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*Taq*IB polymorphism in the *CETP* gene modulates the impact of HC/LF diet on the HDL profile in healthy Chinese young adults☆

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Abstract

The aim of this study was to investigate the interactions of genetic variants in the genes of cholesterol ester transfer protein (*CETP*) and low-density lipoprotein receptor (*LDLR*) with high carbohydrate and low fat (HC/LF) diet on lipid profiles in a young and healthy Chinese Han population. Fifty-six healthy subjects (22.89±1.80 years) were given washout diets of 31% fat and 54% carbohydrate for 7 days, followed by HC/LF diets of 15% fat and 70% carbohydrate for 6 days, with no total energy restriction. Serum lipid profiles at baseline, after washout and following HC/LF diets, as well as *CETP* and *LDLR* polymorphisms were analyzed. Carriers of B2 allele of *CETP Taq*IB polymorphism had significantly higher levels of high density lipoprotein cholesterol (HDL-C) and apo A-I in the whole study population after the diet intervention. Notably, males with *CETP Taq*IB B1B1 experienced significantly increased HDL-C and apo A-I *after* HC/LF diet. Regarding the *LDLR Pvu* II polymorphism, both P1P1 subjects and P2 carriers experienced decreased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels after HC/LF diet with no statistically significant differences between the genotypes. Our results demonstrate that the elevated HDL-C levels after HC/LF diet in healthy Chinese Han youth are associated with *CETP Taq*I B2 allele while males with B1B1 genotype are more susceptible to the influence of HC/LF diet on their HDL-C levels. The decreased TC and LDL-C levels after HC/LF diet are not associated with *LDLR Pvu* II polymorphism.

Keywords: Cholesterol ester transfer protein; Low-density lipoprotein receptor; Polymorphisms; Serum lipids; High-carbohydrate/low-fat diet

1. Introduction

Hypertriacylglycerolemia, characterized by elevated serum levels of triacylglycerol (TG) and lowered concentrations of high density lipoprotein cholesterol (HDL-C), is a well-recognized independent risk factor for coronary artery disease (CAD) [1–3]. Understanding of the mechanism of hypertriacylglycerolemia is crucial for effective prevention and treatment of this disorder and subsequent CAD. Carbohydrate-induced hypertriacylglycerolemia can be an excellent model in investigating hypertriacylglycerolemia in different populations [1,4]. As coronary artery disease is diagnosed mostly after 45 years of age [5], almost all of the previous studies on carbohydrate-induced hypertriacylglycerolemia have been focused on middle-aged or senior subjects. Although the risk of CAD in younger populations has been steadily increasing over the past few decades [6], much less effort has been made in understanding the biochemical mechanisms and gene–environmental interaction in lipid homeostasis in younger populations, especially in young Chinese population.

A lower incidence of CAD in the Chinese population has been well documented [7,8]. This low incidence has been attributed to their more favorable lipid profile, including lower total cholesterol (TC) and higher HDL-C and apolipoprotein A-I/apolipoprotein B-100 (apoA-I/B-100) ratio [9,10], which is most likely a reflection of both genetic and environmental characteristics in the Chinese population. It has been reported that the Chinese population has a diet containing lower fat and higher carbohydrate [11,12]. Therefore, studies on the carbohydrate-induced hypertriacylglycerolemia in young Chinese populations may provide new insight into the development of hypertriacylglycerolemia in a quarter of the world's population.

One of the key proteins involved in lipoprotein remodeling and metabolism is cholesterol ester transfer protein (CETP). This protein enables the transfer of cholesteryl esters in plasma from HDL towards triglyceride-rich lipoproteins in exchange for triglycerides. Several common restriction fragment length polymorphisms (RFLPs) have

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been reported in the CETP gene (*CETP*) locus [13–16]. The most widely studied variant is *Taq*IB, a silent base change affecting the 277th nucleotide in the first intron of *CETP* [13]. It has been well documented that the *Taq*IB polymorphism has been associated with higher HDL-C levels and lower risk of coronary heart disease end points in men with HDL deficiency [13,17,18]. Another key protein involved in the metabolism of lipoproteins containing apolipoproteins (apo) B and E is the low density lipoprotein receptor (LDLR), which transports cholesterol-carrying lipoprotein particles into the cells through endocytosis. The primary ligand for this receptor is low density lipoprotein cholesterol (LDL-C), which contains a single copy of apoB-100, apo E and approximately 65–70% of plasma cholesterol.

A RFLP in the *LDLR* gene detectable with the restriction enzyme *Pvu* II is associated with variations in not only LDL-C [20,21] but also TG , TC, HDL-C and very low density lipoprotein cholesterol (VLDL-C) [19–23]. The *Pvu* II cutting site (CAGCTG) is created by the transition of a CpG to a TpG, within the sequence CAGCCG, at a position 600 bp upstream of the splice acceptor site of exon 16. People who are homozygous for the absence of the *LDLR Pvu* II restriction site (the P1P1 genotype) have a significantly higher total cholesterol level than heterozygotes [20,22,23]. However, the effect of these gene polymorphisms on the plasma lipid profiles in healthy young populations remains to be elucidated.

In this study, we investigated the interactions of genetic variants in the *CETP* and *LDLR* with high carbohydrate and low fat diet on lipid profiles in a young and healthy Chinese Han population. It was found that interactions between *CETP Taq*IB polymorphisms and gender contribute to the heterogeneity in HDL responsiveness to HC/LF diet in healthy Chinese Han youth.

