Lovastatin for Reduction of Leptin in Nondialysis Patients With Type 2 Diabetic Nephropathy

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Introduction. Diabetic Nephropathy (DN) is one of the main complications of diabetes mellitus, mostly ending to end-stage renal disease. Leptin and C-reactive protein (CRP), as inflammatory markers implicated in the progression of DN, increase in diabetes mellitus, while transferrin and albumin, as members of anti-oxidant defense mechanism, are found to decline.

Materials and Methods. In a controlled clinical trial, 65 patients with type 2 DN were assigned to receive lovastatin or placebo, for 3 months, to assess statins’ impact on serum levels of leptin, CRP, transferrin, albumin, and lipid profile.

Results. Serum levels of CRP (3.52 ± 4.16 mg/dL to 2.84 ± 3.06 mg/dL, P = .02), leptin (10.78 ± 8.30 mg/dL to 7.80 ± 5.41 mg/dL, P = .006), low-density lipoprotein cholesterol (116.16 ± 46.54 mg/dL to 85.46 ± 29.22 mg/dL, P < .001), and total cholesterol (199.00 ± 43.33 mg/dL to 164.67 ± 35.19 mg/dL, P < .001) were lowered after lovastatin therapy. Mean serum level of high-density lipoprotein cholesterol increased (40.00 mg/dL to 42.80 mg/dL, P = .005) after the treatment. Lovastatin had no significant effect on albumin and transferrin. Placebo did not change any of the parameters after 3 months.

Conclusions. The effect of statins on the inflammatory markers involved in the development of DN is a new approach to evidence supporting the pleiotropic effect of this drug group.

INTRODUCTION

Diabetic nephropathy (DN) is one of the main complications of diabetes mellitus, especially type 2. Advanced stages of the disease can be a substantial underlying factor for glomerulosclerosis and end-stage renal dysfunction.1,2 Leptin, derived from adipose tissue, is a known mediator in the development of DN. This 167-amino acid protein plays a striking role in body energy homeostasis, regulation of immune function, hematopoiesis, angiogenesis, and bone development.3 Leptin level is likely increased in patients with type 2 diabetes mellitus and correlates positively with insulin resistance.4,5 In an etiologic survey, leptin seemed to induce overexpression of tumor growth factor-β (TGF-β). Rising of TGF-β is associated with collagen type IV augmentation and results in proteinuria, which is the first step to diabetic glomerulopathy. Endothelial cell proliferation and glomerular extracellular accumulation (mesangial expansion) are other pathologic changes found in the later stages of diabetic glomerulopathy, caused...
Diabetes mellitus, like many other diseases such as infectious diseases and cancer, goes with elevated inflammatory factors and decreased antioxidant defense mechanism. Inflammatory status in diabetes accompanies with the decline of anti-inflammatory biomarkers incorporated in antioxidant defense system (albumin and transferrin) and an increase in C-reactive protein (CRP) as a marker of inflammation.

Dyslipidemia, as a known predisposing factor in deterioration of kidney function and cardiovascular disorders in patients with type 2 diabetes mellitus, is of great concern. Statins are found to be effective contributors in improvement of lipid parameters, eg, lowering low-density lipoprotein cholesterol (LDLC) and triglyceride, and raising high-density lipoprotein cholesterol (HDLC). Additionally, statin therapy can be effective in reducing inflammation and glomerular filtration dysfunction in patients with chronic kidney disease. Recent studies manifested that statins, through non-lipid-lowering property (pleiotropic effect), decreased CRP and increased transferrin and albumin as a contributor to anti-oxidant defense mechanism in patients with diabetes mellitus.

To our knowledge there has not yet been any study designed focusing on the impact of statins on leptin levels in patients with type 2 DN. As far as high leptin level is responsible for the degree of inflammation and glomerular dysfunction, statins, through their non-lipid-lowering property, could effectuate leptin levels in DN. Moreover, the effect of statins on transferrin and albumin levels engenders a new approach to inflammation in patients with DN. Hence, we were incited to assess the effect of lovastatin on leptin, transferrin, albumin, CRP, and lipid profile in patients with DN.

