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Sex differences in UGT2B17 expression and activity

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17-dihydroexemestane (17-DHE)

3-hydroxy-cotinine (3-HC)

Abstract

UDP-glucuronosyltransferases (UGTs) are enzymes involved in the metabolism of steroid hormones, carcinogens, cancer chemotherapy agents, and addictive agents from cigarettes. Since the UGT2B family of genes have been linked to hormonal regulation in human cell lines *in vit*ro, we hypothesized that there may be sex-related differences in the expression and activity of these genes in human tissues. To evaluate whether there are sex differences in UGT2B expression and activity, we examined 103 normal human liver specimens for UGT2B expression by real-time PCR and human liver microsome (HLM) *in vitro* glucuronidation activities. Men exhibited approximately 4-fold higher level of expression of UGT2B17 than women (p = 0.007). Consistent with the increased expression of UGT2B17 in men, HLMs from men also had a higher level of glucuronidation activity than women against three UGT2B17 substrates - 3-fold higher for 17-dihydroexemestane (p=0.002); 3-fold higher for 3-hydroxy-cotinine (p<0.001); and 1.5-fold higher for suberoylanilide-hydroxamic-acid (p=0.014). When stratifying by UGT2B17 gene deletion genotype, similar patterns were observed for all three substrates, with HLM from men with the UGT2B17 (+/+) or (+/0) genotypes exhibiting significantly higher levels of glucuronidation activity against all three substrates as compared to HLM from women. These data suggest that men have a higher amount of UGT2B17 glucuronidation activity then women. This sex difference in UGT2B17 gene expression and corresponding protein activity could potentially result in different levels of carcinogen detoxification or drug elimination in men versus women.

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Introduction

UDP-glucuronosyltransferases (UGTs) are phase II enzymes responsible for the metabolism and elimination of a variety of exogenous compounds including drugs, chemotherapeutic agents, and environmental pollutants, and carcinogens, via conjugation with glucuronic acid (Tukey and Strassburg, 2000; Nagar and Remmel, 2006). Glucuronidation is also a major mode of metabolism and excretion of steroid hormones including androgens, estrogens, and their metabolites (Tukey and Strassburg, 2000; Levesque et al., 2001; Belanger et al., 2003; Guillemette et al., 2004; Lepine et al., 2004; Nagar and Remmel, 2006). Although many enzymes participate in maintaining steroid hormone balance, it is postulated that UGTs are vital enzymes modulating the action of steroid hormones, with virtually all individual UGTs exhibiting some level of activity against estrogens and/or androgens (Belanger et al., 2003). While the UGT1A enzymes generally exhibit the highest activity for estrogens, the UGT2B sub-family of enzymes exhibit the highest activity against androgens (Tukey and Strassburg, 2000; Levesque et al., 2001; Belanger et al., 2003; Guillemette et al., 2004; Lepine et al., 2004; Nagar and Remmel, 2006).

Several studies have shown that androgens and estrogens regulate the expression of the UGT2B family of enzymes (Beaulieu et al., 1997; Guillemette et al., 1997; Strasser et al., 1997; Belanger et al., 1998; Hum et al., 1999; Li et al., 1999; Magnanti et al., 2000; Chouinard et al., 2006; Harrington et al., 2006; Hu and Mackenzie, 2009). In human prostate cell lines, the expression of UGTs 2B10, 2B15, and 2B17 was down-regulated by androgens, while UGT2B11 expression was up-

regulated by androgen treatment (Chouinard et al., 2006). Harrington et al showed that in estrogen receptor-positive human breast cancer cells treatment with 17β-estradiol increased expression of UGT2B15 but not other UGT2B enzymes (Harrington et al., 2006). However, using one of the same cell lines (MCF-7), Hu et al demonstrated that expression of UGT2B17, as well as UGT2B15, is induced by 17β-estradiol (Hu and Mackenzie, 2009). Despite the fact that many UGT1A genes also metabolize hormones, no studies have examined the effects of androgens and estrogens on expression of these genes in cells. While no studies of sex-differences in UGT gene expression has yet been performed in human tissues, there have been studies demonstrating that UGT2B15-mediated oxazepam glucuronidation is faster in men than in women (Greenblatt et al., 1980; Court et al., 2002; Court et al., 2004). In addition, a previous study found that there were sex-differences in the expression of UGT genes in mice and that this effect varied by tissue type and by UGT isoform (Buckley and Klaassen, 2007).

Since UGT2B enzymes metabolize many exogenous compounds including a variety of carcinogens, drugs, and cancer chemotherapeutic agents, a sex difference in UGT2B gene expression and corresponding protein activity could potentially result in different levels of carcinogen detoxification or drug elimination in men versus women. Using a panel of normal human liver specimens, the goal of the present study was to examine whether differences exist in the expression and activity of UGT2B enzymes by sex.

Materials and Methods

Chemicals. Exemestane was purchased from Hangzhou HETD Industry Co. LTD (Zhejiang, China), and 17-dihydroexemestane (17-DHE) was synthesized in the Organic Synthesis Core at Penn State University College of Medicine. Suberoylanilide-hydroxamic-acid (SAHA) was also synthesized in this core facility. 3-hydroxy-cotinine (3-HC), 3HC-Glucuronide, and deuterium-labeled internal standards cotinine(methyl-D_3)-3'-O-glucuronide were purchased from Toronto Research Chemicals Inc (North York, Canada). Structures of all compounds are provided in the previous manuscripts (Balliet et al., 2009; Chen et al., 2010; Sun et al., 2010). UDPGA, alamethicin, and β-glucuronidase were purchased from Sigma-Aldrich (St. Louis, MO). Pre-designed and pre-optimized TaqMan Gene Expression Assays were purchased from Applied Biosystems (Foster City, CA). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA) unless specified otherwise.

Tissues. Total RNA from pathologically normal liver tissue from 103 patients (62 men and 41 women) undergoing surgery for hepatocellular carcinoma was obtained and has been described previously (Wiener et al., 2004). Genomic DNA and total RNA was extracted from the same specimens by standard phenol/chloroform or trizol methods, and sufficient liver tissue was also obtained for human liver microsome (HLM) preparation and glucuronidation activity assays for these specimens.

