Hypertriglyceridemia along with hypertension and hyperinsulinemia are the hallmarks of the insulin resistance syndrome, leading to the development of Type-2 diabetes mellitus (13) and cardiovascular diseases (14,28). Triglyceride (TG) metabolism and thereby TG concentration in circulating plasma, and this decrease is often related to its base-line concentrations. Consequently, exercise and/or training have been consistently known to decrease TG concentrations in circulating plasma. Apo C-III inhibits lipoprotein lipase, a key enzyme in hydrolyzing the TG portion of TG-rich lipoproteins (TRL), and thereby results in a delayed removal of TRL in vitro (37) and in vivo (15). Apo C-III also interferes with apo E-mediated remnant removal by displacing apo E from VLDL particles in vivo (1,2,4). In transgenic mice overexpressing human apo C-III, plasma concentrations of human apo C-III were directly proportional to plasma TG concentrations (9,20). On the other hand, targeted gene disruption resulting in suppression of mouse apo C-III expression was associated with reduced plasma TG both in the fasted and in the postprandial state (21,25).

The gene encoding apo-CIII is clustered with genes encoding other apolipoproteins including apo AI and apo AIV. And they are collectively called the apo AI/CIII/AIV gene cluster. The apo AI/CIII/AIV gene cluster located in the long arm of chromosome 11 (24) has been associated with dyslipidemia including hypertriglyceridemia (29). More than 20 polymorphic sites within the gene cluster have been reported (16). Among them, the SstI polymorphism in the 3' untranslated region (UTR) of the apo C-III gene has occurred by a cytosine to guanosine substitution (8), and this genetic variation has been associated with elevated plasma triglyceride, cholesterol, and apo C-III concentrations (16,17,19,27,29,30,36,39).

On the other hand, exercise and/or training have been
treatment in lowering plasma TG concentrations due to its low cost and few side effects. However, the degree of exercise and/or training-induced TG reduction is often related to its baseline concentrations (10).

Consequently, it seems reasonable to ask whether or not the efficacy of exercise training in lowering plasma TG concentrations is dependent on the apo C-III SstI polymorphism. The purpose of this study was to investigate a possible interaction between the apo C-III polymorphism and exercise for plasma TG concentrations in sedentary but apparently healthy and normolipidemic Korean men.

MATERIALS AND METHODS

Participants. A subgroup of 100 Korean men (aged 28.3–41.0 yr) was prescreened by genotypes (S1S1 = 40, S1S2 = 40, S2S2 = 20) from the participants in an ongoing larger-scaled study (N = 400) at our institution. The subgroup was invited to participate in this study. Based on a physician health screening, all subjects were apparently healthy and normolipidemic. All subjects were neither participating in any regular exercise program nor taking any form of medications known to affect the lipoprotein-lipids. Before their participation in the study, interested subjects signed informed consent documents in accordance with procedures of the Institution Human Subject Committee. The subjects were asked to refrain from smoking and alcohol consumption during the entire period of the experiment.

Anthropometric measurements. Weight was assessed with a balance scale and height with a stadiometer, and body mass index was calculated (kg·m⁻²). Waist circumference as an index of central obesity was measured at the umbilicus level using a tape ruler. The anthropometric variables were measured in triplicate by a trained expert. The average values were used.

Lipoprotein-lipid, glucose, and insulin measurements. To minimize any possible effect of the last meal consumed before blood collection, the samples were collected only after 10–12 h overnight fast and in a sitting position. In addition, a 6-d dietary recall was conducted from all the subjects 1 wk before the experiment (data not shown), and they were asked to follow the same dietary pattern throughout the entire period of the study. Each day we also orally checked their adherence to the dietary regimen.

Twenty-four hours before and after the last session of the 6-d exercise intervention, a 20-mL venous blood sample was obtained from the antecubital vein into Vacutainers® containing EDTA. Samples were separated by low speed centrifugation (4500×g × 10 min) and plasma was stored at −70°C until analysis. Plasma glucose concentration was determined in duplicate by measuring cholesterol in the supernatant after precipitation of apo B-containing lipoproteins with magnesium chloride and dextran sulfate (Sigma, St. Louis, MO). The accuracy of the lipid assays was confirmed using the standards (Boehringer Mannheim and Sigma). LDL concentrations were calculated from TC, TG, and HDLC values using the Friedewald formula (LDLC = TC − HDLC − TG/5) (12). The intra-assay coefficients of variation (CV) for glucose, insulin, TC, TG, and HDLC were 0.8%, 1.9%, 3.76%, 4.14%, and 1.98%, respectively.

Determination of the apo C-III SstI gene polymorphism. Genomic DNA was extracted from plasmauffy coats using QiaAmp DNA Blood Mini Kit (Qiagen, Valencia, CA). The extracted DNA was stored at −70°C until analyzed. Two alleles of human apo C-III were determined by a SstI polymorphic SstI (position 371) in the 3' untranslated region of the apo C-III gene by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as previously described elsewhere (18), with modifications.

PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) with a 20-μL reaction volume containing 30–50 ng of genomic DNA, 20 pmol of each primer, and 1X Taq PCR master mix (Qiagen). The Taq PCR master mix provided a final concentration of 2.5 units of Taq DNA polymerase, PCR buffer (1.5 mM MgCl2), and 200 μM each dNTP. PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The primer sequences were as follows; sense 5'-GTG AGG AGA GCA CTG AGA ATA CTG-3' and antisense 5'-GTT CTT TCC AGT AGT TCC CTG-3'.

The PCR products (233-bp) were digested with 4 units of the restriction enzyme SstI (Bethesda Research Laboratory-Gibco), separated by electrophoresis using low melting temperature 2.5% agarose gel (Sigma) and visualized by ethidium bromide staining (Sigma). The S1S1 homozygotes had a single band of a 233-bp fragment. The S2S2 homozygotes had two bands of 158-bp and 75-bp fragments. And the S1S2 heterozygotes had three bands corresponding to the 233-bp, 158-bp, and 75-bp fragments.

Exercise program. Each morning (8:00–9:00 a.m.) subjects in a fasted state arrived in our laboratory and worn a Polar heart rate monitor (POLAR Accurex Plus, Finland), then the subjects participated in an aerobic exercise either on a Quinton treadmill or a Monark bicycle ergometer. A 3- to 5-min warm-up of stretching was followed by either walking and/or running on the treadmill or cycling on the cycle ergometer at an intensity of 70% relative to their own HRmax (THR = [(MHR − RHR) × (0.70)] + (RHR)). During exercise, only water was allowed to help body temperature regulation. Exercise duration varied from 40 to 50 min to elicit 500 kcal each session (23). The energy expenditure was estimated from the heart rate data recorded using the Polar heart rate monitor (6).
TABLE 1. Comparison of the preintervention values in anthropometric, lipid, glucose, and insulin measures across the genotypes (N = 100); mean ± SD.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Genotypes</th>
<th>S1S1 (N = 40)</th>
<th>S1S2 (N = 40)</th>
<th>S2S2 (N = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>35.3 ± 1.2</td>
<td>34.9 ± 1.1</td>
<td>34.7 ± 3.1</td>
<td>0.389</td>
<td></td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>25.4 ± 5.5</td>
<td>25.0 ± 4.6</td>
<td>25.5 ± 3.9</td>
<td>0.878</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>94.8 ± 15.6</td>
<td>92.4 ± 12.9</td>
<td>99.7 ± 13.4</td>
<td>0.173</td>
<td></td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>37</td>
<td>38</td>
<td>38</td>
<td>0.546</td>
<td></td>
</tr>
<tr>
<td>TG (mg·dL⁻¹)¹</td>
<td>123.0 ± 28.2</td>
<td>131.4 ± 34.0</td>
<td>151.0 ± 55.5</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>TC (mg·dL⁻¹)²</td>
<td>163.1 ± 29.5</td>
<td>163.3 ± 30.3</td>
<td>169.0 ± 24.2</td>
<td>0.725</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg·dL⁻¹)</td>
<td>45.8 ± 10.4</td>
<td>44.8 ± 9.7</td>
<td>42.3 ± 10.3</td>
<td>0.457</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg·dL⁻¹)</td>
<td>92.7 ± 25.8</td>
<td>92.2 ± 26.9</td>
<td>96.5 ± 26.2</td>
<td>0.825</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol·L⁻¹)</td>
<td>5.1 ± 0.4</td>
<td>5.1 ± 0.4</td>
<td>5.1 ± 0.4</td>
<td>0.664</td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol·L⁻¹)</td>
<td>107.5 ± 61.7</td>
<td>103.3 ± 49.8</td>
<td>128.1 ± 43.4</td>
<td>0.368</td>
<td></td>
</tr>
</tbody>
</table>

Due to skewed distribution, TG was log₇₀₀⁰ transformed for statistical analysis, but the actual data are presented in the table.

⁰ Bonferroni post hoc test showed S1S1 < S2S2 in the TG concentrations (P = 0.022).

RESULTS

Preintervention values across the genotypes. Table 1 represents the physical characteristics of the participants and the preintervention values of the measured variables across the genotypes. There were no significant differences in anthropometric measures across the genotypes, with a tendency in a higher waist circumference in subjects with the S2S2 genotype than subjects with the S1S1 genotype (P = 0.173). With respect to the preintervention values of lipoprotein-lipids and glucose and insulin, the subjects with the S2S2 genotype had significantly higher fasting TG concentrations than the subjects with the S1S1 genotype (P = 0.022), with no significant differences in any other variables measured across the genotypes. Multiple linear regression analysis showed that waist circumference and the S1S1 genotype in the apo-CIII gene were the only significant predictors for the preintervention fasting TG concentration (Table 2).

