The enterohepatic circulation of bile salts in health and disease
Hulzebos, Christian Victor

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2004

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Cyclosporin A-induced reduction of bile salt synthesis associated with increased plasma lipids in children after liver transplantation


1 Groningen University Institute for Drug Exploration, Center for Liver, Digestive and Metabolic Diseases, Pediatric Gastroenterology, Dept. Pediatrics, University Hospital, Groningen, The Netherlands.
2 Dept. Health Sciences, Faculty of Medical Sciences, University Groningen, The Netherlands.
3 Dept. Hepatobiliary Surgery, University Hospital, Groningen, The Netherlands.
ABSTRACT

**Background:** Hyperlipidemia is a common side effect of Cyclosporin A (CsA) after solid organ transplantation. CsA also markedly reduces the synthesis rate of bile salts in rats and can inhibit biliary bile salt secretion. It is not known, however, whether CsA inhibits the synthesis of bile salts in humans, and whether the hyperlipidemic effects of CsA are related to bile salt metabolism.

**Objectives:** To assess the effects of CsA on the synthesis rate of bile salts and on plasma triglycerides and cholesterol levels in pediatric liver transplant patients.

**Methods:** Before and after discontinuation of CsA treatment after liver transplantation, synthesis rate and pool size of the primary bile salts cholate and chenodeoxycholate were determined using a stable isotope dilution technique and related to plasma lipids.

**Results:** In 6 children (age: 3-16 years) CsA treatment was discontinued at 2 years (median 2.3 years) after liver transplantation. Discontinuation of CsA increased synthesis rate of chenodeoxycholate (+38%, p < 0.001) and cholate (+21%, p < 0.05) and the pool size of chenodeoxycholate (+ 54%, p < 0.001). Discontinuation of CsA decreased plasma levels of cholesterol (-18%, p < 0.05) and triglycerides (-23%, p < 0.05). Bile salt synthesis rate appeared inversely correlated with plasma cholesterol ($r_s=-0.82$, p < 0.01) and plasma triglyceride levels ($r_s=-0.62$, p < 0.05).

**Conclusions:** CsA inhibits bile salt synthesis and increases plasma concentration of cholesterol and triglycerides in pediatric liver transplant patients. Suppression of bile salt synthesis by long-term CsA treatment may contribute to hyperlipidemia and thus to increased risk for cardiovascular disease.
INTRODUCTION

Hypertriglyceridemia and hypercholesterolemia are common side effects of cyclosporin A (CsA), a widely used immunosuppressant after solid organ transplantation\(^1\)\(^-\)\(^3\). Hyperlipidemia contributes to the increased incidence of cardiovascular complications in transplant patients\(^4\)\(^-\)\(^9\). Elucidation of underlying mechanisms may allow identification of potentially preventive and treatment strategies. To date, however, only limited data are available on the mechanism of CsA-associated hyperlipidemia in humans \textit{in vivo}. Data from \textit{in vivo} animal studies and from \textit{in vitro} studies in human hepatoma cells indicated that CsA increases hepatic lipoprotein production and reduces lipoprotein clearance. In mice, CsA treatment increased hepatic VLDL triglyceride secretion\(^10\). In rats, CsA reduced clearance of low-density-lipoprotein (LDL) and down-regulated lipoprotein lipase expression\(^11\). In a human hepatoma cell line CsA reduced LDL receptor activity\(^12\). In addition, LDL uptake could be impaired by incorporation of CsA into the LDL particle\(^13\).

For a long time it has been known that CsA interacts with the enterohepatic cycling of bile salts. CsA inhibits hepatic uptake and hepatobiliary secretion of bile salts\(^14\)\(^-\)\(^17\). CsA has also been demonstrated to inhibit bile salt synthesis. In cultured rat and human hepatocytes and hepatoma cells (HepG2), CsA inhibits bile salt synthesis \textit{in vitro} by inhibition of cholesterol 7\(\alpha\)-hydroxylase (Cyp7a1), considered to be the rate-limiting enzyme in bile salt synthesis, and of mitochondrial sterol-27-hydroxylase (Cyp27), resulting in decreased synthesis rates of cholate and particularly chenodeoxycholate\(^9\);\(^11\);\(^18\)\(^-\)\(^20\). Animal studies confirm that CsA suppresses bile salt synthesis \textit{in vivo}\(^21\)\(^-\)\(^24\).

