DMD Fast Forward. Published on October 18, 2006 as doi:10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Cytochrome P450 and Glutathione *S*-transferase mRNA Expression in Human Fetal Liver Hematopoietic Stem Cells

Jing Shao, Patricia L. Stapleton, Yvonne S. Lin and Evan P. Gallagher

Department of Environmental and Occupational Health Sciences (JS, PLS., EPG.), Department of Pharmaceutics (YSL.), University of Washington, Seattle, WA 98105

Running title: ***CYP* and *GST* expression in fetal stem cells**

Address correspondence to:

Evan P. Gallagher, Ph.D.
School of Public Health and Community Medicine,
Department of Environmental and Occupational Health Sciences,
4225 Roosevelt Way NE, Suite100
University of Washington,
Seattle, WA 98105-6099
Phone (206) 616-4739
Fax: (206) 543-8458
e-mail: *evang3@u.washington.edu*

Number of Text Pages: 21

Number of Tables: 3

Number of Figures: 6

Number of References: 46

Number of Words in:

Abstract: 248

Introduction: 686

Discussion: 1485

Abbreviations: HSC, hematopoietic stem cells; CYP450, cytochrome P450; GST, glutathione-S-transferase; *MLL*, mixed lineage leukemia; PCR, polymerase chain reaction; RT, reverse transcriptase; Q-PCR, quantitative RT-PCR; EROD activity, ethoxyresorufin *O*-deethylase activity.

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

During fetal development, the liver serves as the primary hematopoietic organ in which hematopoietic stem cells (HSC) capable of initiating long-term hematopoiesis comprise a large proportion of the hepatic cell population. Although HSC are potential targets for transplacental chemicals, little is known regarding their xenobiotic biotransformation ability. We quantitated the steady-state mRNA expression of 6 cytochrome P450 (CYP450) and 11 glutathione-S-transferase (GST) isoforms in $CD34^+$-selected HSC isolated from second trimester human fetal liver donors, genotyped donors for polymorphic *hGSTM1* and *hGSTT1* status, and analyzed gene expression in HSC relative to total liver from donors of similar gestational ages. Several CYP isoforms, including *CYP1A1, CYP2E1, CYP3A4,* and *CYP3A5*, were expressed at low levels in HSC (relative mRNA expression *CYP3A5>1A1>2E1>3A4*). *CYP1A2* and *CYP3A7* were not detected in HSC. The *CYP3A4/5* mRNA expression in HSC was accompanied by detectable CYP3A- protein and low midazolam oxidation activity. Several GST isoforms, including *hGSTM1*, *hGSTM2*, *hGSTM4* and *hGSTP1* were significantly higher in HSC as compared to total fetal liver. With the exception of *hGSTA4,* alpha class *GSTs* were not detected in HSC. *GST* expression in HSC was accompanied by substantial GST catalytic activity towards 1-chloro-2,4-dinitrobenzene. In summary, our data indicate that fetal liver $CD34^+$-derived HSC constitutively express several CYP450 isoforms at low levels relative to total hepatic cell populations, but have a higher capacity for GST conjugation reactions through mu and pi class isoforms. The functional ramifications of these observations are discussed relative to the sensitivity of human fetal HSC to transplacental chemical injury.

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

A number of environmental chemicals and drugs have the ability to cross the placental barrier during pregnancy and interact with fetal cell targets (Shu, 1997; Alexander et al., 2001; Woodruff et al., 2004). Because the fetal liver receives a substantial proportion of maternal blood flow during pregnancy, it can be an important target for the exposure to agents that elicit cellular toxicity (Hakkola et al., 1998). However, unlike in adults, the developing fetal liver is not dominated by the presence of hepatocytes, but instead by the presence of hematopoietic cells and precursors, and thus serves as the major site of multi-lineage hematopoiesis (Morrison et al., 1995). Accordingly, a large proportion of the hepatic fetal cell population includes hematopoietic progenitors and hematopoietic stem cells (HSC), which express primitive markers such as $CD34^+$, $CD38^+$ and $CD33^+$, and that can be cell targets for maternally-transferred compounds (Sancewicz-Pach et al., 1997; Moneypenny and Gallagher, 2005). It has been proposed that fetal liver HSC injury during pregnancy may underlie the development of certain hematopoietic disorders that develop after birth (Tavassoli, 1991; Shu, 1997; Alexander et al., 2001; Woodruff et al., 2004).

Our laboratory has shown that $CD34^+$ HSC cultured from human fetal liver are extremely sensitive to the toxicity of certain transplacental chemicals or their metabolites including certain pesticides and anticancer drugs (Moneypenny et al., 2006), and products of oxidative stress (Moneypenny and Gallagher, 2005). In the case of 4-hydroxynonenal, a reactive α,β-unsaturated aldehyde produced *in vivo* and *in utero*, the sensitivity of fetal liver HSC greatly exceeds that of other cell types (Moneypenny and Gallagher, 2005). Furthermore, we have recently shown that exposure to low levels of etoposide, a potent DNA topoisomerase II inhibitor, can cause DNA damage and genetic rearrangements in the mixed

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

lineage leukemia (*MLL*) gene in cultured human fetal liver HSC (Moneypenny et al., 2006). This observation is of particular significance because *MLL* rearrangements are signature lesions in the infant acute leukemias that have been associated with stem cell injury during pregnancy (Armstrong et al., 2002). Collectively, these observations suggest that human fetal HSC may be relevant targets for transplacental chemicals or their metabolites during pregnancy.

Although the mechanistic basis underlying the susceptibility of HSC to chemical toxicity has not been established, given their primitive nature, it is possible that HSC may have a poor ability to detoxify environmental chemicals and drugs via enzymatic biotransformation pathways. In particular, the selective or polymorphic expression of certain cytochrome P450 (CYP450) and glutathione *S*-transferase (GST) isoforms has been associated with susceptibility to a number of drugs and chemicals. For example, the alpha class GST isoform mGSTA3-3 in mice is largely responsible for conferring resistance to the carcinogenic intermediate aflatoxin B_1 8-9 epoxide (Buetler et al., 1992; Eaton and Gallagher, 1994). In addition, elevated fetal CYP2E1 expression has been associated with susceptibility to alcohol teratogenesis (Brzezinski et al., 1999) whereas high CYP1A1 protein expression in lung is associated with increased formation of bulky DNA adducts from tobacco smoke carcinogens and susceptibility for smoking-related lung cancer (Mollerup et al., 2006). While we know that the selective expression of certain xenobiotic metabolizing isoforms can affect *in utero* organotoxicity (Hakkola et al., 1998), there is little information regarding the ability of sensitive $CD34^+$ HSC from human fetal liver to biotransform xenobiotics. The lack of this information represents a data gap in the area of human developmental toxicology and in particular, the origins of hematopoietic disorders that may have an etiology based in part on exposures during pregnancy.

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

In the current study, we have characterized the expression of the predominant *CYP450* and *GST* genes in HSC prepared from a panel of second trimester human fetal livers. Functionality of mRNA expression was confirmed for several gene subfamilies by catalytic activity assays and/or western blotting. In addition, comparisons of gene expression in $CD34^+$ HSC were made to gene expression in the overall fetal liver containing hematopoietic cells and their progenitors as well as hepatocytes in order to better understand the cell origins of *CYP450* and *GST* gene expression during human development, and also the ability for sensitive stem cells to carry out CYP450 and GST-mediated biotransformation.

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

Chemicals and biochemicals

Iscove's Modified Dulbecco's Medium (IMDM), penicillin, streptomycin and heat-inactivated fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). Recombinant human interleukin 3 (IL-3), recombinant human granulocyte colony stimulating factor (G-CSF) and recombinant human stem cell factor (SCF) were obtained from Research Diagnostics Inc. (Flanders, NJ). $CD34^+$ magnetic bead isolation columns were purchased from Miltenyl Biotec (Sunnyvale, CA). Western blotting luminol reagent and horseradish peroxidase-linked goat antirabbit IgG antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Trizol reagent, superscript first strand synthesis kit, formamide, Denhardt's solution, CotI DNA, polyA(72) primer HPLC grade water, TaqMan polymerase, Taq antibody, sequence-specific Q-PCR primers and probes and other molecular biology reagents were purchased from Invitrogen Inc, (Carlsbad, CA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Orlando, FL). Vented culture flasks and 96-well plate were purchased from Corning Inc. (Corning, NY). Trypan blue was purchased from Sigma Chemical Company (St. Louis, MO).

