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# Sex-specific Interaction between APOE Genotype and Carbohydrate Intake Affects Plasma HDL-C Levels: the Strong Heart Family Study

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## Sex-specific interaction between *APOE* genotype and carbohydrate intake affects plasma HDL-C levels: the Strong Heart Family Study

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**Abstract** Low plasma levels of high-density lipoprotein cholesterol (HDL-C) are identified as a risk factor for cardiovascular disease (CVD). Sexual dimorphism, however, is widely reported in both HDL-C and CVD, with the underlying explanations of these sexual differences not fully understood. HDL-C is a complex trait influenced by both genes and dietary factors. Here we examine evidence for a sex-specific effect of *APOE* and the macronutrient carbohydrate on HDL-C, triglycerides (TG) and apoprotein A-1 (ApoA-1) in a sample of 326 male and 423 female participants of the Strong Heart Family Study (SHFS). Using general estimating equations in SAS to account for kinship correlations, stratifying by sex, and adjusting for age, body mass index (BMI) and SHS center, we examine the relationship between *APOE* genotype and carbohydrate intake on circulating levels of HDL-C, TG, and ApoA-1

through a series of carbohydrate-by-sex interactions and stratified analyses. *APOE*-by-carbohydrate intake shows significant sex-specific effects. All males had similar decreases in HDL-C levels associated with increased carbohydrate intake. However, only those females with *APOE-4* alleles showed significantly lower HDL-C levels as their percent of carbohydrate intake increased, while no association was noted between carbohydrate intake and HDL-C in those females without an *APOE-4* allele. These findings demonstrate the importance of understanding sex differences in gene-by-nutrient interaction when examining the complex architecture of HDL-C variation.

**Keywords** *APOE* · Carbohydrate · HDL-C · Lipids · Interaction

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## Introduction

Dietary manipulation of plasma cholesterol levels is widely accepted as a primary step in the treatment of dyslipidemias and prevention of their sequelae of atherosclerosis and cardiovascular disease (CVD). Past dietary recommendations to lower plasma cholesterol emphasized a low-fat diet with a higher intake of complex carbohydrate, yet studies vary in the extent to which either macronutrient influences lipoprotein risk factors [25, 27, 45, 62]. The low-fat diet has not proven universally effective in lowering risk profiles [77], suggesting an underlying genetic variation in dietary response [56]. Moreover, diets high in carbohydrate intake are frequently shown to decrease plasma levels of high-density lipoprotein (HDL) cholesterol (HDL-C) [14, 42] and increase circulating triglycerides (TG) [59]. Such an inverse correlation between plasma triglycerides and HDL-C is widely reported across populations, despite dietary differences [53]. High-density lipoprotein (HDL) is the major lipoprotein of the reverse cholesterol transport system thought to be protective against atherosclerosis. HDL-C is viewed as an indicator of the efficacy of this reverse transport system [12]. The inverse relationship of carbohydrate intake to HDL-C was introduced over 20 years ago [68], with studies indicating that this relationship began in early childhood [72]. Growing evidence supports the presence of the cardio-protective role of higher plasma HDL [3, 49]. Long-term studies now suggest that low-fat, high carbohydrate diets may have exacerbated current problems of obesity, type II diabetes, and the metabolic syndrome [77]. These factors show an association with lower HDL in both males and females [20, 74, 78]. Variation in carbohydrate effects on lipid risk factors may indeed be influenced by the quality of the carbohydrate [59] or other population-specific factors [46, 69]. Although the current dietary recommendations now focus on quality rather than the quantity of carbohydrate, protein and fat intake [1, 4, 41], the results from studies of dietary relationship to plasma lipids remain inconsistent. The issue of sex-specific variation as a factor in the response of plasma lipids to variation in dietary macronutrients appears consistently in many studies, however [16, 40, 58]. We hypothesize that a genetic predisposition and sexual dimorphism may explain some of the variation in response of HDL-C levels to dietary carbohydrate intake [47, 51].

