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Apolipoprotein gene SNPs contribute to sex-dependent differences in blood lipids before and after a high-fat dietary challenge in healthy U.S. adults

By

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THESIS

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SNPs in apolipoprotein genes contribute to sex-dependent differences in blood lipids before and after a high-fat dietary challenge in healthy U.S. adults

Yining Elaine Wang

Abstract

Background

Genetic polymorphisms are known to affect fasting blood lipid levels, but their effects in the context of a high-fat meal challenge are less well characterized. This study investigates the association between SNPs in lipid transport genes and post-prandial blood lipid profiles in healthy adults in the U.S.

Methods

Subjects (n = 349) were 18-66 years of age and balanced by sex (48% male, 52% female). They had body-mass indices (BMI) ranging from 18.5 to 45.0 kg/m^2 and completed the cross-sectional Nutritional Phenotyping Study. We assessed informative single nucleotide polymorphisms (SNPs) in five lipid transport genes (*APOA5*, *APOB*, *APOC3*, *APOE*, and *LDLR*). The association between serum lipid markers and genotypes was evaluated separately for each SNP using an analysis of variance (ANOVA) and pairwise interaction tests adjusted for sex, age, and BMI.

Results

Women carrying the C allele of rs3135506 in *APOA5* and men carrying the C allele of rs429358 in *APOE* had reduced fasting and postprandial HDL-cholesterol levels. The C allele in *APOE* was also correlated with increased LDL-C. The TT genotype of rs2854116 in *APOC3* was associated with elevated total

cholesterol. Additive interactions were detected between *APOA5* and *APOE* and between *APOC3* and *APOE* risk alleles. Nevertheless, the tested SNPs had little impact on the postprandial triglyceride responses to the high-fat challenge meal. Additionally, *APOB* (rs1042034) or *LDLR* (rs2228671) SNPs had no significant effect on serum triglyceride, cholesterol, or free fatty acid levels.

Conclusions

Fasting and postprandial cholesterol levels are strongly correlated with *APOA5*, *APOE*, and *APOC3* SNP genotypes in healthy adults. Sex increases the genetic impact of all tested SNPs on lipid profiles.

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Introduction

Cardiovascular disease (CVD) has been the leading cause of death worldwide for over a decade. Likewise, the global incidence of Type 2 diabetes mellitus has increased dramatically since 1980, creating a worldwide health epidemic [1]. Pathogenic factors underlying both disorders are immune activation and inflammation, and elevated serum triglycerides and cholesterol, which are triggered by excess consumption and accumulation of fat [2]. Dietary fat is transported from the intestinal tract to peripheral tissues via lipoprotein particles that deliver cholesterol and triglycerides to different sites of the body via their specific protein components [3]. See Figure 1 and 2 for details of lipid metabolism pathways. These apolipoproteins play crucial roles in trafficking lipids as they are recognized by distinct cell surface receptors in target tissues. Differences in lipid particle density, size and apolipoprotein content determines how they are routed to different tissues for use [4] (**Table 1**).

The major apolipoproteins (APOA, APOB, APOC, and APOE) are categorized by function [5]. APOAs with subtypes of APOA1, APOA2, APOA4, and APOA5 are primary structural proteins of high-density lipoprotein (HDL). Importantly, APOA5 is also associated with chylomicrons and very low-density lipoprotein (VLDL) to regulate triglyceride homeostasis [6–8]. Patients with APOA5 deficiency are hypertriglyceridemic [9]. On the other hand, transgenic animals overexpressing the human APOA5 have plasma triglyceride concentrations that are decreased by 70% compared to controls [6,7]. In addition, *APOA5* is in a gene cluster with *APOC3*, along with *APOA1* and *APOA4* [8], where the gene cluster has been strongly associated with different lipid levels, such as triglyceride, LDL size, and HDL cholesterol levels in different population[10–12]. APOB is a major structural protein for LDL and all other lipoprotein particles except HDL. APOB100, the full-length form of APOB, binds the LDL receptor (LDLR) to mediate endocytosis of LDL

from the circulation to the liver [13,14]. Loss-of-function *APOB* mutations disrupt lipid metabolism, causing familial hypobetalipoproteinemia and familial ligand-defective APOB100 disease [15]. In contrast, high levels of APOB accompanied with high LDL particle concentrations is strongly associated with atherosclerosis and CVD [16]. APOC subtypes (APOC1, APOC2, and APOC3) are associated with chylomicron, VLDL, and HDL [17] and freely exchange among these lipoprotein particles [5]. APOC1 facilitates the esterification of free cholesterol into cholesterol esters in HDL [14] while APOC2 is a co-factor for lipoprotein lipase (LPL), which promotes triglyceride hydrolysis [18]. On the other hand, APOC3 inhibits the APOC2-mediated activation of LPL [5,14]. Importantly, APOC3 can significantly reduce the clearance rate of LDL by inhibiting the receptor-mediated LDL endocytosis in the liver [19]. Thus, APOC3 has been considered as a powerful indicator of CVD risk and dyslipidemia [20]. APOE is an essential apolipoprotein for cholesterol-rich lipoproteins, such as chylomicron remnants, VLDL, and some HDL [21,22], and it also binds LDLR, mediating LDL uptake in the liver. There are three major coding alleles of APOE (APOE ε2, ε3, and ε4), which have different LDLR binding affinities [23]. Individuals carrying the homozygous *APOE ε4* allele have elevated plasma LDL and an increased risk of atherosclerosis and CVD [21], while those carrying the homozygous *APOE ε4* allele have a high prevalence of type III hyperlipoproteinemia, a disorder with ectopic fat deposits in non-adipose tissue and elevated risk of atherosclerosis and diabetes [24]. LDLR is localized on the cell surface of hepatocytes and binds to APOB100 and APOE for LDL uptake, which clears LDL from the circulation [25,26]. The *LDLR* mutations cause familial hypercholesterolemia, with significantly elevated serum LDL resulting in severe atherosclerosis and CVD [25].

Individuals differ widely in their physiological responses to dietary fat. The magnitude, content, and kinetic features of postprandial serum lipid profiles depend on genetic, sex, and environmental factors [2]. Single-nucleotide polymorphisms (SNPs) in *APO* and *LDLR* genes, such as rs3135506 (*APOA5*), rs1042034

(*APOB*), rs2854116 (*APOC3*), rs429358 (*APOE*), and rs2228671 (*LDLR*) have been associated with fasting dyslipidemia, including elevated total cholesterol, triglycerides, or both, and with increased LDL-C or decreased HDL-C cholesterol levels in the circulation [17,25,27–29]. Unhealthy diets, such as those with high-fat content, contribute to dyslipidemia and increased risk of atherosclerosis and CVD [30]. In addition, quantitative trait locus (QTL) mapping studies of UK Biobank samples indicate significant genotype by sex interactions for apolipoprotein genes, with different implications for disease risk assessment in men and women [31,32].

Although the association between apolipoprotein gene SNPs and dyslipidemia has extensively documented, the contribution of these SNPs to lipid clearance after a high-lipid meal challenge in healthy adults is less well known, especially in healthy U.S. adults stratified by sex, age, and body-mass index (BMI). Moreover, the interactive effects of SNP genotypes on postprandial lipid metabolism in healthy adults is poorly understood. The present study analyzed the contributions of five SNPs in *APOA5* (rs3135506), *APOB* (rs1042034), *APOC3* (rs2854116), *APOE* (rs429358), and *LDLR* (rs2228671) to clinical lipid markers before and after a high-fat liquid meal challenge in healthy U.S. individuals recruited for a human cross-sectional nutritional phenotyping study, who were stratified by sex, age, and BMI.

Materials and methods

Study subjects and dietary challenge

Healthy U.S. adults (*n* = 393) 18-66 years old with BMI from 18.5 to 45.0 kg/m^2 were enrolled in a cross-sectional Nutritional Phenotyping Study (*ClinicalTrials.gov*, ID: NCT02367287) conducted by the United States Department of Agriculture (USDA), Agriculture Research Service (ARS) Western Human Nutrition Research Center (WHNRC) in Davis, California. The upper limit for BMI was modified from 39.9 kg/m^2 in the original sample plan [33] to 45 kg/m^2. Pregnant or lactating women were excluded. Other exclusion criteria were a known egg allergy, recent surgery or hospitalization (minor surgery within 4 wks or major surgery/hospitalization within 4 mos), recent antibiotic treatment (within 4 wks), and daily medication for a diagnosed chronic disease [33]. Recruitment and demographic details for the study population are contained in another report (Kevin Laugero, WHNRC, Davis, CA, *Stress*, under review).

