Increased Serum Apolipoprotein B48 Concentration in Patients with Metabolic Syndrome

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Aim: Postprandial hyperlipidemia is characterized by an increase of chylomicron remnants (CM-R), and is a risk factor for atherosclerosis. Apolipoprotein (apo) B48 exists exclusively in chylomicrons and CM-R, and fasting plasma levels of apo B48 may reflect high postprandial levels of chylomicrons and/or CM-R. We hypothesized that fasting apo B48 levels may be increased in metabolic syndrome.

Methods: We investigated 1,349 inhabitants (528 men and 821 women aged 62.4 ± 12.8 y; mean ± S.D.) of two towns in rural Hokkaido, who underwent health checks in 2005.

Results: The fasting apo B48 level was significantly higher in males than females (geometric mean 1.92; 95% CI 1.80–2.04 μg/mL, vs. 1.69; 95% CI 1.61–1.76 μg/mL; p < 0.001). Ln (apo B48) showed a significant positive correlation with total cholesterol and ln (triglycerides), and a negative correlation with HDL-cholesterol. The correlation between ln (apo B48) and ln (triglycerides) was strong. Apo B48 was significantly higher in men and women with than without metabolic syndrome. Regression analysis revealed that ln (apo B48) was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and ln (triglyceride).

Conclusion: Fasting apo B48 levels are raised in individuals with metabolic syndrome.


Key words; Chylomicrons, Hypertriglyceridemia, Apolipoprotein B48, Metabolic syndrome

Introduction

Postprandial hyperlipidemia, which is characterized by increased levels of chylomicron remnants (CM-R), is considered to be a risk factor for atherosclerosis1, 2. Chylomicrons are assembled in the small intestine and undergo lipolysis by lipoprotein lipase in the plasma to generate CM-R. Because CM-R are then rapidly taken up by the liver, it has been assumed that fasting plasma levels of these particles are very low3.

Postprandial hyperlipidemia is related to metabolic syndrome4-6. This syndrome is characterized by insulin resistance, hypertension, dyslipidemia, and hyperglycemia, and is an important risk factor for atherosclerosis. In metabolic syndrome, dyslipidemia is characterized by hypertriglyceridemia and a low high density lipoprotein (HDL)-cholesterol level. Recent studies have shown that postprandial hyperlipidemia is a major cause of hypertriglyceridemia associated with metabolic syndrome6, 7.

Though CM-R are thought to play an important role in dyslipidemia associated with metabolic syndrome, CM-R concentrations in patients with this syndrome have not yet been reported. Apo B48 exists exclusively in chylomicrons and CM-R. Several methods of measuring the apo B48 concentration in plasma or in triglyceride-rich lipoproteins have been reported6-10. These methods include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)6-10, SDS-PAGE coupled with Western blotting11, 12, and competitive enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies13, 14.
Sakai et al. reported an ELISA for measuring apo B48 in fasting serum that employed a monoclonal antibody against apo B48\(^\text{(15)}\). We also recently developed an ELISA to measure the serum level of apo B48 using another monoclonal antibody\(^\text{(16)}\).

We hypothesized that the fasting plasma level of apo B48 may be increased in patients with metabolic syndrome; therefore, we measured fasting plasma levels of apo B48 in order to evaluate the relationship between CM-R and metabolic syndrome. We also investigated the factors regulating apo B48 levels in fasting plasma.

**Subjects and Methods**

The subjects were 1,349 inhabitants (528 men and 821 women) of two towns in a rural area of Hokkaido, Japan, who underwent routine health checks in 2005. Blood samples were collected from all subjects after an overnight fast. Systolic and diastolic blood pressures were measured at rest in the sitting position. The fasting plasma glucose (FPG) level and plasma levels of total cholesterol, triglycerides, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol were measured by enzymatic methods. Immunoreactive insulin (IRI) was measured by an enzyme immunoassay (EIA). Waist circumference was measured at the level of the umbilicus in the standing position. The HOMA-IR (homeostasis model assessment insulin resistance index) was calculated as FPG × IRI/405, after excluding individuals who had an FPG above 126 mg/dL and/or were on treatment for diabetes\(^\text{(17)}\).