Human liver tissues and isolation of $CD34^+$ HSC

All use of human tissues was approved by the University of Washington Institutional Research Board. Briefly, the human fetal liver tissues (10-20 weeks gestational age) were provided by the University of Washington Birth Defects Research Laboratory and were obtained by elective termination of pregnancy. Some tissues were rinsed in PBS and frozen

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

at -80° prior to total RNA and DNA isolation as described below. Other liver specimens used for $CD34^+$ cell isolations were dissociated under sterile conditions and the resultant total cell population was washed several times in PBS buffer supplemented with 0.3% bovine serum albumin (BSA), 2.5 µg/ml amphotericin B/fungizone and 50 µg/ml gentamicin sulfate. Human fetal liver derived $CD34^+$ HSC were isolated using $CD34^+$ magnetic bead-based separation (Miltenyi Biotec, Sunnyvale, CA). The mononuclear layer was collected by centrifuging over 1.077 g/ml Nycoprep (Life Technologies, Carlsbad, CA) at 400x *g* for 30 min. The $CD34^+$ cells were enriched by magnetic bead separation using the MACS $CD34^+$ isolation (de Wynter et al., 1998), and the purity of the $CD34^+$ HSC averaged >98% as verified by flow cytometry.

After isolation, the $CD34^+$ HSC were seeded at approximately 6250 cells/ml of IMDM containing 15% heat-inactivated FBS, 2 ng/ml IL-3, 1 ng/ml G-CSF, 20 ng/ml SCF, 100 U/ml penicillin and 100 µg/ml streptomycin (Moneypenny and Gallagher, 2005) . The cells were maintained in culture for 7 days, a timeframe which allows for moderate increases in cell number while maintaining a relatively primitive undifferentiated status in a humid chamber at 37 °C in 95% O_2/5% CO_2. HSC under these conditions undergo moderate proliferation and differentiate as a mixed population of several hematopoietic cell lineages. The typical percentage of $CD34^+$, $CD33^+$ and $CD38^+$ on day 7 is approximately 17%, 19% and 68% of total cell populations, respectively, and these populations remain dynamic over the culture period (Moneypenny and Gallagher, 2005). Cell counts and viability were determined over the culture period using a hemocytometer and Trypan blue exclusion, respectively.

Isolation of DNA, RNA and subcellular fractions

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Total genomic DNA from cultured HSC and second trimester fetal livers was extracted using Qiagen DNA extraction kit (Qiagen, Valencia, CA). For the RNA extractions, approximately $4X10^6$ HSC or 200 mg snap frozen fetal liver tissue were used for isolation of total RNA using the Trizol™ reagent according to the manufacturer's instructions. The aqueous layer containing RNA was collected with chloroform, mixed with 70% ethanol and transferred to a Qiagen RNA isolation column. The integrity of the eluted total RNA was verified using an Agilent 2100 Bioanalyzer before we performed quantitative RT-PCR (Q-PCR). For preparation of subcellular fractions, the cultured HSC were collected by centrifugation, washed twice in PBS and resuspended in 300 µl of pre-chilled homogenization buffer containing 250 mM sucrose, 10 mM Tris, 1 mM EDTA, 0.2 mM DTT, and 0.1 mM PMSF, pH 7.4 (added immediately prior to homogenization). The cells were sonicated for 3-5 sec bursts and the homogenates were centrifuged for 15 min at 16,000x*g* at 4°C. The resulting supernatants (S9 fractions containing cytosol and microsomes) were collected and stored at -80°C. The protein concentrations were measured by Bradford Assay using a commercial kit (Sigma Inc, St Louis, MO), with bovine serum albumin used as a standard.

Evaluation of hGSTM1 and hGSTT1 gene deletions

Prior to Q-PCR analysis, HSC and second trimester fetal liver samples were genotyped for *hGSTM1* and *hGSTT1* polymorphic expression/deletion using a multiplexed PCR assay (Sweeney et al., 2000). Briefly, two sets of primers were used to amplify a 215-bp segment of the *hGSTM1* gene and a 480-bp segment of the *hGSTT1* gene. Primers were also included to amplify a 268-bp segment of the ß-globin gene, which was used as a positive control for the PCR. The products were separated by electrophoresis with ethidium bromide-

stained 2.5% agarose gel (ISC BioExpress, Kaysville, UT) and genotyped by visual inspection.

Quantitative PCR analysis of CYP450 and GST mRNA expression

Reverse transcription of 1 to 2 μg total RNA using oligo $d(T)_{15}$ primer and Superscript™ II RNaseH$^-$ (Gibco) was performed according to the manufacturer's instructions. PCR primers and the dual-labeled probes for the specific genes were designed using the primer design software Primer Express™ (Applied Biosystems, Foster City, CA) and verified for specificity using BLAST software. For *CYP2E1* mRNA expression, the gene-specific primers and probe were designed and verified using software by Roche Applied Science (Indianapolis, IN). The PCR primers and/or the corresponding dual-labeled probes were designed to span intron/exon boundaries and are listed in Tables 1 and 2. The probes were labeled at the 5' end with 6-carboxy-fluorescein and at the 3' end with the quenching molecule 6-carboxy-tetramethyl-rhodamine. The PCR mixtures (20 μl final volume) consisted of the appropriate forward and reverse primers (0.35 μM each), 150 nM TaqMan probe and 1X FAST® Universal PCR Master Mix. Gene amplification and detection was measured using the Applied Biosystems 7900HT FAST Real-Time PCR System (Foster City, CA) with the following PCR reaction profile: 1 cycle of 95°C for 15s, 40 cycles of 95°C for 1s, and 62°C for 20s. The relative quantity of target cDNA in each sample was calculated from the experimentally determined C_T value using a standard curve.

Several commonly used housekeeping genes, including GAPDH, β-actin, and 18S RNA, were evaluated for mRNA normalization. A comparison of the steady-state mRNA

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

expression of β-actin and GAPDH revealed that these genes are expressed at markedly higher concentrations in the rapidly dividing HSC relative to total fetal liver, and therefore were not suitable for normalization. The levels of ribosomal 18S RNA expression did not differ among the two groups and was therefore used for subsequent normalization of all *CYP450* and *GST* genes. The measured relative expression levels for the target *CYP450* and *GST* genes were divided by the sample's 18S RNA level and multiplied X 1000 to obtain the normalized mRNA expression values presented in the figures.

CYP and GST catalytic assays

Several CYP and GST catalytic activities were assayed as a means to determine functionality of CYP and GST proteins encoded by the measured mRNAs. Specifically, CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity in HSC S9 fractions was measured using a fluorescent microplate reader (Hahn et al., 1993). To determine the functionality of *CYP3A5* and *CYP3A7* mRNA expression, human fetal HSC were incubated with 12 μM midazolam for 6 hr in a shaking water bath at 37ºC. Each incubation tube contained approximately 20 X 10^6 cells in culture media. Reactions were terminated with the addition of an equal volume of 0.1 M Na_2CO_3, pH 11. ^{15}N-labeled metabolite internal standards were added to the samples and standards before extraction using ethyl acetate. The organic phase was transferred to a clean tube, evaporated to dryness, reconstituted in acetonitrile, and derivatized using *N*-methyl-*N*-(*t*butyl-dimethylsilyl) trifluoroacetamide. Samples and standards were analyzed for 1'-hydroxymidazolam and 4-hydroxymidazolam by negative chemical ionization gas chromatography-mass spectroscopy (Paine et al., 1997). GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) in S9 fractions was

spectrophotometrically measured using 5 mM substrate concentrations in a 96-well microplate reader (Gallagher and Gardner 2002).

Western blotting

Western blotting for the presence of CYP1A- and 3A-reactive proteins in HSC S9 fractions was conducted using human CYP1A2 and CYP3A4 polyclonal antibodies (Oxford Biomedical Research, Oxford, MI) as described (Doi et al., 2002). Forty micrograms of protein from each treatment group were loaded onto a Bio-Rad Criterion 8-16% gradient polyacrylamide gel and size fractionated. The separated proteins were transferred to a PVDF membrane and probed with *anti*-CYP1A2 and *anti*-CYP3A4 polyclonal antibodies, followed by visualization with horseradish peroxidase-linked goat *anti*-rabbit IgG secondary antibody (1:2000 in 1% dry milk/TBS-T). Imaging of the reactive proteins was performed using a BioRaf Fluor-S imager and densitometric scanning.

Statistical analysis

Gene expression data are presented as the mean ± SEM of 6 HSC and 6 fetal liver samples, unless otherwise indicated in those donors that genotyped negative for *hGSTM1* or *hGSTT1*. Differences in *CYP450* and *GST* gene expression among the 6 second trimester liver donors in which tissues were used to isolate $CD34^+$ HSC, and for those donors in which RNA was isolated from total liver, were compared using an unpaired Student's *t*-test (Statview 4.5, Abacus Concepts, Berkeley, CA). Differences in gene expression among the two groups were considered significant at $p \leq 0.05$ or $p \leq 0.001$.

