Original Article

Influence of Fatty Liver on Plasma Small, Dense LDL-Cholesterol in Subjects with and without Metabolic Syndrome

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Aim: Small, dense low density lipoprotein (sLDL) is known as an atherogenic lipoprotein and is often associated with metabolic syndrome (MS). A high frequency of sLDL is found in hypertriglyceridemic subjects. Also, fatty liver (FL) is often associated with MS; therefore, we studied whether the association of FL increases sLDL-cholesterol (C) in subjects with MS.

Methods: In total, 207 patients were enrolled in this study and FL was estimated by echogram. The presence of MS was diagnosed according to the Japanese Guidelines for the Definition of Metabolic Syndrome.

Results: sLDL-C and sLDL-C/LDL-C in the MS group were higher than in the non-MS group. Also, sLDL-C and sLDL-C/LDL-C in the FL group were higher than in the non-FL group. The simple correlation coefficient (r) between plasma triglyceride and sLDL-C or sLDL-C/LDL-C in all subjects was 0.36 and 0.51. In the MS group, r values were 0.32 and 0.52 while, in the non-FL group, r was 0.32 and 0.38, respectively. Two-way ANOVA revealed that FL was a powerful determinant of plasma sLDL-C and sLDL-C/LDL-C, but MS was not. When we divided all subjects into four groups, i.e., MS(-)FL(-), MS(-)FL(+), MS(+)FL(-) and MS(+)FL(+), sLDL-C/LDL-C of MS(+)FL(+) was significantly higher than all other groups.

Conclusion: Association of MS and FL significantly increased sLDL-C and sLDL-C/LDL-C. The significant relationship between sLDL-C/LDL-C and plasma triglyceride in the FL group indicates that FL may produce triglyceride-rich VLDL, a precursor of sLDL, thereby contributing to the appearance of sLDL particles in the plasma of MS patients with FL.


Key words: Small, dense LDL (sLDL), Metabolic Syndrome (MS), Fatty Liver (FL)

Introduction

The definition and diagnostic criteria for metabolic syndrome (MS) in Japan were established in April 20051. It is now well-known that MS is associated with increased coronary artery disease (CAD) events, cardiovascular mortality, and a reduced survival rate2-4. MS is also reported to be a predictor of subclinical atherosclerosis5.

One of the important components of MS is hypertriglyceridemia. It is known that hypertriglyceridemia is often associated with increased small, dense low density lipoprotein (sLDL) or remnant lipoproteins6, 7. Recent studies demonstrated an increased sLDL fraction in MS subjects5, 8, while the mechanisms for the generation of sLDL are not well understood. On the other hand, fatty liver (FL) is often associated with MS and/or hypertriglyceridemia; therefore, it is possi-
ble that the presence of FL in MS subjects may increase the plasma sLDL fraction.

**Aim**

The present study was conducted to examine the influence of FL on serum sLDL-cholesterol (C) level in subjects with and without MS.

**Methods**

Two hundred and seven patients attending the outpatient clinic of the Division of Diabetes, Metabolism and Endocrinology, Toho University Medical Center, Omori Hospital were enrolled in this study. All study protocols and procedures were approved by the Ethics Committee of Toho University Medical Center, Omori Hospital. The study objectives and intended measures were explained to all study patients individually. Written informed consent was obtained from all participants. MS was diagnosed according to the Japanese Guidelines for Metabolic Syndrome. Subjects with secondary MS and fibrate users were excluded. Plasma lipids, including sLDL-C, were measured in all subjects. Waist circumference and blood pressure (in a supine position) were measured. Blood was sampled after an overnight fast. Blood glucose, hemoglobin (Hb) A1c and plasma lipids were measured using standard laboratory methods. The sLDL-C level was measured according to Hirano et al. The presence of FL was estimated by echogram (B-mode ultrasonography). All patients underwent real-time ultrasound scanning performed by two examiners independently who were trained in ultrasound techniques and particularly specialized in abdominal ultrasound examinations. Those who demonstrated an obvious bright liver echo pattern, which signified a discrepancy higher than expected in the echo amplitude between the liver and kidney parenchyma, and having deep attenuation, were diagnosed as having FL. If there were different results in the liver echo pattern between the two examiners, the results by the more skilled and expert examiner were selected.

**Statistical analysis**

All values are expressed as the mean ± SD. Analysis of variance (ANOVA) was used to compare mean values among groups. Simple linear regression analysis was performed to evaluate the relationship between the sLDL-C level and other clinical parameters. Two-way analysis of variance (ANOVA) was performed to examine the influence of FL and MS on the sLDL-C level and sLDL-C per LDL-C ratio. A significant difference was defined as p < 0.05.