For each sex group, we established nine sampling bins to recruit subjects evenly by age and BMI, with three age (18-33, 34-49, 50-65 y) and BMI (18.5-24.99, 25.0-29.99, 30.0-45.0 kg/m^2) criteria. We recruited 19-24 subjects for each bin, except for men with BMI 30-45 kg/m^2 at 18-33 y (n = 13), 34-49 y (n = 17), and 50-65 y (n = 8), women with BMI 30-45 kg/m^2 at 18-33 y (n = 17) and 50-65 y (n = 17), and women with BMI 25-29.9 kg/m^2 at 34-49 y (n = 15).

Each subject visited the WHNRC twice within a 10–14 day period. During the first visit, subjects provided informed consent and were assigned to age and BMI groups. During the second visit, subjects were assessed following a challenge meal test. The night before the test day, each subject was provided the same high carbohydrate meal (approximately 200 g and 873 kcal, consisting 17% kcal from fat, 77% kcal from

carbohydrate, and 7.5% kcal from protein) and asked to eat it by 19 h. Subjects then fasted until the next morning (12 h later), gave a blood sample, and were given the same high-fat liquid challenge meal (approximately 12 fluid oz. and 800 kcal, consisting 60% kcal from fat, 25% kcal from carbohydrates, and 15% kcal from protein). Postprandial blood samples were then drawn 0.5, 3 and 6 h later [33]. Analysis was performed using BMI (kg/m^2) values calculated from height (m) and fasting weight (kg) measurements made during Visit 2.

SNP selection

Five SNPs in *APOA5*, *APOC3*, *APOB*, *APOE*, and *LDLR* were chosen based on their established correlation with lipid-related disease, such as stroke or metabolic syndrome, in genome-wide association studies [29,34–41]; favorable information content (heterozygosity); and high frequency of risk-allele carriers, which would increase detection power. **Table 2** lists SNP and corresponding TaqMan assay IDs, population allele frequencies, nucleotide (codon) changes, physical positions in the human genome (hg38), and the metabolic disease associations. Four SNPs, in *APOA5*, *APOB*, *APOE* and *LDLR*, lead to amino acid variants. The *APOC3* SNP is located upstream of the exon 1 and may regulate transcription [40,42].

Genomic DNA purification and quantification

Blood samples were collected from study subjects at 0.5 h after the challenge meal. Eight milliliters peripheral blood were gently inverted in a PAXgene Blood DNA Tube (Qiagen, Germantown, MD) and stored at -80°C until use. Genomic DNA was purified following maufacturer instructions (Qiagen), measured using a NanoPhotometer P300 (Implen, Westlake Village, CA), and diluted to 25 ng/µL for TaqMan SNP genotyping assays.

SNP genotyping

PCR genotyping assays were performed using TaqMan SNP probe sets (**Table 2**) (ThermoFisher Scientific, Carlsbad, CA), a QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, CA), and 50 ng genomic DNA per reaction. Alleles were called using QuantStudio software. Ambiguous genotype were re-tested in independent PCRs.

Clinical measures

Fasting blood was collected, and serum or plasma was obtained by centrifuging fasting blood samples at 1300 x *g* at 4^{o}C for 10 min. Triglyceride (TG), total cholesterol (TC), HDL-C, LDL-C, and non-esterified free fatty acids (NEFA) were measured using a Cobas Integra 400/800, CHOL2, HDL-C plus 3rd generation, LDLC3 kits (Roche), and a NEFA-HR Series 2 kit (Wako), respectively. All assays were completed on an Integra 400-plus automated Analyzer instrument (Roche).

Statistical analysis

Allele frequencies were determined for each SNP by direct counting. Differences in general characteristics between genotype groups were assessed for significance using Student's *t*-test. A chi-square test was performed and confirmed that the genotypes were in Hardy-Weinberg Equilibrium. Outcome variables were assessed for conformance to the normal distribution via Box-Cox power transformations and transformed if needed, with normality and equal variance test performed; triglyceride values were transformed using natural logarithm, and total cholesterol, HDL-C, LDL-C, and NEFA did not require transformation. The association between lipid parameters and genotypes was tested separately for each SNP with analysis of

variance (ANOVA), adjusted for sex, age, and BMI. Two-factor interactions between SNPs and sex, age, or BMI were also examined. General characteristics are presented as mean ± S.E. with $P < 0.05$ as the significance threshold.

Results

Serum lipid values

The objective of this study was to understand potential genetic contributions of apolipoprotein (*APOA5*, *APOB*, *APOC3*, *APOE*) and *LDLR* genes to clinical lipid measures, including total cholesterol (TC), HDL-C, LDL-C, triglycerides (TG), and non-esterified free fatty acids (NEFA), during the fasting state and after a high-fat liquid challenge meal. A total of 393 subjects were enrolled and completed the nutritional phenotyping study. Genomic DNA samples were available for 349 subjects (88.8% of total) (**Fig. 3**), including 167 men (48%) and 182 women (52%). Among 167 men, 57 (34.1%), 59 (35.3%), and 51 (30.5%) were in 18-33, 34-49, and 50-65-year age groups, respectively. Among 182 women, 63 (34.1%), 58 (31.9%), and 61 (33.5%) were in 18-33, 34-49, and 50-65-year age groups, respectively. A total of 135 (65 men and 70 women), 127 (66 men and 61 women), and 87 (36 men and 51 women) subjects were in BMI groups with 18.5-24.9 (normal weight), 34-29.9 (overweight), and 30-45 (obese) kg/m^2, respectively.

General morphometric features of 349 participants (**Table 3**) were greater in men than women ($P < 0.01$). Fasting blood lipid profiles, including TC, HDL-C, LDL-C, TG, and NEFA levels (**Fig. 4**), for most subjects were within the desirable range for healthy individuals in each age group, based on Lipid Research Clinic (LRC) reference values [43], with TC 170-235 mg/dL for men and 175-250 mg/dL for women; LDL-C 105-165 mg/dL for men and 110-170 mg/dL for women; HDL-C 30-35 mg/dL for men and 40 mg/dL for women, and triglycerides 120-210 mg/dL for men and 115-205 mg/dL for women (**Fig. 4**). Reference values for fasting plasma NEFA concentrations in men and women have not been established, but in this study, we observed that the average values for fasting plasma NEFA concentrations (mEq/L) were 0.29 ± 0.01 for men and 0.35 ± 0.01 for women. Women had 20% higher fasting NEFA concentrations than men ($P < 0.01$). NEFA levels were positively correlated with BMI (0.29 ± 0.01 mEq/L in the 18.5-24.9 kg/m^2 group; 0.31

± 0.01 mEq/L in the 25.0-29.9 group, and 0.39 ± 0.01 mEq/L in the 30.0-45.0 group; $P < 0.0001$), and the correlation between NEFA and age approached significance overall (0.30 ± 0.01 mEq/L in the 18.5-33 y group; 0.33 ± 0.01 mEq/L in the 34-49 y group; and 0.34 ± 0.01 mEq/L in the 50-65 y group; $P = 0.054$). Among the lipid measures examined, only the fasting HDL-C concentration differed significantly between men and women ($P < 0.01$), with women having higher HDL-C than men (**Fig. 4**).

SNP carrier frequency

Among 349 participants, 24 (6.9%) had a CC or CG genotype of rs3135506 (*APOA5*) with C being the risk allele for its association with hypertriglyceridemia and cardiovascular diseases [44] while 91 (26.2%) had a GG or GA genotype of rs1042034 (*APOB*) with G being a risk allele for hyperlipidemia and ischemic stroke [45,46]. In addition, 147 subjects (42.2%) carried the C risk allele of rs2854116 (*APOC3*) that has been reported to be associated with hypertriglyceridemia and nonalcoholic fatty liver disease [47,48]. Moreover, 50 subjects (14.3%) had a CC or CT genotype of rs429358 (*APOE*) with C being the risk allele that has been reported to be associated with cardiovascular and Alzheimer disease [49–51]. Lastly, 33 subjects (9.4%) had a T genotype of rs2228671 (*LDLR*) with T being the risk allele for hypercholesterolemia [52]. These allele and genotype frequencies are consistent with the 1000 Genomes Project database (**Supplemental Table 1**) and evenly distributed across age/BMI/sex groups (**Supplemental Table 2**).

