

# The $-1131T \rightarrow C$ polymorphism in the apolipoprotein A5 gene is associated with postprandial hypertriacylglycerolemia; elevated small, dense LDL concentrations; and oxidative stress in nonobese Korean men<sup>1-3</sup>

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## ABSTRACT

**Background:** Apolipoprotein A5 plays an important role in modulating triacylglycerol metabolism in experimental animal models.

**Objective:** The objective was to determine associations of the common apolipoprotein A5 gene (*APOA5*)  $-1131T \rightarrow C$  polymorphism with postprandial lipemic response and other cardiovascular disease risk factors in humans.

**Design:** Healthy, nonobese subjects [ $n = 158$ ; mean ( $\pm$ SEM) age:  $33.8 \pm 1.2$  y; body mass index (in  $\text{kg}/\text{m}^2$ ):  $23.3 \pm 0.3$ ] were subdivided into 3 genotype groups: *TT* ( $n = 85$ ), *TC* ( $n = 56$ ), and *CC* ( $n = 17$ ). We measured fasting and postprandial lipid concentrations, lipid peroxidation, C-reactive protein concentrations, and DNA damage.

**Results:** Fasting triacylglycerol concentrations in carriers of the *C* allele were higher ( $P < 0.05$ ) than in carriers of the *TT* genotype. No other significant genotype-related differences were observed for any of the other baseline measures. After consumption of a mixed meal, carriers of the *C* allele had significantly greater increases in total chylomicron and VLDL triacylglycerol than did subjects with the *TT* genotype. Moreover, carriers of the *C* allele had higher dense LDL, serum C-reactive protein, and urinary 8-*epi*-prostaglandin  $F_{2\alpha}$  concentrations and more lymphocyte DNA damage. Conversely, we did not find significant genotype-related differences in postprandial glucose, insulin, or free fatty acid measures.

**Conclusions:** Our data confirm the genetic modulation of serum fasting triacylglycerol concentrations by the *APOA5* gene polymorphism and extend this observation to postprandial triacylglycerol concentrations and to markers of oxidation and inflammation. The presence of the *C* allele in the *APOA5* promoter region at position 1131 could be a significant factor contributing to higher cardiovascular disease risk in Koreans independently of common environmental factors. *Am J Clin Nutr* 2004;80:832–40.

**KEY WORDS** Apolipoprotein A5 gene, postprandial lipid response, cardiovascular disease risk, lipid peroxidation, DNA damage, C-reactive protein

## INTRODUCTION

Ischemic heart disease mortality rates in Korea have dramatically increased from 6.8 per 100 000 in 1988 to 13.8 per 100 000 in 1997 (1). This shift has occurred in the context of low serum total cholesterol (192 mg/dL for men) and LDL-cholesterol (114

mg/dL for men) concentrations that have remained relatively unchanged in this population (2). Conversely, Korean men tend to have high mean serum triacylglycerol concentrations ( $\approx 144$  mg/dL), which can be partly attributable to the high carbohydrate intake (66% of calorie intake) in this population (3) and to other factors frequently associated with elevated serum triacylglycerol, such as cigarette smoking, excessive alcohol consumption, being overweight, and lack of exercise (4, 5). In addition, ethnic-specific genetic factors can contribute to the elevated triacylglycerol concentrations. The combination of these factors can contribute to the high serum triacylglycerol concentration and subsequently to the increased cardiovascular disease (CVD) indicated earlier.

Among the genetic factors associated with variability of serum triacylglycerol concentrations in the population, the apolipoprotein A5 gene (*APOA5*) is emerging as a main candidate gene for modulating triacylglycerol metabolism in humans. Several common single-nucleotide polymorphisms (SNPs) were identified at this locus, and they were consistently and significantly associated with plasma triacylglycerol concentrations in white (6, 7), Japanese (8, 9), and Singaporean populations (10). Moreover, these SNPs were associated with increased risk of familial combined hyperlipidemia and coronary artery disease in whites (7, 11). One of these *APOA5* SNPs,  $-1131T \rightarrow C$ , was shown to vary significantly in allele frequency among ethnic groups. Thus, the

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frequency of the less common C allele is much higher in Japanese (0.34) than in whites (0.08) (8), and this observation was extended to Malayan and Chinese ethnicities in Singapore (10). In addition, those previous studies reported significant association of the C allele with increased fasting plasma triacylglycerol concentrations. Paradoxically, Masana et al (12) reported that normolipidemic carriers of the C allele have higher fasting triacylglycerols but lower incremental diurnal triacylglycerolemia, reflecting postprandial lipemia, than do noncarriers. However, only 13 subjects (7 men and 6 women) were carriers of the C allele at the -1131T→C SNP, supporting the need for more extensive studies (12).

The work presented in this paper was carried out in a group of Korean men who genetically and environmentally differ from whites. Our primary goal was to examine the association between the APOA5 -1131T→C SNP and postprandial lipid response. In addition, we examined associations with other novel CVD risk factors, including small, dense LDL; lipid peroxides; C-reactive protein (CRP); and DNA damage of lymphocytes.

