



**HAL**  
open science

## Cytochrome P450 3A activity in mothers and their neonates as determined by plasma 4 $\beta$ -hydroxycholesterol

Hanna Nylén, Sofia Sergel, Lisa Forsberg, Synnöve Lindemalm, Leif Bertilsson, Katarina Wide, Ulf Diczfalusy

► **To cite this version:**

Hanna Nylén, Sofia Sergel, Lisa Forsberg, Synnöve Lindemalm, Leif Bertilsson, et al.. Cytochrome P450 3A activity in mothers and their neonates as determined by plasma 4 $\beta$ -hydroxycholesterol. European Journal of Clinical Pharmacology, 2011, 67 (7), pp.715-722. 10.1007/s00228-010-0984-1 . hal-00661255

**HAL Id: hal-00661255**

**<https://hal.science/hal-00661255>**

Submitted on 19 Jan 2012

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Cytochrome P450 3A activity in mothers and their neonates as determined by plasma 4 $\beta$ -hydroxycholesterol

H Nylén\*<sup>1</sup>, S Sergel\*<sup>2</sup>, L Forsberg<sup>2</sup>, S Lindemalm<sup>3</sup>, L Bertilsson<sup>4</sup>, K Wide\*\*<sup>2</sup>, U Diczfalusy\*\*<sup>1</sup>

\* These two first authors have contributed to the same extent

\*\* These two senior authors have contributed to the same extent

### **Affiliations**

<sup>1</sup> Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital, Huddinge, Sweden

<sup>2</sup> Division of Neuropediatrics, Department of CLINTEC, Karolinska Institutet at Karolinska University Hospital, Huddinge, Sweden

<sup>3</sup> Division of Pediatrics, Department of CLINTEC, Karolinska Institutet at Karolinska University Hospital, Huddinge, Sweden

<sup>4</sup> Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital, Huddinge, Sweden

### **Correspondence**

Ulf Diczfalusy, Clinical Chemistry C1-74, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital, Huddinge, 141 86 Stockholm, tel +46-8-585 812 53, fax + 46-8-585 812 60, E-mail: ulf.diczfalusy@karolinska.se

## **Abstract**

### Purpose

The purpose of this study was to determine the 4 $\beta$ -hydroxycholesterol to cholesterol ratio in mothers and neonates at the time of birth and four months post partum.

### Method

21 mothers and 22 neonates were recruited at the delivery ward at Karolinska University Hospital, Huddinge, Sweden. Blood samples taken from mothers and neonates at birth and four months post partum were analyzed for 4 $\beta$ -hydroxycholesterol and cholesterol.

### Results

The median plasma concentration of 4 $\beta$ -hydroxycholesterol was higher in mothers at delivery (50 ng/mL) compared to healthy non-pregnant women (29 ng/mL). The pregnant women had a higher median cholesterol concentration (6.2 mmol/L) compared to healthy non-pregnant women (4.6 mmol/L) but this could only partly explain the increased 4 $\beta$ -hydroxycholesterol. The major cause is an increased CYP3A activity during pregnancy. The median 4 $\beta$ -hydroxycholesterol/cholesterol ratio $\cdot 10^4$  was elevated in mothers at time of birth compared to non-pregnant women (0.19 and 0.15, respectively) but decreased to 0.15 four months post partum. Neonates had a median 4 $\beta$ -hydroxycholesterol/cholesterol ratio $\cdot 10^4$  (0.19) comparable to adults already at birth, but lower 4 $\beta$ -hydroxycholesterol (12 ng/mL) and cholesterol (1.8 mmol/L) concentrations.

### Conclusion

Pregnancy leads to increased CYP3A enzyme activity as determined by the 4 $\beta$ -hydroxycholesterol/cholesterol ratio. Neonates have low 4 $\beta$ -hydroxycholesterol and cholesterol concentrations but similar total CYP3A activity as adults already at birth.

## **Keywords**

4 $\beta$ -hydroxycholesterol, oxysterols, neonate, CYP3A, pregnancy, enzyme activity

## Introduction

About 30% of the cytochrome P450 enzyme content in liver consists of three members of the cytochrome P450 (CYP) 3A subfamily, i.e. CYP3A4, CYP3A5 and CYP3A7 [1].