Apoproteins are the major protein moiety of plasma lipoproteins and responsible for a variety of roles including stimulation of the synthesis of lipoproteins, cofactors for lipoprotein modulating enzymes, and ligands for lipoprotein receptors. Our candidate gene, apoprotein *E* (*APOE*) has a major polymorphism with three major alleles (*e2*, *e3*, and *e4*). The accepted wild type is *e3* (>60% in all populations studied), with *e2* and *e4* distinguished by a one base-pair

substitution at different locations; *e4*(Cys<sub>112</sub> to Arg) and *e2* (Arg<sub>158</sub> to Cys) [10, 21, 76]. The three resulting isoforms have well-documented variation in functional effects [43, 70]. APOE is a ligand for several cell membrane receptors of the triglyceride-laden low-density lipoproteins chylomicrons and VLDL. APOE also assists in cholesterol efflux from macrophages and mediates uptake of the reverse transport lipoprotein, HDL [11, 49]. Using the binding capacity of E3 as the referent, studies indicates that E2 has an impaired binding, while studies of E4 report somewhat inconsistent results, with E4 binding either similarly to E3 [10] or at an enhanced capacity [2]. Research further suggests preferential binding of E4 to large triglyceride-rich very-low-density lipoproteins (VLDL), while E2 and E3 show preference to the HDL [9].

The plasma lipoprotein response of *APOE* genotype to dietary fat and cholesterol has been extensively examined, while the response to variation in carbohydrate intake has been virtually ignored until recently [57, 58]. Sex-specific associations are documented between *APOE* variants and plasma lipoproteins [17, 57, 65] and between *APOE* variants and the risk of CVD [35]. Current research demonstrates the benefits of examining *APOE* within the context of a sex-stratified approach in order to develop a clear picture of gene action on each of the plasma lipoproteins [73]. Here we examine the sex-specific relationship of *APOE* alleles and the dietary percent of carbohydrate intake on plasma levels of HDL-C, TG, and apoprotein A-1 (ApoA-1), a primary component in regulating the biosynthesis of HDL [60].

## Methods

### Sample

The Strong Heart Study (SHS) is a longitudinal study to determine CVD risk factors among 13 American Indian tribes in three geographical areas across the central plains and the southwest. The Institutional Review Board (IRB) of the Indian Health Service, and the collaborating education institutions approved the study protocol. Initiated in 1989, this study completed baseline exams between 1989 and 1992. The exams consisted of personal interviews, anthropometrics, and physical exams including laboratory tests. Original participants, aged 45–74, included individuals diagnosed for diabetes [28]. The Strong Heart Family Study (SHFS), a component of the SHS, is a family study that has recruited and examined more than 3,800 individuals in multiple extended families. Between 1997 and 1999, a subset of family study participants, including 320 males and 421 females ages 18–88, completed prompted 24-h dietary recalls and gave fasting blood samples for lipid profiles and genotyping [55]. This subset is the sample used for this study.

## Phenotyping

Phenotyping for plasma lipoproteins, apoproteins, triglycerides, insulin and glucose was completed from blood samples drawn after a 12 hour fast. Assays were performed at the MedStar Research Institute, following standard laboratory procedures described elsewhere (<http://strongheart.ouhsc.edu/manual/PhaseV/Vol01.pdf>) [37]. HDL-C, triglycerides (TG), apoprotein A-1, were determined using enzymatic reagents and the Hitachi 717 (Boehringer Mannheim Diagnostics), while LDL-C was determined using the Friedewald equation for individuals with TG < 400 mg/dl. For those participants with TG > 400 mg/dl, the LDL was directly assessed [15, 54]. Fasting insulin was determined through a modified method of the polyethylene glycol-double antibody radioimmunoassay [36], and glucose levels were assayed through the hexokinase method (Glucose-HK: Boehringer Mannheim Diagnostics, Indianapolis, IN), also on the Hitachi 717 [54]. *APOE* phenotypes were determined using 2 h of isoelectric focusing followed by immunoblotting to identify *APOE* bands [31, 33] and used to infer genotypes [48].

Body mass index (BMI) was calculated by Quetelet's index: weight (kg)/height (m)<sup>2</sup> [38]. These measures of weight and height were both sampled with participants wearing light clothing and no shoes [28]. The percent of body fat was determined through an impedance meter and equations based upon total body water [26]. Dietary profiles were collected using an interview-prompted 24 hour recall. Nutrient data were determined from these dietary recalls using nutrient data system [67]. Macronutrient intake was assessed as the percent of total caloric intake and includes the percent of fat (9 kcal/gm), protein and carbohydrate (both 4 kcal/gm). Carbohydrates were not differentiated into complex and simple carbohydrate, and are therefore not categorized for glycemic index [30, 72].

