Estrogen Regulation of the Cytochrome P450 3A Subfamily in Humans

Eric T. Williams, Małgorzata Leyk, Steven A. Wrighton, Peter J. A. Davies, David S. Loose, Gregory L. Shipley, and Henry W. Strobel

Departments of Biochemistry and Molecular Biology (E.T.W., H.W.S.) and Integrative Biology and Pharmacology (P.J.A.D., D.S.L., G.L.S.), Medical School, University of Texas Health Science Center at Houston, Houston, Texas; Department of Statistics, Texas A&M University, College Station, Texas (M.L.); and Department of Drug Disposition, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana (S.A.W.)

Received April 27, 2004; accepted July 21, 2004

ABSTRACT

This study examines the possible role of estrogen in regulating the expression of the human CYP3A subfamily: CYP3A4, CYP3A5, CYP3A7, and CYP3A43. To accomplish this goal, mRNA was quantified from human livers and endometrial samples, and total CYP3A protein levels were evaluated by Western immunoblot analysis of the liver samples. The human endometrial samples were from premenopausal and postmenopausal women. The premenopausal endometrium was either in the proliferative or secretory phase, whereas for the postmenopausal endometrium samples, the women had been treated with either a placebo or estropipate, an estrogen substitute. After analyses, CYP3A4 mRNA was shown to have lower hepatic expression in females than in males. In the endometrium, CYP3A4 and CYP3A43 are down-regulated by estrogen, whereas CYP3A5 is expressed at higher levels during the secretory phase. CYP3A7 was not detected in the endometrium. In addition, the CYP3A subfamily showed increased mRNA expression in the liver as age increased. The expression levels of total CYP3A protein and total CYP3A mRNA showed good correlation. Despite apparent regulation of CYP3A4 mRNA expression by estrogen, the effects of estrogen may be overshadowed by additional regulators of gene expression.

It is known that men and women do not respond equally to certain drugs administered for therapeutic relief (Harris et al., 1995); however, the reasons have yet to be fully identified and appear to be multifaceted. A variety of possibilities may contribute to therapeutic discordance, but a probable explanation is that estrogen directly or indirectly regulates a gene or genes responsible for the differences in response. Of the genes that could cause variations in drug response, a metabolizing enzyme would be a likely candidate. In particular, the cytochrome P450 (P450) superfamily is known for metabolizing a diverse set of substrates with the cytochrome P450 3A (CYP3A) subfamily metabolizing the most diverse range of substrates (Guengerich, 1999). It is possible that an estrogen-regulated P450 of the CYP3A subfamily contributes to the gender dimorphism exhibited in the drug response between women and men.

In humans, four CYP3As are known to be expressed: CYP3A4, CYP3A5, CYP3A7, and CYP3A43. CYP3A4 is the most abundant P450 (Guengerich, 1995) and contributes to the metabolism of the largest percentage of clinically used drugs (Evans and Relling, 1999). CYP3A5 is polymorphically expressed, and in some individuals, CYP3A5 expression can equal that of CYP3A4 (Kuehl et al., 2001). CYP3A7 is known for its expression in fetal liver (Komori et al., 1990); however, expression has also been shown in some adult tissues (Burk et al., 2002). The latest human CYP3A to be discovered, CYP3A43, is thought to have the strongest mRNA expression in the liver and testis (Westlind et al., 2001) or the prostate (Gellner et al., 2001), but the expression of CYP3A43 is significantly lower than that of CYP3A4 (Gellner et al., 2001) and CYP3A5 (Westlind et al., 2001). Some of the substrates known to be metabolized by the CYP3As include erythromycin (Brian et al., 1990), cyclosporine (Kronbach et al., 1988), warfarin (Kaminsky and Zhang, 1997), and 17β-estradiol (Lee et al., 2001).

Conducting in vivo research based on humans is extremely difficult, especially when examining estrogen regulation. Most of the techniques used in animal models are not accept-
able for use on humans. Therefore, many researchers have relied on available tissue resources.

Recent studies of gender-based CYP3A expression in the liver include those of Westlind-Johnsson et al. (2003) and Wolbold et al. (2003). Westlind-Johnsson et al. did not uncover a significant difference between men and women for CYP3A4, CYP3A5, or CYP3A43 mRNA expression; however, Wolbold et al. reported that women had 2-fold higher expression of CYP3A4 mRNA and 3-fold higher protein expression than men.

