DMD Fast Forward. Published on June 10, 2013 as doi:10.1124/dmd.113.051698

Atorvastatin treatment induces uptake and efflux transporters in human liver

Linda Björkhem-Bergman, Helena Bergström, Maria Johansson, Paolo Parini, Mats Eriksson, Anders Rane, Lena Ekström

Division of Clinical Pharmacology (LBB,HB,MJ,AR,LE), Division of Clinical Chemistry (PP), Department of Laboratory Medicine and Department of Medicine, Division of Endocrinology (ME), Karolinska Institutet, Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden

Running title: Atorvastatin induce expression of membrane transporters

Correspondance to:
Linda Björkhem-Bergman (Assoc Prof, MD)
Division of Clinical Pharmacology C1-68,
Karolinska University Hospital, Huddinge
Karolinska Institutet, 141 86, Stockholm
Sweden
e-mail: linda.bjorkhem-bergman@ki.se
Tel: +46 8 585 839 66
Fax: +46 8 585 810 70

Number of pages: 15

Number of Figures: 2

Number of Tables: 1

Number of word in Abstract: 239

Number of words in Introduction: 411

Number of words in Discussion: 1229

Number of References: 44

Abbreviations used: CYP, cytochrome P450 enzyme; OATP, organic anion transporting polypeptide; SREBP-2, sterol regulatory element binding protein 2.

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

The metabolism and disposition of statins are highly dependent on different cytochrome P450 enzymes such as CYP3A4 and CYP2C9 as well as membrane transporters SLCO1B1, SLCO2B1, ABCB1 and ABCG2. Inter-individual gene expression differences among these enzymes may explain part of the variability in tolerance and effect for statin treatment. The aim of the present study was to investigate the effect of statin treatment on these genes in human liver tissue,. Levels of CYP3A4, CYP2C9, SLCO1B1, SLCO2B1, ABCB1 and ABCG2 mRNA in liver tissue from a previously performed clinical trial in 29 patients randomized to treatment with placebo, atorvastatin 80 mg/d or fluvastatin 20mg/d for 4 weeks were measured using quantitative PCR. Atorvastatin treatment (n=10) but not fluvastatin (n=10), resulted in three-fold higher expression of SLCO2B1 compared to placebo-treated (n=9) ($p<0.05$). Atorvastatin, increased the expression of both ABCB1 and ABCG2 more than two-fold ($p<0.05$). There was no difference in CYP2C9, CYP3A4 or SLCO1B1 mRNA expression between patients administered statins or placebo. Premenopausal women (n=8) had higher expression of CYP3A4 ($p<0.05$) and lower expression of CYP2C9 ($p<0.05$) compared to postmenopausal women (n=10) and men (n=11), respectively. Here we show for the first time that atorvastatin treatment leads to increased expression of the membrane transporters SLCO2B1, ABCB1 and ABCG2 in human liver tissue. This may potentially counteract the efficacy of the treatment and our findings may cast light on mechanisms of clinical problems with adverse reactions and drug interactions in statin treatment.

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, are used for treatment of hypercholesterolemia. Large outcome trials have convincingly demonstrated a preventive effect on serious cardiovascular events by statins (Baigent et al., 2005). Today statins are some of the most prescribed drugs in the western world. Several reports have demonstrated that statin-induced muscular side effects such as myalgia are more common than first predicted in clinical trials. In fact, 10 – 15 % of patients prescribed statins may experience muscle symptoms (Bruckert et al., 2005; Joy and Hegele, 2009) (own unpublished results). The statin induced myotoxicity appears to be dose dependent (Silva et al., 2007) but independent of the cholesterol lowering effect (Abd and Jacobson, 2011). Patients experiencing myopathy were found to have higher plasma concentrations of statins and different statin metabolites (Hermann et al., 2006).

Many statins are metabolized predominantly by hepatic cytochrome P450 (CYP) enzymes. CYP3A4 is the main enzyme involved in the phase I metabolism of lovastatin, simvastatin, atorvastatin and cerivastatin, whereas CYP2C9 is involved in the metabolism of fluvastatin (Toda et al., 2009). It would be expected that, given their important role in statin pharmacokinetics, genetic variation in *CYPs* would influence efficacy and safety.

Disposition of a drug is also strongly influenced by different transport processes. Statins are transported into hepatocytes by the organic anion transporting polypeptide (OATP) encoded by the SLCO genes. SLCO1B1 and SLCO2B1 have been shown to be involved in the influx transportation of statins (Kameyama et al., 2005; Grube et al., 2006; Varma et al., 2011) and genetic variation in *SLCOs* has been recognized in statin induced myotoxicity (Puccetti et al.; Link et al., 2008). ATP-binding cassette B1 (ABCB1, also known as P-gp) and G2 (ABCG2, also known as breast cancer resistance protein) are efflux transporters mediating the cellular efflux of a wide variety of xenobiotics including statins (Hochman et al., 2004; Huang et al., 2006; Frishman and Horn, 2008; Keskitalo et al., 2009). Genetic variation in ABCB1 has been shown to affect the pharmacokinetics of active forms of simvastatin and atorvastatin (Keskitalo et al., 2008).

