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A high-carbohydrate diet enhances the adverse effect of the S2 allele of *APOC3 Sst*I polymorphism on the TG/HDL-C ratio only in young Chinese females

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Abstract

Both genetic background and diet have profound effects on plasma lipid profiles. We hypothesized that a high-carbohydrate (high-CHO) diet may affect the ratios of serum lipids and apolipoproteins (apo) differently in subjects with different genotypes of the *Sst*I polymorphism in the apoCIII gene (*APOC3*). Fifty-six healthy university students (27 males and 29 females, 22.89 ± 1.80 years) were given a washout diet of 54% carbohydrate for 7 days, followed by a high-CHO diet of 70% carbohydrate for 6 days without total energy restriction. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apoB100, apoAI, and the *APOC3 Sst*I polymorphism were analyzed. The ratios of serum lipids and apoB100/apoAI were calculated. At baseline, the TG/HDL-C ratio was significantly higher in females, but not in males, with the S2 allele. The differences in the TG/HDL-C ratio between genotypes remained the same after the washout and the high-CHO diet in females. When compared with those before the high-CHO diet, the TC/HDL-C (male S2 carriers: 3.13 ± 1.00 *vs* 2.36 ± 0.65, P = 0.000; male subjects with the S1S1 genotype: 2.97 ± 0.74 *vs* 2.09 ± 0.55, P = 0.000; female S2 carriers: 2.68 ± 0.36 *vs* 2.24 ± 0.37, P = 0.004; female subjects with the S1S1 genotype: 2.69 ± 0.41 *vs* 2.09 ± 0.31, P = 0.000) and LDL-C/HDL-C (male S2 carriers: 1.44 ± 0.71 *vs* 1.06 ± 0.26, P = 0.012; male subjects with the S1S1 genotype: 1.35 ± 0.61 *vs* 1.01 ± 0.29, P = 0.005; female S2 carriers: 1.18 ± 0.33 *vs* 1.00 ± 0.18, P = 0.049; female subjects with the S1S1 genotype: 1.18 ± 0.35 *vs* 1.04 ± 0.19, P = 0.026) ratios were significantly decreased after the high-CHO diet regardless of gender and of genotype of the *APOC3 Sst*I polymorphism. However, in female S2 carriers, the TG/HDL-C (1.38 ± 0.46 *vs* 1.63 ± 0.70, P = 0.039) ratio was significantly increased after the high-CHO diet. In conclusion, the high-CHO diet has favorable effects on the TC/HDL-C and LDL-C/HDL-C ratios regardless of gender and of genotype of the *APOC3 Sst*I polymorphism. Somehow, it enhanced the adverse effect of the S2 allele on the TG/HDL-C ratio only in females.

Key words: High-carbohydrate diet; Lipid ratio; Gene polymorphism; Human *APOC3*

Introduction

Cardiovascular disease (CVD) is recognized as a multifactorial disease, and dyslipidemia accounts for at least 50% of the population-attributable risk (1). Reliable indexes for CVD risk assessment and targets for drug treatment are important to prevent and manage this disease. Conventionally, increases in plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and/or triglyceride (TG) and decreases in high-density lipoprotein cholesterol (HDL-C) were considered to be the major factors causing CVD (2). Clinical guidelines recommended that the primary therapy be targeted to LDL-C reduction, and that the other lipid indexes be used as the secondary or supplementary targets (2). However, accumulating evidence from epidemiological and precisely controlled studies suggests that the HDL-C-related ratios including TG/HDL-C, TC/HDL-C and LDL-C/HDL-C may be superior to conventional lipid parameters as predictors of CVD or therapeutic targets for dyslipidemia (3-6). As the TC/HDL-C ratio is considered to be a more

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sensitive and predictive index of cardiovascular risk than TC, the Canadian Vascular Society has chosen this lipid ratio as a secondary goal of therapy (7). Recent data from observational and interventional studies have suggested that the apolipoprotein B100/apolipoprotein AI (apoB100/apoAI) ratio may be another strong and new CVD marker that is even better than the HDL-C-related ratios in CVD risk assessment (8-10). Each particle of the atherogenic lipoproteins, such as LDL, very low-density lipoprotein (VLDL) and intermediate-density lipoprotein, carries one apoB molecule, so that the concentrations of serum apoB reflect the total atherogenic potentials. On the other hand, apoAI is the major protein component of antiatherogenic lipoprotein, i.e., HDL, and the serum content of apoAI represents the total antiatherogenic potential. Thus, the apoB/apoAI ratio could be a comprehensive and accurate CVD risk maker; the lower the apoB/apoAI ratio, the lower the risk.