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Results

CYP450 mRNA expression and activity in HSC and fetal liver

As observed in Figures 1 and 2, Q-PCR analysis revealed extremely low overall *CYP450* gene expression in the HSC fractions. We were unable to detect *CYP1A2* and *CYP3A7* transcripts, and transcripts encoding *CYP1A1*, *CYP2E1, CYP3A4 and CYP3A5* mRNAs were present at extremely low levels that marginally exceeded the detection limits of Q-PCR. Consistent with other studies, *CYP1A1* mRNA was expressed at extremely low levels in total fetal liver (Yang et al., 1995), and also as previously reported, *CYP1A2* mRNA was not detected in any of the fetal liver preparations (Yang et al., 1995; Hines and McCarver, 2002). As anticipated, *CYP3A7* was the predominant *CYP450* isoform accounting for the majority of *CYP450* content in the fetal liver samples (Figure 2). Transcripts encoding *CYP3A5* were expressed at appreciable levels in the human fetal livers analyzed, whereas transcripts encoding *CYP3A4* were barely detectable (Figure 2). As shown in Figure 2, *CYP3A5* mRNA levels in fetal liver were 70-fold higher than in HSC, whereas *CYP3A4* mRNA levels were 20-fold higher in fetal liver than in HSC.

As observed in Figure 3, the low *CYP1A1* mRNA expression in HSC was not accompanied by the presence of detectable CYP1A-reactive proteins (Figure 3) or EROD activity (Table 3). In contrast, the extremely low *CYP3A4* and *CYP3A5* expression in HSC was accompanied by low, but detectable, CYP3A- protein cross-reactivity (Figure 3). Detectable CYP3A-mediated midazolam oxidation was observed in S9 fractions from two HSC donors analyzed, and the rates were 0.094 and 0.111 pmol/hr/10^6 cells (Table 3). A human adult liver microsomal preparation was used as a positive control in the midazolam

oxidation studies and yielded a rapid rate of CYP3A-mediated oxidation of 729 pmol/min/mg protein. It should be noted, however, that the midazolam oxidation rates in HSC and adult microsomal samples cannot be directly compared due to unit differences (*i.e,* cells vs mg of microsomal protein).

GST mRNA expression and activity in HSC and fetal liver

As observed in Figure 4, HSC did not appreciably express alpha class isoforms *hGSTA1, hGSTA2 or hGSTA3,* whereas low levels of *hGSTA4* were detected by Q-PCR. *hGSTA4* mRNA expression was 5-fold higher in total fetal liver compared to HSC. All alpha class *GST* isoforms analyzed were readily detected in the fetal livers, with the alpha *GST* expression levels being *hGSTA1>hGSTA2>hGSTA4>hGSTA3*. All five mu class *hGSTs* were detected in both HSC and fetal liver and, for the most part, the mu class *hGSTs* were expressed at higher concentrations in HSC (an exception being the relatively low expression of *hGSTM5*, Figure 5). All HSC and fetal liver samples were genotyped for deletion polymorphism in *hGSTM1* gene, and 3 HSC donors and 2 fetal liver donors were negative for the presence of *hGSTM1* alleles and not used in quantitative comparisons of *hGSTM1 mRNA* expression among the two groups. For individuals expressing at least one copy of *hGSTM1*, we did not distinguish the existence of one allele or both alleles. The level of *hGSTM1* in HSC was 32-fold higher than in fetal liver. The level of *hGSTM2* in HSC was 3-fold higher than in fetal liver, whereas *hGSTM3* mRNA transcripts were relatively abundant and similar in HSC and fetal liver (Figure 5). Interestingly, the expression of *hGSTM4* in HSC was the highest among all five mu class *hGSTs* analyzed. In contrast, *hGSTM5* was least abundant mu class isoform and the level of its expression in HSC was 3.3-fold lower than in fetal liver

(Figure 5). Collectively, the level of expression for the five mu class *hGSTs* in HSC was *hGSTM4>hGSTM1≈hGSTM3>hGSTM2>hGSTM5* (Figure 5).

The comparative expression of *hGSTP1* and *hGSTT1* mRNAs in HSC and fetal liver are presented in Figure 6. *hGSTP1* mRNA was expressed at approximately 6-fold higher levels in HSC relative to the fetal liver. Genotyping for the presence/absence of the *hGSTT1* gene revealed that at least one *hGSTT1* allele was present in all samples analyzed. As with *hGSTM1*, we did not distinguish the number of *hGSTT1* alleles that were present in those individuals. As observed, comparative expression of *hGSTT1* mRNA was similar among HSC and in the total fetal liver. The presence of many of the *GST* mRNA isoforms in HSC was accompanied by readily detectable GST activity towards the overall GST substrate CDNB in S9 fractions from the two donors analyzed. As shown in Table 3, the GST-CDNB activity for the two donors was 93 and 166 nmol/min/mg protein (Table 3).

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

There are a very few reports of the literature of biotransformation enzyme expression in HSC, with a few studies centering upon gene expression and catalytic activities using $CD34^+$ cells from bone marrow or peripheral blood (Czerwinski et al., 1997; Bernauer et al., 2000; Kousalova et al., 2004; Czekaj et al., 2005). For example, CYP2E1 is present in $CD34^+$ stem cells derived from human, rat, and rabbit bone marrow, and human $CD34^+$ cells from blood have functional CYP2E1 activity toward chlorzoxazone (Kousalova et al., 2004). In contrast, CYP3A4 protein or 3A-dependent catalytic activities are not present in human $CD34^+$ cells from peripheral blood (Kousalova et al., 2004). However, differences in gene expression, function, and phenotype exist among $CD34^+$ cells isolated from various human tissue sources, thus necessitating caution in extrapolating drug metabolism capabilities of $CD34^+$-derived cells. Although we did not analyze for the expression of all CYP450 and GST isoforms, our goal was to characterize the major isoforms in the cells which participate in xenobiotic metabolism.

CYP3A7 predominates in human fetal liver from early gestation to six months postnatally (de Wildt et al., 1999; Stevens et al., 2003) and contributes to the regulation of retinoic acid metabolism and estriol levels (Kitada et al., 1987; Marill et al., 2000). The lack of detectable *CYP3A7* mRNA in HSC suggests that fetal hepatocytes or other fetal hepatic cell types are the major source of this CYP450 isoform. CYP3A4 expression is extremely low in human fetal liver but increases postnatally (de Wildt et al., 1999; Stevens et al., 2003). We confirmed that *CYP3A4* mRNA is expressed in HSC at lower levels than in the fetal liver, suggesting a poor capacity of CYP3A4-mediated metabolism in HSC. The relatively high expression of *CYP3A5* in HSC, whose protein product shares some substrate similarities with

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

CYP3A4 (Gorski et al., 1994; Huang et al., 2004), indicates that *CYP3A5* may be more important for drug metabolism in fetal liver $CD34^+$-derived HSC. However, the extremely low CYP3A-mediated midazolam oxidation activity observed in two HSC donors along with the relatively low *CYP3A*- mRNA and protein expression suggests a poor capacity for CYP3A-dependent oxidation of transplacental drugs and chemicals in these hematopoietic cells.

CYP1A1 and CYP1A2 proteins are active in the bioactivation of a number of carcinogens and are typically expressed at low levels during development (Yang et al., 1995; Hines and McCarver, 2002). The fact that the low *CYP1A1* mRNA levels in fetal HSC were not accompanied by detectable CYP1A-related proteins by Western blotting or CYP1A1-dependent EROD activity indicate that the low level of *CYP1A1* mRNA does not result in functional protein in these cells. Alternatively, it is possible that the lack of detectable CYP1A- protein in fetal HSC could be explained by alternative *CYP1A1* gene splicing during translation which occurs in rat and human brain (Chinta et al., 2005). Similar to *CYP1A2*, *CYP2E1* is expressed at low levels in fetal liver with upregulation of expression occurring at birth (Mori et al., 2002). CYP2E1 is the high-affinity liver CYP450 isoform involved in the metabolism and activation of several aliphatic and aromatic halogenated hydrocarbons (Hines and McCarver, 2002; Gonzalez, 2005). *CYP2E1*-null mice develop normally and show no overt deleterious phenotype (Gonzalez, 2005), and thus the physiological function of this isoform during development is unknown. Although we did not analyze for CYP2E1-dependent catalytic activities in HSC, the low *CYP2E1* mRNA expression in fetal liver HSC suggests a poor ability of these cells to metabolize and bioactivate CYP2E1 substrates of toxicological significance. Considering that a host of CYP isoforms involved in the bioactivation of chemical carcinogens (*e.g. CYP1A1, CYP1A2, CYP3A7* and *CYP2E1*) display either low expression, or are not detectable in fetal liver HSC, we would hypothesize that these cells

have a poor capacity to bioactivate chemical carcinogens through these pathways. We are currently exploring these questions in catalytic studies.