There is a number of clinical markers, both endo- and exogenous, used to assess the activity of CYP3A, e. g. 6 $\beta$ -hydroxycortisol/cortisol ratio in urine [2][7], plasma midazolam clearance [3][8] and erythromycin breath test [4][9]. However, none of these markers are easy to use in children, elderly, certain vulnerable patient groups or pregnant women. We have previously shown that the cholesterol metabolite 4 $\beta$ -hydroxycholesterol (4b-OHC) in plasma is a marker of CYP3A enzyme activity [5-9][3, 10-13]. The ratio of 4b-OHC/cholesterol instead of 4b-OHC could be used as a marker of CYP3A under conditions where the cholesterol level is expected to change [10-11][14-15]. There is a previous report on a steady increase of total serum cholesterol almost throughout pregnancy (36 weeks) [12][16]. The half lives of both 4b-OHC and cholesterol are long: 17 [8][12] and 72 days [13][18], respectively, making 4b-OHC a useful marker during long term rather than short term studies [14][19]. Another 4-hydroxylated metabolite of cholesterol is 4 $\alpha$ -hydroxycholesterol (4a-OHC) [15][20], which is not formed by CYP3A [9][13]. It is not known if 4a-OHC may be formed enzymatically, but it is known to be formed by autoxidation [15][20].

Pregnancy increases the activity of a number of hepatic enzymes, e. g. CYP3A4 [16-18][21-23], CYP2D6 [18-20][23-25] and CYP2C9 [16][24], while it decreases the activity of some others, such as CYP1A2 [18, 21][23, 26] and CYP2C19 [22][27]. The altered enzyme activities during pregnancy may be due to increased levels of progesterone and estrogen and/or fetal enzyme activity [16][24]. Possibly there is also an effect of the physiological changes that occur, e.g. reduced intestinal movement, altered gastric pH, increased plasma volume, changes in protein binding and increased renal excretion of unchanged drugs [16][24]. However, the mechanisms are not clear and it is likely that different enzymes are affected differently by the above mentioned factors.

There are several chronic conditions for which continuous medication during pregnancy is needed, e. g. epilepsy. For most drugs there is little information about altered dose requirements during pregnancy despite an increasing knowledge about alterations in enzyme activity and increased renal clearance [16][24].

In this study we have monitored the CYP3A activity in mothers and neonates at birth and four months post partum as determined by plasma 4b-OHC.

## **Methods**

### Recruitment of untreated study subjects

21 women, mean age 33 years, range 19-41 years) and their 22 neonates (one pair of fraternal twins, mean birth weight 3699 g, range 2700-4705g) were recruited at the delivery ward at Karolinska University Hospital, Huddinge, Sweden during two months in 2009. The hospital has approximately 5000 deliveries per year and the population in the catchment area consists mainly of Caucasians from Europe and Middle East. The maternal origins of the women in the present study were: Sweden 16, Finland 1, Turkey 1, Iraq 1, Algeria 1, Syria 1, Chile 1; paternal origin: Sweden 16, Algeria 1, Lebanon 1, USA 2, Finland/Germany 1, Iraq 1. Two women were treated with thyroxin for hypothyreosis. The remaining women were not treated with any prescribed or over the counter drugs apart from iron, folic acid and/or multivitamins. Three women had been eating grapefruit occasionally during pregnancy. No one had been using St. John's Wort. Seven women gave birth by elective CS and 14 by vaginal delivery. All neonates (12 girls, 10 boys) had normal Apgar scores, birth weight, birth length and head circumference. pH at delivery ranged 7.16-7.44. All neonates had an uneventful neonatal period and were examined clinically by two of the authors (SS and LF) before they were released from the hospital. There were no abnormal signs in 19 of 22 neonates, while three had neonatal jaundice and one an unspecific heart murmur.

Eleven children were followed up at the age of about 4 months with a clinical examination by SS and LF. None of the children had suffered from any major disease during the first months of life and no one was on drug treatment. Seven children were still at least partly breast fed.

Eleven mothers refused to allow additional blood samples to be drawn from their children for the four month follow up and thus dropped out of the study.

### Recruitment of one carbamazepine-treated mother

A 31 year old woman treated with carbamazepine for epilepsy with general tonic-clonic seizures for the last eight years was also recruited at the delivery ward at Karolinska University Hospital, Huddinge, Sweden. She had been seizure free during the entire pregnancy and one year before. She gave birth by CS due to breech presentation (gestational week 39) to a healthy girl (48 cm, 3570 g, head circumference 36 cm, full Apgar score and normal pH). She and her husband were both of Swedish origin and had one previous child born in 2003.

This mother – child pair dropped out of the study due to parents' refusal of drawing additional blood samples from the child.

All participants gave written informed consent for themselves and their children to participate in the study, which was approved by the ethics committee at Karolinska Institutet, Stockholm.

#### Cohort of healthy adults and non-pregnant healthy women

We used raw data from a previously published study [9][43] to calculate the median values of 4a- and 4b-OHC to cholesterol ratios. The cohort of healthy adults consisted of 49 and 76 healthy men and women, respectively.

#### Blood samples

Venous blood samples were drawn in EDTA containing tubes. After centrifugation the plasma was stored at -70 °C prior to analysis. Mothers and children, elective CS: A venous blood sample was taken from the mother before the surgery. Cord blood was taken immediately after birth. Venous blood samples were also taken from both mothers and children at the four month follow up.