A high-carbohydrate (high-CHO) diet has profound effects on lipid metabolism. Carbohydrates can be converted into fatty acids, TG, cholesterol, and other lipids in the liver and other organs. Previous studies have demonstrated that a high-CHO diet could increase fasting serum TG concentrations (11,12), leading to hypertriglyceridemia, a risk factor of CVD (13). It has also been documented that a high-CHO diet could decrease the risk of CVD by lowering plasma TC and LDL-C concentrations (12). Although the effects of a high-CHO diet on the changes of TC, LDL-C, HDL-C, and TG have been extensively reported in the past several decades, few reports are available about the effects of a high-CHO diet on the HDL-C-related and apoB100/apoAI ratios in subjects with different genotypes of the apolipoprotein gene polymorphisms. Since Chinese populations are accustomed to consuming carbohydrate-enriched diets (14,15), it is necessary to investigate the plasma lipid responses of subjects with a specific genetic background to the high-CHO diets.

ApoCIII plays an important role in the regulation of the metabolism of triglyceride-rich lipoproteins (TRLs) (16). Some polymorphisms in the apoCIII gene (*APOC3*) locus have been found to be closely related to plasma TG concentrations (17-19). The *Sst*I polymorphism is formed by a transversion from C to G and is located in the 3' untranslated region (3'UTR) of this gene (20). A number of studies have reported that the S2 carriers of the *APOC3 Sst*I polymorphism had higher plasma levels of TG (21-26), TC (27,28), LDL-C (27), and lower levels of HDL-C (27). The S2 allele was also associated with elevated plasma concentrations of apoCIII (29,30) and apoB (27,29,30), but not with lower apoAI levels (29). However, the interaction of this polymorphism with the high-CHO diet on serum lipid ratios has not been reported before.

Previous studies have shown that both the high-CHO diet and the *APOC3 Sst*I polymorphism affect the serum lipid and lipoprotein profiles (11,12,21-30). Therefore, we supposed that the different genotypes of the *APOC3 Sst*I polymorphism may have different responses to the high-CHO diet regarding the ratios of serum lipids and apolipoproteins. In the present study, we investigated the interaction of a high-CHO diet with the *APOC3 Sst*I polymorphism on the ratios of serum lipids and apolipoproteins in a young and healthy Chinese population. This may provide experimental evidence for the personalized dietary recommendations in the country with the largest population in the world.

Subjects and Methods

Subjects and diets

A total of 209 university students were recruited as volunteers by advertisement from West China Medical Center, Sichuan University. All were Chinese Han people. Before the dietary intervention, all volunteers completed a medical questionnaire and were subjected to a physiological and biochemical examination. According to the results of the medical survey and physiological and biochemical examination, those who met the inclusion criteria finally entered the study. These criteria included no history of metabolic diseases, understanding of the procedures involved, and providing written consent. Exclusion criteria were: presence of diabetes, dyslipidemia, or cardiovascular, renal, endocrinologic diseases, taking lipid-lowering drugs or hormones, consuming alcohol, smoking, or wide variation in physical activity or sleeping time. Finally, 60 volunteers, who were apparently healthy as indicated by the medical questionnaire and the physiological and biochemical examination, entered the study. Fifty-six subjects (27 males and 29 females) completed the study with good compliance to the end; their baseline characteristics are shown in Table 1.

