Atorvastatin Increases HDL Cholesterol by Reducing Cholesteryl Ester Transfer Protein

Caroline C. van der Hoogt\textsuperscript{1,2,*}, Willeke de Haan\textsuperscript{1,2,*}, Marit Westerterp\textsuperscript{1,2}, Menno Hoekstra\textsuperscript{4}, Geesje M. Dallinga-Thie\textsuperscript{5}, Hans M.G. Princen\textsuperscript{1}, Johannes A. Romijn\textsuperscript{2}, J. Wouter Jukema\textsuperscript{1,3}, Louis M. Havekes\textsuperscript{1,2,3}, Patrick C.N. Rensen\textsuperscript{1,2}

\textsuperscript{1}Netherlands Organization for Applied Scientific Research-Quality of Life, Gaubius Laboratory, P.O. Box 2215, 2301 CE Leiden, The Netherlands; Departments of \textsuperscript{2}General Internal Medicine, Endocrinology, and Metabolic Diseases, and \textsuperscript{3}Cardiology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands; \textsuperscript{4}Leiden/Amsterdam Center for Drug Research, Div. Biopharmaceutics, P.O. box 9502, 2300 RA Leiden, The Netherlands; \textsuperscript{5}Department of Internal Medicine, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

\textsuperscript{*}These authors contributed equally

\textit{Manuscript in preparation}
**Objective** - In addition to lowering low-density lipoprotein (LDL)-cholesterol, statins modestly increase high-density lipoprotein (HDL)-cholesterol in humans. This increase is not seen in mice, a species without cholesteryl ester transfer protein (CETP) expression. Therefore, our aim was to determine whether the increase in HDL depends on CETP expression.

**Methods and Results** - *APOE*^*3-Leiden* (*E3L*) mice, with a human-like lipoprotein profile and a human-like responsiveness to statin treatment, were crossbred with CETP transgenic mice. Whereas atorvastatin-treatment (0.01% in diet) reduced VLDL-cholesterol in both *E3L* and CETP.*E3L* mice (by >80%), HDL-cholesterol increased only in CETP.*E3L* mice (+52%). Atorvastatin down-regulated hepatic CETP expression in CETP.*E3L* mice (-57%; *P*<0.01), and reduced plasma CETP mass (-45%; *P*<0.05) and activity (-57%; *P*<0.01), the latter two when adjusted for HDL-cholesterol. Hepatic expression levels of genes involved in HDL metabolism, such as *Pltp, Abca1, Sr-b1,* and *Apoa1,* were not differently affected by atorvastatin as compared to those in *E3L* mice. Finally, a dose escalation study showed that atorvastatin decreased plasma CETP mass and activity, and increased HDL-cholesterol in a dose-dependent manner.

**Conclusion** - Atorvastatin increases HDL-cholesterol in CETP.*E3L* mice by reducing the CETP-dependent transfer of HDL-cholesterol to (V)LDL, as related to reduced hepatic CETP expression and a reduced plasma (V)LDL pool.
Epidemiological studies have established that a high level of low-density lipoprotein (LDL)-cholesterol is a major cardiovascular risk factor.\textsuperscript{1} In the past decades, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (i.e. statins) have been successfully used to reduce LDL-cholesterol. Statins inhibit this rate-determining enzyme of cholesterol synthesis, which results in hepatic depletion of cholesterol.\textsuperscript{2-3} As a consequence, VLDL production is reduced and the hepatic expression of the LDL receptor (LDLR) is upregulated, leading to decreased plasma cholesterol levels in apoB-containing lipoproteins (i.e. VLDL and LDL).\textsuperscript{4,5} Indeed, a meta-analysis of 25 studies indicated that statins reduce LDL-cholesterol levels by 20-40%.\textsuperscript{6} In addition, statins elevate high-density lipoprotein (HDL)-cholesterol levels by typically 5-15%.\textsuperscript{7-9}

