Long-Term Treatment With EPO Increases Serum Levels of High-Density Lipoprotein in Patients With CKD

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• **Background:** Among lipid abnormalities observed in patients with chronic kidney disease (CKD) is a significant decrease in serum high-density lipoprotein cholesterol (HDL-C) levels. In a previously published randomized control trial, we showed that early erythropoietin (EPO) administration in a predialysis population slowed the progression of CKD. In the present nested substudy, we examine whether EPO has an influence on serum HDL-C levels in comparison to other lipid parameters in this population. **Methods:** Eighty-eight patients with CKD stages 3 and 4 were enrolled in the study. Forty-five patients (group 1) were treated with EPO (50 U/kg/wk), targeting to increase hemoglobin levels to 13 g/dL or greater (>130 g/L). The other patients (group 2) remained without treatment until hemoglobin levels decreased to less than 9 g/dL (<90 g/L). The duration of the study was 12 months. **Results:** At the end of the study, we observed a statistically significant decrease in serum levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides in both groups. However, serum HDL-C levels significantly increased in only group 1 (from 42.5 ± 10.4 to 55.9 ± 8.1 mg/dL [1.10 ± 0.27 to 1.45 ± 0.21 mmol/L]; P < 0.001), whereas they were unchanged in group 2. In addition, a significant decrease in atherogenic LDL-C/HDL-C ratio was observed in only group 1. Importantly, the increase in serum HDL-C levels correlated positively with the increase in hemoglobin values in EPO-treated patients. **Conclusion:** Our results show that EPO treatment of predialysis patients with CKD significantly increases serum HDL-C levels, which may represent an important antiatherogenic effect of this hormone. *Am J Kidney Dis* 48:242-249.

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INDEX WORDS: Renal anemia; erythropoietin (EPO); lipids; chronic kidney disease (CKD).
is influenced significantly by food intake. Although the biochemical mechanisms for these lipid alterations induced by EPO treatment have not been explored adequately, there is evidence that these alterations could be attributed at least in part to the increase in plasma levels of lipoprotein lipase and hepatic TG lipase induced by this hormone. Regarding serum HDL-C levels in patients with CKD and the possible effect of EPO, several studies were conducted mainly in dialysis patients, whereas only 1 study concerned a predialysis population. These studies provided inconsistent results.

The importance of serum HDL-C levels in patients with cardiovascular disease was addressed by various studies. Data provided during the last years showed that decreased HDL-C level may be a risk factor for cardiovascular disease similar in importance to increased LDL-C level. Although the mechanism by which HDL-C reduces the development of atherosclerosis has not been defined with certainty, acceleration of reverse cholesterol transport, as well as antioxidant and anti-inflammatory activities, are likely to have central roles. Thus, interventions that increase HDL-C levels are antiatherosclerotic. Cholesterol ester transfer protein (CETP) has a central role in HDL-C metabolism and regulation of HDL-C levels in serum. CETP exchanges cholesterol esters with TG between HDL-C and apolipoprotein B-containing lipoproteins; thus, it significantly contributes to the reverse cholesterol transport pathway. High levels of CETP activity lead to a decrease in HDL-C levels and an atherogenic lipoprotein profile.

In the present nested substudy performed in the framework of our previously published prospective randomized study, we evaluate the effects of long-term EPO treatment on HDL-C levels in comparison to other lipid parameters in patients with CKD stages 3 and 4.

METHODS

Predialysis patients with renal impairment from any cause other than diabetes mellitus with screening serum creatinine values of 2.0 to 6.0 mg/dL (177 to 530 μmol/L) and hemoglobin levels of 9.0 to 11.6 g/dL (90 to 116 g/L) were eligible for the study. Eighty-eight patients with CKD (38 women, 50 men) with a mean age of 65.5 years (range, 40 to 81 years) were enrolled in the study across 14 participating hospitals in Greece between November 2000 and June 2002. Details on eligibility criteria were reported in our previously published prospective randomized study. The majority of patients had a history of hypertension, whereas 10 patients had biopsy-documented glomerulonephritis, 3 patients had polycystic disease, and 3 patients had obstructive nephropathy caused by nephrolithiasis. The remaining patients had a clinical history of primary chronic glomerulonephritis.

