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The role of ApoCI, LPL and CETP in plasma lipoprotein metabolism - studies in mice

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Citation

Hoogt, C. C. van der. (2006, November 28). *The role of ApoCI, LPL and CETP in plasma lipoprotein metabolism - studies in mice*. Retrieved from <https://hdl.handle.net/1887/5414>

Version: Corrected Publisher's Version

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Note: To cite this publication please use the final published version (if applicable).



Atorvastatin Increases HDL Cholesterol by Reducing Cholesteryl Ester Transfer Protein

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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*, *Apoa1*, *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.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.^{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).^{4,5} Indeed, a meta-analysis of 25 studies indicated that statins reduce LDL-cholesterol levels by 20-40%.⁶ In addition, statins elevate high-density lipoprotein (HDL)-cholesterol levels by typically 5-15%.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.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.^{11,12} Accordingly, CETP-deficient subjects display increased HDL-cholesterol levels¹³ and also inhibition of CETP activity by small-molecule inhibitors leads to increased HDL-cholesterol levels.¹⁴⁻¹⁷

Treatment of patients with combined hyperlipidemia with atorvastatin resulted in increased levels of relatively CE-rich large HDL_{2a} with a concomitant decrease in CE-poor small HDL_{3c}.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.¹⁸ Simvastatin treatment of normolipidemic subjects also resulted in an increase in HDL-cholesterol (+8.3%), with a concomitant reduction in CETP concentration (-26%).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%).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.

*APOE*3-Leiden (E3L)* transgenic mice are an established model for hyperlipidemia and atherosclerosis^{21,22} and display a human-like lipoprotein profile.^{23,24} In contrast to treatment of wild-type and other hyperlipidemic mouse lines,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.²⁸ However, in contrast to humans, in *E3L* mice HDL-cholesterol levels were not increased by atorvastatin treatment.^{28,29} Of note is that *E3L* mice, like other mice, do not express CETP,³⁰ whereas humans do.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 crossbred with transgenic mice expressing human *CETP* under control of the natural flanking regions (*E3L.CETP* mice).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 sequences³² were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and crossbred with hemizygous *E3L* mice²² 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[®]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 described³³ 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.³³

CETP Activity and Mass Determination

CETP activity in plasma was measured as the transfer of [³H]cholesteryl oleate ([³H]CO) from exogenous LDL to HDL as described elsewhere.³⁴ CETP activity was calculated as $\mu\text{mol CE transfer per ml plasma per h}$. Plasma CETP mass was analyzed by a two-antibody sandwich immunoassay as described previously.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 $\mu\text{g/ml}$) 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; Biotrend 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 NucleoSpin® 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*³⁶ and *Sr-b1*³⁷ have been described previously. Primers for *Abca1*, *Apoa1*, *Hmgcoa reductase*, and *Pltp* are listed in table 1. Expression levels were normalized, using HPRT and cyclophilin as housekeeping genes.^{37,38} Hepatic SR-BI protein was determined by immunoblot analysis as described previously.³⁹

Table 1. Primers for quantitative real-time PCR analysis

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Hmgcoa reductase</i>	CCGGCAACAACAAGATCTGTG	ATGTACAGGATGGCGATGCA
<i>Abca1</i>	CCCAGAGCAAAAAGCGACTC	GGTCATCATCACTTTGGTCCTTG
<i>Apoa1</i>	GGAGCTGCAAGGGAGACTGT	TGCGCAGAGAGTCTACGTGTGT
<i>Pltp</i>	TCAGTCTGCGCTGGAGTCTCT	AAGGCATCACTCCGATTGTC

Abca1, ATP-binding cassette transporter a1; *Apoa1*, apolipoprotein a1; *Hmgcoa reductase*, hydroxymethylglutaryl coenzyme A reductase; *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. *P*-values less than 0.05 were considered statistically significant.

Results

Atorvastatin Increases HDL-Cholesterol in Mice Expressing CETP

Treatment of male *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 *CETP.E3L* mice by -31% (2.9±1.0 vs. 4.3±0.8 mM; *P*<0.05). In *CETP.E3L* mice, atorvastatin also caused a strong reduction in (V)LDL-cholesterol (-88%; Fig. 1B). Moreover, whereas HDL-cholesterol levels were unaffected in *E3L* mice, atorvastatin administration increased HDL-cholesterol (+52%; Fig. 1B) in *CETP.E3L* mice.