Low HDL-cholesterol has been confirmed as a strong and independent risk factor for cardiovascular disease in a meta-analysis of four prospective studies. An increase in HDL-cholesterol of 1 mg/dl resulted in a 2-3\% decrease in cardiovascular risk.\textsuperscript{10} One of the key players in HDL-metabolism is cholesteryl ester transfer protein (CETP), a hydrophobic plasma glycoprotein. CETP transfers neutral lipids (e.g. triglycerides [TG] and cholesteryl esters [CE]) between lipoproteins, resulting in the net flux of CE from HDL towards apoB-containing lipoproteins in exchange for TG.\textsuperscript{11,12} Accordingly, CETP-deficient subjects display increased HDL-cholesterol levels\textsuperscript{13} and also inhibition of CETP activity by small-molecule inhibitors leads to increased HDL-cholesterol levels.\textsuperscript{14-17}

Treatment of patients with combined hyperlipidemia with atorvastatin resulted in increased levels of relatively CE-rich large HDL\textsubscript{2a} with a concomitant decrease in CE-poor small HDL\textsubscript{3c}.\textsuperscript{18} This was associated with a minor reduction in CETP mass and a decrease in total CETP-mediated CE transfer from HDL to apoB-containing lipoproteins.\textsuperscript{18} Simvastatin treatment of normolipidemic subjects also resulted in an increase in HDL-cholesterol (+8.3\%), with a concomitant reduction in CETP concentration (-26\%).\textsuperscript{19} Likewise, in type 2 diabetic subjects carrying the CETP TaqIB polymorphism, the increase in HDL-cholesterol (+7.2\%) after atorvastatin treatment is correlated with the reduction in CETP mass (-18\%).\textsuperscript{20} Although these results indicate that the effects of statin treatment on HDL-cholesterol levels are related to a reduction in CETP-mediated transfer of CE, a causal relationship between statin-induced reduced CETP activity and increased HDL-cholesterol levels has not been proven as yet.

\textit{APOE}\textsuperscript{*3-}Leiden (E3L) transgenic mice are an established model for hyperlipidemia and atherosclerosis\textsuperscript{21,22} and display a human-like lipoprotein profile.\textsuperscript{23,24} In contrast to treatment of wild-type and other hyperlipidemic mouse lines,\textsuperscript{25-27} administration of atorvastatin to E3L mice resulted in reductions in total cholesterol (TC) levels, by lowering apoB-containing lipoproteins, as observed in humans.\textsuperscript{28} However, in contrast to humans, in E3L mice HDL-cholesterol levels were not increased by atorvastatin treatment.\textsuperscript{28,29} Of note is that E3L mice, like other mice, do not express CETP,\textsuperscript{30} whereas humans do.\textsuperscript{31} Therefore, the aim of this study was to evaluate whether the effect of statin treatment on HDL-cholesterol levels would depend on CETP expression. Hereto, E3L mice were crossed with transgenic mice expressing human CETP under control of the natural flanking regions (E3L.CETP mice).\textsuperscript{32} Whereas HDL-cholesterol was not affected in E3L mice, atorvastatin indeed increased HDL-cholesterol levels in CETP. E3L mice. In addition, hepatic CETP mRNA expression, and plasma CETP mass and
activity were reduced. From these results we conclude that atorvastatin increases HDL-cholesterol by reducing CETP expression and activity.

Materials and Methods

Animals
Hemizygous human CETP transgenic (CETP) mice, expressing a human CETP mini-gene under the control of natural flanking sequences\textsuperscript{32} were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and crossbred with hemizygous E3L mice\textsuperscript{22} at our Institutional Animal Facility to obtain E3L and CETP.E3L littermates (C57Bl/6J background). Mice were housed under standard conditions in conventional cages and had free access to food and water. Mice were fed a semi-synthetic diet containing 15\% [w/w] fat (Hope Farms, Woerden, The Netherlands), supplemented with either 0.1\% or 0.25\% (w/w) cholesterol (Sigma, St. Louis, MO, USA) for two weeks. Subsequently, the mice received the same diet with or without atorvastatin (Lipitor\textsuperscript{®}-20, Pfizer B.V., Capelle a/d IJssel, The Netherlands). Experiments were performed after 4 h of fasting at 12:00 pm with food withdrawn at 8:00 am, unless indicated otherwise. The institutional Ethical Committee on Animal Care and Experimentation has approved all experiments.