SNP association with fasting serum lipid levels

Four of the five tested SNPs cause amino acid substitutions and thus may have a dominant effect on protein function. We therefore applied a dominant model to analyze the association between risk alleles at each SNP with clinical lipid measures, such as CC + CG vs. GG for rs3135506 (*APOA5*); GG + AG vs. AA for rs1042034 (*APOB*); CC + CT vs. TT for rs2854116 (*APOC3*) and rs429358 (*APOE*); and CT + TT vs. CC

for rs2228671 (*LDLR*). As shown in **Table 4**, the adjusted mean (corrected for sex, age, and BMI) fasting LDL-C levels were higher by 8% (8.8 mg/dL; $P < 0.05$) in the subjects carrying the CC or CT genotype of rs429358 (*APOE*). Conversely, the adjusted mean fasting HDL-C levels were reduced in these subjects by 7% (3.7 mg/dL; $P < 0.05$). Moreover, a significant association was detected between fasting TC levels and the *APOC3* SNP. Subjects carrying the CC or CT genotype of rs2854116 (*APOC3*) had higher TC levels by 5% (8.0 mg/L; $P < 0.05$) than those carrying the TT genotype. Fasting LDL-C levels were also elevated in subjects with high TC levels, but the *APOC3* association did not reach statistical significance ($P = 0.06$). No correlation could be established between *APOA5*, *APOB* and *LDLR* SNPs and fasting TG, TC, HDL-C, LDL-C or NEFA levels, although the adjusted mean for fasting HDL-C levels were lower by 7% (4.0 mg/dL; $P = 0.08$) in individuals carrying the dominant C risk allele of the *APOA5* SNP. Likewise, no significant association was detected between *APOE* and *APOC3* SNPs and fasting TG or NEFA levels (**Table 4**).

Apolipoprotein genotype-sex interactions have been reported for HDL-C levels [53]. We, therefore, investigated whether sex-specific changes in HDL-C are associated with apolipoprotein and *LDLR* SNP genotypes. As shown in **Table 5**, women had significantly higher fasting HDL-C levels than men regardless of their tested genotypes ($P < 0.01$) except for the CC or CG genotype of rs3135506 (*APOA5*; $P > 0.05$; **Table 5**). Women carrying the CC or CG risk genotype of rs3135506 had ~17% (9 mg/dL) lower HDL-C than GG homozygotes ($P < 0.05$). However, this difference was not detected in men ($P > 0.05$). Specifically, women carrying the C risk allele of rs3135506 had similar HDL-C levels as men. Moreover, men carrying the C risk allele of rs429358 (*APOE*) had significantly lower HDL-C levels (~12%; 5 mg/dL) than those with the TT genotype (**Table 5**) whereas this effect was not noted in women. Taken together, sex appeared to increase the association between rs3135506 and rs429358 genotypes with HDL-C levels ($P < 0.05$ after adjusting for age and BMI).

APOA5 and *APOE* SNP effects on postprandial HDL-C levels after lipid challenge

To further analyze the association between *APOA5* and *APOE* SNPs and HDL-C metabolism, we performed an ANOVA between genotypes and HDL-C levels in men and women at four timepoints – 0, 0.5, 3 and 6 hours after lipid challenge (**Fig. 5**). Women carrying the dominant C risk allele of the *APOA5* SNP had significantly decreased HDL-C levels at all times compared to those with the GG genotype, by ~17% (9 mg/dL, $P < 0.05$). Likewise, men carrying the risk dominant C risk allele of the *APOE* SNP had lower levels of HDL-C than non-carriers by ~12% (5 mg/dL, $P < 0.05$) before and after lipid challenge. Together, *APOA5* (rs3135506) and *APOE* (rs429358) risk alleles are negatively correlated with HDL cholesterol concentrations in a sex-dependent manner ($P < 0.05$ for the genotype-by-sex interaction after adjusting for age and BMI). However, the kinetics of postprandial HDL-C clearance after lipid challenge was not affected by the *APOA5* and *APOE* SNP genotypes in men or women, despite their effect on baseline HDL-C levels.

APOC3 and *APOE* SNP effects on postprandial TC and LDL-C levels after lipid challenge

As noted above, *APOC3* and *APOE* genotypes are correlated with fasting TC and LDL-C concentrations (**Table 4**). To further investigate this relationship, we assessed postprandial TC and LDL-C levels in the study population following lipid challenge. As shown in **Fig. 6A**, male and female subjects carrying the *APOC3* C risk allele had significantly higher TC concentrations than non-carriers at 0 and 0.5-hour timepoints post lipid challenge by ~4% (8 mg/dL after adjusting for age and BMI). TC levels remained high in risk allele carriers at 3 and 6-h timepoints but did not reach statistical significance ($P = 0.05$ and 0.08, respectively). Moreover, the *APOE* SNP-mediated difference in fasting LDL-C levels (**Table 4**) was mainly contributed by men ($P < 0.05$ for the genotype-by-sex interaction after adjusting for age and BMI). As shown in **Fig. 6B**, men carrying the C risk allele had ~7% (8 mg/dL) higher LDL-C levels than those

with the TT genotype ($P < 0.05$) and this difference was maintained postprandially. A similar trend was observed in women before and after lipid challenge, but no statistical significance was detected ($P > 0.05$). As noted above, the postprandial clearance of TC and LDL-C after lipid challenge was not correlated with *APOC3* or *APOE* SNP genotypes (**Fig. 6A, B**).

Additive effects of *APOA5*/*APOE* and *APOC3*/*APOE* SNPs on lipid markers

Since the risk genotypes of *APOA5* ($P = 0.08$) and *APOE* ($P < 0.05$) SNPs (**Table 4**) were each negatively correlated with fasting HDL-C levels, we next examined whether subjects who carry risk alleles at both loci showed more extreme blood lipid levels, i.e. whether risk alleles have additive effects on TC, HDL-C, LDL-C, TG and NEFA levels before and after lipid challenge. As shown in **Table 6**, among 349 subjects, 18 carried both risk alleles of *APOA5* and *APOE* SNPs and 224 were non-carriers. An additive effect of *APOA5* and *APOE* risk alleles was observed for HDL-C (~10-12% or ~6 mg/dL) for all timepoints. However, it was not statistically significant after the data were adjusted for age, sex and BMI ($P > 0.05$) (**Table 6**). Importantly, fasting LDL-C was significantly increased by ~12% (15 mg/dL) in subjects carrying both *APOA5* and *APOE* risk alleles, compared to the non-carriers ($P < 0.05$). The difference was consistent at all postprandial timepoints ($P < 0.05$), suggesting a baseline effect on LDL-C levels. Nevertheless, other lipid measures, such as TC, TG, and NEFA, were not significantly affected by the combination of the risk alleles of *APOA5* and *APOE* (**Table 6**). Lastly, subjects carrying the double risk alleles at *APOA5* and *APOE* had similar postprandial clearance patterns for HDL-C and LDL-C as subjects carrying the non-risk alleles at both loci (**Table 6**).

Additive effects of *APOC3* and *APOE* risk alleles on lipid markers were also evaluated in fasting and postprandial states. As shown in **Table 7**, carriers of the double risk alleles (CC or CT for both *APOC3* and

APOE; n = 21) had significantly lower NEFA concentrations 6 h after lipid challenge (decreased by ~24% or 0.11 mEq/L; $P < 0.01$ after adjusting for age, sex, and BMI) than non-carriers (n = 130), but other lipid markers were not altered between these groups.

Discussion

In this study, we draw an association between SNPs in lipid transport genes and clinical lipid-metabolic parameters, including the clearance kinetics of blood lipids following a liquid high-fat meal. Three of five clinical lipid markers measured (total cholesterol, HDL-C, and LDL-C) were significantly associated with tested SNPs in *APOA5*, *APOE*, and *APOC3*, which are key components of HDL, LDL and TGRL particles, respectively.