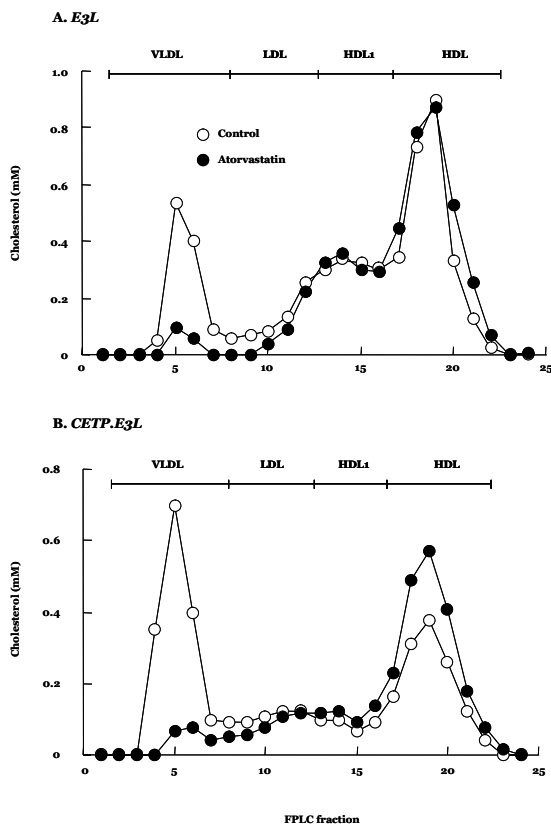


Figure 1. Effect of atorvastatin on the distribution of cholesterol over lipoproteins. Male *E3L* (A) and *CETP.E3L* (B) mice received a diet containing 0.25% (w/w) cholesterol without (open circles) or with (closed circles) 0.01% (w/w) atorvastatin for 6 weeks. Plasmas of the various mouse groups were pooled (n=6 per group). Lipoproteins were separated by FPLC, and fractions were analyzed for cholesterol.

Atorvastatin Decreases Hepatic CETP mRNA Expression and Plasma CETP Mass and Activity

In line with previous observations in *E3L* mice,⁴⁰ 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^{41,42} and reduced hepatic ABCA1 levels⁴³ are associated with decreased HDL-cholesterol levels, these effects on mRNA expression cannot contribute to the increase in HDL-cholesterol in *CETP.E3L* mice.

Table 2. Effect of atorvastatin on hepatic mRNA expression in *E3L* and *CETP.E3L* transgenic mice

	<i>E3L</i> mice		<i>CETP.E3L</i> mice	
	Control	Atorvastatin	Control	Atorvastatin
<i>Hmgcoa reductase</i>	1.00±0.24	2.46±0.32*	1.00±0.18	2.80±0.52*
<i>Pltp</i>	1.00±0.18	1.34±0.25	1.00±0.22	1.69±0.65
<i>Abca1</i>	1.00±0.15	0.41±0.10*	1.00±0.06	0.55±0.10*
<i>Sr-b1</i>	1.00±0.14	0.70±0.16	1.00±0.12	0.73±0.07
<i>Apoa1</i>	1.00±0.20	0.87±0.10	1.00±0.21	0.99±0.07
<i>CETP</i>	n.d.	n.d.	1.00±0.12	0.43±0.09**

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.