MATERIALS AND METHODS
A single-blinded, parallel group, placebo-controlled clinical trial was carried out to assess the effect of 3 months of treatment with lovastatin, 20 mg/dL, daily, on the plasma levels of leptin, CRP, transferrin, albumin, fasting blood glucose, urea, creatinine, estimated glomerular filtration rate (GFR), and lipid profile (triglyceride, total cholesterol, LDLC, and HDLC), along with 24-hour urine volume, 24-hour urine protein, and 24-hour urine creatinine in patients with DN. Sixty-five patients with type 2 DN (GFR, 30 mL/min to 60 mL/min, calculated by the Cockcroft-Gault formula; proteinuria, > 500 mg/d) were recruited. The study population included men with DN, aged between 25 and 60 years. Patients with the following conditions were excluded: smoking habits, extreme physical activity (> 3 hours exercise per week), uncontrolled cardiovascular (diagnosed by symptoms and electrocardiogram) or liver disease (abnormal liver function tests), history of diabetic complications (such as ketoacidosis, hyperosmolar coma, and diabetic foot), poorly controlled type 2 diabetes (hemoglobin A1c > 7.5%), acute myocardial infarction, malnutrition, acute or chronic infection (diabetic foot, hepatitis, etc), hospitalization in the past 6 months, and nonadherence to regular life style (such as regular diet, drugs, and exercise). Women were not enrolled for inflammatory acute-phase markers are altered by female sexual hormones.

The study was carried out from February 2006 to March 2008. The ethics committee of Tabriz University of Medical Sciences approved the study protocol and all patients gave written informed consent.

Patients with hypertension were treated with 50 mg/d to 150 mg/d of captopril or 10 mg/d to 40 mg/d of enalapril during the study. Blood pressure was controlled to be lower than 130/90 mm Hg and the blood glucose was kept between 70 mg/dL and 170 mg/dL by oral hypoglycemic agents or insulin injection therapy. Thirty patients were assigned to receive 20 mg/dL/d of lovastatin (Hakim Pharma, Tehran, Iran) and 35 patients received placebo. Peripheral blood samples were drawn after an overnight fasting and stored at -70°C, before and after 3 months of treatment with lovastatin or placebo. Twenty-four-hour urine was collected and assessed for volume, protein, and creatinine at the beginning and end of the study.

Serum levels of total cholesterol, triglyceride, and HDLC were quantified with an automated chemical analyzer (Abbott analyzer, Abbott Laboratories, Abbott Park, Chicago, Illinois, USA). Serum LDLC was calculated by the Friedewald equation. An ultrasensitive solid phase enzyme-linked immunosorbent assay (DRG instruments GmbH, Marburg, Germany; Lot No, RN-28874) was used to determine serum CRP. Transferrin and leptin levels were measured by enzyme-linked immunosorbent assay (ELA-Vendor, batch...
No, RD-1505).

The results were expressed as mean ± standard deviation, and statistical analysis was performed using the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA). The differences in quantitative variables were tested with paired samples $t$ test and correlations with the Pearson correlation test. $P$ values less than .05 were assumed significant.

RESULTS

Demographic characteristics for both groups are presented in Table 1. Meaningful reduction in serum levels of CRP (3.52 ± 4.16 mg/dL to 2.84 ± 3.06 mg/dL, $P = .02$), leptin (10.78 ± 8.30 mg/dL to 7.80 ± 5.41 mg/dL, $P = .006$), LDL C (116.16 ± 46.54 mg/dL to 85.46 ± 29.22 mg/dL, $P < .001$), and total cholesterol (199.00 ± 43.33 mg/dL to 164.67 ± 35.19 mg/dL, $P < .001$) were noticeable after 3 months of treatment with lovastatin; nevertheless, the mean level of HDL C increased prominently (40.00 ± 5.30 mg/dL to 42.80 ± 5.15 mg/dL, $P = .005$). Plasma concentrations of transferrin, albumin, blood urea nitrogen, creatinine, and triglyceride, as well as 24-hour urine volume and 24-hour urine protein-creatinine ratio were not markedly influenced by lovastatin (Table 2). Placebo did not change any of the parameters after 3 months.