Diabetes status was defined by three categories: those without diabetes, those diagnosed with diabetes and those with impaired glucose tolerance, ascertained by fasting glucose and insulin levels. The categories were in accordance with the World Health Organization criteria of 1980 and detailed in a strong heart study publication by Kataoka et al. [32].

## Statistical analyses

Data were initially examined with multivariate analyses in SPSS 11.5, analyzing both unadjusted lipoprotein measures (HDL and TG log-transformed) and standardized residuals of lipids after adjustment by the basic model. Dietary intake was categorized into sex-stratified tertiles of macronutrient intake. Results were then verified within the SAS software system (SAS Institute, version 8.0, Cary, NC) in

order to implement general estimating equations (GEE). These GEE models and mixed model analysis of variance (ANOVA) allow tests of association while accounting for the correlation among members within common pedigrees. We are reporting results obtained using GEE models in this paper.

*APOE* allele frequencies were estimated by gene counting, and the familial-based data were evaluated for Mendelian inconsistencies and cleaned of pedigree errors using PREST and the effects of genotyping error using Simwalk II, both described in detail elsewhere, [54, 71, 75] and (<http://strongheart.ouhsc.edu/manual/PhaseV/>). Individuals were grouped into two categories, those without an *e4* allele and those with an *e4* allele. As the frequency of individuals with at least one *e2* allele was minimal and similar for both males and females, they were consolidated within the corresponding study categories, with or without *e4*. Therefore, genotype *e3/2* was included with *e3/3*, and *e4/2* included with may *e3/4* and *e4/4*. No homozygote *e2/2* was noted in the sample.

We assessed the association between carbohydrate intake, genotype, and their interaction on HDL-C, TG and APOA-I, using mixed model analysis of variance for continuous, normally distributed traits and generalized estimating equations (GEE) for categorical traits. The GEE method estimates within-pedigree similarity of residuals to reestimate regression parameters and thus calculate more accurate standard errors [22]. We specified an exchangeable correlation structure for these analyses, which should have been slightly conservative. Analyses were completed on the sex-stratified analyses and tests of genotype-by-sex interaction, diet-by-sex, genotype-by-diet, and genotype-by-diet-by-sex interaction were performed. We used two levels of covariate adjustment: (a) base model: SHS center, age, BMI; and (b) a fully adjusted model that includes the base model adjustment factors, with the additional factors of body fat, fasting insulin, glucose levels and diabetes status. Continuous outcomes were first examined for adherence to distributional assumptions (including approximate normality of error terms conditional on covariates and homoscedasticity) and appropriate transformations were made when necessary. Specifically, HDL-C and TG were natural log-transformed. *P* values ≤ 0.05 were deemed statistically significant.

## Results

### Descriptive statistics

Table 1 present the descriptions of this Strong Heart Family Study sample as a whole, with adiposity and

**Table 1** Characteristics of Strong Heart Family participants

Trait	Mean (SE)	Mean (SE)	<i>P</i> value <sup>e</sup>
Age (years)	39.5 (1.3)	40.1 (1.2)	0.60
BMI <sup>a</sup>	32.9 (1.0)	34.1 (0.9)	<b>0.027</b>
BF <sup>b</sup> (%)	31.2 (1.1)	43.3 (1.1)	<b>&lt;0.001</b>
FG (mg/dl)	123.2 (5.2)	125.8 (5.0)	0.72
FI (mIU/l)	26.9 (3.3)	28.4 (3.2)	0.075
Diabetes status			
Diabetic (%)	20.7	24.9	0.052
IGT (%)	9.9	6.7	0.073
Lipids			
TC (mg/dl)	180.9 (3.4)	174.0 (3.3)	<b>0.008</b>
HDL (mg/dl)	42.0 (1.5)	45.2 (1.5)	<b>&lt;0.001</b>
HDL/AHA <sup>c</sup>			
<40 (%)	54.8	44.6	0.075
41–59 (%)	36.6	43.6	0.095
>60 (%)	8.6	11.8	0.26
LDL (mg/dl)	117.1 (2.4)	109.1 (2.2)	<b>&lt;0.001</b>
TG (mg/dl)	136.6 (5.3)	123.0 (4.9)	<b>0.029</b>
APOA-1 (mg/dl)	1.3 (0.03)	1.37 (0.03)	<b>&lt;0.001</b>
Dietary <sup>d</sup>			
Total (Kcal)	2,303.6 (58.7)	1,899.1(52.5)	<b>&lt;0.001</b>
Carb (%)	50.3 (0.9)	52.8 (0.8)	<b>0.005</b>
Prot (%)	14.4 (0.3)	13.9 (0.3)	0.65
Fat (%)	34.2 (0.7)	33.9 (0.7)	0.24

