Title: SNPs in apolipoproteins contribute to sex-dependent differences in blood lipids before and after a dietary challenge in healthy U.S. adults

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Abstract

Background
The effect of genetic polymorphisms on fasting blood lipid levels have been widely studied but the effects of these within the context of a meal challenge remain less characterized. The current study aimed to investigate the association of SNPs in lipoprotein-related genes with blood lipid levels in healthy adults in the U.S.

Methods
Men and women (n = 393) between 18-66 years of age with BMIs ranging from 18.5-45 kg/m² completed the cross-sectional Nutritional Phenotyping Study. Among them, 349 subjects (men = 167, 48%; women = 182, 52%) gave consent for genotyping. SNPs in APOA5, APOB, APOC3, APOE, and LDLR were assessed.

Results
Reduced blood HDL-cholesterol levels were associated with the C allele of the APOA5 SNP (rs3135506) in women and the C allele of the APOE SNP (rs429358) in men in both fasting and postprandial states. The C allele of the APOE SNP was also correlated with increased LDL-C levels. The APOC3 rs2854116 TT genotype was associated with elevated total cholesterol in both sexes. Nevertheless, these SNPs had little impact on the postprandial triglyceride responses to the high-fat challenge meal. Additionally, no significant effects of SNPs in APOB (rs1042034) and LDLR (rs2228671) on triglycerides, cholesterol, or non-esterified free fatty acids levels were found.

Conclusions
Cholesterol levels are strongly correlated with genotypes of the tested SNPs in APOA5 and APOE in a sex-dependent manner during fasting and postprandial states of healthy adults.
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Introduction

The pathogenesis of cardiovascular diseases (CVDs), being the leading cause of death globally for over a decade and Type 2 diabetes mellitus creating a worldwide health epidemic [1], is closely related to immune activation and inflammation, along with elevated triglycerides and cholesterol, which are triggered by the consumption and accumulation of fat [2]. In lipid metabolism, the process of transporting dietary fat from the intestinal tract to peripheral tissues is accomplished by lipoprotein particles that deliver cholesterol and triglycerides to different sites of the body [3]. In this process, apolipoproteins play crucial roles in transporting lipids as they are recognized by a host of cell surface receptors in target tissues. Taken together, the differences in lipoprotein particle densities, particle sizes, and the types of apolipoproteins on the particles help determine where these particles are taken up by different tissues for usage [4].

Apolipoproteins have been categorized based on their function: APOA, APOB, APOC, and APOE [5]. APOA has four subtypes, APOA1, APOA2, APOA4, and APOA5, which are known as the primary structural proteins of high-density lipoprotein (HDL). APOA5 has been shown to regulate triglyceride homeostasis [6, 7]. While present in HDL, this apolipoprotein is also associated with chylomicrons and very low-density lipoprotein (VLDL), but not with low-density lipoprotein (LDL) [8]. Previous studies had shown that mice overexpressing the human APOA5 had a 70% reduction in plasma triglycerides when compared to control mice [6, 7]. Patients with APOA5 deficiency are hypertriglyceridemic [9].

APOB is a major structural protein for all lipoprotein particles, except HDL. Due to RNA editing, two APOB isoforms exist, APOB48 and APOB100. APOB48 shares 48% of the amino acid sequences of APOB100, but it lacks the C-terminal LDL receptor binding region of APOB100. The two isoforms are functionally and metabolically different. APOB48 is involved in the formation of chylomicrons in the gut, thus it is fundamental in the uptake of exogenous lipids, whereas APOB100 is one of the key components for the synthesis of VLDL and LDL in the endogenous pathway [10]. APOB100 is also a ligand for the LDL receptor (LDLR) which mediates endocytosis of LDL. Genetic mutations in APOB result in disorders
of lipid metabolism, familial hypobetalipoproteinemia and familial ligand-defective APOB100 [11]. High APOB-containing LDL particle levels are strongly associated with atherosclerosis and CVDs [12].

APOC subtypes include APOC1, APOC2, and APOC3 that can be freely exchangeable among lipoprotein particles and closely linked to chylomicron, VLDL and HDL [5]. APOC1 facilitates the esterification of free cholesterol into cholesterol esters in HDL [10]. APOC2 is a co-factor for lipoprotein lipase (LPL), which promotes triglyceride hydrolysis [13]. On the other hand, APOC3 inhibits the APOC2-mediated activation of LPL [5, 10]. APOC3 can significantly reduce the clearance rate of APOB-containing lipoprotein particles by inhibiting the receptor-mediated endocytosis [14]. Thus, APOC3 has been considered as a powerful indicator for CVD risk and dyslipidemia [15].

APOE is an essential apolipoprotein for cholesterol-rich lipoproteins, such as VLDL and cholesterol-rich HDL [16, 17]. There are three major allelic types, ε2, ε3, and ε4, for the APOE gene. Regardless of allelic differences among the three versions of the APOE gene, the primary function of APOE is as a ligand for the LDL receptor-mediated uptake of APOE-containing lipoproteins. However, the allelic differences of APOE affect the capacity of interactions between APOE-containing lipoproteins and the LDL receptor. Individuals carrying homozygous APOE4 alleles have elevated plasma LDL and an increased risk of atherosclerosis and CVDs [16]. In contrast, APOE2 has a reduced binding ability to the LDLR and APOE2 homozygosity is associated with a high prevalence of type III hyperlipoproteinemia, a disorder that results in ectopic fatty deposits and elevates the risk of atherosclerosis [18].