In the present study, a 13-day dietary intervention included 7 days of the washout diet as a washout period and 6 days of the high-CHO diet. Previous studies (31) have indicated that serum lipids could reach a new steady state after 5 days of dietary intervention. Therefore, a regime of 7 days of washout control diet followed by 6 days of high-CHO intervention diet was adopted in this study. As shown in Table 2, the washout diet contained 54% carbohydrate, 31% fat, and 15% protein, and the high-CHO diet contained 70% carbohydrate, 15% fat, and 15% protein of the total energy. The ingredients of the meals were from foods consumed by local people daily and all meals were prepared by the Department of Nutrition, West China Hospital, Sichuan University. Three daily meals were given to the volunteers who always ate together in a group at the student canteen at Huaxi campus of Sichuan University. The offered meals were breakfast at 7:00 to 8:00 am, lunch at 11:30 to 12:30, and dinner at 5:00 to 6:00 pm. The composition of each meal changed every day, but each meal had constant ratios of carbohydrate, protein, and fat as part of total energy. A daily dietary log was used to assess the compliance of each participant to the dietary intervention. There was no total energy restriction. Calories from food

intake were not restricted for each meal. The volunteers ordered their meals according to their estimations of how much they needed to eat for satiation as usual in their daily life. However, subjects were instructed not to take any other food or drink, except water. The study protocol was approved by the Human Research Ethics Committee of Sichuan University (No. 3011011).

Measurements of serum lipids and apolipoproteins

On the mornings of the day when the washout diet was started, of the day when the high-CHO diet was started and of the day after the high-CHO diet, 12-h fasting venous blood samples were collected. Serum was prepared by centrifugation at 3000 *g* for 15 min and immediately used for the measurement of serum lipids and apolipoproteins. Enzymatic methods were used to determine the serum concentrations of TG and TC. HDL-C concentrations were measured after precipitation of apoB-containing lipoproteins with phosphotungstic-Mg^{2+} (Biosino, China). LDL-C concentrations were determined by the polyvinyl sulfate (Biosino) precipitation method using a semi-automated biochemistry analyzer BT-224 (Biotecnica, Italy). Immunoturbidimetry assays were used to determine the serum levels of apoB100 and apoAI. All biochemical parameters were measured three times, and the mean value of the three measurements was used for the calculation of the ratios of serum lipids and apolipoproteins.

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using a DNAout kit (Tiandz, China). The DNA fragments containing the *Sst*I site of *APOC3* were amplified by PCR in a 25-µL reaction mixture containing 1 µL forward primer of 5'-GGTGACCGATG GCTTCAGTTCCCTGA-3' and 1 µL reverse primer of 5'-CAGAAGGTGG ATAGAGCGCTGGCC-3' (Sangon Biotech, China). The cycling conditions were 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 58°C for 40 s, and 72°C for 40 s, with a final extension at 72°C for 5 min. One microliter PCR product was digested at 37°C for 16 h with 1 U *Sst*I restriction enzyme (New England BioLabs, USA). Digested PCR products were analyzed on 2% agarose gel and visualized by ethidium bromide (GBC BIO-Tech, China) staining. The wild-type allele lacking the *Sst*I cutting site was defined as S1, whose hydrolyte was an intact 428-bp DNA fragment, and the allele containing the *Sst*I cutting site was defined

Table 1. Anthropometric and biochemical characteristics of the study subjects.

Variables	Males (N = 27)	Females (N = 29)	All (N = 56)
Age (years)	22.96 ± 1.95	22.83 ± 1.67	22.89 ± 1.80
Weight (kg)	62.99 ± 11.67	50.91 ± 7.07*	56.73 ± 11.26
Body mass index (kg/m^2)	21.86 ± 4.13	20.27 ± 2.58	21.04 ± 3.48
Waist circumference (cm)	74.87 ± 10.01	67.54 ± 6.24*	71.14 ± 9.02
Waist-to-hip ratio	0.89 ± 0.06	0.83 ± 0.04*	0.86 ± 0.06
Heart rate (bpm)	72.30 ± 10.49	74.07 ± 9.31	73.21 ± 9.85
Systolic blood pressure (mmHg)	117.04 ± 14.02	104.66 ± 6.40*	110.63 ± 12.36
Diastolic blood pressure (mmHg)	75.93 ± 12.25	67.93 ± 7.14*	71.79 ± 10.64
Triglyceride (mg/dL)	89.12 ± 55.68	65.22 ± 24.29*	76.52 ± 43.43
Total cholesterol (mg/dL)	135.47 ± 45.47	155.56 ± 28.34	145.87 ± 38.59
HDL-C (mg/dL)	55.50 ± 15.62	71.20 ± 12.46*	63.63 ± 16.04
LDL-C (mg/dL)	64.32 ± 44.09	73.20 ± 30.82	68.92 ± 37.71
Apolipoprotein AI (mg/dL)	193.63 ± 26.41	213.76 ± 15.90	204.64 ± 23.39
Apolipoprotein B100 (mg/dL)	65.88 ± 22.73	69.34 ± 18.38	67.77 ± 20.33
Glucose (mg/dL)	4.00 ± 0.53	4.01 ± 0.54	4.01 ± 0.53
Insulin (µU/mL)	4.80 ± 3.72	5.37 ± 2.29	5.11 ± 3.03