Recent *in vitro* studies indicate that statins affect the gene expression of SLCOs and ABCs genes (Rodrigues et al., 2006; Rodrigues et al., 2009; Hoffart et al., 2012). However, the statins´ impact on

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

CYPs, SLCOs and ABCs expression in human liver tissues has not been studied. Our study was designed to study the hepatic gene expression of CYP2C9, CYP3A4, SLCO1B1, SLCO2B1, ABCB1 and ABCG2 in patients administrated with 80 mg atorvastatin or 20 mg fluvastatin for 4 weeks.

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Material and methods

Study population

In this study we used hepatic tissue material from a previously performed clinical study (Parini et al., 2008). In this study premenopausal female, postmenopausal female and male patients, scheduled for elective cholecystectomy because of uncomplicated gallstone disease, had been enrolled. Each of the 3 patient groups was randomized to 3 treatment arms: placebo, fluvastatin 20 mg/d or atorvastatin 80 mg/d for 4 weeks before surgery. Twenty-nine RNA samples were available from the original study; placebo (N=9), atorvastatin (N=10) and fluvastatin (N=10). The demographic data of these subjects are shown in Table 1 including the cholesterol, LDL and HDL values at baseline and change during the study period in the different treatment arms. Unfortunately we had no data whether the premenopausal women were taking contraceptives or if postmenopausal women were taking hormonal replacement therapy, which would have been of interest for this study. A biopsy was obtained from the left lobe of the liver at surgery and the specimen was immediately frozen in liquid nitrogen. Inclusion criteria and surgical procedure are described in details elsewhere (Parini et al., 2008). Written informed consent was obtained from all patients before inclusion into the study, which was approved by the Human Ethics Committee of Karolinska Institutet and by the Swedish Medical Products Agency.

Quantitative PCR

Stored RNA prepared from the liver biopsies (Parini et al., 2008) was used. cDNA was synthesized using 1 µg RNA and first strand synthesis kit with a random hexamer primer (GE healthcare, Uppsala). The mRNA levels of CYP2C9, CYP3A4, SLCO2B1, SLCO1B1, ABCB1 and ABCG2 were determined by real-time PCR using Taqman® Gene Expression Assays from Applied Biosystems Assay ID CYP2C9: Hs01682803_mH, CYP3A4: Hs00604506_m1, SLCO1B1: Hs00272374_m1, SLCO2B1: Hs00200670_m1, ABCB1: Hs00184500_m1 and ABCG2: Hs01053790_m1. 18S rRNA was chosen as an endogenous control gene (catalog number #4310893E, Applied Biosystems).

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Quantitative real-time PCR was performed using 7500 Fast Applied Biosystems. Reaction mixture contained 2xTaqman reaction mix (Applied Biosystems), and 1 µl cDNA template (corresponding to 500ng/µl) in a total volume of 15 µl. Thermal cycling conditions included activation at 95 C (10 min) followed by 40 cycles each of denaturation at 95 C (15 s) and annealing/elongation at 60 C (1 min). Each reaction was performed in triplicates and no-template controls were included in each experiment. A control sample (placebo) was employed as a calibrator and the ddCt formula was used as described by Livak (Livak and Schmittgen, 2001).

Statistical analysis

The statistical analysis of the mRNA expression was performed using GraphPad Prism software version 5 (San Diego, CA, USA). Differences among means were analyzed by one-way ANOVA followed by Bonferroni post-hoc test comparing each statin treatment with placebo. Results are presented as mean ±SD in the text and the figures shows mean, 95% CI and min and max values in box whisker plots. Correlation between gene expression and LDL reduction were calculated using Spearman rank

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Results

The mRNA expression of CYP, SLCO and ABC patients treated with statins or placebo

There was no difference in CYP2C9 and CYP3A4 mRNA expression between patients administered statins or placebo as shown in fig 1a and 1b. The inter-individual variation in CYP expression was large. A 225- and 101-fold variation in the relative mRNA expression of CYP2C9 and CYP3A4, respectively, was observed.

There was no difference in SLCO1B1 mRNA expression between patients treated with statins and placebo, fig 1c. Atorvastatin, but not fluvastatin, significantly increased the expression of SLCO2B1 3-fold ($p<0.05$), fig 1d. The mean expression for SLCO2B1 was 1.13 (±0.43), 3.29 (±2.27) and 0.84 (±0.72) for placebo, atorvastatin and fluvastatin, respectively.

Atorvastatin, significantly increased the expression of both ABCB1 and ABCG2 2.4- and 2.2 -fold, respectively ($p<0.05$), fig 1e and 1f. The mean expression of ABC1B1 and ABCG2 was 1.77 (±0.91) and 1.74 (±0.69) in placebo group as compared to 4.25 (±3.98) and 3.70 (±2.55) in atorvastatin treated group. Fluvastatin treatment lead to a minor significant decrease in ABCB1 mRNA expression (0.90±0.41) compared to placebo (1.77±0.91), fig 1e.

The inter-individual variation in the gene expression of the transporters were between 15-40 fold.

Gender and CYP3A4and CYP2C9 mRNA expression

Women in the premenopausal age (28-41 years old) expressed significantly lower levels of CYP2C9 compared to males. In contrast, women in premenopausal age expressed significantly higher CYP3A4 mRNA levels than postmenopausal women (55-76 years old) as shown in figure 2.