Our laboratory, as well as others, have shown that several alpha-, mu-, and pi- class GST proteins are abundant in human fetal liver tissues (Mera et al., 1994; Gallagher and Gardner, 2002). In the current study, *GST* mRNA expression profiles in HSC markedly contrasted those observed for the various CYP450s in that a number of *GST*s were expressed at higher levels than in fetal liver. Several of the human fetal donors analyzed for *hGSTM1* polymorphisms exhibited gene deletions, with the percentage of gene deletion consistent with general Caucasian population (50%) (Dagnino-Subiabre et al., 2000; Hayes et al., 2005). In *hGSTM1* null individuals, the absence of functional hGSTM1-1 protein could potentially impact the ability of cells to metabolize PAH metabolites formed *in utero*. Of particular note was the relatively higher expression of *hGSTM1*, *hGSTM2*, and *hGSTM4* mRNAs in HSC relative to the total fetal liver. In fact, $CD34^+$ HSC are likely major origins of mu class GST during second trimester in fetal liver, thus indicating an important function for these proteins in HSC. Little is known regarding the substrate specificity for mu class GST isoforms other than hGSTM1-1. hGSTM2-2 conjugates 1,2-dichloro-4-nitrobenzene, aminochrome, and dopa *O*-quinone (Dagnino-Subiabre et al., 2000; Hayes et al., 2005) with GSH. There is less known about the preferred substrates of hGSTM3-3, hGSTM4-4 and hGSTM5-5, although these isoforms can conjugate for general GST substrate CDNB (Patskovsky et al., 2000).

Of note was our observation of high expression of *hGSTP1* mRNA in HSC, which exceeded that observed in fetal liver. As discussed, hGSTP1-1 and hGSTA1-1 are the major human fetal liver GST isoforms. Interindividual differences in hGSTP1-1 protein expression

have been linked to susceptibility against the mutagenic action of a number of carcinogens and their intermediates (reviewed in (Hayes and Pulford, 1995; Eaton and Bammler, 1999)). When considered in the context of the absence, or relatively low expression of CYP1A1 and other bioactivating CYP450 isoforms, we would hypothesize that human HSC would not form bulky DNA adducts from certain carcinogens. It was also previously reported that at least one theta-like GST is present at second trimester in human fetal liver (Mera et al., 1994). Our results demonstrate that *hGST1* mRNA is expressed at appreciable levels in the total fetal liver and also in the HSC sub-populations. The hGSTT1-1 protein is active in the metabolism of several pesticides and may partially protect fetal liver HSC from transplacental pesticide exposure (Hayes and Pulford, 1995). However, there does not appear to be a single class of chemicals preferentially metabolized by hGST1-1, and this isoform bioactivates certain industrial agents such as methyl bromide and methylene chloride to genotoxic metabolites which may affect susceptibility to genotoxic injury from these compounds (Hallier et al., 1993; Kempkes et al., 1996).

With the exception of *hGSTA4*, the alpha class GSTs were not appreciably expressed in HSC. The low, but detectable *hGSTA4* expression in HSC is consistent with our earlier report of low expression of *hGSTA4* mRNA in 70 human tissues and human cell lines (Gallagher and Gardner, 2002). Of the alpha class GSTs, hGSTA4-4 is the high affinity isoform that conjugates 4-hydroxynonenal (Hubatsch et al., 1998). The extremely low expression of *hGSTA4* mRNA in HSC is consistent with the sensitivity of these cells to 4-hydroxynonenal toxicity (Moneypenny and Gallagher, 2005). We also previously reported relatively high expression of *hGSTA1* mRNA and hGSTA1-1 catalytic activities in second trimester human fetal liver (Gallagher and Gardner, 2002). It is of interest that hGSTA1 is not expressed in fetal liver HSC, as this alpha class GST has comparatively high constitutive

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

expression in most human tissues (Gallagher and Gardner, 2002). It has been reported that *hGSTA1* mRNA is not expressed in $CD34^+$ cells derived from human bone marrow (Czerwinski et al., 1997). Because the hGSTA1-1, hGSTA2-2 and hGSTA4-4 proteins are the dominant GST isoforms that protect against oxidative damage (Hayes and Pulford, 1995; Hubatsch et al., 1998), lack of these isoforms in HSC suggests that GST may not constitute an important pathway of protection against byproducts of oxidative stress in these cells. hGSTA3-3 is expressed in steroidogenic tissues and catalyzes the GSH-dependent isomerization of steroids (Johansson and Mannervik, 2001). Thus, hGSTA3-3 may play a role in regulation of steroid hormone biosynthesis. Polymorphisms in the coding region of *hGSTA3* may modulate the effectiveness of hGSTA3-3 in steroid hormone synthesis (Johansson and Mannervik, 2001; Tetlow et al., 2004). If the primary role of this GST isoform lies in steroid biosynthesis, we would not expect to find appreciable *hGSTA3* mRNA expression in primitive HSC due to hematopoietic functionality of these cells.

In summary, our studies indicate an overall low expression profile in human fetal liver HSC for many CYP450 isoforms which confer chemical metabolic capabilities in other tissues. In contrast, we observed relatively high expression of certain *GST* isoforms and functional GST catalytic activity indicating that HSC have the potential to conjugate reactive intermediates and thus reduce the potential for DNA damage in these sensitive cells. Ongoing studies in our laboratory are directed towards a better understanding the role of xenobiotic metabolizing and conjugating enzymes in the susceptibility of HSC to chemicals of relevance *in utero*.

References

Alexander FE, Patheal SL, Biondi A, Brandalise S, Cabrera ME, Chan LC, Chen Z, Cimino G, Cordoba JC, Gu LJ, Hussein H, Ishii E, Kamel AM, Labra S, Magalhaes IQ, Mizutani S, Petridou E, de Oliveira MP, Yuen P, Wiemels JL and Greaves MF (2001) Transplacental chemical exposure and risk of infant leukemia with MLL gene fusion. *Cancer Res* **61:**2542-2546.

Armstrong SA, Staunton JE, Silverman LB, Pieters R, den Boer ML, Minden MD, Sallan SE, Lander ES, Golub TR and Korsmeyer SJ (2002) MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet* **30:**41-47.

Bernauer U, Vieth B, Ellrich R, Heinrich-Hirsch B, Janig GR and Gundert-Remy U (2000) CYP2E1 expression in bone marrow and its intra- and interspecies variability: approaches for a more reliable extrapolation from one species to another in the risk assessment of chemicals. *Arch Toxicol* **73:**618-624.

Brzezinski MR, Boutelet-Bochan H, Person RE, Fantel AG and Juchau MR (1999) Catalytic activity and quantitation of cytochrome P-450 2E1 in prenatal human brain. *J Pharmacol Exp Ther* **289:**1648-1653.

Buetler TM, Slone D and Eaton DL (1992) Comparison of the aflatoxin B1-8,9-epoxide conjugating activities of two bacterially expressed alpha class glutathione S-transferase isozymes from mouse and rat. *Biochem Biophys Res Commun* **188:**597-603.

Chinta SJ, Kommaddi RP, Turman CM, Strobel HW and Ravindranath V (2005) Constitutive expression and localization of cytochrome P-450 1A1 in rat and human brain: presence of a splice variant form in human brain. *J Neurochem* **93:**724-736.

Czekaj P, Wiaderkiewicz A, Florek E and Wiaderkiewicz R (2005) Tobacco smoke-dependent changes in cytochrome P450 1A1, 1A2, and 2E1 protein expressions in fetuses, newborns, pregnant rats, and human placenta. *Arch Toxicol* **79:**13-24.

Czerwinski M, Kiem HP and Slattery JT (1997) Human CD34+ cells do not express glutathione S-transferases alpha. *Gene Ther* **4:**268-270.