In *APOA5*, we showed that the risk allele of rs3135506 is significantly associated with blood HDL-C levels in a sex-dependent manner. Specifically, the dominant risk C allele was correlated with lower HDL-C levels in women (**Fig. 5**). In general, women have higher HDL-C than men [54]. Our results demonstrate that this sex difference is diminished in women carrying the risk allele of rs3135506. Clinically, HDL-C is considered to be "good cholesterol" with protective effects against coronary heart disease [55–58]. It acts by transporting cholesterol from extrahepatic tissues, including steroidogenic tissues and arteries, to the liver [55–58]. These data are the first to show an association between rs3135506 genotypes and HDL-C levels in healthy women before and after a meal challenge. The rs3135506 C allele could thus act as an independent cardiovascular risk factor in women.

APOA5 is thought to activate proteoglycan-bound lipoprotein lipase in the vascular wall indirectly, by suppressing angiopoietin-like protein 3/8 (ANGPTL3/8) inhibition, which is a complex that drastically increase ANGPTL3 inhibition of LPL in the skeletal muscle, resulting in increased circulating triglycerides [8,59,60]. Accordingly, null mutations in *APOA5* lead to high serum TG levels [9]. Therefore, its role in TG metabolism and the therapeutic modification of TG levels have been major research topics. Surprisingly,

in the current study cohort, we did not detect an association between the rs3135506 risk allele and TG levels in healthy adults or lower HDL-C in healthy women. APOA5, via the rs3135506 variant, may profoundly modulate HDL-C metabolism, independently from its direct impact on blood triglyceride levels. A functional study has shown that as the hydrophilic serine is changed to hydrophobic tryptophan (Ser19Trp), it alters the signal peptide function of the third common *APOA5* haplotype by changing the insertion angle of APOA5 into the lipid bilayer[61]. Therefore, as one potential mechanism, the *APOA5* variant (Ser19Trp) may directly affect the maturation of HDL particles. First, APOA5 is a critical component of HDL particles even though it is less abundant than other apolipoproteins [62]. Second, circulating TG levels directly reflect the TG content of triglyceride-rich lipoproteins (TGRL) [63]. When TG levels rise, APOA5 translocates to TGRL. Conversely, as triglycerides in TGRL are hydrolyzed, APOA5 slowly returns to HDL[64]. Lastly, APOA5 directly stimulates the activity of lecithin-cholesterol acyltransferase (LCAT), which promotes cholesterol efflux [65]. APOA5 may thus modulate HDL and cholesterol metabolism; the exact mechanisms will require structural and functional studies.

In *APOE*, we found that the risk C allele of rs429358 could significantly increase LDL-C levels in both fasting and postprandial states (**Table 4** and **Fig. 6**). Most importantly, this risk allele also had a negative impact on HDL-C levels in men (**Table 5**). Moreover, when risk alleles of rs429358 and rs3135506 were combined, the difference in LDL-C levels between risk allele carriers and non-carriers increased significantly (**Table 6**). It is well known that elevated LDL-C is a risk factor for coronary heart disease [66]. APOE is a key ligand for LDLR [67] and thus reduces the risk of atherosclerosis by clearing chylomicrons and VLDL remnant particles from the circulation. *APOE* alleles are correlated with LDL-C levels, increasing in order *APOE ε2* < *ε3* < *ε4* [68]. The *APOE ε4* haplotype includes the C allele of rs429358 and C allele of rs7412. The encoded APOE ε4 isoform binds preferentially to VLDL whereas the APOE ε3

isoform binds preferentially to HDL [69,70]. This property is strongly associated with an atherogenic lipoprotein phenotype in APOE ε4 carriers. Together, our results suggest that the C allele of rs429358 has a detrimental effect on cholesterol metabolism, especially in men, and hence men carrying this risk allele have a higher risk of cardiovascular disease than non-carriers.

In *APOC3*, our results revealed associations between rs2854116 genotype and total cholesterol levels and, to a lesser extent, fasting and postprandial LDL-C levels (**Fig. 6**). In a previous meta-analysis, the rs2854116 risk allele was positively associated with TG levels and negatively associated with HDL-C levels [71]. APOC3 protein is primarily found in cholesterol-rich particles, such as chylomicron, VLDL, and LDL, and it inhibits lipoprotein lipase [17]. Our findings suggest APOC3 may be a key regulator of cholesterol metabolism, as well as TG metabolism [71]. This observation is supported by genetic data from a northern French population linking rs2854116 to elevated LDL-C in women [72]. However, the molecular mechanism for the association remains unclear. The rs2854116 variant is located upstream of the *APOC3* transcription start site and may thus influence mRNA expression. If the risk allele decreased the abundance APOC3 protein, this would increase the conversion of VLDL to LDL particles, via reduced inhibition of lipoprotein lipase [47], and decrease hepatic uptake of VLDL remnants – thus elevating total cholesterol and LDL-C levels in serum.

Interestingly, we observed an inverse association between serum NEFA levels and combined *APOC3* (rs2854116) and *APOE* (rs429358) risk alleles 6-h after lipid challenge (**Table 7**). Reduced circulating NEFA may reflect a lower capacity to oxidize fat and mobilize NEFA from adipose tissue to the bloodstream, leading to metabolic deterioration [73,74]. Indeed, metabolically healthy obese individuals with high fasting NEFA levels are less likely to develop type 2 diabetes than insulin-resistant individuals [75].

Accordingly, the reduction in 6-h circulating NEFA levels we observed suggests that subjects carrying double *APOC3* and *APOE* risk alleles have a lower capacity to oxidize fat, which may increase their risks for obesity as well as type 2 diabetes.

Although *APOA5* and *APOC3* have been reported to be in the *APOA1*/*APOC3*/*APOA4*/*APOA5* gene cluster on human chromosome 11q23 in a genetic interval of ~60kb [8,76,77], the effects of *APOA5* have also been reported to be independent from the effect of *APOC3* [77], as a study had shown that the two gene regions had been separated by historical recombination events [78], and they are not in linkage disequilibrium [78,79], which this current study has supported the previous finding as the statistically significant effects of the SNPs in *APOA5* and *APOC3* were different, with former had a significant effect on HDL-C and the latter had a significant effect on cholesterol and LDL-C. As a result, the results of SNPs in *APOA5* and *APOC3* were evaluated independently in this study, rather than as a gene cluster.

Our study reveals strong associations between SNPs in lipid transport genes and cholesterol profiles. However, some limitations confound the findings. First, the cohort size was relatively small, which limits its power, despite dramatic differences between subject bins. Second, other relevant serum phenotypes – lipoprotein particle size, which affects the specific function of particles within each subtype [80,81] and plasma phospholipid-transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) levels, which critically mobilize lipids [82] – were not measured. Future studies, with larger cohorts and expanded metrics, may thus provide further information connecting SNP genotypes and lipoprotein subtypes.

Conclusions

We identified significant associations between SNPs rs3135506 (*APOA5*), rs429358 (*APOE*) and rs2854116 (*APOC3*) and cholesterol metabolism, including *APOA5* and *APOC3* SNPs previously shown to influence TG levels. Moreover, we show that *APOA5* and *APOE* SNPs effects on HDL-C and LDL-C levels are sex-dependent. The apolipoprotein SNP variants have greatest impact in the fasting state. To our knowledge, the current study is the first to investigate the effect of apolipoprotein alleles on blood lipid profiles in healthy adults challenged with a liquid high-fat diet. Our findings offer new insights regarding the roles of *APOA5*, *APOE* and *APOC3* in modulating cholesterol and NEFA metabolism. Accordingly, individuals carrying non-risk alleles for tested *APOA5*, *APOE* and *APOC3* SNPs may have reduced genetic risk of cardiovascular disease. More studies are needed to assess sex-specific genetic risks for cardiovascular disease.