Among the exclusion criteria were the presence of an easily correctable cause of anemia; transfusion dependency; the presence of systemic diseases, infections, or clinical inflammatory conditions; uncontrollable hypertension; proteinuria with protein greater than 2 g/24 h; serum albumin level less than 3.5 g/dL (<35 g/L); hepatic insufficiency; active hepatitis; chronic alcoholism; congestive heart failure; severe obesity; history of seizures or thrombotic episodes; known hypersensitivity to EPO alfa; and use of EPO in the previous 6 months. We should emphasize that patients were not being administered drugs that affect plasma lipid levels (androgens, lipid-lowering drugs, cortisol, diuretics others than furosemide, and aluminum hydroxide or sevelamer hydrochloride [as phosphate binders]), and they did not have disorders that affect lipid metabolism (diabetes mellitus, hypothyroidism).

Monitoring of blood pressure and treatment of hypertension were performed according to established standards aimed at blood pressure levels less than 130/85 mm Hg by using restricted salt intake and appropriate drug therapy, not including angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, to avoid potential EPO resistance. Use of β-blockers in small doses for purposes other than antihypertensives was permitted. Protein intake generally was restricted to less than 1 g/kg/d based on appropriate dietary instructions given to patients. No extra-low-protein diets were used in the study, whereas obese patients were advised to lose weight. The duration of this substudy was 12 months. Forty-five patients (group 1) were administered EPO subcutaneously (EPO alfa; Eprex; Janssen-Cilag Pharmaceutical, Athens, Greece) in dose of 50 U/kg body weight once per week, whereas 43 patients (group 2) were not treated with EPO until hemoglobin levels decreased to less than 9 g/dL (<90 g/L). Further details of the EPO treatment protocol are described in the previous published study. Patients were randomly assigned by using a computerized sequence kept at the coordinating center at the University of Ioannina. After a patient consented to randomization, it was performed centrally.

Ethical committees of the hospitals approved the study, and all patients gave informed consent for the investigation.

Laboratory Measurements

We assessed lipid profiles at baseline and after 2, 4, 6, 9, and 12 months by determination of total cholesterol, TG, HDL-C, LDL-C, and lipoprotein(a) (Lp[a]) levels. In all participants, blood samples were obtained after a 14-hour overnight fast. Plasma and serum were prepared and stored at −80°C for analysis of laboratory parameters.

Concentrations of total cholesterol, HDL-C, and TG were determined automatically on the Olympus AU 600 Clinical Chemistry Analyzer (Olympus Diagnostica, Hamburg Germany). LDL-C was calculated by using the Friedewald formula. Lp(a) was quantified by means of an enzyme immunoassay (sandwich enzyme-linked immunosorbent as-
Laboratory parameters

Forward approach. Comparisons of discrete and continuous or missed appointment, we used the last-observation-carried-the case of missing values because of death, loss to follow-up, between them was analyzed by using Pearson correlation. In test for association between parameters. The relationship of variance adjusting for baseline measurements. Univariate subgroups defined by baseline parameters. We used analysis assigned. We assessed whether results were different in

465 nm and emission wavelength of 535 nm.21 fluorescence spectrometer at an excitation wavelength of

hour of incubation at 37°C, CETP activity was determined EDTA, pH 7.4) were used as the source of CETP. After 1 plasma diluted 1:1 with sample buffer (10 mmol/L of Tris-HCl, 150 mmol/L of sodium chloride, and 2 mmol/L of EDTA, pH 7.4) were used as the source of CETP. After 1 hour of incubation at 37°C, CETP activity was determined by the increase in fluorescence intensity measured in a fluorescence spectrometer at an excitation wavelength of 465 nm and emission wavelength of 535 nm.21

say; Macra Lp[a]; Terumo Medical Corp Diagnostic Division, Elkton, MD).

At the mentioned times, hematocrit, hemoglobin, serum creatinine, and serum albumin also were measured. Creatinine clearance was determined by using the Cockcroft-Gault formula, whereas 24-hour urine was collected for determination of proteinuria.

