Pharmacokinetics of bupropion and its pharmacologically active metabolites in pregnancy

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List of non-standard abbreviations: AUC$_{ss}$, area under the curve at steady state; BID, two times a day; BUP, bupropion; CL/F, apparent oral clearance; CL, clearance; EB, erythrohydrobupropion; EM, extensive metabolizer; GFR, glomerular filtration rate; IM, intermediate metabolizer; LC-MS, liquid chromatography – mass spectrometry; M.R., metabolic ratio; OHBUP, hydroxybupropion; PK, pharmacokinetics; PM, poor metabolizer; SNP, single nucleotide polymorphism; SR, sustained release; TB, threo hydrobupropion; TID, trice daily; QD, once a day; UM, ultra-rapid metabolizer; 11βHSD1, 11β-hydroxysteroid dehydrogenase
Abstract

Bupropion sustained release (SR) is used to promote smoking cessation in males and non-pregnant females. However, its efficacy as a smoking cessation aid during pregnancy is not reported. The pregnancy-associated changes in maternal physiology may alter the pharmacokinetics and pharmacodynamics of bupropion, and consequently, its efficacy in pregnant smokers. Therefore, the aims of this study were to determine the steady-state pharmacokinetics of bupropion during pregnancy and the effect of functional genetic variants of \textit{CYP2B6} and \textit{CYP2C19} on bupropion pharmacokinetics in pregnant women. Plasma and urine concentrations of bupropion and its metabolites hydroxybupropion (OHBUP), threohydrobupropion, and erythrohydrobupropion were determined by liquid chromatography-mass spectrometry. Subjects were genotyped for 5 non-synonymous SNPs that result in 7 \textit{CYP2B6} alleles, namely *2, *3, *4, *5, *6, *7, and *9, and for \textit{CYP2C19} variants *2, *3, and *17. The present study reports that the isoform-specific effect of pregnancy on bupropion-metabolizing enzymes along with the increase of renal elimination of the drug could collectively result in a slight decrease in exposure to bupropion in pregnancy. On the other hand, pregnancy-induced increase in \textit{CYP2B6}-catalyzed bupropion hydroxylation did not impact the plasma levels of OHBUP, probably due to a higher rate of OHBUP glucuronidation and renal elimination associated with pregnancy. Therefore, exposure to OHBUP, a pharmacologically active metabolite of the bupropion, appears to be similar to that of the non-pregnant state. The predicted metabolic phenotypes of \textit{CYP2B6}*6 and variant alleles of \textit{CYP2C19} in pregnancy are similar to those in the non-pregnant state.
Introduction

Bupropion sustained release (SR), an antidepressant, is used clinically in a standardized dose of 150 mg twice a day to promote smoking cessation in males and non-pregnant females (Raupach and van Schayck, 2011). However, its efficacy as a smoking cessation aid for pregnant smokers is not reported.

The pharmacokinetic data for bupropion in humans reported in the literature were obtained from males and non-pregnant females after single or multiple doses (Benowitz et al., 2013; Laizure et al., 1985). Bupropion is extensively metabolized via multiple pathways (Jefferson et al., 2005); three major metabolites of the drug in plasma, namely, hydroxybupropion (OHBUP), threohydrobupropion (TB) and erythrohydrobupropion (EB), are pharmacologically active (Laizure et al., 1985). OHBUP is half as potent as the parent drug, while TB and EB have lower activity (Jefferson et al., 2005; Golden et al., 1988). At steady state, the plasma level of OHBUP greatly exceeds that of the parent drug; therefore, OHBUP is thought to be the major contributor to the pharmacologic activity of bupropion (Golden et al., 1988).

CYP2B6 is the principal enzyme catalyzing the formation of OHBUP from bupropion in liver (Hesse et al., 2000), and the formation of TB and EB is catalyzed by hepatic 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) and carbonyl reductases (Molnari et al., 2012). In addition, CYP2C19 contributes to hydroxylation of bupropion and its metabolites, TB and EB (Zhu et al., 2014). Both CYP2B6 and CYP2C19 genes are highly polymorphic and some of the single nucleotide polymorphisms (SNPs) have functional consequences (http://www.cypalleles.ki.se/). Specifically, the CYP2B6*6 allele of CYP2B6 represents the combination of 516Q>T and 785A>G SNPs and is
associated with reduced protein expression and enzymatic activity (Zanger et al., 2013). 

CYP2B6 variants are associated with altered plasma concentrations of OHBUP (Benowitz et al., 2013; Høiseth et al., 2015), and bupropion (Kirchheiner et al., 2003).

The positive correlation between levels of OHBUP and response to smoking cessation treatment with bupropion was previously reported (Zhu et al., 2012). On the other hand, carriers of the CYP2B6*6 variant, which is associated with slower metabolism of bupropion, have higher abstinence rates than wild type-allele carriers (Lee et al., 2007). Therefore, it appears that the levels of bupropion and its metabolite, OHBUP, could affect the quit rate in smokers treated with bupropion for cessation.

During pregnancy, women experience numerous physiological changes that could affect the pharmacokinetic profile of bupropion (Loebstein et al., 1997). Pregnancy-induced increases in hepatic flow may accelerate bupropion metabolic clearance (Loebstein et al., 1997). Furthermore, in vitro studies suggest the upregulation of hepatic CYP2B6 and downregulation of CYP2C19 by increased production of progestational hormones (Dickman and Isoherranen, 2013; Anderson et al., 2005; Mwinyi et al., 2010). These in vitro findings were corroborated by observations in vivo: clearance of CYP2B6 substrates, namely methadone and efavirenz, was higher in pregnancy (Wolff et al., 2005; Olagunju et al., 2015), while clearance of the CYP2C19 substrate proguanil was decreased (McGreedy et al., 2003).