Quantitative serum level of CRP before drug administration had a significant positive correlation with leptin serum levels ($r = 0.465$, $P = .01$) and a significant negative correlation with transferrin serum levels ($r = -0.361$, $P = .05$) in the lovastatin group (Figure). The positive correlation between serum CRP and leptin levels remained significant after administration of lovastatin ($r = 0.391$, $P = .03$). The control group demonstrated a positive correlation between CRP and leptin, both

<table>
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<tr>
<th>Characteristic</th>
<th>Lovastatin Group</th>
<th>Placebo Group</th>
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<tbody>
<tr>
<td>Mean age, y</td>
<td>54.47 ± 6.11</td>
<td>50.53 ± 8.25</td>
</tr>
<tr>
<td>Mean body mass index, kg/m²</td>
<td>25.55</td>
<td>25.72</td>
</tr>
<tr>
<td>Mean systolic blood pressure, mm Hg</td>
<td>139.72</td>
<td>137.11</td>
</tr>
<tr>
<td>Mean diastolic blood pressure, mm Hg</td>
<td>76.00</td>
<td>73.46</td>
</tr>
<tr>
<td>Mean duration of diabetes, y</td>
<td>9.77 ± 3.11</td>
<td>8.77 ± 3.46</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>13 (43.3)</td>
<td>12 (40.0)</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of Participants in Lovastatin and Placebo Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lovastatin Group</th>
<th>Placebo Group</th>
</tr>
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<tbody>
<tr>
<td>Plasma</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.41</td>
<td>4.47</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>175.3</td>
<td>152.1</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>199.0†</td>
<td>164.7†</td>
</tr>
<tr>
<td>LDL C, mg/dL</td>
<td>116.2†</td>
<td>85.5†</td>
</tr>
<tr>
<td>HDL C, mg/dL</td>
<td>40.0†</td>
<td>42.8†</td>
</tr>
<tr>
<td>Urea, mg/dL</td>
<td>55.2</td>
<td>51.8</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.66</td>
<td>1.61</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>5.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>157.83</td>
<td>151.53</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>3.52†</td>
<td>2.84†</td>
</tr>
<tr>
<td>Transferrin, mg/dL</td>
<td>241.26</td>
<td>227.47</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>10.78†</td>
<td>7.80†</td>
</tr>
<tr>
<td>GFR, mL/min/1.73 m²</td>
<td>54.78</td>
<td>54.57</td>
</tr>
</tbody>
</table>

| Parameter                     | Before           | After         | Before           | After          |
|-------------------------------|------------------|---------------|
| Plasma                        | Before           | After         | Before           | After          |
| 24-hour volume, ml/d          | 2138             | 2229          | 2217             | 2260           |
| 24-hour protein, mg/d         | 681              | 618           | 675              | 683            |
| 24-hour creatinine, mg/d      | 1218             | 1239          | 1246             | 1297           |

Table 2. Mean Levels of Recorded Parameters in Lovastatin and Placebo Groups*

*LDL C indicates low-density lipoprotein cholesterol; HDL C, high-density lipoprotein cholesterol; GFR, glomerular filtration rate; and CRP, C-reactive protein.

†$P < .01$
before \((r = 0.468, P = .005)\) and after \((r = 0.416, P = .01)\) placebo administration and no significant correlation between CRP and transferrin either before or after taking placebo. No significant correlation was found between leptin and transferrin serum levels in either group.

**DISCUSSION**

Statins are the first-line lipid-lowering therapy in diabetic patients. They reduce lipid production by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.\(^24,25\) Recent studies investigated the anti-inflammatory aspect of this drug group beyond its lipid lowering property,\(^19,26\) though no trial has reported the effect of statins on leptin, transferrin, albumin, and CRP in patients with type 2 DN. This study showed that a 3-month administration of lovastatin significantly reduced serum levels of total cholesterol, LDL, and leptin and raised serum levels of HDL in patients with DN. Recent data demonstrated that statins lower total cholesterol, LDL, and triglyceride and significantly increase HDL levels.\(^14,15,27,28\) Nevertheless, in our study, no significant change in triglyceride levels was seen.