2. Methods and materials

2.1. Subjects

Volunteers were recruited via advertisement seeking healthy young students in West China Medical Center, Sichuan University. Recruitment criteria included no history of metabolic disease, understanding of the procedures involved, and providing written consent. Volunteers with diabetes or cardiovascular, renal, or endocrinological diseases were excluded. Volunteers who took lipid-lowering drugs, hormones, consumed alcohol, smoked, or whose physical activity or sleeping time varied widely were also excluded. A total of 209 university students were recruited and 60 of these who met the above criteria finally entered the study. They were all apparently healthy, as indicated by the medical questionnaire and physical examination. All of them were Chinese Han people. Fifty-six subjects (27 males and 29 females) completed the study with good compliance. Their baseline characteristics are shown in Table 1. All volunteers were asked to maintain sleeping and physical activity in a constant manner during the study.

2.2. Study design

This is a study of dietary intervention. Previous studies have shown that after 5-7 days of HC/LF diet, serum triacylglycerol reaches a new steady state and remains constant throughout the period of the diet [24,25]. Therefore, a regime of a 7-day washout diet followed by a 6-day intervention was adopted for this study. The study protocol was approved by the Human Research Ethics Committee of Sichuan University.

Table 1
Demographic and biochemical characteristics of the study subjects

Variables	All (n=56)	Males (n=27)	Females (n=29)
Age, y	22.9±1.8	23.0±2.0	22.8±1.7
TG, mg/dl	76.5±43.4	89.1±55.7	65.2±24.3 *
TC, mg/dl	145.9±38.6	135.5±45.5	155.6±28.3
HDL-C, mg/dl	63.6±16.0	55.5±15.6	71.2±12.5 *
LDL-C, mg/dl	68.9±37.7	64.3±44.1	73.2±30.8
ApoA-I, mg/dl	204.6±23.4	193.6±26.4	213.8±15.9 *
ApoB-100, mg/dl	67.8±20.3	65.9±22.7	69.3±18.4

Values are expressed as mean±SD.
* P<.05 compared with that of the males (ANOVA).

2.3. Diets

The meals were composed of breakfast (received at 7:00–8:00 a.m.), lunch (received at 11:30 a.m.–12:30 p.m.) and dinner (received at 5:00–6:00 p.m.). The foods of each meal were changed every day. However, each meal had constant ratios of carbohydrate, protein, and fat. The washout diet contained 54% carbohydrate, 15% protein, and 31% fat. The HC/LF diet contained 70% carbohydrate, 15% protein, and 15% fat. All the meals were prepared from foods consumed by local people daily and were provided by the Department of Nutrition, West China Hospital, Sichuan University. No restriction of total energy intake was imposed for each meal. All the subjects ate to their satiation as usual in their daily life, though subjects were instructed not to take any other food or drink, except water. A daily dietary log was used to assess the compliance of each subject to the study design.

2.4. Blood collection

On the mornings of the first day of the study, the day starting the HC/LF diet, and the day after the HC/LF diet was completed; 12 hour-fasting venous blood samples were collected between 7:00 and 8:00 a.m., and the subject's weight and height were recorded.

2.5. Laboratory analysis

Serum was prepared by centrifugation of blood samples at 3000*g* for 15 min at 4°C. Multiple aliquots of each sample were stored in cryovials at −20°C until the end of the study when all samples were analyzed. Serum TG, TC, and glucose were measured enzymatically using a semi-automated biochemistry analyzer (BT-224). HDL-C was determined enzymatically after precipitation of apo B-containing lipoproteins with phosphotungstic-Mg^{2+}. LDL-C was quantified by the polyvinyl sulfate precipitation method using a semi-automated biochemistry analyzer (BT-224). apoB-100 and apoA-I were measured by an immunoturbidimetry assay with a Hitachi 7070 Analyzer and insulin concentration was determined by electrochemical luminescence with a Roche E170 Analyzer. The inter- and intra-assays coefficients of variation were less than 6%. Each analyte of a given sample was measured three times, and the average value of three measurements was used for statistical analysis.

2.6. DNA extraction and genotyping

Variations of *CETP Taq*IB and *LDLR Pvu* II were analyzed by polymerase chain reaction (PCR) and RFLP analysis [18]. Genomic DNA was isolated from white blood cells using a DNAout kit (Tiandz, Mianyang, China). *CETP Taq*IB genotype was determined by amplifying a 535 bp fragment of intron 1 of the gene by PCR followed by *Taq*IB digestion. The resulting DNA fragments are 174 and 361 bp in length for the B1 allele and an intact 535-bp fragment for the B2 allele. For *LDLR Pvu* II genotyping, amplification of an 1148-bp fragment of the 15th intron of this gene was carried out using PCR, followed by digestion with *Pvu* II. The P2 allele has two fragments of 951 and 197 bp, respectively, while the P1 allele is characterized by one band of 1148 bp.

2.7. Statistical analysis

The results are expressed as mean±S.D. unless otherwise stated. Normality in each group was tested using Shapiro-Wilk test. For positively skewed distribution (e.g. TGs), a log power transformation was applied. The means of variables were compared among subjects with different genotype before or after HC/LF diet by one-way analysis of variance (ANOVA). Two-tailed paired t tests were performed to analyze the statistical significance of the changes of the variables before and after HC/LF diet in the whole study population and in each genotype subgroups. Statistical significance was defined as P<.05.

3. Results

3.1. Biochemical and molecular characterization of the study population

In this study, isoenergetic design was not adopted because it does not reflect the real energy intake of people as the amount of energy intake is governed by the individual's satiation and cannot always be kept isoenergetic in real life. In addition, the variation of energy intake is also an important factor that determines the lipids response to HC/LF diet.

The demographic and biochemical characteristics of the study subjects are summarized in Table 1. Among the 60 volunteers admitted, 56 completed the study. Of these 56, two subjects missed a lunch in the third day of washout diet and ate their own meal. These two participants followed all the other dietary intervention and their data were not excluded.