Data are reported as means ± SD. HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol. *P < 0.05 compared to males (unpaired *t*-test).

Table 2. Composition of the diets administered to the volunteers of the study as determined by chemical analysis.

Ingredients	Washout diet (7 days)	High-CHO diet (6 days)
Protein (% of total energy)	15.8 ± 1.8	16.2 ± 1.6
Carbohydrate (% of total energy)	54.1 ± 2.4	70.1 ± 2.8
Fiber (g/day)	11.6 ± 2.3	15.4 ± 3.6
Fatty acids (% of total energy)	30.1 ± 3.6	13.8 ± 1.4
Saturated fatty acids (% of total energy)	7.5 ± 0.9	3.6 ± 0.5
Monounsaturated fatty acids (% of total energy)	16.1 ± 1.4	7.3 ± 0.8
Polyunsaturated fatty acids (% of total energy)	6.4 ± 1.5	2.8 ± 0.3
Fatty acid composition		
Palmitic fatty acids (16:0) (% of total fatty acids)	15.9 ± 4.4	18.9 ± 5.8
Stearic fatty acids (18:0) (% of total fatty acids)	6.9 ± 1.3	7.4 ± 0.9
Palmitoleic fatty acids (16:1) (% of total fatty acids)	2.1 ± 0.7	2.0 ± 0.4
Oleic fatty acids (18:1) (% of total fatty acids)	30.7 ± 6.5	32.1 ± 3.7
Linoleic fatty acids (18:2) (% of total fatty acids)	13.2 ± 3.3	17.0 ± 5.1

Data are reported as means ± SD. Calories from food intake were not restricted for each meal. The volunteers ate to satiation as usual in their daily life. The high-carbohydrate diets (high-CHO) were supplied by the Department of Nutrition, West China Hospital, Sichuan University.

as S2, whose hydrolytes were two DNA fragments of 308 and 120 bp.

Statistical analysis

Data are reported as means ± standard deviation (SD) unless otherwise stated. Normality of the data in each group was tested using the Shapiro-Wilk test. A log power transformation was applied to data with a positively skewed distribution (e.g., TG, TG/HDL-C). The genotype and allele frequencies were compared between males and females by the chi-square test. The mean values of the variables obtained before and after the high-CHO diet were compared by the paired *t*-test. The mean values of the variables were compared between different genotypes by one-way analysis of variance (ANOVA). The mean values of the variables were compared between males and females, or between males and females with the same genotype by the unpaired *t*-test. Statistical significance was defined at 0.05.

Results

Frequencies of genotypes and alleles of the *APOC3 Sst*I polymorphism

Of the 56 subjects studied, 32 (57.1%) were wild-type homozygotes (S1S1), 20 (35.7%) were heterozygotes (S1S2), and 4 (7.1%) were mutant homozygotes (S2S2). The frequency of the major allele S1 was 75%, and the frequency of the minor allele S2 was 25%. No significant differences in genotype frequency ($P = 0.189$, $\chi^2 = 1.722$) or allele frequency ($P = 0.275$, $\chi^2 = 1.192$) were found between males and females. No deviation from the Hardy-Weinberg equilibrium was found in the distribution of the genotypes ($P = 0.743$, $\chi^2 = 0.107$).

Baseline ratios of lipids and apolipoproteins according to the *APOC3 Sst*I genotype

Due to the small group size of the homozygotes for the minor allele, the heterozygotes and homozygotes carrying the S2 allele were combined and referred to as the S2 carriers for statistical analysis. As shown in Table 3, there were no significant differences in the ratios between the S2 carriers and the subjects with the S1S1 genotype in the study population as a whole. When gender was taken into account, the female S2 carriers had a significantly higher ratio of TG/HDL-C ($P = 0.009$) than the females with the S1S1 genotype, whereas no significant associations were observed in males.

