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Pharmacokinetics of Bupropion and Its Pharmacologically Active Metabolites in Pregnancy

Valentina M. Fokina,[1] Meixiang Xu,[1] Erik Rytting, Sherif Z. Abdel-Rahman, Holly West, Cheryl Oncken, Shannon M. Clark, Mahmoud S. Ahmed, Gary D.V. Hankins, and Tatiana N. Nanovskaya

Department of Pharmacology and Toxicology (V.M.F.), Maternal-Fetal Pharmacology and Biodevelopment Laboratories, Department of Obstetrics and Gynecology (M.X., E.R., S.Z.A.-R., M.S.A., T.N.N.), and Department of Obstetrics and Gynecology (H.W., S.M.C., G.D.V.H.), University of Texas Medical Branch, Galveston, Texas; and University of Connecticut Health Center, Farmington, Connecticut (C.O.)

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ABSTRACT

Bupropion sustained release is used to promote smoking cessation in males and nonpregnant 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 *CYP2B6* and *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 five nonsynonymous single-nucleotide polymorphisms that result in seven *CYP2B6* alleles, namely **2*, **3*, **4*, **5*, **6*, **7*, and **9*, and for *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. In contrast, pregnancy-induced increase in 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 nonpregnant state. The predicted metabolic phenotypes of *CYP2B6*6* and variant alleles of *CYP2C19* in pregnancy are similar to those in the nonpregnant state.

Introduction

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

The pharmacokinetic (PK) data for BUP in humans reported in the literature were obtained from males and nonpregnant females after single or multiple doses (Laizure et al., 1985; Hsyu et al., 1997; Benowitz et al., 2013). BUP 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, whereas TB and EB have lower activity (Golden et al., 1988; Jefferson et al., 2005). 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 BUP (Golden et al., 1988).

CYP2B6 is the principal enzyme catalyzing the formation of OHBUP from BUP in liver (Hesse et al., 2000), and the formation of TB and EB is catalyzed by hepatic 11β-hydroxysteroid dehydrogenase 1 and carbonyl reductases (Molnari and Myers, 2012). In addition, CYP2C19 contributes to hydroxylation of BUP 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 and Klein, 2013). *CYP2B6* variants are associated with altered plasmaconcentrations of OHBUP (Benowitz et al., 2013; Høiseth et al., 2015) and BUP (Kirchheiner et al., 2003).

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[1]V.M.F. and M.X. contributed equally to the preparation of this manuscript.

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ABBREVIATIONS: AUC_{ss}, area under the plasma concentration-time curve for a dose interval at steady state; BID, twice per day; BUP, bupropion; CL/F_{ss}, apparent steady state oral clearance; CL_R, renal clearance; EB, erythrohydrobupropion; EM, extensive metabolizer; IM, intermediate metabolizer; LC-MS, liquid chromatography–mass spectrometry; OHBUP, hydroxybupropion; PK, pharmacokinetic; PM, poor metabolizer; QD, once per day; SNP, single-nucleotide polymorphism; SR, sustained release; TB, threohydrobupropion; UGT, uridine glucuronosyl transferase; UM, ultrarapid metabolizer; UTMB, University of Texas Medical Branch.

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

During pregnancy, women experience numerous physiologic changes that could affect the PK profile of BUP (Loebstein et al., 1997). Pregnancy-induced increases in hepatic flow may accelerate BUP 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 (Anderson, 2005; Mwinyi et al., 2010; Dickmann and Isoherranen, 2013). 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), whereas clearance of the CYP2C19 substrate proguanil was decreased (McGready et al., 2003).

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

About 10% of the BUP 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 BUP 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 BUP metabolites.

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

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

Materials and Methods

Subjects. This was a prospective, opportunistic study conducted at the University of Texas Medical Branch (UTMB). Eligible participants were pregnant women taking BUP to treat depression who agreed to participate in the 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 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 UTMB. All subjects were compensated for participation.

