Significance of Measuring Serum Concentrations of Remnant Lipoproteins and Apolipoprotein B-48 in Fasting Period

Itsuko Sato¹, Yuichi Ishikawa², Ai Ishimoto², Shiho Katsura², Atsushi Toyokawa², Fujio Hayashi¹, Seiji Kawano¹, Yoshio Fujioka³, Shizuya Yamashita⁴, and Shunichi Kumagai¹, ⁵

¹Department of Clinical Laboratory, Kobe University Hospital, Kobe, Japan
²Faculty of Health Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
³Faculty of Nutrition, Kobegakuin University, Kobe, Japan
⁴Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan
⁵Department of Clinical Pathology and Immunology, Kobe University Graduate School of Medicine, Kobe, Japan

Aim: To characterize lipid profiles conveniently in the fasting period to detect postprandial hyperlipidemic subjects, we measured the concentrations of lipids, including remnant lipoproteins and apoB-48, before and after loading the test meal in 24 normolipidemic subjects.

Methods: We examined remnant-like particle-cholesterol and -triglyceride (RLP-C, RLP-TG) by the immune adsorption method, RemL-C by the newly developed homogeneous method, and apoB-48 by chemiluminescence enzyme immunoassay.

Results: After loading, TG, RemL-C, RLP-C, RLP-TG, and apoB-48 concentrations were elevated. Twenty subjects had only a slight elevation of TG (low TG group) after loading, while 4 subjects showed apparent increase of TG (more than 150 mg/dL, high TG group). In the fasting period, the high TG group had significantly higher serum concentrations of TG and RemL-C than the low TG group. Although not significant, RLP-C, RLP-TG and apoB-48 concentrations in the high TG group were also higher than in the low TG group. After loading, serum concentrations of TG, RemL-C, RLP-C, RLP-TG, and apoB-48 increased significantly more in the high TG group than in the low TG group.

Conclusion: In conclusion, TG, RemL-C, RLP-C, RLP-TG, and apoB-48 concentrations in the fasting period may be suitable for detecting postprandial hyperlipidemic subjects.


Key words: Postprandial hyperlipidemia, RLP-C, RemL-C, Small, dense LDL-C

Introduction

Since Zilversmit's proposal of the significance of postprandial hyperlipidemia, many studies have investigated the role of remnant lipoproteins in the pathogenesis of atherosclerosis, and have identified a delay in their removal from blood as an independent risk factor. In fact, high concentrations of remnant-like particle (RLP)-cholesterol (RLP-C) predict coronary events in patients with CAD, independent of traditional coronary risk factors.

In normal humans, the postprandial hyperlipidemic period is about 4–6 hours, but in individuals with certain dyslipidemia, this period may be increased beyond 6 hours. Of note, impaired removal of chylomicron remnants in the liver potentially induces longer retention times for these lipoproteins in blood circulation. Moreover, it is important to recognize that dietary lipid is transferred to various parts of the body via plasma lipoproteins after food ingestion.

In the postprandial period, we can observe the elevation of triglyceride (TG)-rich lipoproteins, which include chylomicrons, very-low-density lipoproteins...
Nascent chylomicrons, synthesized by enterocytes, have a high TG-to-cholesterol mass ratio, and consist primarily of apolipoprotein (apo) B-48 and apoA-I. After acquiring apoC-II and apoE, chylomicrons bind to lipoprotein lipase (LPL), which induces lipolysis of TG in chylomicrons. TG depletion results in a size reduction, and is referred to as chylomicron remnants, or “remnants”. Finally, chylomicron remnants are cholesteryl ester-rich and retain apoB-48 and apoE. VLDL produced by hepatocytes are also hydrolyzed by LPL like chylomicrons, and become VLDL remnants containing apoB-100 and apoE.

Although it has been difficult clinically to distinguish exogenous lipids (chylomicrons and their remnants) from endogenous lipids (VLDL and their remnants), it recently became possible to conveniently measure the serum B-48 concentration. In the present study, to characterize the lipid profiles conveniently in the fasting period to detect postprandial hyperlipidemic subjects, we measured the concentrations of lipids, including remnant lipoproteins and apoB-48, before and after loading the test meal in normolipidemic subjects.