Tables

Table 1 Descriptions of apolipoproteins [83]

Apolipoprotein	MW	Primary Source	Lipoprotein Association	Function
Apo A-I	28,000	Liver, Intestine	HDL, chylomicrons	Structural protein for HDL, Activates LCAT
Apo A-II	17,000	Liver	HDL, chylomicrons	Structural protein for HDL, Activates hepatic lipase
Apo A-IV	45,000	Intestine	HDL, chylomicrons	Unknown
Apo A-V	39,000	Liver	VLDL, chylomicrons, HDL	Promotes LPL mediated TG lipolysis
Apo B-48	241,000	Intestine	Chylomicrons	Structural protein for chylomicrons
Apo B-100	512,000	Liver	VLDL, IDL, LDL, Lp (a)	Structural protein, Ligand for LDL receptor
Apo C-I	6,600	Liver	Chylomicrons, VLDL, HDL	Activates LCAT
Apo C-II	8,800	Liver	Chylomicrons, VLDL, HDL	Co-factor for LPL
Apo C-III	8,800	Liver	Chylomicrons, VLDL, HDL	Inhibits LPL and uptake of lipoproteins
Apo E	34,000	Liver	Chylomicron remnants, IDL, HDL	Ligand for LDL receptor
Apo (a)	250,000-800,00	Liver	Lp (a)	Inhibits plasminogen activation

Table 2 SNPs used in this study

Gene	SNP ID	TaqMan assay ID	Nucleotide substitution	Frequency[a]	Codon substitution	Genome position (GRCh38.p12)	Associated diseases with null mutations
APOA5	rs3135506	C_25638153_10	C>G	C=0.056 (279/5008)	S[TCG]>W[TGG]	chr11:116,791,691	Familial hypertriglyceridemia
APOB	rs1042034	C_7615376_20	G>A	G=0.370 (1855/5008)	S[AGT]>N[AAT]	chr2:21,002,409	Familial hypercholesterolemia II
APOC3	rs2854116	C_12081482_20	C>T	T=0.452 (2262/5008)	n.a.[b]	chr11:116,829,453	Hypertriglyceridemia Nonalcoholic fatty liver disease
APOE	rs429358	C_3084793_20	C>T	C=0.151 (754/5008)	R[CGC]>C[TGC]	chr19:44,908,684	Hyperlipoproteinemia type III
LDLR	rs2228671	C_27208873_10	C>T	T=0.057 (285/5008)	C[TGC]>C[TGT]	chr19:11,100,236	Familial hypercholesterolemia I

a. Based on the data from the 1000 Genomes Project (https://www.internationalgenome.org/). *b*. Upstream Transcript Variant. Risk alleles are underlined. The bases affecting the condon substitution has been indicated in the red. The ">" indicates the substitution of bases, where SNPs for *APOA5* (rs3135506), *APOB* (rs1042034), *APOC3* (rs2854116), and *APOE* (rs429358) have replaced the risk alleles with non-risk alleles, and SNP for *LDLR* (rs2228671) has replaced the non-risk allele with risk-allele.

Table 3 General characteristics of study participants

	Men	Women
N	167 (48%)	182 (52%)
Age (y)	39.7±14.0 (38.0; 18.0-65.0)	40.6±13.7 (41.0; 19.0-65.0)
Height (cm)[a]	177.9±7.6 (177.4; 161.8-201.7)	163.6±6.9 (163.9; 146.3-180.6)
Weight (kg)[a]	86.2±17.8 (84.2; 50.3-175.2)	74.6±16.2 (71.9; 44.9-121.2)
Body mass index (kg/m^2)	27.1±4.7 (26.2; 18.2-43.9)	27.8±5.3 (27.1; 18.0-43.3)
Waist circumference (cm)†	88.7±12.8 (86.0; 63.3-138.4)	82.6±12.2 (80.4; 60.8-119.7)

Data are shown as number (percentage) and metric data as mean ± SD (median; range). *a*, statistically significant between men and women ($P < 0.05$).

Table 4 Fasting blood lipid concentrations based on SNPs in the examined genes

Gene & genotype (n)	TG (mg/dL)	Total cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	NEFA (mEq/L)
APOA5					
CC (1) + CG (46)	90.69±5.20	179.3±4.67	52.04±1.88	116.7±4.23	0.31±0.02
GG (302)	87.72±1.92	174.2±1.81	55.78±0.96	108.7±1.64	0.32±0.01
% change of the C allele	3.27	2.84	-7.19	6.86	-3.23
APOB					
GG (27) + AG (127)	88.57±2.80	174.7±2.57	56.43±1.10	108.9±2.33	0.32±0.01
AA (192)	87.74±2.48	175.0±2.30	54.39±0.98	110.4±2.08	0.32±0.01
% change of the G allele	0.95	-0.17	3.62	-1.38	0
APOC3					
CC (63) + CT (167)	87.74±2.25	177.5±2.07	55.95±0.90	111.8±1.89	0.33±0.01
TT (117)	88.30±3.19	169.5±2.93	54.01±1.27	105.7±2.67	0.30±0.01
% change of the C allele	-0.64	4.51*	3.47	5.46	9.09
APOE					
CC (4) + CT (92)	93.08±3.74	179.1±3.28	52.62±1.41	116.2±2.96	0.33±0.01
TT (253)	86.30±2.11	173.2±1.99	56.29±0.85	107.4±1.80	0.31±0.01
% change of the C allele	7.28	3.29	-6.97*	7.57*	6.06
LDLR					
TT (3) + CT (59)	88.98±4.43	178.8±4.05	57.73±1.74	110.8±3.69	0.32±0.02
CC (286)	87.94±2.02	174.0±1.87	54.74±0.80	109.5±1.70	0.32±0.01
% change of the T allele	1.17	2.68	5.18	1.18	0

Data are presented as mean ± SE. *, $P < 0.05$. Triglyceride (TG) values were transformed to the natural logarithm scale for analysis. Other values were analyzed without transformation. Data were adjusted for sex, age, and body-mass index (BMI). The percent (%) change of each lipid level due to carrying the risk allele of each SNP was calculated by dividing the value of the difference between two genotypes of the SNP by the value of the risk allele and multiplying the answer by 100. HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, non-esterified fatty acids.

Table 5 Fasting serum HDL-C based on SNPs in the examined genes, by sex

Men		Women	
Gene & genotype (n)	HDL-C (mg/dL)	Gene & genotype (n)	HDL-C (mg/dL)
APOA5		*APOA5*	
CC (0) + CG (25)	50.57±2.72	CC (1) + CG (21)	52.88±2.87
GG (142)	49.00±1.13	GG (160)	61.91±1.06[a]
% change of the C allele	3.10%	% Change of the C allele	-17.08[c]
APOB		*APOB*	
GG (12) + AG (60)	49.16±1.60	GG (15) + AG (67)	63.28±1.50[a]
AA (93)	49.51±1.40	AA (99)	58.60±1.38[a]
% change of the G allele	-0.71	% Change of the G allele	7.40
APOC3		*APOC3*	
CC (28) + CT (80)	50.53±1.31	CC (35) + CT (87)	61.10±1.23[a]
TT (58)	46.94±1.79	TT (59)	60.24±1.78[a]
% change of the C allele	7.10	% Change of the C allele	1.41
APOE		*APOE*	
CC (2) + CT (38)	45.22±2.16	CC (2) + CT (54)	59.45±1.82[a]
TT (127)	50.50±1.21	TT (126)	61.43±1.21[a]
% change of the C allele	-11.68[b]	% Change of the C allele	-3.33
LDLR		LDLR	
TT (2) + CT (29)	50.61±2.45	TT (1) + CT (30)	64.46±2.45[a]
CC (135)	48.89±1.17	CC (151)	60.06±1.11[a]
% change of the T allele	3.40	% Change of the T allele	6.83

Data were adjusted for age and BMI in each sex group and are presented as mean ± SE. *a*, $P < 0.01$ between men and women of the indicated genotype; *b*, $P < 0.05$ and *c*, $P < 0.01$ between genotypes of the indicated gene. The percent (%) change of HDL-C levels due to carrying the risk allele of each SNP was calculated by dividing the value of the difference between two genotypes of the SNP by the value of the risk allele and multiplying the answer by 100.