## SUBJECTS AND METHODS

### Subjects

One hundred fifty-eight healthy men were recruited from volunteers who responded to advertisements for a nutrition study conducted by the Clinical Nutrition Research Team at Yonsei University in 2003. The age range was 20–55 y, and the body mass index (BMI; in kg/m<sup>2</sup>) range was 18.0–30.0. All subjects had normal results on glucose tolerance tests and normal electrocardiograms. None of them were taking any medication or had clinical evidence of CVD or cancer. Written informed consent was obtained from all subjects, and the protocol was approved by the Ethical Committee of the Yonsei University. Subjects were asked to refrain from performing strenuous exercise or drinking alcoholic beverages 24 h before the meal tolerance test. They were also instructed to avoid eating or drinking anything except water during the test period.

### Meal tolerance test

A 6-h postprandial lipemia response test was carried out starting at 0830 after an overnight fast of >12 h. A standardized test meal was prepared in the metabolic ward with the use of common food items. It consisted of a sandwich containing white bread, lettuce, ham, and soybean oil-based mayonnaise. The energy content, calculated from the computerized database Korean food-code, based on food-composition tables from the National Rural Living Science Institute (6th ed, 2000) in Korea, was 608 kcal (2.54 MJ), representing the average caloric intake from a traditional breakfast. Fat represented 41.4% (28 g) of the calories, carbohydrates made up 45.4% (69 g), and 13.2% (20 g) was derived from protein. In contrast, the macronutrient composition of the subjects' usual diet was that of a typical diet with cooked refined rice, consumed by a substantial number of Koreans, consisting of about 20% from fat, 57% of energy from carbohydrate, 15% from protein, and 7% from others (mainly alcohol).

### Blood collection

Venous blood samples were obtained from the forearm and collected into EDTA-treated and plain tubes during fasting (baseline) and at 2, 3, 4, and 6 h after breakfast for assessment of

glucose, insulin, free fatty acids (FFAs), triacylglycerols, chylomicron triacylglycerol, and VLDL triacylglycerol. Tubes were immediately placed on ice until they arrived at the analytic laboratory (within 1–3 h) and were stored at -70 °C (for glucose, insulin, FFAs, and triacylglycerol measurements) and at 4 °C (for chylomicron and VLDL separation).

### APOA5 genotyping

Genomic DNA was extracted from 5 mL whole blood with the use of a commercially available DNA isolation kit (WIZARD Genomic DNA purification kit; Promega Corp, Madison, WI) according to the manufacturer's protocol. -1131T→C genotyping was performed by SNP-IT assays with the use of single primer extension technology (SNPstream 25K System; Orchid Biosystems, Princeton, NJ). The results of yellow or blue or both color developments were analyzed with enzyme-linked immunosorbent assay reader, and the final genotype calls were made with the QCRView program (Orchid Biosystems, Princeton, NJ).

### Anthropometric and blood pressure measurements

Body weight and height were measured in the morning while the participants were unclothed and without shoes. BMI was calculated as body weight (in kg) divided by height<sup>2</sup> (in m). Waist and hip circumferences were combined into waist-to-hip ratio, representing a commonly used surrogate of body fat distribution. Blood pressure was read from the left arm while subjects remained seated. An average of 3 measurements was recorded for each subject.

### Total and visceral fat areas at fourth lumbar vertebra

We performed computerized tomography scanning with the use of a General Electric High Speed Advantage 9800 scanner (Milwaukee) to measure fat areas at the level of the fourth lumbar (L4) vertebra. Each computerized tomography slice was analyzed for the cross-sectional area of fat with the use of a density-control program available in the standard General Electric computer software. Limits for total abdominal fat density at the L4 level were selected between the range of -150 and -50 Hounsfield units. Total abdominal fat area was divided into visceral and subcutaneous fat areas to calculate specific fat areas.

### Measurement of serum lipid profile and apolipoproteins A-I and B

Fasting serum total cholesterol and triacylglycerol concentrations were measured with the use of commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd, Tokyo). After precipitation of serum chylomicron, LDL, and VLDL with dextran sulfate-magnesium, HDL cholesterol left in the supernatant fluid was measured by using an enzymatic method. LDL cholesterol was estimated indirectly with the use of the Friedewald formula for subjects with serum triacylglycerol concentrations < 4.52 mol/L (400 mg/mL). Serum apolipoproteins A-I and B were determined by using turbidometry at 340 nm with a specific antiserum (Roche, Basel, Switzerland).

### Glucose, insulin, and free fatty acids

Glucose was measured by using a glucose oxidase method with the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin was measured by using radioimmunoassays

with commercial kits from Immuno Nucleo Corporation (Stillwater, MN). FFAs were analyzed with the use of a Hitachi 7150 autoanalyzer (Hitachi Ltd). Responses of glucose, insulin, and FFAs to the fat challenge were calculated with the use of the trapezoidal method as area under the curve (AUC).

### Lipoprotein fraction analysis by density gradient ultracentrifugation

To collect chylomicron triacylglycerol, 1.006-g/mL density solution was added into plasma. Centrifugation was carried out in a Beckman 50.4Ti rotor at 10 000 rpm and 4 °C for 30 min with the use of a Beckman LE8 ultracentrifuge (Beckman Coulter, Fullerton, CA). To collect VLDLs, 2 mL plasma was adjusted to 1.182 g/mL and overlaid with a discontinuous gradient (density: 1.0988–1.0588 g/mL). Centrifugation was carried out in a Beckman SW 40 rotor at 35 000 rpm and 23 °C for 18 h with the use of a Beckman LE8 ultracentrifuge.