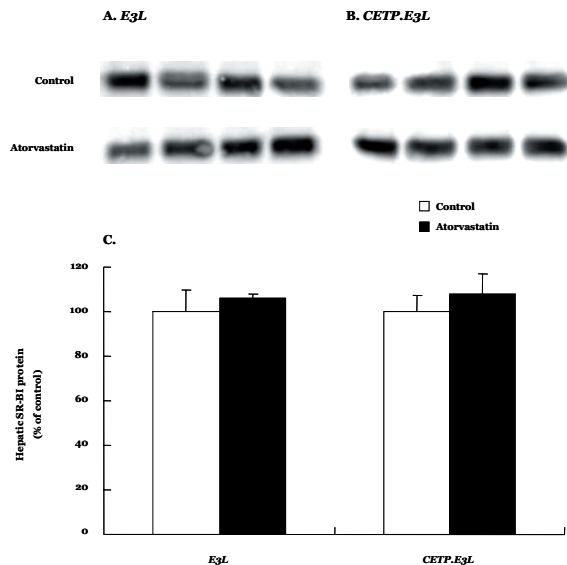


Figure 2. Effect of atorvastatin on hepatic SR-BI protein levels. *E3L* and *CETP.E3L* male mice received a diet containing 0.25% (w/w) cholesterol with or without 0.01% (w/w) atorvastatin for 6 weeks. Livers were isolated after cervical dislocation. SR-BI protein was determined by immunoblot analysis 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.⁴⁴ Atorvastatin tended to reduce hepatic *Sr-b1* expression in both *E3L* (-30%; n.s.) and *CETP.E3L* mice (-27%; n.s.). Since *Sr-b1* expression does not correlate well with protein mass,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 *CETP.E3L* mice are not explained by differences in SR-BI expression.

Increased levels of apoAI are positively correlated with HDL-cholesterol.⁴⁶ However, mRNA levels of *Apoa1* were not affected in both types of mice as compared to their controls. In addition, atorvastatin did not increase apoAI plasma levels in *E3L* and in *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 *E3L* and *CETP.E3L* mice. The main discriminatory factor between both types of

Table 3. Effect of atorvastatin on plasma apoAI protein levels and plasma CETP mass and activity levels in *E3L* and *CETP.E3L* transgenic mice

	<i>E3L</i> mice		<i>CETP.E3L</i> mice	
	Control	Atorvastatin	Control	Atorvastatin
ApoAI				
(mg/dl)	77±41	85±42	75±25	49±14
CETP mass				
(µg/ml)	n.d.	n.d.	25±8	22±8
(µg CETP/µmol HDL-cholesterol)	n.d.	n.d.	20±6	11±4*
CETP activity				
(µmol CE/ml/h)	n.d.	n.d.	0.63±0.18	0.45±0.11
(µmol CE/h/µmol HDL-cholesterol)	n.d.	n.d.	0.44±0.15	0.19±0.08**

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, 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. **P*<0.05; ***P*<0.01 compared to control.

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.¹² *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 $\mu\text{mol CE/ml/h}$) and in controls (0.56 ± 0.19 $\mu\text{mol CE/ml/h}$). 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 affect²⁷ or even increase²⁶ plasma total cholesterol levels in apoE-deficient mice, and LDL receptor deficient mice also hardly show a response to statin treatment.²⁵ In contrast, *E3L* mice respond to statin treatment with respect to lowering of apoB-containing lipoproteins and reduction of atherosclerosis development similarly as humans.⁴⁰ 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 mice³⁰ 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. *Apoa1* deficient mice have reduced HDL levels⁴⁷ and inversely, human *APOA1* transgenic mice show an increase in HDL.⁴⁶ Therefore, an increase in *Apoa1* expression might account for the increased HDL-cholesterol levels. However, atorvastatin did not increase hepatic *Apoa1* expression or plasma apoAI levels either in *E3L* mice nor in *CETP.E3L* mice. Lipid-