Mothers and children, vaginal delivery: Venous blood samples were taken from both mothers and children at time of neonatal screening when the children were two-three days old. Venous blood was taken from both mothers and children at the four month follow up.

#### Analysis of 4a- and 4b-OHC

4a- and 4b-OHC were determined by isotope-dilution GC-MS as described previously [9][43]. The between day variations (CV) for 4a- and 4b-OHC was 10.8% (at 7.2 ng/mL) and 4.9% (at 26.5 ng/mL), respectively. 4a- and 4b-OHC concentrations of 1 ng/mL correspond to 2.5 nmol/L.

#### Analysis of cholesterol

Cholesterol was determined by an enzymatic method (CHOD-PAPP) run on a Roche/Hitachi Modular instrument. The between day variation was 1.3% (at 5 mmol/L).

#### CYP3A5 genotype

DNA was prepared from whole blood taken from mothers and children in EDTA containing tubes at the four month follow up (QIAamp DNA Blood Midi Kit (Qiagen Ltd, Crawley, UK). Samples were analysed for the

single nucleotide polymorphism A6986G (CYP3A5\*3) as described before [23][28]. When the CYP3A5\*3 allele was not present the allele was designated CYP3A5\*1.

#### Statistical analysis

All statistical calculations were done using the Statistica software, version 8 (StatSoft Inc., Tulsa, OK, USA).

The concentration distribution of 4b-OHC is non-normally distributed which is the reason for the use of Mann-Whitney U test and Wilcoxon Matched Pairs Test in the statistical analyses.

### **Results**

#### Plasma levels of 4b-OHC in mothers and children

Plasma concentrations of 4b-OHC were higher in mothers at time of delivery than in a cohort of healthy non-pregnant women (median 50.0 ng/mL, quartile range, QR=10.7, n=21 and median 28.9 ng/mL, QR=14.4, n=76 [9][13], respectively). Plasma levels of 4b-OHC in mothers at time of delivery and four months post partum are presented in Table 1.

At birth all children had lower plasma 4b-OHC than their mother (Figure 1, upper panel) and also lower than a cohort of healthy adults (median 18.8 ng/mL, QR=8.6, n=22 and median 27.2 ng/mL, QR=13.8, n=125 (12), respectively). The plasma levels 4b-OHC in children increased significantly between birth and the four month follow up (Table 2).

#### Plasma levels of 4a-OHC in mothers and children

Children born by vaginal delivery had twice the plasma levels of 4a-OHC at the time of birth when compared to children born by elective CS (30.2 and 14.1 ng/mL, respectively. Table 2 and Figure 1, lower panel). All children born by vaginal delivery had higher plasma levels of 4a-OHC than their mother which is in contrast with the children born by elective CS, who all had lower plasma 4a-OHC than their mother.

Four months after birth the plasma levels of 4a-OHC in children born by vaginal delivery decreased to the same level as in children born by elective CS (30.2 to 15.1 ng/mL. Table 2).

#### Plasma levels of cholesterol in mothers and children

All mothers had significantly higher plasma levels of cholesterol at the time of delivery than a cohort of healthy non-pregnant women (medians 6.2 and 4.6 mmol/L, respectively. Table 3). Four months post partum the cholesterol levels had decreased to the same levels as in non-pregnant healthy women (Table 3).

Children born by elective CS had a median plasma level of cholesterol of 1.8 mmol/L at birth which had increased to 4.4 mmol/L four months later (Table 2). Unfortunately, we have no data on plasma levels of cholesterol at birth in children born by vaginal delivery due to limited sample volumes. However, four months later the cholesterol levels were similar to the levels in children born by CS (4.0 mmol/L).

#### 4b-OHC/cholesterol ratios in mothers and children

Mothers had significantly higher 4b-OHC/cholesterol ratios  $\cdot 10^4$  around delivery when compared to a cohort of non pregnant healthy women (median 0.19 and 0.15, respectively. Table 3). Four months post partum there was no longer any significant difference between the 4b-OHC/cholesterol ratios in mothers and non-pregnant healthy women.

There was no difference in 4b-OHC/cholesterol ratios  $\cdot 10^4$  at birth between children born by elective CS and a cohort of 125 healthy adults [9][13] (0.19 and 0.15, respectively).

At the age of four months children (n=11) had a significantly higher 4b-OHC/cholesterol ratio  $\cdot 10^4$  than the cohort of healthy adults (0.20 and 0.15, respectively. Mann Whitney,  $p < 0.01$ ). The cohort of 125 healthy adults consisted of 49 men and 76 women, with median concentrations of 4b-OHC and cholesterol 24,2 ng/mL and 4.30 mmol/L, respectively in men and 28.9 ng /mL and 4.58 mmol/L respectively in women. The 4b-OHC/cholesterol ratio  $\cdot 10^4$  in men and women were 0.14 and 0.16, respectively.