Correlation analysis

The ABCB1 and ABCG2 gene expression correlated significantly ($r=0.72$, $p<0.0001$) in this study population. There was a strong a correlation between SLCO2B1 mRNA and ABCB1 levels

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

(r=0.70,p<0.0001) and ABCG2 (r=0.70, p<0.0001) mRNA levels. There was no correlation between the CYP and SLCO genes.

The percentage decrease in LDL in the statin treated patients was negatively associated with SLCO2B1 (r=-0.83, p=0.0005), ABCB1 (r=-0.53, p=0.05) and ABCG2 (r=-0.74, p=0.004). For SLCO1B1 and CYP genes no correlations were found.

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

Here we show that atorvastatin has a large impact on the hepatic gene expression of SLCO2B1, ABC1B1 and ABCG2 in man. A 3- to 4- fold induction of the SLCO2B1 and ABC genes was observed after 4 weeks´ daily treatment with 80 mg atorvastatin. There are no previous *in vivo* studies investigating the impact of statins on the hepatic gene expression of these transporters. In human hepatocytes, atorvastatin exposure was associated with approximately 2-fold induction of ABCB1 and SLCO1B1 genes (Hoffart et al., 2012). Similarly, other *in vitro* studies investigating the expression profile of these genes after atorvastatin exposure in human hepatoma cells (HepG2) have demonstrated an up-regulation of the expression of SLCO2B1 and ABCG2 (Rodrigues et al., 2009), and a down-regulation of the ABCB1 expression (Rodrigues et al., 2006). However in these cell experiments supra-physiological concentrations of statins were used that can never be achieved in humans (Bjorkhem-Bergman et al., 2011). In addition to hepatic studies, it has been shown that atorvastatin treatment for 4 weeks reduced the ABCB1 mRNA expression in mononuclear peripheral blood cells of hypercholesterolemic individuals (Rodrigues et al., 2006). These results indicate that statins may exert tissue specific regulatory mechanisms.

A limitation with this study is that fluvastatin was given at low dose (20 mg) whereas atorvastatin was given at high doses (80 mg). Even though both are in the accepted therapeutic dose range (20-80 mg/day for fluvastatin and 10-80mg/day for atorvastatin), it is possible that a higher dose of fluvastatin would have had an effect on the transporters studied herein. Both active atorvastatin and its inactive lactone metabolite are substrates of SLCO and ABC transporters, whereas fluvastatin is a poor substrate due to it hydrophobicity (Hochman et al., 2004; Chen et al., 2005; Kameyama et al., 2005). Our results indicate that atorvastatin induces several steps in its own elimination, which may further contribute to the inter-individual variability in disposition and therapeutic efficacy of atorvastatin, as previously suggested (Hoffart et al., 2012). In addition to the genes studied here, the expression and activity of other drug metabolizing enzymes may be involved in the elimination of statins. Recent studies indicate that UGTs play an important role in the clearance of atorvastatin (Riedmaier et al., 2010; Stormo et al., 2013). Unfortunately, there are no material left to perform additional gene studies.

It is also important to address that the ultimate outcome is the activity of these enzymes/transporters and that the gene expression not always corresponds to the enzyme activity. For CYP3A4 there is a strong correlation between mRNA levels and enzyme activity whereas for CYP2C9 only weak correlations could be discerned (Rodriguez-Antona et al., 2001). It is likely that the enzyme activities of the proteins studied here would correlate to a higher degree with LDL reduction than can be shown when studying the mRNA levels.

In a meta-analysis of 18 prospective randomized controlled trials the highest rate of muscular adverse effects was found to be associated with use of atorvastatin and the lowest risk with use of fluvastatin (Silva et al., 2006). Pharmacokinetic differences may partly explain this observation. The half-life of atorvastatin is 15-30 h compared to fluvastatin which has a half-life of only 0.5-2.3 h. The statins are metabolized by different CYPs. Fluvastatin is metabolized by CYP2C9 (Toda et al., 2009), whereas atorvastatin is metabolized mainly by CYP3A4. Consequently, the co-use of other pharmacologic agents affecting the activity of CYP3A4 is associated with increased risk of myotoxicity. Many patients not tolerating atorvastatin (or any other CYP3A metabolizing statin) may escape side effects when turning to fluvastatin.

The inter-individual variation in the hepatic gene expression of CYP2C9 and CYP3A4 appears to be large as shown in other studies (Furuya et al., 1991; Wortham et al., 2007). There were no up-regulation of CYP2C9 or CYP3A4 in the statin treated subjects, in contrast to previously in vitro studies showing that atorvastatin up-regulates CYP-enzymes in primary hepatocytes (Kocarek et al., 2002; Monostory et al., 2009). However, in these experiments very high statin concentrations were used; 10 -30 μM; to be compared with 1-10 nM that are achieved in human serum during statin treatment (Bjorkhem-Bergman et al., 2011). On the other hand, the large variability in CYP-expression between the subjects in this study, in addition to the lack of data about hormonal therapy in women and the few samples analysed warrant caution in the interpretation of the results. Previous

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

studies have shown that the gene expression and activity of CYP3A is higher in women than in men (Wolbold et al., 2003; Bjorkhem-Bergman et al., 2013). The reason for this sex difference is not clear, but the sex specific hormonal pattern has been suggested to be involved (Sarkar et al., 2003; Isoherranen and Thummel, 2013; Papageorgiou et al., 2013). In line with this we could see reduced CYP3A4 expression in post-menopausal women, who have lower estrogen levels than premenopausal women. However, we could not see any difference in CYP3A4-expression between men and women in this study, probably due to the limited number of subjects. In contrast to CYP3A4, CYP2C9 has been shown to be down-regulated by estradiol (Mwinyi et al., 2011). This is consistent with our results showing lower levels of hepatic CYP2C9 expression in premenopausal women than in men (Fig 2).