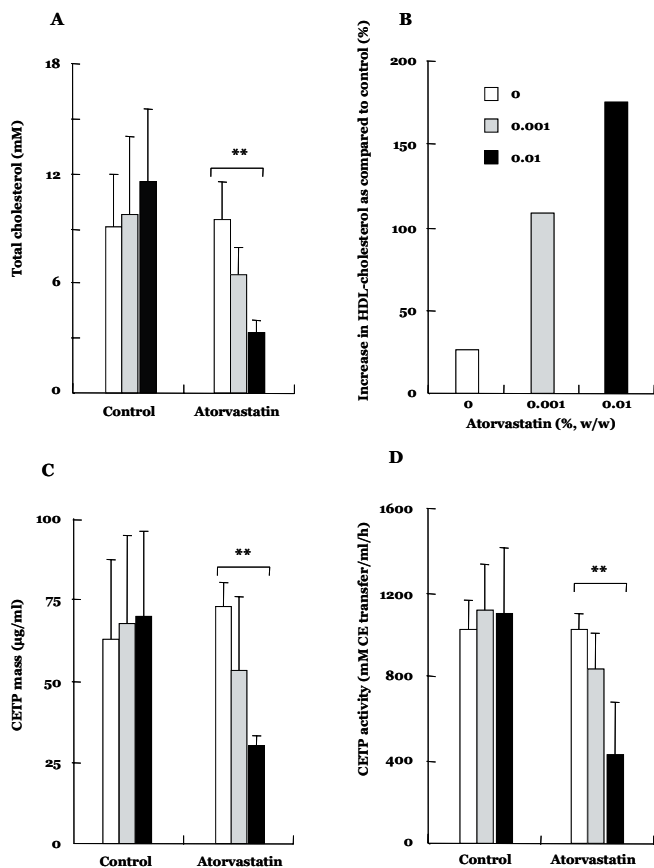


Figure 3. Effect of atorvastatin on plasma cholesterol, CETP mass, and CETP activity. Female *CETP.E3L* mice received a diet containing 0.1% (w/w) cholesterol (open bars; n=6) or this diet supplemented with 0, 0.001, or 0.01% (w/w) atorvastatin (closed bars; n=5) for 2 weeks. Plasma cholesterol (A), the increase in HDL-cholesterol as compared to control mice (B), CETP mass (C), and CETP activity (D) were determined. Statistical analysis was performed using univariate trend analysis (** $P < 0.01$).

poor apoAI is subsequently lipidated via ABCA1. Since overexpression of human *ABCA1* increases HDL-cholesterol levels in mice,⁴⁸ whereas the disruption of *Abca1* gene leads to a deficiency in HDL-cholesterol,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,^{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.⁴⁴ 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 direct⁵⁰ and in the SR-BI-mediated⁵¹ 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 expression³² via an LXR responsive element in the *CETP* promoter,⁵² 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 LXR α . In addition, the CETP promoter activity is affected by several other regulatory transcription factors,¹² 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.^{53,54} However, a high residual cardiovascular risk still remains.^{55,56} 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.⁸ 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.¹⁴ 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 (2001Do32). We thank L.C. van der Zee-van Vark, C.M. van der Hoogen, and E. Hoegee-de Nobel for excellent technical assistance.

References

1. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Ann Intern Med.* 1971;74:1-12.
2. Brown MS and Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science.* 1986;232:34-47.
3. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam Clin Pharmacol.* 2005;19:117-125.
4. Knopp RH. Drug treatment of lipid disorders. *N Engl J Med.* 1999;341:498-511.
5. Vega GL and Grundy SM. Effect of statins on metabolism of apo-B-containing lipoproteins in hypertriglyceridemic men. *Am J Cardiol.* 1998;81:36B-42B.
6. Wilt TJ, Bloomfield HE, MacDonald R, Nelson D, Rutks I, Ho M, Larsen G, McCall A, Pineros S, Sales A. Effectiveness of statin therapy in adults with coronary heart disease. *Arch Intern Med.* 2004;164:1427-1436.
7. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA.* 2001;285:2486-2497.
8. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med.* 2004;350:1495-1504.
9. Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, Crowe T, Howard G, Cooper CJ, Brodie B, Grines CL, DeMaria AN. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA.* 2004;291:1071-1080.
10. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR, Jr., Bangdiwala S, Tyroler HA. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* 1989;79:8-15.
11. Hesler CB, Swenson TL, Tall AR. Purification and characterization of a human plasma cholesteryl ester transfer protein. *J Biol Chem.* 1987;262:2275-2282.
12. Le Goff W, Guerin M, Chapman MJ. Pharmacological modulation of cholesteryl ester transfer protein, a new therapeutic target in atherogenic dyslipidemia. *Pharmacol Ther.* 2004;101:17-38.
13. Yamashita S, Hui DY, Wetterau JR, Sprecher DL, Harmony JA, Sakai N, Matsuzawa Y, Tarui S. Characterization of plasma lipoproteins in patients heterozygous for human plasma cholesteryl ester transfer protein (CETP) deficiency: plasma CETP regulates high-density lipoprotein concentration and composition. *Metabolism.* 1991;40:756-763.