Table 6 Lipid concentrations after dietary challenge in subjects carrying both risk alleles of *APOA5* and *APOE*

Gene & genotype (n)	Time (h) after dietary challenge			
	0	0.5	3	6
APOA5/APOE, GG/TT (224)[a]				
Triglycerides (mg/dL)	87.05±2.3	97.30±2.6	178.5±5.5	148.3±4.7
Total cholesterol (mg/dL)	172.8±2.11	180.8±2.24	174.3±2.16	176.9±2.20
HDL-C (mg/dL)	56.07±0.88	58.20±0.91	53.35±0.88	52.48±0.88
LDL-C (mg/dL)	107.1±1.90	111.2±1.99	102.4±1.84	104.8±1.90
NEFA (mEq/L)	0.32±0.01	0.23±0.01	0.26±0.01	0.56±0.01
APOA5/APOE, CC+CG/CC+CT (18)[b]				
Triglycerides (mg/dL)	93.09±9.0	101.8±9.9	199.7±22.8	170.6±19.9
% change of the risk alleles	6.49	4.42	10.62	13.07
Total cholesterol (mg/dL)	182.2±7.85	191.7±8.30	185.9±8.00	189.1±8.09
% change of the risk alleles	5.16	5.69	6.24	6.45
HDL-C (mg/dL)	49.99±3.28	52.96±3.35	47.90±3.25	47.30±3.23
% change of the risk alleles	-12.16	-9.89	-11.38	-10.95
LDL-C (mg/dL)	122.0±7.07	127.0±7.36	117.8±6.83	119.3±6.99
% change of the risk alleles	12.21[c]	12.44[*]	13.07[c]	12.15[*]
NEFA (mEq/L)	0.30±0.03	0.21±0.03	0.27±0.03	0.52±0.04
% change of the risk alleles	-6.67	-9.52	3.70	-7.69

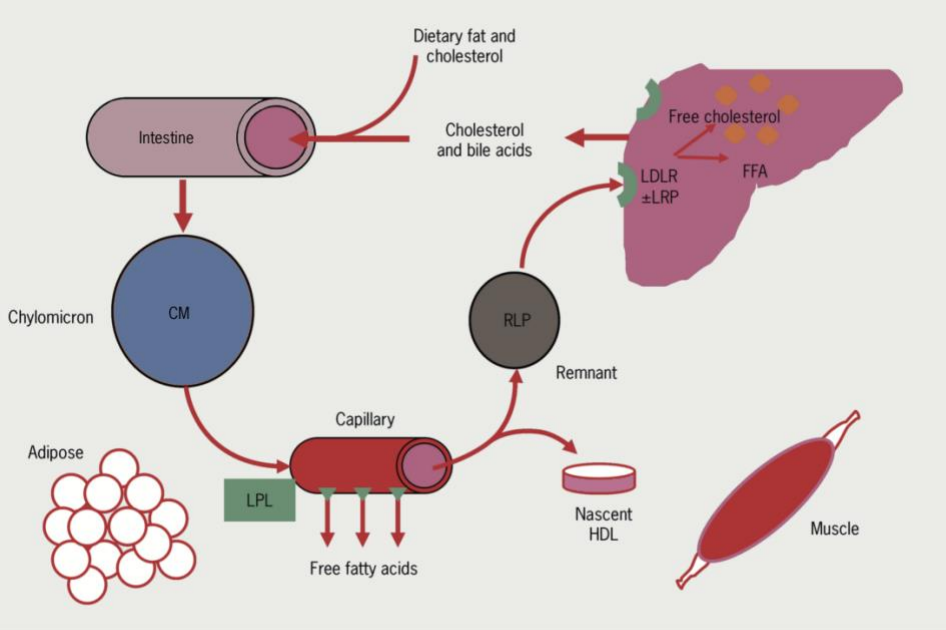
Data were adjusted for sex, age, and BMI and are presented as mean ± SE. *a*, subjects carrying both non-risk alleles of *APOA5* and *APOE*; *b*, subjects carrying both risk alleles of *APOA5* and *APOE*. *, $P < 0.05$. The percent (%) change of each lipid level due to carrying the combination of the risk alleles of *APOA5* and *APOE* was calculated by dividing the value of the difference between CC+CG/CC+CT and GG/TT genotypes of *APOA5*/*APOE* by the value of CC+CG/CC+CT genotype and multiplying the answer by 100.

Table 7 Lipid concentrations after dietary challenge in subjects carrying both risk alleles of *APOC3* and *APOE*

Gene & genotype (n)	Time (h) after dietary challenge			
	0	0.5	3	6
APOC3/APOE, TT/TT (130)[a]				
Triglycerides (mg/dL)	87.14±2.94	97.17±3.45	176.2±7.02	148.6±6.53
Total cholesterol (mg/dL)	178.0±2.98	186.0±3.15	179.1±3.09	181.6±3.13
HDL-C (mg/dL)	55.78±1.30	58.00±1.36	52.90±1.29	52.35±1.30
LDL-C (mg/dL)	112.6±2.80	116.7±2.91	107.8±2.73	109.6±2.80
NEFA (mEq/L)	0.33±0.01	0.24±0.01	0.25±0.01	0.56±0.01
APOC3/APOE, CC+CT/CC+CT (21)[b]				
Triglycerides (mg/dL)	86.48±7.53	96.38±9.08	172.3±17.66	140.5±15.72
% change of the risk alleles	-0.76	-0.82	-2.26	-5.77
Total cholesterol (mg/dL)	184.1±7.70	193.7±8.35	186.6±7.93	189.3±8.02
% change of the risk alleles	3.31	3.98	4.02	4.07
HDL-C (mg/dL)	55.00±3.36	57.59±3.61	53.36±3.12	52.22±3.33
% change of the risk alleles	-1.42	-0.71	0.86	-0.25
LDL-C (mg/dL)	121.5±7.23	127.1±7.72	117.7±7.01	119.7±7.16
% change of the risk alleles	7.33	8.18	8.41	8.44
NEFA (mEq/L)	0.29±0.03	0.23±0.03	0.21±0.02	0.45±0.03
% change of the risk alleles	-13.79	-1.72	-15.96	-24.28**

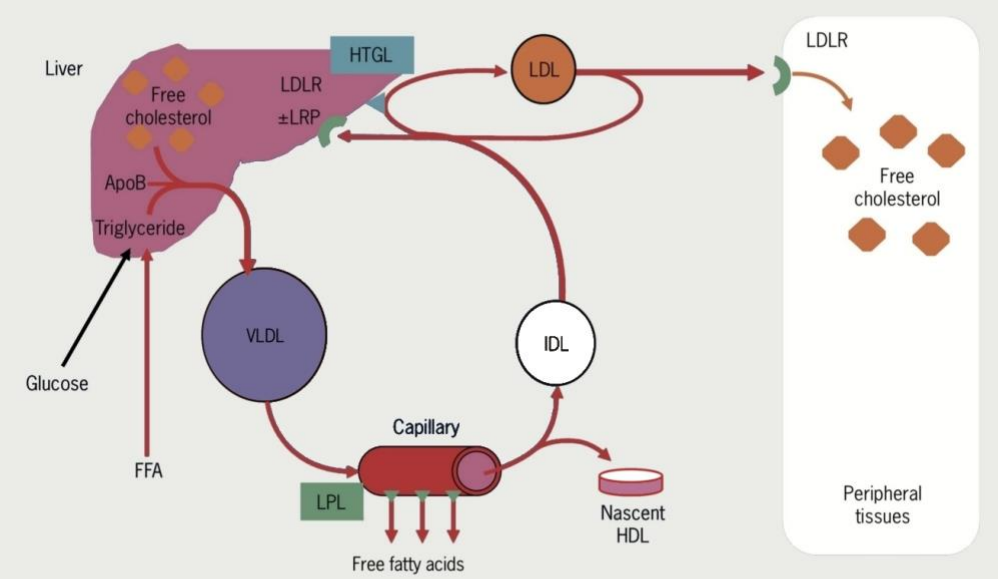
Data were adjusted to sex, age, and BMI and are presented as mean ± SE. *a*, subjects carrying both non-risk alleles of *APOC3* and *APOE*; *b*, subjects carrying both risk alleles of *APOC3* and *APOE*. **, $P < 0.01$. The percent (%) change of each lipid level due to carrying the combination of the risk alleles of *APOC3* and *APOE* was calculated by dividing the value of the difference between CC+CT/CC+CT and TT/TT genotypes of *APOC3*/*APOE* by the value of CC+CT/CC+CT genotype and multiplying the answer by 100.