The classical tissue for assaying P450 is the liver, since it is the major site of detoxification for the body and the principal location of drug metabolism. Comparing male and female livers will not directly identify genes that are regulated in part by estrogen; however, knowing gender differences in mRNA expression may suggest possible candidates for further investigation.

The endometrium may be a better tissue to investigate in terms of responsiveness to estrogen. It is not a primary site for drug metabolism; nevertheless, the CYP3As are present in the endometrium and may play an endogenous role. For premenopausal endometrium, studies of CYP3A mRNA expression include studies by Schuetz et al. (1993), Hukkanen et al. (1998), and Sarkar et al. (2003). Schuetz et al. reported that CYP3A7 was expressed higher in the secretory phase than the proliferative phase. Hukkanen et al. detected CYP3A4 and CYP3A5 but did not explore differences between phases, and CYP3A7 was not detected. Sarkar et al. found a significant difference between the two phases for CYP3A7, but not CYP3A4.

Similar to the results reported for CYP3A expression in liver, the literature discussing CYP3A expression in premenopausal endometrium is equally contradictory. Hukkanen et al. (1998) did not detect CYP3A7 expression; however, Schuetz et al. (1993) and Sarkar et al. (2003) did detect CYP3A7 expression, but they contradict each other in terms of the phase in which CYP3A7 is expressed at higher levels. Sarkar et al. showed that CYP3A7 was expressed at higher levels in the proliferative phase, whereas Schuetz et al. found higher expression during the secretory phase than during the proliferative phase.

The current study provides a more inclusive exploration into the relationship between estrogen and the human CYP3A subfamily by examining the expression of the CYP3A forms utilizing samples from the liver, premenopausal endometrium, and postmenopausal endometrium with and without exogenous estrogen treatment. Additionally, in the current study, the effect of age on CYP3A expression has also been analyzed.

Materials and Methods

**Human Liver and Endometrial Samples.** A total of 27 liver samples, 20 postmenopausal endometrial samples, and 13 premenopausal endometrial samples were obtained from various sources.

Of the human liver samples, 20 (HL1–HL20) were obtained under approved protocols from the Medical College of Wisconsin or the Indiana University School of Medicine, six (HL21–HL26) from Steve Strom at the University of Pittsburgh, and one (HL27) from the International Institute for the Advancement of Medicine (IIAM; Exton, PA). Most of the livers originated from transplant sources, although four originated from biopsy. All liver samples were from persons that died of accidental causes.

For each liver sample, 100 mg of whole liver was added to 1 ml of RNA STAT-60 reagent (CS-111; Tel-Test Inc., Friendswood, TX). The samples were homogenized until no debris was visible and allowed to sit at room temperature for 5 min. After adding 200 μl of chloroform, the mixtures were shaken for 15 s and allowed to sit at room temperature for 2 to 3 min. Then, the samples were centrifuged at 4°C for 15 min. The clear supernatant fractions were transferred to clean tubes, mixed with 500 μl of isopropanol, and allowed to sit at room temperature for 5 to 10 min. Next, the mixtures were centrifuged at 4°C for 10 min, and the supernatant fractions were discarded. The remaining pellets were washed with 1 ml of 75% ethanol and centrifuged at 4°C for 5 min. The supernatant fractions were discarded, and the pellet was dried and resuspended in water treated with diethyl pyrocarbonate.

The endometrial samples were processed as specified by Deng et al. (2003). All samples were DNase I-treated and stored at −80°C until analysis.

**Quantitative Real-Time PCR.** All liver and endometrial RNA samples were quantified using quantitative real-time PCR with an ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA).

The primer and probe sets were designed to overlap the exon/exon junctions of mRNA using the Primer Express software (Applied Biosystems). Regions of high homology between the human CYP3As were excluded during the design. The probes contain a 5′-6-FAM (5-carboxyfluorescein) and a 3′ TAMRA (5-carboxytetramethylrhodamine). The standards were oligonucleotides spanning the region of the amplifier, three bases upstream of the forward primer to three bases downstream of the reverse primer. The primers, probes, and standards utilized in this study are listed in Supplemental Material Table 1 (Supplemental tables are available at http://jpet.aspetjournals.org) and were synthesized by SeqWright (Houston, TX). Integrated DNA Technologies (Coralville, IA), or BioSource International (Camarillo, CA).