<sup>a</sup> BMI = kg/m<sup>2</sup>

<sup>b</sup> BF = body fat (% of body composition)

<sup>c</sup> HDL-AHA = risk categories % of population

<sup>d</sup> Dietary traits = percent of total daily caloric intake

<sup>e</sup> *P* values were determined using generalized linear mixed models (continuous outcomes) and generalized estimating equations (categorical outcomes), which controlled for the correlation among members in the same pedigrees

FG = fasting glucose, FI = fasting insulin—% of sample, Diabetes status = diabetes and IGT (impaired glucose tolerance) % of population

diabetes related traits, lipid profiles, and dietary data for both males and females. Participants' ages ranged from 18–89 years, with a mean of 39 years for both males and females. A total of 54% of the males and 62% of the females had BMI scores  $\geq 30$  (data not shown), indicating a substantial proportion of obese individuals in this sample [34]. As expected, the percent of body fat was higher in females than males. Sexual dimorphism was also present in the plasma lipids, with females exhibiting significantly higher circulating levels of HDL-C and APOA-1 and lower levels of TG, LDL-C and total cholesterol (TC). Dietary differences between males and females were statistically significant only for total caloric intake and carbohydrate percent of total intake, with females taking in fewer

**Table 2** Frequency of APOE alleles and genotypes in both males and females

	Males ( <i>n</i> = 321)		Females ( <i>n</i> = 423)	
	<i>n</i>	Frequency	<i>n</i>	Frequency
APOE alleles				
e2	13	0.02	15	0.02
e3	539	0.84	732	0.87
e4	90	0.14	101	0.12
Genotypes				
2/2	0	0.000	0	0.000
2/3	11	0.003	13	0.003
3/3	225	0.701	315	0.745
3/4	78	0.243	89	0.210
4/2	2	0.006	1	0.002
4/4	5	0.002	5	0.001

calories but a higher percent in carbohydrate than males. The percent of individuals experiencing diabetes or impaired glucose tolerance was 30.6 for males, 31.6 for females (Table 1).

*APOE* allele and genotype frequencies are shown in Table 2. Further delineation of characteristics between *APOE* study categories identified distinct genotypic differences (Table 3), using least squared means to account for the covariates within the data. Males carrying the *e4* allele had significantly increased BMI and body fat, without a significant difference in total calorie intake or nutrient intake. Levels of fasting insulin were higher in males with *e4*. In contrast, no significant differences were noted in females for adiposity-related characteristics or diabetes-related traits. Both males and females carrying an *e4* allele exhibited lower HDL-C and APOA-1 levels, however, the effects of genotype were more pronounced in AHA HDL risk categories in females than males.

#### Main effect of diet

In the initial analyses with SPSS, only carbohydrate intake remained consistently associated with any lipid measure, and specifically only with HDL-C (data not shown). We changed to SAS with GEE to accommodate any effect for pedigrees and identified the main effect of carbohydrate intake on HDL-C and TG variation to be statistically significant in males only (see Table 4). Findings were consistent for carbohydrate intake regardless of covariate adjustment, in both the base model and the fully adjusted model (additionally adjusted for body fat, diabetes status, fasting insulin and glucose levels). Carbohydrate intake was not associated with APOA-1 variation in either males or females.

## Main effect of genotype

Genotypic differences were noted in HDL-C and APOA-1 levels, with significantly lower levels in both males and females carrying the *e4* allele (Table 3). After the adjustment for covariates, only APOA-1 remained significantly different for both males and females, while the main effect of *APOE* remained statistically significant for HDL-C in females only. No effect of *APOE* genotype was significant for TG levels (Table 4).