Plasma Lipid and Lipoprotein Analysis
Plasma was obtained via tail vein bleeding as described\textsuperscript{33} and assayed for total cholesterol (TC) using the enzymatic kit 236691 (Roche Molecular Biochemicals, Indianapolis, IN, USA). The distribution of lipids over plasma lipoproteins was determined by fast-performance liquid chromatography (FPLC) as described previously.\textsuperscript{33}

CETP Activity and Mass Determination
CETP activity in plasma was measured as the transfer of [\textsuperscript{3}H]cholesteryl oleate ([\textsuperscript{3}H]CO) from exogenous LDL to HDL as described elsewhere.\textsuperscript{34} CETP activity was calculated as \textmu mol CE transfer per ml plasma per h. Plasma CETP mass was analyzed by a two-antibody sandwich immunoassay as described previously.\textsuperscript{35}

ApoAI Plasma Concentration
Plasma apoAI concentrations were determined using a sandwich ELISA. Hereto, rabbit anti-mouse apoAI polyclonal antibody (ab20453; Abcam plc, Cambridge, UK) was coated overnight onto Costar strips (Costar, Inc., New York, NY, USA) (3 \textmu g/ml) at 4\°C and incubated with diluted mouse plasma (dilution 1:400000) for 90 min at 37\°C. Subsequently, goat anti-mouse apoAI antibody (600-101-196; Rockland Immunochemicals, Inc., Gilbertsville, PA, USA; dilution 1:3000) was added and incubated for 90 min at 37\°C. Finally, horse radish peroxidase (HRP)-conjugated rabbit anti-goat IgG antibody (605-4313; Rockland; dilution 1:15000) was added and incubated for 90 min at 37\°C, and HRP was detected by incubation with tetramethylbenzidine (Organon Teknika, Boxtel, The Netherlands) for 15 min at room temperature. Purified mouse apoAI (A23100m; Biodesign International, Saco, Maine, USA) was used as a standard.
Hepatic mRNA Expression, SR-BI Protein and Lipid Analysis
Livers were isolated after cervical dislocation. Total RNA was isolated using the Nu-
cleoSpin® RNA II kit (Macherey-Nagel, Düren, Germany) as recommended by the 
manufacturer. RNA expression was determined in duplicate by real-time PCR on a 
MyiQ Single-Color real-time PCR detection system (Bio-Rad Laboratories, Hercules, 
CA, U.S.A.). Primers for CETP\textsuperscript{36} and Sr-b\textsuperscript{37} have been described previously. Primers 
for Abca\textsubscript{1}, Apoa\textsubscript{1}, Hmgcoa reductase, and Pltp are listed in table 1. Expression levels 
were normalized, using HPRT and cyclophilin as housekeeping genes.\textsuperscript{37,38} Hepatic SR-
BI protein was determined by immunoblot analysis as described previously.\textsuperscript{39}

Table 1. Primers for quantitative real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5'-3')</th>
<th>Reverse primer (5'-3')</th>
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<tbody>
<tr>
<td>Hmgcoa reductase</td>
<td>CCGGCAACAAACAGATCTGTG</td>
<td>ATGTACAGGATGGCGATGCA</td>
</tr>
<tr>
<td>Abca\textsubscript{1}</td>
<td>CCCAGAGCAAAAGCGACTC</td>
<td>GGTCATCATCAGTGGCTCCTG</td>
</tr>
<tr>
<td>Apoa\textsubscript{1}</td>
<td>GGAGTGCAAGGGAGACTGT</td>
<td>TGCAGAGAGTCGCTG</td>
</tr>
<tr>
<td>Pltp</td>
<td>TCAGTCGCTGGAGTCTCT</td>
<td>AAGGATCAGTGGAGT</td>
</tr>
</tbody>
</table>