There was a large variability in the statin-induced effect between the subjects as demonstrated in Table 1. Some studies have shown that genetic variations in SLCO1B1 and ABCG2 may affect the efficacy of statins (Generaux et al., 2011; Rodrigues et al., 2011) which could potentially explain the variability observed here. However, it should also be noted that others have failed to find any associations with these genetic variations and response to statins (Deshmukh et al., 2012). It is interesting that our correlation analysis of drug-transporter specific mRNA levels revealed that the gene expression of efflux (ABCB1 and ABCG2) as well as the uptake drug transporter SLCO2B1 was negatively correlated with percent reduction in LDL after statin treatment. The findings that both influx and efflux transporters are up-regulated is difficult to interpret and would expect to cancel out each other. However, the results suggest that the up-regulation of efflux-transporters are dominating since there is a negative correlation with LDL-reduction, indicating lower intracellular concentrations of statins in the hepatocytes.

The co-comitant regulation of expression of SLCO2B1 and ABCB1/G2 transporters indicate that these genes are regulated to large extent by the same hepatic transcription factors. Atorvastatin has been suggested to activate PXR and CAR, two nuclear receptors responsible for inducing transcriptional

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

activity of various genes involved in absorption, distribution, metabolism and excretion of drugs in hepatocytes (Monostory and Dvorak, 2011; Hoffart et al., 2012). Moreover, a decrease in cholesterol results in an activation of sterol regulatory element binding protein 2 (SREBP-2) (Brown and Goldstein, 2009) resulting in an increase in the gene expression of HMGCR and LDLR in the atorvastatin samples (Parini et al., 2008). It is possible that SREBP-2 regulates the expression of the transporters (Kobayashi et al., 2013). However, further studies are warranted to elucidate the mechanism behind the atorvastatin specific regulation of these genes in the liver.

In conclusion, we have for the first time demonstrated an induction of treatment with atorvastatin on efflux and uptake transporters in human liver that may potentially counteract the efficacy of the treatment. Our findings may cast light on mechanisms of therapeutic problems with adverse reactions and drug interactions in statin treatment.

Authors contributions:

Participated in research design: LBB, HB, PP, ME, AR, LE

Conducted experiments: HB, MJ, PP, ME, LE

Performed data analysis: LBB, HB, MJ, PP, ME, LE

Wrote or contributed to the writing of the manuscript: LBB, MJ, PP, ME, AR, LE

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

References

Abd TT and Jacobson TA (2011) Statin-induced myopathy: a review and update. *Expert Opin Drug Saf* **10:**373-387.

Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, and Simes R (2005) Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* **366:**1267-1278.

Bjorkhem-Bergman L, Lindh JD, and Bergman P (2011) What is a relevant statin concentration in cell experiments claiming pleiotropic effects? *Br J Clin Pharmacol* **72:**164-165.

Bjorkhem-Bergman L, Nylen H, Norlin AC, Lindh JD, Ekstrom L, Eliasson E, Bergman P, and Diczfalusy U (2013) Serum Levels of 25-Hydroxyvitamin D and the CYP3A Biomarker 4beta-Hydroxycholesterol in a High-Dose Vitamin D Supplementation Study. *Drug Metab Dispos* **41:**704-708.

Brown MS and Goldstein JL (2009) Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J Lipid Res* **50 Suppl:**S15-27.

Bruckert E, Hayem G, Dejager S, Yau C, and Begaud B (2005) Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients--the PRIMO study. *Cardiovasc Drugs Ther* **19:**403-414.

Chen C, Mireles RJ, Campbell SD, Lin J, Mills JB, Xu JJ, and Smolarek TA (2005) Differential interaction of 3-hydroxy-3-methylglutaryl-coa reductase inhibitors with ABCB1, ABCC2, and OATP1B1. *Drug Metab Dispos* **33:**537-546.

Deshmukh HA, Colhoun HM, Johnson T, McKeigue PM, Betteridge DJ, Durrington PN, Fuller JH, Livingstone S, Charlton-Menys V, Neil A, Poulter N, Sever P, Shields DC, Stanton AV, Chatterjee A, Hyde C, Calle RA, Demicco DA, Trompet S, Postmus I, Ford I, Jukema JW, Caulfield M, and Hitman GA (2012) Genome-wide association study of genetic determinants of LDL-c response to atorvastatin therapy: importance of Lp(a). *J Lipid Res* **53:**1000-1011.

Frishman WH and Horn J (2008) Statin-drug interactions: not a class effect. *Cardiol Rev* **16:**205-212.

Furuya H, Meyer UA, Gelboin HV, and Gonzalez FJ (1991) Polymerase chain reaction-directed identification, cloning, and quantification of human CYP2C18 mRNA. *Mol Pharmacol* **40:**375-382.