Study Protocol. Subjects in this opportunistic study received the following formulations and dosages of BUP: immediate-release, 100 mg three times daily (Mylan Pharmaceuticals, Cononsburg, PA); sustained-release (SR), 150 mg once per day (QD; Teva Pharmaceuticals USA Inc., North Wales, PA; Actavis Inc., Parsippany, NJ) and 150 mg twice per day (BID; Actavis Inc.; GlaxoSmithKline, Philadelphia, PA; Watson Laboratories Inc., Corona, CA); and extended release, 300 mg QD (Actavis Inc.; Zydus Pharmaceuticals Inc., Pennington, NJ). 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 was noted. Blood for genotyping was collected in BD Vacutaner ethylenediaminetetraacetic acid purple-top tubes. All samples were stored at −80°C until analysis.

Plasma and Urine Sample Analysis. The concentrations of BUP, 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 BUP 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 BUP metabolites were processed with and without glucuronide deconjugation using a modified method of Petsalo et al. (2007). The glucuronides of OHBUP, EB, and TB were quantified as the difference between the concentrations of the nonconjugated (free) drug and the total. The validation of the LC-MS methods was performed following the US Food and Drug administration guideline (http://www.fda.gov/downloads/Drugs/GuidanceCompilanceRegulatoryInformation/Guidances/ucm070107.pdf); detailed description of BUP and its metabolites assayed in plasma and urine is provided in Supplemental Materials and Methods 1. The concentrations of creatinine in serum were determined in the biochemical laboratories of UTMB.

***CYP2B6* and *CYP2C19* Genotyping.** Subjects were genotyped for five nonsynonymous SNPs that result in the seven 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 polymerase chain reaction–restriction fragment length polymorphism methods reported previously (Lang et al., 2001) and allele discrimination assays using TaqMan probes (Thermo Fisher Scientific, 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 noncompartmental analysis (Kinetica software version 5.0; Thermo Scientific). Area under the plasma concentration-time curve for a dose interval at steady state (AUC_{ss}) served as the main measure of exposure to BUP 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_{ss} values were computed as the sum of $AUC_{0\text{-}n}$ and $AUC_{n\text{-}\tau}$ where n is the last measured time point. The apparent steady state oral clearance (CL/F_{ss}) of BUP was estimated as dose/AUC_{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_{ss} for OHBUP over that of the parent drug, corrected for mol. wt. difference. Clearance via reductive metabolic pathways was estimated in a similar fashion as TB/BUP and EB/BUP metabolic ratios. Renal clearance of BUP and its metabolites was calculated as:

$$[(\text{urine concentration, ng/mL})/(\text{average plasma concentration, ng/mL})] \times [(\text{urine volume, mL})/(\text{does interval, hours})].$$

The molar percentage of BUP dose excreted as the parent drug and metabolites was calculated as (total excreted, mg)/(dose, mg) × 100%, corrected for the mol. wt. difference. Creatinine clearance was estimated using the Cockcroft-Gault formula:

$$0.85 \times [140 - \text{age (year)}]/[\text{serum creatinine (mg/dL)}] \times [\text{prepregnancy weight (kg)}/72].$$

Statistical Analysis. Results are presented as mean values ± S.D. Pairwise statistical comparisons were conducted using Wilcoxon signed rank test (SPSS Statistics, version 23; IBM, Armonk, NY). Mann–Whitney *U* test (SPSS Statistics, version 23; IBM) 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, nine subjects (32%) completed one PK visit, 12 (43%) completed two PK visits, six (21%) completed three PK visits, and one subject completed four PK visits. Sixteen subjects were prescribed BUP SR 150 mg BID, five subjects took BUP SR 150 mg QD, three subjects took BUP immediate-release 100 mg three times daily, and two subjects took BUP extended release 300 mg QD. Having the same dose/formulation of BUP was an essential criterion for adequate paired comparison of PK parameters.