Subjects and Methods

Subjects and Physical Examination

We recruited healthy subjects \(n=24\); male/female, 11/13; mean \pm standard deviation (SD), 21.5 \pm 1.2 years old) who had never been treated or taken any drugs at least 3 months before the study. All subjects gave their informed consent to participate in the study. The study protocol was carried out according to the Declaration of Helsinki.

Blood pressure was measured twice by the same observer using a standard mercury sphygmomanometer after the subject had rested in a supine position for 30 min. Waist circumference of subjects was measured. Body mass index (BMI) was calculated by dividing body weight by the square of the height (kg/m^2).

Study Protocol

We used a test meal containing carbohydrate, fat, and protein, which was developed for the assessment of both postprandial hyperglycemia and hyperlipidemia by the Japanese Diabetes Society (Test meal A). This test meal consists of cream of chicken soup, biscuit, and custard pudding. The total calories is 450 kcal, including carbohydrate 57.6 g (51.4% in energy balance), protein 17.2 g (15.3%), fat 16.6 g (33.3%), which is a slightly higher percentage of fat than in the usual Japanese breakfast (20–25%). Blood samples were obtained at 9–10 AM after a 12-hour fast and 1, 2, 4, 6, and 8 hours after ingestion of the test meal.

Blood Sampling and Analysis

Serum concentrations of TG, total cholesterol, high-density lipoprotein (HDL)-cholesterol (HDL-C) and low-density lipoprotein (LDL)-cholesterol (LDL-C) were determined by enzymatic methods (Kyowa Medex, Tokyo, Japan); plasma oxidized LDL concentration by enzyme-linked immunosorbent assay (Kyowa Medex); serum concentrations of apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE by turbidimetric immunoassay methods (Nittobo, Tokyo, Japan); serum apoB-48 concentration by chemiluminescence enzyme immunoassay (Fujirebio, Tokyo, Japan); serum apoB-48 and apoE concentrations by enzyme immunoassay methods (MetaboLead RemL-C, Kyowa Medex); serum concentration of RLP-C and RLP-TG by the immune adsorption method (JIMRO II, Otsuka Pharmaceutical, Tokyo, Japan); serum remnant lipoprotein cholesterol (RemL-C) concentration by homogeneous assay (MetaboLead RemL-C, Kyowa Medex); serum high sensitivity C-reactive protein (hs-CRP) concentration by nephelometry method (Dade Behring, Deerfield, IL); plasma glucose concentration and glycosylated hemoglobin A1C (HbA1C) by HPLC; and serum insulin concentrations by enzyme immunoassay, respectively.

Agarose Gel Electrophoresis Analysis

Samples were subjected to lipoprotein analysis using agarose gel electrophoresis (Rapid Electrophoresis; Helena Laboratories, Beaumont, Texas), with 15 min of electrophoresis at 400 volts and 20°C. After staining with cholesterol and TG reagent, elution profiles were analyzed by an automatic densitometer, Chol/Trig Combo™ (Helena Kenkyusho, Saitama, Japan). Contents of cholesterol and TG in each fraction were calculated with total lipids and the area under the curve according to the report by Kido et al. Moreover, the ratios of cholesterol to TG in HDL and LDL fractions were calculated and compared with those in healthy volunteers reported previously (mean \pm SD; HDL, 5.8 \pm 2.0; LDL, 4.9 \pm 1.3).