CETP activity also was determined in plasma samples of all patients at baseline, as well as after 6 and 12 months, by means of a fluorometric assay using a commercial kit by Roar Biomedical Inc (New York, NY). Two microliters of plasma diluted 1:1 with sample buffer (10 mmol/L of Tris-HCl, 150 mmol/L of sodium chloride, and 2 mmol/L of EDTA, pH 7.4) were used as the source of CETP. After 1 hour of incubation at 37°C, CETP activity was determined by the increase in fluorescence intensity measured in a fluorescence spectrometer at an excitation wavelength of 465 nm and emission wavelength of 535 nm.21

Statistical Analysis

All analyses are based on intention to treat, with patients analyzed in the arm to which they originally were randomly assigned. We assessed whether results were different in subgroups defined by baseline parameters. We used analysis of variance adjusting for baseline measurements. Univariate and multivariate linear regression models also were used to test for association between parameters. The relationship between them was analyzed by using Pearson correlation. In the case of missing values because of death, loss to follow-up, or missed appointment, we used the last-observation-carried-forward approach. Comparisons of discrete and continuous variables between groups were performed by using chi-square test with continuity correction and t-test, respectively. Analyses were conducted using SPSS, version 11.5 (SPSS Inc, Chicago, IL). All P are 2 tailed.

RESULTS

Demographic characteristics and laboratory parameters of the study population are listed in Table 1. There were no differences between the 2 patient groups in demographic characteristics and laboratory parameters.

As expected, after 12 months of follow-up, a statistically significant increase in hematocrit (from 30.8% ± 1.4% to 38.4% ± 1.5%; P < 0.001) and hemoglobin levels (from 10.1 ± 0.5 to 12.9 ± 0.4 g/dL [101 ± 5 to 129 ± 4 g/L]; P < 0.001) was observed in patients in group 1 treated with EPO, whereas these parameters were not altered during follow-up in patients in group 2. Decreases in renal function after 12 months of follow-up were greater in untreated patients than in those treated with EPO (Table 2). Decreases in blood pressure was well controlled in most patients in both groups.
The most important observation of the present study is that in patients with CKD treated with EPO, serum HDL-C levels progressively increased to 55.9 ± 8.1 mg/dL (1.45 ± 0.21 mmol/L; P < 0.001) 12 months after the initiation of EPO therapy, a phenomenon not observed in EPO-untreated patients in group 2 (Fig 1; Table 3). The difference in HDL-C levels between the 2 groups after 12 months of follow-up was statistically significant (P < 0.001). Univariate analysis showed that total weekly EPO doses at 6 and 12 months were not an important factor for the increase in HDL-C levels. Conversely, multivariate analysis showed that hemoglobin levels were a strong independent factor for HDL-C levels (P = 0.04), and there was a significant positive correlation between these parameters at 12 months (r = 0.417; P = 0.02; Fig 2).

A significant decrease in serum total cholesterol, TG, and LDL-C levels was observed during follow-up in both patient groups (Table 3). The increase in HDL-C levels observed in patients in group 1 during follow-up had as a consequence a progressive significant decrease in atherogenic LDL-C/HDL-C ratio, a phenomenon not observed in patients in group 2 (Table 3). Thus, 12 months after the initiation of the study, this ratio was significantly lower in EPO-treated patients compared with patients in group 2 (2.31 ± 0.28 versus 3.21 ± 0.93; P = 0.0001).

In an effort to investigate the mechanism for the increase in HDL-C levels induced by EPO, we determined the activity of CETP, the protein that has a critical role in reverse cholesterol transport and significantly influences serum HDL-C levels. As listed in Table 3, CETP activity did not change during follow-up in EPO-treated or EPO-untreated patients with CKD.

**DISCUSSION**

The most important observation of the present study is that long-term EPO treatment in predialysis patients leads to an increase in serum HDL-C levels, which correlates positively with the EPO-induced increase in hemoglobin values. The elevation in serum HDL-C levels has as a consequence the significant improvement in atherogenic LDL-C/HDL-C ratio. Furthermore, during follow-up, a significant decrease in serum total cholesterol, LDL-C, and TG levels was observed in both patient groups; thus, this finding seems to be unrelated to EPO treatment.