**Results**

Among 207 subjects, 111 had MS (66 male and 45 female), while 96 did not (34 and 62). In the MS group, 75 had FL, while 36 did not. In the non-MS group, 28 had FL while 68 did not (Table 1). The sLDL-C level and sLDL-C per LDL-C ratio in the MS group were significantly higher than in the non-MS group (Fig. 1, upper panel). When the subjects were divided according to an association of FL, both sLDL-C level and sLDL-C per LDL-C ratio were higher in the FL group than those in the non-FL group (Fig. 1, lower panel). When the subjects were divided into 4 groups according to the association of FL and/or MS, both the sLDL-C level and sLDL-C per LDL-C ratio were the highest in the FL and MS groups (Table 2). The simple correlation coefficients (r) between plasma triglyceride and the sLDL-C level or sLDL-C per LDL-C ratio in all subjects were 0.36 and 0.51, respectively (Fig. 2). When the subjects were divided to those with MS, the r was 0.32 and 0.52, respectively (Fig. 3, upper panel), while in the non-MS group, the r was 0.36 and 0.40, respectively (Fig. 3, lower panel). When the subjects were limited to those without FL, the r was 0.32 and 0.52, respectively (Fig. 4, upper panel). When the subjects were limited to those without FL, the r was 0.32 and 0.38, respectively (Fig. 4, lower panel). Two-way ANOVA revealed that the presence of FL was a significant determinant of the serum sLDL-C level and serum sLDL-C per LDL-C ratio but the presence of MS was not (Table 3 and 4).

**Discussion**

Plasma LDL-C is the most important risk factor for CAD events, while plasma LDL is composed of multiple discrete subclasses, differing in size and...
density. Initial studies conducted by Krauss and Burke demonstrated that two distinct LDL subclass phenotypes can be distinguished on the basis of the LDL particle distribution separated by gradient gel electrophoresis (GGE). Pattern A consists of a major peak greater than 25.5 nm, whereas in pattern B this is less than 25.5 nm. An investigation of LDL subfractions in normotriglyceridemic men revealed that small LDL can be a coronary risk factor in subjects without apparent hyperlipidemia. LDL composition was examined in diabetic, myocardial infarction survivors. Decreased cholesterol loading in LDL and an increased number of LDL particles were also marked in this group of patients, indicating the predominance of sLDL in myocardial infarction survivors with type 2 diabetes.

Table 2. Small, dense LDL-cholesterol, LDL-cholesterol and small, dense LDL-cholesterol per LDL-cholesterol in four groups

<table>
<thead>
<tr>
<th></th>
<th>MS(-)FL(-)</th>
<th>MS(-)FL(+)</th>
<th>MS(+FL(-))</th>
<th>MS(+FL(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>68</td>
<td>28</td>
<td>36</td>
<td>75</td>
</tr>
<tr>
<td>sLDL-C (mg/dL)</td>
<td>33.9 ± 18.2a</td>
<td>42.6 ± 18.6b</td>
<td>39.8 ± 19.8b</td>
<td>48.0 ± 25.5b</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>127.9 ± 32.3a</td>
<td>137.5 ± 45.8a</td>
<td>134.2 ± 31.7a</td>
<td>130.9 ± 36.9a</td>
</tr>
<tr>
<td>sLDL-C/LDL-C</td>
<td>0.27 ± 0.12a</td>
<td>0.31 ± 0.10a</td>
<td>0.30 ± 0.12a</td>
<td>0.37 ± 0.15b</td>
</tr>
</tbody>
</table>

MS: metabolic syndrome, FL: fatty liver, a, b: there are significant differences between data not sharing the same superscript (p<0.05 by Tukey's method). Data are expressed as the mean ± SD.
Fig. 2. Relationship between plasma triglyceride and small, dense LDL-cholesterol or small, dense LDL-cholesterol per LDL-cholesterol ratio in all subjects. The vertical scale indicates plasma triglyceride (TG) and the horizontal scale small, dense LDL-cholesterol and small, dense LDL-cholesterol per LDL-cholesterol ratio.

Fig. 3. Relationship between plasma triglyceride and small, dense LDL-cholesterol or small, dense LDL-cholesterol per LDL-cholesterol ratio in metabolic syndrome subjects (upper panel) and non-metabolic syndrome subjects (lower panel). The vertical scale indicates plasma triglyceride (TG) and the horizontal scale small, dense LDL-cholesterol and small, dense LDL-cholesterol per LDL-cholesterol ratio.
**Table 3.** The influence of metabolic syndrome or fatty liver on small, dense LDL-cholesterol estimated by two-way ANOVA

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>SSq</th>
<th>VR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor of MS</td>
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<td>1422.78</td>
<td>3.03</td>
<td>0.082</td>
</tr>
<tr>
<td>Factor of FL</td>
<td>1</td>
<td>3109.66</td>
<td>6.63</td>
<td>0.010*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>2.04</td>
<td>0.004</td>
<td>0.947</td>
</tr>
<tr>
<td>Residual</td>
<td>203</td>
<td>95106.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole</td>
<td>206</td>
<td>102308.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df: degree of freedom, SSq: sum of squares, VR: variance ratio, MS: metabolic syndrome, FL: fatty liver