Bupropion and OHBUP are only moderately bound to plasma proteins (84% and 77%, respectively) (GlaxoSmithKline, data on file); therefore, pregnancy-associated declines in plasma albumin (<15%) and alpha-1-acid glycoprotein (50%) (Olagunju et al., 2012) should not alter the fraction of unbound bupropion and OHBUP. In addition,
the high lipophilicity of bupropion suggests its preferential distribution into the tissue over the plasma compartment; therefore, pregnancy-induced increases in body water should not affect bupropion biodisposition.

About 10% of the bupropion dose is recovered in the urine as the unchanged drug or as its free or glucuronidated OHBUP, TB and EB metabolites (Jefferson et al., 2005; Gufford et al., 2016). A recent study identified uridine glucuronosyl transferase (UGT) isoforms 2B7 and 1A9 as the primary enzymes catalyzing the glucuronidation of bupropion metabolites (Gufford et al., 2016). Pregnancy-associated upregulation of UGT enzymes (Anderson, 2005) along with the increase in renal blood flow in pregnancy (Costantine, 2014) could accelerate renal elimination of bupropion metabolites.

Taken together, it appears that while the effect of pregnancy-induced changes in plasma volume and plasma protein concentrations on the pharmacokinetics of bupropion is unlikely, changes in renal function, hepatic flow and pregnancy-associated induction of CYP2B6 and reduced activity of CYP2C19 could affect the pharmacokinetic profile of bupropion in pregnancy.

Therefore, the primary aim of this study was to determine the pharmacokinetics of bupropion during pregnancy. The secondary aim was to explore the association between CYP2B6 and CYP2C19 genotypes and the metabolism of bupropion during pregnancy. The data would provide evidence on the magnitudes of the effects of genetics and pregnancy on the biodisposition of bupropion in pregnancy.
Material and methods

Subjects

This was a prospective, opportunistic study conducted at the University of Texas Medical Branch (UTMB). Eligible participants were pregnant women taking bupropion to treat depression who agreed to participate in the pharmacokinetic (PK) studies in pregnancy and postpartum. Decisions about diagnosis and treatment were made by the subjects’ own healthcare provider(s) and were independent of participation in this study. The eligible participants were 18 years of age or older and in a pregnancy window of 10-14 weeks (early pregnancy), 22-26 weeks (mid-pregnancy), and 34-38 weeks (late pregnancy). Women were excluded from participation if there was anemia with hematocrit of less than 28% or a prior history of or current medical examination consistent with the presence of clinically significant alterations in hepatic, renal or gastrointestinal functions. All procedures involving human subjects were conducted according to the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines in agreement with the declaration of Helsinki. All women were enrolled with written informed consent under a protocol that was reviewed and approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB). All subjects were compensated for participation.

Study protocol

Subjects in this opportunistic study received the following formulations and dosages of bupropion: immediate-release (IR), 100 mg three times daily TID (Mylan), sustained-release (SR), 150 mg once a day (QD) (Teva, Actavis) and 150 mg twice a day (BID) (Actavis, GSK, Watson lab), and extended release (XL), 300 mg QD (Actavis,
Zydus). Prior to the PK study all subjects completed at least 4 days of a dosing diary and were therefore presumed to be at steady state. Serial blood samples were collected prior to dosing (0 hours) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and up to 10, 12 and 24 hours, depending on the respective dosing intervals. All blood samples were collected in heparinized BD Vacutaner® tubes, and plasma was separated immediately by centrifugation. Urine samples were collected within the same dose interval as the blood samples. All urine output was collected and the volume noted. Blood for genotyping was collected in BD Vacutaner® EDTA purple-top tubes. All samples were stored at -80°C until analysis.

**Plasma and urine sample analysis**

The concentrations of bupropion, OHBUP, EB and TB in plasma were determined simultaneously using a modified liquid chromatography – mass spectrometry (LC-MS/MS) method developed and validated in our laboratory as previously reported (Wang et al., 2012). The concentrations of bupropion and its metabolites, namely OHBUP, TB and EB, in urine were determined separately using a modified LC-MS method (Wang et al., 2010). The urine samples for quantification of bupropion metabolites were processed with and without glucuronide deconjugation using a modified method of Petsalo et al (Petsalo et al., 2007). The glucuronides of OHBUP, EB and TB were quantified as the difference between the concentrations of the non-conjugated (free) drug and the total. The validation of the LC-MS methods was performed following the U.S. Food and Drug administration guideline (FDA, 2001), detailed description of bupropion and its metabolites assayed in plasma and urine is
provided in Supplemental methods 1. The concentrations of creatinine in serum were
determined in the biochemical laboratories of UTMB.

**CYP2B6 and CYP2C19 genotyping**

Subjects were genotyped for 5 non-synonymous SNPs that result in the 7 common CYP2B6 variant alleles, namely CYP2B6*2 (64C>T), CYP2B6*3 (777C>A), CYP2B6*4 (785A>G), CYP2B6*5 (1459C>T), CYP2B6*6 (516G>T and 785A>G), and CYP2B6*7 (516G>T, 785A>G and 1459C>T). SNPs were identified following the PCR-RFLP methods reported previously (Lang et al., 2001) and allele discrimination assays using TaqMan probes (Thermo Fisher Scientific Inc., Waltham, MA); the specifics are provided in the Supplemental materials and methods section 1. The most common alleles of CYP2C19 are the “loss-of-function” variants CYP2C19*2 (681G>A, rs4244285) and *3 (636G>A; rs4986893), and the “gain-of-function” variant CYP2C19*17 (-806C>T; rs12248560) (Fricke-Galindo et al., 2016). The identification of these alleles in our subjects was conducted as described by Zhu et al., 2014; details are provided in Supplemental materials and methods section 2.