A relationship between bile salt metabolism and plasma lipid concentrations has emerged from various studies. Bile salts inhibit VLDL secretion in rat and human hepatocytes \textit{in vitro} and, to a limited extent, in rodents \textit{in vivo}\(^25\). The relationship between bile salt biosynthesis and VLDL formation in humans \textit{in vivo} seems bi-directional; treatment with cholestyramine results in increased hepatic VLDL secretion\(^26\), whereas an increased formation of plasma triglycerides, as occurs in hyperlipoproteinemia, is associated with an augmented bile salt formation\(^27\). Familial hypertriglycidemia is associated with bile salt malabsorption\(^26\);\(^29\), and treatment with the bile salt chenodeoxycholate decreases plasma triglyceride concentrations\(^26\);\(^30\).

In the present study we aimed to elucidate whether CsA treatment affects bile salt synthesis in humans \textit{in vivo}, and whether possible effects on bile salt synthesis are related to CsA-associated hyperlipidemia.

In our center, CsA treatment is discontinued in pediatric liver transplant patients at least 2 years after transplantation, provided that histology of liver biopsy does not suggest the presence of rejection, including severe fibrosis or signs of cholestasis. Recently, we reported the results of this regimen with respect to incidence of rejection, graft survival and kidney function in preliminary form\(^31\). Determination of bile salt kinetics and plasma lipid levels before and after CsA discontinuation provides a unique opportunity to determine effects of CsA on bile salt synthesis rate and plasma lipid levels. Our results indicate that CsA reversibly reduced primary bile salt synthesis rate in children after liver transplantation and...
that the reduction is strongly, inversely correlated with plasma lipid levels.

**SUBJECTS AND METHODS**

**Patient characteristics**

The study protocol was approved by the Medical Ethics Committee of the University Hospital Groningen and included informed consent by the parents and the children. The study group included 6 children (4 males, 2 females; age: 3-16.5 yr.). Indications for OLT were end-stage liver disease caused by biliary atresia (n = 5) and recurrent cholangitis with secondary biliary cirrhosis after surgical removal of a choledochal cyst (n=1). Five patients received a whole liver graft, and one a partial graft. Retransplantation had been performed in 1 patient at 10 months after the primary transplantation because of secondary biliary cirrhosis (ischemic type of biliary lesions and recurrent cholangitis). All patients received a hepatico-jejunostomy on a Roux-and-Y loop. Immunosuppressive medication after liver transplantation consisted of CsA (trough levels from 6 months posttransplant of ~100 ng/mL), prednisolon (0.2 mg·kg⁻¹·days⁻¹, alternate day dosing) and azathioprine (2 mg·kg⁻¹·day⁻¹). CsA treatment was discontinued after a median duration of 2.3 years (range: 2.1-8.7 yr.) after OLT in case of histological (liver biopsy) and biochemical (serum) absence of rejection. Immunosuppressive medication after discontinuation of CsA consisted of prednisolon (0.4 mg·kg⁻¹·days⁻¹, alternate day dosing) and azathioprine (2.5 mg·kg⁻¹·day⁻¹). Two children used received ursodeoxycholate (15 mg·kg⁻¹·day⁻¹) for a cholestatic episode in the past. Other medication consisted of nifedipine (n=2), magnesiumsulphate/ gluconate (n=5), budesonide nasal spray (n=1), alpha-calcidol (n=5), calciumcarbonate/ -lactogluconate (n=4), ferrofumarate (n=2), and captopril (n=1).