### LDL subfraction analysis by density gradient ultracentrifugation

A discontinuous salt gradient was devised which permitted isolation of LDL subfractions directly from plasma within 48 h according to the method of Griffin et al (13). Total LDL (density: 1.019–1.063 g/mL) was isolated by sequential density gradient ultracentrifugation. Three LDL subfractions (LDL<sub>1</sub>, density: 1.025–1.034 g/mL; LDL<sub>2</sub>, density: 1.034–1.044 g/mL; and LDL<sub>3</sub>, density: 1.044–1.060 g/mL) were quantified in fresh plasma by nonequilibrium density ultracentrifugation. Lipoprotein mass was determined by the sum of the following components: total cholesterol, triacylglycerol, free cholesterol, and phospholipids, which were determined with the use of enzymatic colorimetric test kits (Roche Diagnostic GmbH, Mannheim, Germany; Wako Chemicals GmbH, Neuss, Germany), esterified cholesterol, which was calculated from the difference between total cholesterol and free cholesterol, and apolipoproteins, which were determined by using a modified Lowry method (14).

### Urine collection and 8-*epi*-prostaglandin F<sub>2α</sub> and plasma malondialdehyde

Urine was collected after a 12-h fast in polyethylene bottles containing 1% butylated hydroxytoluene before blood collection. The tubes were immediately covered with aluminum foil and stored at –70 °C until analysis. 8-*epi*-prostaglandin F<sub>2α</sub> (8-*epi*-PGF<sub>2α</sub>) was measured with the use of an enzyme immunoassay (BIOXYTECH urinary 8-*epi*-PGF<sub>2α</sub> Assay kit; OXIS International Inc, Portland, OR). The resulting color reaction was read with the use of a Victor 2 (Perkin Elmer Life Sciences, Turku, Finland) at 650 nm. Urinary creatinine was determined by using the alkaline picrate (Jaffe) reaction (15), and urinary 8-*epi*-PGF<sub>2α</sub> concentrations were expressed as pmol/mmol creatinine. Plasma malondialdehyde was assayed according to the fluorometric method described by Miller et al (16).

### Alkaline comet assay for DNA damage

For the comet assay, 120 μL whole blood was mixed with 900 μL phosphate-buffered saline and poured gently over 150 μL lymphocyte separation solution (Histopaque-1077; Sigma-Aldrich Korea Ltd, Yong-In, Korea). After centrifugation at 1450 rpm and 4 °C for 4 min, lymphocytes were removed by

pipette and transferred to another tube. DNA damage was analyzed as described by Green et al (17).

### Plasma C-reactive protein

Plasma CRP concentrations were measured on an Express Plus autoanalyzer (Chiron Diagnostics Co, Walpole, MA) with the use of a commercially available high-sensitivity kit, CRP-Latex (II) X2 (Seiken Laboratories Ltd, Tokyo) (18).

### Assessment of food intake and physical activity level

Information about habitual food intake was obtained with the use of a 24-h recall method and a semiquantitative food-frequency questionnaire. We used the 24-h recall data to carry out our analyses, and the food-frequency questionnaire was used to verify that the 24-h recall was representative of the usual dietary pattern. Nutrient intake data were calculated as mean values from the same database as referred to earlier [National Rural Living Science Institute (6th ed, 2000)]. Total calorie expenditure (in kcal/d) was calculated from activity patterns, including basal metabolic rate, physical activity for 24 h (19), and specific dynamic action of food. Basal metabolic rate for each subject was calculated with the Harris-Benedict equation (20).

### Statistical analysis

We used SPSS version 11.0 for WINDOWS (SPSS Inc, Chicago) for all our statistical analyses. The main goal of this study was to investigate the effect of *APOA5* on postprandial lipid response, LDL phenotype, lipid peroxidation, and DNA damage. Subjects were categorized into the following 3 genotype groups: 1) *TT* group (*n* = 85) as a control group, 2) *TC* group (*n* = 56), and 3) *CC* group (*n* = 17). To investigate the genotype effect on the postprandial triacylglycerol response, we first performed multiple regression analysis with relevant variables such as age, BMI, HDL cholesterol, fasting triacylglycerol concentration, and *APOA5* genotype. Genotype-related differences were tested with the use of analysis of variance followed by the Tukey test or the Kruskal-Wallis test. We also performed analysis of variance for repeated measures to find the interaction between genotypes and time during postprandial lipid response with the use of covariants such as age and BMI affecting triacylglycerol concentration and AUC. The postprandial total triacylglycerol as well as triacylglycerol concentrations in chylomicron and VLDL were plotted as changes in concentration over the fasting value (taking fasting values as zero) to normalize for the differences in baseline values. In addition, we used an unpaired *t* test when comparing the differences between *TT* homozygotes and *C* carriers. Each variable was examined for normal distribution, and significantly skewed variables were log transformed. For descriptive purposes, mean values are presented on untransformed and unadjusted variables. Results are expressed as means ± SEs. A two-tailed value of *P* < 0.05 was considered statistically significant.