14. Brousseau ME, Schaefer EJ, Wolfe ML, Bloedon LT, Digenio AG, Clark RW, Mancuso JP, Rader DJ. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *N Engl J Med.* 2004;350:1505-1515.
15. Clark RW, Sutfin TA, Ruggeri RB, Willauer AT, Sugarman ED, Magnus-Aryitey G, Cosgrove PG, Sand TM, Wester RT, Williams JA, Perlman ME, Bamberger MJ. Raising high-density lipoprotein in humans through inhibition of cholesteryl ester transfer protein: an initial multidose study of torcetrapib. *Arterioscler Thromb Vasc Biol.* 2004;24:490-497.
16. de Grooth GJ, Kuivenhoven JA, Stalenhoef AF, de Graaf J, Zwinderman AH, Posma JL, van Tol A, Kastelein JJ. Efficacy and safety of a novel cholesteryl ester transfer protein inhibitor, JTT-705, in humans: a randomized phase II dose-response study. *Circulation.* 2002;105:2159-2165.
17. Kuivenhoven JA, de Grooth GJ, Kawamura H, Klerkx AH, Wilhelm F, Trip MD, Kastelein JJ. Effectiveness of inhibition of cholesteryl ester transfer protein by JTT-705 in combination with pravastatin in type II dyslipidemia. *Am J Cardiol.* 2005;95:1085-1088.
18. Guerin M, Lassel TS, Le Goff W, Farnier M, Chapman MJ. Action of atorvastatin in combined hyperlipidemia : preferential reduction of cholesteryl ester transfer from HDL to VLDL1 particles. *Arterioscler Thromb Vasc Biol.* 2000;20:189-197.
19. Ahnadi CE, Berthezene F, Ponsin G. Simvastatin-induced decrease in the transfer of cholesterol esters from high density lipoproteins to very low and low density lipoproteins in normolipidemic subjects. *Atherosclerosis.* 1993;99:219-228.
20. van Venrooij FV, Stolk RP, Banga JD, Sijmonsma TP, van Tol A, Erkelens DW, Dallinga-Thie GM. Common cholesteryl ester transfer protein gene polymorphisms and the effect of atorvastatin therapy in type 2 diabetes. *Diabetes Care.* 2003;26:1216-1223.
21. Groot PH, van Vlijmen BJ, Benson GM, Hofker MH, Schiffelers R, Vidgeon-Hart M, Havekes LM. Quantitative assessment of aortic atherosclerosis in APOE*3 Leiden transgenic mice and its relationship to serum cholesterol exposure. *Arterioscler Thromb Vasc Biol.* 1996;16:926-933.
22. van den Maagdenberg AM, Hofker MH, Krimpenfort PJ, de B, I, van Vlijmen B, van der BH, Havekes LM, Frants RR. Transgenic mice carrying the apolipoprotein E3-Leiden gene exhibit hyperlipoproteinemia. *J Biol Chem.* 1993;268:10540-10545.
23. van Vlijmen BJ, van den Maagdenberg AM, Gijbels MJ, van der BH, HogenEsch H, Frants RR, Hofker MH, Havekes LM. Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-Leiden transgenic mice. *J Clin Invest.* 1994;93:1403-1410.
24. van Vlijmen BJ, Mensink RP, 't Hof HB, Offermans RF, Hofker MH, Havekes LM. Effects of dietary fish oil on serum lipids and VLDL kinetics in hyperlipidemic apolipoprotein E*3-Leiden transgenic mice. *J Lipid Res.* 1998;39:1181-1188.
25. Bisgaier CL, Essenburg AD, Auerbach BJ, Pape ME, Sekerke CS, Gee A, Wolle S, Newton RS. Attenuation of plasma low density lipoprotein cholesterol by select 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in mice devoid of low density lipoprotein receptors. *J Lipid Res.* 1997;38:2502-2515.
26. Quarfordt SH, Oswald B, Landis B, Xu HS, Zhang SH, Maeda N. In vivo cholesterol kinetics in apolipoprotein E-deficient and control mice. *J Lipid Res.* 1995;36:1227-1235.
27. Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S, Rosa R, Hermanowski-Vosatka A, Wang PR, Zhang D, Peterson L, Detmers PA, Chao YS, Wright SD. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. *Arterioscler Thromb Vasc Biol.* 2001;21:115-121.
28. Verschuren L, Kleemann R, Offerman EH, Szalai AJ, Emeis SJ, Princen HM, Kooistra T. Effect of low dose atorvastatin versus diet-induced cholesterol lowering on atherosclerotic lesion progression and inflammation in apolipoprotein E*3-Leiden transgenic mice. *Arterioscler Thromb Vasc Biol.* 2005;25:161-167.