For each sequence quantitated, four parameters were measured: 5-log dilution series of the oligonucleotide standards, no template controls (NTC), unknown samples, and no amplification controls (NAC) per unknown sample. The NTC omits RNA to ensure contaminant-free reagents, whereas the NAC omits the reverse transcriptase to ensure the lack of DNA contamination. All standards, NTCs, and unknown samples were conducted in triplicate with one NAC per unknown sample.

<table>
<thead>
<tr>
<th>N</th>
<th>Age</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>10</td>
<td>56.6 ± 3.97</td>
</tr>
<tr>
<td>ERT</td>
<td>10</td>
<td>53.1 ± 4.56</td>
</tr>
</tbody>
</table>

ERT, estrogen-replacement therapy; AA, African-American; C, Caucasian.
per unknown sample. The assays used reagents provided in the Superscript One-Step RT-PCR with platinum Taq kit (catalog no. 10928-042; Invitrogen, Carlsbad, CA). A total of 50 μl was aliquoted to each well and consisted of 1× reaction mix (a buffer with 0.4 mM each deoxynucleoside-5′-tri-phosphate and 2.4 mM magnesium sulfate), an additional 1.8 mM magnesium sulfate, 800 nM each of forward and reverse primers, and 200 nM probe. The standards, samples, and NTCs contained 1 μl of RT/Taq mixture, whereas the NACs contained 1 μl of platinum Taq DNA polymerase (catalog no. 10966-018; Invitrogen).

The template used for the standards was a serial 10-fold dilution of the oligonucleotide standard ranging from 100 aM to 1 pM (quantity calculation described below). The NTC contained water instead of the oligonucleotide standard. For the liver samples, a template of 100 ng of RNA was used for each well containing samples and NAC. On the other hand, the endometrial samples had a template of 10 ng of RNA for the β-actin assay and 40 ng of RNA for the CYP3A transcripts quantitated.

Each assay was tested for cross-reactivity using the assay mixture outlined above. The template mixture for the CYP3A4 assay contained the full-length CDNA for CYP3A5, CYP3A7, and CYP3A43. As a positive control, 1 ng of the full-length CYP3A4 cDNA was used as the template, whereas the negative control had no template. Likewise, the other CYP3A assays were tested for cross-reactivity. Only in the positive control reactions did amplification occur, suggesting no cross-reactivity among assays.

The results are reported in quantity of transcripts. By using the molecular weight of the standards, the number of grams to achieve the desired number of standard templates was calculated. ABI Prism’s software then used the quantity of the standards to calculate the desired number of standard templates was calculated. ABI Prism’s software then used the quantity of the standards to calculate the desired number of standard templates.

**Western Immunoblot Analysis.** From the human liver samples, microsomes were prepared as previously described (Kalsotra et al., 2002). The protein concentration of the microsomes was determined using the BCA protein assay kit from Pierce (catalog no. 23225; Rockford, IL). A total of 50 μg of each sample was loaded onto 4 to 20% gradient gels (catalog no. 161-1105EDU; Bio-Rad, Hercules, CA) and electrophoresed at 100 V until the dye-front reached the end of the gel. The gels were transferred to pure nitrocellulose membranes using the suggested protocol supplied by the semidry electrophoretic transfer cell from Bio-Rad (catalog no. 170-3940). The membranes were blocked overnight using 5% (w/v) evaporated milk in Tris-buffered saline containing 0.0005% (v/v) Tween 20. The membranes were probed at a 1:1000 dilution using the CYP3A4 polyclonal antibody from Research Diagnostics (RD1-CYP3A4ab; Flanders, NJ) as the primary antibody for 2 h and at 1:1250 using a goat anti-rabbit horseradish peroxidase conjugate (catalog no. 170-5046; Bio-Rad) as the secondary antibody for 1 h. SuperSignal West Pico chemiluminescence (catalog no. 34080; Pierce) was used for detection in the ChemiGenius2 (Syngene, Frederick, MD). GeneTools 3.04b by Syngene quantitated the bands.

For separation of the individual CYP3A isoforms, CYP3A4 (catalog no. 456207; BD Testset, Bedford, MA), CYP3A5 (catalog no. 456235; BD Testset), and CYP3A7 (catalog no. 456237; BD Testset) were purchased. CYP3A43 was excluded since it is not commercially available. All enzymes were loaded either individually or mixed.