## RESULTS

### Frequency of the *APOA5* –1131T→C polymorphism

The *APOA5* –1131T→C genotype distribution among the 158 subjects examined was as follows: 85 men were homozygous for the *T* allele (*TT*), 56 were heterozygous for the *C* allele (*TC*),



**TABLE 1**Anthropometric values, blood pressure measures, and habitual macronutrient intakes by apolipoprotein A5 (−1131) genotype group<sup>1</sup>

	<i>TT</i> ( <i>n</i> = 85)	<i>TC</i> ( <i>n</i> = 56)	<i>CC</i> ( <i>n</i> = 17)
Age (y)	33.1 ± 0.87	33.9 ± 1.19	37.1 ± 2.61
BMI (kg/m <sup>2</sup> )	23.2 ± 0.29	23.5 ± 0.37	23.3 ± 0.47
Waist (cm)	82.9 ± 0.83	84.5 ± 1.10	84.7 ± 1.71
Waist-to-hip ratio	0.85 ± 0.01	0.86 ± 0.01	0.88 ± 0.01
Fourth lumbar vertebra (cm <sup>2</sup> )			
Total fat	188.5 ± 9.55	203.7 ± 11.1	199.8 ± 14.5
Visceral fat	73.2 ± 3.92	68.9 ± 5.54	86.0 ± 8.30
Blood pressure (mm Hg)			
Systolic	122 ± 1.5	122 ± 1.8	128 ± 3.4
Diastolic	75 ± 1.3	74 ± 1.4	76 ± 2.6
Tobacco consumption (cigarettes/d)	10.9 ± 1.36	8.45 ± 1.41	8.55 ± 2.36
Alcohol intake (g/d)	17.1 ± 3.99	24.5 ± 5.67	14.6 ± 3.40
TEE (kcal/d)	2563 ± 29	2508 ± 51	2580 ± 63
TCI (kcal/d)	2604 ± 36	2600 ± 66	2550 ± 82
Protein (%)	15.1 ± 0.33	15.2 ± 0.36	15.6 ± 0.66
Carbohydrate (%)	56.1 ± 0.91	57.4 ± 0.99	56.9 ± 1.88
Fat (%)	23.0 ± 1.92	18.9 ± 2.69	23.0 ± 2.36
Alcohol (%)	5.81 ± 1.50	8.65 ± 2.03	4.37 ± 1.44
TEE/TCI	0.99 ± 0.01	0.97 ± 0.01	1.02 ± 0.04

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ . TEE, total energy expenditure; TCI, total calorie intake. There were no significant differences between the genotype groups (one-way ANOVA with the Tukey test or the Kruskal-Wallis test).

and 17 were homozygous for the *C* allele (*CC*). These frequencies did not deviate significantly from Hardy-Weinberg equilibrium. The *C* allele frequency was 0.28, much greater than that reported in whites ( $\approx 0.08$ ) (21).

#### Basal behavioral, anthropometric, and clinical characteristics of the participants

The baseline characteristics of the subjects according to the *APOA5* genotype are shown in **Table 1**. All subjects had similar habitual dietary macronutrient intake, total energy expenditure, and socioeconomic status. We did not observe significant differences for age, BMI, waist circumference, visceral and total fat areas at the L4 level, blood pressure, and consumption of cigarettes and alcohol among subjects with the *TT*, *TC*, and *CC* genotypes. As indicated in **Table 2**, the *APOA5* genotype had a significant effect on fasting triacylglycerol concentrations but not on serum concentrations of total, LDL cholesterol, HDL cholesterol, apolipoprotein A-I, or apolipoprotein B.

During the postprandial period, triacylglycerol concentrations at 0, 2, 3, 4, and 6 h in the subjects with the *TT* genotype were significantly different from those in the subjects with the *TC* and

*CC* genotypes (data not shown). However, there were no significant differences in triacylglycerol concentrations between subjects in the *TC* and *CC* groups. In addition, the mean triacylglycerol changes from baseline to 2, 3, 4, and 6 h were not different between the *TC* and *CC* groups. Therefore, we combined the *TC* and *CC* groups and compared postprandial lipid metabolism between carriers of the *C* allele (*TC* + *CC*) and *TT* homozygotes.

#### Identification of predictors of postprandial triacylglycerol response

To identify significant predictors of postprandial lipid response, we carried out stepwise multiple regression analyses. Age, BMI, HDL cholesterol, fasting triacylglycerol, and *APOA5* genotype were used as independent variables, and postprandial triacylglycerol AUC was the dependent variable. The most significant predictor of postprandial lipemic response was fasting triacylglycerol concentration followed by HDL-cholesterol concentrations as shown in **Table 3**. As expected, there was a high correlation between fasting triacylglycerol concentrations and postprandial triacylglycerol area ( $R = 0.949$ ,  $P = 0.001$ ; data not

