Original Article

Effects of Different Statin Treatments on Small Dense Low-Density Lipoprotein in Patients with Metabolic Syndrome

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Aim: To compare the effects of different low-density lipoprotein (LDL) cholesterol-lowering statin treatments on small dense LDL (sd-LDL) in hypercholesterolemic patients with metabolic syndrome (MetS).

Methods: Forty hypercholesterolemic MetS patients ≥30 years of age were randomized to rosuvastatin (n=17) or other statins (n=23) groups. In the other statins group, those taking atorvastatin (n=12) were also evaluated separately. Statin doses were 10 mg/day rosuvastatin, 20 mg/day atorvastatin, 40 mg/day simvastatin, and 40 mg/day pravastatin. Treatment duration was planned to be 8 weeks. Sd-LDL levels were assessed at baseline and at the completion of treatment.

Results: After treatment, sd-LDL levels were significantly reduced in all 3 groups (from 29.6 ± 24.8 mg/dL to 8.9 ± 8.5 mg/dL in the rosuvastatin group, p=0.001; from 26.2 ± 15 mg/dL to 14.8 ± 9.6 mg/dL in the atorvastatin group, p=0.02; and from 29.1 ± 16.5 mg/dL to 14.7 ± 11.2 mg/dL in the other statins group, p=0.0001). There was no significant difference in the mean percent changes among groups.

Conclusion: Significant reduction in sd-LDL levels was observed after 8 weeks of statin treatment in hypercholesterolemic patients with MetS. This effect was similar for all statins and can be considered a class effect.


Key words: Metabolic syndrome, Statin, Small-dense low-density lipoprotein cholesterol

Introduction

Metabolic syndrome (MetS) is the clustering of cardiometabolic risk factors characterized by abdominal obesity, hypertension, dyslipidemia, hyperglycemia, prothrombosis and proinflammatory conditions¹. MetS dyslipidemia is characterized by high triglyceride, reduced high-density lipoprotein (HDL)-cholesterol (C), and elevated small dense (sd) low-density lipoprotein (LDL)-C, which is also known as the atherogenic lipid triad².

Being a very strong indicator of atherosclerotic vascular events, LDL-C is mainly composed of 2 fractions, namely large-buoyant (lb)-LDL and sd-LDL, the latter known to have greater atherogenicity than the former³. In the Stanford Five Cities Project, Physician’s Health Survey and the Quebec Cardiovascular Study, sd-LDL was reported to be associated with increased cardiovascular risk⁴-⁶. It has been suggested that treatments affecting LDL sub-groups reduce the risk for atherosclerosis, and that the measurement of LDL-particle size is beneficial in the treatment and clinical assessment of patients at high risk for coronary artery disease⁷.

The effects of statins on sd-LDL as potent inhibitors of the enzyme hydroxy-methyl-glutaryl coenzyme-A-reductase are generally controversial and it has been proposed that novel statins, having a more potent LDL-C-lowering effect, are more effective in the modification of LDL size and sub-groups⁸. A
drug that lowers triglycerides more effectively and causes a greater increase in HDL-C can be expected to have a greater effect on sd-LDL.\textsuperscript{9}

The aim of the present study was to determine whether there is a difference between the effects of different statin treatments used for lowering LDL-C in patients with MetS on sd-LDL, which is one of the most important components of dyslipidemia in these patients.

**Materials and Methods**

Patients ≥30 years of age being followed up at the outpatient clinic of the Internal Medicine Department in Göztepe Training and Research Hospital and fulfilling the criteria below were consecutively included in the study. The local ethics committee granted approval for the study, and written informed consent was obtained from all patients prior to their inclusion. This study was conducted in accordance with the Declaration of Helsinki.

**Inclusion Criteria**

Patients diagnosed with MetS who were to receive lipid-lowering treatment.

**Exclusion Criteria**

Patients with severe renal disease or renal dysfunction (creatinine >1.5 mg/dL), chronic liver disease or liver dysfunction (alanine amino transferase >1.5 times the upper normal limit), inflammatory muscle disease or any laboratory finding suggesting a muscle disorder (creatine phosphokinase >3 times the upper normal limit), coronary artery disease, severe heart failure, severe chronic airway disease or cancer, except for non-melanoma skin cancer, antidiabetic treatment modification within the last 3 months, statin or fibrate use within the last 3 months, conditions known to have an influence on metabolism or immunity (hypo-hyperthyroidism, life-style changes within the last month, immunosuppressive treatment or initiation/cessation of hormone replacement treatment within the last month), drug and substance dependency, and a history of stroke or acute coronary syndrome within the last month, drug and substance dependency, and a history of stroke or acute coronary syndrome within the last month, especially for the last 3 months.