PK of BUP and Its Metabolites during Pregnancy and Postpartum. Table 1 shows the paired estimated PK parameters of BUP and its metabolites for eight subjects in middle and late pregnancy, and for 12 subjects in late pregnancy and postpartum (lactating or non/postlactation period depending on availability). Only postlactating PK parameters were used for subjects 2 and 8, who participated during both postpartum studies (lactating and non/postlactating).

Paired analysis did not reveal any difference in the mean apparent oral clearance of BUP (CL/F_{ss}) between the tested treatment windows, with or without adjustment to weight (Table 1). However, we observed that the mean value of AUC_{ss} of BUP in late pregnancy was slightly lower than that of postpartum (654 ± 301 ng × h/ml versus 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_{ss} of OHBUP in mid- versus late pregnancy comparisons or late pregnancy versus postpartum (Table 1).

The mid-pregnancy mean value for TB AUC_{ss} was slightly higher than that in late pregnancy, although the results were not statistically significant (4843 ± 3196 ng × h/ml versus 3911 ± 2896 ng × h/ml, P = .068; Table 1). No difference in TB AUC_{ss} was observed in late pregnancy as compared with the nonpregnant state. However, the TB/BUP metabolic ratio in late pregnancy was slightly higher than that of postpartum (6.79 ± 3.60 versus 5.21 ± 3.10, P = .06; Table 1).

The mean values for EB AUC_{ss} and EB/BUP metabolic ratio in mid-pregnancy were higher than those of late pregnancy (EB AUC_{ss}: 759 ± 447 ng × h/ml versus 541 ± 370 ng × h/ml, P < .05; EB/BUP: 1.33 ± 0.65 versus 1.06 ± 0.57, P < .05; Table 2). Although we observed lower EB AUC_{ss} in late pregnancy than postpartum (621 ± 387 ng × h/ml versus 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 BUP and Its Metabolites. Data on the excretion of BUP 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 versus 166 ± 33 mL/min, P < .05), whereas the mean value of creatinine clearance in late pregnancy was higher as compared with that of postpartum (175 ± 38 mL/min versus 128 ± 23 mL/min, P < .05).

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

No statistically significant differences in the fractions of BUP dose eliminated in the urine as unchanged drug or as unconjugated metabolites OHBUP, TB, and EB were observed between the groups. The percentage of BUP dose recovered in the urine as unchanged drug in late pregnancy was slightly below that of postpartum (0.51 ± 0.59% versus 0.87 ± 1.01%, P = .059; Table 2). A similar trend was observed in late pregnancy versus postpartum comparison of the percentage of the drug dose excreted in urine in a form of unconjugated EB (0.76 ± 0.68% versus 1.00 ± 0.76%, P = .062; Table 2). Moreover, the percentage of

TABLE 1

Paired estimated PK parameters for BUP during mid-pregnancy compared with late pregnancy; and late pregnancy compared with postpartum

Data presented as mean ± S.D. Mid-pregnancy, 22–26 weeks of gestation; late pregnancy, 34–38 weeks of gestation.

	Parameter	Mid-pregnancy (n = 8)	Late Pregnancy (n = 8)	Late Pregnancy (n = 12)	Postpartum (n = 12)
BUP	AUC_{ss} BUP (ng × h/ml)	640 ± 263	554 ± 214	654 ± 301	775 ± 291
	CL/F_{ss} (L/h)	359 ± 389	321 ± 152	259 ± 117	208 ± 93
	CL/F_{ss} (L/h/kg)	4.37 ± 4.41	3.74 ± 2.29	3.10 ± 1.27	2.85 ± 1.79
OHBUP	AUC_{ss} OHBUP (ng × h/ml)	9008 ± 3191	10,092 ± 3865	9499 ± 3893	9857 ± 6032
	OHBUP/BUP M.R.	22.5 ± 28.1	21.3 ± 10.7	17.7 ± 10.7	14.1 ± 8.60
TB	AUC_{ss} TB (ng × h/ml)	4843 ± 3196	3911 ± 2896	4105 ± 2564	4164 ± 3232
	TB/BUP M.R.	7.91 ± 4.01	7.58 ± 4.63	6.79 ± 3.60	5.21 ± 3.10
EB	AUC_{ss} EB (ng × h/ml)	759 ± 447 *	541 ± 370	621 ± 387	871 ± 586
	EB/BUP M.R.	1.33 ± 0.65 *	1.06 ± 0.57	1.01 ± 0.51	1.10 ± 0.59