Unlike the apolipoproteins above, LDLR is localized on the cell surface for lipoprotein uptake that directly affects LDL clearance from the circulation [19]. Generally, LDLR binds cholesterol-rich lipoproteins, such as APOB100 and APOE [20]. Genetic mutations in LDLR lead to familial hypercholesterolemia with significantly elevated circulating lipoproteins, which initiates and accelerates atherosclerosis and CVDs [19].

Individual responses to dietary fat can differ in magnitudes and the kinetic behavior is dependent on both genetic and environmental factors [2]. To study the association of single-nucleotide polymorphisms (SNPs) in apolipoproteins and LDLR with the commonly used blood lipid markers, including triglycerides,
cholesterol, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and non-esterified free fatty acids (NEFA), we genotyped individuals recruited for a human cross-sectional nutritional phenotyping study conducted by the United States Department of Agriculture, Agriculture Research Service Western Human Nutrition Research Center on the University of California Davis campus in Davis, California. We found that fasting serum HDL-cholesterol (HDL-C) concentrations were associated with SNPs in APOA5 (rs3135506) in women and in APOE (rs429358) in men. The presence of the risk allele of the APOE SNP also increased serum LDL-C levels in both sexes. Total cholesterol levels were correlated with a SNP in APOC3 (rs2854116) in a sex-independent manner. However, these SNPs had little impact on the triglyceride appearance or removal in the circulation after a high-fat liquid meal challenge.
Materials and methods

Study subjects and dietary challenge

Three hundred ninety-three healthy U.S. adults aged 18-66 years old with BMI ranges of 18.5 to 45.0 kg/m² were enrolled for a cross-sectional Nutritional Phenotyping Study (ClinicalTrials.gov, ID: NCT02367287). It should be acknowledged that the original sampling plan had an upper limit of BMI at 39.9 kg/m² [21], but the study was modified to increase the upper BMI to 45 kg/m². Pregnant or lactating women were excluded from the study. Other exclusion criteria included a known allergy to egg, recent surgeries or hospitalization (minor surgeries for the last 4 weeks or major surgeries/hospitalization for last 4 months), antibiotic treatments for last 4 weeks. Individuals who take daily medication for a diagnosed chronic disease at the time of the study were also excluded [21]. Details of recruitment and demographic characteristics of the overall population are contained within another report on this study under consideration for publication in Stress: International Journal on the Biology of Stress (personal communication, Dr. Kevin Laugero, USDA/ARS/WHNRC, Davis CA).

For each sex group, 9 sampling bins were established to recruit subjects evenly among these bins. Three age bins, 18-33, 34-49, and 50-65 y were established, each with BMI 18.5-24.99, 25.0-29.99, or 30.0-45.0 kg/m². Upon completion of the study, 19-24 subjects were recruited for each sampling bin with the exception of these: men with BMI 30-45 kg/m² at 18-33 y (n = 13), 34-49 y (n = 17), and 50-65 y (n = 8) and women with BMI 30-45 kg/m² at 18-33 y (n = 17) and 50-65 y (n = 17) as well as women with BMI 25-29.9 kg/m² at 34-49 y (n = 15).

The study included two visits to WHNRC scheduled within a period of 10–14 days. Visit 1 included the informed consent procedure and a screening of vital signs to ensure the volunteers fell within expected ranges for the study. was the preliminary screening visit that included an in-person informed consent form procedure, and ensured the vital signs of the volunteers fell within the expected ranges of the study. Visit 2 was the challenge meal test day. The night before the test day, the subject was provided a high carbohydrate meal (17% kcal from fat, 77% kcal from carbohydrate, and 7.5% kcal from protein) and asked to eat it by
19:00 h. The subject arrived fasted (12 h) the next morning and blood was collected before a high-fat liquid challenge meal (60% kcal from fat, 25% kcal from carbohydrates, and 15% kcal from protein) was given. Multiple blood draws were then conducted postprandially at 0.5-, 3- and 6-h [21]. Heights (m) and weights (kg) were recorded in Visit 1 and Visit 2. BMI (kg/m²) was then calculated from the averages of heights and the fasted weight from Visit 2.

**SNP selection**

Five SNPs in *APOA5, APOC3, APOB, APOE*, and *LDLR* were chosen in this study. We chose these SNPs based on their strong correlations with lipid metabolism-related diseases, such as stroke or metabolic syndrome supported by many genome-wide association and meta-analysis studies [22-30], and their relative high risk allele carrier frequencies which would increase detection power for this particular study that had relatively small participant numbers. **Table 1** lists the SNP IDs for the chosen SNPs and their corresponding TaqMan assay IDs, allele frequencies in populations, nucleotide changes, codon changes, physical positions in the human genome, and the associated metabolic diseases. Four of the chosen SNPs in *APOA5, APOB, APOE*, and *LDLR* lead to missense amino acid changes in the respective protein. The SNP in *APOC3* is located upstream of the *APOC3* coding sequence with a potential role in regulation of gene expression of *APOC3* [29, 31].

**Genomic DNA purification and quantification**

Eight milliliters of whole blood were collected in a PAXgene Blood DNA Tube (Qiagen, Germantown, MD) from study subjects at 0.5 h after the challenge meal was given. Collected blood was gently inverted in the PAXgene blood DNA tube and immediately stored at -80°C until use. Genomic DNA was then purified from the whole blood using a PAXgene Blood DNA Kit according to the manufacturer’s instructions (Qiagen). The concentrations of DNA were measured using a NanoPhotometer™ P300 (Implen, Westlake Village, CA, USA). All DNA samples had a A260/A280 ratio greater than 1.6, indicating the
purity of nucleic acids was suitable for genotyping. DNA was then diluted to 25 ng/µL for TaqMan SNP genotyping with sterilized double distilled water for subsequent TaqMan SNP genotyping assays.