**Table 4.** Influence of metabolic syndrome or fatty liver on small, dense LDL-cholesterol per LDL-cholesterol estimated by two-way ANOVA

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>SSq</th>
<th>VR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor of MS</td>
<td>1</td>
<td>0.03</td>
<td>1.88</td>
<td>0.17</td>
</tr>
<tr>
<td>Factor of FL</td>
<td>1</td>
<td>0.12</td>
<td>6.97</td>
<td>0.008*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.01</td>
<td>1.06</td>
<td>0.30</td>
</tr>
<tr>
<td>Residual</td>
<td>203</td>
<td>3.61</td>
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<tr>
<td>Whole</td>
<td>206</td>
<td>3.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df: degree of freedom, SSq: sum of squares, VR: variance ratio, MS: metabolic syndrome, FL: fatty liver

**Fig. 4.** Relationship between plasma triglyceride and small, dense LDL-cholesterol or small, dense LDL-cholesterol per LDL-cholesterol ratio in fatty liver subjects (upper panel) and those in non-fatty liver subjects (lower panel). The vertical scale indicates plasma triglyceride (TG) and the horizontal scale small, dense LDL-cholesterol and small, dense LDL-cholesterol per LDL-cholesterol ratio.
A previous study based on gradient gel electrophoresis demonstrated that subjects with metabolic syndrome have sLDL. In a recent cross-sectional study the number of sLDL particles determined by nuclear magnetic resonance spectroscopy was found to be greater in patients with MS and to increase with the number of components of MS. In addition, Cali et al. reported that the presence of FL was associated with a pronounced dyslipidemic profile characterized by large VLDL and small, dense LDL (sLDL).

In this study, we found that the sLDL-C level was elevated in not only MS but also in FL subjects. It is now a matter of debate how the presence of FL increases the population of sLDL. There is substantial evidence that the accumulation of intra-abdominal fat with type 2 diabetes, insulin resistance or MS may increase free fatty acid release from adipocytes and elevated plasma glucose. The liver may increase triglyceride production utilizing increased free fatty acid and glucose from the plasma, leading to the generation of FL. As a consequence of increased hepatic triglyceride synthesis, there may be an increased production of triglyceride-rich (large) very low density lipoprotein (VLDL) in FL. This triglyceride-rich VLDL can be a precursor of sLDL, i.e., triglyceride-rich VLDLs are converted to triglyceride-enriched LDLs, which are the favored substrate for hepatic lipase and are transformed into smaller LDL by lipase-mediated triglyceride hydrolysis.

In the present study, when the subjects were limited to those with FL, the simple correlation coefficient (r) between plasma triglyceride and sLDL-C per LDL-C ratio was 0.52, which was much higher than in subjects without FL (r = 0.38). Since the sLDL-C per LDL-C ratio may indicate the peak size of the LDL fraction and therefore the increase of this ratio in the MS with FL group may indicate the increased population of sLDL particles without an increase of large LDL particles, these results may indicate that FL is a very important factor for the generation of small LDL in hypertriglycerideremia and supports the above proposed mechanisms for the generation of sLDL.

Furthermore, two-way ANOVA revealed that FL is a significant determinant of the serum sLDL-C level and serum sLDL-C per LDL-C ratio. These results may support the above proposal for a generation system of sLDL.

In this study, MS was not a significant determinant of either the plasma sLDL-C level or sLDL-C per LDL-C ratio, contrary to previous studies. Since the plasma triglyceride level is known as one of the important determinants of LDL size, the inclusion of normotriglyceridemic subjects in the MS group in this study can be an explanation for the above results.

Finally, it is possible that statin administration may modify the plasma sLDL-C level and sLDL-C per LDL-C ratio; therefore, we re-analyzed the data after excluding statin users. However, the results of linear regression analysis, ANOVA and two-way ANOVA were the same before and after excluding statin users (data not shown).

**Limitations**

The diagnosis of fatty liver in this study was based on an abdominal ultrasound examination but not on MRI, and we have no liver biopsy data; thus, the exact lipid content in the liver of each subject is not available in this study.

**Conclusion**

The present study revealed that in the presence of FL there was a very good correlation between plasma triglyceride and the sLDL-C level per LDL-C ratio. This may support the proposed generation mechanism of sLDL in hypertriglycerideremic subjects. Furthermore, the correlation between sLDL-C per LDL-C ratio and plasma triglyceride was stronger than that between the sLDL-C level and plasma triglyceride. Since the sLDL-C per LDL-C ratio may indicate the peak size of LDL particles, LDL peak size is more influenced by the presence of FL than the absolute value of serum sLDL-C. Finally, according to two-way ANOVA, the presence of FL is a significant determinant of serum sLDL-C per LDL-C ratio.

**References**


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