**Table 2. Renal Function Parameter Measurements in Both Groups at Baseline and After 12 Months**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After 12 Months</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>3.27 ± 0.99</td>
<td>3.81 ± 1.43</td>
<td>Not significant</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>25.7 ± 9.1</td>
<td>21.9 ± 9.4</td>
<td>Not significant</td>
</tr>
<tr>
<td>Urinary protein (g/24 h)</td>
<td>0.66 ± 0.39</td>
<td>0.62 ± 0.48</td>
<td>Not significant</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>3.39 ± 0.82</td>
<td>5.07 ± 2.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>22.3 ± 6.0</td>
<td>16.1 ± 6.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urinary protein (g/24 h)</td>
<td>0.57 ± 0.36</td>
<td>0.55 ± 0.30</td>
<td>Not significant</td>
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</tbody>
</table>

NOTE. To convert serum creatinine in mg/dL to μmol/L, multiply by 88.4; creatinine clearance in mL/min to mL/s, multiply by 0.01667.

**Fig 1.** Bar graph shows serum HDL-C levels in patients with CKD during follow-up. To convert HDL-C in mg/dL to mmol/L, multiply by 0.02586.
In an effort to investigate the mechanism by which the HDL-C levels increase in patients treated with EPO, we determined plasma CETP activity in our patients with CKD. CETP has an important role in reverse cholesterol transport and significantly influences serum HDL-C levels. CETP exchanges cholesterol esters with TG between HDL-C and apolipoprotein B–containing lipoproteins, thus leading to a decrease in serum HDL-C levels.30 Increased CETP activity is associated with low serum HDL-C levels, as well as with an overall atherogenic profile.31 Because HDL-C levels are related inversely to coronary artery disease risk, it is hypothesized that inhibition of CETP activity would lead to an increase in serum HDL-C levels.32,33

Based on this assumption, several inhibitors of CETP currently are under investigation in large-scale clinical studies.20 According to our results, baseline CETP activity in patients with CKD was not influenced by EPO treatment. Consequently, CETP may not be involved in the increase in HDL-C levels observed in patients treated with EPO. Another mechanism that leads to increased HDL-C levels is enhanced hydrolysis of TG-rich lipoproteins that is associated with the decrease in serum TG levels.34 However, it is unlikely that this mechanism could account for the increase in HDL-C levels observed in the present study because, according to our results, TG levels decreased significantly.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>232.5 ± 32.8</td>
<td>230.4 ± 36.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>148.4 ± 40.9</td>
<td>152.4 ± 47.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>42.5 ± 10.4</td>
<td>44.9 ± 9.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>160.2 ± 32.5</td>
<td>155.4 ± 32.7</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>4.05 ± 1.52</td>
<td>3.61 ± 1.08</td>
<td>Not significant</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>8.05</td>
<td>9.02</td>
<td>Not significant</td>
</tr>
<tr>
<td>CETP (nmol/mL/h)</td>
<td>19.9 ± 7.5</td>
<td>20.3 ± 6.9</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

NOTE. To convert serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin and hemoglobin in g/dL to g/L, multiply by 10; total cholesterol, HDL-C, and LDL-C in mg/dL to mmol/L, multiply by 0.02586; TG in mg/dL to mmol/L, multiply by 0.01229; creatinine clearance in mL/min to mL/s, multiply by 0.01667.

*Between baseline and after 12 months.
†P = 0.0001 compared with the respective values of group 2.

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Because HDL-C levels are related inversely to coronary artery disease risk, it is hypothesized that inhibition of CETP activity would lead to an increase in serum HDL-C levels and thus to a decrease in risk for coronary artery disease.32,33

Based on this assumption, several inhibitors of CETP currently are under investigation in large-scale clinical studies.20 According to our results, baseline CETP activity in patients with CKD was not influenced by EPO treatment. Consequently, CETP may not be involved in the increase in HDL-C levels observed in patients treated with EPO. Another mechanism that leads to increased HDL-C levels is enhanced hydrolysis of TG-rich lipoproteins that is associated with the decrease in serum TG levels.34 However, it is unlikely that this mechanism could account for the increase in HDL-C levels observed in the present study because, according to our results, TG levels decreased significantly.

![Increase of HDL-chol levels, %](image1)

![Increase of Hb levels, %](image2)

**Fig 2.** Correlation between increase in hemoglobin (Hb) levels and increase in HDL-C levels in patients with CKD treated with EPO.
decreased equally in EPO-treated and untreated patients with CKD.