Pre- to postexercise Δ scores across the genotypes. Table 3 represents the comparisons of the Δ scores in lipoprotein-lipids, glucose, and insulin across the genotypes. With respect to Δ TG scores, ANCOVA showed that compared with the subjects with the S1S1 genotype, the subjects with the S2S2 genotype had a greater reduction in TG concentration (P = 0.047) after adjusted for its preintervention values, with no significant differences between the other two genotypes. But there were no significant changes in body weight, body mass index, and waist circumference after the 6-d exercise program (data not shown).

DISCUSSION

Apo C-III plays a role in TG metabolism by inhibiting the hydrolysis of TG by LPL and/or by interfering apo E-mediated clearance of lipoproteins by hepatocytes (15). Consequently, apo C-III concentration has been positively associated with TG concentration in circulating plasma (11). In addition, the SstI polymorphism in the apo C-III gene has been associated with elevated apo C-III (29) and hypertriglyceridemia in several human populations including Caucasian, Japanese, Arabic, and Asian-Indian populations (7,26,33,35,39).

In the present study, we examined whether the SstI polymorphism in the apo C-III gene would modulate the response of fasting plasma TG concentrations to a 6-d moderate exercise intervention. With respect to the preintervention lipids, glucose and insulin, the SstI polymorphism was significantly associated with the TG concentration such that the subjects with the S2S2 genotype had a significantly higher TG concentration than the subjects with the S1S1 genotype. The variation in the TG concentration was accounted for by both the SstI polymorphism and central obesity (i.e., waist circumference) up to 20%. As a possible explanation, visceral adiposity indicative of the central obesity has been positively associated with plasma TG concentration (22). However, how the visceral adiposity across the SstI polymorphism determine TG concentrations remains to be investigated.

TABLE 2. Linear regression model for the preintervention plasma TG concentrations (N = 100); mean ± SD.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Predictor Variables</th>
<th>Block</th>
<th>R²</th>
<th>Adjusted R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>Waist circumference</td>
<td>2</td>
<td>0.18</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Genotype</td>
<td>3</td>
<td>0.21</td>
<td>0.20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TABLE 3. Comparison of the change scores in lipoprotein-lipids, glucose, and insulin concentrations across the genotypes, adjusted for the preintervention values (N = 100); mean ± SD.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Genotypes</th>
<th>S1S1 (N = 40)</th>
<th>S1S2 (N = 40)</th>
<th>S2S2 (N = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg·dL⁻¹)⁴</td>
<td>1.9 ± 30.8</td>
<td>1.2 ± 25.5</td>
<td>0.8 ± 23.9</td>
<td>0.938</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg·dL⁻¹)</td>
<td>0.0 ± 0.6</td>
<td>0.1 ± 0.4</td>
<td>0.0 ± 0.4</td>
<td>0.361</td>
<td></td>
</tr>
</tbody>
</table>

Due to skewed distribution, TG was log₇₀₀⁰ transformed for statistical analysis, but the actual data are presented in the table.

⁴ Bonferroni post hoc test showed that compared to S1S2 homozygotes, S2S2 homozygotes had a greater reduction in fasting TG concentration (P = 0.047).
Of particular interest, this was the first study to report that the efficacy of exercise program in lowering the preintervention fasting TG concentration was genotype dependent of the SstI polymorphism in the apo C-III gene. Compared with the subjects with the S1S1 genotype, the subjects with the S2S2 genotype had a greater reduction than the subjects with the S1S1 genotype. Consequently, the finding of the current study suggests that exercise might be especially effective for those who had elevated TG concentrations (10,23). Second, the SstI polymorphism may also have some influence on mRNA stability (29,30). Third, the S2 allele is also in disequilibrium with two polymorphisms located in the insulin response element (IRE) of the apo C-III gene (8), in which apo C-III is down-regulated by insulin via the IRE (3).

REFERENCES


On the other hand, it has been consistently reported that exercise and/or training improve lipids profiles secondary to an enhanced lipolysis of TRL by postheparin LPL in circulating plasma (38). Postheparin LDL appears to be increased in athletes after physical training and after a single bout of exercise (34). Unfortunately, however, neither hepatic synthesis of apo C-III nor LPL content and activity are available in the current study. Nevertheless, the current finding suggests that the S2S2 genotype might lead to an enhanced hydrolysis of TRL and or an enhanced apo E-mediated clearance of TRL by hepatocytes in response to exercise. However, how the SstI polymorphism modulates the LPL activity and/or apo E-mediated removal of TRL by hepatocytes remains to be clarified.

Caution is necessary in interpreting the current findings because of some limitations in this study: 1) the physiologic significance of the S2 variant has not been reported; 2) no control group was available to reflect any possible circadian rhythm in lipids; and 3) in order to eliminate the possibility of a chance finding, the current finding is yet to be confirmed in other studies.

In conclusion, this study suggests that the SstI polymorphism in the apo C-III gene may prove to be useful for determining who is most susceptible to intervention by exercise in lowering fasting plasma TG concentrations.

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