Figures



Key: CM = chylomicron; FFA = free fatty acids; HDL = high-density lipoprotein cholesterol; LDLR = low density lipoprotein receptor; LPL = lipoprotein lipase; LRP = LDL receptor-related protein; RLP = remnant lipoprotein

Fig. 1. Intestinal (exogenous pathway) of lipid metabolism[84]. Lipids are absorbed from the gut, where triglycerides are broken down into free fatty acids, monoacylglycerols and cholesterol, which are resynthesized into triglyceride-rich lipoproteins, i.e. chylomicrons. Lymphatic and blood vascular systems transport these lipoproteins to liver and other tissues. Chylomicron fat is delivered to adipose tissue via lipoprotein lipase as free fatty acids. Once chylomicron remnants become small enough, they are taken up by the liver via APOE binding to the remnant receptor (LRP) or low-density lipoprotein receptor (LDLR) on the surface of hepatocytes. Remaining surface phospholipids and apolipoproteins join the HDL pool as the 'nascent HDL.'



Key: ApoB = apolipoprotein B; FFA = free fatty acids; HDL = high-density lipoprotein cholesterol; HTGL = hepatic triglyceride lipase; IDL = intermediate-density lipoprotein cholesterol; LDLR = low-density lipoprotein receptor; LPL = lipoprotein lipase; LRP = LDL receptor-related protein; VLDL = very low-density lipoprotein cholesterol

Fig. 2. Hepatic (endogenous pathway) of lipid metabolism[84]. Liver fatty acids, stored or newly synthesized via *de novo* lipogenesis, are re-esterified into triglycerides, which are are combined with cholesterol to form VLDL. These particles travel to peripheral tissues, including adipocytes and muscle, and supply fat for catabolism during the fasting state. In adipose tissue, VLDL triglycerides are converted to fatty acid via lipoprotein lipase as they are imported. As VLDL particles are depleted of fat content and their diameter is reduced, they turn into IDL, which are subsequently recycled in the liver – or converted into LDL by hepatic lipase and taken by LDLR on peripheral tissues for local cholesterol needs.

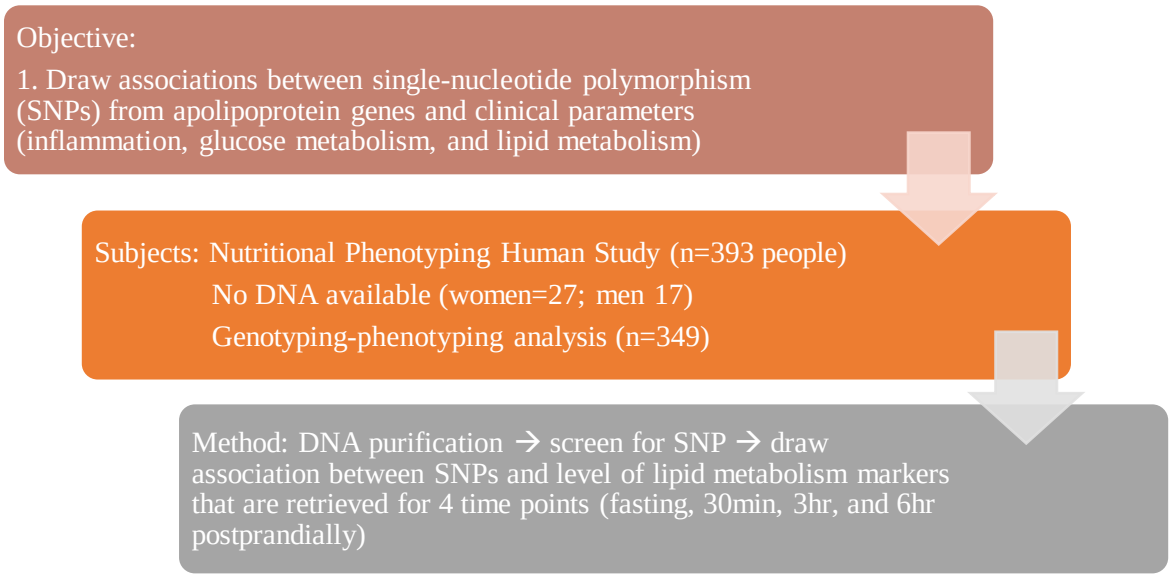


Fig. 3 Study flow chart

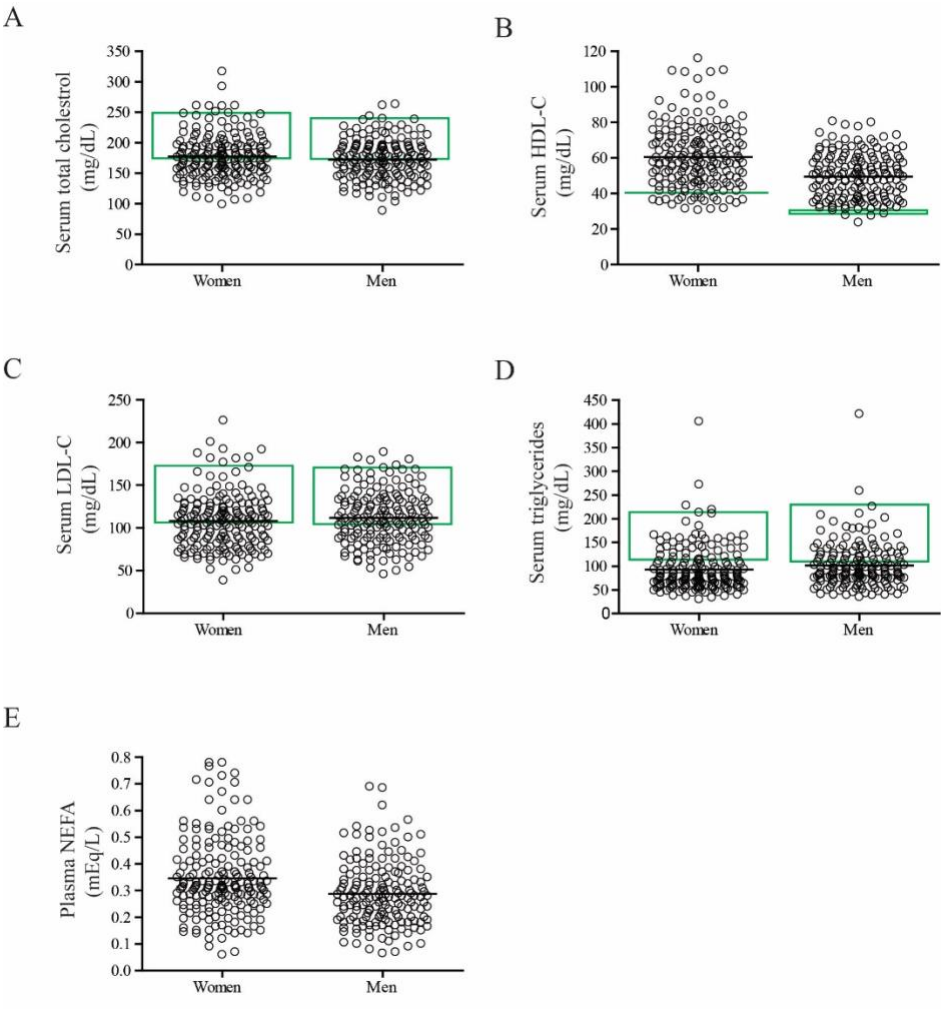
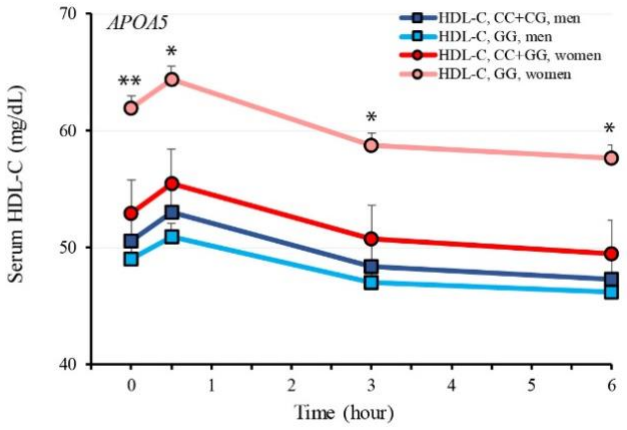