## Interaction between diet and genotype

Because sex-specific effects of diet and genotype were observed, we assessed the evidence for diet-by-genotype interaction in males and females separately. Interaction between carbohydrate intake and *APOE* genotype in variation in HDL-C levels was observed in females only, in both the base and fully adjusted models (Table 4). Plasma HDL-C levels of females without an *e4* allele showed no association with carbohydrate intake, whereas females

**Table 3** Characteristics of individuals stratified by APOE genotypes

Trait	Males		<i>P</i> <sup>c</sup>	Females		<i>P</i> <sup>c</sup>
	Without <i>e4</i> ( <i>n</i> =236)	With <i>e4</i> ( <i>n</i> =85)		Without <i>e4</i> ( <i>n</i> =329)	With <i>e4</i> ( <i>n</i> =94)	
	LS-Mean (SE)	LS-Mean (SE)		LS-Mean (SE)	LS-Mean (SE)	
Age (year)	39.3 (0.9)	36.5 (1.6)	0.12	40.6 (1.3)	39.5 (1.8)	0.51
BMI <sup>a</sup>	32.3 (0.9)	34.6 (1.2)	<b>0.009</b>	33.8 (1.2)	35.6 (1.4)	0.12
BF <sup>b</sup> (%)	30.9 (1.1)	33.3 (1.3)	<b>0.012</b>	42.9 (1.2)	44.1 (1.4)	0.18
FG (mg/dl)	115.9 (4.1)	122.8 (6.3)	0.24	130.0 (7.2)	126.9 (8.8)	0.79
FI (mIU/l)	28.8 (4.2)	33.4 (5.1)	0.042	24.8 (1.4)	26.3 (2.7)	0.25
Diabetes status						
Diabetic (%)	18.6	26.0	0.17	25.2	23.4	0.63
IGT <sup>c</sup> (%)	10.6	7.0	0.33	6.4	6.4	0.99
Lipids						
TC (mg/dl)	180.0 (3.6)	178.8 (4.9)	0.74	176.0 (3.7)	175.6 (4.8)	0.92
HDL (mg/dl)	43.7 (1.7)	39.5 (2.1)	<b>0.029</b>	45.3 (1.6)	41.6 (1.9)	<b>0.012</b>
HDL-AHA						
<40 (%)	51.7	61.2	0.13	41.6	53.4	<b>0.004</b>
41–59 (%)	37.3	35.2	0.56	44.4	40.4	0.15
>60 (%)	10.6	3.5	0.11	13.4	6.4	<b>0.024</b>
LDL (mg/dl)	115.4 (3.0)	118.6 (4.1)	0.44	109.9 (2.2)	113.2 (3.2)	0.29
TG (mg/dl)	130.2 (7.3)	141.8 (10.1)	0.10	126.8 (5.0)	121.4 (7.7)	0.62
APOA-1 (mg/dl)	1.33 (0.03)	1.21 (0.03)	<b>&lt;0.001</b>	1.38 (0.04)	1.29 (0.04)	<b>0.004</b>
Dietary <sup>d</sup>						
Total (Kcal)	2,373 (85)	2,112 (128)	0.075	1,914 (50)	1,816 (91)	0.65
Carb (%)	50.2 (1.0)	50.4 (1.5)	0.88	52.2 (0.8)	53.2 (1.3)	0.45
Prot (%)	14.3 (0.4)	15.0 (0.6)	0.46	14.0 (0.3)	13.8 (0.5)	0.67
Fat (%)	34.3 (0.9)	32.9 (1.3)	0.28	34.4 (0.6)	33.8 (1.0)	0.57

Genotypes pooled into two groups: those without *e4* and those with *e4* allele. *P* values indicate significant differences between genotypes, within sex

<sup>a</sup> BMI = kg/m<sup>2</sup>

<sup>b</sup> BF = body fat

<sup>c</sup> IGT = impaired glucose tolerance

<sup>d</sup> Dietary traits = percent of total daily Kcal intake

<sup>e</sup> Significant *P* ≤ 0.05 in generalized linear mixed models (continuous outcomes) or generalized estimating equations (categorical outcomes), which controlled for the correlation among members in the same pedigrees

FG = fasting glucose, FI = fasting insulin

**Table 4** *P* values for tests of main effects and interaction of sex, *APOE* and carbohydrate, with table representing results of maximum model (basic adjustment and adjustment for measures of diabetes, body fat, glucose and fasting insulin)

	Gender × Carb. × <i>ApoE</i> interaction <sup>a</sup>	Carb. × <i>ApoE</i> interaction <sup>b</sup>	Carb. main effects <sup>c</sup>	<i>ApoE</i> main effects <sup>c</sup>
Log(HDL)				
Sex-combined	<b>0.0002</b>			
Males		0.14	<b>0.029</b>	0.17
Females		<b>0.0003</b>	<b>0.11</b>	<b>0.0006</b>
Log(ApoA1)				
Sex-combined	0.060			
Males		0.084	0.14	<b>&lt;0.0001</b>
Females		0.063	0.56	<b>0.0023</b>
Log(trigs)				
Sex-combined	0.58			
Males		0.47	<b>0.018</b>	0.54
Females		0.92	0.53	0.60