Statistical Analysis

Values are expressed as the mean \pm SD. Statistical significance of data was evaluated using either the Mann-Whitney \(U\)-test or Welch’s \(t\)-test. Correlations between apoB-48 and other parameters were calculated using the formula for Pearson’s correlation coefficient. Responses to the test meal were compared by analysis of variance (ANOVA) for repeated measures.
Table 1. Glucose and lipid parameters before and after loading the test meal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>6h</th>
<th>8h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>89.4 ± 4.7</td>
<td>104.5 ± 19.2**</td>
<td>89.2 ± 11.5</td>
<td>85.3 ± 4.8**</td>
<td>85.2 ± 4.5**</td>
<td>85.1 ± 5.0**</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>6.1 ± 2.9</td>
<td>53.7 ± 27.3**</td>
<td>24.6 ± 16.4**</td>
<td>5.1 ± 2.2</td>
<td>4.1 ± 1.3*</td>
<td>3.7 ± 1.4*</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>65.6 ± 25.5</td>
<td>86.9 ± 38.2*</td>
<td>95.7 ± 47.4*</td>
<td>77.1 ± 32.6</td>
<td>60.7 ± 19.2</td>
<td>52.2 ± 16.8*</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>182.6 ± 32.2</td>
<td>181.5 ± 33.8</td>
<td>179.8 ± 32.4</td>
<td>181.4 ± 30.8</td>
<td>184.2 ± 31.7</td>
<td>189.5 ± 33.3</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>100.0 ± 25.6</td>
<td>97.8 ± 25.7</td>
<td>96.8 ± 25.4</td>
<td>97.9 ± 24.4</td>
<td>101.1 ± 25.2</td>
<td>104.3 ± 26.2</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>70.0 ± 14.3</td>
<td>67.8 ± 14.2</td>
<td>67.2 ± 13.1</td>
<td>68.0 ± 14.1</td>
<td>70.1 ± 14.9</td>
<td>72.5 ± 15.2</td>
</tr>
<tr>
<td>RemL-C (mg/dL)</td>
<td>3.5 ± 1.6</td>
<td>3.9 ± 1.9</td>
<td>4.0 ± 2.1</td>
<td>3.7 ± 2.1</td>
<td>3.1 ± 1.3</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>RLP-C (mg/dL)</td>
<td>3.1 ± 1.2</td>
<td>4.4 ± 2.0*</td>
<td>4.7 ± 2.6*</td>
<td>3.8 ± 1.7</td>
<td>3.0 ± 1.3</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td>RLP-TG (mg/dL)</td>
<td>15.8 ± 2.6</td>
<td>24.8 ± 14.6*</td>
<td>30.4 ± 23.6*</td>
<td>20.1 ± 8.7</td>
<td>15.2 ± 0.7*</td>
<td>15.1 ± 0.2*</td>
</tr>
<tr>
<td>Sd-LDL-C (mg/dL)</td>
<td>21.4 ± 8.9</td>
<td>17.3 ± 5.8</td>
<td>17.1 ± 6.9</td>
<td>17.0 ± 5.7</td>
<td>17.5 ± 5.9</td>
<td>17.8 ± 5.8</td>
</tr>
<tr>
<td>Oxidized LDL (U/mL)</td>
<td>6.7 ± 4.9</td>
<td>6.1 ± 4.4</td>
<td>6.6 ± 5.5</td>
<td>6.5 ± 4.8</td>
<td>6.8 ± 4.7</td>
<td>7.1 ± 4.9</td>
</tr>
<tr>
<td>ApoA-I (mg/dL)</td>
<td>164.0 ± 27.0</td>
<td>161.8 ± 28.0</td>
<td>161.3 ± 25.0</td>
<td>162.5 ± 26.7</td>
<td>165.5 ± 26.7</td>
<td>168.2 ± 28.1</td>
</tr>
<tr>
<td>ApoA-II (mg/dL)</td>
<td>38.9 ± 7.1</td>
<td>38.5 ± 7.4</td>
<td>38.0 ± 6.9</td>
<td>38.5 ± 7.0</td>
<td>38.7 ± 7.1</td>
<td>39.3 ± 7.1</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>67.5 ± 14.2</td>
<td>66.3 ± 14.8</td>
<td>66.2 ± 14.0</td>
<td>66.8 ± 13.6</td>
<td>68.5 ± 13.9</td>
<td>70.5 ± 14.2</td>
</tr>
<tr>
<td>ApoB-48 (μg/mL)</td>
<td>3.2 ± 2.1</td>
<td>6.2 ± 3.1**</td>
<td>6.1 ± 3.4**</td>
<td>5.2 ± 2.9**</td>
<td>3.6 ± 2.1</td>
<td>3.0 ± 1.7</td>
</tr>
<tr>
<td>ApoC-II (mg/dL)</td>
<td>3.1 ± 1.1</td>
<td>3.2 ± 1.2</td>
<td>3.2 ± 1.2</td>
<td>3.2 ± 1.1</td>
<td>3.2 ± 1.1</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td>ApoC-III (mg/dL)</td>
<td>9.4 ± 2.5</td>
<td>9.9 ± 2.6</td>
<td>9.5 ± 2.6</td>
<td>9.3 ± 2.4</td>
<td>9.1 ± 2.3</td>
<td>9.3 ± 2.4</td>
</tr>
<tr>
<td>ApoE (mg/dL)</td>
<td>4.6 ± 1.0</td>
<td>4.5 ± 1.0</td>
<td>4.4 ± 1.0</td>
<td>4.4 ± 1.0</td>
<td>4.3 ± 0.9</td>
<td>4.4 ± 0.9</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD. **p<0.05, *p<0.01 (vs. 0 time) by Mann-Whitney U-test. TG: triglyceride, TC: total cholesterol, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol, RemL-C: remnant lipoprotein cholesterol measured with “Metabo-Lead RemL-C”, RLP-C: remnant-like particle-cholesterol measured with “JIMRO II”, RLP-TG: remnant-like particle-triglyceride, Sd-LDL-C: small, dense-LDL-cholesterol, Apo: apolipoprotein.