**Anthropometric Measurements**

Sitting blood pressure was measured in both arms after at least 10 minutes of rest with an appropriate mercury sphygmomanometer using the Phase I and Phase V Korotkoff sounds. A second measurement was made in the arm with the higher reading and the mean of the two measurements was recorded. Waist circumference was measured at the plane between anterior superior iliac spines and lower costal margins at the narrowest part of the waistline while the patient was standing and in slight expiration.

**Biochemical Measurements**

Blood samples obtained at baseline and at the end of treatment following 12 hours of overnight fasting were immediately centrifuged (2500 rpm) and the sera were separated. Glucose, total-C, HDL-C, and triglyceride levels were determined by enzymatic methods with the Olympus AU 5200 auto-analyzer. LDL-C was calculated using the Friedewald formula\textsuperscript{11}. The LDL sub-fraction was analyzed by a polycrylamide gel tube electrophoresis system (Liprint\textsuperscript{TM} LDL System; Quantimetrix Corp, Redondo Beach, CA, USA)\textsuperscript{12}.

**Statistical Analysis**

Data were analyzed with the NCSS (Number Cruncher Statistical System) 2007 package. In addition to descriptive statistics (mean, standard deviation), the independent samples t-test was used for inter-group comparisons, paired samples t-test for inter-group comparisons, paired samples t-test for...
Clinical characteristics of the study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rosuvasatin (n = 17) Before Treatment</th>
<th>Rosuvasatin (n = 17) After Treatment</th>
<th>Other Statins (atorvastatin, simvastatin, pravastatin) (n = 23) Before Treatment</th>
<th>Other Statins (atorvastatin, simvastatin, pravastatin) (n = 23) After Treatment</th>
<th>Atorvastatin (n = 12) Before Treatment</th>
<th>Atorvastatin (n = 12) After Treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>146.2 ± 25.2</td>
<td>132.4 ± 14.8</td>
<td>0.03</td>
<td>142.6 ± 13.9</td>
<td>135.2 ± 7.9</td>
<td>0.002</td>
<td>140 ± 17.6</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>87.1 ± 10.5</td>
<td>84.1 ± 8.9</td>
<td>0.181</td>
<td>88.9 ± 10</td>
<td>84.6 ± 8.7</td>
<td>0.036</td>
<td>85.4 ± 11.6</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>97.8 ± 9.1</td>
<td>95.9 ± 8.9</td>
<td>0.021</td>
<td>97.4 ± 8.9</td>
<td>95.7 ± 8.2</td>
<td>0.009</td>
<td>96.4 ± 9.1</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>166.6 ± 77.6</td>
<td>131.7 ± 46.5</td>
<td>0.006</td>
<td>142.7 ± 70.1</td>
<td>134.5 ± 67.5</td>
<td>0.578</td>
<td>151.8 ± 71.1</td>
</tr>
<tr>
<td>Total-C (mg/dL)</td>
<td>272.1 ± 55.7</td>
<td>174.5 ± 33.3</td>
<td>0.0001</td>
<td>260.1 ± 42.4</td>
<td>171.7 ± 29.7</td>
<td>0.0001</td>
<td>245.5 ± 27.5</td>
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<tr>
<td>Triglyceride (mg/dL)</td>
<td>223.1 ± 80.4</td>
<td>161 ± 42.5</td>
<td>0.003</td>
<td>211.3 ± 74</td>
<td>179.3 ± 66</td>
<td>0.042</td>
<td>210.1 ± 66.9</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>47.1 ± 11.1</td>
<td>45.6 ± 9.6</td>
<td>0.552</td>
<td>47.2 ± 12.4</td>
<td>43.8 ± 11.4</td>
<td>0.066</td>
<td>45.2 ± 11</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>179.3 ± 50.7</td>
<td>96.2 ± 34.9</td>
<td>0.0001</td>
<td>173.5 ± 35.5</td>
<td>92.3 ± 25.4</td>
<td>0.0001</td>
<td>161.2 ± 26.3</td>
</tr>
<tr>
<td>Sd-LDL (mg/dL)</td>
<td>29.6 ± 24.8</td>
<td>8.9 ± 8.5</td>
<td>0.001</td>
<td>29.1 ± 16.5</td>
<td>14.7 ± 11.2</td>
<td>0.001</td>
<td>26.2 ± 15</td>
</tr>
<tr>
<td>Sd-LDL/LDL-C</td>
<td>0.16 ± 0.13</td>
<td>0.09 ± 0.07</td>
<td>0.006</td>
<td>0.17 ± 0.09</td>
<td>0.16 ± 0.13</td>
<td>0.287</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>Total-C/HDL-C</td>
<td>6.1 ± 2.1</td>
<td>4 ± 1.2</td>
<td>0.0001</td>
<td>5.8 ± 1.6</td>
<td>4.1 ± 1</td>
<td>0.0001</td>
<td>5.7 ± 1.5</td>
</tr>
<tr>
<td>Non-HDL (mg/dL)</td>
<td>225 ± 55.8</td>
<td>128.9 ± 33.8</td>
<td>0.0001</td>
<td>212.9 ± 40.1</td>
<td>128 ± 28.7</td>
<td>0.0001</td>
<td>200.3 ± 29.9</td>
</tr>
<tr>
<td>Triglyceride/HDL-C</td>
<td>5.1 ± 2.2</td>
<td>3.7 ± 1.4</td>
<td>0.01</td>
<td>5 ± 2.7</td>
<td>4.5 ± 2.1</td>
<td>0.277</td>
<td>5.2 ± 2.7</td>
</tr>
</tbody>
</table>