Glucuronidation Assays. Microsome preparation and *in vitro* glucuronidation activity assays against three UGT2B17 substrates were performed previously as described (Balliet et al., 2009; Chen et al., 2010; Sun et al., 2010). Briefly, glucuronidation activities of HLM against 17-DHE, 3-HC, or SAHA were determined after an initial incubation of HLM (2.5-25 µg protein) with alamethicin (50 µg/mg protein) for 15 min in an ice bath. Incubations (10-50 µL) were subsequently performed at 37°C for 1 h in 50 mmol/L Tris buffer (pH 7.5), 10 mmol/L $MgCl_2$, 4 mmol/L UDPGA, and 9.4 µmol/L to 8 mmol/L of substrate for rate determinations, while 4-150 µM 17-dihydroexemestane were used for kinetic analysis by HLM. Reactions were terminated by the addition of the same volume as the initial reaction of cold acetonitrile. For 3-HC, reactions were first spiked with 5 µl of internal standard a cotinine-(methyl-D_3)-3'-O-glucuronide, at a concentration of 10 ppm and 3-HC reactions were terminated by the addition of cold acetonitrile/methanol (75%/25% [v/v]). Reactions were centrifuged at 12,000 - 16,000 *g* for 10 min at 4°C, and supernatants were collected for analysis on ultra-pressure liquid chromatography (UPLC) or UPLC-MS-MS.

UPLC and UPLC-MS-MS conditions. 17-DHE glucuronidation was analyzed as previously described (Sun et al., 2010) using a Waters ACQUITY UPLC System (Milford, MA) with a 1.7 µ ACQUITY UPLC BEH C18 analytical column (2.1 mm $\times$ 50 mm, Waters, Ireland) in series with a 0.2 µm Waters assay frit filter (2.1 mm, Waters, USA). The gradient elution conditions, using a flow rate of 0.3 ml/min, were as follows: starting with 19% acetonitrile and 81% buffer A (5 mM ammonium acetate, pH 5.0) for 1 min, a subsequent linear gradient to 75% acetonitrile/25% buffer over 2 min was

performed and then maintained at 75% acetonitrile for 2 min. Exemestane-17-*O*-glucuronide was confirmed by its stability in 1M NaOH but sensitivity to the treatment of β-glucuronidase. In addition, incubation products (up to 5 µL) were loaded onto an UPLC/MS/MS for confirmation of exemestane-17-*O*-glucuronide formation. By using a positive mode, the parent compound $[M+H]^+$ peak and their glucuronide $[M\text{-}Gluc.+H]^+$ peak were characterized.

SAHA glucuronidation assays were also analyzed by UPLC, as previously described (Balliet et al., 2009) with a gradient elution starting with 5.6% buffer B (100% acetonitrile) and 94.4% buffer A [10 mmol/L ammonium acetate (pH 5.0) and 10% acetonitrile]; a linear gradient to 72% buffer B over 3 min was performed. The flow rate was maintained at 0.3 mL/min. The amount of glucuronide formed was determined based on the ratio of SAHA-glucuronide versus unconjugated SAHA after calculating the area under the curve for the SAHA and SAHA-glucuronide peaks using the known amount of SAHA to each reaction as the reference. SAHA-glucuronide was confirmed by sensitivity to β-glucuronidase and mass spectrometry.

3-HC glucuronidation was analyzed as previously described (Chen et al., 2010) using an Acquity LC-MS-MS system (Waters Corporation, Milford, MA, USA), consisting of an Acquity UPLC pump, an auto sampler, an ACQUITY UPLC BEH HILIC (2.1 mm X 100 mm, 1.7 µm particle size; Waters Corp.) column at 45ºC, and an Acquity TQ tandem mass spectrometer (Waters). UPLC was performed at a flow rate of 0.5 ml/min using the following conditions: 1.5 min in 20% solvent A, a linear gradient for 0.5 min to 100% solvent A, and 2 min in 100% solvent A, where solvent A is 5 mM NH_4AC (pH 5.7) and 50% acetonitrile (v/v), and solvent B is 5 mM NH_4AC (pH 5.7) and 90% acetonitrile

(v/v). After each run the column were washed and reconditioned with a 2 minutes in 100% A and a 2 min in 20% solvent A at flow rate of 1 ml/min. The injection volume of each prepared sample was 5 µL. The Waters Acquity TQ tandem mass spectrometer was equipped with an electrospray ionization (ESI) probe operated in the positive-ion mode, with capillary voltage at 0.64 kV. Nitrogen was used as both the cone and desolvation gases with flow rates maintained at 20 and 760 L/h, respectively. Ultra-pure argon was used as the collision gas with a flow rate of 0.1 L/h for collision-induced dissociation. The source and desolvation gas temperatures were 140 and 450ºC, respectively. For the assay of 3HC-Gluc in 3HC glucuronidation assay samples, the mass spectrometer was operated in the multiple reaction monitoring mode (MRM) and the concentration of 3HC-Gluc were determined. The dwell time for each ion was 100 ms with 5 ms of inter-scan-delay. The cone voltage and collision energy were 35 V and 15 volt respectively for both 369 > 193 (3HC-Gluc) and 372>196 (internal standard) ion transitions. Standard curves were constructed by plotting the ratio of 3HC-Gluc peak area to peak area of its deuterium-labeled internal standards versus analyte concentration ranged from 0.2 ppm to 25 ppm. 3HC-Gluc concentrations were determined by measuring the peak area ratios of 3HC-Gluc to its internal standard and then calculating 3HC-Gluc concentration from the standard curve using Waters' MassLynx software. The 3HC-Gluc formation rate was accordingly calculated.

Expression and Genotype Assays. Reverse-transcription PCR was performed using the Superscript II kit (Invitrogen) on 103 liver RNA samples to obtain cDNA. Real-time PCR gene expression assays were performed on 32 frequently-used endogenous

control genes for a subset of our data set (>5%) in order to choose an appropriate control in our liver samples. As peptidylprolyl isomerase A (PPIA) exhibited the least variability out of all 32 endogenous control genes (including β-actin and GAPDH), it was used as the endogenous control for further analysis of UGT expression. Pre-designed and pre-optimized TaqMan Gene Expression Assays were used in real-time PCR reactions carried out on the ABI 7900 HT Sequence Detection System (gene expression assays for UGT2Bs: Hs02383831_s1 for UGT2B4, Hs02556232_s1 for UGT2B7, Hs02556282_s1 for UGT2B10, Hs01894900_gH for UGT2B11, Hs00870076_s1 for UGT2B15, Hs00854486_sH for UGT2B17, and Hs00852540_s1 for UGT2B28). Each reaction was performed in triplicate according to manufacturer's instructions. SDS 2.2.2 software was used to evaluate real-time PCR reactions and to determine the threshold cycle (Ct), which is defined as the cycle at which PCR amplification reaches a value significantly greater than the baseline. This software was also used to calculate the relative expression of each mRNA by the ΔCt method, which is defined as the value obtained by subtracting the Ct value of PPIA mRNA from the Ct value of the target mRNA. The amount of target UGT mRNA relative to PPIA mRNA was presented as $2^{-\Delta CT}$, as traditionally performed (Nishimura and Naito, 2006). The UGT2B17 deletion polymorphism was genotyped by real-time PCR with allelic discrimination as previously described (Gallagher et al., 2007).