Generaux GT, Bonomo FM, Johnson M, and Doan KM (2011) Impact of SLCO1B1 (OATP1B1) and ABCG2 (BCRP) genetic polymorphisms and inhibition on LDL-C lowering and myopathy of statins. *Xenobiotica* **41:**639-651.

Grube M, Kock K, Oswald S, Draber K, Meissner K, Eckel L, Bohm M, Felix SB, Vogelgesang S, Jedlitschky G, Siegmund W, Warzok R, and Kroemer HK (2006) Organic anion transporting polypeptide 2B1 is a high-affinity transporter for atorvastatin and is expressed in the human heart. *Clin Pharmacol Ther* **80:**607-620.

Hermann M, Bogsrud MP, Molden E, Asberg A, Mohebi BU, Ose L, and Retterstol K (2006) Exposure of atorvastatin is unchanged but lactone and acid metabolites are increased several-fold in patients with atorvastatin-induced myopathy. *Clin Pharmacol Ther* **79:**532-539.

Hochman JH, Pudvah N, Qiu J, Yamazaki M, Tang C, Lin JH, and Prueksaritanont T (2004) Interactions of human P-glycoprotein with simvastatin, simvastatin acid, and atorvastatin. *Pharm Res* **21:**1686-1691.

Hoffart E, Ghebreghiorghis L, Nussler AK, Thasler WE, Weiss TS, Schwab M, and Burk O (2012) Effects of atorvastatin metabolites on induction of drug-metabolizing enzymes and membrane transporters through human pregnane X receptor. *Br J Pharmacol* **165:**1595-1608.

Huang L, Wang Y, and Grimm S (2006) ATP-dependent transport of rosuvastatin in membrane vesicles expressing breast cancer resistance protein. *Drug Metab Dispos* **34:**738-742.

Isoherranen N and Thummel KE (2013) Drug Metabolism and Transport During Pregnancy: How Does Drug Disposition Change during Pregnancy and What Are the Mechanisms that Cause Such Changes? *Drug Metab Dispos* **41:**256-262.

Joy TR and Hegele RA (2009) Narrative review: statin-related myopathy. *Ann Intern Med* **150:**858-868.

Kameyama Y, Yamashita K, Kobayashi K, Hosokawa M, and Chiba K (2005) Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+C1007G, by using transient expression systems of HeLa and HEK293 cells. *Pharmacogenet Genomics* **15:**513-522.

Keskitalo JE, Kurkinen KJ, Neuvoneni PJ, and Niemi M (2008) ABCB1 haplotypes differentially affect the pharmacokinetics of the acid and lactone forms of simvastatin and atorvastatin. *Clin Pharmacol Ther* **84:**457-461.

Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ, and Niemi M (2009) ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther* **86:**197-203.

Kobayashi M, Gouda K, Chisaki I, Asada K, Ogura J, Takahashi N, Konishi T, Koshida Y, Sasaki S, Yamaguchi H, and Iseki K (2013) Regulation of multidrug resistance protein 2 (MRP2, ABCC2) expression by statins: Involvement of SREBP-mediated gene regulation. *Int J Pharm*.

Kocarek TA, Dahn MS, Cai H, Strom SC, and Mercer-Haines NA (2002) Regulation of CYP2B6 and CYP3A expression by hydroxymethylglutaryl coenzyme A inhibitors in primary cultured human hepatocytes. *Drug Metab Dispos* **30:**1400-1405.

Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, and Collins R (2008) SLCO1B1 variants and statin-induced myopathy--a genomewide study. *N Engl J Med* **359:**789-799.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:**402-408.

Monostory K and Dvorak Z (2011) Steroid regulation of drug-metabolizing cytochromes P450. *Curr Drug Metab* **12:**154-172.

Monostory K, Pascussi JM, Szabo P, Temesvari M, Kohalmy K, Acimovic J, Kocjan D, Kuzman D, Wilzewski B, Bernhardt R, Kobori L, and Rozman D (2009) Drug interaction potential of 2-((3,4-dichlorophenethyl)(propyl)amino)-1-(pyridin-3-yl)ethanol (LK-935), the novel nonstatin-type cholesterol-lowering agent. *Drug Metab Dispos* **37:**375-385.

Mwinyi J, Cavaco I, Yurdakok B, Mkrtchian S, and Ingelman-Sundberg M (2011) The ligands of estrogen receptor alpha regulate cytochrome P4502C9 (CYP2C9) expression. *J Pharmacol Exp Ther* **338:**302-309.

Papageorgiou I, Grepper S, and Unadkat JD (2013) Induction of Hepatic CYP3A Enzymes by Pregnancy-Related Hormones: Studies in Human Hepatocytes and Hepatic Cell Lines. *Drug Metab Dispos* **41:**281-290.

Parini P, Gustafsson U, Davis MA, Larsson L, Einarsson C, Wilson M, Rudling M, Tomoda H, Omura S, Sahlin S, Angelin B, Rudel LL, and Eriksson M (2008) Cholesterol synthesis inhibition elicits an integrated molecular response in human livers including decreased ACAT2. *Arterioscler Thromb Vasc Biol* **28:**1200-1206.

Puccetti L, Ciani F, and Auteri A Genetic involvement in statins induced myopathy. Preliminary data from an observational case-control study. *Atherosclerosis* **211:**28-29.