Dagnino-Subiabre A, Cassels BK, Baez S, Johansson AS, Mannervik B and Segura-Aguilar J (2000) Glutathione transferase M2-2 catalyzes conjugation of dopamine and dopa o-quinones. *Biochem Biophys Res Commun* **274:**32-36.

de Wildt SN, Kearns GL, Leeder JS and van den Anker JN (1999) Cytochrome P450 3A: ontogeny and drug disposition. *Clin Pharmacokinet* **37:**485-505.

de Wynter EA, Buck D, Hart C, Heywood R, Coutinho LH, Clayton A, Rafferty JA, Burt D, Guenechea G, Bueren JA, Gagen D, Fairbairn LJ, Lord BI and Testa NG (1998) CD34+AC133+ cells isolated from cord blood are highly enriched in long-term culture-

initiating cells, NOD/SCID-repopulating cells and dendritic cell progenitors. *Stem Cells* **16:**387-396.

Doi AM, Patterson PE and Gallagher EP (2002) Variability in aflatoxin B(1)-macromolecular binding and relationship to biotransformation enzyme expression in human prenatal and adult liver. *Toxicol Appl Pharmacol* **181:**48-59.

Eaton DL and Bammler TK (1999) Concise review of the glutathione S-transferases and their significance to toxicology. *Toxicol Sci* **49:**156-164.

Eaton DL and Gallagher EP (1994) Mechanisms of aflatoxin carcinogenesis. *Annu Rev Pharmacol Toxicol* **34:**135-172.

Gallagher EP and Gardner JL (2002) Comparative expression of two alpha class glutathione S-transferases in human adult and prenatal liver tissues. *Biochem Pharmacol* **63:**2025-2036.

Gonzalez FJ (2005) Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. *Mutat Res* **569:**101-110.

Gorski JC, Hall SD, Jones DR, VandenBranden M and Wrighton SA (1994) Regioselective biotransformation of midazolam by members of the human cytochrome P450 3A (CYP3A) subfamily. *Biochem Pharmacol* **47:**1643-1653.

Hahn ME, Lamb TM, Schultz ME, Smolowitz RM and Stegeman JJ (1993) Cytochrome P4501A induction and inhibition by 3,3',4,4'-tetrachlorobiphenyl in an Ah receptor-containing fish hepatoma cell line (PLHC-1). *Aquatic Toxicology* **26:**185-208.

Hakkola J, Pelkonen O, Pasanen M and Raunio H (1998) Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit: role in intrauterine toxicity. *Crit Rev Toxicol* **28:**35-72.

Hallier E, Langhof T, Dannappel D, Leutbecher M, Schroder K, Goergens HW, Muller A and Bolt HM (1993) Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. *Arch Toxicol* **67:**173-178.

Hayes JD, Flanagan JU and Jowsey IR (2005) Glutathione transferases. *Annu Rev Pharmacol Toxicol* **45:**51-88.

Hayes JD and Pulford DJ (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* **30:**445-600.

Hines RN and McCarver DG (2002) The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. *J Pharmacol Exp Ther* **300:**355-360.

Huang W, Lin YS, McConn DJ, 2nd, Calamia JC, Totah RA, Isoherranen N, Glodowski M and Thummel KE (2004) Evidence of significant contribution from CYP3A5 to hepatic drug metabolism. *Drug Metab Dispos* **32:**1434-1445.

Hubatsch I, Ridderstrom M and Mannervik B (1998) Human glutathione transferase A4-4: an alpha class enzyme with high catalytic efficiency in the conjugation of 4-hydroxynonenal and other genotoxic products of lipid peroxidation. *Biochem J* **330 (Pt 1):**175-179.

Johansson AS and Mannervik B (2001) Human glutathione transferase A3-3, a highly efficient catalyst of double-bond isomerization in the biosynthetic pathway of steroid hormones. *J Biol Chem* **276:**33061-33065.

Kempkes M, Wiebel FA, Golka K, Heitmann P and Bolt HM (1996) Comparative genotyping and phenotyping of glutathione S-transferase GSTT1. *Arch Toxicol* **70:**306-309.

Kitada M, Kamataki T, Itahashi K, Rikihisa T and Kanakubo Y (1987) P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16 alpha-hydroxylase of dehydroepiandrosterone 3-sulfate. *J Biol Chem* **262:**13534-13537.

Kousalova L, Anzenbacherova E, Baranova J, Anzenbacher P, Skoumalova I and Vondrakova J (2004) Presence of cytochrome P450 enzymes in human CD34+ haematopoietic progenitor cells. *Gen Physiol Biophys* **23:**251-257.

Marill J, Cresteil T, Lanotte M and Chabot GG (2000) Identification of human cytochrome P450s involved in the formation of all-trans-retinoic acid principal metabolites. *Mol Pharmacol* **58:**1341-1348.

Mera N, Ohmori S, Itahashi K, Kiuchi M, Igarashi T, Rikihisa T and Kitada M (1994) Immunochemical evidence for the occurrence of Mu class glutathione S-transferase in human fetal livers. *J Biochem (Tokyo)* **116:**315-320.

Mollerup S, Berge G, Baera R, Skaug V, Hewer A, Phillips DH, Stangeland L and Haugen A (2006) Sex differences in risk of lung cancer: Expression of genes in the PAH bioactivation pathway in relation to smoking and bulky DNA adducts. *Int J Cancer* **119:**741-744.

Moneypenny CG and Gallagher EP (2005) 4-Hydroxynonenal inhibits cell proliferation and alters differentiation pathways in human fetal liver hematopoietic stem cells. *Biochem Pharmacol* **69:**105-112.

Moneypenny CG, Shao J, Song Y and Gallagher EP (2006) MLL rearrangements are induced by low doses of etoposide in human fetal hematopoietic stem cells. *Carcinogenesis* **27:**874-881.

Mori Y, Koide A, Kobayashi Y, Morimura K, Kaneko M and Fukushima S (2002) Effect of ethanol treatment on metabolic activation and detoxification of esophagus carcinogenic N-nitrosamines in rat liver. *Mutagenesis* **17:**251-256.

Morrison SJ, Uchida N and Weissman IL (1995) The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol* **11:**35-71.

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD and Thummel KE (1997) Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* **283:**1552-1562.

Patskovsky YV, Patskovska LN and Listowsky I (2000) The enhanced affinity for thiolate anion and activation of enzyme-bound glutathione is governed by an arginine residue of human Mu class glutathione S-transferases. *J Biol Chem* **275:**3296-3304.

Sancewicz-Pach K, Groszek B, Pach D and Klys M (1997) Acute pesticides poisonings in pregnant women. *Przegl Lek* **54:**741-744.

Shu XO (1997) Epidemiology of childhood leukemia. *Curr Opin Hematol* **4:**227-232.

Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ and Zaya MJ (2003) Developmental expression of the major human hepatic CYP3A enzymes. *J Pharmacol Exp Ther* **307:**573-582.

Sweeney C, Farrow DC, Schwartz SM, Eaton DL, Checkoway H and Vaughan TL (2000) Glutathione S-transferase M1, T1, and P1 polymorphisms as risk factors for renal cell carcinoma: a case-control study. *Cancer Epidemiol Biomarkers Prev* **9:**449-454.

Tavassoli M (1991) Embryonic and fetal hemopoiesis: an overview. *Blood Cells* **17:**269-281; discussion 282-266.

Tetlow N, Coggan M, Casarotto MG and Board PG (2004) Functional polymorphism of human glutathione transferase A3: effects on xenobiotic metabolism and steroid biosynthesis. *Pharmacogenetics* **14:**657-663.

Woodruff TJ, Axelrad DA, Kyle AD, Nweke O, Miller GG and Hurley BJ (2004) Trends in environmentally related childhood illnesses. *Pediatrics* **113:**1133-1140.

Yang HY, Namkung MJ and Juchau MR (1995) Expression of functional cytochrome P4501A1 in human embryonic hepatic tissues during organogenesis. *Biochem Pharmacol* **49:**717-726.

Footnotes

Financial support. This work was supported in part by grants from NIH (R01-ES09427, P30-ES07033), the USEPA STAR program (R-827441), the National Oceanic and Atmospheric Administration Coastal Ocean Program (NA05NS4781253), the University of Washington Royalty Research Fund, the University of Washington Birth Defects Research, (NIH- HD000836) and by the NIH Roadmap/National Center for Research Resources (K12-8K12RR023265-02).

Reprint request. Evan P. Gallagher, 4225 Roosevelt Way NE, Suite100, Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA 98105-6099

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Figure legends

Figure 1. Comparative *CYP1A1, CYP1A2 and CYP2E1* gene expression in human HSC and fetal livers. (A) *CYP1A1*, (B) *CYP1A2* and (C) *CYP2E1* mRNA expression. Data represent mean ± SEM of 6 individuals with all gene expression data normalized to the levels of 18S RNA in each sample. Asterisks denote significant differences in gene expression among HSC and fetal liver samples at $p \leq 0.001$.