**SNP genotyping**

TaqMan SNP probe sets for rs3135506 (Assay ID: C_25638153_10, APOA5), rs1042034 (Assay ID: C_7615376_20, APOB), rs2854116 (Assay ID: C_12081482_20, APOC3), rs429358 (Assay ID: C_3084793_20, APOE), rs2228671 (Assay ID: C_27208873_20, LDLR) were purchased from ThermoFisher Scientific (Carlsbad, CA, USA). TaqMan genotyping reactions were performed using a TaqMan SNP assay-based PCR (ThermoFisher Scientific) in an Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System according to the manufacturer’s instructions. Fifty ng of genomic DNA was used for each PCR reaction. Allelic discrimination assays were performed using QuantStudio™ Real-Time PCR software (ThermoFisher Scientific). All ambiguous genotypes were repeated in independent PCR reactions.

**Clinical measures**

Fasting blood was collected and serum or plasma was obtained by centrifugation at 1300x g at 4°C for 10 min. Lipid-related markers of cardiovascular diseases, including TG, total cholesterol (TC), HDL-C, LDL-C, and NEFA were measured using a Cobas Integra 400/800 kit (Roche), a Cobas CHOL2 kit (Roche), a Cobas HDL-C plus 3rd generation kit (Roche), a Cobas LDLC3 kit (Roche), and a Wako HR Series NEFA-HR (2) kit (Wako), respectively. All assays were completed on an auto-analyzer, Integra 400+ instrument (Roche).

**Statistical analysis**

Allele frequency was determined by direct counting. Differences in general characteristics between genotype groups were assessed for significance using Student’s *t*-test. Outcome variables were assessed for
conformance to the normal distribution and transformed if needed; triglycerides were transformed using natural logarithm, and total cholesterol, HDL-C, LDL-C, and NEFA did not require transformation. The association between lipid parameters and genotype was tested separately for each SNP with analysis of variance (ANOVA), adjusted for sex, age category, and BMI categories. Two-factor interactions between SNP and sex, age category, or BMI category were also examined. The general characteristics were presented as mean ± S.E. $P < 0.05$ was considered significant.
Results

General results

The objective of this study was to understand potential genetic contributions of apolipoprotein genes, including *APOA5*, *APOB*, *APOC3*, and *APOE*, and the *LDLR* to clinical lipid measures, such as total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), and non-esterified free fatty acids (NEFA), during fasting as well as 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 the genetic association study in 349 subjects (88.8% of the total enrolled subjects) (Fig. 1), including 167 men (48%) and 182 women (52%). Among 167 men, 57 (34.1%), 59 (35.3%), and 51 (30.5%) were in the age groups of 18-33, 34-49, and 50-65 years old, respectively. Among 182 women, 63 (34.1%), 58 (31.9%), and 61 (33.5%) were in the age groups of 18-33, 34-49, and 50-65 years old, respectively. A total of 135 subjects were in the BMI 18.5-24.9 kg/m² (normal weight group, 65 men and 70 women), 127 subjects in the BMI 25-29.9 kg/m² (overweight group, 66 men and 61 women), and 87 in the BMI 30-45 kg/m² (obese group, 36 men and 51 women).

The general characteristics, including age, height, weight, BMI, and waist circumference are shown for the 349 participants in Table 2. Higher weight, height, and waist circumference were present in men than women ($P <0.01$). Fasting blood lipid profiles, including TC, HDL-C, LDL-C, TG, and NEFA from the 349 subjects are presented in Fig. 2. Most of subjects had fasting lipid values within the desirable ranges for healthy individuals in the respective age group in the U.S. based on the lipid reference values obtained from the Lipid Research Clinic (LRC) Program Population Studies [32], in which the reference values for total cholesterol was 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. 2). The 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 the fasting plasma NEFA concentrations (mEq/L) were $0.29 \pm 0.01$ for
men and 0.35 ± 0.01 for women. Women had approximately 20% higher fasting NEFA concentrations than men ($P < 0.01$). The NEFA levels were also positively associated with BMI (0.29 ± 0.01 mEq/L in the BMI 18.5-24.9 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 age-dependent increases 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; $P = 0.054$). Moreover, among the lipid measures examined, significant difference in the fasting concentrations of HDL-C was noticeable between men and women ($P < 0.01$). As expected, women had higher HDL-C than men (Fig. 2).

**SNP carrier frequency**

Among the 349 subjects studied, 6.88% of the total had a CC or CG genotype of rs3135506 ($APOA5$) with the dominant C genotype being the risk allele for its association with hypertriglyceridemia and cardiovascular diseases [33] while 26.16% of the subjects had a risk GG or GA genotype of rs1042034 ($APOB$) for its correlation to hyperlipidemia and ischemic stroke [34, 35]. In addition, 42.22% of the study subjects carried the dominant C risk allele of rs2854116 ($APOC3$) for either hypertriglyceridemia or nonalcoholic fatty liver disease [36, 37]. Moreover, 14.33% of the subjects had a CC or CT genotype of rs429358 ($APOE$) with the dominant C genotype being the risk allele for coronary heart disease and Alzheimer's disease [38, 39]. Lastly, 9.34% of the subjects had a T genotype of rs2228671 ($LDLR$) with the dominant T genotype being the risk allele for hypercholesterolemia [40]. The comparison of allelic frequencies of the tested SNPs with the ones in the database of the 1000 Genomes Project (https://www.genome.gov/27528684/1000-genomes-project) is presented in **Supplemental Table 1**. The allele frequencies of the SNPs revealed in the current study were in agreement with the frequencies derived from the global population. In addition, these observed SNP genotypes were evenly distributed across all age/BMI/sex groups (**Supplemental Table 2**).
Association of SNPs in *APOA5, APOB, APOC3, APOE,* and *LDLR* with fasting blood levels of TC, HDL-C, LDL-C, TG, and NEFA