#### 4a-OHC/cholesterol ratios in mothers and children

Mothers (n=21) had significantly higher 4a-OHC/cholesterol ratios both at time of delivery and four months post partum when compared to a cohort of non-pregnant healthy women (Table 3).

Children born by elective CS had a significant decrease in the 4a-OHC/cholesterol ratio  $\cdot 10^4$  between birth and the four month follow up ((0.20 to 0.09, n=6, Table 2). However, the ratio  $\cdot 10^4$  was still significantly higher in the children (n=11) than in a cohort of healthy adults (0.09, QR=0.03, n=11 and 0.03, QR=0.01, n=125, respectively. Mann Whitney  $p < 0.001$ ). The 4a-OHC/cholesterol ratio  $\cdot 10^4$  for the 76 healthy women was 0.03.

#### Plasma levels of 4b-, 4a-OHC, cholesterol and 4b- and 4a-OHC/cholesterol ratios after carbamazepine treatment during pregnancy

A mother treated with carbamazepine during the entire pregnancy had a very high plasma level of 4b-OHC two days after an elective CS (392 ng/mL) when compared to untreated mothers (elective CS; 53.2 ng/mL, Table 1).

The level of 4b-OHC in cord blood plasma of the child was higher as compared to children born by elective CS of untreated mothers (76 ng/mL and 12.0 ng/mL, respectively).

The carbamazepine treated mother had a plasma level of 4a-OHC comparable to untreated mothers giving birth by elective CS (18.9 and 16.8 ng/mL, respectively).

The newborn child had a cord blood plasma level of 4a-OHC comparable to children born by elective CS of untreated mothers (19.1 and 14.1 ng/mL, respectively). The carbamazepine treated mother and her child had cholesterol levels of 5.5 and 1.9 mmol/L, respectively.

The 4b-OHC/cholesterol ratio·10<sup>4</sup> in this mother was about nine-fold higher than in untreated mothers (1.8 and 0.19, respectively). The child had a close to five-fold higher 4b-OHC/cholesterol ratio·10<sup>4</sup> when compared to children of untreated mothers (0.99 and 0.19, respectively).

## Discussion

We have previously shown that 4b-OHC is a marker of CYP3A activity and that there is a weak correlation between 4b-OHC and cholesterol [7][3], indicating that it is the CYP3A activity and not the cholesterol concentration which determines the level of 4b-OHC. The use of the 4b-OHC/cholesterol ratio instead of 4b-OHC has been suggested for conditions where the cholesterol concentration is expected to change [10][14]. Pregnancy is one such a condition [12, 24][16, 29].

In the present study we show that pregnancy led to increased CYP3A enzyme activity as determined by higher plasma 4b-OHC/cholesterol ratios in mothers at the time of delivery compared to a cohort of non-pregnant healthy women. The increased enzyme activity during pregnancy has been shown previously using midazolam as a CYP3A marker [17][22]. In that study it was suggested that the CYP3A enzyme activity had returned to baseline within 6-10 weeks post partum which is in line with the present study where there was no difference between 4b-OHC/cholesterol ratios in mothers four months post partum and non-pregnant healthy women.

Other markers of CYP3A enzyme activity have also shown increased enzyme activity during pregnancy, e. g. the dextromethorphan/3-hydroxymorphinan ratio in 24 h urine [18][23] and the 6β-hydroxycortisol/cortisol ratio in 4 h urine [25][30]. However, the dextromethorphan/3-hydroxymorphinan ratio in plasma [19][24] and the 6β-hydroxycortisol/cortisol ratio in spot urine [26][34] showed marginal or no effect on the CYP3A activity during pregnancy.

This may be a result of the different substrate specificities of the different CYP3A enzymes. Midazolam is mainly a marker of CYP3A4 [27][32], while the 4b-OHC/cholesterol ratio and 4b-OHC are markers of the

combined activity of CYP3A4 and CYP3A5 [7, 28][3, 33]. In the present study, only three mothers and three children had one functional CYP3A5\*1 allele, thus expressing CYP3A5. The CYP3A5\*3 allele was determined in only 10 mothers and 11 children due to high drop out rate at the four month follow up when the genotyping was scheduled. Due to the small data set, statistical analyses were not performed.