Data under the threshold of RLP-C (<2.0 mg/dL) or RLP-TG (<15 mg/dL) were treated as 2.0 mg/dL or 15 mg/dL, respectively. Statistical analysis was performed using Stat Flex ver.5.0 software (Artec, Osaka, Japan). Two-tailed values of p<0.05 were considered significant.

**Results**

**Characteristics of Subjects**

Subject characteristics are as follows (mean ± SD): BMI, 20.7 ± 1.7 kg/m²; waist circumferences, 72.3 ± 4.2 cm in men and 65.7 ± 4.5 cm in women; HbA1c, 4.9 ± 0.2%; hsCRP, 0.04 ± 0.02 mg/dL.

**Fasting and Postprandial Concentrations of Lipids in Total Subjects**

Table 1 shows the changes of lipid concentrations before and after loading the test meal in all subjects. In the fasting period (time 0), concentrations of all parameters were within normal limits or low ranges; however, there were significant correlations between apoB-48 concentration and TG, RemL-C, RLP-C, RLP-TG, apoC-II, or apoC-III concentration (Table 2), indicating that intestine-derived lipoproteins were present in the circulation and had characteristics of remnants even in the fasting period in normolipemic subjects.

After loading the test meal, TG, RLP-C, RLP-TG, and apoB-48 concentrations elevated significantly compared with before loading (Table 1). TG, RLP-C, and RLP-TG concentrations peaked at 2 hours, and were restored to the baseline within 4 hours. ApoB-48 concentrations peaked at 1 hour, and returned to basal levels at 6 hours. RemL-C concentrations also peaked at 2 hours and were restored within 6 hours, but this elevation had no significance. On the other hand, the concentrations of TC, HDL-C, LDL-C, sd-LDL-C, oxidized LDL, apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE were not elevated. Sd-LDL-C concentrations decreased below the basal levels during the study without statistical significance.

**Comparison of Fasting Lipid Concentrations between High and Low TG Groups**

In the results of the loading test, we noticed that some subjects showed apparent increases of TG at 2 hours as peak values, and others showed only a slight elevation. We therefore established two groups by TG values at 2 hours, and designated subjects with <150 mg/dL of TG (n=20) as the low TG group and subjects with >150 mg/dL of TG (n=4) as the high TG group.
group. The peak concentrations of TG in the low TG group were 78.4 ± 27.6 mg/dL, whereas those in the high TG group were 182.5 ± 33.7 mg/dL.

First, we compared the profiles and lipid parameters in the fasting period between the two groups (Table 3). The high TG group had significantly higher serum concentrations of TG and RemL-C than the low TG group. Although not significant, RLP-C, RLP-TG and apoB-48 concentrations in the high TG group were also higher than in the low TG group. There were no significant differences in the profiles, including age, BMI, and abdominal circumference between the two groups.

Comparison of Postprandial Concentrations between High and Low TG Groups

Fig. 1 demonstrates the sequential changes of parameters in lipids before and after loading the test meal in each group. Serum concentrations of TG, RemL-C, RLP-C, RLP-TG, and apoB-48 significantly increased in the high TG group than in the low TG group, especially from 1 to 4 hours. In contrast, although sd-LDL-C concentrations were significantly higher in the high TG group, they gradually decreased after loading. There were no significant differences in serum concentrations of TC, LDL-C, HDL-C, and other apolipoproteins. Concentrations of glucose, insulin, and oxidized LDL also did not alter (data not shown).