Adjustments made for basic model, then incremental additions with measures of diabetes, body fat, and fasting insulin and glucose levels. Interaction models adjusted for all lower order terms. Basic model adjusted for age, SHS center, BMI; Maximum model adjustment for 2 diabetes variables (s3adadm and s3adaifg), body fat (s3fat), glucose (s3g0) and fasting insulin (s3insu). Models with interactions are adjusted for all lower order terms

<sup>a</sup> Performed on sex-combined sample

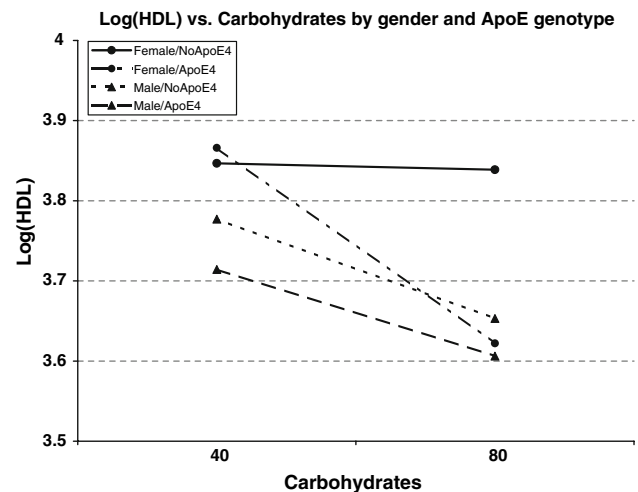
<sup>b</sup> Performed on sex-stratified samples

<sup>c</sup> Performed on sex-stratified samples only if *P* value for carb × *APOE* interaction was not significant in the previous model

carrying an *e4* allele demonstrated a highly significant association. In contrast, an inverse relationship between HDL-C and carbohydrate intake was noted in males, but no effect modification by *APOE* genotype was observed (Fig. 1). No effect of interaction was noted in either sex for TG or APOA-1 levels, regardless of covariate adjustment.

## Discussion

Sexual dimorphism of HDL-C levels and CVD is widely reported in many populations, yet underlying mechanisms for these differences are not fully understood. Controlling for age, SHS center and BMI, fasting blood sugar and insulin levels, and diabetic status, we identified an interaction between sex, *APOE* genotype, and carbohydrate intake on plasma HDL-C levels. This gene-by-macronutrient interaction was restricted to the female SHFS participants. As dietary manipulation of plasma lipids is the initial strategy in CVD prevention, identifying factors which affect circulating HDL-C may support the ability to implement more effective individually-tailored dietary intervention.



**Fig. 1** Log(HDL) versus carbohydrates by gender and *ApoE* genotype

HDL remains an important risk modifier in cardiovascular diseases. Beyond its putative role of sequestering and transporting cholesterol and other detrimental products back to the liver for disposal, HDL is believed to inhibit endothelial inflammation and promote production of endothelial nitric oxide (NO) and prostacyclins [39]. Variation in HDL levels is believed to be primarily associated with variation in clearance, except in dietary studies which have identified that fat intake affects HDL production or transport rates [61]. Although we cannot identify the specific metabolic effects on HDL-C variation here, as plasma levels of APOA-1 are not associated with carbohydrate intake, our findings suggest that catabolism may be the more likely mechanism affecting HDL-C variation in this study. Moreover, with carbohydrate intake not the traditionally studied macronutrient for association with *APOE*, our findings demonstrate the importance of including carbohydrate intake in a sex-specific context, when examining the genetic and dietary influence underlying HDL-C variation.