**Study protocol**

After informed consent was obtained, the patients were subjected to maximal 2 study periods before and after discontinuation of CsA. To exclude the presence of rejection, liver biopsy was performed 10 weeks (range 5-20) prior to CsA withdrawal. Discontinuation of CsA occurred in 7 days after increasing the doses of prednisolon and azathioprine. The median interval of the two study periods after withdrawal of CsA was 4 weeks (range: 1-5) and 14 weeks (range: 7-29), respectively. On day 1 of a study period, after an overnight fast, a baseline blood sample was collected into an EDTA-containing tube, after which the patients received a tetradeuterated bile salt solution (oral ingestion or administration via a nasogastric tube) containing 50 mg [²H₄]-cholate and 50 mg [²H₄]-chenodeoxycholate dissolved in 80 mL 0.25% NaHCO₃. Subsequently, blood samples (0.5 mL) in EDTA-containing tubes were taken at 10, 24, 32, 48, and 72 hours after administration of the bile salt solution. Plasma was obtained by centrifugation at 4000 rpm for 10 minutes and stored at -20 °C until analysis. During the 3 days of the test, patients ate their regular meals and medications. In case of diarrhea the study was not initiated.
Cyclosporin A reduces bile salt synthesis and increases plasma lipids

Materials

[2,2,4,4-2H4]-cholate and [2,2,4,4-2H4]-chenodeoxycholate ([2H4]-CA and [2H4]-CDCA, isotopic purity 98%) were obtained from Isotech Inc. (Miamisburg, OH). Cholylglycine hydrolase from Clostridium perfringens (welchii) was purchased from Sigma Chemicals (St. Louis, MO). Pentfluorobenzylbromide (PFB) was purchased from Fluka Chemie (Buchs, Neu-Ulm, Switzerland). All other chemicals and solvents used were of the highest purity commercially available.

Analytical procedures

Plasma alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AP), γ-glutamyltranspeptidase (GGT), bilirubin, total cholesterol, HDL-cholesterol and triglycerides were determined by routine laboratory techniques. LDL-cholesterol was calculated according to Friedewald et al.32. Total bile salts in plasma were determined by an enzymatic fluorimetric assay using 3α-hydroxysteroid dehydrogenase33. Whole blood concentrations of CsA were determined by use of an enzymatic multiplied immunoassay technique (EMIT)34.

Gas-liquid chromatography electron capture negative chemical ionization mass spectrometry

Isotope enrichment of plasma bile salts was determined by gas chromatography mass spectrometry (GC-MS) using a Finnigan SSQ7000 Quadrupole GC-MS (Finnigan MAT, San José)35. GC separation was performed on a 15 m x 0.25 mm column, 0.25 µm film thickness (AT-5MS, Alltech Associates, Inc., Deerfield, IL) and details of the running program and detection were identical to the method previously developed and described35.

Calculations

Isotope dilution technique.

Enrichment of CA and CDCA was defined as the increase of M4-CA/ M0-CA relative to baseline measurements after administration of [2H4]-CA and expressed as the natural logarithm of atom % excess (ln APE) value6. The decay of ln APE in time was calculated by linear regression analysis. From this linear decay curve the fractional turnover rate (FTR) and pool size of CA were determined. The FTR (day⁻¹) equals the slope of the regression line. The pool size (µmol.kg⁻¹) was determined according to the formula: ((D - b·100) / eᵃ) - D)/ BW, where “D” is the administered amount of label, “b” is the isotopic purity, “a” is the intercept on the y-axis of the ln APE versus time curve, and “BW” bodyweight (kg). Cholate synthesis rate (µmol.kg⁻¹.day⁻¹) was then calculated by multiplying pool size and FTR.

Statistical analysis.

Results are presented as means ± standard deviation or as medians when considered appropriate. To test the null hypothesis (no effect of CsA discontinuation) the number of patients whose measurements without CsA all exceeded those with CsA was used as test statistic. Paired statistical analysis was performed in which each study subject served as its own control. Exact one-sided p-values are presented. Based on in vitro and in vivo data on effects of CsA on lipid
and bile salt metabolism we tested the hypothesis that CsA withdrawal would increase bile salt synthesis and reduce plasma lipid levels\textsuperscript{2-4,10-13,18-24}. Correlations between parameters were calculated using Spearman rank correlation coefficient ($r_s$). Level of significance for all statistical analyses was set at $p < 0.05$. Analysis was performed using SPSS 10 for Windows software (SPSS, Chicago, IL).