**Data analysis**

The PK parameters were computed using non-compartmental analysis (Kinetica software version 5.0, Thermo Scientific, Waltham, MA). Area under the plasma concentration-time curve for a dose interval at steady state (AUC<sub>ss</sub>) served as the main measure of exposure to bupropion and its metabolites. For the subjects whose plasma sampling times were terminated prior to the end of the respective dosing intervals, the remaining plasma concentration values were extrapolated from the best fit curve, and the AUC<sub>ss</sub> values were computed as the sum of AUC<sub>0-n</sub> and AUC<sub>n+1</sub>, where \( n \) is the last
measured time point. The apparent steady state oral clearance (CL/F\textsubscript{ss}) of bupropion was estimated as dose/AUC\textsubscript{ss} with and without normalization to the actual body weight (kg). The activity of CYP2B6 was estimated using the OHBUP/BUP metabolic ratio in plasma, calculated as a ratio of AUC\textsubscript{ss} for OHBUP over that of the parent drug, corrected for molecular weight difference. Clearance via reductive metabolic pathways was estimated in a similar fashion as TB/BUP and EB/BUP metabolic ratios. Renal clearance (CL\textsubscript{R}) of bupropion and its metabolites was calculated as ((urine concentration, ng/mL)/(average plasma concentration, ng/ml)) X ((urine volume, mL)/(dose interval, hrs). The molar percentage of bupropion dose excreted as the parent drug and metabolites was calculated as (total excreted, mg)/(dose, mg)*100%, corrected for the molecular weight difference. Creatinine clearance was estimated using the Cockcroft-Gault formula, 0.85*(140-age [yr])/(serum creatinine [mg/dL])*(pre-pregnancy weight [kg]/72).

**Statistical analysis**

Results are presented as mean values ± standard deviation. Pairwise statistical comparisons were conducted using Wilcoxon signed rank test (SPSS Statistics, version 23, IBM Corp., Armonk, NY). The Mann-Whitney-U test (SPSS Statistics, version 23, IBM Corp., Armonk, NY) was used to compare PK data obtained from pregnant subjects homozygous for the CYP2B6 wild type allele and carriers of the CYP2B6*6 allele, as well as to conduct comparisons between the metabolizer phenotypes of CYP2C19. P values < .05 were considered statistically significant. Post-hoc analysis of statistical power was conducted using G*Power 3.1.9.2. (Faul et al., 2009).
Results

Subjects

Twenty-nine subjects volunteered to participate in this opportunistic study. One subject was excluded from analysis due to deviations from the study protocol. Characteristics of the remaining 28 subjects are shown in Supplemental table 1. At enrollment, the subjects had a mean age of 29.2 ± 6.9 (21-39) years, the mean gestational age was 27.5 ± 8.5 (13.1-38.0) weeks, and the average body weight was 86.8 ± 24.6 (50.4-168.8) kg. The majority of subjects were white/non-Hispanic (57%) and white/Hispanic (36%). Five subjects (18%) were enrolled during the early window, 11 (39%) during the middle window, and 12 (43%) during the late window. Depending on the time of enrollment and compliance, 9 subjects (32%) completed one PK visit, 12 (43%) completed two PK visits, 6 (21%) completed three PK visits, and 1 subject completed four PK visits. Sixteen subjects were prescribed bupropion SR 150 mg BID, five subjects took bupropion SR 150 mg QD, three subjects took bupropion IR 100 mg TID, and two subjects took bupropion XL 300 mg QD. Having the same dose/formulation of bupropion was an essential criterion for adequate paired comparison of PK parameters.

Pharmacokinetics of bupropion and its metabolites during pregnancy and postpartum

Table 1 shows the paired estimated PK parameters of bupropion and its metabolites for 8 subjects in middle and late pregnancy, and for 12 subjects in late pregnancy and postpartum (lactating or non-/post-lactation period depending on
availability). Only post-lactating PK parameters were used for subjects 2 and 8, who participated during both postpartum studies (lactating and non-/post-lactating).

Paired analysis did not reveal any difference in the mean apparent oral clearance of bupropion (CL/F<sub>ss</sub>) between the tested treatment windows, with or without adjustment to weight (Table 1). However, we observed that the mean value of AUC<sub>ss</sub> of bupropion in late pregnancy was slightly lower than that of postpartum (654 ± 301 ng*h/ml vs 775 ± 291 ng*h/ml, \(P = 0.099\), Table 1). Furthermore, data analysis did not reveal any differences in the mean values of either OHBUP/BUP metabolic ratio or AUC<sub>ss</sub> of OHBUP in mid- vs late pregnancy comparisons or late pregnancy vs postpartum (Table 1).

The mid-pregnancy mean value for TB AUC<sub>ss</sub> was slightly higher than that in late pregnancy, although the results were not statistically significant (4843 ± 3196 ng*h/ml vs 3911 ± 2896 ng*h/ml, \(P = .068\), Table 1). No difference in TB AUC<sub>ss</sub> was observed in late pregnancy as compared to the non-pregnant state. However, the TB/BUP metabolic ratio in late pregnancy was slightly higher than that of postpartum (6.79 ± 3.60 vs 5.21 ± 3.10, \(P = .06\), Table 1).