SBP: systolic blood pressure, DBP: diastolic blood pressure, WC: waist circumference, FPG: fasting plasma glucose, C: cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, Sd: small dense

repeated group measures and for correlations between variables, and the Chi-square test was used for qualitative data comparisons. Stepwise regression analysis was used to determine the variables affecting sd-LDL. The results were evaluated within a 95% confidence interval (CI) and at a significance level of $p < 0.05$.

**Results**

Forty patients were included in the study. After randomization, the rosuvasatin group included 17 patients (5 male, 12 female; mean age: 53.94 ± 10.62 years) while the other statins group had 23 patients (atorvastatin was administered to 12 patients, simvastatin to 10 patients, and pravastatin to 1 patient; 7 male, 6 female; mean age: 51.96 ± 9.34 years). In the other statins group, those taking atorvastatin ($n = 12$; 4 male, 8 female; mean age: 51.08 ± 6.69 years) were also evaluated separately. Anthropometric and biochemical characteristics of the groups are shown in Table 1.

The groups were similar in terms of age, gender, co-morbid conditions, baseline systolic and diastolic blood pressure, waist circumference, fasting plasma glucose, total-C, triglyceride, HDL-C, LDL-C, and sd-LDL levels

**Anthropometric Parameters**

At the end of treatment, compared to baseline, systolic blood pressure ($p = 0.03$) and waist circumference ($p = 0.021$) had decreased in the rosuvasatin group, and systolic and diastolic blood pressure ($p = 0.002$ and $p = 0.036$, respectively) and waist circumference ($p = 0.009$) had decreased in the other statins group.

**Biochemical Parameters**

After treatment, sd-LDL levels were significantly reduced in all 3 groups (from 29.6 ± 24.8 mg/dL to 8.9 ± 8.5 mg/dL in the rosuvasatin group, $p = 0.001$; from 26.2 ± 15 mg/dL to 14.8 ± 9.6 mg/dL in the atorvastatin group, $p = 0.02$; and from 29.1 ± 16.5 mg/dL to 14.7 ± 11.2 mg/dL in the other statins group, $p = 0.0001$). Significant decreases were observed in fasting plasma glucose ($p = 0.006$), total-C ($p = 0.0001$), triglyceride ($p = 0.003$), LDL-C ($p = 0.0001$), sd-LDL/LDL-C ($p = 0.006$), total-C/HDL-C ($p = 0.0001$), non-HDL ($p = 0.0001$), and triglyceride/HDL-C ($p = 0.01$) in the rosuvasatin group; in total-C ($p = 0.0001$), LDL-C ($p = 0.0001$), total-C/HDL-C ($p = 0.001$), and non-HDL ($p = 0.0001$) of the atorvastatin group; and in total-C ($p = 0.0001$), triglyceride ($p = 0.042$), LDL-C ($p = 0.0001$), total-C/HDL-C ($p = 0.0001$), and non-HDL ($p = 0.0001$) in the other statins group.