Analysis of Lipid by Electrophoretogram

Fig. 2 demonstrates representative cases of densitometric scanning patterns of electrophoretogram and lipid data before and after loading the test meal in the high and low TG groups. Sample A belongs to the high TG group. Fractions of chylomicrons and VLDL

Table 2. Correlation between apoB-48 concentration and other parameters in fasting period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>0.236</td>
<td>0.2661</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>0.042</td>
<td>0.8448</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>0.791</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>0.287</td>
<td>0.1743</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>0.301</td>
<td>0.1526</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>-0.081</td>
<td>0.7065</td>
</tr>
<tr>
<td>RemL-C (mg/dL)</td>
<td>0.811</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RLP-C (mg/dL)</td>
<td>0.768</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RLP-TG (mg/dL)</td>
<td>0.745</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sd-LDL-C (mg/dL)</td>
<td>0.367</td>
<td>0.0776</td>
</tr>
<tr>
<td>Oxidized LDL (mg/dL)</td>
<td>-0.150</td>
<td>0.4832</td>
</tr>
<tr>
<td>ApoA-I (mg/dL)</td>
<td>0.016</td>
<td>0.9395</td>
</tr>
<tr>
<td>ApoA-II (mg/dL)</td>
<td>0.290</td>
<td>0.1693</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>0.282</td>
<td>0.1820</td>
</tr>
<tr>
<td>ApoC-III (mg/dL)</td>
<td>0.689</td>
<td>0.0002</td>
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<tr>
<td>ApoC-II (mg/dL)</td>
<td>0.534</td>
<td>0.0071</td>
</tr>
<tr>
<td>ApoE (mg/dL)</td>
<td>0.189</td>
<td>0.3764</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.253</td>
<td>0.2321</td>
</tr>
</tbody>
</table>

Correlations between apoB-48 and other parameters were calculated using the formula for Pearson's correlation coefficient. TG: triglyceride, TC: total cholesterol, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol, RemL-C: remnant lipoprotein cholesterol measured with “MetaboLead RemL-C”, RLP-C: remnant-like particle-cholesterol measured with “JIMRO II”, RLP-TG: remnant-like particle-triglyceride, Sd-LDL-C: small, dense-LDL-cholesterol, Apo: apolipoprotein, hs-CRP: high sensitivity-C-reactive protein.

Table 3. Comparison of clinical characteristics and fasting concentrations of glucose and lipid parameters between two groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>High TG group (n=4)</th>
<th>Low TG group (n=20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>22.0 ± 2.5</td>
<td>21.4 ± 0.9</td>
<td>0.6695</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0 ± 2.2</td>
<td>20.4 ± 1.5</td>
<td>0.2975</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>73.5 ± 5.7</td>
<td>67.8 ± 5.2</td>
<td>0.1334</td>
</tr>
<tr>
<td>Hs-CRP (mg/dL)</td>
<td>0.06 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.1655</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.0 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>0.3018</td>
</tr>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>83.0 ± 4.8</td>
<td>91.0 ± 3.6</td>
<td>0.0719</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>6.3 ± 2.6</td>
<td>6.1 ± 3.0</td>
<td>0.9600</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>109.5 ± 15.8</td>
<td>56.9 ± 16.1</td>
<td>0.0044</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>196.5 ± 14.6</td>
<td>179.9 ± 34.3</td>
<td>0.1460</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>110.3 ± 16.6</td>
<td>98.0 ± 26.8</td>
<td>0.2982</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>67.5 ± 26.7</td>
<td>70.5 ± 11.5</td>
<td>0.8477</td>
</tr>
<tr>
<td>RemL-C (mg/dL)</td>
<td>5.9 ± 1.3</td>
<td>3.0 ± 1.2</td>
<td>0.0148</td>
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<tr>
<td>RLP-C (mg/dL)</td>
<td>4.9 ± 1.4</td>
<td>2.8 ± 0.8</td>
<td>0.0694</td>
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<tr>
<td>RLP-TG (mg/dL)</td>
<td>19.5 ± 5.4</td>
<td>15.6 ± 2.7</td>
<td>0.2744</td>
</tr>
<tr>
<td>Sd-LDL-C (mg/dL)</td>
<td>30.7 ± 13.0</td>
<td>17.9 ± 5.6</td>
<td>0.1602</td>
</tr>
<tr>
<td>Oxidized LDL (U/mL)</td>
<td>4.9 ± 1.7</td>
<td>7.0 ± 5.2</td>
<td>0.1630</td>
</tr>
<tr>
<td>ApoA-I (mg/dL)</td>
<td>163.8 ± 49.1</td>
<td>164.0 ± 22.5</td>
<td>0.9930</td>
</tr>
<tr>
<td>ApoA-II (mg/dL)</td>
<td>44.0 ± 10.4</td>
<td>37.9 ± 6.0</td>
<td>0.3609</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>75.8 ± 10.2</td>
<td>65.8 ± 14.5</td>
<td>0.1865</td>
</tr>
<tr>
<td>ApoB-48 (µg/mL)</td>
<td>6.1 ± 2.6</td>
<td>2.6 ± 1.5</td>
<td>0.0957</td>
</tr>
<tr>
<td>ApoC-II (mg/dL)</td>
<td>3.6 ± 1.1</td>
<td>2.9 ± 1.1</td>
<td>0.3734</td>
</tr>
<tr>
<td>ApoC-III (mg/dL)</td>
<td>11.3 ± 3.7</td>
<td>9.1 ± 2.1</td>
<td>0.3531</td>
</tr>
<tr>
<td>ApoE (mg/dL)</td>
<td>5.1 ± 1.1</td>
<td>4.5 ± 0.9</td>
<td>0.4477</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD, Welch's t test. High group: the peak triglyceride (TG) concentration ≥150 mg/dL, low group: peak TG concentration <150 mg/dL, BMI: body mass index, HbA1c: glycosylated hemoglobin A1c, TC: total cholesterol, RemL-C: remnant lipoprotein cholesterol measured with “MetaboLead RemL-C”, RLP-C: remnant-like particle-cholesterol measured with “JIMRO II”, RLP-TG: remnant-like particle-triglyceride, Sd-LDL-C: small, dense-LDL-cholesterol, Apo: apolipoprotein.
increased 1 hour after loading, peaked at 2 hours, and gradually decreased. Sample B belongs to the low TG group. We observed a slight increase of the chylomicron fraction 2 hours after loading. Thus, the peak values of TG and lipoprotein profiles in the postprandial period were different between the two cases, although fasting TG concentrations in both cases were around 80 mg/dL.