Riedmaier S, Klein K, Hofmann U, Keskitalo JE, Neuvonen PJ, Schwab M, Niemi M, and Zanger UM (2010) UDP-glucuronosyltransferase (UGT) polymorphisms affect atorvastatin lactonization in vitro and in vivo. *Clin Pharmacol Ther* **87:**65-73.

Rodrigues AC, Curi R, Britto LR, Rebbechi IM, Hirata MH, Bertolami MC, Bernik MM, Dorea EL, and Hirata RD (2006) Down-regulation of ABCB1 transporter by atorvastatin in a human hepatoma cell line and in human peripheral blood mononuclear cells. *Biochim Biophys Acta* **1760:**1866-1873.

Rodrigues AC, Curi R, Genvigir FD, Hirata MH, and Hirata RD (2009) The expression of efflux and uptake transporters are regulated by statins in Caco-2 and HepG2 cells. *Acta Pharmacol Sin* **30:**956-964.

Rodrigues AC, Perin PM, Purim SG, Silbiger VN, Genvigir FD, Willrich MA, Arazi SS, Luchessi AD, Hirata MH, Bernik MM, Dorea EL, Santos C, Faludi AA, Bertolami MC, Salas A, Freire A, Lareu MV, Phillips C, Porras-Hurtado L, Fondevila M, Carracedo A, and Hirata RD (2011) Pharmacogenetics of OATP Transporters Reveals That SLCO1B1 c.388A>G Variant Is Determinant of Increased Atorvastatin Response. *Int J Mol Sci* **12:**5815-5827.

Rodriguez-Antona C, Donato MT, Pareja E, Gomez-Lechon MJ, and Castell JV (2001) Cytochrome P-450 mRNA expression in human liver and its relationship with enzyme activity. *Arch Biochem Biophys* **393:**308-315.

Sarkar MA, Vadlamuri V, Ghosh S, and Glover DD (2003) Expression and cyclic variability of CYP3A4 and CYP3A7 isoforms in human endometrium and cervix during the menstrual cycle. *Drug Metab Dispos* **31:**1-6.

Silva M, Matthews ML, Jarvis C, Nolan NM, Belliveau P, Malloy M, and Gandhi P (2007) Meta-analysis of drug-induced adverse events associated with intensive-dose statin therapy. *Clin Ther* **29:**253-260.

Silva MA, Swanson AC, Gandhi PJ, and Tataronis GR (2006) Statin-related adverse events: a meta-analysis. *Clin Ther* **28:**26-35.

Stormo C, Bogsrud MP, Hermann M, Asberg A, Piehler AP, Retterstol K, and Kringen MK (2013) UGT1A1*28 is Associated with Decreased Systemic Exposure of Atorvastatin Lactone. *Mol Diagn Ther*.

Toda T, Eliasson E, Ask B, Inotsume N, and Rane A (2009) Roles of different CYP enzymes in the formation of specific fluvastatin metabolites by human liver microsomes. *Basic Clin Pharmacol Toxicol* **105:**327-332.

Varma MV, Rotter CJ, Chupka J, Whalen KM, Duignan DB, Feng B, Litchfield J, Goosen TC, and El-Kattan AF (2011) pH-sensitive interaction of HMG-CoA reductase inhibitors (statins) with organic anion transporting polypeptide 2B1. *Mol Pharm* **8:**1303-1313.

Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, Eichelbaum M, Schwab M, and Zanger UM (2003) Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* **38:**978-988.

Wortham M, Czerwinski M, He L, Parkinson A, and Wan YJ (2007) Expression of constitutive androstane receptor, hepatic nuclear factor 4 alpha, and P450 oxidoreductase genes determines interindividual variability in basal expression and activity of a broad scope of xenobiotic metabolism genes in the human liver. *Drug Metab Dispos* **35:**1700-1710.

Footnotes

Funding: Financial support was provided through the regional agreement on training and clinical research (ALF) between Karolinska Institutet and Stockholm County Council, the Swedish Foundation for Clinical Pharmacology and Pharmacotherapy, unrestricted grants from Pfizer, Swedish Heart and Lung Foundation, Magnus Bergwall Stiftelse, Karolinska Institutet and the Swedish Science Council (04496 AR).

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legends

Figure 1.

Relative expression of A) CYP2C9 B) CYP3A4 C) SLCO1B1 and D) SLCO2B1 E) ABCB1 F) ABCG2 in the liver obtained from individuals treated with placebo (N=9), atorvastatin (ATV) (N=10) or fluvastatin (FLU) (N=10) for 4 weeks. The boxes shows mean, 95% CI and min and max values. ATV treatment increased the mRNA levels of ABCB1, ABCG2 and SLCO2B1. FLU decreased the mRNA levels of ABCB1.

Figure 2

Relative mRNA expression of A) CYP2C9 and B) CYP3A4 in premenopausal women (N=8, age 28-41 years), postmenopausal women (N=10, age 55-76 years) and males (N=11, age 32-68 years). The boxes shows mean, 95% CI and min and max values.CYP2C9 was higher in males as compared to premenopausal women, whereas CYP3A4 is significantly higher in premenopausal women as compared to women postmenopausal.