Apolipoprotein B48 was measured by EIA\(^\text{(16)}\). Briefly, a 96-well microtiter plate (Nalge Nunc International, Japan) was coated with an anti-apoB-48 monoclonal antibody (4C8) by overnight incubation at 4°C. After washing the microtiter plate with phosphate-buffered saline, 50-μL aliquots of 100-fold-diluted serum or plasma (diluted with 0.05 mol/L Tris-HCl buffer, pH 7.5, 0.15 mol/L NaCl, and 0.1% Triton X-100) were added in duplicate to the wells and the plate was incubated at room temperature (20–25°C) for 1 hr. Aliquots (50 μL) of the apoB-48 standard (2.5 ng/mL to 160 ng/mL; 7-point calibration curve) were incubated in the same way. After the plate was washed three times, 50 μL biotin-conjugated anti-apoB-48/B-100 (ICN Pharmaceuticals Inc., USA) diluted in 0.01 mol/L phosphate buffer (pH 7.2) with 0.15 mol/L NaCl and 0.1% bovine serum albumin was added to each well and incubated with gentle shaking at room temperature for 1 hr. After the plate was washed, 50 μL horseradish peroxidase-conjugated avidin solution was added followed by incubation at room temperature for 30 min. After the plate was washed, 50 μL chromogenic substrate solution was added to each well and incubated with shaking at room temperature for 20 min until the color developed. Then 50 μL of stop solution was added to each well and plate was read at 450 nm using a Spectra-Fluor-Plus plate reader (Tecan, USA).

Metabolic syndrome was defined according to Japanese criteria\(^\text{(18)}\). Briefly, a waist circumference of more than 85 cm in men and 90 cm in women combined with more than one of the following factors led to a diagnosis of metabolic syndrome: plasma triglycerides > 150 mg/dL and/or HDL cholesterol < 40 mg/dL, systolic blood pressure > 130 mmHg and/or diastolic blood pressure > 85 mmHg, and FPG > 110 mg/dL. Some subjects were taking medications, but subjects with or without medications were grouped together for this study.

The mean ± SD or median with interquartile range is shown to summarize the characteristics of the study subjects by sex. Between-group comparisons of the means and median were performed by unpaired t-test and the Wilcoxon rank-sum test, respectively. The relationship of serum lipids and lipoproteins with apo B48 was examined by correlation and multiple regression analysis. Pearson's correlation coefficients were calculated for the correlation. Stepwise multiple regression analysis was used to determine independent predictors of apo B48, with p-to-enter and p-to-retain set at 0.10 each. Statistical significance was declared if the two-sided p value was less than 0.05. Statistical analyses were performed using JMP software (SAS Institute, Cary, NC).

**Results**

The age of all subjects, men and women was 62.4 ± 12.8 years, 64.2 ± 12.8 years and 61.2 ± 12.7 years (mean ± S.D.), respectively. The mean body mass index (BMI) did not differ significantly between men and women (23.9 ± 3.1 vs. 23.5 ± 3.6, respectively). Plasma levels of total cholesterol, HDL-cholesterol and LDL-cholesterol were significantly lower (p < 0.001) in men than in women, whereas the values of apo B48, triglycerides, and FPG were significantly higher (p < 0.001) (Table 1). Fig. 1 shows the distribution of apo B48 in men and women. The mean apo B48 level was 1.92 μg/mL in men and 1.69 μg/mL in women.