\textit{Abca1}, ATP-binding cassette transporter a\textsubscript{1}; \textit{Apoa1}, apolipoprotein a\textsubscript{1}; \textit{Hmgcoa reductase}, hydroxymethylglutaryl coenzyme A 
reductase; \textit{Pltp}, phospholipid transfer protein

Statistical Analysis
All data are presented as means ± SD unless indicated otherwise. Data were analyzed 
using the unpaired Student’s t test unless indicated otherwise. \textit{P}-values less than 0.05 
were considered statistically significant.

Results
\textit{Atorvastatin Increases HDL-Cholesterol in Mice Expressing CETP}
Treatment of male \textit{E3L} mice, on a diet containing 0.25% (w/w) cholesterol, with 
atorvastatin (0.01%, w/w) caused a reduction in TC by -25% (3.8±1.2 vs. 5.1±0.9 mM) 
(data not shown). This effect was reflected by a strong decrease in (V)LDL-cholesterol 
(-86%), whereas HDL-cholesterol was not affected (Fig. 1A). Atorvastatin induced a 
similar decrease in TC in \textit{CETP.E3L} mice by -31% (2.9±1.0 vs. 4.3±0.8 mM; \textit{P}<0.05). 
In \textit{CETP.E3L} mice, atorvastatin also caused a strong reduction in (V)LDL-cholesterol 
(-88%; Fig. 1B). Moreover, whereas HDL-cholesterol levels were unaffected in \textit{E3L} 
mice, atorvastatin administration increased HDL-cholesterol (+52%; Fig. 1B) in \textit{CETP. E3L} mice.
Atorvastatin Decreases Hepatic CETP mRNA Expression and Plasma CETP Mass and Activity

In line with previous observations in E3L mice, atorvastatin increased the expression of Hmgcoa reductase both in E3L (2.5-fold; \( P < 0.05 \)) and in CETP.E3L mice (2.8-fold; \( P < 0.05 \)) (Table 2). This is probably caused by an attempt to compensate for the decrease in cholesterol formation upon statin administration.

Since differences in genes encoding proteins that are crucially involved in HDL metabolism may account for the increase in HDL-cholesterol in CETP.E3L mice upon atorvastatin treatment, we examined the effect of atorvastatin on hepatic expression of Pltp, Abca1, Sr-b1, Apoa1, and CETP (Table 2).

The expression of Pltp, involved in transfer of phospholipids between lipoproteins, was slightly but not significantly increased in both E3L (+34%) and CETP.E3L (+69%) mice upon treatment. In addition, the expression of Abca1, which is an important determinant for HDL formation, was reduced in E3L (-59%; \( P < 0.05 \)) and in CETP.E3L (-45%; \( P < 0.05 \)) mice. Since increased plasma PLTP activity and reduced hepatic ABCA1 levels are associated with decreased HDL-cholesterol levels, these effects on mRNA expression cannot contribute to the increase in HDL-cholesterol in CETP.E3L mice.
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Table 2. Effect of atorvastatin on hepatic mRNA expression in E3L and CETP.E3L transgenic mice

<table>
<thead>
<tr>
<th></th>
<th>E3L mice</th>
<th>CETP.E3L mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Atorvastatin</td>
</tr>
<tr>
<td>Hmgcoa reductase</td>
<td>1.00±0.24</td>
<td>2.46±0.32*</td>
</tr>
<tr>
<td>Pltp</td>
<td>1.00±0.18</td>
<td>1.34±0.25</td>
</tr>
<tr>
<td>Abca1</td>
<td>1.00±0.15</td>
<td>0.41±0.10*</td>
</tr>
<tr>
<td>Sr-b1</td>
<td>1.00±0.14</td>
<td>0.70±0.16</td>
</tr>
<tr>
<td>Apoa1</td>
<td>1.00±0.20</td>
<td>0.87±0.10</td>
</tr>
<tr>
<td>CETP</td>
<td>n.d.</td>
<td>n.d.</td>
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</table>