The mean values for EB AUC<sub>ss</sub> and EB/BUP metabolic ratio in mid pregnancy were higher than those of late pregnancy (EB AUC<sub>ss</sub>: 759 ± 447 ng*h/ml vs 541 ± 370 ng*h/ml, \(P < .05\); EB/BUP: 1.33 ± 0.57 vs 1.06 ± 0.57, \(P < .05\), Table 2). Although we observed lower EB AUC<sub>ss</sub> in late pregnancy than postpartum (621 ± 387 ng*h/ml vs 871 ± 586 ng*h/ml, \(P = .05\), Table 1), no difference was revealed in the corresponding mean values of EB/BUP metabolic ratios (Table 1).

**Urinary elimination of bupropion and its metabolites**
Data on the excretion of bupropion and its metabolites in the urine are shown in Table 2. The mean value of creatinine clearance in mid-pregnancy was higher than that in late pregnancy (185 ± 45 mL/min vs 166 ± 33 mL/min, \( P < .05 \)), while the mean value of creatinine clearance in late pregnancy was higher as compared to that of postpartum (175 ± 38 mL/min vs 128 ± 23 mL/min, \( P < .05 \)).

Comparisons of renal clearance of the drug and its metabolites in late pregnancy vs postpartum did not reveal any differences (Table 2). Moreover, no difference was observed in renal clearance of OHBUP, EB and TB in mid- vs late pregnancy comparisons, while renal clearance of bupropion in mid-pregnancy was slightly elevated as compared to late pregnancy (23.1 ± 12.5 mL/min vs 9.06 ± 5.80 mL/min, \( P = .068 \), Table 2)

No statistically significant differences in the fractions of bupropion dose eliminated in the urine as unchanged drug or as unconjugated metabolites OHBUP, TB and EB, were observed between the groups. The percentage of bupropion dose recovered in the urine as unchanged drug in late pregnancy was slightly below that of postpartum (0.51 ± 0.59% vs 0.87 ± 1.01%, \( P = .059 \), Table 2). A similar trend was observed in late pregnancy vs postpartum comparison of the percentage of the drug dose excreted in urine in a form of unconjugated EB (0.76 ± 0.68% vs 1.00 ± 0.76%, \( P = .062 \), Table 2). Moreover, the percentage of bupropion dose excreted as free TB and free EB metabolites in mid-pregnancy tended to exceed the percentages excreted in late gestation (for TB-free, 15.9 ± 11.1 % vs 6.37 ± 6.71 %, \( P = .068 \), and for EB-free 1.71 ± 1.54% vs 0.47 ± 0.48%, \( P = .068 \), Table 2).
In pregnancy, 89 ± 9% of total OHBUP eliminated in the urine was excreted in a glucuronidated form, while TB and EB conjugates accounted for 28 ± 20% and 46 ± 23% of total excreted TB and EB, respectively. The fraction of bupropion eliminated as OHBUP-glucuronide was higher in late pregnancy than postpartum (13.8 ± 15.7% vs 6.25 ± 5.47%, \( P < .05 \), Table 2). Likewise, the fraction of bupropion recovered in the urine as TB-glucuronide in late pregnancy was higher than that of postpartum (3.10 ± 2.20% vs 1.00 ± 1.15%, \( P < .05 \), Table 3). In addition, OHBUP-glucuronide as % of bupropion dose recovered in urine in late pregnancy slightly exceeded that of mid-pregnancy (11.7 ± 8.10% vs 7.97 ± 4.47%, \( P = .068 .05 \), Table 2). However, TB-glucuronide recovered in urine as % of bupropion dose in mid-pregnancy did not differ from that in late pregnancy (Table 2). The results showed no difference in the urinary excretion of EB-glucuronide in pregnancy and postpartum.

**CYP2B6 and CYP2C19 genetic variants and pharmacokinetics of bupropion in pregnancy**

Bupropion pharmacokinetic parameters were compared among the pregnant subjects with and without genetic variant alleles of CYP2B6 and CYP2C19. We aimed to conduct the comparisons in early, middle and late pregnancy separately in order to minimize the effect of gestational age-associated changes. However, comparative analysis within the early pregnancy group was not possible due to an insufficient number of subjects (n=5, Supplemental table 1). In the remainder of the pregnancy groups, bupropion clearance and metabolic ratios were examined irrespective of the drug dosing; while comparisons of the urinary excretion data and AUCss of bupropion
and its metabolites were restricted to those subjects treated with the same dose of the drug, 150 mg BID.

Thirteen pregnant women participated in the PK study during mid-pregnancy, and twenty-one participated during late pregnancy (Supplemental table 1). The following CYP2B6 genotype combinations were observed in the study subjects: in mid-pregnancy (n=10 total), five subjects were of *1/*1 wild-type for CYP2B6, three were *1/*6, one was *6/*6 and one was *1/*5. In late pregnancy (n=19 total): eleven were *1/*1, four were *1/*6, two were *6/*6, one was *1/*9 and one was *4/*4 (Supplemental table 1).

Based on the CYP2B6 allele frequencies in both groups, we compared the PK parameters between carriers of *6 (which confers reduced activity) and wild type carriers (Supplementary table 2, Figure 1A-C).