**Statistical Analyses.** In the liver samples, gender and age were examined for effects. The data were transformed to normality using the Box-Cox transformation. Power transformations of −0.25, logarithm, −0.25, and logarithm were used in the case of CYP3A4, CYP3A5, CYP3A7, and CYP3A43, respectively.

A previous study using human liver samples was reported by Westlind-Johnsson et al. (2003); therefore, these data were also examined for gender and age effects utilizing the same statistical methods used to analyze the new data presented in this study. Power transformations of 0.25, 0.25, 0.25, and 0.50 were used in the case of CYP3A4, CYP3A5, CYP3A43, and pregnane X receptor (PXR), respectively.

For any CYP3A mRNA expression in the human liver that showed both an age and gender effect, a nonparametric test (Kruskal-Wallis) was conducted to confirm the parametric results. This analysis divided the human liver samples into two groups: females 55 years of age and under versus males of all ages and females over the age of 55. The age of 55 years was chosen as a conservative approximation of the median age for menopause.

In the endometrial samples, differences in mRNA expression were tested between proliferative and secretory stages in premenopausal individuals, as well as the placebo and estriproprate treatments in the postmenopausal individuals. For CYP3A5, only data from premenopausal individuals were available due to the lack of available postmenopausal samples. Power transformations of 0.5, logarithm, and −0.25 were used in the case of CYP3A4, CYP3A5, and CYP3A43, respectively. Differences between different stages and treatments were tested using Scheffe’s test for multiple comparisons.

For all the above analyses, in the SAS PROC MIXED procedure (SAS Institute, Cary, NC), compound symmetry covariance structure was used on observations from the same individual. We also standardized each observed level of RNA by the average level of β-actin in each individual.

The mRNA expression levels of all four enzymes collected in this study were combined for each sample and analyzed using a power transformation of −0.25. The Kruskal-Wallis analysis described above was also conducted on the combined data. The total protein expression levels were analyzed using a power transformation of −0.25 and analyzed by the Kruskal-Wallis analysis described above. Spearman’s correlation was conducted between the total mRNA expression and the total protein expression. A p value less than 0.05 is considered to be statistically significant.

**Results**

In this study, RNA samples from two human tissues were analyzed as well as protein expression from the liver. The first tissue analyzed was a comparison of human liver samples from males and females for CYP3A mRNA expression levels. The second tissue analyzed was the human endometrium.

A total of 27 human liver samples were collected. For each sample, the mRNA was quantified by quantitative real-time PCR for β-actin, CYP3A4, CYP3A5, CYP3A7, and CYP3A43 mRNA expression. Each CYP3A was normalized against β-actin, and the results are listed in Supplemental Material Table 2.

Statistical analyses were conducted on the CYP3A4 mRNA levels of the liver samples for age and gender effects. For CYP3A4 mRNA, age and gender effects were found to be statistically significant covariates, as shown in Table 2, with younger females (Fig. 1A) expressing lower mRNA levels than younger males (Fig. 1B). For CYP3A7 and CYP3A43,

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Significant p values from statistical analyses of human liver CYP3A mRNA expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
</tr>
<tr>
<td></td>
<td>Age x gender</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>Age</td>
</tr>
<tr>
<td>CYP3A7</td>
<td>Age</td>
</tr>
<tr>
<td>CYP3A43</td>
<td>Age</td>
</tr>
</tbody>
</table>

NT, not tested.

* This study.

Westlind-Johnsson et al. (2003).
only an age effect was found to be statistically significant. For these three genes, mRNA expression was found to increase with age, as seen in Fig. 1, A, B, D, and E. The estimated model for each is given in Supplemental Material Table 3 and shown in Fig. 1. On the other hand, no significant age or gender effect was found for CYP3A5, as shown in Fig. 1C.

As confirmation of the results obtained from the CYP3A4 regression analyses in this study, the Kruskal-Wallis non-parametric test was used on the mRNA expression levels of hepatic CYP3A4. The data divided into two groups, as described under Materials and Methods, reveals a significant result ($p = 0.0190$) that women 55 years and younger had a much lower expression of CYP3A4 mRNA. More samples, particularly from younger females, would allow for a refinement of the statistical model.