M.R., metabolic ratio, defined as the ratio of AUCs, corrected for mol. wt.
*P < .05.

TABLE 2

Urinary excretion of BUP and its metabolites over a dose interval. Paired analysis: mid-pregnancy versus late pregnancy, and late pregnancy versus postpartum

Data presented as mean ± S.D. Mid-pregnancy, 22–26 weeks of gestation; late pregnancy, 34–38 weeks of gestation.

Parameter		Mid-pregnancy (n = 4)	Late Pregnancy (n = 4)	Late Pregnancy (n = 11)	Postpartum (n = 11)
Creatinine clearance[a]	(mL/min)	185 ± 45*	166 ± 33	175 ± 38*	128 ± 23
Renal clearance	CL_R BUP (mL/min)	23.1 ± 12.5	9.06 ± 5.80	17.2 ± 19.0	38.9 ± 77.7
	CL_R OHBUP (mL/min)	3.77 ± 3.19	1.34 ± 0.22	2.98 ± 3.58	3.08 ± 3.44
	CL_R TB (mL/min)	72.1 ± 48.3	34.6 ± 12 6	56.8 ± 50.0	49.1 ± 32.1
	CL_R EB (mL/min)	50.9 ± 40.3	20.4 ± 7.13	32.5 ± 32.4	27.8 ± 18.3
% of dose recovered as	BUP	0.59 ± 0.24	0.25 ± 0.24	0.51 ± 0.59	0.87 ± 1.01
	OHBUP-free	1.20 ± 1.03	0.53 ± 0.17	1.27 ± 1.63	1.47 ± 1.98
	OHBUP-glucuronide	7.97 ± 4.47	11.69 ± 8.10	13.8 ± 15.7*	6.25 ± 5.47
	TB-free	15.9 ± 11.1	6.37 ± 6.71	10.0 ± 9.52	8.30 ± 6.30
	TB-glucuronide	1.07 ± 0.73	0.82 ± 0.83	3.10 ± 2.20*	1.00 ± 1.15
	EB-free	1.71 ± 1.54	0.47 ± 0.48	0.76 ± 0.68	1.00 ± 0.76
	EB-glucuronide	2.55 ± 1.93	4.00 ± 3.88	0.56 ± 0.40	0.42 ± 0.37

[a]The number of subjects in paired analysis of estimated renal creatinine clearance was the same as in Table 1.
*$P < .05$.

BUP 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% versus 6.37 ± 6.71%, P = .068, and for EB-free, 1.71 ± 1.54% versus 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, whereas TB and EB conjugates accounted for 28 ± 20% and 46 ± 23% of total excreted TB and EB, respectively. The fraction of BUP eliminated as OHBUP glucuronide was higher in late pregnancy than postpartum (13.8 ± 15.7% versus 6.25 ± 5.47%, $P < .05$; Table 2). Likewise, the fraction of BUP recovered in the urine as TB glucuronide in late pregnancy was higher than that of postpartum (3.10 ± 2.20% versus 1.00 ± 1.15%, $P < .05$; Table 2). In addition, OHBUP glucuronide as percentage of BUP dose recovered in urine in late pregnancy slightly exceeded that of mid-pregnancy (11.7 ± 8.10% versus 7.97 ± 4.47%, P = .068; Table 2). However, TB glucuronide recovered in urine as percentage of BUP 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 PK of BUP in Pregnancy.** BUP PK 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 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, BUP clearance and metabolic ratios were examined irrespective of the drug dosing, whereas comparisons of the urinary excretion data and AUC_{ss} of BUP 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 21 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): 11 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 (Fig. 1; Supplemental Table 2).