DMD Fast Forward. Published on June 10, 2013 as DOI: 10.1124/dmd.113.051698
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1: Demographic data of the study cohort. Numbers of women in premenopausal age (28-41 years old), postmenopausal women (55-76 years old) and male (32-68 years old). For age, BMI, cholesterol (chol.), LDL and HDL the numbers show median values and min and max within parenthesis in the different treatment arms.

	All (n=29)	Placebo (n=9)	Atorvastatin (n=10)	Fluvastatin (n=10)
Premenopausal women/ postmen women /male	8 / 10 / 11	3 / 3 / 3	2 / 4 / 4	3 / 3 / 4
Age (years)	55 (28-76)	55 (28-68)	57 (35-76)	50 (30-72)
BMI (kg/m^2)	26.6 (20.9-39.4)	26.6 (20.9-39.4)	25.6 (22.2-36.0)	28.4 (23.0-34.6)
Cholesterol baseline, mmol/L	5.4 (3.3-8.8)	5.1 (3.3-8.8)	5.5 (5.0-7.8)	5.1 (4.0-7.1)
Chol. change during study (min, max)		-5% (+12, -16)	-41% (-33, -65)	-14% (+2, -35)
LDL baseline, mmol/L	3.3 (1.6-6.1)	3.3 (1.6-6.1)	3.4 (2.9-5.5)	3.3 (2.9-4.8)
LDL change in during study (min, max)		-7% (+6, -19)	-65% (-48, -78)	-21% (+3, -58)
HDL baseline, mml/L	1.6 (0.8-1.9)	1.3 (1.0-1.9)	1.6 (1.1-1.7)	1.1 (0.8-1.9)
HDL change % during study (min, max)		0% (+10, -21)	0% (+20, -40)	-12% (+18, -31)