**TABLE 2**Fasting serum lipid and apolipoprotein concentrations by apolipoprotein A5 (−1131) genotype group<sup>1</sup>

	<i>TT</i> ( <i>n</i> = 85)	<i>TC</i> ( <i>n</i> = 56)	<i>CC</i> ( <i>n</i> = 17)
Triacylglycerols (mg/dL)	113.7 ± 6.61 <sup>b</sup>	142.1 ± 11.4 <sup>a</sup>	169.2 ± 30.2 <sup>a</sup>
Total cholesterol (mg/dL)	188.5 ± 3.77	190.9 ± 5.03	194.5 ± 8.72
LDL cholesterol (mg/dL)	115.3 ± 3.17	111.1 ± 4.00	114.9 ± 5.93
HDL cholesterol (mg/dL)	50.1 ± 1.10	51.3 ± 2.48	45.7 ± 2.16
Apolipoprotein A-I (mg/dL)	134.4 ± 2.03	134.4 ± 3.01	134.7 ± 4.06
Apolipoprotein B (mg/dL)	80.4 ± 2.19	84.2 ± 2.94	91.8 ± 4.93

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ . Values in the same row with different superscript letters are significantly different,  $P < 0.05$  (ANOVA with the Tukey test or the Kruskal-Wallis test).

**TABLE 3**Factors from multiple regression analyses for predicting fasting and postprandial triacylglycerol responses in nonobese, healthy men<sup>1</sup>

Dependent variable	Steps	Independent variable	$\beta$ coefficients	Standardized $\beta$ coefficients	<i>P</i>
Postprandial triacylglycerol area <sup>2</sup>	1	Fasting triacylglycerol	0.919	0.933	0.001
	2	HDL <sup>3</sup>	-0.00269	-0.068	0.010
Fasting triacylglycerol <sup>4</sup>	1	BMI	0.007	0.361	0.001
	2	<i>APOA5</i> genotype	0.224	0.281	0.001
	3	HDL	-0.007	-0.177	0.013
Postprandial triacylglycerol area <sup>5</sup>	1	BMI	0.071	0.340	0.001
	2	<i>APOA5</i> genotype	0.268	0.249	0.001
	3	HDL	-0.010	-0.247	0.001

<sup>1</sup> *APOA5*, apolipoprotein A5 gene. All dependent variables were log transformed.<sup>2</sup> The independent variables were age, BMI, HDL, fasting triacylglycerol, and *APOA5* genotype.<sup>3</sup> HDL had no correlation with any other variables except fasting and postprandial triacylglycerol responses.<sup>4</sup> The independent variables were age, BMI, HDL, and *APOA5* genotype.<sup>5</sup> The independent variables were age, BMI, HDL, and *APOA5* genotype (omitting fasting triacylglycerol).

shown), whereas HDL cholesterol was correlated only with fasting triacylglycerols (data not shown). Next, we performed additional stepwise multiple regression analyses with the use of fasting triacylglycerol concentration as dependent variable and age, BMI, HDL cholesterol, and *APOA5* genotype as independent variables. These analyses showed that BMI was the best significant predictor, followed by *APOA5* genotype. We also performed another stepwise multiple regression analysis with the use of postprandial triacylglycerol area as dependent variable and age, BMI, HDL cholesterol, and *APOA5* genotype as independent variables, omitting fasting triacylglycerol concentration from the list of potential independent variables. From this test, we found that BMI was the best significant predictor, followed by *APOA5* genotype and HDL concentration in that order. Therefore, our results show that *APOA5* genotype affects both the fasting and postprandial triacylglycerol concentration and response.

#### Association of the *APOA5* -1131T→C SNP with LDL subfractions, C-reactive protein, lipid peroxide concentrations, and DNA damage

Carriers of the *C* allele showed lower LDL<sub>2</sub> and higher LDL<sub>3</sub> concentrations than did subjects with the *TT* genotype (Table 4). There was no difference in concentration and proportion of the

larger LDL<sub>1</sub> between *TT* homozygotes and *C* carriers. The mean LDL<sub>3</sub> concentration in carriers of the *C* allele was 95 mg/dL, 68% higher than that measured in subjects with the *TT* genotype. Moreover, carriers of the *C* allele had higher concentrations of CRP and 8-*epi*-PGF<sub>2 $\alpha$</sub>  and more DNA damage than did subjects with the *TT* genotype (Figure 1).

#### Comparison of postprandial lipemia, glucose, insulin, and free fatty acids between carriers of the *C* allele and subjects with the *TT* genotype

Total plasma, chylomicron, and VLDL triacylglycerol concentrations before and after the mixed meal tolerance test for men in the *TT* and *TC* + *CC* genotype groups are shown in Figure 2. Significant time and genotype effects were observed for each one of those measures; however, we did not identify significant gene  $\times$  time interactions for any of the variables examined (Figure 2). Postprandial AUCs for total triacylglycerol (41%), chylomicron triacylglycerol (41%), and VLDL triacylglycerol (82%) were significantly higher in *C* carriers than in subjects with the *TT* genotype.

Mean changes for total, chylomicron, and VLDL triacylglycerol concentrations adjusted for baseline concentrations for each of the 2 allele groups are shown in Figure 3. Significant time effects were observed for all these measures. In both total and chylomicron triacylglycerol, significant genotype effects were shown, but we did not observe significant gene  $\times$  time interactions. There were no significant allele effects observed in VLDL triacylglycerol. Postprandial incremental AUCs for total triacylglycerol (32%), chylomicron triacylglycerol (28%), and VLDL triacylglycerol (76%) in *C* carriers were significantly higher than in subjects with the *TT* genotype (Figure 3). No significant differences were observed in fasting concentrations and AUCs of serum glucose, insulin, and FFAs during the mixed meal tolerance test between *C* carriers and subjects with the *TT* genotype (Table 5).