Figure 2. Comparative *CYP3A4*, *CYP3A5* and *CYP3A7* gene expression in human HSC and fetal livers. (A) *CYP3A4*, (B) *CYP3A5* and (C) *CYP3A7* mRNA expression. Data represent mean ± SEM of 6 individuals with all gene expression data normalized to the levels of 18S RNA in each sample. Asterisks denote significant differences in gene expression among HSC and fetal liver samples at $p \leq 0.001$.

Figure 3. Western blotting of CYP1A- and 3A- immunoreactive proteins in HSC. HSC S9 (40 µg each blot) were probed using *anti* human CYP1A2 and CYP3A4 antibodies. Liver microsomes from an adult donor (HAL, 5 µg for CYP3A- and 20 µg for CYP1A- detection) were used as a positive control for the Western blots.

Figure 4. Comparative alpha class *GST* gene expression in human HSC and fetal livers. (A) *hGSTA1*, (B) *hGSTA2,* (C) *hGSTA3* and (D) *hGSTA4* mRNA expression. Data represent mean ± SEM of 6 individuals with all gene expression data normalized to the levels of 18S RNA in each sample. Asterisks denote significant differences in gene expression among HSC and fetal liver samples at $p \leq 0.001$.

Figure 5. Comparative mu class *GST* gene expression in human HSC and fetal livers. (A) *hGSTM1*, (B) *hGSTM2*, (C) *hGSTM3*, (D) *hGSTM4* and (E) *hGSTM5* mRNA expression. Data represent mean ± SEM of 6 individuals, except for polymorphic *hGSTM1* where at least one allele was present in n=3 HSC donors and n=4 fetal liver donors. All gene expression data was normalized to the levels of 18S RNA. Asterisks denote significant differences in gene expression among HSC and fetal liver samples at $p \leq 0.001$.

Figure 6. Comparative *hGSTP1 and hGSTT1* gene expression in human HSC and fetal livers. (A) *hGSTP1* and (B) *hGSTT1* mRNA expression. Data represent the mean ± SEM of 6 individuals and all gene expression data were normalized to the levels of 18S RNA in each sample. Asterisks denote significant differences in gene expression among HSC and fetal liver samples at $p \leq 0.05$.

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. Primer pairs and probes used to measure human *CYP* genes by quantitative PCR

Gene (Genebank accession number)	**Type of oligo**	**Sequence (5'→3')**	**Position**
CYP1A1	primer (forward)	accttccgacactcttccttcg	1245-1266
(XM_113272)	primer (reverse)	cagatgggttgacccatagcttct	1398-1375
	probe	cttcaccatcccccacagcacaacaag	1271-1297
CYP1A2	primer (forward)	ccttccgacactcctccttcttg	1188-1210
(NM_000761)	primer (reverse)	gggatgtagaagccattcagcg	1269-1248
	probe	cttcaccatcccccacagcacaacaag	1213-1239
CYP2E1	primer (forward)	caagccattttccacagga	1289-1307
(AF182276)	primer (reverse)	caacaaaagaaacaactccatgc	1361-1339
	probe	tgctggag	1319-1326
CYP3A4	primer (forward)	cacagatccccctgaaattaagctta	1516-1541
(NM_017460)	primer (reverse)	aaaattcaggctccacttacggtg	1621-1598
	probe	aggacttcttcaaccagaaaaacccgttgttct	1544-1576
CYP3A5	primer (forward)	acagatccccttgaaattagacacg	1497-1521
(NM_000777)	primer (reverse)	cttagggttccatctcttgaatcca	1586-1562
	probe	aaggacttcttcaaccagaaaaacccattgttcta	1523-1557
CYP3A7	primer (forward)	tcagaacttctccttcaaaccttgtaa	1485-1511
(NM_000765)	primer (reverse)	gcctttagaacaatgggtttttctgtta	1583-1556
	probe	aaacacagatccccctgaaattacgctttggag	1514-1546
Beta-actin	primer (forward)	aaccccaaggccaaccg	372-388
(X00351)	primer (reverse)	agggatagcacagcctgga	466-448
	probe	atgacccagatcatgtttgagaccttcaacac	396-427
GAPDH	primer (forward)	tcctgcaccaccaactgctt	503-522
(BC08351)	primer (reverse)	gaggggccatccacagtctt	627-608
	probe	actcatgaccacagtccatgccatcac	571-597
18S	primer (forward)	ctcaacacgggaaacctcac	1247-1266
(X03205)	primer (reverse)	cgctccaccaactaagaacg	1356-1337
	probe	ggtggtgg	1321-1328

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2. Primer pairs and probes used to measure human *GST* genes by quantitative PCR.

Gene(Genebank accession number)	**Type**	**Sequence (5'→3')**	**Position**
hGSTA1	primer (forward)	gactccagtcttatctccagcttcc	616-640
(NM_145740)	primer (reverse)	tgcttcttctaaagatttctcatccat	753-727
	probe	tgaaggccctgaaaaccagaatcagcaa	647-674
hGSTA2	primer (forward)	tggaagagcttgactctagccttatt	613-638
(BC002895)	primer (reverse)	ggctgccaggctgtagaaac	720-701
	probe	ctgaaggccctgaaaaccagaatcagtaacc	654-684
hGSTA3	primer (forward)	ctctactatgtggaagagcttgactcca	569-596
(NM_000847)	primer (reverse)	tgcatctgcgggaggctt	709-692
	probe	tgaaggccctgaaaaccagaatcagcaa	621-648
hGSTA4	primer (forward)	aatcagctgagccttgcagatg	563-584
(NM_001512)	primer (reverse)	ttgctgccaggttcaaggaat	720-700
	probe	cctccaggaatacacagtga	652-671
hGSTM1	primer (forward)	aaagtacttggaggaactccctgaaa	429-454
(NM_000561)	primer (reverse)	gctcaaatatacggtggaggtcaa	568-545
	probe	aaacaagatcacttttgtagattt	504-527
hGSTM2	primer (forward)	aaatgctgaagctctactcacagtttc	445-471
(NM_000848)	primer (reverse)	ggctcaaatacttggtttctctcaag	562-537
	probe	aggtgatcttgtccccaaga	510-491
hGSTM3	primer (forward)	ggacaactgaaacaattctccatgt	540-564
(NM_000849)	primer (reverse)	ccaagatatcataggtgagaaaatcca	636-610
	probe	tgagcttttccccggcaa	603-586
hGSTM4	primer (forward)	ttctcacagttcctggggaaga	721-742
(NM_000850)	primer (reverse)	gctcaaatatacggtggaggtcaa	823-800
	probe	aggtgatcttgtctccaacaa	772-752
hGSTM5	primer (forward)	aaatacttggaggaactccctgaaa	446-470
(NM_000851)	primer (reverse)	gtcaaggacatcataggcaagga	565-543
	probe	aggtgatcttgtctcctgc	533-515
hGSTP1	primer (forward)	ccagaaccagggaggcaaga	434-453
(NM_000852)	primer (reverse)	gaggcgccccacatatgct	581-563
	probe	tgggagaccagatctccttcgctgactac	463-491
hGSTT1	primer (forward)	tccttactggtcctcacatctcctt	470-494
(NM_000853)	primer (reverse)	cttgggtcggccttcgaa	570-553
	probe	acgggatgcatcagctccgtgatg	533-510

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Table 3. CYP and GST catalytic activities in HSC from two donors

Donor	EROD[a] (pmol/min/mg)	Midazolam[b] (pmol/hr/10^6 cells)	GST-CDNB[a] (nmol/min/mg)
1	<2	0.094	93
2	<2	0.111	166

[a] CYP1A-mediated EROD and GST-CDNB activities were measured in S9 fractions as described in the Methods. The practical detection limit for EROD assay was 2 pmol/min/mg S9 protein. [b] CYP3A-dependent midazolam activities were assayed in cultured HSC as described in the Methods.

DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 1

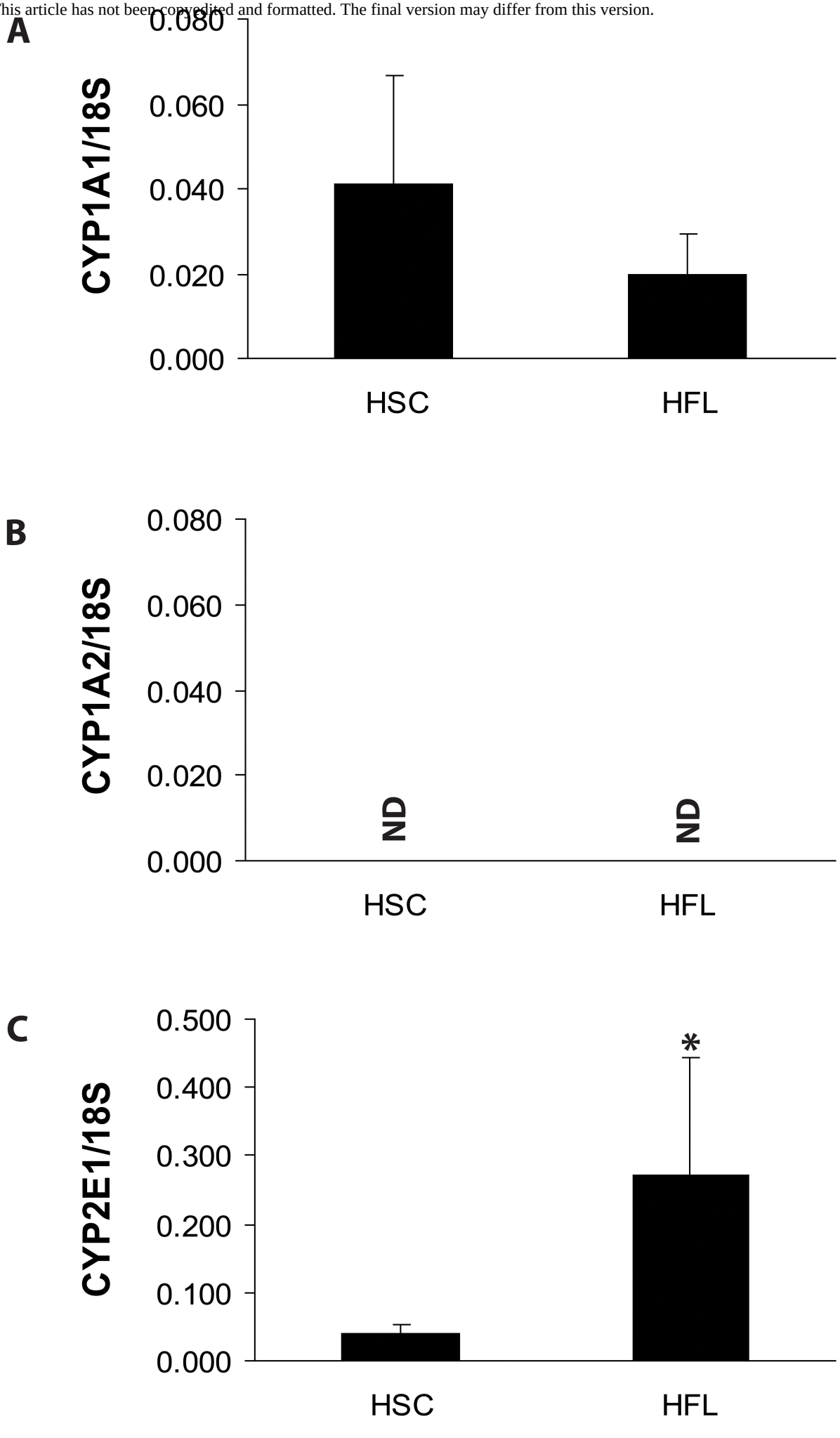
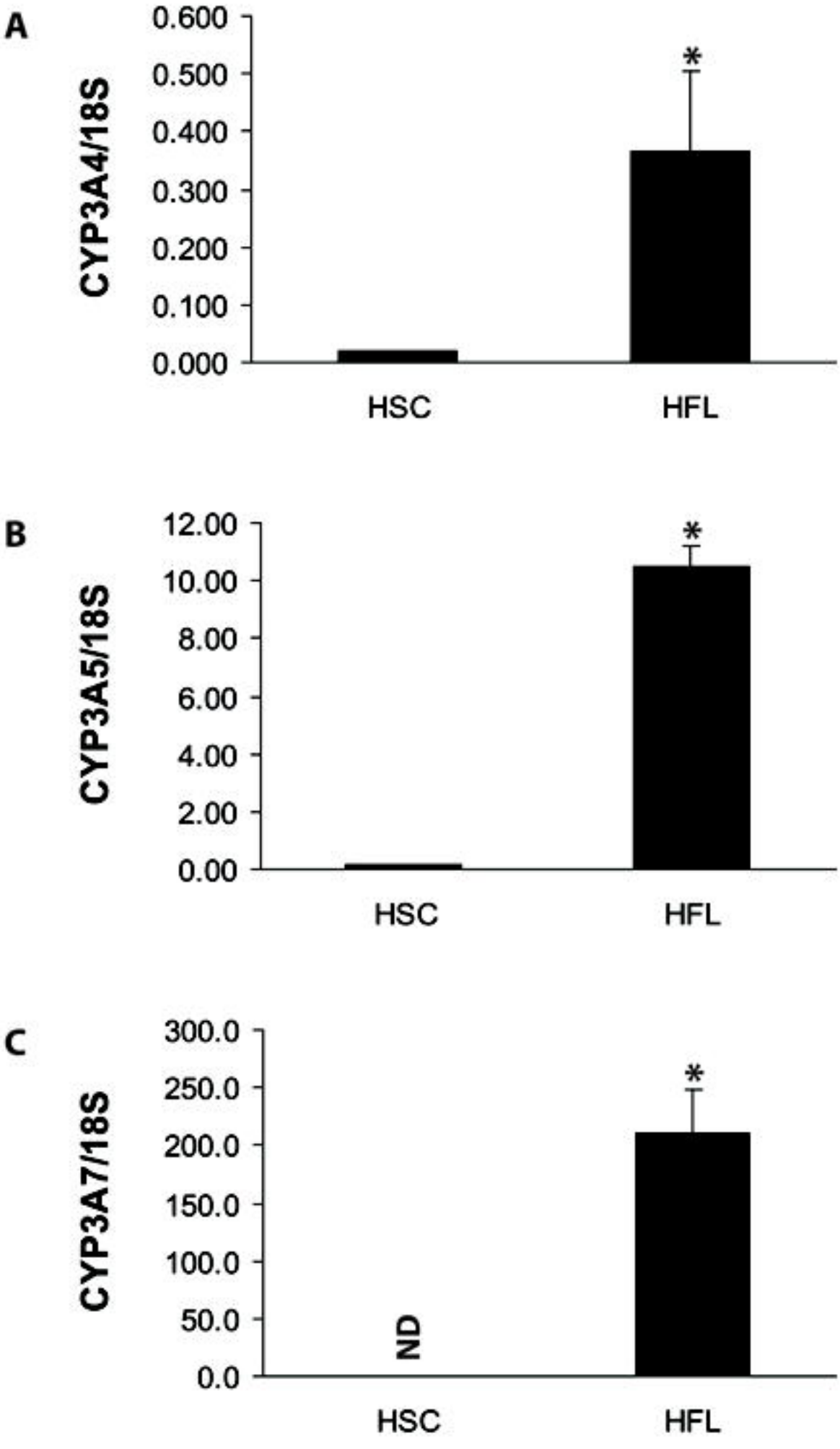


Figure 2



DMD Fast Forward. Published on October 18, 2006 as DOI: 10.1124/dmd.106.012757
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3