Most of the tested SNPs (4 out 5) were expected to have a dominant effect on protein function due to missense amino acid changes. Thus, a dominant nucleotide model was adopted for each SNP by analyzing the association of the SNPs 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 3, the adjusted mean corrected for sex, age, and BMI for fasting LDL-C concentrations were higher by 8% (8.8 mg/dL; *P* < 0.05) in the subjects carrying the CC or CT genotype of rs429358 (*APOE*). On the other hand, the adjusted mean for fasting HDL-C were reduced in these subjects by 7% (3.7 mg/dL; *P* < 0.05). Moreover, a significant association was detected for the SNP in *APOC3* for fasting total cholesterol levels. Subjects carrying the CC or CT genotype of rs2854116 (*APOC3*) had higher total cholesterol by 5% (8.0 mg/L; *P* < 0.05) than those carrying the TT genotype. Additionally, fasting LDL-C levels were elevated in these subjects with high total cholesterol levels; but the level of increase did not reach statistical significance (*P* = 0.06). Interestingly, no correlation of examined SNPs in *APOA5, APOB* and *LDLR* could be established with TG, TC, HDL-C, LDL-C, and NEFA levels in the fasting state 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 in the *APOA5* SNP. Moreover, no significant association of the tested SNPs in *APOE* and *APOC3* with fasting levels of TG and NEFA could be found (Table 3).

Genotype-sex interactions of apolipoproteins with HDL-C levels have been reported [41]. We, therefore, investigated sex-specific changes in HDL-C levels that were associated with the tested genotypes of the apolipoprotein genes and LDLR. As shown in Table 4, 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 4). Women carrying the CC or CG risk genotype of rs3135506 had ~17% (9 mg/dL) lower HDL-C than those with the GG genotype (*P* < 0.05). However, no difference between rs3135506 genotypes was observed in men (*P* > 0.05). Specifically, women carrying the C
dominant risk allele of rs3135506 had HDL-C levels similar to men. On the other hand, men carrying the C allele of rs429358 (APOE) had significantly lower HDL-C levels (~12%; 5 mg/dL) than those with the TT genotype (Table 4). No significant difference between genotypes of rs429358 was observed in women. Importantly, women in both the TT and CC + CT genetic groups of rs429358 had significantly higher HDL-C levels than men carrying the same genotypes.

Tracing the association of the SNPs in APOA5 and APOE with serum HDL-C during lipid clearance after the high-fat liquid meal challenge

To further analyze the association of SNPs in APOA5 and APOE with the metabolic pattern of HDL-C, an ANOVA test was performed to draw associations between the SNPs and the levels of HDL-C at four different time points in men and women, which were 0- (before the challenge meal); 0.5-, 3-, and 6-h after the challenge meal (Fig. 3). Our results demonstrated that women carrying the dominant C genotype (risk allele) of the APOA5 SNP had significantly decreased HDL-C levels across all time-points by ~17% (9 mg/dL) compared to those with the GG genotype. Moreover, the risk C allele carriers of APOE in men had lower levels of HDL-C than the non-carriers by ~12% (5 mg/dL) before and after the meal challenge. Taken together, these results demonstrated that the risk alleles of APOA5 (rs3135506) and APOE (rs429358) SNPs negatively affected cholesterol concentrations in HDL particles in the fasting state and after a meal in women and men, respectively. However, the dynamic state of HDL-C level was not affected by the genotypes of both APOA5 and APOE SNPs after the high-fat challenge in both sexes, suggesting the baseline effect of the SNPs on HDL-C levels.

Tracing the association of the SNPs in APOC3 and APOE with serum TC and LDL-C concentrations during lipid clearance after high-fat liquid meal challenge

In the current study, interactions of the tested SNPs in APOC3 and APOE with blood TC or LDL-C concentrations were revealed in the fasting state (Table 3). We further assessed the postprandial states of TC or LDL-C levels in different genotypic and sex groups following the dietary lipid challenge. As shown
in Fig. 4A, in both men and women, the subjects with the dominant C genotype (risk allele) of the APOC3 SNP had significantly higher age- and BMI-adjusted blood TC concentrations (~ 4%; 8 mg/dL) than those with the TT genotype before and 30 min after the dietary challenge. The TC remained at the higher levels in these subjects carrying the risk allele at 3- and 6-h after the challenge; but, these did not reach statistical significance ($P = 0.05$ and 0.08 for the 3-h and 6-h time points, respectively). Moreover, we found that the APOE SNP-mediated difference in fasting LDL-C levels (Table 3) was mainly contributed by men. As shown in Fig. 4B, men carrying the dominant C allele had ~7% (8 mg/dL) higher LDL-C levels than those with the TT allele ($P < 0.05$) and this difference was maintained postprandially. A similar trend of the impact of the APOE SNP on LDL-C levels was also observed in women before and after the dietary challenge. However, no statistical significance was detected ($P > 0.05$). Again, it was noted that the dynamics of blood cholesterol content (TC or LDL-C) was not influenced by the APOC3 or APOE SNP after the high-fat diet challenge as demonstrated by the similarity in the curves of both genotypic groups throughout the postprandial period examined (Fig. 4A and B).