In mid-pregnancy, the OHBUP/BUP metabolic ratio tended to be lower in **6* carriers than in wild-type, (9.46 ± 4.4 versus 32.8 ± 34.0, P = .086; Fig. 1A), which is consistent with the reduced metabolic phenotype of *CYP2B6*6* allele. Although no difference was observed in OHBUP AUC_{ss} (Fig. 1B, mid-pregnancy), the BUP AUC_{ss} trended higher in **6* carriers in mid-pregnancy (742 ± 114 ng × h/ml versus 414 ± 225 ng × h/ml, P = .077; Fig. 1C). The mid-pregnancy AUC_{ss} 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 versus 2553 ± 2084 ng × h/ml, for TB, P = .077, and 1119 ± 393 ng × h/ml versus 477 ± 340 ng × h/ml for EB, $P < .05$; Supplemental Table 2). Neither comparison in late pregnancy revealed any differences (Fig. 1; Supplemental Table 2). 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), 10 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, PK parameters obtained from extensive metabolizers (EM) and ultrarapid 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/ultrarapid (EM + UM) group in both mid- and late pregnancy (mid-pregnancy, 11.6 ± 3.16 versus 6.58 ± 3.33, $P < .05$; late pregnancy, 11.6 ± 3.16 versus 6.58 ± 3.33, $P < .05$; Fig. 2A; Supplemental Table 3). We observed higher AUC_{ss} TB in PM + IM than in EM + UM in late pregnancy (5773 ± 2517 ng × h/ml versus 2333 ± 1313 ng × h/ml, $P < .05$; Fig. 2B; Supplemental Table 3); however, no difference in the AUC_{ss} of TB was observed between the groups in mid-pregnancy (Fig. 2B). Moreover, no difference in BUP AUC_{ss} 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 versus 0.95 ± 0.56, P = .088;

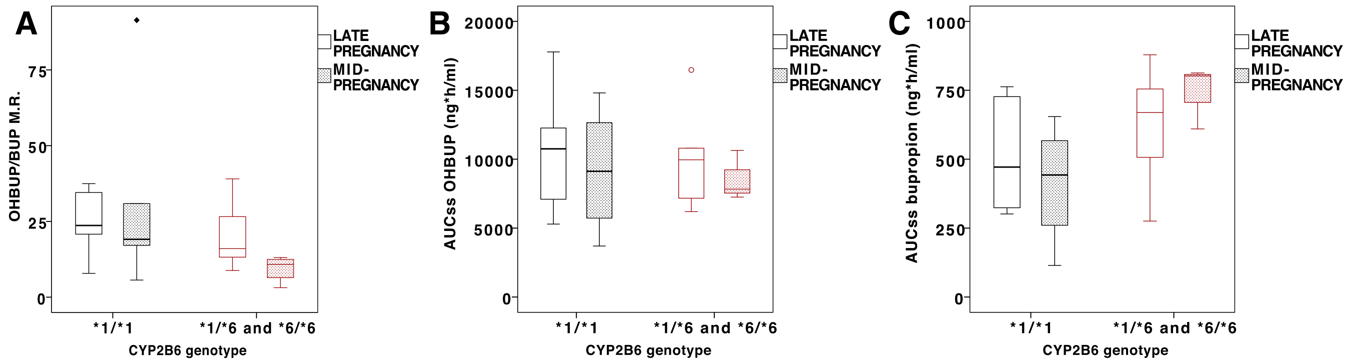

Fig. 1. The effect of *CYP2B6*6* variant allele on the selected PK parameters of BUP in mid- and late pregnancy: OHBUP/BUP metabolic ratio (M.R.) (A), AUC_{ss} of OHBUP (B) and AUC_{ss} of BUP (C). M.R., defined as the ratio of AUCs, corrected for mol. wt.; mid-pregnancy, 22–26 weeks of gestation; late pregnancy, 34–38 weeks of gestation.