**Fig. 3** shows the changes in TG concentrations of chylomicrons and VLDL, and the ratios of cholesterol to TG in HDL and LDL before and after loading the test meal. In the left panel of sample A, the higher the TG concentrations of chylomicrons and VLDL, the lower the ratios of cholesterol to TG in HDL and LDL decreased slightly.

**Discussion**

In this study we analyzed serum lipids and apolipoproteins in fasting and postprandial periods among 24 young normolipidemic subjects. When we divided subjects into two groups according to the peak values of TG after loading, we found that the high TG group showed higher concentrations of TG, RemL-C, RLP-C, RLP-TG, and apoB-48 in the fasting period, although some parameters did not show significant differences. These concentrations were obviously elevated after loading in the high TG groups.

It is clear that an increase in chylomicrons and their remnants derived from the intestine is observed in the postprandial period; however, some investigators have reported that VLDL and their remnants derived from the liver are also increased, and it is
Fig. 2. Densitometric scanning electrophoretogram patterns of samples before and after loading the test meal. Elution profiles were analyzed by an automatic densitometer, Chol/Trig Combo™.

Sample A: Representative elution profile in the group with >150 mg/dL triglyceride (TG) concentration elevation after loading the test meal.
Sample B: Representative elution profile in the group with <150 mg/dL TG concentration elevation before and after loading the test meal.

Fig. 3.
Changes in triglyceride (TG) levels of very-low-density lipoproteins (VLDL) and chylomicrons, and the ratios of cholesterol to TG of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) before and after loading the test meal. Open column: TG content of VLDL, closed column: TG content of chylomicrons, closed circle: ratios of cholesterol to TG of HDL, closed square: ratio of cholesterol to TG of LDL, dashed line: ratio of cholesterol to TG of HDL of the mean-2SD in control subjects, dotted line: ratio of cholesterol to TG of LDL of the mean-2SD in control subjects.
controversial which is predominant in exogenous and endogenous lipids in the postprandial period\(^\text{18, 22, 23}\). Although the detailed mechanism remains unknown, the postprandial increase in VLDL and their remnants may be caused by reduced clearance, which was a result of competition by chylomicrons for the removal of triglycerides by lipoprotein lipase, or increased hepatic secretion of VLDL\(^\text{22}\). Receptor-mediated mechanisms are the predominant pathway by which chylomicron remnants are taken up by hepatocytes, and the LDL receptor pathway is thought to be the major mechanism for the uptake of both remnants with apoE as a ligand\(^\text{12, 24, 25}\). Impairment of or competition for the removal of both remnants in the liver may also potentially induce an increased retention time of these lipoproteins in the blood circulation. Thus, both remnant concentrations may be elevated in the postprandial period. In this study there was a remarkable elevation of apoB-48, which is a component of chylomicrons and chylomicron remnants, corroborating that lipoproteins derived from the intestine increase in the postprandial period, especially from 1 to 4 hours. ApoB concentrations (most derived from the liver) did not alter remarkably, suggesting that the postprandial increase in VLDL and their remnants may be small in young normolipidemic subjects.