E3L and CETP.E3L male mice were fed a cholesterol-containing diet (0.25%) with or without 0.01% (w/w) atorvastatin. After 6 weeks, livers were collected to determine mRNA expression. Values are expressed as means ± S.E.M. relative to control mice (n=4 per group). n.d., not detectable. *P<0.05; **P<0.01 compared to control.

Figure 2. Effect of atorvastatin on hepatic SR-BI protein levels. E3L and CETP.E3L male mice received a diet containing 0.25% (w/w) cholesterol with or without 0.01% (w/w) atorvastatin for 6 weeks. Livers were isolated after cervical dislocation. SR-BI protein was determined by immunoblot analysis in E3L (A) and CETP.E3L (B) mice. Intensity of bands were determined by pixel counting and calculated relative to the control mice (C). Values are means ± S.E.M. (n=4 per group).
SR-BI is involved in the selective uptake of HDL-CE, and a reduction might thus result in increased HDL-cholesterol.\footnote{44} Atorvastatin tended to reduce hepatic \textit{Sr-b1} expression in both \textit{E3L} (-30\%; n.s.) and \textit{CETP.E3L} mice (-27\%; n.s.). Since \textit{Sr-b1} expression does not correlate well with protein mass,\footnote{45} hepatic SR-BI protein levels were also determined. Immunoblot analysis showed that SR-BI protein was not affected by atorvastatin as compared to control mice (Fig. 2). Therefore, the atorvastatin-mediated increase in HDL-cholesterol in \textit{CETP.E3L} mice are not explained by differences in SR-BI expression.

Increased levels of apoAI are positively correlated with HDL-cholesterol.\footnote{46} However, mRNA levels of \textit{Apoa1} were not affected in both types of mice as compared to their controls. In addition, atorvastatin did not increase apoAI plasma levels in \textit{E3L} and in \textit{CETP.E3L} mice (Table 3), thereby excluding a role of apoAI in the atorvastatin-mediated increase in HDL-cholesterol.

Altogether, atorvastatin caused similar effects on the expression of these genes in both \textit{E3L} and \textit{CETP.E3L} mice. The main discriminatory factor between both types of

\begin{table}
\centering
\caption{Effect of atorvastatin on plasma apoAI protein levels and plasma CETP mass and activity levels in \textit{E3L} and \textit{CETP.E3L} transgenic mice}
\begin{tabular}{lcccc}
\hline
 & \textit{E3L} mice & & \textit{CETP.E3L} mice & \\
 & Control & Atorvastatin & Control & Atorvastatin \\
\hline
ApoAI & & & & \\
\textit{(mg/dl)} & 77±41 & 85±42 & 75±25 & 49±14 \\
CETP mass & & & & \\
\textit{(µg/ml)} & n.d. & n.d. & 25±8 & 22±8 \\
\textit{(µg CETP/µmol HDL-cholesterol)} & n.d. & n.d. & 20±6 & 11±4* \\
CETP activity & & & & \\
\textit{(µmol CE/ml/h)} & n.d. & n.d. & 0.63±0.18 & 0.45±0.11 \\
\textit{(µmol CE/h/µmol HDL-cholesterol)} & n.d. & n.d. & 0.44±0.15 & 0.19±0.08** \\
\hline
\end{tabular}
\end{table}