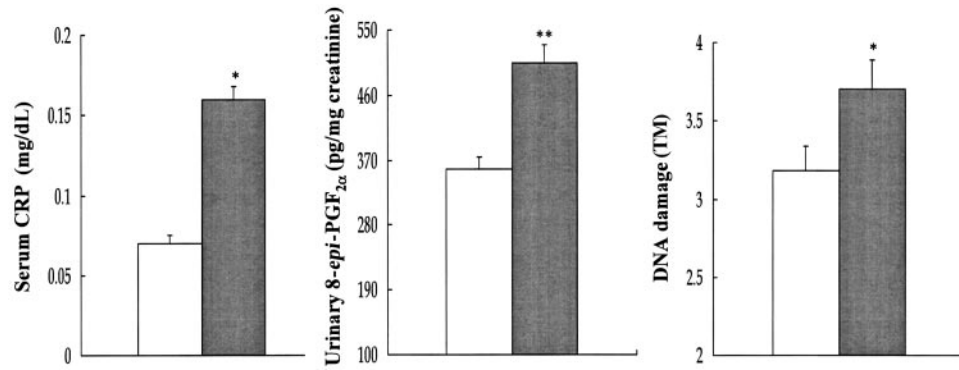
#### DISCUSSION

Our results indicate that the *APOA5* -1131T→C polymorphism has a significant influence on both fasting triacylglycerol and postprandial lipemic response after a mixed meal tolerance test. In the present study, fasting triacylglycerol concentrations in

**TABLE 4**Fasting concentrations of plasma LDL subfractions in men according to apolipoprotein A5 (-1131) genotypes<sup>1</sup>

	<i>TT</i> ( <i>n</i> = 85)	<i>TC</i> + <i>CC</i> ( <i>n</i> = 73)
LDL <sub>1</sub>		
(%)	17.6 $\pm$ 1.89	17.5 $\pm$ 2.20
(mg/dL)	39.5 $\pm$ 3.67	41.0 $\pm$ 4.40
LDL <sub>2</sub>		
(%)	60.6 $\pm$ 3.09	46.8 $\pm$ 3.53 <sup>2</sup>
(mg/dL)	147.6 $\pm$ 11.9	115.6 $\pm$ 10.4 <sup>3</sup>
LDL <sub>3</sub>		
(%)	27.1 $\pm$ 3.51	35.6 $\pm$ 4.58 <sup>3</sup>
(mg/dL)	56.3 $\pm$ 9.75	94.8 $\pm$ 14.0 <sup>3</sup>

<sup>1</sup> All values are  $\bar{x} \pm$  SEM.<sup>2,3</sup> Significantly different from *TT* genotype group (independent *t* test):<sup>2</sup> *P* < 0.01, <sup>3</sup> *P* < 0.05.



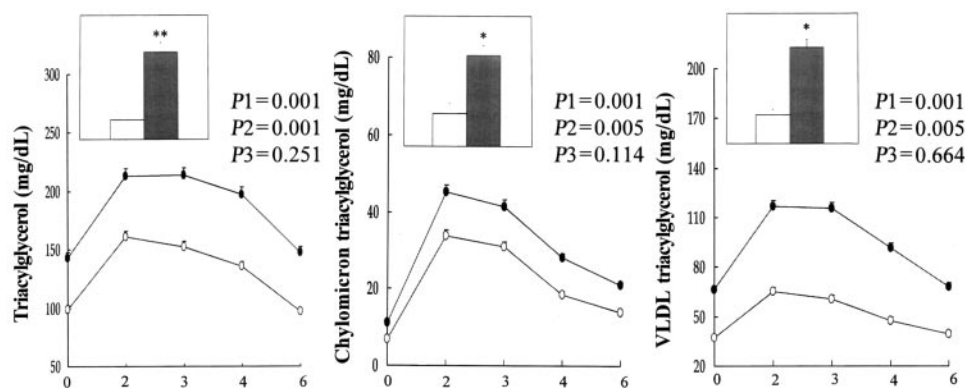
**FIGURE 1.** Mean ( $\pm$ SEM) C-reactive protein (CRP) concentrations, lipid peroxide [urinary 8-*epi*-prostaglandin F<sub>2α</sub> (8-*epi*-PGF<sub>2α</sub>)] concentrations, and DNA damage in lymphocytes from *TT* homozygotes (□; *n* = 85) and carriers of the *C* allele (■; *n* = 73). CRP values were tested after log transformation. TM, tail moment [percentage of DNA in the tail  $\times$  tail length (in  $\mu$ m)]. \*,\*\*Significantly different from *TT* homozygotes: \**P* < 0.05, \*\**P* < 0.01.

subjects with the *CC* and *TC* genotypes were 49% and 25% higher than those in subjects carrying the *TT* genotype. The difference in triacylglycerol concentrations between *TC* and *CC* genotypes was not statistically significant, probably because of the relatively small number of *CC* homozygous subjects, in conjunction with the larger variability expected in higher triacylglycerol concentrations. Because environmental factors, such as sex, calorie and carbohydrate intakes, BMI, visceral fat accumulation, and cigarette and alcohol consumption, were not significantly different among the 3 genotype groups, our results support the importance of the *APOA5* -1131T $\rightarrow$ C polymorphism in determining fasting triacylglycerol concentrations in Korean men, above and beyond the contribution of environmental factors characteristic of this population.