In mid-pregnancy, the OHBUP/BUP metabolic ratio tended to be lower in *6 carriers than in wild type, (9.46 ± 4.4 vs 32.8 ± 34.0, P = .086, Figure 1A), which is consistent with the reduced metabolic phenotype of CYP2B6*6 allele. Although no difference was observed in OHBUP AUCss (Figure 1B, mid-pregnancy), the BUP AUCss trended higher in *6 carriers in mid-pregnancy (742 ± 114 ng*h/ml vs 414 ± 225 ng*h/ml, P = .077, Figure 1C). The mid-pregnancy AUCss of TB and EB also appeared to be higher in *6 carriers than in subjects homozygous for the wild-type allele (7263 ± 3116 ng*h/ml vs 2553 ± 2084 ng*h/ml, for TB, P = .077, and 1119 ± 393 ng*h/ml vs 477 ± 340 ng*h/ml for EB, P < .05, Supplemental table 2). Neither comparison in late pregnancy revealed any differences (Supplemental table 2, Figure 1A-C). Moreover, no differences in urinary excretion data were observed between the *6 carriers and those
with the wild type CYP2B6 variant in mid- or late pregnancy comparisons (Supplemental table 2)

The observed CYP2C19 genotype combinations are presented in Supplemental table 1 and were as follows: in mid-pregnancy (n=12 total): four subjects were *1/*1 wild-type for CYP2C19, two were *1/*17, one was *17/*17, four were *1/*2, and one was *2/*2. In late pregnancy (n=19 total): ten were *1/*1, two were *1/*17, five were *1/*2, one was *2/*17 and one was *2/*2 (Supplemental table 1). The subjects were stratified in two groups based on their metabolic phenotypes (Scott et al., 2013). Thus, pharmacokinetic parameters obtained from “extensive” metabolizers (EM) and “ultra-rapid” metabolizers (UM), namely *1/*1, *1/*17, and *17/*17 carriers, were compared with those of “poor” metabolizers (PM) and “intermediate” metabolizers (IM), namely *2/*2 and *1/*2, including *2/*17 (Supplemental table 3).

The TB/BUP metabolic ratio in the “poor/intermediate” metabolizers (PM+IM) group was higher than in the “extensive/ultra-rapid” (EM+UM) group in both mid- and late pregnancy (mid-pregnancy, 11.6 ± 3.16 vs 6.58 ± 3.33, P < .05, late pregnancy, 11.6 ± 3.16 vs 6.58 ± 3.33, P < .05, Figure 2A, Supplemental table 3). We observed higher AUCss TB in PM+IM than in EM+UM in late pregnancy (5773 ± 2517 ng*h/ml vs 2333 ± 1313 ng*h/ml, P < .05, Figure 2B, Supplemental table 3); however, no difference in the AUCss of TB was observed between the groups in mid-pregnancy (Figure 2B). Moreover, no difference in BUP AUCss was revealed between PM+IM and EM+UM groups in both mid- and late pregnancy comparisons.

In a similar pattern, the EB/BUP metabolic ratio in PM+IM group was higher than in the EM+UM group in both mid- and late pregnancy, although statistical significance
was not attained in mid-pregnancy comparisons (mid-pregnancy, 1.64 ± 0.46 vs 0.95 ± 0.56, \(P = .088\); late pregnancy, 1.57 ± 0.34 vs 1.03 ± 0.52, \(P < .05\), Figure 2C, Supplemental table 2). The EB AUC\textsubscript{ss} in the PM+IM group was slightly higher than in the EM+UM group in late pregnancy (782 ± 350 ng\textsuperscript{*}h/ml vs 403 ± 273 ng\textsuperscript{*}h/ml, \(P = .055\), Figure 2D, Supplemental table 3); however, no difference was observed in the mean value of EB AUC\textsubscript{ss} in mid-pregnancy comparisons.

The percentage of bupropion dose recovered in urine of PM+IM as conjugated TB in late pregnancy was 5.15 ± 2.17% and was higher than that of EM+UM (1.80 ± 1.18%, \(P < .05\), Supplemental table 3). In addition, the percentage of bupropion dose recovered as unconjugated OHBUP and TB in the urine of PM+IM subjects in late pregnancy slightly exceeded that of the EM+UM group, although statistical significance was not reached (OHBUP-free: 0.71 ± 0.42% vs 0.35 ± 0.16%, \(P = .068\); TB-free: 9.69 ± 6.01% vs 2.21 ± 1.41%, \(P = .068\), Supplemental table 3).

**Discussion**

The typical bupropion SR dose for promoting cessation from smoking is 150 mg BID for 7-12 weeks in non-pregnant smokers. Bupropion is extensively metabolized, and its major product OHBUP contributes to the drug’s anti-smoking properties. Pregnancy induced physiological changes in the activity of hepatic enzymes metabolizing bupropion—as well as increased hepatic blood flow and increased renal plasma flow—can alter the pharmacokinetics of bupropion.

The OHBUP/BUP metabolic ratio has been historically used as a measure of CYP2B6 activity in bupropion hydroxylation. Several studies suggest that the activity of CYP2B6 primarily affects the OHBUP levels but not bupropion (Zhu et al., 2012;
Benowitz et al., 2013; Høiseth et al., 2015). The pregnancy-induced upregulation of CYP2B6 has been suggested based on *in vitro* and *in vivo* studies (Anderson et al., 2005; Olagubju et al., 2015). However, in our study we did not observe any significant changes in the OHBUP/BUP metabolic ratio and OHBUP AUC<sub>ss</sub> in pregnancy as compared to the non-pregnant state.

