The −1131T→C polymorphism in the apolipoprotein A5 gene is associated with postprandial hypertriglyceridemia; elevated small, dense LDL concentrations; and oxidative stress in nonobese Korean men1–3

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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→C polymorphism with postprandial lipemic response and other cardiovascular disease risk factors in humans.
Design: Healthy, nonobese subjects [n = 158; mean (±SEM) age: 33.8 ± 1.2 y; body mass index (in kg/m²): 23.3 ± 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 F₂α, 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.

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 (~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 triglycerol, 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 triglycerol concentrations. The combination of these factors can contribute to the high serum triglycerol concentration and subsequently to the increased cardiovascular disease (CVD) indicated earlier.

Among the genetic factors associated with variability of serum triglycerol concentrations in the population, the apolipoprotein A5 gene (APOA5) is emerging as a main candidate gene for modulating triglycerol metabolism in humans. Several common single-nucleotide polymorphisms (SNPs) were identified at this locus, and they were consistently and significantly associated with plasma triglycerol 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→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/m²) 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 assay reader, and the final genotype calls were made with the QCReview 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 height² (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 antisemum (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 (LDL₁, density: 1.025–1.034 g/mL; LDL₂, density: 1.034–1.044 g/mL; and LDL₃, 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 F₂α 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 F₂α (8-epi-PGF₂α) was measured with the use of an enzyme immunoassay (BIOXYTECH urinary 8-epi-PGF₂α Assay kit; OXIS International Inc, Portland, OR). The resulting color reaction was read with the use of a Victor 2 (Perkin Elmer Life Sciences, Turka, Finland) at 650 nm. Urinary creatinine was determined by the alkaline picrate (Jeffe) reaction (15), and urinary 8-epi-PGF₂α 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

<table>
<thead>
<tr>
<th></th>
<th>TT (n = 85)</th>
<th>TC (n = 56)</th>
<th>CC (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33.1 ± 0.87</td>
<td>33.9 ± 1.19</td>
<td>37.1 ± 2.61</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 0.29</td>
<td>23.5 ± 0.37</td>
<td>23.3 ± 0.47</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>82.9 ± 0.83</td>
<td>84.5 ± 1.10</td>
<td>84.7 ± 1.71</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.85 ± 0.01</td>
<td>0.86 ± 0.01</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>Fourth lumbar vertebra (cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat</td>
<td>188.5 ± 9.55</td>
<td>203.7 ± 11.1</td>
<td>199.8 ± 14.5</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>73.2 ± 3.92</td>
<td>68.9 ± 5.54</td>
<td>86.0 ± 8.30</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>122 ± 1.5</td>
<td>122 ± 1.8</td>
<td>128 ± 3.4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75 ± 1.3</td>
<td>74 ± 1.4</td>
<td>76 ± 2.6</td>
</tr>
<tr>
<td>Tobacco consumption (cigarettes/d)</td>
<td>10.9 ± 1.36</td>
<td>8.45 ± 1.41</td>
<td>8.55 ± 2.36</td>
</tr>
<tr>
<td>Alcohol intake (g/d)</td>
<td>17.1 ± 3.99</td>
<td>24.5 ± 5.67</td>
<td>14.6 ± 3.40</td>
</tr>
<tr>
<td>TEE (kcal/d)</td>
<td>2563 ± 29</td>
<td>2508 ± 51</td>
<td>2580 ± 63</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>15.1 ± 0.33</td>
<td>15.2 ± 0.36</td>
<td>15.6 ± 0.66</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>56.1 ± 0.91</td>
<td>57.4 ± 0.99</td>
<td>56.9 ± 1.88</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>23.0 ± 1.92</td>
<td>18.9 ± 2.69</td>
<td>23.0 ± 2.36</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>5.81 ± 1.50</td>
<td>8.65 ± 2.03</td>
<td>4.37 ± 1.44</td>
</tr>
<tr>
<td>TEE/TCI</td>
<td>0.99 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>1.02 ± 0.04</td>
</tr>
</tbody>
</table>

All values are ± 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 (≈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 shown).