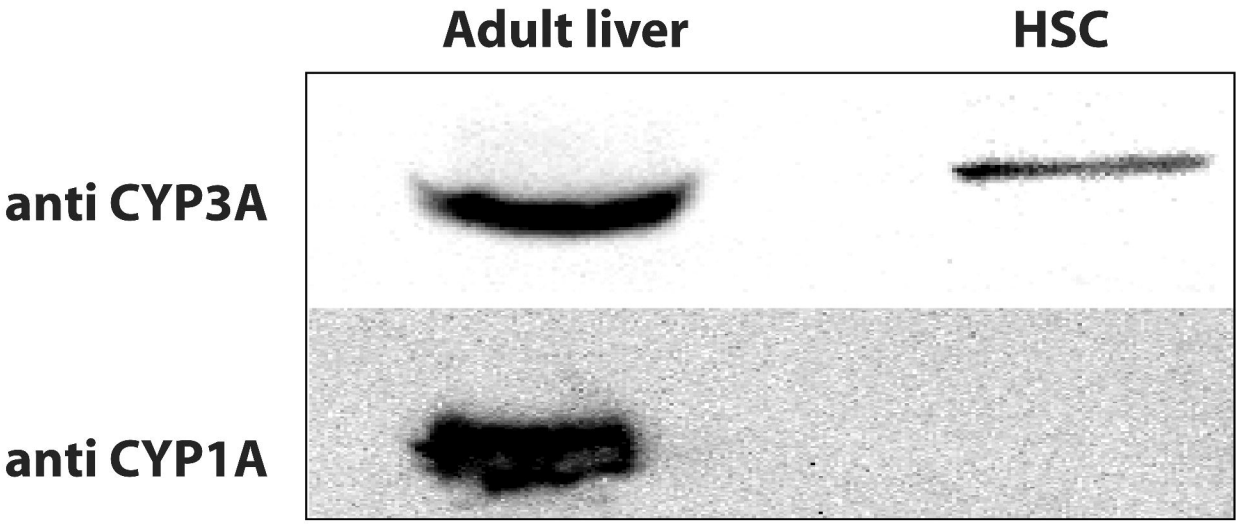


Figure 4

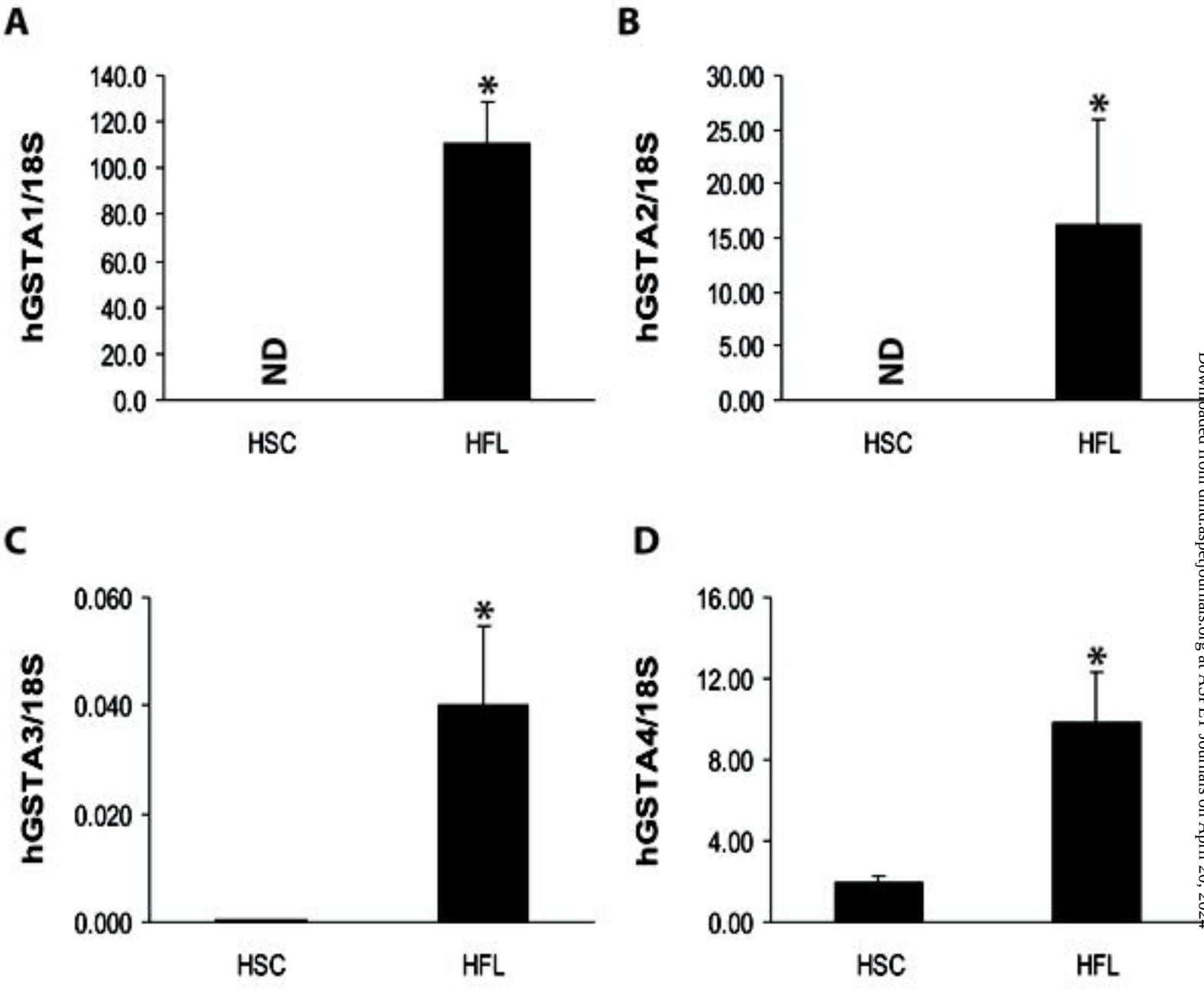


Figure 5

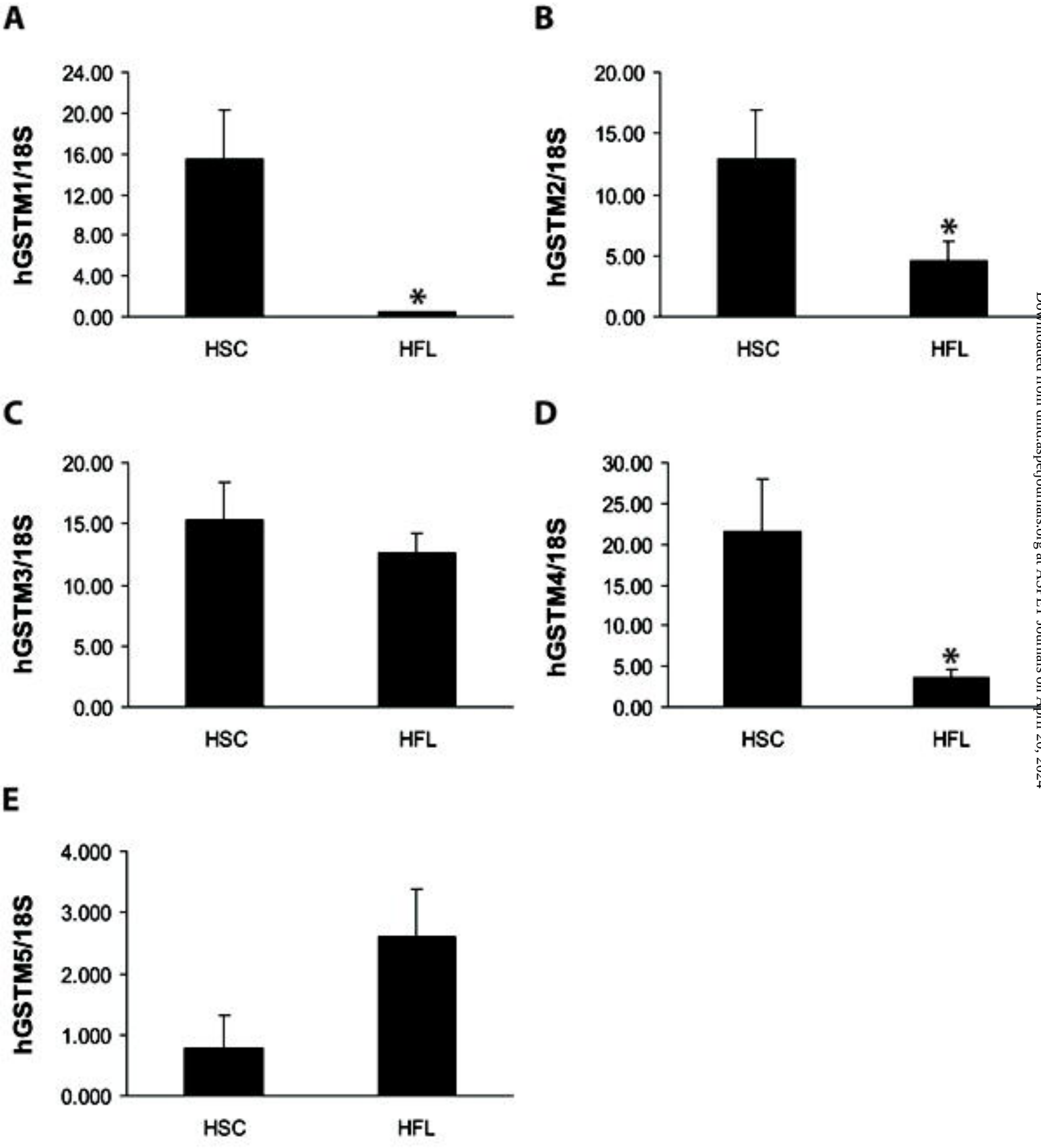


Figure 6