Statistical Analysis. The non-parametric Mann-Whitney test was used to determine statistical significance of expression of UGT2B genes between men and

women. Kinetic constants were determined using the Michaelis-Menten Model in Graphpad Prism 5 software. The equation for this model is as below:

$$Y = Vmax*X/(Km + X)$$

Where X represents substrate concentration, and Y represents enzyme velocity. All other statistical analyses were conducted using SPSS 15.0 statistical software (SPSS 15.0, SPSS Inc., Chicago IL).

Results

The levels of mRNA expression of the seven UGT2B genes (UGTs 2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28) in a series (n=103) of normal human liver specimens are shown in Table 1. All seven UGT2B genes were expressed at detectable levels in human liver, with UGT2B4 expressed at the highest level, followed in order by UGTs 2B15>2B10>2B7>2B17>2B11>2B28. UGT2B expression was different in livers from men versus women, with the order being 2B4>2B15>2B10>2B7>2B17>2B11>2B28 in men and 2B4>2B15>2B10>2B7>2B28 >2B17>2B11 in women. UGT2B17 exhibited a 3.6-fold higher level of expression in liver specimens from men than in liver specimens from women (p=0.007; Table 1). While UGT2B4 expression was observed to be marginally higher in liver specimens from women (1.9-fold; p=0.074), no other UGT2B gene exhibited significant differences in expression between men and women.

The UGT2B17 gene deletion allele is present at an allelic frequency of 0.30 in Caucasians (Murata et al., 2003; Wilson et al., 2004; Gallagher et al., 2007). When stratifying UGT2B17 expression by UGT2B17 deletion genotype and sex (Figure 1), liver specimens from men exhibited significantly higher levels of expression of UGT2B17 then liver specimens from women. Male livers exhibited approximately 4-fold higher levels of expression of UGT2B17 than females for individuals with either 1 (p-value=0.014) or 2 (p-value <0.001) copies of UGT2B17. As expected, liver specimens from both women and men with the UGT2B17 deletion genotype [(0/0)] exhibited no expression of UGT2B17, and specimens from both women and men with the (+/0) or

(+/+) UGT2B17 genotypes exhibited significantly higher levels of UGT2B17 gene expression than specimens from individuals with no genomic copies of UGT2B17 (*p*-value <0.001 for both).

Since UGT2B17 was the only UGT2B gene that displayed significant sex differences in gene expression, glucuronidation activity assays were performed in HLM against UGT2B17 substrates to determine if the increased expression in men resulted in increases in glucuronidation activity in men. UGT2B17 was previously shown to exhibit the highest glucuronidation activity of any hepatic UGT tested against 17-DHE, 3-HC, and SAHA (Balliet et al., 2009; Chen et al., 2010; Sun et al., 2010), so those substrates were the focus of the glucuronidation studies by sex in this study. When stratifying by sex, significantly higher amounts of exemestane-17-*O*-glucuronide formation were observed in individuals with either 1 or 2 copies of UGT2B17 then in individuals with 0 copies of UGT2B17 in both women (*p* =0.045 and 0.001, respectively) and men (*p*<0.001 for both; Figure 2A). Men exhibited a 4.5-fold higher level of glucuronidation activity against 17-DHE than women for individuals with 1 copy of UGT2B17 [(+/-); *p* =0.001] and a 3.0-fold higher level of glucuronidation activity against 17-DHE than women for individuals for individuals with 2 copies of UGT2B17 [(+/+); *p*=0.002; Figure 2A]. There was no difference between men and women in the amount of exemestane-17-*O*-glucuronide formation in individuals who did not have any copies [(0/0)] of UGT2B17 (*p*=0.773).

A similar pattern was observed against additional UGT2B17 substrates, 3-HC and SAHA. While no significant difference in 3-HC-glucuronide formation was observed between individuals with 1 copy of UGT2B17 and individuals with 0 copies of UGT2B17

in women, this difference was significant in men (p<0.001). A marginal difference in 3-HC-glucuronide formation was observed in HLM from women with 2 copies of UGT2B17 versus HLM from women with 0 copies of UGT2B17 (p=0.061); this difference was significant in men (p <0.001). A 3.3-fold higher level of 3-HC-glucuronide formation activity was observed in HLM from men than women for subjects with 1 copy of UGT2B17 [(+/-); p=0.001]. This difference was 2.7-fold higher for HLM from subjects with 2 copies of UGT2B17 [(+/+), p<0.001; Figure 2B]. There was no difference between HLM from men versus women in the amount of 3-HC-glucuronide formation for subjects homozygous deleted (0/0) for UGT2B17.

For SAHA, significant differences in SAHA-glucuronide formation were not observed between HLM from women with 1 or 2 copies of UGT2B17 versus HLM from women with 0 copies of UGT2B17 (Figure 2C). However, a significant difference in SAHA-glucuronide formation was observed in HLM from men with 1 or 2 copies of UGT2B17 versus HLM from men with 0 copies of UGT2B17 (p<0.001 for both). HLM from men exhibited a 1.5-fold higher level of glucuronidation of SAHA than HLM from women for individuals with 2 copies of UGT2B17 [(+/+), p=0.014] and a 1.6-fold higher level of glucuronidation activity than HLM from women with 1 copy of UGT2B17 [(+/-), p=0.046]. There was no difference between HLM from men versus HLM from women in the amount of SAHA-glucuronide formation in individuals who did not have any copies [(0/0)] of UGT2B17.

To determine if the sex difference in glucuronidation activity was also manifested as a change in kinetics, kinetic analysis was performed against 17-DHE as an example UGT2B17 substrate for 3 men and 3 women that each had 2 copies of UGT2B17 (+/+).

A representative concentration curve for exemestane-17-*O*-glucuronide formation from HLM is shown in Figure 3A. Similar to the pattern observed in the HLM glucuronidation screening assays, a significant (p=0.017) 3-fold increase in Vmax/Km was observed for exemestane-17-*O*-glucuronide formation in UGT2B17 (+/+) HLM from men compared to HLM from women (Figure 3D). This difference was due to a significantly lower V_{max} in HLM from women than men (p=0.027; Figure 3C); no difference was observed in K_m (Figure 3B).