**RESULTS**

**Patient characteristics and effects of chronic CsA treatment on parameters of liver function.**

Table 1 shows the clinical data and biochemical parameters of the included patients. Discontinuation of CsA significantly increased bodyweight (+5%; paired measurements, $p < 0.01$) at 5 weeks (median) after withdrawal of the drug and slightly decreased plasma alkaline phosphatase activity. Alanine transaminase activity increased, but remained below the upper level of normal after discontinuation of CsA. Other parameters of liver graft function were comparable before and after discontinuation of CsA.

**Effects of chronic CsA treatment on plasma lipid concentrations.**

To establish the effects of CsA on lipid metabolism, plasma concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were measured. Figure 1A shows that plasma levels of total cholesterol were significantly reduced by 18% ($p < 0.05$) after discontinuation of CsA. This decrease consisted predominantly of a decrease in LDL-cholesterol levels (-27%, $p < 0.05$), whereas HDL-cholesterol levels were not significantly affected (data not shown). Discontinuation of CsA also decreased mean plasma levels of triglycerides (-23%; $p < 0.05$, Figure 1B).

**Table 1.** Patient characteristics and plasma parameters of liver function during CsA treatment and after CsA discontinuation.

<table>
<thead>
<tr>
<th></th>
<th>CsA treatment</th>
<th>CsA discontinued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>11.3 ± 5.6</td>
<td>11.6 ± 5.6*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>39.6 ± 19.7</td>
<td>41.2 ± 20.4*</td>
</tr>
<tr>
<td>Years after OLT</td>
<td>3.2 ± 0.7</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>Bile salts (mmol/L)</td>
<td>25 ± 22</td>
<td>22 ± 13</td>
</tr>
<tr>
<td>Total bilirubin (mmol/L)</td>
<td>20 ± 6</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>15 ± 8</td>
<td>22 ± 9*</td>
</tr>
<tr>
<td>Aspartate transaminase (U/L)</td>
<td>28 ± 4</td>
<td>32 ± 10</td>
</tr>
<tr>
<td>γ-glutamyltranspeptidase (U/L)</td>
<td>10 ± 3</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>193 ± 53</td>
<td>137 ± 53*</td>
</tr>
<tr>
<td>Cyclosporin (mg/L)</td>
<td>104 ± 33</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Means ± standard deviation of patient characteristics and liver function parameters in pediatric liver transplant patients before and after discontinuation of CsA (n = 6, paired; N.D., not detectable; *: $p < 0.01$).
Cyclosporin A reduces bile salt synthesis and increases plasma lipids

Effects of chronic CsA treatment on bile salt metabolism.

To obtain an integrated view on the effects of CsA on bile salt metabolism we studied parameters of bile salt kinetics, i.e., bile salt synthesis rate, pool size, and fractional turnover rate using a recently developed stable isotope dilution, applicable for small (50µL) plasma volumes. Figure 2 shows that discontinuation of CsA significantly increased total bile salt synthesis rate (18 ± 11 vs. 24 ± 7 µmol.kg⁻¹.day⁻¹, CsA-treated vs. CsA discontinued, resp.; p < 0.01). Discontinuation of CsA treatment increased synthesis rates of chenodeoxycholate (8 ± 3 vs. 10 ± 4 µmol.kg⁻¹.day⁻¹, CsA-treated vs. CsA discontinued, resp.; p < 0.001) and of that of cholate (10 ± 8 vs. 13 ± 4 µmol.kg⁻¹.day⁻¹, CsA-treated vs. CsA discontinued, resp.; p < 0.001).
Chapter 4

Figure 3. Pool sizes of cholate (black bars) and chenodeoxycholate (white bars) in pediatric liver transplant patients before (CsA+) and after discontinuation (CsA-) of CsA. Data are means ± standard deviation of 6 paired measurements. *: p < 0.05.