Recently, a similar study was reported by Westlind-Johnsson et al. (2003). In their study, the mRNA levels for CYP3A4, CYP3A5, CYP3A43, and PXR were quantified in the human liver after normalization against human acidic ribosomal phosphoprotein. The results of the Westlind-Johnsson et al. study were analyzed in a similar manner to
the data generated in this study. An age effect was found to be statistically significant for CYP3A4, CYP3A5, and CYP3A43, as shown in Table 2. CYP3A7 was not quantified in the Westlind-Johnsson et al. study. Similar to the results reported here, the data from Westlind-Johnsson et al. show that the level of mRNA increases with age. The estimated models are given in Supplemental Material Table 3 and shown in Fig. 2. No statistically significant age or gender effect was found for PXR.

A total of 27 human livers were obtained and assayed for the mRNA levels of all four human CYP3A forms (Supplemental Material Table 2) with β-actin as the normalizer. In the study conducted by Westlind-Johnsson et al. (2003), 46 human livers were assayed for CYP3A4, CYP3A5, CYP3A43, and PXR mRNA with human acidic ribosomal phosphoprotein as the normalizer. Since these two studies used different genes for normalization, the results were not combined.

In addition to the human liver samples, CYP3A mRNA levels from human endometrial samples were analyzed. Two sets of samples from human endometria were obtained, premenopausal and postmenopausal. The premenopausal samples were separated into proliferative and secretory phases. A few samples were found to be inactive and atrophic, suggesting the possibility that the women were undergoing menopause. Other samples were found to have progestational effects, suggesting that this group of women were using oral contraceptives. The endometrial samples that were inactive or showed progestational effects were excluded from analyses. Supplemental Material Table 4 shows the levels of CYP3A gene expression, normalized to β-actin, for the samples that were included and excluded from statistical analyses.

The postmenopausal samples were divided into two treatment groups, either placebo or estropipate. To determine which human CYP3A genes should be quantified, a pool of 10 randomized samples for initial testing was generated for both groups; the results are shown in Supplemental Material Table 5. After quantitating the pool samples, a new group of 10 randomized samples were analyzed for both groups and normalized to β-actin; the results are shown in Supplemental Material Table 6. Clinical information regarding each group is compiled in Table 1. For expression levels for CYP3A4 mRNA, the placebo-treated endometrium was higher than the estropipate-treated endometrium and premenopausal (both phases) endometrium, as shown in Fig. 3A. The multiple comparison adjusted p values were determined for placebo- versus estropipate-treated (p = 0.0213), placebo-treated versus proliferative phase (p < 0.0001), and placebo-treated versus secretory phase (p = 0.0019). Verification of the results was obtained using the Kruskal-Wallis nonparametric test, which gave a statistically significant p value (p = 0.0003). For CYP3A5 (Fig. 3B), the proliferative phase was significantly lower than the secretory phase (p = 0.0058). The Kruskal-Wallis nonparametric test that was used as...
confirmation of CYP3A5 had a statistically significant $p$ value ($p = 0.0087$). No statistical differences were found for CYP3A43 (Fig. 3C). Table 3 provides the estimated means and standard errors for these determinations.

From the human liver samples gathered in this study, all mRNA expression levels were combined for each sample and analyzed in a fashion similar to the analysis for CYP3A4. Both age ($p = 0.0051$) and gender ($p = 0.0498$) effects were found to be significant, but the interaction between the two was not statistically significant. The Kruskal-Wallis analysis for two groups was significant ($p = 0.0290$).

The results presented thus far reveal associations between age and/or gender effects and mRNA expression levels for human CYP3A subfamily members as determined by quantitative real-time PCR. To extend the results seen with mRNA expression to protein expression, the separation of individual CYP3A isoforms was attempted, as shown in Fig. 4. The CYP3A isoforms were not separated by electrophoresis, which is consistent with previous results reported (Domanski et al., 2001) using different conditions. Therefore, the total CYP3A protein expression was examined for associations with age and/or gender effects. Using similar statistical analyses for total protein expression, as had been conducted for total mRNA expression, yielded no statistically significant relationships with age and/or gender. However, the Spearman's correlation coefficient (0.62149), which is a measure of the degree of relatedness between variables, was significant ($p = 0.0005$) for the relationship between total protein and total mRNA expression. The human endometrium samples were not analyzed due to limited quantity of samples obtainable at collection.

**Discussion**

The focus of this study was to determine whether estrogen regulates the expression of the human CYP3A subfamily. To accomplish this goal, liver and endometrial samples were assayed for the mRNA levels for CYP3A4, CYP3A5, CYP3A7, and CYP3A43 and normalized against $\beta$-actin. The mRNA of the liver was then compared with liver protein expression.