Additive effects of the SNPs in APOA5/APOE and APOC3/APOE on the lipid measures

Since the risk genotypes of SNPs in APOA5 ($P = 0.08$) and APOE ($P < 0.05$) (Table 3) displayed negative correlations with HDL-C levels at fasting, we next examined whether the carriers for both risk alleles of APOA5 and APOE showed a difference in lipid measures, i.e., a potential additive effect of the risk alleles on the levels of TC, HDL-C, LDL-C, TG and NEFA before and after the dietary lipid challenge. As shown in Table 5, among the total 349 subjects, 18 of them were carriers for both risk alleles of the APOA5 and APOE SNPs while 224 of them were non-carriers for both genotypes. Surprisingly, although an additive effect of risk alleles of APOA5 and APOE was observed for HDL-C throughout four time points (0-, 0.5-, 3-, and 6-h) by ~10-12% (~6 mg/dL after the dietary challenge), it was not statistically significant after adjustments for age, sex and BMI ($P > 0.05$), indicating that age, sex, and BMI had an impact on HDL-C levels (Table 5), but not the genotypes of the tested SNPs. Importantly, fasting LDL-C was significantly increased by ~12% (15 mg/dL) in subjects carrying both risk alleles of APOA5 and APOE compared to the
non-carriers ($P < 0.05$). The difference was kept consistent postprandially ($P < 0.05$), suggesting the effect at the baseline might be the causal 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 5). Lastly, subjects carrying the double risk alleles of APOA5 and APOE had a similar dynamic state of HDL-C and LDL-C levels, i.e. the difference in the lipid concentrations between the two tested genotypes remained similar after dietary lipid challenge (Table 5).

Additive effects of the risk alleles of the APOC3 and APOE SNPs on lipid measures were also evaluated in both fasting and postprandial states. As shown in Table 6, carriers of the double risk alleles (CC or CT for both APOC3 and APOE; $n = 21$) had significant lower NEFA concentrations 6-h after the dietary challenge (decreased ~24% or 0.11 mEq/L; $P < 0.01$ after adjusting for age, sex, and BMI) than the non-carriers ($n = 130$). Nonetheless, the presence of the double risk alleles of APOC3 and APOE SNPs had limited impact on other tested lipid measures before and after the challenge meal.
Discussion

In this study, we aimed to draw an association between lipid-metabolic parameters and SNPs in lipid metabolically related genes and to assess the variation in lipid metabolic patterns followed by the administration of a liquid high-fat diet. Of all the clinical lipid markers studied, total cholesterol, HDL-C, and LDL-C were the ones that have significant associations with the tested SNPs in \textit{APOA5, APOB,} and \textit{APOC3}, genes that are heavily involved in lipid metabolism.

Interestingly, the tested SNP in \textit{APOA5} was significantly associated with blood HDL-C levels in a sex-dependent manner. Specifically, the dominant risk C allele of the SNP in \textit{APOA5} was associated with lower HDL-C levels in women (\textbf{Fig. 3}). It is known that HDL-C levels differ between men and women. In general, women have higher HDL-C than men [42]. Our results demonstrate that this sex difference is diminished in women carrying the risk allele of the SNP in \textit{APOA5}. Clinically, HDL-C is known as “the good cholesterol” as studies have shown the protective effect of HDL-C against coronary heart disease [43-46]. It is commonly recognized that HDL-C exerts its protective effects towards arteries and the heart by transporting cholesterol away from extrahepatic tissues, particularly steroidogenic tissues. By doing so, it removes cholesterol from the arteries to the liver [43-46]. This apparent protective effect of HDL-C on CVDs, on the other hand, could be counteracted by high levels of LDL-C and/or triglycerides [47]. Moreover, the cardioprotective effect of HDL-C towards CVDs has been challenged with some studies, which indicate that an inverse relationship between plasma HDL-C concentrations and CVDs cannot be established in individuals who have a loss-of-function scavenger receptor BI (SR-BI, a hepatocellular receptor for HDL-C uptake) [48] or the presence of APOC3 on the HDL particles [49] due to inflammatory effects of APOC3 on the particles [50]. Other observational studies also demonstrated that the linear inverse relationship of HDL-C with CVDs can only be established in men and women with HDL-C levels in the lower range of the reference value [51, 52]. When HDL-C concentration reaches to a plateau level, such as 90 mg/dL in men and 75 mg/dL in women, the inverse relationship between HDL-C levels and CVDs
disappears [51]. Nevertheless, because the risk of high HDL-C levels on CVDs is generally lower than that of the low HDL-C, low HDL-C concentrations likely remain to be one of the most significant HDL-C biomarkers that is highly associated with the increased risk of CVDs [45, 53] with the exception of other variables, such as genetic, lifestyle, and other characteristics of HDL metabolism of an individual [53, 54].

It is known that APOA5 is an activator of lipoprotein lipase [7]. Disruption of APOA5 is associated with high blood TG levels [9]. The SNP APOA5 rs3135506 is associated with the combination of higher triglycerides and lower HDL-C profiles in different populations [55, 56]. Moreover, this SNP has been correlated with metabolic syndromes due to its association with dyslipidemia [55]. In fact, besides rs3135506, other SNPs in or upstream of APOA5, including rs662799, rs2075291, and rs201079485, have been linked to dyslipidemia, including HDL-C, LDL-C, and TG [57-61]. Until now, the emphasis of APOA5 has been on its association with the metabolism of triglycerides. This is the first report to show an association of rs3135506 with HDL-C levels in healthy women before and after a meal challenge. Nevertheless, the molecular pathway that links the effect of APOA5 on potential modulation of blood HDL-C levels is still largely unknown.