Data of sample A (high TG group) demonstrated that delayed clearance of TG-rich lipoproteins in the postprandial period may be detected even in normolipidemic subjects. Impaired removal of TG-rich lipoproteins may induce the change of cholesterol and TG composition in LDL and HDL via the mechanism by which cholesteryl ester transfer protein is mediated\(^\text{26, 27}\); the higher the TG concentrations of chylomicrons and VLDL, the lower the ratios of cholesterol to TG of HDL and LDL. Thus, our data suggest that it may be possible to characterize lipid profiles conveniently in the fasting period by measuring TG, RemL-C, RLP-C, RLP-TG, and apoB-48 concentrations to detect postprandial hyperlipidemic subjects.

In the postprandial period, the elevation of RemL-C concentration did not reach a significant level, although the elevation period of RemL-C is similar to those of TG, RLP-C, and RLP-TG. These results may be due to the different measuring methods. RLP-C and RLP-TG were measured by the immune adsorption method\(^\text{17}\), while RemL-C is measured by a newly developed and convenient assay for remnant lipoproteins\(^\text{18}\). This assay utilizes surfactant and phospholipase-D to directly solubilize and degrade remnants. As such, it can be performed with an automated clinical analyzer in a short time\(^\text{18}\). There was reportedly a strong correlation between RemL-C and RLP-C concentrations in patients with coronary artery disease\(^\text{28}\); however, our results (Table 1) suggest that differences in sensitivity for exogenous and endogenous lipoproteins between both methods may exist. The method for RLP-C and RLP-TG may be more sensitive to exogenous remnants, while RemL-C may be suitable for endogenous remnants. This hypothesis is compatible with previous reports\(^\text{18, 28}\). When we compared the two groups, the different character between RLP-C and RemL-C became clear (Table 2 and Fig. 1). The high TG group had significantly higher fasting TG and RemL-C concentrations. After loading, RLP-C, RLP-TG, and apoB-48 also became significant parameters. It can be deduced that, in the fasting period, exogenous remnants in postprandial hyperlipidemic subjects may decrease to a similar level to that in normal subjects, and endogenous remnants may remain at a significantly higher level.

There are some limitations of the present study as follows: as the study was performed with a small number of normal young subjects, only 4 individuals had the peak value of TG over 150 mg/dL as the high group. We need further examination with a larger number of normal and hyperlipidemic subjects in order to verify the TG value of 150 mg/dL, to identify the most dangerous lipid profile(s) involving remnants, apoB-48, and other parameters, and to clarify the significant differences between RLP-C and RemL-C. In addition, analyses of apoCs in remnant lipoproteins and LPL are necessary because these enzyme and proteins interfere with the apoE-mediated uptake of remnants and lipolysis\(^\text{29, 30}\). Here, since we did not examine LPL activity and protein mass measurement, we could not determine whether subjects in the high TG group have heterozygous LPL deficiency\(^\text{31}\). Of note, fasting sd-LDL-C concentration was higher in the high TG group. Interestingly, sd-LDL-C concentrations gradually decreased after loading. Here, we have no data to explain why sd-LDL-C in the high TG group but not in the low TG group gradually decreased after loading. Recently, Ogita et al. have reported that serum sd-LDL-C concentrations decreased after the 75 g oral glucose tolerance test and suggested that insulin can be a key modulator of sd-LDL-C concentrations\(^\text{32}\). In conclusion, TG, RLP-C, RLP-TG, RemL-C, and apoB-48 concentrations in the fasting period may be suitable to detect and characterize postprandial hyperlipidemia in normolipidemic subjects. In future, it is necessary to reveal which parameter or combination is useful to identify postprandial hyperlipidemia with a large-scale study.
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