Effects of the high-carbohydrate diet on the ratios of serum lipids and apolipoproteins in the subjects with different genotypes of the *APOC3 Sst*I polymorphism

As shown in Table 4, the S2 carriers had a significantly higher ratio of TG/HDL-C ($P = 0.008$) than the subjects with the S1S1 genotype after the high-CHO diet in the study population as a whole. Compared with those before the high-CHO diet, both TC/HDL-C ($P = 0.000$ for both the S2 carriers and the subjects with the S1S1 genotype) and LDL-C/HDL-C ($P = 0.002$ for the S2 carriers and $P = 0.000$ for the subjects with the S1S1 genotype) decreased significantly after the high-CHO diet regardless of genotype. TG/HDL-C ($P = 0.011$) increased and apoB100/apoAI ($P = 0.006$) decreased significantly only in the S2 carriers.

When gender was taken into account, the females with the S1S1 genotype had a significantly lower ratio of TG/HDL-C ($P = 0.013$) than the males with the same genotype before the high-CHO diet. The female S2 carriers had a higher ratio of TG/HDL-C than the females with the S1S1 genotype before ($P = 0.016$) or after ($P = 0.013$) the high-CHO diet. When compared with those before the high-CHO diet, both TC/HDL-C ($P = 0.000$ for both the male S2 carriers and the male subjects with the S1S1 genotype; $P = 0.004$ for the female S2 carriers and $P = 0.000$ for the female subjects with the S1S1 genotype) and LDL-C/HDL-C ($P = 0.012$ for the male S2 carriers, $P = 0.005$ for the male subjects with the S1S1 genotype, $P = 0.049$ for the female S2 carriers, and $P = 0.026$ for the female subjects with the S1S1 genotype) decreased significantly after the high-CHO diet regardless of gender or genotype of the *APOC3 Sst*I

Table 3. Lipid and apolipoprotein ratios of the cohort at baseline.

Variables	Males		Females		All	
	S1S1 (N = 13)	S2 carriers (N = 14)	S1S1 (N = 19)	S2 carriers (N = 10)	S1S1 (N = 32)	S2 carriers (N = 24)
Age (years)	23.69 ± 1.93	22.29 ± 1.77	22.68 ± 1.86	23.10 ± 1.29	23.09 ± 1.92	22.62 ± 1.61
TG/HDL-C	1.66 ± 1.87	1.82 ± 1.28	0.80 ± 0.28	1.29 ± 0.66*	1.13 ± 1.23	1.60 ± 1.08
TC/HDL-C	2.38 ± 0.55	2.75 ± 0.74	2.18 ± 0.33	2.27 ± 0.50	2.26 ± 0.42	2.55 ± 0.68
LDL-C/HDL-C	1.24 ± 0.57	1.30 ± 0.70	1.05 ± 0.42	1.00 ± 0.43	1.12 ± 0.48	1.18 ± 0.61
ApoB100/apoAI	0.33 ± 0.12	0.36 ± 0.15	0.33 ± 0.07	0.31 ± 0.10	0.33 ± 0.09	0.34 ± 0.13

Data are reported as means ± SD. TG = triglyceride; HDL-C = high-density lipoprotein cholesterol; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; ApoB100 = apolipoprotein B100; apoAI = apolipoprotein AI. *$P < 0.05$ compared to the subjects with the S1S1 genotype (ANOVA). No significant differences were detected between males and females with the same genotype (unpaired *t*-test).

Table 4. Lipid and apolipoprotein ratios of the subjects before and after the high-carbohydrate (high-CHO) diet according to the *APOC3 Sst*I genotype.