Consistent with the findings of Pennachio et al (21) and Nabika et al (8) we did not find significant differences in HDL-cholesterol concentrations between men with the *TT* genotype and men with the *TC* or *CC* genotype. However, another study by Endo et al (9) reported lower HDL-cholesterol concentrations associated with the *TC* or *CC* genotypes. These interstudy differences could be related to sex differences in the expression of this association. In this regard, Evans et al (7) recently reported that the HDL-lowering effect of the -1131T $\rightarrow$ C polymorphism is less in men than in women.

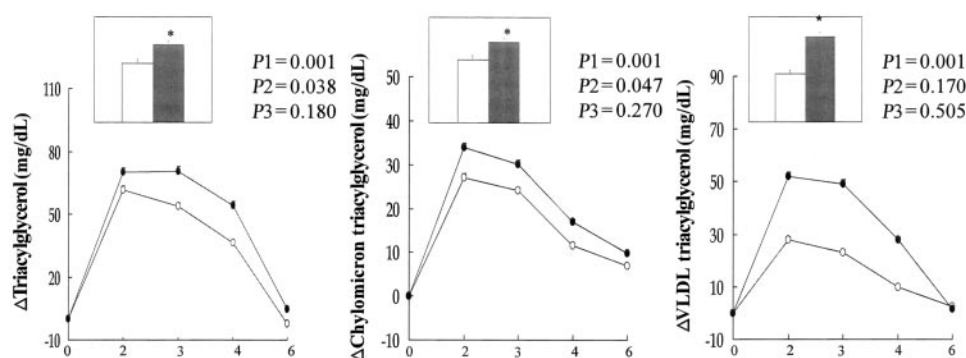
*APOA5* was reported to enhance the LPL-mediated lipolytic conversion of triacylglycerol-rich lipoproteins, thereby stimulating remnant formation and hepatic clearance in hyperlipidemic mice (22). Because the -1131T $\rightarrow$ C polymorphism might have a functional role in the expression of the *APOA5* gene (9, 22), the presence of the *C* allele might influence triacylglycerol concentrations by decreasing *APOA5* activity (6). In our study, carriers of the *C* allele showed impaired clearance of postprandial triacylglycerol-rich lipoproteins, which might relate to the decrease in their LPL-mediated clearance motivated by the decreased *APOA5* function. However, Martin et al (23) recently reported that postprandial triacylglycerol responses after adjusting for fasting triacylglycerol were not significantly different between subjects with the *TT* and *TC* genotypes. These different findings might be due to the interstudy differences in age, fasting triacylglycerol concentrations, or the composition of the habitual diets.

During the postprandial period, triacylglycerol-enriched particles could become substrates for the triacylglycerol-lipolytic activity of hepatic lipase (24, 25), which would result in their remodeling, giving rise to small, dense LDL (LDL<sub>3</sub>) (24, 26–29). In this study, carriers of the *C* allele showed significantly higher mean concentrations of LDL<sub>3</sub> than did subjects with the *TT*



**FIGURE 2.** Mean ( $\pm$ SEM) postprandial concentrations of total triacylglycerol, chylomicron triacylglycerol, and VLDL triacylglycerol after a mixed meal tolerance test in *TT* homozygotes (□, *n* = 85) and carriers of the *C* allele (●, *n* = 73) before adjustment for each fasting concentration. Bars represent the area under the curve responses for each genotype group. *P*<sub>1</sub>, time effect; *P*<sub>2</sub>, genotype effect; *P*<sub>3</sub>, interaction between genotype and time (repeated-measures ANOVA for all). \*,\*\*Significantly different from *TT* homozygotes: \**P* < 0.01, \*\**P* < 0.001.





**FIGURE 3.** Mean ( $\pm$ SEM) changes ( $\Delta$ ) from baseline in total triacylglycerol, chylomicron triacylglycerol, and VLDL triacylglycerol concentrations after a mixed meal tolerance test in *TT* homozygotes ( $\circ$ ,  $\square$ ;  $n = 85$ ) and carriers of the *C* allele ( $\bullet$ ,  $\blacksquare$ ;  $n = 73$ ). Bars represent the incremental area under the curve responses for each genotype group.  $P_1$ , time effect;  $P_2$ , genotype effect;  $P_3$ , interaction between genotype and time (repeated-measures ANOVA for all). \*Significantly different from *TT* homozygotes,  $P < 0.05$ .

genotype. This is an important observation, considering that subjects with small, dense LDL concentrations  $> 100$  mg/dL were found to have a 7-fold increase in CVD risk (30). Visceral fat accumulation (31) and insulin resistance (32) were reported to be associated with elevation in small, dense LDL concentrations. In this study, we did not observe significant differences in visceral fat areas, fasting insulin concentrations, and postprandial insulin AUC between genotypic groups. Therefore, we propose that these factors were not likely to be responsible for the marked difference in  $LDL_3$  concentrations between carriers of the *C* allele and *TT* homozygotes.