**Intergroup Comparisons**

After treatment, there was no significant difference between groups with respect to mean percent changes in anthropometric and biochemical parameters (Tables 2, 3).

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Table 1. Clinical characteristics of the study groups
Sd-LDL levels were positively correlated with total-C ($p=0.0001$), triglyceride ($p=0.0001$), LDL-C ($p=0.0001$), sd-LDL/LDL-C (0.0001), total-C/HDL-C ($p=0.0001$), non-HDL ($p=0.0001$), and triglyceride/HDL-C ($p=0.0001$), and negatively correlated with HDL-C ($p=0.004$) (Table 4). In multivariate analysis, sd-LDL levels were correlated with triglyceride (Standardized Coefficients; 0.24, $p=0.006$) and total-C/HDL-C (standardized coefficients; 0.61, $p=0.0001$) levels.

### Safety

All patients completed the treatment. No serious adverse events requiring therapy cessation were observed during the study period. At the end of treatment, no patients had alanine aminotransferase or creatinine phosphokinase enzyme levels exceeding 3 times the upper normal limit.

### Discussion

In the present study, 8 weeks of statin treatment was found to significantly reduce serum sd-LDL levels in hyperlipidemic patients with MetS. This effect was similar for all statins used in the study, and no significant difference was noted among rosuvastatin, atorvastatin and other statins.

In addition to the quantity of LDL-C, which is known as a very strong marker of atherosclerotic vascular events, its quality has also been reported to have...
Correlation between sd-LDL and clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>sd-LDL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.095</td>
<td>0.402</td>
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<tr>
<td>Diastolic blood pressure</td>
<td>0.057</td>
<td>0.618</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.053</td>
<td>0.641</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.139</td>
<td>0.219</td>
<td></td>
</tr>
<tr>
<td>Total-C</td>
<td>0.561</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.562</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.315</td>
<td>0.004</td>
<td></td>
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<tr>
<td>LDL-C</td>
<td>0.569</td>
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<tr>
<td>Total-C/HDL-C</td>
<td>0.738</td>
<td>0.0001</td>
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<tr>
<td>Non-HDL</td>
<td>0.634</td>
<td>0.0001</td>
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</tr>
<tr>
<td>Sd-LDL/LDL-C</td>
<td>0.877</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Triglyceride/ HDL-C</td>
<td>0.583</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

C: cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein

Table 4. Correlation between sd-LDL and clinical characteristics

a great impact on cardiovascular risk\textsuperscript{13}. Sd-LDL, described as a significant cardiovascular risk factor by ATPIII, is accepted as a good predictor of cardiovascular events and coronary heart disease progression\textsuperscript{14,16}. The atherogenic dyslipidemia pattern, accepted to be due to insulin resistance in patients with MetS, is characterized by high triglyceride, low HDL-C, and increased sd-LDL particle levels\textsuperscript{2}. Although LDL-C levels in patients with MetS were not found to be higher than in the general population, qualitative LDL-C abnormalities, such as sd-LDL, are commonly observed in these patient groups, with reports that LDL particle measurement may be a useful tool to identify candidates for intensive lipid-lowering treatment\textsuperscript{17,18}.

It has been stated that the therapeutic modulation of LDL size and subgroups would provide significant benefits in the risk reduction of cardiovascular events\textsuperscript{13}. Since atherogenic dyslipidemia generally appears prior to clinical manifestations of MetS, treatment strategies have focused on the pharmacological approach, with reports that patients with atherogenic dyslipidemia may benefit from statins\textsuperscript{19}. Moreover, different statins have been described to have different effects on sd-LDL\textsuperscript{8}. The aims of the present study were to compare the outcomes of different statin treatments used to lower LDL-C in patients with MetS on sd-LDL, which is one of the most important components of dyslipidemia in these patients, and to test whether this can be regarded as a class effect. For this purpose, the effects of different statin treatments on sd-LDL were compared in 40 hypercholesterolemic patients with MetS. Despite a significant sd-LDL reduction in all groups after treatment, no significant difference was observed in inter-group comparisons. These results suggest that the sd-LDL-lowering effects of different statins in patients with MetS can be regarded as a class effect.