As shown in Fig. 1, the distribution of apo B48 was skewed to the left. Data were therefore normalized by logarithmic transformation for further statistical analysis. The triglyceride and HOMA-IR data were
The ln (apo B48) showed a weak positive correlation with total cholesterol, and a weak negative correlation with HDL-cholesterol (Fig. 2). In addition, ln (apo B48) and ln (triglycerides) showed a strong positive correlation \( (r=0.53 \text{ in men and } r=0.48 \text{ in women}). \)

Ln (apo B48) also showed a strong positive correlation with ln (HOMA-IR) (Fig. 2). The fasting apo B48 level was significantly higher in both men and women with metabolic syndrome than without (Table 2).

**Discussion**

In this study, we measured plasma apo B48 levels with a novel ELISA. According to previous reports, the fasting plasma apo B48 concentration ranges between

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Table 1. Characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Men ((n=524))</th>
<th>Women ((n=819))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(^a)</td>
<td>64.2 ± 12.8</td>
<td>61.2 ± 12.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))(^a)</td>
<td>23.9 ± 3.1</td>
<td>23.5 ± 3.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Waist (cm)(^a)</td>
<td>85.9 ± 8.7</td>
<td>83.1 ± 10.7</td>
<td>0.04</td>
</tr>
<tr>
<td>MS with/without</td>
<td>397/125</td>
<td>741/78</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>T. chol (mg/dL)(^a)</td>
<td>192.4 ± 31.1</td>
<td>204.4 ± 30.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TG (mg/dL)(^b)</td>
<td>99 (74–140)</td>
<td>83 (63–117)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HDL-C (mg/dL)(^a)</td>
<td>53.9 ± 13.2</td>
<td>62.1 ± 14.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)(^a)</td>
<td>106.2 ± 27.6</td>
<td>115.8 ± 27.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)(^b)</td>
<td>138 (124–153)</td>
<td>135 (117–151)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP (mmHg)(^b)</td>
<td>78 (70–86)</td>
<td>74.5 (66–83)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>FPG (mg/dL)(^a)</td>
<td>102.9 ± 23.4</td>
<td>95.0 ± 18.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IRI ((\mu)U/mL)(^b)</td>
<td>3.9 (2.6–5.8)</td>
<td>4.1 (2.8–5.8)</td>
<td>0.38</td>
</tr>
<tr>
<td>HOMA-IR (mg/dL × (\mu)U/mL)(^b)</td>
<td>0.924 (0.602–1.387)</td>
<td>0.909 (0.636–1.341)</td>
<td>0.02</td>
</tr>
<tr>
<td>ApoB 48 ((\mu)g/mL)(^b)</td>
<td>1.80 (1.20–3.10)</td>
<td>1.61 (1.14–2.45)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SD

\(^b\)Median (25th and 75th interquartile range)

\(p\)-values were based on paired t-test and Wilcoxon rank-sum test for mean and median, respectively.
Our data (men: 1.92 μg/mL; women: 1.69 μg/mL (mean value)) were also in this range, and were similar to the level reported by Sakai et al. for normolipidemic subjects \( (5.2 \pm 3.8 \text{ mg/mL}) \) using a similar ELISA with another monoclonal antibody against apo B48\(^{15}\). Among the methods available to measure apo B48, ELISA systems based on monoclonal antibodies are valuable because they are simple and quantitative methods.

In this study, we measured fasting plasma levels of apo B48. It has been suggested that a high fasting apo B48 level reflects high postprandial concentrations of chylomicrons and/or CM-R\(^{12}\); therefore, we assumed that a high fasting plasma level of apo B48 indicated the existence of postprandial hyperlipidemia.

The B48 concentration was higher among men than women (Fig. 1). Sakai et al. previously found that men also had higher apo B48 levels than women among normolipidemic subjects\(^{15}\). These results may indicate that women show more rapid catabolism of chylomicrons and/or CM-R, or less intestinal fat absorption, or both.

Apo B48 showed a significant and strong correlation with triglycerides (Fig. 2). Cortner et al. reported that delayed catabolism of CM-R leads to hypertriglyceridemia\(^3\). Since very low density lipoprotein (VLDL) and VLDL remnants are considered the main contributors to plasma triglyceride concentration, the close relationship between apo B48 and triglyceride levels indicates that delayed catabolism of CM-R leads to the accumulation of VLDL or VLDL remnants. It is interesting that the plasma concentration of apo B48, which is far lower than that of apo B100 (0.15–0.2 vs.