Discussion

Previous studies have suggested that UGT2B genes may be hormonally regulated in human cell lines (Beaulieu et al., 1997; Guillemette et al., 1997; Strasser et al., 1997; Belanger et al., 1998; Hum et al., 1999; Li et al., 1999; Magnanti et al., 2000; Chouinard et al., 2006; Harrington et al., 2006; Hu and Mackenzie, 2009). In the present study, normal human liver specimens from men exhibited 4-fold higher levels of expression of UGT2B17 than liver specimens from women. This correlation between expression and sex corresponded with microsomal fractions from the same specimens displaying a 1.5 - 4.5-fold increase in glucuronidation activity against three different UGT2B17 substrates in specimens from males as compared to specimens from females. Significant male-specific increases in both liver UGT2B17 expression and liver glucuronidation activity against UGT2B17 substrates were observed in specimens from subjects who carried one [(+/0)] or two [(+/+)] genomic copies of UGT2B17; the levels of glucuronidation activity were lower against all three substrates with no sex difference observed for HLM from individuals completely deleted for the UGT2B17 gene [(0/0)]. This corresponded with the fact that no expression of UGT2B17 was observed in all specimens in UGT2B17 (0/0) subjects and suggest that hepatic UGT2B17 may be up-regulated in males versus females in subjects with one or more intact copies of UGT2B17.

Previous studies have demonstrated that several UGTs, including UGT2B17, were down-regulated by androgens in human prostate cancer cell lines (Beaulieu et al., 1997; Guillemette et al., 1997; Belanger et al., 1998; Chouinard et al., 2006). In

addition, Harrington et al showed that in estrogen receptor-positive human breast cancer cells, treatment with 17β-estradiol increased expression of UGT2B15 but not other UGT2B enzymes, including UGT2B17 (Harrington et al., 2006). However, using one of the same cell lines (MCF-7), Hu et al demonstrated that the expression of UGT2B17 and UGT2B15 are induced by 17β-estradiol (Hu and Mackenzie, 2009). The data from the present study suggests that men have a higher amount of UGT2B17 mRNA expression and glucuronidation activity then women, which is consistent with a hormonal regulatory mechanism for UGT2B17 in humans. However there are additional mechanisms that could account for this difference including sex-dependent imprinting via epigenetic mechanisms. Since UGT2B17 has been shown to be down-regulated by androgens in prostate and up-regulated by estrogens in breast, we would expect women to have higher UGT2B17 expression than men. However, in human liver we found that women have lower UGT2B17 expression and activity then men, which may indicate that these regulatory effects vary by tissue type. Consistent with our study in humans, a recent study found that there were sex-differences in the expression of UGT genes in mice and that this effect varied by tissue type and UGT isoform (Buckley and Klaassen, 2007).

In the present study, no significant differences in expression of UGT2B enzymes other than UGT2B17 were observed in liver specimens from males versus females. However, Court et al. have detected a sex difference in the glucuronidation of a UGT2B15 substrate, oxazepam (Court et al., 2004). Although there is no difference in UGT2B15 mRNA levels in the present study, it is possible that previously unexamined

variation in environmental factors, such as drugs or dietary compounds, could be involved in UGT2B15 regulation.

The primers and probes used in the gene expression assay for UGT2B28 (Applied Biosystems) are located on exon 1 of the UGT2B28 gene. One of the two inactive variants of the UGT2B28 gene is lacking much of exon 1, however the other inactive variant still contains exon 1 (Levesque et al., 2001). Therefore, the gene expression assay performed for UGT2B28 in this study is likely detecting one active (type I) and one inactive (type II) UGT2B28 variant, but not the other inactive variant of UGT2B28 (type III). Depending upon the relative expression of type I vs type II variant UGT2B28 in liver in different individuals, this could result in aberrant expression findings for the active UGT2B28 variant in the present study. Further studies examining the relative expression of UGT2B28 variants may be required to better assess this possibility.

In addition to hormone metabolism, UGT2B17 also metabolizes many exogenous compounds including cancer chemotherapeutic agents like SAHA (Balliet et al., 2009), a major metabolite of the aromatase inhibitor exemestane, 17-DHE (Sun et al., 2010), and metabolites of nicotine like 3-HC (Chen et al., 2010). The sex difference in glucuronidation activity was more pronounced when assaying activity against 17-DHE and 3-HC than against SAHA. In addition, the difference in glucuronidation activity due to UGT2B17 deletion genotype in both males and females was most pronounced against 17-DHE>3-HC>SAHA. This is consistent with the fact that UGT2B17 plays a more primary role in 17-DHE and 3-HC glucuronidation then in SAHA glucuronidation (Balliet et al., 2009; Chen et al., 2010; Sun et al., 2010). Kinetic studies have indicated

that there is only one additional hepatic enzyme that glucuronidates 17-DHE (UGT1A4) and that UGT2B17 exhibits a 17-fold higher level of activity than that enzyme (Sun et al., 2010). In contrast, there are two other hepatic enzymes that glucuronidate both 3-HC and SAHA, with UGT2B17 exhibiting a 5-8-fold higher level of activity than UGTs 2B7 or UGT1A9 against 3-HC (Chen et al., 2010), and only a 2-fold greater glucuronidation activity than UGT1A9 against SAHA (Balliet et al., 2009).

This is the first study to determine whether there are sex differences in the expression of UGT enzymes in human tissue. Unlike the hormonal regulation observed previously *in vitro*, UGT2B17 was the only UGT that exhibited sex-specific differences in expression in human liver, a difference that was substantiated in studies examining hepatic glucuronidation activities against three UGT2B17 substrates from the same series of liver specimens. These data are consistent with a hormonal regulatory mechanism for UGT2B17 in humans and suggest that men have a higher amount of UGT2B17 glucuronidation activity then women. This sex difference in hepatic gene expression and activity for UGT2B17 could have significant pharmacogenetic implications in terms of the metabolism of UGT2B17 substrates including carcinogens as well as drugs including cancer therapeutic agents. Studies of metabolite levels *in vivo* will be required to better assess this possibility.

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Footnotes

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Figure Legends

Figure 1. UGT2B17 gene expression by sex and deletion genotype in human liver specimens. Real time PCR of UGT2B17 from 103 human liver cDNA specimens was performed in triplicate. The y-axis displays the amount of UGT2B17 gene expression relative to PPIA (presented as $2^{-\Delta CT}$). Sex (F, M) and UGT2B17 deletion genotype [(+/+), (+/0), (0/0)] is shown on the x-axis. Only significant comparisons are shown (p-value < 0.05).

Figure 2. Human liver microsome activity against UGT2B17 substrates by sex and genotype. Glucuronidation assays were performed against three UGT2B17 substrates as previously described (Balliet et al., 2009; Chen et al., 2010; Sun et al., 2010). Sex (F, M) and UGT2B17 deletion genotype [(+/+), (+/0), (0/0)] is shown on the x-axis. The y-axis displays the HLM glucuronidation activity against 17-DHE (Panel 2A); 3-HC (Panel 2B); and SAHA (Panel 2C). Only significant comparisons are shown (*p*-value < 0.05).

