronutrients and *ANGPTL4*[E40K] is due to imperfect assessment of dietary exposures. However, the fact that the main effects for the relations between macronutrient intake and plasma lipids were significant and in directions predicted by many controlled feeding studies [7,22] suggests that for our purposes, diet was adequately measured. Nevertheless, additional study of this research question in cohorts with more comprehensive measures of dietary intake is warranted.

Ours is the first study to evaluate interactions between dietary macronutrient intake and *ANGPTL4*[E40K]. It is important that similarly designed analyses be conducted in other cohorts to verify whether the interaction observed in the current analysis is real or the result

of chance. It may be of key importance that future work evaluate whether the gender differences observed in our sample were due to underlying biologic differences between men and women or due to differences in dietary patterns and food sources of carbohydrate between men and women.

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Fig. 1.

Interaction between E40K genotype, gender, and %energy from carbohydrate for HDL-C (mg/dL). Adjusted for variables in model 1: energy intake (kcal/d), age (y), center, physical activity level (Baecke score), alcohol intake (g/d), smoking status (current/former/never), cigarette years, education level (up to and including grade school, high school without diploma, high school graduate, vocational school, college graduate, graduate school/professional school), and BMI. Values were obtained by taking mean HDL-C of each ANGPTL4[E40K] genotype (separately for men and women) and calculating expected change in HDL-C according to 1- and 2-SD changes in %energy from carbohydrate. For example, for GG men, mean HDL-C was 42.2 mg/dL. If the corresponding stratum-specific regression coefficient for the relation between %energy from carbohydrate and HDL-C (-0.108) is multiplied by the SD for %energy from carbohydrate intake (11.7%), the result is 44.7 mg/dL. The same procedure was followed for a 2-SD increase, and for 1- and 2-SD decreases in %energy from carbohydrate.

Table 1

Baseline characteristics of 3815 White men and 4696 White women from the Atherosclerosis Risk in Communities (ARIC) study stratified by *ANGPTL4*[E40K] genotype^a

	Men		<i>p</i>	Women		<i>p</i>
	G/G	A/A + G/A		G/G	A/A + G/A	
<i>N</i>	3667	148		4496	200	
Age	54.6 ± 0.1	54.5 ± 0.5	0.80	53.7 ± 0.1	54.0 ± 0.4	0.45
Education (% >high school degree)	82.8	85.7	0.36	85	87	0.32
Smoking status (% current)	22.3	23.7	0.70	23.8	21.5	0.45
Cigarette years	439 ± 8	477 ± 41	0.36	215 ± 5	208 ± 24	0.79
Physical activity level (% in Q5)	21.3	20.0	0.71	12.6	13.3	0.79
BMI (kg/m ²)	27.2 ± 0.1	27.0 ± 0.3	0.62	26.3 ± 0.1	25.8 ± 0.4	0.21
Alcohol intake (g/d)	5.5 ± 0.1	7.3 ± 0.7	0.01	2.2 ± 0.1	2.4 ± 0.3	0.57
Systolic blood pressure (mmHg)	118.8 ± 0.3	118.8 ± 1.3	1.00	115.8 ± 0.3	115.3 ± 1.2	0.67
Total cholesterol (mg/dL)	209 ± 0.6	209 ± 3.0	0.94	216.3 ± 0.6	209.5 ± 2.9	0.02
LDL-C (mg/dL)	139.8 ± 0.6	139.4 ± 2.9	0.90	134.4 ± 0.6	127.6 ± 2.8	0.02
HDL-C (mg/dL)	42.2 ± 0.2	46.6 ± 1.0	<0.0001	58.0 ± 0.2	61.9 ± 1.2	0.001
TG (mg/dL)	140.8 ± 1.4	115.7 ± 1.2	0.0006	119.7 ± 1.0	99.9 ± 4.7	<0.0001
Insulin	80.2 ± 0.9	70.2 ± 4.7	0.04	67.0 ± 0.8	63.2 ± 3.6	0.31
Glucose (mg/dL)	100.7 ± 0.1	100.9 ± 0.7	0.81	96.6 ± 0.1	96.9 ± 0.6	0.65
Prevalent coronary heart disease (%)	7.5	4.3	0.15	1.5	1.0	0.61
Prevalent hypertension (%)	24.9	22.1	0.46	22.9	22.3	0.84
Medications with secondary lipid effects (% current users)	18.1	15.0	0.35	20.9	21.4	0.85
Hormone therapy (% current users)	–	–		21.1	21.0	0.98
Nutrients ^b						
Energy (kcal/d)	1778 ± 11	1768 ± 54	0.85	1492 ± 8	1499 ± 38	0.86
Protein (% of energy)	17.1 ± 0.1	17.6 ± 0.3	0.12	18.5 ± 0.1	18.7 ± 0.3	0.60
Carbohydrate (% of energy)	48.5 ± 0.1	46.7 ± 0.7	0.02	49.9 ± 0.1	50.3 ± 0.6	0.58
Total fat (% of energy)	34.2 ± 0.1	34.7 ± 0.5	0.32	33.0 ± 0.1	32.5 ± 0.5	0.25
Saturated fat (% of energy)	12.6 ± 0.05	12.9 ± 0.2	0.14	12.2 ± 0.05	12.0 ± 0.2	0.57
MUFA (% of energy)	13.2 ± 0.05	13.4 ± 0.2	0.42	12.5 ± 0.04	12.2 ± 0.2	0.13
PUFA (% of energy)	5.2 ± 0.02	5.1 ± 0.1	0.39	5.1 ± 0.02	5.0 ± 0.1	0.48