Variables	Males		Females		All	
	S1S1 (N = 13)	S2 carriers (N = 14)	S1S1 (N = 19)	S2 carriers (N = 10)	S1S1 (N = 32)	S2 carriers (N = 24)
Age (years)	23.69 ± 1.93	22.29 ± 1.77	22.68 ± 1.86	23.10 ± 1.29	23.09 ± 1.92	22.62 ± 1.61
TG/HDL-C						
Before	1.64 ± 0.95	1.84 ± 1.29	1.01 ± 0.31^{+}	1.38 ± 0.46$^{\#}$	1.26 ± 0.71	1.65 ± 1.04
After	1.41 ± 0.59	2.04 ± 1.40	1.15 ± 0.29	1.63 ± 0.70$^{\#*}$	1.25 ± 0.45	1.87 ± 1.16$^{\#*}$
TC/HDL-C						
Before	2.97 ± 0.74	3.13 ± 1.00	2.69 ± 0.41	2.68 ± 0.36	2.80 ± 0.58	2.94 ± 0.82
After	2.09 ± 0.55**	2.36 ± 0.65**	2.09 ± 0.31**	2.24 ± 0.37*	2.09 ± 0.42**	2.31 ± 0.55**
LDL-C/HDL-C						
Before	1.35 ± 0.61	1.44 ± 0.71	1.18 ± 0.35	1.18 ± 0.33	1.25 ± 0.47	1.33 ± 0.59
After	1.01 ± 0.29*	1.06 ± 0.26*	1.04 ± 0.19*	1.00 ± 0.18*	1.03 ± 0.23**	1.04 ± 0.23*
ApoB100/apoAI						
Before	0.35 ± 0.17	0.38 ± 0.17	0.31 ± 0.07	0.30 ± 0.09	0.33 ± 0.12	0.34 ± 0.15
After	0.35 ± 0.17	0.35 ± 0.16*	0.31 ± 0.08	0.29 ± 0.10	0.33 ± 0.12	0.32 ± 0.14*

Data are reported as means ± SD. TG = triglyceride; HDL-C = high-density lipoprotein cholesterol; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; ApoB100 = apolipoprotein B100; apoAI = apolipoprotein AI. $^{\#}P < 0.05$ compared to male and female subjects and to the subjects as a whole with the S1S1 genotype (ANOVA). *P < 0.05 and **P < 0.001 compared to the value before the high-CHO diet for males, for females and for the subjects as a whole (paired *t*-test). $^{+}P < 0.05$ compared to males with the same genotype before or after the high-CHO diet (unpaired *t*-test).

polymorphism. A significant elevation of TG/HDL-C (P = 0.039) was observed only in the female carriers of the S2 allele and a significant reduction of apoB100/apoAI (P = 0.012) was observed only in the male carriers of the S2 allele after the high-CHO diet intervention.

Discussion

ApoCIII is a major component of TRLs and has several functions in the regulation of the metabolism of TRLs. It inhibits the activities of lipoprotein lipase and hepatic lipase (32,33), two enzymes that metabolize TGs in TRLs and facilitate their clearance from the circulation. It impairs the hepatic uptake of TRL remnants, probably by interfering with the interaction of apoE in the lipoproteins with LDL receptors. Recent evidence has shown that apoCIII might stimulate VLDL assembly and secretion (34). Mutations at several loci have been found within or around the *APOC3* gene. The most extensively studied one is the *Sst*I polymorphism located in the 3'UTR of the gene (20). Associations of the minor allele S2 with higher levels of TG (21-26) and lower levels of HDL-C (27) have been reported in various ethnic groups. However, the interactions of this genetic variant with a high-CHO diet on the ratios of serum lipids and apolipoproteins have not been reported before. In the present study, the baseline level of TG/HDL-C of the S2 carriers was significantly higher than that of the subjects with the S1S1 genotype in females but not in males (Table 3). This result suggests that the *Sst*I polymorphism in *APOC3* is associated with the TG/HDL-C ratio and this association is gender-dependent. In addition, the TG/HDL-C ratio of the S2 carriers before or after the high-CHO diet was significantly higher than that of the subjects with the S1S1 genotype only in females but not in males (Table 4). These results indicate that the S2 allele of the *Sst*I polymorphism in *APOC3* is consistently associated with a higher TG/HDL-C ratio in females on different diets. On the other hand, the TG/HDL-C ratio in the females with the S1S1 genotype was significantly lower than that in the males with the same genotype after the washout diet. This result implies that, in the present study, the S1 allele had a favorable effect on the TG/HDL-C ratio in females on the washout diet.