Figure 4. Correlation between fractional turnover rates and pool sizes for chenodeoxycholate (A) and cholate (B) in pediatric liver transplant patients before (closed circles) and after (open circles) discontinuation of CsA.

vs. CsA discontinued, resp.; p < 0.05) significantly. Figure 3 shows that total bile salt pool size increased after discontinuation of CsA, but this difference did not reach statistical significance (47 ± 20 vs. 60 ± 26 µmol·kg⁻¹, CsA-treated vs. CsA discontinued, resp.; NS). Discontinuation of CsA significantly increased CDCA pool size (23 ± 13 vs. 36 ± 19 µmol·kg⁻¹, CsA-treated vs. CsA discontinued, resp.; p < 0.001), but not CA pool size (24 ± 10 vs. 25 ± 12 µmol·kg⁻¹, CsA-treated vs. CsA discontinued, resp.; NS).

Fractional turnover rate of CDCA, i.e., the portion of the CDCA pool that is newly synthesized per day was not significantly changed (0.40 ± 0.21 vs. 0.35 ± 0.15 day⁻¹, CsA-treated vs. CsA discontinued, resp.; NS), whereas that of CA increased after discontinuation of CsA (0.45 ± 0.33 vs. 0.62 ± 0.32 day⁻¹, CsA-treated vs. CsA discontinued, resp.; p < 0.05).

Analogous with previous studies, Figures 4A and 4B show that an inverse
Cyclosporin A reduces bile salt synthesis and increases plasma lipids

Figure 5. Correlation between plasma levels of cholesterol and total bile salt synthesis rate (5A) and plasma triglycerides and total bile salt synthesis rate (5B) in pediatric liver transplant patients before (closed circles) and after (open circles) discontinuation of CsA.

A

B

relationship was observed between the bile salt pool size and the fractional turnover rate for CA and CDCA. This relationship was more pronounced for CDCA ($r_s = -0.72$, $p < 0.01$) than for CA ($r_s = -0.58$, $p = 0.05$). No significant correlation existed between bile salt synthesis rates and bile salt pool sizes of cholate and chenodeoxycholate (data not shown).

**Relationship between bile salt synthesis and plasma lipid levels.**
From results described above, it could already be appreciated that CsA treatment increased plasma lipid levels and inhibited bile salt synthesis (Figure 5). Bile salt synthesis rate appeared inversely correlated with plasma cholesterol ($r_s = -0.82$, $p < 0.01$) as well as with triglyceride levels ($r_s = -0.62$, $p < 0.05$). Of the two primary bile salts measured, synthesis rate of chenodeoxycholate showed a stronger correlation with plasma lipid levels than that of cholate (cholesterol: $r_s = -0.78$, $p < 0.01$ for CDCA and $r_s = -0.68$, $p < 0.05$ for CA; triglycerides: $r_s = -0.79$, $p < 0.01$ for CDCA and $r_s = -0.56$, $p = 0.06$ for CA).

**DISCUSSION**

The present study demonstrates that CsA treatment significantly affects bile salt kinetics and plasma lipid levels in pediatric liver transplant patients. Discontinuation of CsA decreases plasma levels of cholesterol, LDL-cholesterol, and triglycerides by ~20%. These effects appeared strongly related to an increased synthesis rate of bile salts, especially to that of chenodeoxycholate. The results indicate that CsA increases the plasma triglyceride and cholesterol levels...
in humans *in vivo* in association with inhibition of bile salt synthesis.