TABLE 2

Fasting serum lipid and apolipoprotein concentrations by apolipoprotein A5 (−1131) genotype

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

All values are ± 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).
Factors from multiple regression analyses for predicting fasting and postprandial triacylglycerol responses in non-obese, healthy men

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Steps</th>
<th>Independent variable</th>
<th>β coefficients</th>
<th>Standardized β coefficients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postprandial triacylglycerol area</td>
<td>1</td>
<td>Fasting triacylglycerol</td>
<td>0.919</td>
<td>0.933</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>HDL</td>
<td>-0.00269</td>
<td>-0.068</td>
<td>0.010</td>
</tr>
<tr>
<td>Fasting triacylglycerol</td>
<td>1</td>
<td>BMI</td>
<td>0.007</td>
<td>0.361</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>APOA5 genotype</td>
<td>0.224</td>
<td>0.281</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>HDL</td>
<td>-0.007</td>
<td>-0.177</td>
<td>0.013</td>
</tr>
<tr>
<td>Postprandial triacylglycerol area</td>
<td>1</td>
<td>BMI</td>
<td>0.071</td>
<td>0.340</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>APOA5 genotype</td>
<td>0.268</td>
<td>0.249</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>HDL</td>
<td>-0.010</td>
<td>-0.247</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1 APOA5, apolipoprotein A5 gene. All dependent variables were log transformed.
2 The independent variables were age, BMI, HDL, fasting triacylglycerol, and APOA5 genotype.
3 HDL had no correlation with any other variables except fasting and postprandial triacylglycerol responses.
4 The independent variables were age, BMI, HDL, and APOA5 genotype.
5 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 LDL₁ and higher LDL₃ concentrations than did subjects with the TT genotype (Table 4). There was no difference in concentration and proportion of the larger LDL₁ between TT homozygotes and C carriers. The mean LDL₁ 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-PGF₂α 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 × 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 × 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...
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/L1152T3C 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→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→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 (LDL3) (24, 26–29). In this study, carriers of the C allele showed significantly higher mean concentrations of LDL3 than did subjects with the TT genotype.
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 LDL₃ 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 LDL₃ 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. 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 LDL₃ concentrations between carriers of the C allele and TT homozygotes.

TABLE 5

<table>
<thead>
<tr>
<th>Fasting serum concentrations of and areas under the postprandial curves for glucose, insulin, and free fatty acids according to apolipoprotein A5 (–1131) genotype groups¹</th>
<th>TT (n = 85)</th>
<th>TC + CC (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose (mg/dL)</strong></td>
<td>90.1 ± 1.43</td>
<td>90.0 ± 1.40</td>
</tr>
<tr>
<td><strong>Insulin (μIU/mL)</strong></td>
<td>7.76 ± 0.48</td>
<td>8.50 ± 0.61</td>
</tr>
<tr>
<td><strong>FFAs (μEq/L)</strong></td>
<td>469.8 ± 30.0</td>
<td>537.6 ± 38.6</td>
</tr>
<tr>
<td><strong>Response area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glucose (mg·h/dL)</strong></td>
<td>521.7 ± 6.36</td>
<td>522.6 ± 5.48</td>
</tr>
<tr>
<td><strong>Insulin (μIU·h/mL)</strong></td>
<td>58.8 ± 3.60</td>
<td>60.4 ± 5.21</td>
</tr>
<tr>
<td><strong>FFAs (μEq·h/L)</strong></td>
<td>3055.8 ± 166.8</td>
<td>3444.4 ± 202.4</td>
</tr>
</tbody>
</table>

¹ All values are x ± SEM. FFAs, free fatty acids; Eq, equivalent. There were no significant differences between the TT group and the TC + CC group (independent t test).

species. Consequently, their production is enhanced in the presence of oxidative stress (34). The measurement of urinary 8-epi-PGF₂α, is a precise method for assessing oxidative stress (35), and in our population carriers of the C allele had higher urinary 8-epi-PGF₂α, 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.

FIGURE 3. Mean (±SEM) changes (Δ) from baseline in total triacylglycerol, chylomicron triacylglycerol, and VLDL triacylglycerol concentrations after a mixed meal tolerance test in TT homozygotes (○, □; n = 85) and carriers of the C allele (●, △; n = 73). Bars represent the incremental area under the curve responses for each genotype group. P₁, time effect; P₂, genotype effect; P₃, interaction between genotype and time (repeated-measures ANOVA for all).

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
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 contributions 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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