Figure 3. Kinetic analysis for human liver microsome activity against 17-DHE by sex. Panel A, Representative concentration curve for exemestane-17-O-glucuronide formation from HLM; Panel B-D, Kinetic analyses of HLMs from 6 individuals (3 females and 3 males) against 17-DHE (all of whom had 2 copies of UGT2B17 [(+/+)]. Sex (F, M) is shown on the x-axis. The y-axis displays the mean and standard error for the K_m (Panel B), V_{max} (Panel C), and V_{max}/K_m (Panel D), against 17-DHE.

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Table 1. Sex differences in UGT2B gene expression in human liver.

Gene	mRNA Expression Men (n=62) ($2^{-\Delta CT}$ Mean ± SE)*	mRNA Expression Women (n=41) ($2^{-\Delta CT}$ Mean ± SE)*	P-value**
UGT2B4	5.14 ± 0.24	9.90 ± 2.14	0.074
UGT2B7	1.16 ± 0.05	1.14 ± 0.07	0.866
UGT2B10	2.28 ± 0.11	2.68 ± 0.26	0.573
UGT2B11	0.014± 0.001	0.037 ± 0.021	0.339
UGT2B15	3.71 ± 0.55	3.35 ± 0.39	0.178
UGT2B17	0.424 ± 0.057	0.119± 0.029	**0.007**
UGT2B28	0.006 ± 0.001	0.126 ± 0.119	0.856