Novel statins with more potent LDL-C-lowering effects\textsuperscript{20} are known to demonstrate partial differences in their non-LDL effects compared to previous statins\textsuperscript{8}. Ai et al\textsuperscript{21} found, in 271 hyperlipidemic patients, that 6 weeks of maximal-dose rosvastatin (40 mg/day) and atorvastatin (80 mg/day) treatments provided significant and beneficial change throughout the lipoprotein particle spectrum, and that rosvastatin was significantly more effective in reducing sd-LDL levels than atorvastatin (−53% vs. −46%). In the present study, sd-LDL levels were reduced by 51.5% in the rosvastatin group, by 32.3% in the atorvastatin group, and by 44.1% in the other statins group. Although no significant differences were found among these groups, the absolute reduction in sd-LDL in the rosvastatin group (from 29.6 mg/dL to 8.9 mg/dL) was higher than in the other statins group (from 29.1 mg/dL to 14.7 mg/dL) and the atorvastatin group (from 26.2 mg/dL to 14.8 mg/dL). Differences between the two studies can be attributed to the discrepancy between the selected patient group and the presence of atherogenic dyslipidemia in MetS patients.

It has been reported that the sd-LDL-lowering effects of statin treatment may be due to the reduction of total-LDL levels rather than their effects on LDL size\textsuperscript{22,23}. Tokuno et al\textsuperscript{24} observed that 3 months of pitavastatin treatment in type-2 diabetes reduced sd-LDL by 26% and LDL by 25%, while micronized phenofibrate reduced sd-LDL by 23% without any effect on LDL. They reported that the statin-related decrease in sd-LDL was associated with the reduction in LDL-C and apolipoprotein B, while fibrate-related decrease was associated with the reduction in triglyceride levels. In the present study, a LDL-C decrease of approximately 45% in all 3 groups supports the hypothesis that the effects of statin treatment on sd-LDL are mainly through LDL-C-lowering.

It is well known that sd-LDL levels have an inverse relationship with HDL-C and a direct relationship with triglyceride, which is known to be related to the MetS-associated cardiovascular risk increase\textsuperscript{7}. In the present study, the positive correlation between sd-LDL and triglyceride levels and the negative correlation between sd-LDL and HDL-C was in accordance with this fact.

Despite being regarded as a significant cardiovascular risk factor by ATPIII, sd-LDL appears prior to clinical manifestations of MetS, treat-
cular risk factor, sd-LDL cannot be routinely measured in every laboratory. Easier measurement methods have been reported to accurately determine LDL size. For instance, the triglyceride/HDL-C ratio, which can be measured by an inexpensive and simple method, has been stated to be a marker for LDL size. In the present study, the significant correlation between triglyceride/HDL-C ratio and the sd-LDL supports this hypothesis.

The restricted number of patients and the relatively short follow-up duration were the limitations of this study; therefore, it seems very difficult to rule out the possibility that the lack of statistical significance among 3 groups was simply because the sample size was too small. Another drawback of our study was that the relation between sd-LDL and insulin resistance, an important element contributing to induction of sd-LDL, was not evaluated. In this study, the reduction in sd-LDL may be related to the decrease in insulin resistance; however, insulin resistance was not assessed. Therefore, we are unable to draw conclusions regarding the possible effects of statins on insulin resistance. Although the significant decrease in fasting blood glucose compared to baseline in the rosuvastatin group may indicate a decrease in insulin resistance, various factors modifying the level of blood glucose control may also play a role; therefore, the significant decrease in fasting blood glucose in the rosuvastatin group does not necessarily indicate that the drug decreases insulin resistance, causing a further reduction in sd-LDL. The triglyceride/HDL-C ratio, a potential indicator of insulin resistance, was significantly lower in the rosuvastatin group, which may be an effect of rosuvastatin on lipid parameters or may be the result of an insulin resistance-decreasing effect. Firmer conclusions can only be drawn from the results of a study specifically designed to assess insulin resistance.

In conclusion, the present study demonstrated that 8 weeks of statin treatment in hypercholesterolemic patients with MetS significantly reduces serum sd-LDL levels. This effect was similar for all statins used and can be considered a class effect.

References
4) Gardner CD, Fortmann SP, Krauss RM: Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA, 1996; 276: 875-881
sity lipoprotein cholesterol is a useful marker of metabolic syndrome in patients with coronary artery disease. J Atheroscler Thromb, 2007; 14: 202-207