The plasma 4b-OHC concentration was lower in neonates than in healthy adults. This may be due to the low plasma cholesterol level in the newborns which increased rapidly and more than doubled during the first four months. The 4b-OHC/cholesterol ratio in neonates was comparable to that of healthy adults already at birth and did not change during the next four months. A previous study on CYP3A4 activity in infants reported a low activity at birth and progressively increasing activity reaching 72% of that in adults after one year [29-30][34-35]. Similar findings have been reported by others [31-32][36-37]. The difference in CYP3A activity determined by 4b-OHC or different probe drugs may be explained by different substrate specificities of the CYP3A members. There is no information at present whether CYP3A7 catalyzes the conversion of cholesterol to 4b-OHC in vivo. CYP3A7 is abundant in fetal and neonate livers and may account for more than 50% of total P450 [33][38]. If CYP3A7 is an active cholesterol 4 $\beta$ -hydroxylase the 4b-OHC levels determined would be a sum of CYP3A4, CYP3A5 and CYP3A7 activity. A postnatal decrease in CYP3A7 activity and a concomitant increase in CYP3A4 activity would thus not be possible to monitor with 4b-OHC since the total CYP3A activity would stay approximately constant.

The plasma levels of cholesterol were significantly higher in mothers compared to the cohort of non-pregnant women. Four months post partum the cholesterol levels had declined and were not significantly different from the level in the cohort of non-pregnant women. There are previous reports on a steady increase of total serum cholesterol almost throughout pregnancy [12][46].

An interesting finding in the present study is the increased level of 4a-OHC at birth in children born by vaginal delivery. After four months the 4a-OHC level had decreased to the same level found in children born by CS. 4a-OHC is known to be formed by cholesterol autoxidation [9][43] during oxidative stress, but it is not known if it can be formed enzymatically as well. There are inconsistencies in the literature on the oxidative effect on mother and child by uncomplicated vaginal delivery or CS [34-40][39-45], possibly due to the use of different markers. The elevated levels of 4a-OHC in children born by vaginal delivery may be a consequence of increased oxidative stress during birth. At present nothing is known about potential effects of elevated 4a-OHC levels. If the increased level of 4a-OHC in children born vaginally is due to cholesterol autoxidation, 4b-OHC is expected to

be formed to a similar degree by this mechanism. It is noteworthy that the children born by vaginal delivery had higher 4b-OHC levels at birth than children born by CS.

One possible weakness of our study is the time difference between samples taken from mothers and neonates with elective CS and vaginal delivery; i.e. at birth and two days after birth, respectively. However, rapid changes in the levels of 4b-OHC and cholesterol during a two day period are unlikely due to half lives of several days for both substances [8, 13][12,18]. It should also be noted that the number of observations is small which limits the strength of the statistical analyses.

In conclusion, pregnancy led to increased CYP3A enzyme activity in mothers compared to non-pregnant women, as determined by elevated plasma 4b-OHC/cholesterol ratios.

Neonates had lower 4b-OHC and cholesterol concentrations than adults but the 4b-OHC/cholesterol ratio was comparable to that of adults. 4b-OHC is an endogenous CYP3A marker that can be used in situations where probe drugs can not be given, e.g. in pregnant women and in infants. Comparisons of 4b-OHC and drug metabolism are needed to validate 4b-OHC as a CYP3A marker in neonates.

### **Acknowledgements**

This study was supported by grants from The Swedish Research Council, Medicine (3902), Karolinska Institutets Forskningsstiftelser, the Regional Agreement on Medical Training and Clinical Research (ALF) between Stockholm County Council and the Karolinska Institute (ALF 581107) and Svenska Läkaresällskapet.

### **Conflict of interest/Disclosure**

The authors declare no conflict of interest.

### **References**

- 1 Eichelbaum M, Burk O (2001) CYP3A genetics in drug metabolism. *Nat Med* 7: 285-287
- 2 Galteau MM, Shamsa F (2003) Urinary 6 $\beta$ -hydroxycortisol: a validated test for evaluating drug induction or drug inhibition mediated through CYP3A in humans and in animals. *Eur J Clin Pharmacol* 59: 713-733
- 3 Streetman DS, Bertino JS, Nafziger AN (2000) Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogen* 10: 187-216
- 4 Rivory LP, Slaviero KA, Hoskins JM, Clarke SJ (2001) The erythromycin breath test for the prediction of drug clearance. *Clin Pharmacokinet* 40: 151-158
- 5 Bodin K, Andersson U, Rystedt E, Ellis E, Norlin M, Pikuleva I, Eggertsen G, Björkhem I, Diczfalusy U (2002) Metabolism of 4 $\beta$ -hydroxycholesterol in humans. *J Biol Chem* 277: 31534-31540
- 6 Kanebratt KP, Diczfalusy U, Bäckström T, Sparve E, Bredberg E, Böttiger Y, Andersson TB, Bertilsson L (2008) Cytochrome P450 induction by rifampicin in healthy subjects: determination using the Karolinska cocktail and the endogenous CYP3A4 marker 4 $\beta$ -hydroxycholesterol. *Clin Pharmacol Ther* 84: 589-594
- 7 Diczfalusy U, Miura J, Roh HK, Mirghani RA, Sayi J, Larsson H, Bodin KG, Allqvist A, Jande M, Kim JW, Aklillu E, Gustafsson LL, Bertilsson L (2008) 4 $\beta$ -hydroxycholesterol is a new endogenous CYP3A