***** Gene expression values are presented as each UGT mRNA relative to PPIA mRNA as $2^{-\Delta CT}$.

** Mann-Whitney test was used to determine statistical significance of expression of UGT2B genes between sexes.

Figure 1

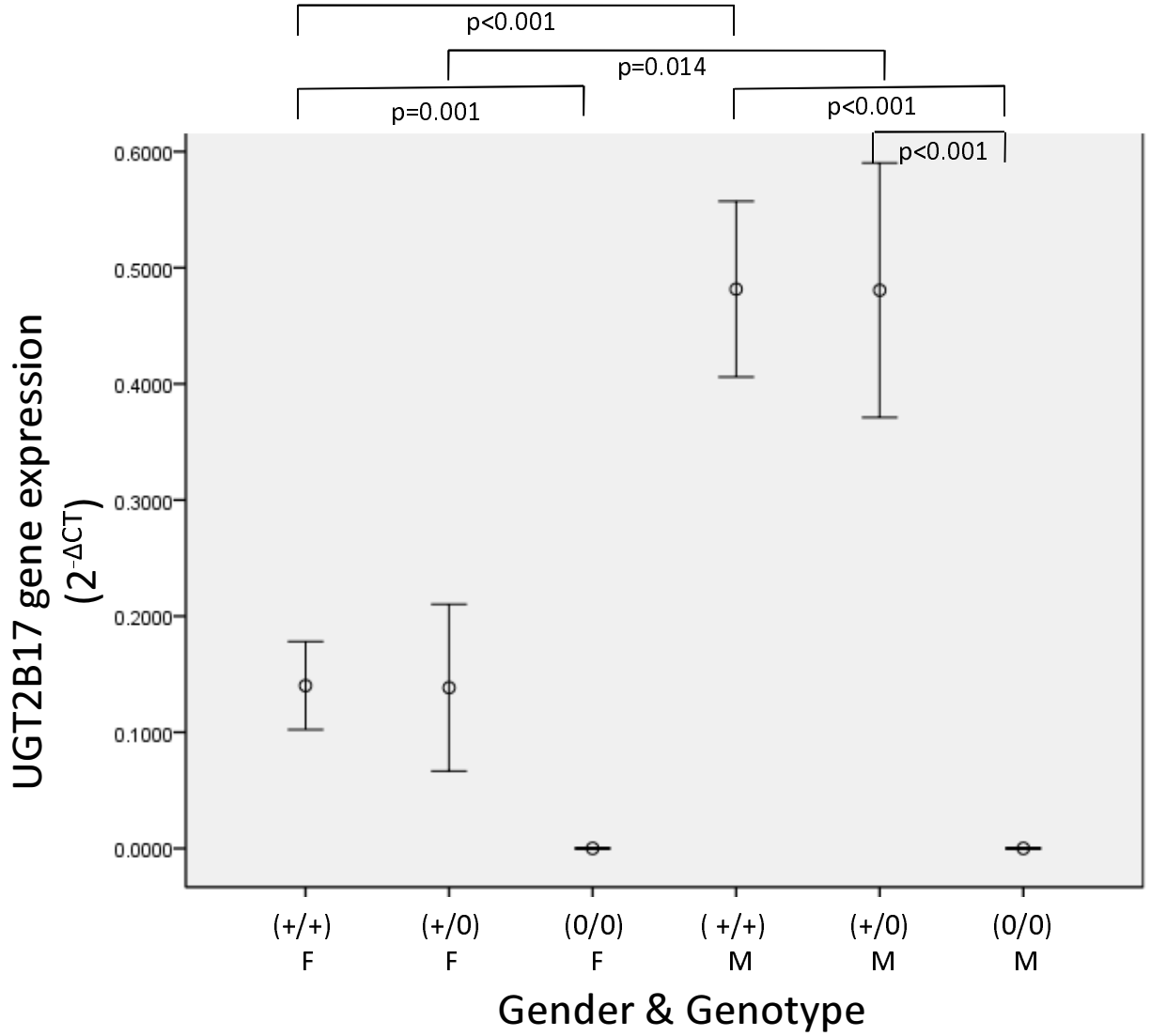


Figure 2A

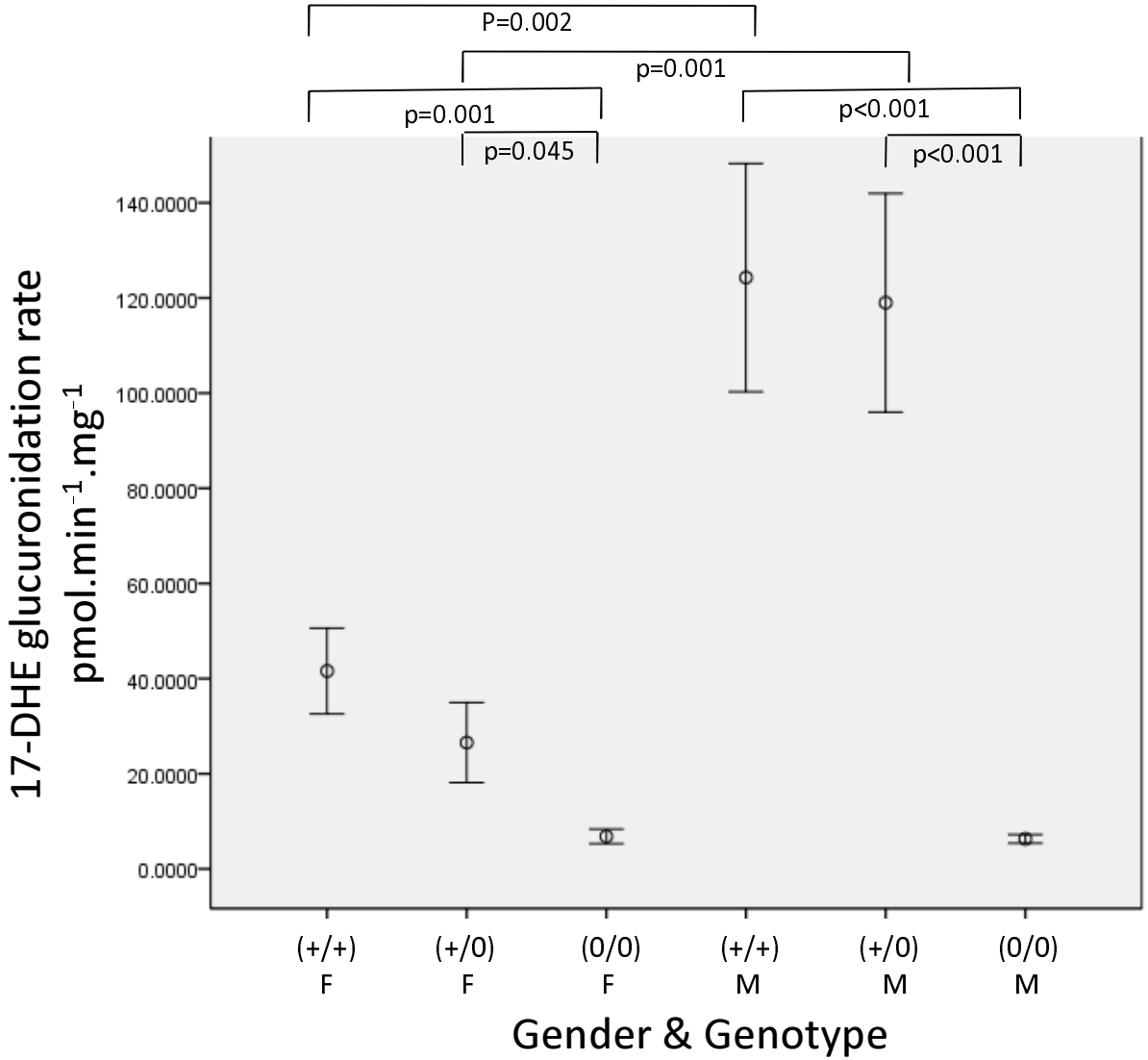


Figure 2B

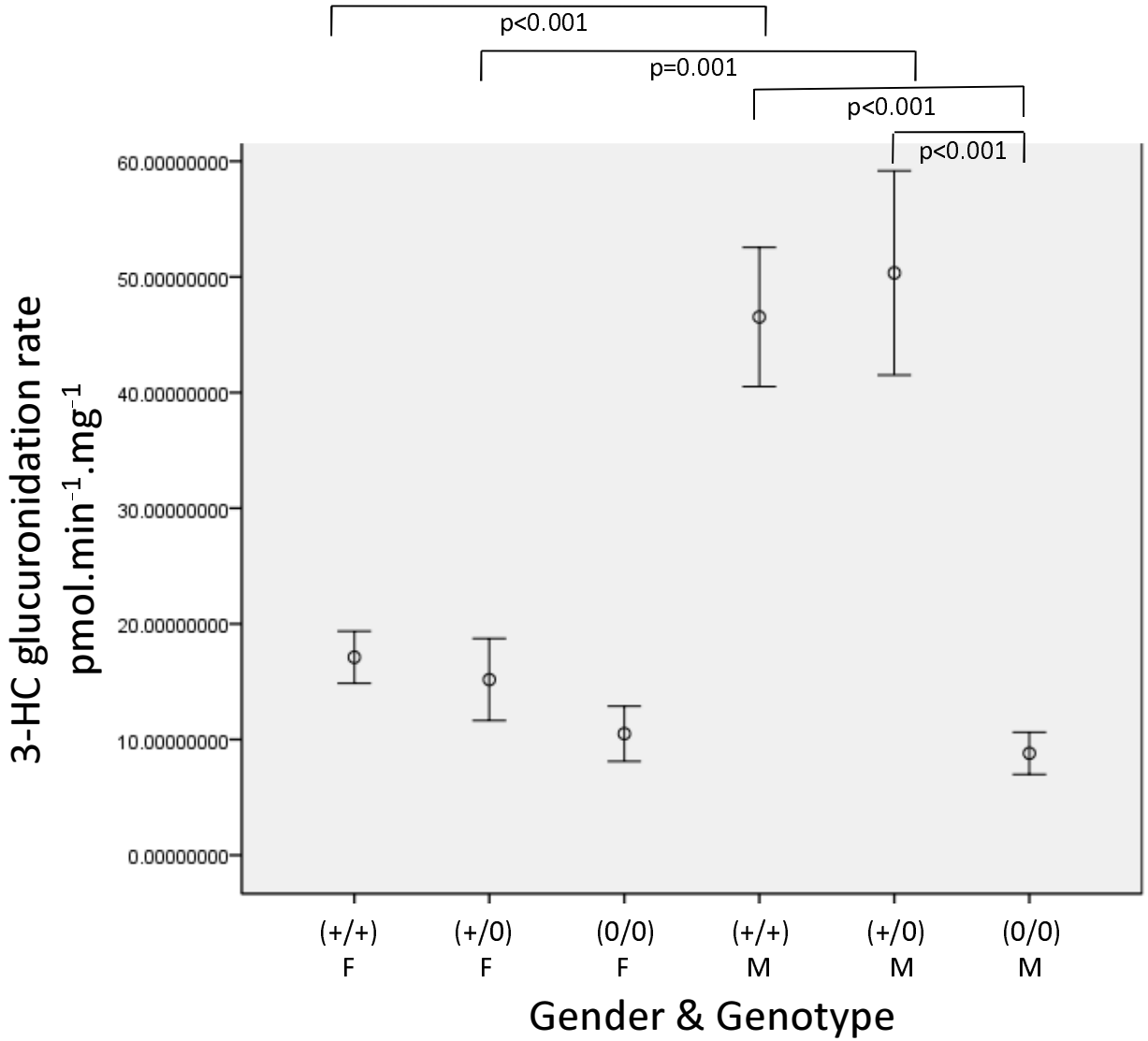


Figure 2C

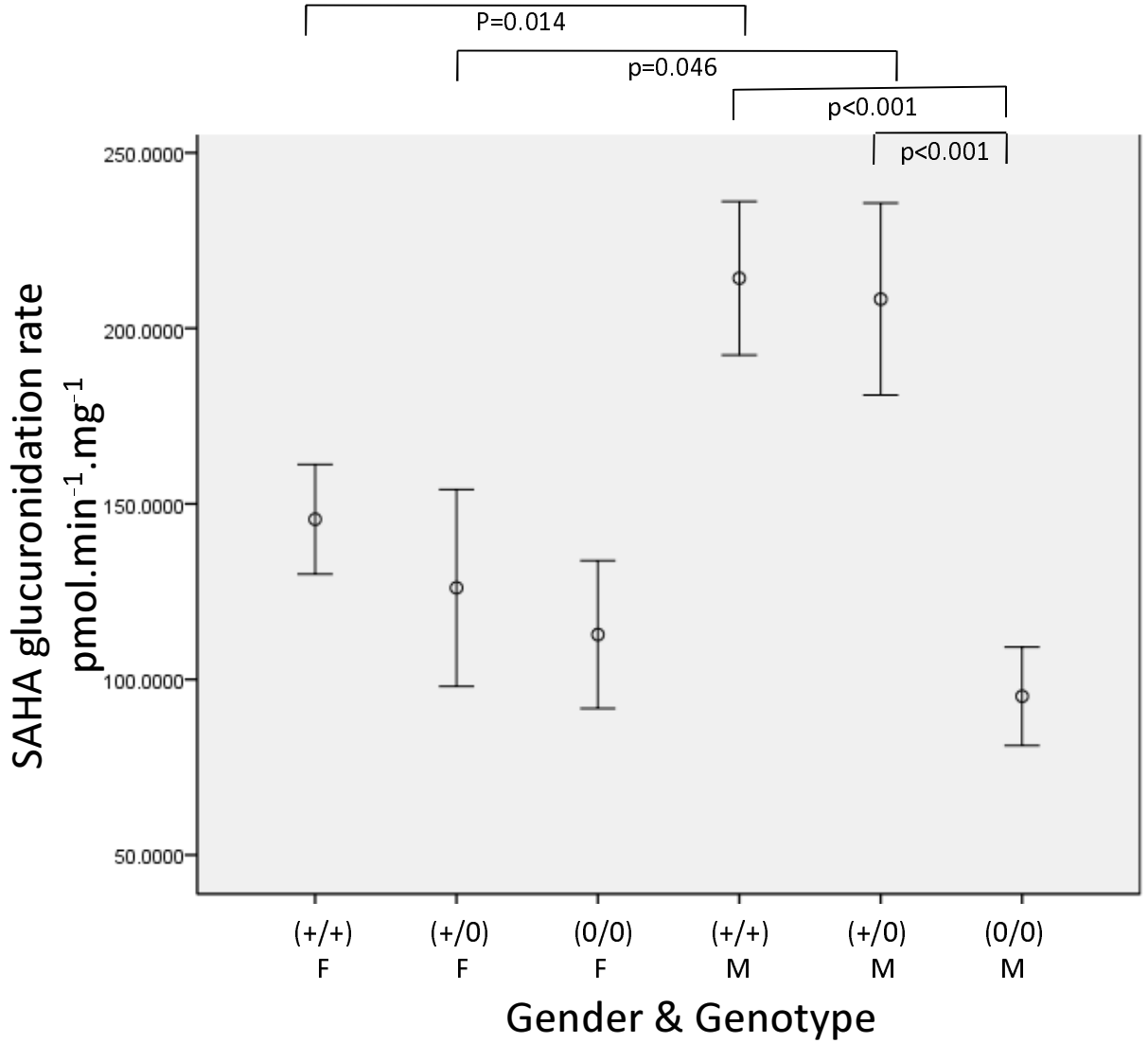
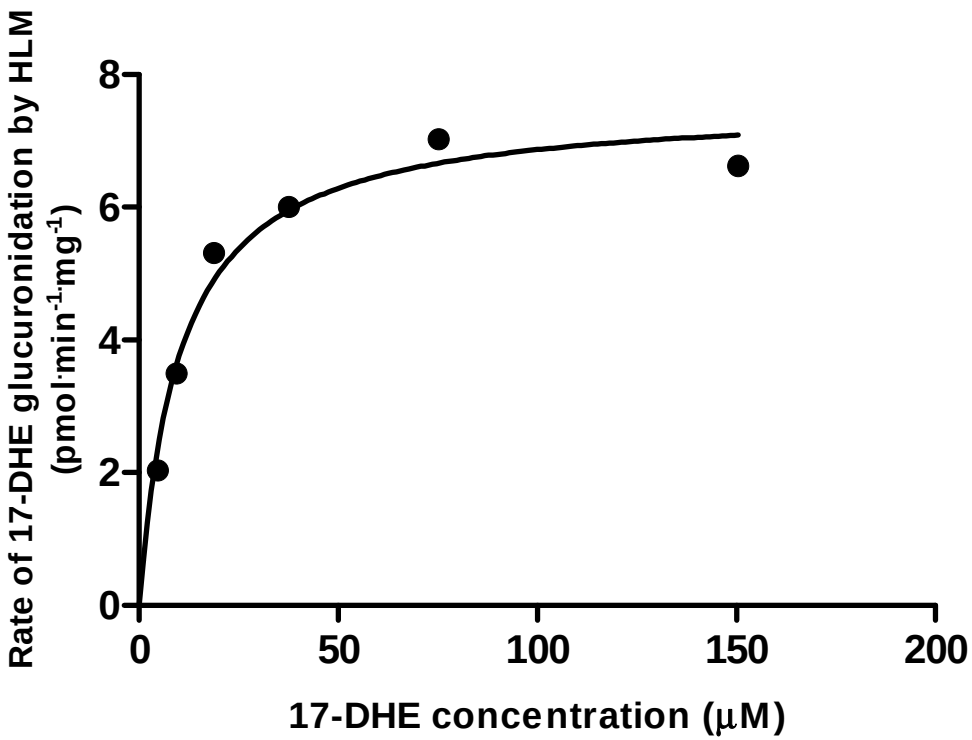