Statins can inhibit mesangial cell proliferation through influencing LDL, oxidized LDL, high levels of glucose, and many other factors mediating the progression of kidney disease.\(^1,29\) Available studies demonstrate the role of leptin in glomerulosclerosis and development of kidney damage in diabetic patients. The potential impact of leptin on renal pathophysiology reveals that high leptin level induces endothelial cell proliferation, TGF-\(\beta_1\), and collagen type IV overproduction and mesangial overexpansion. These changes discriminate leptin as a causal factor for development of DN.\(^3,5-7,30\) However, leptin level rise assuages glomerular injury in at least certain types of DN.\(^3,5-7,31\) The effect of statins on leptin levels seems to be ambiguous and needs further research. In the study by von Eynatten and colleagues, atorvastatin, 40 mg/d, did reduce leptin levels in type 2 diabetic patients,\(^32\) while Chu and colleagues showed that atorvastatin (10 mg/d, 20 mg/d, and 40 mg/d) did not affect leptin levels in hyperlipidemic type 2 diabetes mellitus.\(^33\) Gannagé-Yared and colleagues found that a 12-week treatment with pravastatin, 40 mg/d, does not change leptin and adiponectin levels in healthy volunteers.\(^34\) The result by Gouni-Berthold and colleagues also demonstrated that simvastatin therapy, 40 mg/d, did not change leptin levels in healthy men.\(^35\) Statin treatment significantly decreased plasma lipids and leptin levels in coronary heart disease patients.\(^26\) In a study on
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hypercholesterolemic rabbits, atorvastatin, 2.5 mg/kg/d, decreased serum leptin levels by reducing leptin mRNA expression.  

Here in this study, leptin levels were significantly decreased in patients with type 2 DN. Meanwhile, all of our patients received angiotensin-converting enzyme inhibitors as an antihypertensive or antiproteinuric agent during the study. Angiotensin II, like leptin, promotes fibroses in diabetic kidneys by decreasing TGF-β.  

Treatment by angiotensin-converting enzyme inhibitors may have a similar effect as statin on reducing leptin level.  

Inflammatory biomarkers are associated risk factors participating in the pathophysiology of morbidity and mortality in diabetic patients.  

Diabetes mellitus, as an inflammatory condition, is associated with decreased serum levels of negative acute-phase proteins (eg, transferrin and albumin) and increased positive acute-phase proteins (eg, CRP and leptin).  

Transferrin, as a prototype of negative acute-phase protein, was correlated negatively with CRP, as the prototype of positive acute phase protein, before lovastatin therapy. Statin therapy through its pleiotropic character can decline CRP and improve serum albumin level though lovastatin made no statistically significant changes in transferrin and albumin in our study. El Haggan and colleagues found positive correlation between CRP and leptin in early phase of post kidney transplantation. We found the same relationship between leptin and CRP pretreatment and posttreatment with lovastatin.  

Limitations of our study point to the need for further research. In particular, it would be beneficial to carry out similar studies with larger patient populations, administration of higher doses of statins, longer durations of therapy, and inclusion of both men and women. Given the menstrual cycle in females causing changes in blood levels of a number of inflammatory factors including c reactive protein, in this study we only recruited the male population. Since we have the evident effect of lovastatin on leptin in the men population, one can include the women in future studies to expand the understanding of this effect.  

CONCLUSIONS  
The effect of lovastatin on reducing leptin as a proinflammatory factor in pathogenesis of DN favors the so called “pleiotropic effect” of statins. Positive correlation of CRP with leptin and its negative correlation with transferrin confirm leptin as an inflammatory and transferrin as a negative inflammatory marker. The impact of statins on serum albumin level and transferrin needs further research and is still drawn in uncertainty.  

CONFLICT OF INTEREST  
None declared.

REFERENCES