- marker: relationship to CYP3A5 genotype, quinine 3-hydroxylation and sex in Koreans, Swedes and Tanzanians. *Pharmacogenet Genomics* 18: 201-208
- 8 Diczfalusy U, Kanebratt KP, Bredberg E, Andersson TB, Böttiger Y, Bertilsson L (2009) 4 $\beta$ -hydroxycholesterol as an endogenous marker for CYP3A4/5 activity. Stability and half-life of elimination after induction with rifampicin. *Br J Clin Pharmacol* 67: 38-43
- 9 Bodin K, Bretillon L, Aden Y, Bertilsson L, Broome U, Einarsson C, Diczfalusy U (2001) Antiepileptic drugs increase plasma levels of 4 $\beta$ -hydroxycholesterol in humans: evidence for involvement of cytochrome P450 3A4. *J Biol Chem* 276: 38685-38689
- 10 Yang Z, Rodrigues AD (2010) Does the long plasma half-life of 4 $\beta$ -hydroxycholesterol impact its utility as a cytochrome P450 3A (CYP3A) metric? *J Clin Pharmacol* doi: 10.1177/0091270009360041
- 11 Tomalik-Scharte D, Lütjohann D, Doroshyenko O, Frank D, Jetter A, Fuhr U (2009) Plasma 4 $\beta$ -hydroxycholesterol: An endogenous CYP3A metric? *Clin Pharmacol Ther* 86: 147-153
- 12 Mazurkiewicz JC, Watts GF, Warburton FG, Slavin BM, Lowy C, Koukkou E (1994) Serum lipids, lipoproteins and apolipoproteins in pregnant non-diabetic patients. *J Clin Pathol* 47: 728-731
- 13 Chobanian AV, Burrows BA, Hollander W (1962) Body cholesterol metabolism in man. II. Measurement of the body cholesterol miscible pool and turnover rate. *J Clin Invest* 41: 1738-1744
- 14 Diczfalusy U, Nylén H, Elander P, Bertilsson L (2010) 4 $\beta$ -Hydroxycholesterol, an endogenous marker of CYP3A4/5 activity in humans. *Br J Clin Pharmacol* doi: 10.1111/j.1365-2125.2010.03773.x
- 15 Breuer O, Dzeletovic S, Lund E, Diczfalusy U (1996) The oxysterols cholest-5-ene-3 $\beta$ ,4 $\alpha$ -diol, cholest-5-ene-3 $\beta$ ,4 $\beta$ -diol and cholestane-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ -triol are formed during in vitro oxidation of low density lipoprotein, and are present in human atherosclerotic plaques. *Biochim Biophys Acta* 1302 145-152
- 16 Anderson GD (2005) Pregnancy-induced changes in pharmacokinetics: A mechanistic-based approach. *Clin Pharmacokinet* 44: 989-1008
- 17 Hebert MF, Easterling TR, Kirby B, Carr DB, Buchanan ML, Rutherford T, Thummel KE, Fishbein DP, Unadkat JD (2008) Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: A University of Washington Specialized Center of Research Study. *Clin Pharmacol Ther* 84: 248-253
- 18 Tracy TS, Venkataramanan R, Glover DD, Caritis SN (2005) Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. *Am J Obstet Gynecol* 192: 633-639
- 19 Wadelius M, Darj E, Frenne G, Rane A (1997) Induction of CYP2D6 in pregnancy. *Clin Pharmacol Ther* 62: 400-407
- 20 Högstedt S, Lindberg BS, Regårdh CG, Moström U, Rane A (1990) The rhesus monkey as a model for studies of pregnancy induced changes in metoprolol metabolism. *Pharmacol Toxicol* 66: 32-36
- 21 Tsutsumi K, Kotegawa T, Matsuki S, Tanaka Y, Ishii Y, Kodama Y, Kuranari M, Miyakawa I, Nakano S (2001) The effect of pregnancy on cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activities in humans. *Clin Pharmacol Ther* 70: 121-125
- 22 McGready R, Stepniowska K, Seaton E, Cho T, Cho D, Ginsberg A, Edstein MD, Ashley E, Looareesuwan S, White NJ, Nosten F (2003) Pregnancy and use of oral contraceptives reduces the biotransformation of proguanil to cycloguanil. *Eur J Clin Pharmacol* 59: 553-557
- 23 Mirghani RA, Sayi J, Aklillu E, Allqvist A, Jande M, Wennerholm A, Eriksen J, Herben VM, Jones BC, Gustafsson LL, Bertilsson L (2006) CYP3A5 genotype has significant effect on quinine 3-hydroxylation in Tanzanians, who have lower total CYP3A activity than a Swedish population. *Pharmacogenet Genomics* 16: 637-645
- 24 Neary RH, Kilby MD, Kumpatula P, Game FL, Bhatnagar D, Durrington PN, O'Brien PM (1995) Fetal and maternal lipoprotein metabolism in human pregnancy. *Clin Sci (Lond)* 88: 311-318
- 25 Kosel BW, Beckerman KP, Hayashi S, Homma M, Aweeka FT (2003) Pharmacokinetics of nelfinavir and indinavir in HIV-1-infected pregnant women. *AIDS* 17: 1195-1199
- 26 Nakamura H, Hasegawa A, Kimura M, Yamagata S, Nakasa H, Osada H, Sekiya S, Ohmori S, Kitada M (1999) Comparison of urinary 6 $\beta$ -hydroxycortisol/cortisol ratio between neonates and their mothers. *Br J Clin Pharmacol* 47: 31-34
- 27 Fromm MF, Schwilden H, Bachmakov I, König J, Bremer F, Schuttler J (2007) Impact of the CYP3A5 genotype on midazolam pharmacokinetics and pharmacodynamics during intensive care sedation. *Eur J Clin Pharmacol* 63: 1129-1133
- 28 Gebeyehu E, Engidawork E, Bijnsdorp A, Aminy A, Diczfalusy U, Aklillu E (2010) Sex and CYP3A5 genotype influence total CYP3A activity: high CYP3A activity and a unique distribution of CYP3A5 variant alleles in Ethiopians. *Pharmacogenomics J* doi: 10.1038/tpj.2010.16
- 29 Johnson TN, Tucker GT, Rostami-Hodjegan A (2008) Development of CYP2D6 and CYP3A4 in the first year of life. *Clin Pharmacol Ther* 83: 670-671