\textit{E3L} and \textit{CETP.E3L} male mice were fed a cholesterol-containing diet (0.25\%) with or without 0.01\% (w/w) atorvastatin. After 6 weeks, plasma apoAI levels and plasma CETP mass and activity levels were determined. Values are expressed as means ± S.D. (n=5 per group). n.d., not detectable. *\textit{P}<0.05; **\textit{P}<0.01 compared to control.
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mice after atorvastatin treatment is a -57% reduction in hepatic CETP expression in the CETP.E3L mice (P<0.01) (Table 2), whereas CETP expression could of course not be detected in E3L mice. The decrease in CETP expression was accompanied by a trend towards reduction in plasma CETP mass (-12%) and activity (-29%) (Table 3). Apart from mRNA, also plasma HDL-cholesterol is a determinant of CETP levels. CETP activity was predominantly found on HDL, since precipitation of the apoB-containing lipoproteins did not affect CE transfer activities in atorvastatin-treated mice (0.37±0.15 µmol CE/ml/h) and in controls (0.56±0.19 µmol CE/ml/h). Therefore, CETP was adjusted for HDL-cholesterol, which led to significant reductions of -45% in CETP mass (P<0.05) and -57% in CETP activity (P<0.01) (Table 3).

Atorvastatin Dose-Dependently Decreases CETP Mass and Activity

To determine whether the effects of atorvastatin on HDL-cholesterol and CETP levels are dose-dependent, female CETP.E3L mice were fed a diet containing 0.1% (w/w) for two weeks, randomized according to plasma cholesterol levels, and successively received the diet supplemented with 0.001% and 0.01% of atorvastatin (w/w) for two weeks. Atorvastatin dose-dependently decreased plasma cholesterol up to -71% (P<0.01) at the highest concentration (Fig. 3A). This was accompanied by a dose-dependent increase in HDL-cholesterol up to 176% (Fig. 3B) and dose-dependent reductions in plasma CETP mass up to -57% (P<0.05) (Fig. 3C) and CETP activity up to -61% (P<0.05) (Fig. 3D).

Discussion

Statins do not affect or even increase plasma total cholesterol levels in apoE-deficient mice, and LDL receptor deficient mice also hardly show a response to statin treatment. In contrast, E3L mice respond to statin treatment with respect to lowering of apoB-containing lipoproteins and reduction of atherosclerosis development similarly as humans. To investigate whether the statin-induced elevation of HDL-cholesterol in humans would depend on CETP expression, we crossbred E3L mice with CETP transgenic mice. We found that atorvastatin increased HDL-cholesterol in CETP.E3L mice, which was not observed in E3L littermates. This was accompanied by decreased hepatic CETP mRNA expression levels with concomitant reductions in plasma CETP mass and activity.

Although steady-state HDL-cholesterol was not affected by atorvastatin in E3L mice, it was increased in CETP.E3L mice. Apparently, lack of CETP expression in mice prevents the atorvastatin-induced increase in HDL-cholesterol. However, several additional key players in HDL-metabolism might be affected differently in CETP.E3L as compared to E3L mice, and thus participate in the HDL-cholesterol raising effect.

ApoAI is a prerequisite for the formation of HDL particles. ApoAI deficient mice have reduced HDL levels and inversely, human APOA1 transgenic mice show an increase in HDL. Therefore, an increase in ApoAI expression might account for the increased HDL-cholesterol levels. However, atorvastatin did not increase hepatic ApoAI expression or plasma apoAI levels either in E3L mice nor in CETP.E3L mice. Lipid-
poor apoAI is subsequently lipidated via ABCA1. Since overexpression of human ABCA1 increases HDL-cholesterol levels in mice,\textsuperscript{48} whereas the disruption of Abca1 gene leads to a deficiency in HDL-cholesterol,\textsuperscript{49} the decreased Abca1 expression in E3L and CETP. E3L mice as we observed upon atorvastatin treatment cannot explain the elevation of HDL in CETP.E3L mice. PLTP plays an important role in the remodeling of HDL. A slight but non-significant increase in Pltp expression was observed both in E3L and in CETP.E3L mice. Since adenoviral mediated gene-transfer of PLTP to the liver results in a dose-dependent reduction of HDL-cholesterol,\textsuperscript{41,42} this excludes Pltp expression as a cause for the increased HDL levels upon atorvastatin treatment in CETP.E3L mice.
Finally, hepatic SR-BI forms the most important pathway for selective HDL-CE uptake from plasma in mice. We found that atorvastatin did not affect hepatic SR-BI protein levels in both types of mice. Taken these results together, the raise in HDL-cholesterol in CETP.E3L mice can not be explained by atorvastatin-mediated effects on apoAI, ABCAI, PLTP, or SR-BI.