## Genotype effects

*APOE* polymorphisms and their distinct isoforms are the most widely studied of the apoproteins. The Strong Heart Study population exhibits a higher frequency of the wild type *e3* and lower frequencies of *e2* and *e4* than seen in many populations worldwide [32, 50], yet a profile similar to other American and Mexican Indian populations [8, 19]. Acting as the major ligand for clearance of triglyceride-rich lipoproteins and HDL through cellular receptors, *APOE* function may vary depending upon the lipoprotein with which it is connected and include additional anti-atherogenic properties [60, 63]. Dallongeville et al. meta-analyses of studies from 17 countries evaluated the relationship of



*APOE* with plasma cholesterol. Reporting consistent findings in the relationships, the authors suggested variation in amplitude of effect between populations. No clear association was noted between HDL-C and those *APOE* phenotypes carrying one allele of *e2*, however, HDL-C variation between individuals with *e3/3*, *e3/4*, and *e4/4* was reported. Additionally, aside from the expected association of *e2* with elevated TG, these authors also reported a significant association of higher TG and lower HDL-C with the heterozygote *e3/4* genotypes than in lipid levels of homozygotes *e3/3* and *e4/4*. Moreover, they stressed that these relationships remained consistent in a variety of populations including children, diabetics, obese, and hyperlipidemic individuals [7]. However, while this study did include both males and females, it did not address sex-specific variation. Hallman's nine country study reported consistent associations of *APOE* genotype with total cholesterol levels among the countries, despite assumed dietary differences. No direct measure of any dietary variable was included in that study, nor were the data sex-stratified [21].

Kataoka et al. [32] study examining the relationship of *APOE* polymorphisms to plasma lipids in the original Strong Heart Study individuals ( $n = 4,410$ ), reported sex-specific variation associated with *APOE* polymorphisms. Genotype was not significantly associated with HDL-C variation in males (ages 45–64), however, in females, a significant trend for HDL-C levels was noted in all female subgroups studied: normal and diabetic, premenopausal and menopausal. HDL-C was higher in females carrying an *e2* and lower in individuals carrying an *e4* than for females with *e3/3* genotypes. Genotype variation was noted for TG differences in males only, with *e2* and *e4* having higher TG levels than males homozygous for *e3/3* genotype in both normal and diabetic individuals. Dietary factors were not considered in the study [32].

Many other studies document sex-specific effects of *APOE* on plasma lipids in a variety of populations, although various lipid effects are not consistent throughout the studies [13, 18, 44, 50, 52, 64, 66]. The complexity of the interrelatedness of the lipid system with its constant remodeling of lipoproteins, makes study comparisons difficult. The relationship of *APOE* to lipids has been shown to be age and context dependent, with increased heterogeneity of variance in older participants attributable to cumulative environmental effects reflecting greater variation in diet, exercise and other factors. Unique environmental variation is offered as an explanation for some inconsistencies [52]. A recent review article proposed that gender and hyperglycemia were two of the primary environmental factors affecting the *APOE*-HDL-C relationship [18], which points to variation in carbohydrate intake and metabolism as moderating factors in HDL-C variation.

## Dietary effects

The varied relationships between carbohydrate intake and plasma HDL-C have long been acknowledged in the literature [42, 68]. The recent focus on macronutrient quality emphasizing complex carbohydrates, to include both soluble and insoluble dietary fibers, has sparked renewed interest in physiological responses to carbohydrate intake. Traditional dietary studies examining nutrient association with lipid profiles concentrated on fatty acids and cholesterol intake, and most studies examined males only [58]. Few studies have quantified sex-specific variation directly, choosing instead to adjust for any sex differences and suggest that the discrepancies between studies may be based on unidentified sex variation in dietary response [5]. This approach of adjusting for sex differences has been challenged for genetic studies by Stengard et al. [73] who suggest that pooling sex data results in findings which do not adequately reflect the genotype effects on phenotypic variation in either sex.

Differences between sexes were reported in the National Health and Nutrition Examination Study (NHANES III, 1988–1994), with higher carbohydrate intake associated with lower HDL in males and elevated TG in females, BMI in both males and females, and glucose levels in males only [80]. In our study, the main effect of carbohydrate intake was significantly associated with lower HDL-C and higher TG in males only, after adjustment for BMI and traits associated with glucose levels.

## Interaction of *APOE* and carbohydrate

Polymorphisms of *APOE* have been shown to affect variation in plasma lipid profiles when associated with dietary nutrients. Traditionally, studies have examined cholesterol and fat intake and indicated that individuals with an *e4* allele have greater plasma lipid sensitivity in LDL and TG to dietary variation [18]. Most reported studies of diet-*APOE* interaction are documented in males only [58]. Our study compared males and females of similar backgrounds and families to identify a sex-specific *APOE* genotype vulnerability to carbohydrate effects on HDL-C levels in females only, a vulnerability which remained consistent after adjusting for center, age, adiposity and glucose altering traits. We used additional analyses on these data, defining carbohydrate intake in sex-stratified tertiles and applying a general linear model in SPSS 11.5 to substantiate these findings (results not shown).