From our study, it is reasonable to propose that APOA5, particularly with the SNP rs3135506, could profoundly modulate the metabolism of HDL-C, even before its direct impact on blood triglyceride levels. One potential hypothesis is that the APOA5 SNP can mechanistically impact the levels of HDL-C by affecting the maturation of HDL. First, APOA5 is highly associated with the HDL particles even though present in relatively low concentrations compared to other apolipoproteins [62]. Second, it is known that TG levels directly correspond to the TG content in triglyceride-rich lipoproteins (TGRL) [63]. But there may be a direct correlation of low HDL with high TG. When TG contents rise, the majority of APOA5 is found in TGRL rather than HDL. Conversely, APOA5 slowly moves back to HDL when TG contents decrease in TGRL after hydrolysis [64]. Lastly, APOA5 enhances the activity of lecithin-cholesterol acyltransferase (LCAT) that promotes cholesterol efflux [65]. Although all of the above evidence supports that APOA5 may modulate HDL and cholesterol metabolism, the underlying mechanism by which APOA5 regulates blood HDL-C concentration still needs to be further elucidated by structural and functional studies.
It is well acknowledged that increased LDL-C level is a risk factor for coronary heart disease [66]. In this study, we found that the risk C allele of the SNP rs429358 in APOE could significantly increase LDL-C levels in both fasting and postprandial states (Table 3 and Fig. 4). Most importantly, this risk allele also had a negative impact on HDL-C levels in men (Table 4). Moreover, a combination of the risk alleles of the APOA5 and APOE SNPs significantly augmented the difference in LDL-C levels between the risk allele carriers and non-carriers (Table 5). Studies from others have shown that APOE acts as a ligand for the LDL-receptor [67]. Thus, it plays an anti-atherogenic role in reducing the risk of cardiovascular disease by mediating VLDL remnant clearance. It has also been shown that the impact of APOE on LDL-C levels is associated with different isoforms of APOE, which is in the order of APOE-ε2, APOE-ε3 and APOE-ε4 with APOE-ε2 having the least impact, APOE-ε3 considered as normal, and APOE-ε4 being detrimental [68]. The C allele of the SNP rs429358 is one of the determining elements for the APOE-ε4 isoform along with the C allele of the SNP rs7412. APOE-ε4 preferentially binds to VLDL while APOE-ε3 to HDL. This propriety of binding to VLDL over HDL by APOE-ε4 is closely associated with an atherogenic lipoprotein phenotype in APOE-ε4 carriers. Together, our results suggest that the C allele of the SNP rs429358 has a detrimental effect on cholesterol metabolism, especially in men, and hence men carrying this risk allele may be at a higher risk of cardiovascular disease.

Apart from the finding mentioned above, this study also revealed the association of APOC3 rs2854116 with total cholesterol during fasting and postprandially (Fig. 4). Additionally, the current study suggests that this SNP might affect fasting LDL-C levels ($P = 0.07$) in women. In a meta-analysis study, the risk allele of rs2854116 was shown to be positively associated with TG levels while negatively associated with HDL-C levels [69]. Additionally, APOC3 is primarily associated with cholesterol-rich lipoproteins, such as chylomicron, VLDL, and LDL [70]. Therefore, as suggested by the current study, it could be a key regulator of cholesterol metabolism, in addition to TG metabolism [69]. This theory is also supported by a genetic study performed in a northern French population which reported a correlation of rs2854116 to elevated LDL-C levels in women [71]. The molecular mechanism of the association remains
unclear. The tested SNP is located upstream of the gene transcription start site of *APOC3*. Thus, a plausible explanation would be that the SNP might negatively influence the *APOC3* expression leading to increased rates of conversion of VLDL to LDL particles due to a reduced inhibition of APOC3 to the lipoprotein lipase-mediated lipolysis [36]. This could subsequently elevate circulating total cholesterol and LDL-C likely induced by a decrease of hepatic uptake of VLDL remnants.

Interestingly, an inverse association of blood NEFA concentrations with the genotypes of the combination of the risk alleles of *APOC3* and *APOE* was found 6-h after the administration of the challenge meal (Table 6). A number of studies have suggested that reduced circulating NEFA is correlated with a lower ability to oxidize fat and subsequently liberate NEFA into plasma from adipose tissue leading to metabolic deterioration [72, 73]. It is also reported that metabolically healthy obese individuals with high fasting NEFA levels are less likely to develop type 2 diabetes than their insulin-resistant counterparts [74]. Thus, a reduction in 6-h circulation NEFA levels found in the current study may suggest less fat oxidation in subjects carrying the double risk alleles of the SNPs in *APOC3* and *APOE*, which may increase risks of obesity as well as type 2 diabetes in these individuals.

Although strong associations between SNPs and cholesterol profiles were correlated in this study, there were limitations in the current study that confounded the findings. First, the population size assessed in this study was relatively small, which may hinder significant association of the tested SNPs with TG metabolism as there were dramatic inter-individual differences among the subjects. Second, other phenotypic measurements, such as the sizes of lipoproteins, which contribute to specific functions of their subtypes, and plasma transfer proteins for lipoproteins, including plasma phospholipid-transfer protein (PLTP) and cholesteryl ester transfer protein (CETP), were not measured. Thus, this study could not provide further information on the effects of SNPs on lipoprotein subtypes.