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**Fig. 2.** Correlation of ln (apo B48) with total cholesterol, ln (triglycerides), HDL-cholesterol, and ln (HOMA-IR) in men and women.

**Table 2.** Apo B48 levels according to metabolic syndrome in men and women

<table>
<thead>
<tr>
<th>Sex</th>
<th>Metabolic syndrome</th>
<th>N</th>
<th>Geometric mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>(−)</td>
<td>397</td>
<td>1.76 (1.65–1.89)</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>125</td>
<td>2.50 (2.17–2.88)</td>
</tr>
<tr>
<td>Women</td>
<td>(−)</td>
<td>741</td>
<td>1.64 (1.57–1.72)</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>78</td>
<td>2.19 (1.86–2.59)</td>
</tr>
</tbody>
</table>

CI, confidence interval

\( p \)-value was based on unpaired \( t \)-test.
Table 3. Stepwise multiple regression analysis of ln (apoB48) in relation to serum lipids, lipoproteins, and glucose-related parameters (n = 1,089)

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>S.E.</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.0069</td>
<td>0.0013</td>
<td>-5.12</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.0213</td>
<td>0.0599</td>
<td>-3.55</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.0066</td>
<td>0.0021</td>
<td>3.13</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.0068</td>
<td>0.0022</td>
<td>-3.15</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-0.0047</td>
<td>0.0020</td>
<td>-2.28</td>
</tr>
<tr>
<td>ln (Triglycerides)</td>
<td>0.5520</td>
<td>0.0715</td>
<td>7.72</td>
</tr>
<tr>
<td>ln (HOMA-IR)</td>
<td>0.0583</td>
<td>0.0337</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Sex, SBP, DBP, total cholesterol, HDL cholesterol, LDL cholesterol, apo E, ln (triglyceride), and ln (IRI) were also included as explanatory variables in the model, but they did not remain in the final model.

100–120 mg/dL), has such a significant relationship with the VLDL or VLDL remnant level. This may because the triglyceride content of CM-R is very high when compared to VLDL or VLDL remnants.

Apo B48 was also positively correlated with HOMA-IR (Fig. 2), which is a marker of insulin resistance, and the apo B48 level was significantly higher in subjects with metabolic syndrome than without (Table 2). These results indicate that apo B48 increases in the presence of insulin resistance and/or metabolic syndrome. Since insulin resistance is considered to be involved in the development of metabolic syndrome, insulin sensitivity might influence the level of apo B48. It has been reported that insulin resistance shows a negative correlation with lipoprotein lipase mRNA expression and activity in adipose tissue. Thus, defects of lipoprotein lipase may cause the accumulation of apo B48 particles. In fact, it has been reported that insulin resistance might lead to postprandial hyperlipidemia.

Because our subjects with metabolic syndrome showed higher fasting plasma concentrations of apo B48, there is a possibility that CM-R may play a role in the increased risk of atherosclerosis related to this syndrome. Vine et al. reported that impaired postprandial metabolism of apo B48 led to atherosclerosis in rats with metabolic syndrome. On the other hand, Velero et al. reported that the fasting apo B48 level does not predict the risk of coronary heart disease. Thus, whether the fasting apo B48 level influences the risk of atherosclerosis remains to be determined.

Multiple regression analysis revealed that apo B48 was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride. Gender does not affect apo B48 by this method, which may be due to an other factor related to gender (i.e., LDL cholesterol or HDL cholesterol) having a strong association with apo B48.

In conclusion, the fasting plasma level of apo B48 was correlated with the serum triglyceride concentration, and apo B48 levels were higher in rural Japanese subjects with metabolic syndrome than those without; however, further studies of other populations are needed to confirm these results.

References
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