Several studies have shown little variation in lipid patterns between hyperglycemic and diabetic groups when comparing *APOE* genotypes across quintiles of glucose levels. One, however, reported *APOE*-serum glucose interaction affecting variation in HDL-C and APOA-1 in

both males and females, and TG in males only [18]. Adjusting for the basic model and each glucose altering trait separately did not alter our outcome (Table 4), and APOA-1 variation was not affected by carbohydrate intake in either males or females in any of our models.

The association pattern of HDL-C with *APOE* polymorphisms in our study reflected Kataoka et al's findings in females, and only when carbohydrate was a factor. We believe that these findings reinforce the importance of further examining the relationship of carbohydrate intake to HDL variation. Most importantly, these findings support our hypothesis that genetic predisposition and sexual dimorphism are in fact present in the response of HDL-C to carbohydrate intake (Fig. 1).

Two issues must be noted here: (a) the between sex variation noted in individuals carrying the common *e3*, and (b) within sex HDL-C variation of *APOE* genotypes seen in females. The first might be explained through sexual dimorphism noted in lipid metabolism, with females showing a greater sensitivity to insulin's antilipolytic effects, and suppression of postprandial plasma fatty acid concentrations to compensate for their higher basal fatty acid flux [47]. The second issue may be connected to *e4* as an effective ligand for triglyceride-rich lipoproteins (TRL), and high levels of APOE in HDL-C appearing to accelerate the lipoprotein's catabolism [29]. Animal research suggests a further option of variation in *APOE* isoforms during recycling of (TRL), a process stimulated by HDL. While APOE3 is readily resupplied to plasma HDL particles during TRL recycling, thus serving to maintain HDL levels and further enhance hepatic clearance of remnants, APOE4 is believed to accumulate intracellularly. Therefore, APOE4 is not readily recycled and reattached to HDL particles, thereby resulting in a decrease of HDL as well as decreasing recycling of TRL [24]. Addressing these specific issues is beyond the scope of this paper, yet our findings may suggest a sex-specific component at that level. The Heeren study did not address sex-specific variation. Determining HDL-C subfractions in future research to address such questions as whether the preferential binding of *APOE* isoforms and variation in recycling of these isoforms is present in American Indian populations, or how carbohydrate affects variation in specific HDL-C subfractions with and without association to *APOE* genotypes, may identify underlying explanations for our results. Furthermore, such information may also indicate whether dietary lowering of HDL-C actually reduces its cardioprotective properties.

Metabolically active adipose tissue may have contributed to our findings. Body fat was significantly higher in both males and females with *APOE4* allele, despite lower caloric intake. Our adjustment for body fat cannot account for variation in levels of adipokines [82] or the HDL scavenger receptor class B type I (SR-BI) which is highly

expressed in adipose tissue [6]. Furthermore, sexual dimorphism has been widely documented in both adipose tissue levels and patterns, its hormones leptin and adiponectin [81, 82], and energy homeostasis [79] all of which are influenced by nutritional status and affect energy balance, carbohydrate and lipid metabolism and insulin resistance [23, 47].

In this study we are limited by a cross-sectional approach, the lack of specific information of HDL subfractions and the subjective approach of recall as a measure of dietary exposure. However, while cross-population studies suggesting consistency among populations of *APOE* effects on lipid variation in the face of inferred dietary differences [21], this study incorporates a quantified dietary variable and a sex-stratified approach in a larger population. Whether the findings of this study reporting sex-specific variation of HDL-C as an effect of carbohydrate-*APOE* polymorphism interaction are applicable to other populations will depend on future research. Our findings demonstrate the importance of understanding sex differences in gene-by-nutrient interaction when examining the complex architecture of HDL-C variation.

## Summary

In conclusion, we identified sex-specific gene-by-nutrient effects on HDL-C in American Indian participants of the SHFS. Males, regardless of their *APOE* genotype, consistently demonstrated an inverse relationship between carbohydrate intake and plasma HDL-C levels. In contrast, genotype-by-carbohydrate-specific associations were shown in females. Only those females with an *e4* allele demonstrated the inverse relationship between carbohydrate intake and HDL-C levels widely documented in many populations, whereas those females without an *e4* allele showed no relationship of HDL-C to increased levels of carbohydrate intake.

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