late pregnancy, 1.57 ± 0.34 versus 1.03 ± 0.52, $P < .05$; Fig. 2C; Supplemental Table 2). The EB AUC_{ss} in the PM + IM group was slightly higher than in the EM + UM group in late pregnancy (782 ± 350 ng × h/ml versus 403 ± 273 ng × h/ml, $P = .055$; Fig. 2D; Supplemental Table 3); however, no difference was observed in the mean value of EB AUC_{ss} in mid-pregnancy comparisons.

The percentage of BUP 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 BUP 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% versus 0.35 ± 0.16%, $P = .068$; TB-free: 9.69 ± 6.01% versus 2.21 ± 1.41%, $P = .068$; Supplemental Table 3).

Discussion

The typical BUP SR dose for promoting cessation from smoking is 150 mg BID for 7–12 weeks in nonpregnant smokers. BUP is extensively metabolized, and its major product OHBUP contributes to the drug's antismoking properties. Pregnancy-induced physiologic changes in the activity of hepatic enzymes metabolizing BUP—as well as increased hepatic blood flow and increased renal plasma flow—can alter the PK of BUP.

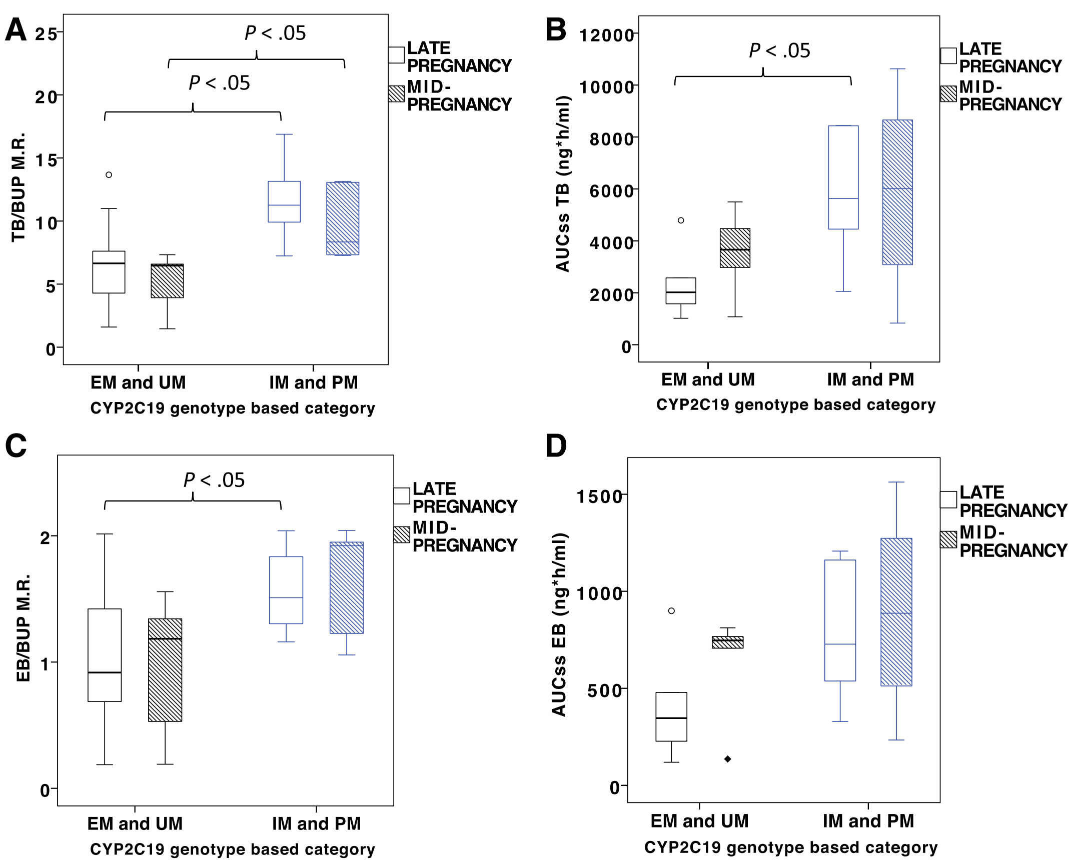

Fig. 2. The effect of CYP2C19 metabolic phenotype on the selected PK parameters of bupropion in mid- and late pregnancy: TB/BUP M.R. (A), AUC_{ss} of TB (B), EB/BUP metabolic ratio (M.R.) (C), AUC_{ss} of EB (D). M.R., defined as the ratio of AUCs, corrected for mol. wt.; mid-pregnancy, 22–26 weeks of gestation; late pregnancy, 34–38 weeks of gestation.

The OHBUP/BUP metabolic ratio has been historically used as a measure of CYP2B6 activity in BUP hydroxylation. Several studies suggest that the activity of CYP2B6 primarily affects the OHBUP levels, but not BUP (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, 2005; Olagunju et al., 2015). However, in our study, we did not observe any significant changes in the OHBUP/BUP metabolic ratio and OHBUP AUC_{ss} in pregnancy as compared with the nonpregnant state.

Furthermore, 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 BUP in pregnancy. However, CYP2C19 also contributes to the hydroxylation of BUP and its metabolites, TB and EB (Chen et al., 2010; Zhu et al., 2014). We observed that, relative to late pregnancy, the AUC_{ss} 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* (McGready et al., 2003). Hence, this could contribute to an increased EB/BUP metabolic ratio in mid-pregnancy. The slight increase in the AUC_{ss} 