Thus, our data indicate that the reduction in hepatic CETP mRNA is the primary cause of the statin-induced increase in HDL-cholesterol. Both the decrease in plasma CETP activity and the reduction in the available CE-acceptor particles (i.e. VLDL) can account for a reduction in CETP transfer activity, which in its turn causes the increase in HDL-cholesterol. In addition to its transfer activity, CETP was implicated in the direct and in the SR-BI-mediated HDL-CE uptake by hepatocytes. Inhibition of these uptake pathways may also contribute to the increase in HDL-cholesterol.

The atorvastatin-induced down-regulation of CETP expression may be caused by a reduction in plasma cholesterol levels. Since cholesterol feeding of CETP transgenic mice increases hepatic CETP mRNA expression via an LXR responsive element in the CETP promoter, the mechanism underlying the atorvastatin-induced down-regulation of CETP expression might conversely be related to a reduction in LXR signaling, as the reduction in plasma cholesterol may result in a decrease in oxysterols, the natural ligands of LXRα. In addition, the CETP promoter activity is affected by several other regulatory transcription factors, which alone or in combination with others could be responsible for decreased transcription.

Clinical studies have established that statins improve the survival rate of patients with hypercholesterolemia and coronary artery disease by lowering LDL-cholesterol in addition to pleiotropic anti-inflammatory effects. However, a high residual cardiovascular risk still remains. Even with aggressive atorvastatin treatment in the PROVE-IT (Pravastatin or Atorvastatin Evaluation and Infection Therapy) study, the risk remained 60-70% despite greater protection against death or major cardiovascular events. A more pronounced increase in HDL levels might further reduce the events. Therefore a combination therapy of atorvastatin and a small-molecule CETP inhibitor, torcetrapib, is currently tested in humans. The results so far are promising since combination therapy in subjects with low HDL-cholesterol (<1 mM), increased HDL-cholesterol by +61% and decreased LDL-cholesterol by -17%. Studies on the effect of combination therapy on atherosclerosis development in CETP.E3L mice might provide valuable and timely evidence about the benefit that can be expected from such a therapy, while results from long-term clinical studies using cardiovascular disease endpoints are awaited.

In conclusion, our results show that atorvastatin increases HDL-cholesterol in CETP.E3L mice by reducing the hepatic CETP expression and plasma CETP activity. We postulate that the increase in HDL after atorvastatin treatment in humans is also caused by a reduction in CETP activity. Further reduction of CETP activity beyond that achieved by statins might result in a more pronounced increase in HDL and provide additional beneficial effects regarding reduction of cardiovascular risk. CETP.E3L mice constitute a useful model to test such strategies.
Acknowledgements

This work was performed in the framework of the Leiden Center for Cardiovascular research LUMC-TNO, and supported by the Leiden University Medical Center (Gisela Thier Fellowship to P.C.N.R.), the Netherlands Organization for Scientific Research (NWO grant 908-02-097 and NWO VIDI grant 917.36.351 to P.C.N.R.), the Netherlands Heart Foundation (NHS grant 2003B136 to P.C.N.R.), and the Center for Medical Systems Biology (project 115). J.W.J. is an established clinical investigator of the Netherlands Heart Foundation (2001D032). We thank L.C. van der Zee-van Vark, C.M. van der Hoogen, and E. Hoeye-de Nobel for excellent technical assistance.

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