**Conclusions**

We identified significant associations of rs3135506 (*APOA5*), rs429358 (*APOE*), and rs2854116 (*APOC3*) with cholesterol metabolism although previously the two SNPs in *APOA5* and *APOC3* have been mostly
recognized as influencers of TG levels. Moreover, we showed that the impact of the *APOA5* and *APOE* SNPs on HDL-C and LDL-C levels were sex-dependent. Our results also suggest that the impact of the SNP variants in the above apolipoproteins might be particularly important for the fasting state. To the best of our knowledge, the current study is the first to investigate the effect of apolipoprotein allelic variants on blood lipid profiles in healthy adults challenged with a liquid high-fat diet. The findings from the study suggest potential new insights of the roles of *APOA5*, *APOE*, and *APOC3* in modulating cholesterol metabolism, which demonstrated that individuals who carry the non-risk alleles of the tested SNPs of *AOPA5*, *APOE*, and *APOC3* may have reduced genetic risks of cardiovascular disease. The current study may also shed the light on the need for more studies on the assess of sex-specific risks of cardiovascular disease.

**Abbreviations:**

*APOA5*: apolipoprotein A5  
*APOB*: apolipoprotein B  
*APOC3*: apolipoprotein C3  
*APOE*: apolipoprotein 4  
**HDL-C**: high-density lipoprotein-cholesterol  
**LDL-C**: low-density lipoprotein-cholesterol  
**TC**: total cholesterol  
**TG**: triglycerides  
**TGRL**: triglyceride-rich lipoproteins  
**NEFA**: non-esterified free fatty acids  
**CVDs**: cardiovascular diseases
Declarations

Ethics approval and consent to participate
The study was registered on ClinicalTrials.gov (ID: NCT02367287) and received ethical approval from the University of California Davis Institutional Review Board. This study was carried out at the USDA/Agriculture Research Service/Western Human Nutrition Research Center at Davis, CA. Generally healthy people living near Davis, CA were invited to participate in this cross-sectional study. Details of the study were explained to and discussed with participants, and those who agreed to the terms of the study provided informed consent during the first study visit.

Consent for publication
Consent for publication was obtained from all subjects in this study.

Availability of data and materials
Datasets generated during the current study are available from Dr. Liping Huang (USDA/ARS/Western Human Nutrition Research Center, liping.huang@usda.gov) or Dr. Charles B. Stephensen (charles.stephensen@usda.gov) upon reasonable request.

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Authors’ contributions
LH conceived the project, designed scientific objectives, analyzed the data, created figures and tables, participated in drafting the manuscript, and final approval of the manuscript. YEW performed genomic DNA purification, quantification; carried out SNP genotyping and analysis, and drafted the manuscript. CPK optimized protocols for genomic DNA purification and was involved in blood DNA purification, quantification, SNP genotyping, and edited the manuscript. LW organized and supervised the measurements of clinical markers and participated in manuscript editing. CBS was the project administrator and principal investigator, supervised the project, reviewed and edited the manuscript. BB was the co-principal investigator, supervised the project, reviewed and edited the manuscript. JN was the co-investigator for the project and reviewed and edited the manuscript. NK was the co-investigator for the project and reviewed and edited the manuscript. ELB managed and supervised the subject administration for the study.

Competing interests
The authors declare that they have no competing interests.
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## Tables
### Table 1 SNPs used in this study

| Gene | SNP ID | TaqMan assay ID | Nucleotide Change | Frequency$^a$ | Codon change | Genome position (GRCh38.p12) | Associated diseases with null mutations |
|------|--------|-----------------|-------------------|--------------|--------------|-------------------------------|----------------------------------------|
| APOA5 | rs3135506 | C_25638153_10 | C>G               | C=0.056 (279/5008) | S[TGC]>W[TGG] | chr11:116791691 | Familial hypertriglyceridemia |
| APOB  | rs1042034 | C_7615376_20   | G>A               | G=0.370 (1855/5008) | S[AGT]>N[AAT] | chr2:21002409 | Familial hypercholesterolemia II |
| APOC3 | rs2854116 | C_12081482_20  | C>T               | T=0.452 (2262/5008) | n.a.$^b$     | chr11:116829453 | Hypertriglyceridemia |
| APOE  | rs429358  | C_3084793_20   | C>T               | C=0.151 (754/5008) | R[CGC]>C[TGC] | chr19:44908684 | Nonalcoholic fatty liver disease |
| LDLR  | rs2228671 | C_27208873_10  | C>T               | T=0.057 (285/5008) | C[TGC]>C[TGT] | chr19:11100236 | Hyperlipoproteinemia type III |

$^a$. Based on the data from the 1000 Genomes Project (https://www.internationalgenome.org/).

$^b$. Upstream Transcript Variant.
| Characteristic              | 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)                | 177.9±7.6 (177.4; 161.8-201.7) | 163.6±6.9 (163.9; 146.3-180.6) |
| Weight (kg)                | 86.2±17.8 (84.2; 50.3-175.2) | 74.6±16.2 (71.9; 44.9-121.2) |
| Body mass index (kg/m²)    | 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 3: 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^a | 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^a | 7.57^a | 6.06 |
| **LDLR**            |            |                           |               |               |              |
| CT (59) + TT (3)    | 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 ± S.E. $a$, $P < 0.05$. Triglyceride (TG) values were transformed to the natural logarithm scale for analysis. Other analyses were conducted without transformation. Data were adjusted for sex, age, and BMI.
Table 4 Fasting serum HDL-C based on SNPs and sexes in the examined genes