The interactions of the *Sst*I polymorphism in *APOC3* with the high-CHO diet on the ratios of serum lipids and apolipoproteins of the subjects with different genotypes were also investigated in the present study. Conceivably, other genetic and environmental factors affecting lipoprotein metabolisms would remain constant for each individual, especially during such a short period of 6 days on the high-CHO diet. Therefore, the differences in changes of ratios upon the high-CHO diet intervention were most likely attributed to the specific genetic background of the individuals. The TG/HDL-C ratio after the high-CHO diet was significantly higher than that before the high-CHO diet only in the female carriers of the S2 allele but not in the females with the S1S1 genotype (Table 4). Considering the result that the TG/HDL-C ratio of the S2 carriers was consistently and significantly higher than that of the subjects with the S1S1

genotype only in the females before or after the high-CHO diet (Table 4), we may conclude that the high-CHO diet enhances the adverse effect of the S2 allele on the TG/HDL-C ratio in females.

To our knowledge, there are no published studies on the gender-dependent association of the *Sst*I polymorphism in *APOC3* with the TG/HDL-C ratio. However, several studies (26,27) have demonstrated that there were gender-dependent associations of the *APOC3 Sst*I polymorphism with the plasma lipids, including TG, TC, HDL-C, LDL-C, and apoB. In a cohort study of American Caucasians, Russo et al. (27) reported that the S2 allele of the *APOC3 Sst*I polymorphism was associated with lower concentrations of HDL-C and higher concentrations of TG only in males, indicating that the males with the S2 allele tended to have a higher TG/HDL-C ratio. Conversely, in a cohort study of Asian Taiwanese, Huang et al. (26) reported that the S2 allele of the *APOC3 Sst*I polymorphism was associated with elevated TG only in females. Therefore, the females with this allele tended to have a higher TG/HDL-C ratio in this population. Taken together with the results of the present study, these data indicate that the association of the *Sst*I polymorphism in *APOC3* with the TG/HDL-C ratio is not only dependent on gender, but also on ethnicity.

One explanation for the fact that only females with the S2 allele of the *Sst*I polymorphism in *APOC3* were negatively influenced by the high-CHO diet could be their higher levels of estrogen. High-CHO diets can adversely increase the plasma concentrations of TGs (11,12), which are mainly hydrolyzed by lipoprotein lipase, the key enzyme in the hydrolysis of TGs in blood. However, high levels of estrogen in females significantly inhibit the activity of lipoprotein lipase and retard the hydrolysis of TGs by this enzyme (35).

Although previous investigators have reported that the S2 allele was associated with increased level of TC and decreased level of HDL-C (27,28), no significant differences were found in the TC/HDL-C and LDL-C/HDL-C ratios at baseline between S2 carriers and subjects with the S1S1 genotype. This result might be explained by the small sample size of 56 subjects and by the young age of the subjects (22.89 ± 1.80 years), since almost all previous studies were carried out on middle-aged or senior subjects (27,28). After the high-CHO diet, all subjects regardless of gender or genotype experienced significant decreases in the TC/HDL-C and LDL-C/HDL-C ratios determined before the diet. This may have been due to the fact that the marked decreases in the TC/HDL-C and LDL-C/HDL-C ratios after the high-CHO diet masked the effects of the genetic background and gender on the changes of the ratios in the young and healthy cohort. It is well known that the Chinese population is characterized by a lower incidence of CVD (36-38), more favorable lipid profiles including lower levels of TG, TC, and LDL-C and higher level of HDL-C, and a diet containing low fat and high carbohydrate (14,15). It is possible that the Chinese population has adapted to the high-CHO diet over the long time of biological evolution. Therefore, the TC/HDL-C and LDL-C/HDL-C ratios experienced marked decreases after a short-term high-CHO intake.

Favorable effects of the high-CHO diet were observed on the TC/HDL-C and LDL-C/HDL-C ratios regardless of gender or genotype of the *APOC3 Sst*I polymorphism in a young and healthy Chinese cohort. However, the high-CHO diet enhanced the adverse effect of the S2 allele on the TG/HDL-C ratio in the females of this cohort. Our results may provide experimental evidence for personalized dietary recommendations in the country with the largest population in the world.

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