Further, the EB/BUP metabolic ratio in mid-pregnancy exceeded that of late pregnancy. Moreover, the TB/BUP metabolic ratio was slightly higher in late pregnancy than in postpartum, although not statistically significant. These data suggest an increase in the reductive metabolism of bupropion in pregnancy. However, CYP2C19 also contributes to the hydroxylation of bupropion and its metabolites, TB and EB (Zhu et al., 2014; Chen et al., 2010). We observed that relative to late pregnancy, the AUC<sub>ss</sub> of EB was higher in mid-pregnancy, suggesting a decreased rate of EB metabolism in mid-pregnancy, possibly due to pregnancy-induced downregulation of *CYP2C19* (McGreedy et al., 2003). Hence, this could contribute to an increased EB/BUP metabolic ratio in mid-pregnancy. The slight increase in the AUC<sub>ss</sub> of TB in mid-pregnancy relative to late pregnancy was not statistically significant, and had no effect on the corresponding TB/BUP ratios. We cannot discount the potential decrease in CYP2C19-mediated metabolism of bupropion during pregnancy; however, the accelerated CYP2B6-catalyzed hydroxylation of bupropion could possibly counterbalance bupropion metabolic clearance. As a net result, we detected no significant changes in the AUC<sub>ss</sub> of bupropion in pregnancy (slight decrease in late pregnancy vs postpartum, *P* = .099); and no effect of pregnancy on the bupropion CL/F<sub>ss</sub> was observed.
Another factor that could affect the pharmacokinetics of bupropion is urinary excretion of the drug and its metabolites due to pregnancy induced increase in renal plasma flow. Our findings indicated a slight increase in the renal clearance of bupropion in mid-pregnancy relative to late pregnancy, which is probably associated with the peaking increment of glomerular filtration rate (GFR) around the second trimester of pregnancy (Hnat et al., 2009). Moreover, the higher percent of dose excreted as unconjugated TB and EB in mid-pregnancy as compared to late pregnancy could reflect the higher plasma levels (and consequently AUC<sub>ss</sub>) of TB and EB in mid-pregnancy, in addition to the increased GFR. However, the results in the urine excretion of the drug and its metabolites in mid- vs late pregnancy comparisons were not statistically significant. The small sample size leads to low statistical power for some analysis. A potential carryover of the drug and its metabolites from the previous dose(s) was a limitation of the urine PK analysis in our study.

We measured the fraction of bupropion recovered in the urine as the metabolites OHBUP, TB and EB, in their free forms or as glucuronide conjugates. Out of the OHBUP, TB and EB metabolites quantified in the urine of pregnant subjects, OHBUP was the most appreciably conjugated, followed by TB and EB. Relative to postpartum, the percent of bupropion dose excreted as TB- and OHBUP-glucuronides was higher in late pregnancy, which was consistent with the hormone-mediated upregulation of several hepatic UGT enzymes during pregnancy, particularly in late trimester (Jeong et al., 2008; Abernethy et al., 1982; Ohman et al., 2008). The current sample size for late pregnancy vs postpartum comparisons (n=12) was sufficient to achieve 93% statistical power for the TB-glucuronide data analysis, although for the OHBUP-glucuronide it was
43%. We did not measure the concentrations of conjugated metabolites in plasma, and that was one of the limitations in our study. However, the increased elimination rate of TB and OHBUP in their conjugated forms could contribute to the higher clearance of these metabolites. Therefore, it is possible that with pregnancy-induced upregulation of CYP2B6, the increase in the formation of OHBUP could not be observed due to a higher rate of OHBUP glucuronidation and its subsequent excretion. Likewise, the decrease in TB metabolism due to a pregnancy-associated downregulation of CYP2C19, along with an increase in TB clearance via glucuronidation would result in no evident changes in TB levels during late pregnancy.

In the second part of our study, we investigated the influence of functional polymorphisms of CYP2B6 and CYP2C19 on bupropion biodisposition in pregnancy, irrespective of pregnancy-induced changes. This was investigated to collectively understand the effects of both genetics and pregnancy on the pharmacokinetics of bupropion. Bupropion and its metabolites exhibit linear pharmacokinetics at steady state (Findlay et al., 1981); therefore, the influence of genotype on bupropion CL/Fss and OHBUP/BUP, TB/BUP and EB/BUP metabolic ratios were examined irrespective of dosing.

Our results showed higher TB/BUP and EB/BUP metabolic ratios in pregnant CYP2C19 PM+IM subjects in both mid- and late pregnancy groups, and higher TB and EB AUCss in these subjects in late pregnancy only. These results are consistent with the effect of CYP2C19 polymorphism on TB and EB in non-pregnant subjects (Zhu et al., 2014). However, it appears that CYP2C19 metabolizer phenotype did not influence the levels of bupropion, and consequently, its AUCss and CL/Fss in pregnant subjects in our
study. Small sample size in both mid- and late pregnancy groups limited the power of statistical analysis. Moreover, the sample size in the mid-pregnancy group was insufficient to observe the effect of CYP2C19 metabolizer phenotypes on the AUCs of TB and EB as was detected in late pregnancy. In addition, our sample size in both mid- and late pregnancy was insufficient to differentiate individually between the different CYP2C19 metabolic phenotypes, namely, PM, IM, EM, and UM.

In our study we did not observe any significant effect of the CYP2B6*6 variant on OHBUP/BUP metabolic ratio, and either BUP or OHBUP AUCs in pregnancy. However, the slight decrease in OHBUP/BUP metabolic ratio in carriers of CYP2B6*6 as compared to wild type carriers in mid-pregnancy suggest that in pregnant women, the CYP2B6*6 variant is associated with a reduced rate of bupropion hydroxylation, as observed in men and non-pregnant women (Benowitz et al, 2013). Of note, in the mid-pregnancy group, the AUCss of EB was higher in *6 carriers than in subjects without that variant. The results could indicate an imbalance of CYP2B6 and CYP2C19 genotypes in these individuals. In addition to the insufficient sample size, a limitation we acknowledge is that we did not genotype for the CYP2B6*18 reduced activity variant allele which is present exclusively in individuals of African descent, with an allele frequency of 4-7% (Zanger et al., 2013). There were only two African American pregnant subjects in our study, and neither of these two subjects was included in the CYP2B6 variant allele comparisons (Supplemental table 1).