	Men		<i>p</i>	Women		<i>p</i>
	G/G	A/A + G/A		G/G	A/A + G/A	
Fiber (g/d)	17.9 ± 0.1	17.8 ± 0.5	0.74	17.3 ± 0.1	17.7 ± 0.4	0.41
Foods (servings/wk)						
Whole grains	9.9 ± 0.2	9.9 ± 0.8	0.94	9.3 ± 0.1	10.6 ± 0.5	0.02
Refined grains ^c	12.7 ± 0.2	11.4 ± 0.8	0.11	9.5 ± 0.1	9.0 ± 0.5	0.30
Fish ^d	1.9 ± 0.03	1.9 ± 0.2	0.77	2.2 ± 0.1	2.1 ± 0.2	0.87
Nuts	3.2 ± 0.2	11.4 ± 0.8	0.11	2.6 ± 0.1	2.5 ± 0.2	0.75

^aExcluding non-fasting participants, participants with diabetes, participants taking lipid-lowering meds, and participants with extreme alcohol intake (>20 g alcohol/d for women; >30 g alcohol/d for men). Presented values are mean ± S.E.M., except in the case of triglycerides and insulin which were transformed to the natural log scale for analyses and are presented as geometric means (95% CI).

^bWith the exception of energy, all variables are adjusted for total kcal/d.

^cIncludes refined grain cereals, rice, pasta, bread, and snacks.

^dIncludes light meat fish, dark meat fish, canned tuna fish, and seafood.

Table 2Regression coefficients for difference in lnTG or HDL-C per 5% difference in energy from macronutrients^a

All participants	White men (<i>n</i> = 3815)		White women (<i>n</i> = 4696)	
	lnTG	HDL-C	lnTG	HDL-C
Carbohydrate	0.03 ± 0.005 [*]	−0.58 ± 0.11 [*]	0.01 ± 0.004 [*]	−0.70 ± 0.13 [*]
Total fat	−0.04 ± 0.01 [*]	0.79 ± 0.14 [*]	−0.02 ± 0.005 [*]	0.89 ± 0.17 [*]
Saturated fat	−0.08 ± 0.01 [*]	1.9 ± 0.31 [*]	−0.04 ± 0.01 [*]	2.3 ± 0.37 [*]
Monounsaturated fat	−0.09 ± 0.01 [*]	1.5 ± 0.32 [*]	−0.04 ± 0.01 [*]	1.8 ± 0.38 [*]
Polyunsaturated fat	−0.10 ± 0.03 [*]	0.89 ± 0.62 ^{NS}	−0.04 ± 0.02 [†]	0.35 ± 0.73 ^{NS}
n-3 polyunsaturated fat	−0.55 ± 0.23 [†]	17.9 ± 5.2 [*]	0.15 ± 0.15 ^{NS}	10.7 ± 5.1 [†]

^a $\beta \pm$ S.E. adjusted for energy intake (kcal/d), age (y), center, physical activity level (Baecke score), alcohol intake (g/d), smoking status (current/former/never), cigarette years, education level (up to and including grade school, high school without diploma, high school graduate, vocational school, college graduate, graduate school/professional school), and BMI.

^{*} $p < 0.001$.

[†] $p < 0.05$.

NS $p > 0.05$.

Table 3
Interaction between percent of energy from carbohydrate and E40K genotype with respect to HDL-C concentrations in White men and White women^a

G/G n = 3609	p for β	G/A and A/A n = 147	p for β	P _{interaction}
β Regression coefficients for HDL-C per 5% change in energy from CHO in <i>White men</i>				
-0.54 $\pm$ 0.11	<0.0001	-1.80 $\pm$ 0.54	0.001	0.04
G/G n = 4420	p for β	G/A and A/A n = 195	p for β	P _{interaction}
β Regression coefficients for HDL-C per 5% change in energy from CHO in <i>White women</i>				
-0.69 $\pm$ 0.13	<0.0001	-0.92 $\pm$ 0.70	0.19	0.69

p for 3-way interaction among gender, genotype, and % energy from CHO = 0.15.

^a β Regression coefficients per 5% change in energy from CHO adjusted for energy intake (kcal/d), age (y), center, physical activity level (Baecke score), alcohol intake (g/d), smoking status (current/former/never), cigarette years, education level (up to and including grade school, high school without diploma, high school graduate, vocational school, college graduate, graduate school/professional school), and BMI.