| Gene & genotype (n) | HDL-C (mg/dL) | Gene & genotype (n) | HDL-C (mg/dL) |
|---------------------|--------------|---------------------|--------------|
| **Men**             |              | **Women**           |              |
| **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±1.06a  |
| 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±1.50a  |
| AA (93)             | 49.51±1.40   | AA (99)             | 58.60±1.38a  |
| 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±1.23a  |
| TT (58)             | 46.94±1.79   | TT (59)             | 60.24±1.78a  |
| 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±1.82a  |
| TT (127)            | 50.50±1.21   | TT (126)            | 61.43±1.21a  |
| Change (%) of the C allele | -11.68%b | Change (%) of the C allele | -3.33%     |
| **LDLR**            |              | **LDLR**            |              |
| CT (29) + TT (2)    | 50.61±2.45   | CT (30) + TT (1)    | 64.46±2.45a  |
| CC (135)            | 48.89±1.17   | CC (151)            | 60.06±1.11a  |
| 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 ± S.E. \( a, P < 0.01 \) between men and women of the indicated genotype; \( b, P < 0.05 \) and \( c, P < 0.01 \) between genotypes of the indicated gene.
Table 5 Lipid concentrations after dietary challenge in subjects carrying both risk alleles of \textit{APOA5} and \textit{APOE}

| Gene & genotype (n) | Time (h) after dietary challenge |
|---------------------|----------------------------------|
|                     | 0  | 0.5 | 3  | 6  |
| \textit{APOA5}/\textit{APOE}, GG/TT (224)\textsuperscript{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 |

| \textit{APOA5}/\textit{APOE}, CC+CG/CC+CT (18)\textsuperscript{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\textsuperscript{c} | 12.44\textsuperscript{c} | 13.07\textsuperscript{c} | 12.15\textsuperscript{c} |
| 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 to sex, age, and BMI and are presented as mean ± S.E. \textsuperscript{a}, subjects carrying both non-risk alleles of \textit{APOA5} and \textit{APOE}; \textsuperscript{b}, subjects carrying both risk alleles of \textit{APOA5} and \textit{APOE}. \textsuperscript{c}, \( P < 0.05 \).
Table 6 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$^c$ |
Data were adjusted to sex, age, and BMI and are presented as mean ± S.E. 

- **a**, subjects carrying both non-risk alleles of APOC3 and APOE. 
- **b**, subjects carrying both risk alleles of APOC3 and APOE. 
- **c**, $P < 0.01$
Figures
Fig. 1. Study flow chart.
Fig. 2. Distributions of fasting lipid measures in subjects. Each open circle represents a subject and the black line is the mean of the dataset. The green line or box represents the reference values for 18.5- to 65-year-old categories obtained from the Lipid Research Clinic (LRC) Program Population Studies [32]. The 90th percentile (triglycerides), 75th percentile (cholesterol and LDL-C), and 10th percentile (HDL-C) of the LRC datasets in different age groups were used for determining the reference values. Lower (triglycerides, total cholestrol, and LDL-C) or higher (HDL-C) concentrations than the reference values are desired for reducing the risk of coronary artery disease.
Fig. 3. Effects of the SNPs APOA5 rs23135506 and APOE rs429358 on blood HDL-C levels after the administration of a high-fat challenge meal. A. The SNP APOA5 rs3135506. Subjects were divided into two genotypic and sex groups (n = 25 and 22 for the CC + CG genotypes in men and women, respectively; n = 142 and 160 for the GG genotype in men and women, respectively). B. The SNP APOE rs429358. Subjects were divided into two genotypic groups (n = 40 and 56 for the CC + CT genotypes in men and women, respectively; n = 127 and 126 for the TT genotype). All data were adjusted for age and BMI and are presented as mean ± S.E. **, P < 0.01 between the genotypic groups within the same sex group.
Fig. 4. Effects of the SNPs APOC3 rs2854116 and APOE rs429358 on blood TC and LDL-C levels after the administration of the high-fat challenge meal. A. The SNP APOC3 rs2854116. Subjects were grouped into two genotypic and sex categories: the CC + CT genotypes (n = 108 for men and 122 for women) and the TT genotype (n = 58 for men and 59 for women). B. The SNP APOE rs429358. Subjects were grouped into two genotypic and sex categories: the CC + CT genotypes (n = 40 for men and 56 for women) and the TT genotype (n = 127 for men and 126 for women). All data were adjusted for age and BMI and are presented as mean ± S.E. *, P < 0.05 between the genotypic groups within the same sex group. P = 0.05 between the two genotypic groups within the same sex group. TC = total cholesterol.
**Supplemental Table 1** Comparison of the observed SNP frequencies between this study and the 1000 genome project

| SNP ID | *APOA5* | *APOB*<sup>a</sup> | *APOC3*<sup>a</sup> | *APOE* | *LDLR*<sup>a</sup> |
|--------|---------|------------------|------------------|--------|------------------|
|        | rs3135506 | rs1042034 | rs2854116 | rs429358 | 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<sup>b</sup> | 5.57 94.43 | 37.04 62.96 | 54.83 45.17 | 15.06 84.94 | 5.69 94.31 |

<sup>a</sup>, Genotypes were not determined: *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); *LDLR*, 1 man (Subject ID 9024). <sup>b</sup>, Global population.
### 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/m²); LDLR, 1 man (Subject ID 9024) in the groups of 34-49-year-old and BMI 18.5-24.9 kg/m². The Chi-square test was used to determine the genotypic distribution among categorical variables. No significance was found for the distribution among the categories.