Due to the limited number of participants in our study, we could not investigate the impact of CYP2B6 and CYP2C19 polymorphism on the magnitude of pregnancy-induced changes in the pharmacokinetics of bupropion and its metabolites.
Nevertheless, it appears that decreased activity of CYP2C19 due to pregnancy, along with loss-of-function variants of \textit{CYP2C19} could contribute to higher steady-state exposure to TB and EB during pregnancy. The TB and EB metabolites of bupropion have an inhibitory effect on the CYP2D6 enzyme (Parkinson et al., 2010), which is upregulated during pregnancy (Ke et al., 2013; Ryu et al., 2015). Therefore, possible drug-drug interactions of bupropion and CYP2D6 substrates cannot be discounted in pregnancy, particularly in instances when dose adjustment of CYP2D6-metabolized medications is considered (Ryu et al., 2015).

In summary, we reported the effect of pregnancy on the pharmacologic profile of bupropion, as well as the impact of \textit{CYP2B6} and \textit{CYP2C19} functional polymorphisms on bupropion biodisposition during mid- and late pregnancy. It appears that the pregnancy-induced increase in CYP2B6-catalyzed bupropion hydroxylation did not impact the plasma levels of OHBUP in pregnancy, probably due to a higher rate of OHBUP glucuronidation and renal elimination associated with pregnancy. Therefore, while maternal exposure to bupropion could be slightly decreased in pregnancy, the exposure to its pharmacologically active metabolite OHBUP appears similar to that of the non-pregnant state. The predicted metabolic phenotypes of \textit{CYP2B6*6} and variant alleles of \textit{CYP2C19} in pregnancy are similar to those in the non-pregnant state. The association of the \textit{CYP2B6*6} variant with quit rates among pregnant smokers treated with bupropion for smoking cessation remains to be investigated.
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Contributed new reagents or analytical tools: Fokina

Performed data analysis: Fokina, Xu, Rytting, Abdel-Rahman, Ahmed, Hankins, Nanovskaya

Wrote or contributed to the writing of the manuscript: Fokina, Xu, Rytting, Abdel-Rahman, Oncken, West, Clark, Ahmed, Hankins, Nanovskaya
References


Zhu AZ, Zhou Q, Cox LS, Ahluwalia JS, Benowitz NL, Tyndale RF. Gene variants in CYP2C19 are associated with altered in vivo bupropion pharmacokinetics but not
Footnotes:

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Figure 1. The effect of CYP2B6*6 variant allele on the selected pharmacokinetic parameters of bupropion in mid- and late pregnancy: OHBUP/BUP M.R. (A), AUC$_{ss}$ of OHBUP (B) and AUC$_{ss}$ of bupropion. AUC$_{ss}$, area under the curve at steady state; BUP, bupropion; OHBUP, hydroxybupropion; M.R., metabolic ratio, defined as the ratio of AUCs, corrected for molecular weight; Mid-pregnancy, 22-26 weeks of gestation; Late pregnancy, 34-38 weeks of gestation
Figure 2. The effect of CYP2C19 metabolic phenotype on the selected pharmacokinetic parameters of bupropion in mid- and late pregnancy: TB/BUP M.R. (A), AUC\textsubscript{ss} of TB (B), EB/BUP M.R. (C), AUC\textsubscript{ss} of EB (D). AUC\textsubscript{ss}, area under the curve at steady state; BUP, bupropion; TB, Threohydrobupropion; EB, Erythrohydrobupropion; M.R., metabolic ratio, defined as the ratio of AUCs, corrected for molecular weight; EM, extensive metabolizer; UM, ultrarapid metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; Mid-pregnancy, 22-26 weeks of gestation; Late pregnancy, 34-38 weeks of gestation
Table 1. Paired estimated pharmacokinetic parameters for bupropion during mid-pregnancy compared to late pregnancy; and late pregnancy compared to postpartum.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mid-pregnancy (n=8)</th>
<th>Late pregnancy (n=8)</th>
<th>Late pregnancy (n=12)</th>
<th>Postpartum (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUP AUC&lt;sub&gt;ss&lt;/sub&gt; BUP (ng·h/ml)</td>
<td>640 ± 263</td>
<td>554 ± 214</td>
<td>654 ± 301</td>
<td>775 ± 291</td>
</tr>
<tr>
<td>CL/F&lt;sub&gt;ss&lt;/sub&gt; (L/h)</td>
<td>359 ± 389</td>
<td>321 ± 152</td>
<td>259 ± 117</td>
<td>208 ± 93</td>
</tr>
<tr>
<td>CL/F&lt;sub&gt;ss&lt;/sub&gt; (L/h/kg)</td>
<td>4.37 ± 4.41</td>
<td>3.74 ± 2.29</td>
<td>3.10 ± 1.27</td>
<td>2.85 ± 1.79</td>
</tr>
<tr>
<td>OHBUP AUC&lt;sub&gt;ss&lt;/sub&gt; OHBUP (ng·h/ml)</td>
<td>9008 ± 3191</td>
<td>10092 ± 3865</td>
<td>9499 ± 3893</td>
<td>9857 ± 6032</td>
</tr>
<tr>
<td>OHBUP/BUP M.R.</td>
<td>22.5 ± 28.1</td>
<td>21.3 ± 10.7</td>
<td>17.7 ± 10.7</td>
<td>14.1 ± 8.60</td>
</tr>
<tr>
<td>TB AUC&lt;sub&gt;ss&lt;/sub&gt; TB (ng·h/ml)</td>
<td>4843 ± 3196</td>
<td>3911 ± 2896</td>
<td>4105 ± 2564</td>
<td>4164 ± 3232</td>
</tr>
<tr>
<td>TB/BUP M.R.</td>
<td>7.91 ± 4.01</td>
<td>7.58 ± 4.63</td>
<td>6.79 ± 3.60</td>
<td>5.21 ± 3.10</td>
</tr>
<tr>
<td>EB AUC&lt;sub&gt;ss&lt;/sub&gt; EB (ng·h/ml)</td>
<td>759 ± 447 *</td>
<td>541 ± 370</td>
<td>621 ± 387</td>
<td>871 ± 586</td>
</tr>
<tr>
<td>EB/BUP M.R.</td>
<td>1.33 ± 0.65 *</td>
<td>1.06 ± 0.57</td>
<td>1.01 ± 0.51</td>
<td>1.10 ± 0.59</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation

AUC<sub>ss</sub>, area under the curve at steady state; BUP, Bupropion; OHBUP, Hydroxybupropion; TB, Threohydrobupropion; EB, Erythrohydrobupropion; M.R., metabolic ratio, defined as the ratio of AUCs, corrected for molecular weight

Mid-pregnancy, 22-26 weeks of gestation; Late pregnancy, 34-38 weeks of gestation

*P < .05
Table 2. Urinary excretion of bupropion and its metabolites over a dose interval. Paired analysis: mid-pregnancy versus late pregnancy, and late pregnancy versus postpartum.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mid-pregnancy (n=4)</th>
<th>Late pregnancy (n=4)</th>
<th>Late pregnancy (n=11)</th>
<th>Postpartum (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>185 ± 45 *</td>
<td>166 ± 33</td>
<td>175 ± 38 *</td>
<td>128 ± 23</td>
</tr>
<tr>
<td>Renal clearance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL\text{R} BUP (mL/min)</td>
<td>23.1 ± 12.5</td>
<td>9.06 ± 5.80</td>
<td>17.2 ± 19.0</td>
<td>38.9 ± 77.7</td>
</tr>
<tr>
<td>CL\text{R} OHBUP (mL/min)</td>
<td>3.77 ± 3.19</td>
<td>1.34 ± 0.22</td>
<td>2.98 ± 3.58</td>
<td>3.08 ± 3.44</td>
</tr>
<tr>
<td>CL\text{R} TB (mL/min)</td>
<td>72.1 ± 48.3</td>
<td>34.6 ± 12.6</td>
<td>56.8 ± 50.0</td>
<td>49.1 ± 32.1</td>
</tr>
<tr>
<td>CL\text{R} EB (mL/min)</td>
<td>50.9 ± 40.3</td>
<td>20.4 ± 7.13</td>
<td>32.5 ± 32.4</td>
<td>27.8 ± 18.3</td>
</tr>
<tr>
<td>% of dose recovered as</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUP</td>
<td>0.59 ± 0.24</td>
<td>0.25 ± 0.24</td>
<td>0.51 ± 0.59</td>
<td>0.87 ± 1.01</td>
</tr>
<tr>
<td>OHBUP-free</td>
<td>1.20 ± 1.03</td>
<td>0.53 ± 0.17</td>
<td>1.27 ± 1.63</td>
<td>1.47 ± 1.98</td>
</tr>
<tr>
<td>OHBUP-glucuronide</td>
<td>7.97 ± 4.47</td>
<td>11.69 ± 8.10</td>
<td>13.8 ± 15.7 *</td>
<td>6.25 ± 5.47</td>
</tr>
<tr>
<td>TB-free</td>
<td>15.9 ± 11.1</td>
<td>6.37 ± 6.71</td>
<td>10.0 ± 9.52</td>
<td>8.30 ± 6.30</td>
</tr>
<tr>
<td>TB-glucuronide</td>
<td>1.07 ± 0.73</td>
<td>0.82 ± 0.83</td>
<td>3.10 ± 2.20 *</td>
<td>1.00 ± 1.15</td>
</tr>
<tr>
<td>EB-free</td>
<td>1.71 ± 1.54</td>
<td>0.47 ± 0.48</td>
<td>0.76 ± 0.68</td>
<td>1.00 ± 0.76</td>
</tr>
<tr>
<td>EB-glucuronide</td>
<td>2.55 ± 1.93</td>
<td>4.00 ± 3.88</td>
<td>0.56 ± 0.40</td>
<td>0.42 ± 0.37</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation

BUP, Bupropion; OHBUP, Hydroxybupropion; TB, Threohydrobupropion; EB, Erythrohydrobupropion; CL\text{R}, renal clearance; Mid-pregnancy, 22-26 weeks of gestation; late pregnancy, 34-38 weeks of gestation

\(^a\) The number of subjects in paired analysis of estimated renal creatinine clearance was the same as in Table 2.

\(*P < .05\)
Figure 1

- A: CYP2B6 genotype
  - *1/*1
  - *1/*6 and *6/*6

- B: AUCss OHBUP (ng*h/ml)
  - Mid-Pregnancy
  - Late Pregnancy

- C: AUCss bupropion (ng*h/ml)
  - *1/*1
  - *1/*6 and *6/*6

Figure 1
Figure 2

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