29. Delsing DJ, Jukema JW, van de Wiel MA, Emeis JJ, van der LA, Havekes LM, Princen HM. Differential effects of amlodipine and atorvastatin treatment and their combination on atherosclerosis in ApoE*3-Leiden transgenic mice. *J Cardiovasc Pharmacol.* 2003;42:63-70.
30. Jiao S, Cole TG, Kitchens RT, Pflieger B, Schonfeld G. Genetic heterogeneity of lipoproteins in inbred strains of mice: analysis by gel-permeation chromatography. *Metabolism.* 1990;39:155-160.
31. Ha YC and Barter PJ. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comp Biochem Physiol B.* 1982;71:265-269.
32. Jiang XC, Agellon LB, Walsh A, Breslow JL, Tall A. Dietary cholesterol increases transcription of the human cholesteryl ester transfer protein gene in transgenic mice. Dependence on natural flanking sequences. *J Clin Invest.* 1992;90:1290-1295.
33. Berbee JF, van der Hoogt CC, Sundararaman D, Havekes LM, Rensen PC. Severe hypertriglyceridemia in human APOC1 transgenic mice is caused by apoC-I-induced inhibition of LPL. *J Lipid Res.* 2005;46:297-306.
34. Speijer H, Groener JE, van Ramshorst E, van Tol A. Different locations of cholesteryl ester transfer protein and phospholipid transfer protein activities in plasma. *Atherosclerosis.* 1991;90:159-168.
35. Niemeijer-Kanters SD, Dallinga-Thie GM, Ruijter-Heijstek FC, Algra A, Erkelens DW, Banga JD, Jansen H. Effect of intensive lipid-lowering strategy on low-density lipoprotein particle size in patients with type 2 diabetes mellitus. *Atherosclerosis.* 2001;156:209-216.
36. Gautier T, Masson D, Jong MC, Pais de Barros JP, Duverneuil L, Le Guern N, Deckert V, Dumont L, Bataille A, Zak Z, Jiang XC, Havekes LM, Lagrost L. Apolipoprotein CI overexpression is not a relevant strategy to block cholesteryl ester transfer protein (CETP) activity in CETP transgenic mice. *Biochem J.* 2005;385:189-195.
37. Out R, Hoekstra M, de Jager SC, de Vos P, van der Westhuyzen DR, Webb NR, van Eck M, Biessen EA, van Berkel TJ. Adenovirus-mediated hepatic overexpression of scavenger receptor class B type I accelerates chylomicron metabolism in C57BL/6J mice. *J Lipid Res.* 2005;46:1172-1181.
38. Post SM, Groenendijk M, Solaas K, Rensen PC, Princen HM. Cholesterol 7 α -hydroxylase deficiency in mice on an APOE*3-Leiden background impairs very-low-density lipoprotein production. *Arterioscler Thromb Vasc Biol.* 2004;24:768-774.
39. van Eck M, Twisk J, Hoekstra M, Van Rij BT, Van der Lans CA, Bos IS, Kruijt JK, Kuipers F, van Berkel TJ. Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. *J Biol Chem.* 2003;278:23699-23705.
40. Delsing DJ, Post SM, Groenendijk M, Solaas K, van der BH, van Duyvenvoorde W, de Wit EC, Bloks VW, Kuipers F, Havekes LM, Princen HM. Rosuvastatin reduces plasma lipids by inhibiting VLDL production and enhancing hepatobiliary lipid excretion in ApoE*3-leiden mice. *J Cardiovasc Pharmacol.* 2005;45:53-60.
41. Ehnholm S, van Dijk KW, van 't HB, van der ZA, Olkkonen VM, Jauhainen M, Hofker M, Havekes L, Ehnholm C. Adenovirus mediated overexpression of human phospholipid transfer protein alters plasma HDL levels in mice. *J Lipid Res.* 1998;39:1248-1253.
42. Foger B, Santamarina-Fojo S, Shamburek RD, Parrot CL, Talley GD, Brewer HB, Jr. Plasma phospholipid transfer protein. Adenovirus-mediated overexpression in mice leads to decreased plasma high density lipoprotein (HDL) and enhanced hepatic uptake of phospholipids and cholesteryl esters from HDL. *J Biol Chem.* 1997;272:27393-27400.
43. Oram JF and Heinecke JW. ATP-binding cassette transporter A1: a cell cholesterol exporter that protects against cardiovascular disease. *Physiol Rev.* 2005;85:1343-1372.
44. Out R, Hoekstra M, Spijkers JA, Kruijt JK, van Eck M, Bos IS, Twisk J, van Berkel TJ. Scavenger receptor class B type I is solely responsible for the selective uptake of cholesteryl esters from HDL by the liver and the adrenals in mice. *J Lipid Res.* 2004;45:2088-2095.