Figure 3A



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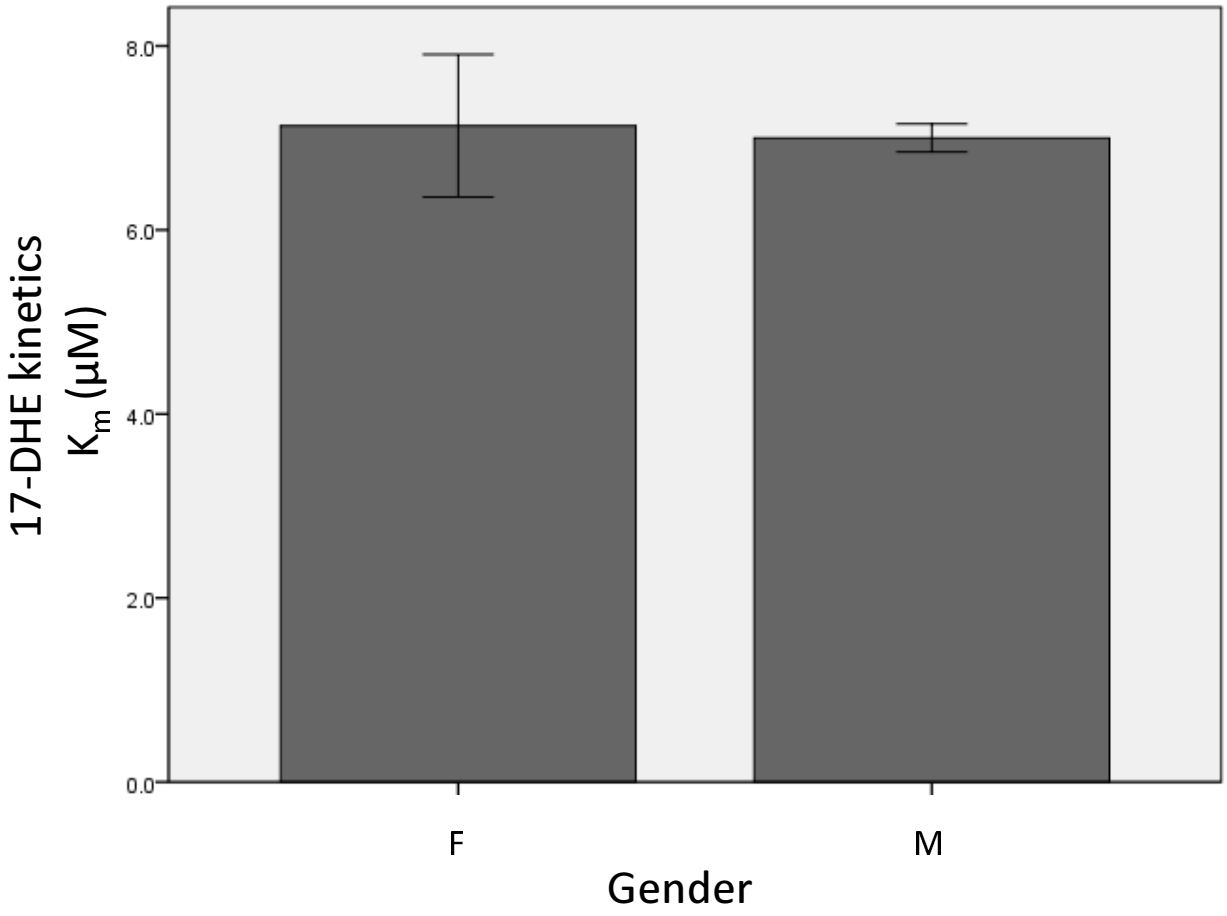

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Figure 3C

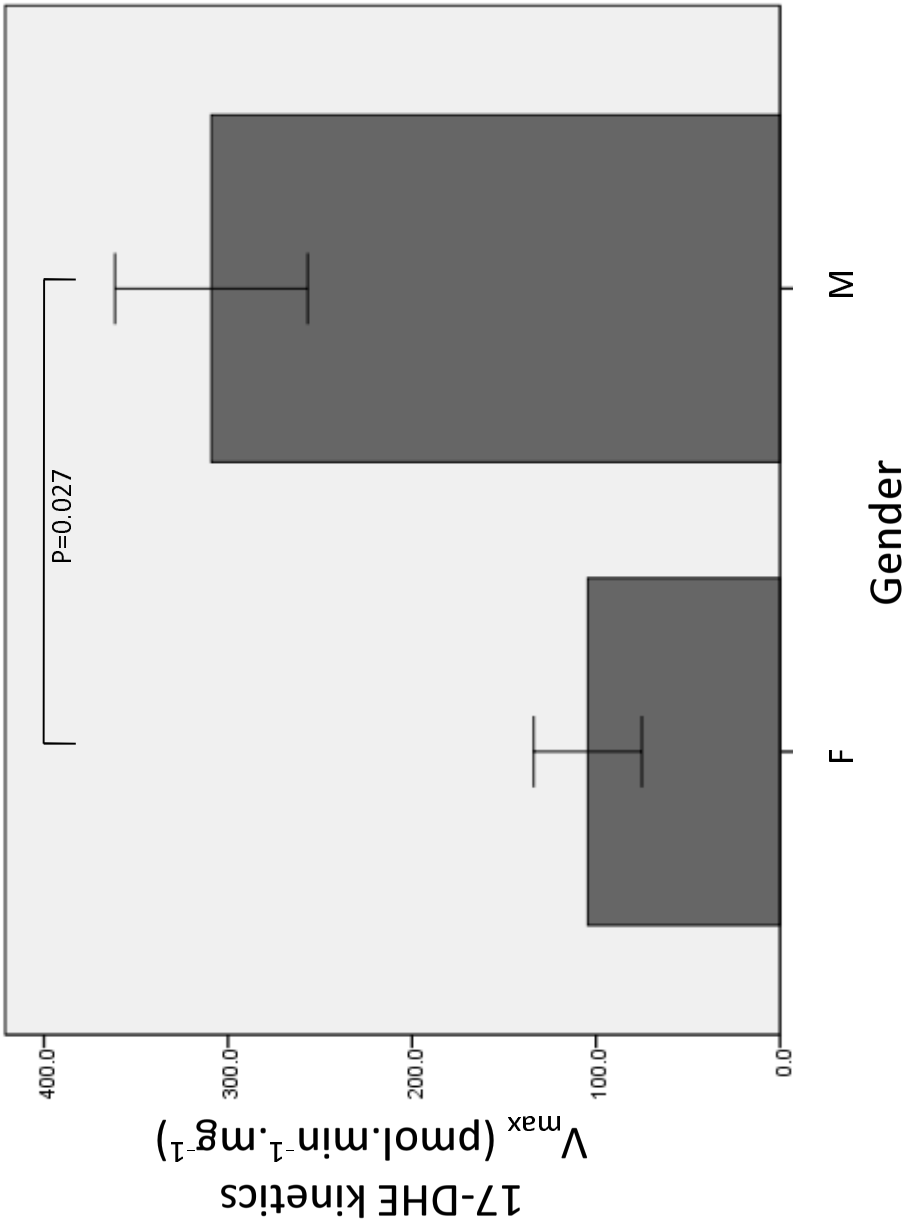

Figure 3D