- 30 Blake MJ, Gaedigk A, Pearce RE, Bomgaars LR, Christensen ML, Stowe C, James LP, Wilson JT, Kearns GL, Leeder JS (2007) Ontogeny of dextromethorphan O- and N-demethylation in the first year of life. *Clin Pharmacol Ther* 81: 510-516
- 31 Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, Zaya MJ (2003) Developmental expression of the major human hepatic CYP3A enzymes. *J Pharmacol Exp Ther* 307: 573-582
- 32 Treluyer JM, Bowers G, Cazali N, Sonnier M, Rey E, Pons G, Cresteil T (2003) Oxidative metabolism of amprenavir in the human liver. Effect of the CYP3A maturation. *Drug Metab Dispos* 31: 275-281
- 33 Ring JA, Ghabrial H, Ching MS, Smallwood RA, Morgan DJ (1999) Fetal hepatic drug elimination. *Pharmacol Ther* 84: 429-445
- 34 Fogel I, Pinchuk I, Kupferminc MJ, Lichtenberg D, Fainaru O (2005) Oxidative stress in the fetal circulation does not depend on mode of delivery. *Amer J Obstet Gynecol* 193: 241-246
- 35 Nabhan AF, El-Din LB, Rabie AH, Fahmy GM (2009) Impact of intrapartum factors on oxidative stress in newborns. *J Matern Fetal Neonatal Med* 22: 867-872
- 36 Inanc F, Kilinc M, Kiran G, Guven A, Kurutas E, Cikim IG, Akyol O (2005) Relationship between oxidative stress in cord blood and route of delivery. *Fetal Diagn Ther* 20: 450-453
- 37 Raijmakers MTM, Roes EM, Steegers EAP, van der Wildt B, Peters WHM (2005) Umbilical glutathione levels are higher after vaginal birth than after cesarean section. *J Perinat Med* 31: 520-522
- 38 Paamoni-Keren O, Silberstein T, Burg A, Raz I, Mazor M, Saphier O (2007) Oxidative stress as determined by glutathione (GSH) concentrations in venous cord blood in elective cesarean delivery versus uncomplicated vaginal delivery. *Arc Gynecol Obstet* 276: 43-46
- 39 Vakilian K, Ranjbar A, Zarganjfard A, Mortazavi M, Vosough-Ghanbari S, Mashaiee S, Abdollahi M (2009) On the relation of oxidative stress in delivery mode in pregnant women; A toxicological concern. *Toxicol Mech Methods* 19: 94-99
- 40 Schulpis KH, Lazaropoulou C, Vlachos GD, Partsinevelos GA, Michalakakou K, Gavrili S, Gounaris A, Antsaklis A, Papassotiriou I (2007) Maternal-neonatal 8-hydroxy-deoxyguanosine serum concentrations as an index of DNA oxidation in association with the mode of labour and delivery. *Acta Obstet Gynecol Scand* 86: 320-326

## Figure Legend

Figure 1

Plasma levels of 4b-OHC (upper panel) and 4a-OHC (lower panel) in mothers and neonates. Elective CS = open squares. Vaginal delivery = filled diamonds. All concentrations in ng/mL.