A carbohydrate-rich diet was also suggested to be one of the possible causes of the higher  $LDL_3$  concentrations in Koreans than in Northern Europeans (33). Again, we did not detect significant differences in usual carbohydrate intakes between carriers of the *C* allele and *TT* homozygotes. Thus, the raised concentrations of  $LDL_3$  in participants of this study might be related to high concentrations of fasting triacylglycerol and postprandial lipemia associated with the genetic variation at the *APOA5* locus.

A novel and important contribution from our study relates to the association between the *APOA5* locus and isoprostane values. Isoprostanes are a family of eicosanoids produced mainly by nonenzymatic oxidation of arachidonic acid by reactive oxygen

species. Consequently, their production is enhanced in the presence of oxidative stress (34). The measurement of urinary 8-*epi*-PGF $_{2\alpha}$  is a precise method for assessing oxidative stress (35), and in our population carriers of the *C* allele had higher urinary 8-*epi*-PGF $_{2\alpha}$ , consistent with higher oxidative stress, which might be related to their elevated fasting triacylglycerol concentration and postprandial lipemia. In fact, Jang et al (36) recently showed the enhancement in the oxidative stress and lipid peroxidation in subjects with more pronounced or exaggerated postprandial lipemia after the mixed meal tolerance test.

In addition, oxidative stress can adversely affect the antioxidant system (37, 38), resulting in increased DNA damage (39). Evaluation of DNA damage in lymphocytes was used as a biological marker of oxidative stress in the detection, monitoring, and prognosis of chronic degenerative diseases such as atherosclerosis (39, 40). Differences in the extent of DNA damage in the normal population were reported to depend not only on aging but also on visceral fat accumulation, eating, and smoking habits (36, 41, 42). In this study, no differences were observed regarding age, visceral fat area, caloric intake, alcohol consumption, and smoking habits between carriers of the *C* allele and *TT* homozygotes. Therefore, the higher oxidative stress observed in carriers of the *C* allele might cause greater DNA damage than in subjects with the *TT* genotype. Likewise, the higher oxidative stress has the potential to promote the initiation and to maintain the progression of chronic inflammation and the development of atherosclerosis (32). Concentrations of CRP, a known inflammatory marker of atherothrombotic vascular disease, were higher in carriers of the *C* allele than in subjects with the *TT* genotype. Interestingly, Genoux et al (43) suggested that *APOA5* is an inflammatory responsive gene and constitutes a link between inflammation and triacylglycerol-associated CVD risk, and our data support this notion.

In summary, the modulation of serum fasting and postprandial triacylglycerol concentrations by the *APOA5* locus was clearly established in this study in Korean men. We showed that, independently of other measured environmental factors, carriers of the *C* allele in the *APOA5* promoter region at position 1131 may be at increased CVD risk because of higher fasting triacylglycerol; small, dense LDL concentrations; oxidative stress; and postprandial lipemia. Because the frequency of the *C* allele at the 1131 locus is much higher in Koreans than in whites, this gene

**TABLE 5**

Fasting serum concentrations of and areas under the postprandial curves for glucose, insulin, and free fatty acids according to apolipoprotein A5 (−1131) genotype groups<sup>†</sup>

	<i>TT</i> ( $n = 85$ )	<i>TC + CC</i> ( $n = 73$ )
Fasting concentration		
Glucose (mg/dL)	90.1 $\pm$ 1.43	90.0 $\pm$ 1.40
Insulin ( $\mu$ IU/mL)	7.76 $\pm$ 0.48	8.50 $\pm$ 0.61
FFAs ( $\mu$ Eq/L)	469.8 $\pm$ 30.0	537.6 $\pm$ 38.6
Response area		
Glucose (mg $\cdot$ h/dL)	521.7 $\pm$ 6.36	522.6 $\pm$ 5.48
Insulin ( $\mu$ IU $\cdot$ h/mL)	58.8 $\pm$ 3.60	60.4 $\pm$ 5.21
FFAs ( $\mu$ Eq $\cdot$ h/L)	3055.8 $\pm$ 166.8	3444.4 $\pm$ 202.4

<sup>†</sup> All values are  $\bar{x} \pm$  SEM. FFAs, free fatty acids; Eq, equivalent. There were no significant differences between the *TT* group and the *TC + CC* group (independent *t* test).



variant might have a significant influence on the population-attributable risk. Further studies are needed to establish the mechanisms of action associated with the presence of this allelic variation. Moreover, given the number of other loci and environmental factors known to influence fasting and postprandial triacylglycerol concentrations, it will be highly relevant to examine how the effect of this APOA5 polymorphism is modified by the presence of other genetic and environmental factors. ☞

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All the authors were involved in the development of the study protocol and the experimental design. Sample collection and experiments were performed by YJ, JYK, and HC. DNA analysis was performed by JEL. Data were analyzed by OYK and JMO. JHL wrote the draft manuscript with contribution from JMO and YJ. All the authors read, commented on, and contributed to the submitted and revised manuscripts. None of the authors had any conflicts of interest in relation to the materials presented in this paper.

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