45. Silver DL. A carboxyl-terminal PDZ-interacting domain of scavenger receptor B, type I is essential for cell surface expression in liver. *J Biol Chem.* 2002;277:34042-34047.
46. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature.* 1991;353:265-267.
47. Plump AS, Erickson SK, Weng W, Partin JS, Breslow JL, Williams DL. Apolipoprotein A-I is required for cholesteryl ester accumulation in steroidogenic cells and for normal adrenal steroid production. *J Clin Invest.* 1996;97:2660-2671.
48. Singaraja RR, Bocher V, James ER, Clee SM, Zhang LH, Leavitt BR, Tan B, Brooks-Wilson A, Kwok A, Bissada N, Yang YZ, Liu G, Tafuri SR, Fievet C, Wellington CL, Staels B, Hayden MR. Human ABCA1 BAC transgenic mice show increased high density lipoprotein cholesterol and ApoAI-dependent efflux stimulated by an internal promoter containing liver X receptor response elements in intron 1. *J Biol Chem.* 2001;276:33969-33979.
49. McNeish J, Aiello RJ, Guyot D, Turi T, Gabel C, Aldinger C, Hoppe KL, Roach ML, Royer LJ, de Wet J, Brocardo C, Chimini G, Francone OL. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc Natl Acad Sci U S A.* 2000;97:4245-4250.
50. Gauthier A, Lau P, Zha X, Milne R, McPherson R. Cholesteryl ester transfer protein directly mediates selective uptake of high density lipoprotein cholesteryl esters by the liver. *Arterioscler Thromb Vasc Biol.* 2005;25:2177-2184.
51. Collet X, Tall AR, Serajuddin H, Guendouzi K, Royer L, Oliveira H, Barbaras R, Jiang XC, Francone OL. Remodeling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I. *J Lipid Res.* 1999;40:1185-1193.
52. Luo Y and Tall AR. Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. *J Clin Invest.* 2000;105:513-520.
53. Libby P and Aikawa M. Mechanisms of plaque stabilization with statins. *Am J Cardiol.* 2003;91:4B-8B.
54. Yoshida M. Potential role of statins in inflammation and atherosclerosis. *J Atheroscler Thromb.* 2003;10:140-144.
55. Ballantyne CM, Olsson AG, Cook TJ, Mercuri MF, Pedersen TR, Kjekshus J. Influence of low high-density lipoprotein cholesterol and elevated triglyceride on coronary heart disease events and response to simvastatin therapy in 4S. *Circulation.* 2001;104:3046-3051.
56. Sacks FM, Tonkin AM, Shepherd J, Braunwald E, Cobbe S, Hawkins CM, Keech A, Packard C, Simes J, Byington R, Furberg CD. Effect of pravastatin on coronary disease events in subgroups defined by coronary risk factors: the Prospective Pravastatin Pooling Project. *Circulation.* 2000;102:1893-1900.