To the best of our knowledge, this is the first study to demonstrate that CsA inhibits bile salt synthesis in pediatric liver transplant patients. Although for long it has been known that CsA interacts with bile formation in humans, *i.e.*, cholelithiasis and cholestasis have repeatedly been reported in patients on CsA therapy[^36^], data on bile salt synthesis and pool size in humans chronically treated with CsA are scarce[^37^]. Our observation that CsA reduces bile salt synthesis is in agreement with *in vitro* and *in vivo* animal data. CsA acutely inhibits bile salt synthesis in cultured rat and human hepatocytes and in rats[^18^,^21^,^22^,^24^,^38^]. Accordingly, protein levels but not mRNA levels of cholesterol 7α-hydroxylase Cyp7a1, the rate-limiting enzyme in bile salt synthesis, have been shown to be markedly downregulated in livers of CsA-treated rats[^11^,^24^]. Previously, it was hypothesized that a reduced bile salt synthesis upon CsA treatment contributed to the concomitantly observed reduction in bile salt pool size[^21^,^22^,^38^]. Yet, in a recent study, we found that the cholate pool size was unaffected in CsA-treated rats despite a significantly reduced cholate synthesis upon CsA treatment[^24^]. The unaffected cholate pool size in the pediatric liver transplant patient presented here is in agreement with our data in CsA-treated rats. Interestingly, maintenance of cholate pool size in CsA-treated rats was associated with increased expression of the apical sodium-dependent bile salt transporter (Asbt) protein expression in the distal ileum, considered pivotal in intestinal bile salt absorption[^11^,^24^]. Various studies support the concept that regulation of the bile salt pool size is not only regulated by hepatic biosynthesis in response to fecal loss of bile salts, but that intestinal events can influence the bile salt pool size independently[^11^,^24^,^39^-^41^]. We can not completely exclude an effect of the temporarily increased steroid dosing on bile salt synthesis. Yet, comparison between the first and second period after CsA withdrawal did not give any sustained indication of a rebound effect (data not shown), whereas the steroid dose had already been tapered again.

Long-term treatment with CsA increases plasma levels of triglycerides and VLDL- and LDL-cholesterol[^3^,^42^,^43^]. HDL-cholesterol levels have been reported to decrease or, as in the present study, to remain unaffected[^44^-^46^]. The incidence and extent of hyperlipidemia varies among the numerous reports, which may be related to differences in underlying diseases, time after transplantation, fasting versus non-fasting lipid measurements, or in nutritional status of patients[^42^-^47^]. Elucidation of the mechanism by which CsA raises plasma lipid levels may allow the identification of preventive and therapeutic options. Several hypotheses reminiscent on concepts of impaired clearance and/or increased production of lipoproteins have been proposed for the CsA-associated increase in LDL cholesterol. Raine *et al.* suggested that hepatic LDL uptake was impaired by incorporation of CsA into the LDL particle[^13^]. LDL uptake could also be diminished by impaired LDL receptor activity. In agreement with this, CsA suppresses the LDL receptor activity in HepG2 cells possibly by affecting the free intracellular cholesterol pool[^12^]. Yet, it is not clarified whether CsA decreases the clearance of LDL from plasma, since data from *in vivo* and *in vitro* studies are conflicting[^48^]. Another explanation for the CsA-associated hypertriglyceridemia could reside in an impaired expression or activity of the lipoprotein lipase enzyme as observed in numerous *in vivo* studies in animals as well as in humans[^11^,^49^-^51^]. Apart from
Cyclosporin A reduces bile salt synthesis and increases plasma lipids

a reduced activity of peripheral lipoprotein lipase, a reduced activity of hepatic lipase may contribute to a lower lipolytic activity as has been described in renal transplant patients on CsA therapy. Alternatively, the CsA-associated hyperlipidemia could result from increased hepatic production of cholesterol and triglyceride-rich lipoproteins. In HepG2 cells, CsA dose- and time-dependently decreased apo-B secretion, suggesting that the elevated plasma LDL-cholesterol levels are not caused by hepatic overproduction of apoB-100-containing lipoproteins. Yet, CsA treatment increased VLDL triglyceride secretion in mice in the presence of increased plasma lipid levels. Considering the strong inverse correlation between bile salt synthesis and triglycerides, bile salts could also be involved in CsA-induced hyperlipidemia. Recently, it has been shown that bile salts, i.e., transhepatic bile salt fluxes, are inversely related with VLDL-triglyceride concentration and hepatic triglyceride secretion in vivo in rodents. The present study does not allow addressing the transhepatic flux in the patients, so that its potential involvement in the CsA-induced hypertriglyceridemia is unclear.