It is well known that inflammation has an important role in CKD.\textsuperscript{35} According to previously published results, such inflammatory mediators as lipopolysaccharide and cytokines inhibit cholesterol efflux from cells by decreasing expression of the adenosine triphosphate–binding cassette A1 (ABCA1) gene.\textsuperscript{36} This effect leads to the decrease in reverse cholesterol transport and consequently the decrease in serum HDL-C levels.\textsuperscript{37} Furthermore, it was suggested that EPO expresses anti-inflammatory activities.\textsuperscript{38} Thus, we may hypothesize that the elevation in HDL-C levels observed in our patients with CKD administered EPO could be attributed to the anti-inflammatory activities of EPO.\textsuperscript{38}

Alternatively, the increase in serum HDL-C levels could be attributed to the improvement in tissue oxygenation in patients with CKD administered EPO. This suggestion is supported by the positive correlation between the increase in serum HDL-C levels and the increase in hemoglobin values observed in EPO-treated patients. On a biochemical and pathophysiological basis of view, the increased tissue oxygenation may have as a consequence an increase in activity of ABCA1 or other enzymes involved in HDL maturation and lead to the increase in HDL-C levels, such as lecithin cholesterol acyl transferase. This hypothesis currently is under investigation in our laboratory. Furthermore, the EPO-induced improvement in tissue oxygenation also could increase the exercise capacity of our patients. Although physical activity of our patients was not estimated in the present study, we cannot exclude the possibility that it may have contributed to the increase in serum HDL-C levels observed in patients administered EPO. Several studies provided strong evidence that the increase in physical activity leads to increased HDL-C levels, and it currently is considered one of the most important nonpharmacological strategies recommended by clinical guidelines to increase serum HDL-C levels.\textsuperscript{39}

It is well established that HDL is a potent antiatherogenic lipoprotein. The cardioprotective effects of HDL have been attributed to its role in reverse cholesterol transport, its beneficial effects on endothelium, and its antioxidant and anti-inflammatory activities.\textsuperscript{39} Recent observational, biological, and clinical evidence strongly suggests that HDL is a promising target of therapeutic intervention. Consequently, the increase in serum HDL-C levels in patients with CKD administered EPO could be considered an important antiatherogenic effect of this hormone. Among HDL constituents that significantly contribute to the antioxidant and anti-inflammatory activities of this lipoprotein is platelet-activating factor acetylhydrolase.\textsuperscript{40} We recently showed that long-term therapy with EPO in patients with CKD stages 3 to 4 significantly increased the enzyme activity associated with HDL, a phenomenon primarily attributed to the EPO-induced enhancement of enzyme secretion from peripheral blood monocytes.\textsuperscript{34} Consequently, based on these results, we may suggest that long-term therapy with EPO in patients with CKD may be athero-protective in at least 2 ways, ie, by enhancing HDL-C serum levels and increasing activity of the HDL-associated platelet-activating factor acetylhydrolase.

In conclusion, the present study shows that EPO administration to patients with CKD significantly increases serum HDL-C levels and improves atherosclerotic LDL-C/HDL-C ratio. This beneficial effect of EPO administration on HDL-C levels may contribute to the decreased progression seen in individuals with CKD whose anemia was corrected with EPO therapy, a hypothesis that needs further investigation.

ACKNOWLEDGMENT

The authors thank Aleka Papageorgiou for skilled secretarial assistance.

APPENDIX

The following investigators participated in this multicenter study in Greece: University Hospital, Ioannina: K.C. Siamopoulos; General Hospital G. Hatzikosta, Ioannina: M. Pappas; Hippocrates Hospital, Thessaloniki: D. Memmos; Evangelismos Hospital, Athens: N. Nikolopoulos; Laikon General Hospital, Athens: C. Stathakis; General Hospital, Kavala: K. Kalaitzidis; Thriaseion Hospital, Athens: J. Malegos; General Hospital, Larissa: I. Stafanidis; Papageorgiou Hospital, Thessaloniki: G. Sakellariou; General Hospital, Volos: C. Sirganiis; General Hospital, Veria: D. Tsakiris; General Hospital, Edessa: N. Zoumbardis; Papanikolaou...
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