Fig 1

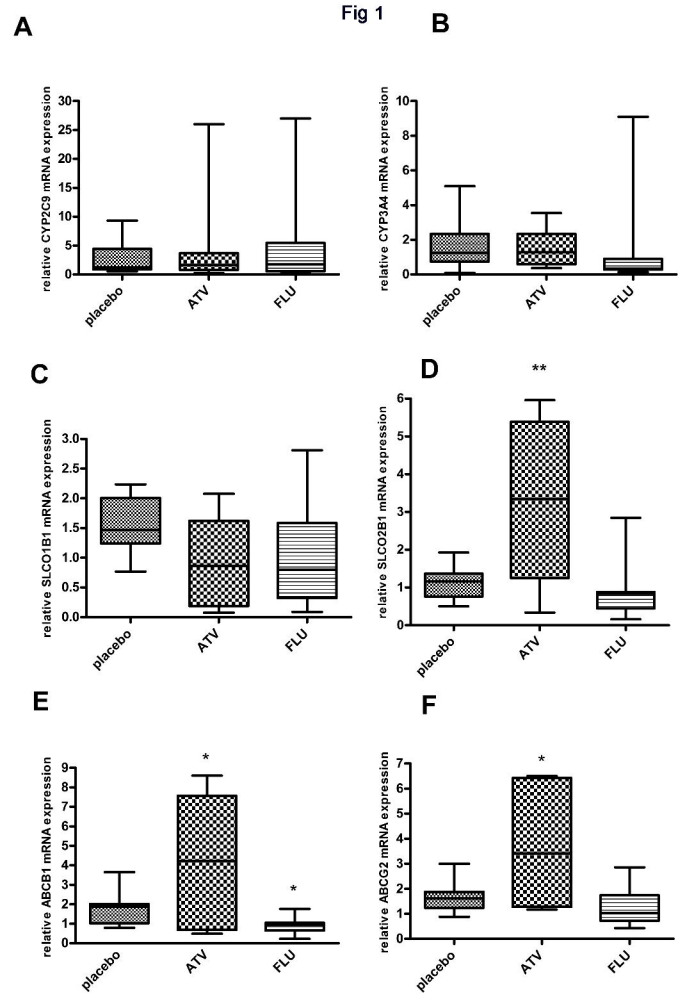
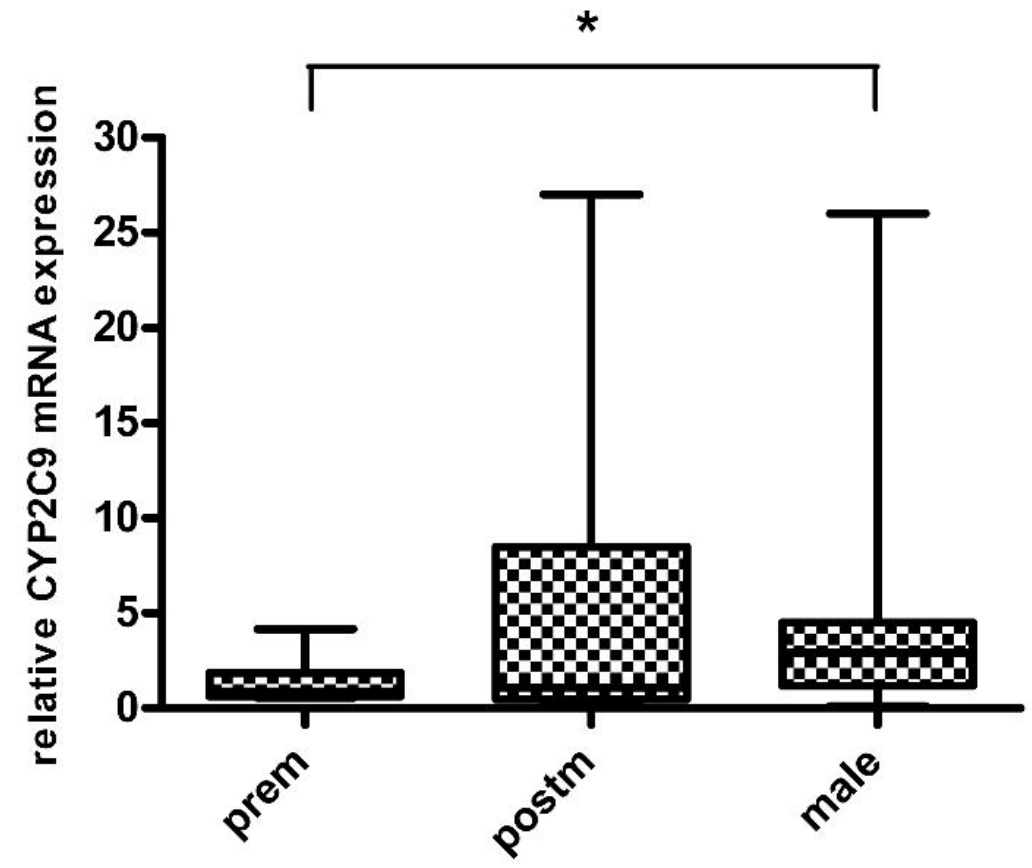
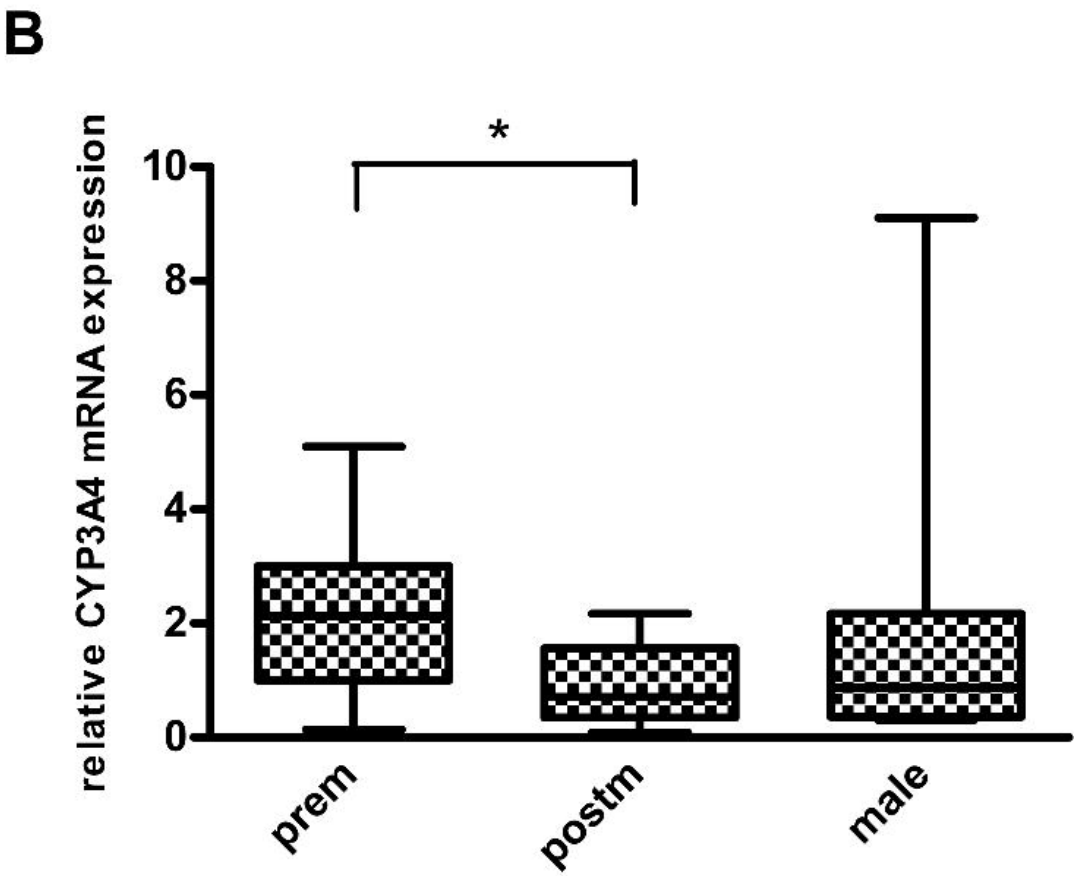

A
B
C
D
E
F
relative CYP2C9 mRNA expression
relative CYP3A4 mRNA expression
relative SLCO1B1 mRNA expression
relative SLCO2B1 mRNA expression
relative ABCB1 mRNA expression
relative ABCG2 mRNA expression
placebo
ATV
FLU
**
*

Fig 2


A
*
relative CYP2C9 mRNA expression
30
25
20
15
10
5
0
prem
postm
male


B
*
relative CYP3A4 mRNA expression
10
8
6
4
2
0
prem
postm
male