It is tempting to speculate that the strong quantitative association between bile salt synthesis and plasma lipid levels is due to interaction between bile salt and lipid metabolism. Conversion of cholesterol into bile salts and their subsequent fecal excretion provides the major route for elimination of excess cholesterol. Recently, it has become clear that bile salts exert regulatory actions on expression of specific genes involved in bile salt and lipid metabolism via activation of nuclear hormone receptors, e.g., the farnesoid X-receptor (FXR; NR1H4). Bile salts, such as cholate, chenodeoxycholate and their conjugates are natural ligands for FXR. Bile salt-activated FXR controls expression of several genes considered crucial in maintenance of bile salt and cholesterol homeostasis. Bile salt-activated FXR inhibits transcription of the Cyp7a1 gene, encoding cholesterol 7α-hydroxylase which catalyzes the first and rate-limiting step in bile salt synthesis. In addition, FXR appears to control a variety of genes involved in control of plasma lipid levels. In agreement with this is the observation that upon FXR activation with a nonsteroidal ligand for FXR (GW4064) plasma triglyceride levels decreased dose-dependently in Fischer rats. Yet, in the FXR knockout mice, an increased bile salt synthesis rate is associated with high plasma lipid levels, which makes the exact role of FXR elusive at the moment. Effects of CsA on plasma lipids may, at least in part, be mediated by bile salts. We propose that the CsA-induced reduction in hepatic bile salt synthesis may increase hepatic cholesterol content and thus reduce cholesterol clearance from plasma. This concept is supported by the data presented in this study, which demonstrate that CsA causes a sustained but reversible inhibition of bile salt synthesis in vivo in humans associated with increased plasma lipids.

A substantial number of pediatric liver transplant patients have lipid abnormalities that may contribute to atherosclerosis. To treat posttransplant hyperlipidemia several options exist. Replacing CsA by tacrolimus (FK506) as primary immunosuppressive drug reduces hyperlipidemia. Dietary therapy, weight reduction and administration of HMG-CoA reductase inhibitors have also been shown to effectively lower plasma lipid levels in organ transplant recipients. In the pediatric liver transplant patients reported in this study,
the absolute values of plasma lipids were in the normal (cholesterol) – high (triglycerides) range (median percentile corrected for age: P35 and P95, respectively) and discontinuation of CsA resulted in low (cholesterol) – normal (triglycerides) lipid levels. The present study suggest that discontinuation of CsA, according to the present protocol lowers plasma lipid levels\textsuperscript{31,69}. Observational data show that there is no threshold below which lower plasma lipid levels are not associated with a lower risk of cardiovascular disease\textsuperscript{70}. The strength of the relation between plasma lipid levels and coronary heart disease is weaker with increasing age, stressing the importance of controlling lipid levels at a young age\textsuperscript{70}.

In conclusion, CsA inhibits bile salt synthesis and increases plasma concentration of cholesterol and triglycerides in pediatric liver transplant patients. Suppression of bile salt synthesis by long-term CsA treatment may contribute to hyperlipidemia and thus to increased risk on cardiovascular disease.

**ACKNOWLEDGEMENTS**

The authors are indebted to Theo Boer and Renze Boverhof for their skillful assistance in the analysis described in this manuscript. This project has been supported by the Foundation “De Drie Lichten” in The Netherlands.
Cyclosporin A reduces bile salt synthesis and increases plasma lipids

REFERENCES


Cyclosporin A reduces bile salt synthesis and increases plasma lipids


67. Neal DA, Gimson AE, Gibbs P, Alexander GJ. Beneficial effects of converting liver transplant recipients from cyclosporine to tacrolimus on blood pressure, serum lipids,