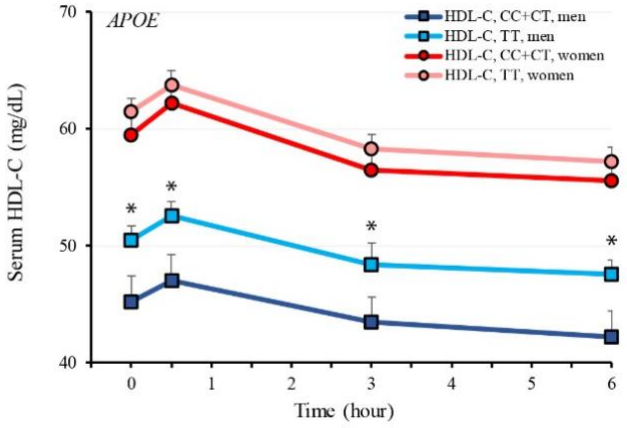
Fig. 4. Fasting lipid measures in subjects. Each open circle represents one subject and the black line is the mean. The green line or box show reference values for 18.5- to 65-year-old categories obtained from the Lipid Research Clinic (LRC) Program Population Studies [43]. The 90th percentile (triglycerides), 75th percentile (cholestrol, LDL-C), and 10th percentile (HDL-C) of LRC data from different age groups were used to determine reference values. Lower (triglycerides, total cholestrol, LDL-C) or higher (HDL-C) concentrations than reference values are desired for reducing coronary artery disease risk.

A



# of Subjects		
	Men	Women
CC+CG	25	22
GG	142	160

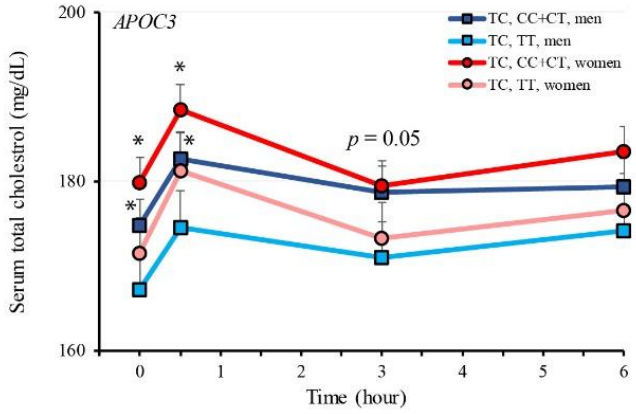
B



# of Subjects		
	Men	Women
CC+CT	40	56
TT	127	126

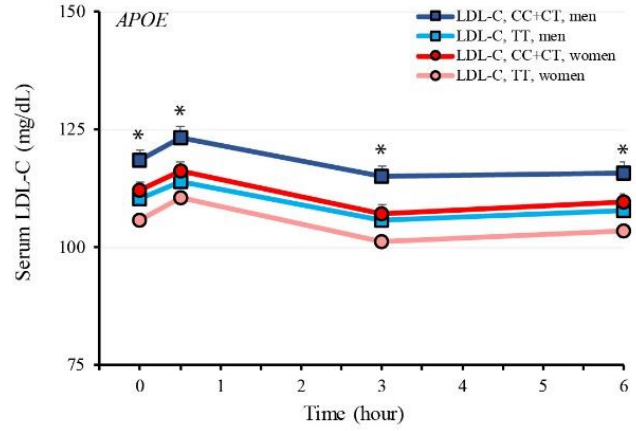
Fig. 5. Effects of [A] *APOA5* rs23135506 and [B] *APOE* rs429358 on blood HDL-C levels after lipid challenge. Subjects were divided into genotypic groups by sex. All data were adjusted for age and BMI, and are presented as mean ± SE. *, $P < 0.05$ and **, $P < 0.01$ for comparisons between genotypic groups within the same sex. Baseline values differ significantly between groups, but the clearance kinetics are similar.

A



# of Subjects		
	Men	Women
CC+CT	108	122
TT	58	59

B



# of Subjects		
	Men	Women
CC+CG	25	22
GG	142	160

Fig. 6. Effects of [A] *APOC3* rs2854116 and [B] *APOE* rs429358 on blood TC and LDL-C levels after lipid challenge. Subjects were divided into genotypic groups by sex. All data were adjusted for age and BMI, and are presented as mean ± SE. *, $P < 0.05$ for comparisons between genotypic groups within the same sex. TC, total cholestrol.

Supplemental Table 1 Comparison between SNP frequencies in this study and the 1KGP

SNP ID	*APOA5* rs3135506		*APOB*[a] rs1042034		*APOC3*[a] rs2854116		*APOE* rs429358		*LDLR*[a] rs2228671	
Allele	C (%)	G (%)	G (%)	A (%)	C (%)	T (%)	C (%)	T (%)	T (%)	C (%)
This study	6.88	93.12	26.16	73.84	42.22	57.78	14.33	85.67	9.34	90.66
1000 genome[b]	5.57	94.43	37.04	62.96	54.83	45.17	15.06	84.94	5.69	94.31

a, Genotypes were determined for all 349 participants with 2 alleles per participant except at *APOB*, 2 men (subject IDs 6100 and 6103) and 1 woman (subject ID 8085); *APOC3*, 1 man (subject ID 9049) and 1 woman (subject ID 9067); and *LDLR*, 1 man (subject ID 9024). *b*, Global population. No statistical difference in the SNP frequencies was found between the current cohort and the global population revealed by the 1000 genome project (1KGP, $n = 1000$). The risk alleles are underlined.

Supplemental Table 2 Distribution of the observed SNP genotype in sex/age/BMI groups

Gene	*APOA5*		*APOB*[a]		*APOC3*[a]		*APOE*		*LDLR*[a]	
Genotype	CC+CG % (n)	GG % (n)	AG+GG % (n)	AA % (n)	CC+CT % (n)	TT % (n)	CC+CT % (n)	TT % (n)	CT+TT % (n)	CC % (n)
Men	15.0 (25)	85.0 (142)	43.6 (72)	56.4 (93)	65.1 (108)	34.9 (58)	24.0 (40)	76.0 (127)	18.7 (31)	81.3 (135)
Women	12.1 (22)	87.9 (160)	45.3 (82)	54.7 (99)	67.4 (122)	32.6 (59)	30.8 (56)	69.2 (126)	17.0 (31)	83.0 (151)
Age (y):										
18-33	15.8 (19)	84.2 (101)	45.8 (55)	54.2 (65)	65.0 (78)	35.0 (42)	29.2 (35)	70.8 (85)	18.3 (22)	81.7 (98)
34-49	13.8 (16)	86.2 (100)	46.6 (54)	53.4 (62)	64.9 (74)	35.1 (40)	28.4 (33)	71.6 (83)	22.6 (26)	77.4 (89)
50-65	10.6 (12)	89.4 (101)	40.9 (45)	59.1 (65)	69.0 (78)	31.0 (35)	24.8 (28)	75.2 (85)	12.4 (14)	87.6 (99)
BMI (kg/m^2)										
18.5-24.9	11.1 (15)	88.9 (120)	49.6 (67)	50.4 (68)	65.7 (88)	34.3 (46)	25.2 (34)	74.8 (101)	17.2 (23)	82.8 (111)
25.0-29.9	14.2 (18)	85.8 (109)	45.2 (56)	54.8 (68)	69.3 (88)	30.7 (39)	29.9 (38)	70.1 (89)	18.1 (23)	81.9 (104)
30.0-45.0	16.1 (14)	83.9 (73)	35.6 (31)	64.4 (56)	62.8 (54)	37.2 (32)	27.6 (24)	72.4 (63)	18.4 (16)	81.6 (71)

a, Genotypes were not determined: *APOB*, 2 men (subject IDs 6100 and 6103) and 1 woman (subject ID 8085) in the groups of 50-65-year-old and BMI 25.0-29.9 kg/m^2; *APOC3*, 1 man (subject ID 9049) in the groups of 34-49-year-old and BMI 30-45 kg/m^2) and 1 woman (subject ID 9067) in the groups of 34-49-year-old and BMI 18.5-24.9 kg/m^2); *LDLR*, 1 man (subject ID 9024) in the groups of 34-49-year-old and BMI 18.5-24.9 kg/m^2. No significant differences were found for genotype distributions among category groups (Chi-square test).

Disclosures

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