Table 1

Plasma levels of 4b-, 4a-OHC, cholesterol, 4b-, and 4a-OHC/cholesterol ratios in mothers

		Elective CS			Vaginal delivery			p-value
		median	QR	n	median	QR	n	
4b-OHC	delivery	53.2	32.5	7	48.0	10.6	14	n.s.
	4 months	28.6 *	3.7	5	40.5 <sup>a</sup>	7.8	5	0.032
4a-OHC	delivery	16.8	7.3	7	13.5	2.6	14	0.031
	4 months	11.5 *	3.7	5	10.1 *	3.9	5	n.s.
cholesterol	delivery	7.5	2.9	7	6.0	1.1	14	n.s.
	4 months	5.2 *	1.0	5	4.7 *	2.5	5	n.s.
4b-OHC/chol	delivery	0.19	0.06	7	0.20	0.08	14	n.s.
	4 months	0.12 <sup>a</sup>	0.01	5	0.21 <sup>a</sup>	0.07	5	n.s.
4a-OHC/chol	delivery	0.06	0.01	7	0.05	0.02	14	n.s.
	4 months	0.05 <sup>a</sup>	0.01	5	0.05 <sup>a</sup>	0.002	5	n.s.

4b-OHC and 4a-OHC in ng/mL, cholesterol in mmol/L, 4b-OHC/chol and 4a-OHC are multiplied by  $10^4$ . QR = quartile range.

Vaginal delivery: Blood samples were taken at the neonatal screening two-three days after delivery. p-values from Mann Whitney U test. The change in concentrations of 4a-OHC, 4b-OHC and cholesterol from delivery to four months later were statistically tested by Wilcoxon Matched Pairs. p-values are reported by \* ( $p < 0,05$ ), <sup>a</sup> ( $p > 0,05$ ) and in text in section "Plasma levels of cholesterol in mothers and children".

Table 2

Plasma levels of 4b-, 4a-OHC, cholesterol, 4b-, and 4a-OHC/cholesterol ratios in children

		Elective CS			Vaginal delivery			p-value
		median	QR	n	median	QR	n	
4b-OHC	birth	12.0	5.4	8	20.2	4.7	14	0.003
	4 months	43.0 *	16.0	6	25.3 *	16.0	5	n.s.
4a-OHC	birth	14.1	3.5	8	30.2	9.9	14	0.00001
	4 months	16.4 <sup>a</sup>	6.4	6	15.1 *	1.6	5	n.s.
cholesterol	birth	1.8	0.8	8	-	-	-	-
	4 months	4.4 *	0.2	6	4.0	0.6	5	0.030
4b-OHC/chol	birth	0.19	0.07	8	-	-	-	-
	4 months	0.24 <sup>a</sup>	0.10	6	0.18	0.11	5	n.s.
4a-OHC/chol	birth	0.20	0.04	8	-	-	-	-
	4 months	0.09 *	0.04	6	0.97	0.01	5	n.s.

4b-OHC and 4a-OHC in ng/mL, cholesterol in mmol/L, 4b-OHC/chol and 4a-OHC are multiplied by  $10^4$ . QR = quartile range.

Vaginal delivery: Blood samples were taken at the neonatal screening two-three days after birth.

p-values from Mann Whitney U test.

The change in concentrations of 4a-OHC, 4b-OHC and cholesterol from delivery to four months later were statistically tested by Wilcoxon Matched Pairs. p-values are reported by \* ( $p < 0,05$ ), <sup>a</sup> ( $p > 0,05$ ) and in text in section "Plasma levels of cholesterol in mothers and children".

Table 3; Cholesterol levels, 4b- and 4a-OHC/cholesterol ratios in mothers at delivery and in non-pregnant healthy women

		mothers			women, non-pregnant			p-value
		median	QR	n	median	QR	n	
4b-OHC/chol ( $\cdot 10^4$ )	delivery	0.19	0.06	21				0.0009
	4 months	0.15 <sup>a</sup>	0.09	10	0.15	0.07	76	n. s.
4a-OHC/chol ( $\cdot 10^4$ )	delivery	0.06	0.01	21				<0.00001
	4 months	0.05 <sup>*</sup>	0.01	10	0.03	0.01	76	0.001
cholesterol (mmol/L)	delivery	6.2	1.7	21				<0.00001
	4 months	5.2 <sup>*</sup>	2.5	10	4.6	0.9	76	n. s.

QR = quartile range. p-values from Mann Whitney U test. The change in 4b- and 4a-OHC / cholesterol-ratios and cholesterol from delivery to four months later were statistically tested by Wilcoxon Matched Pairs. p-values are reported by <sup>\*</sup> (p<0.05), <sup>\*\*</sup> (p<0.01) and <sup>a</sup> (p>0.05).