The increase in liver mRNA expression with age is consistent across all human CYP3A isoforms. The reason for this observation is not obvious, but one possibility is a tie to a common function of the CYP3A isoforms, the ability to me-

**TABLE 3**

Estimated means and standard errors of human endometrium mRNA expression

<table>
<thead>
<tr>
<th></th>
<th>Pre-menopause</th>
<th>Post-menopause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proliferative</td>
<td>Secretary</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>3.464</td>
<td>3.232</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>4.502</td>
<td>16.050</td>
</tr>
<tr>
<td>CYP3A43</td>
<td>5.737</td>
<td>9.794</td>
</tr>
</tbody>
</table>

NT, not tested.
tabolize exogenous compounds. Some of the exogenous substances are known to up-regulate the expression of CYP3A4 through activation of PXR (Goodwin et al., 1999). The possibility for increased PXR activation with age exists despite the fact that PXR mRNA expression was not found to increase with age, as shown by the data from the Westlind-Johnsson et al. study (Fig. 2D). The increase in PXR activation may be the result of an increase in medications as age increases; thus, no increase in PXR mRNA expression would be observed. This increase in medications taken as one ages would be expected in the general population. However, according to the known medical histories of the liver donors used in the current study, no such difference was evident. Thus, PXR activation causing the increase in CYP3A expression as age increases cannot be confirmed.

A reason the CYP3As increase with age may lie in the source of the liver samples obtained. Most of the livers were obtained from transplantation, others from biopsy, but the source is not known for all samples. Therefore, the liver samples may have been handled differently depending upon the source. Of the samples with known sources, the samples of people aged 58 and younger were from transplantation, and the samples of people aged 59 and older were from biopsy. Our observations suggest a difference in mRNA expression quantified for each CYP3A versus the source of the sample; however, upon analysis, the difference was not statistically significant. Therefore, it is unlikely that the source of the samples had any significant effects on the expression levels of the CYP3As as a function of age.

In liver, CYP3A4 showed both gender and age dependencies. The results show that postmenopausal women express CYP3A4 mRNA at equal levels with men, which supports the notion that estrogen down-regulates CYP3A4 mRNA expression and is consistent with the results of the human endometrial samples in this study. Another study (Wolbold et al., 2003) reports differing findings, but did not examine age. In the current study, the mean age for their female control group is 2003 (suppressed CYP3A4 expression) and is consistent with the results of the human endometrium (Fig. 2D). The increase in PXR activation may be the result of an increase in medications as age increases; thus, no increase in PXR mRNA expression would be observed. This increase in medications taken as one ages would be expected in the general population. However, according to the known medical histories of the liver donors used in the current study, no such difference was evident. Thus, PXR activation causing the increase in CYP3A expression as age increases cannot be confirmed.

A reason the CYP3As increase with age may lie in the source of the liver samples obtained. Most of the livers were obtained from transplantation, others from biopsy, but the source is not known for all samples. Therefore, the liver samples may have been handled differently depending upon the source. Of the samples with known sources, the samples of people aged 58 and younger were from transplantation, and the samples of people aged 59 and older were from biopsy. Our observations suggest a difference in mRNA expression quantified for each CYP3A versus the source of the sample; however, upon analysis, the difference was not statistically significant. Therefore, it is unlikely that the source of the samples had any significant effects on the expression levels of the CYP3As as a function of age.

According to this study, CYP3A5 expression is significantly higher in the secretory phase than the proliferative phase of the endometrium (Fig. 3B). Another study reported CYP3A5 expression in premenopausal endometrium without exploring differences between phases (Hukkanen et al., 1998).

Also, in conjunction, these explanations are plausible.

The current study did not detect CYP3A7 mRNA in the endometrium (Fig. 3C) since the endometrial samples from the placebo-treated, postmenopausal women appear to have higher mRNA expression than those of the estrogen-treated postmenopausal and premenopausal women, although the changes are not significant. CYP3A43 could be expressed in higher levels in the proliferative rather than the secretory phase, although additional studies are necessary for a more definitive answer.
Estrogen Regulation of the Human CYP3A Subfamily


In conclusion, estrogen may be important for tissue-specific expression of the CYP3As. The literature is divided on the specific expression of the CYP3As. The literature is divided on the specific expression of the CYP3As. The literature is divided on the specific expression of the CYP3As. The literature is divided on the specific expression of the CYP3As. The literature is divided on the specific expression of the CYP3As.