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 BUP during pregnancy; however, the accelerated CYP2B6-catalyzed hydroxylation of BUP could possibly counterbalance BUP metabolic clearance. As a net result, we detected no significant changes in the AUC_{ss} of BUP in pregnancy (slight decrease in late pregnancy versus postpartum, $P = .099$); and no effect of pregnancy on the BUP CL/F_{ss} was observed.

Another factor that could affect the PK of BUP 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 BUP in mid-pregnancy relative to late pregnancy, which is probably associated with the peaking increment of glomerular filtration rate around the second trimester of pregnancy (http://www.glowm.com/section_view/item/157). Moreover, the higher percentage of dose excreted as unconjugated TB and EB in mid-pregnancy as compared with late pregnancy could reflect the higher plasma levels (and consequently AUC_{ss}) of TB and EB in mid-pregnancy, in addition to the increased glomerular filtration rate. However, the results in the urine excretion of the drug and its metabolites in mid- versus 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 BUP recovered in the urine as the metabolites OHBUP, TB, and EB, in their free forms or as glucuronide conjugates. 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 percentage of BUP 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 (Abernethy et al., 1982; Jeong et al., 2008; Ohman et al., 2008). The current sample size for late pregnancy versus 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 BUP biodisposition in pregnancy, irrespective of pregnancy-induced changes. This was investigated to collectively understand the effects of both genetics and pregnancy on the PK of BUP. BUP and its metabolites exhibit linear PK at steady state (Findlay et al., 1981); therefore, the influence of genotype on BUP CL/F_{ss} and OHBUP/BUP, TB/BUP, and EB/BUP metabolic ratios was 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 AUC_{ss} in these subjects in late pregnancy only. These results are consistent with the effect of *CYP2C19* polymorphism on TB and EB in nonpregnant subjects (Zhu et al., 2014). However, it appears that CYP2C19 metabolizer phenotype did not influence the levels of BUP and, consequently, its AUC_{ss} and Cl/F_{ss} 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 with wild-type carriers in mid-pregnancy suggests that, in pregnant women, the *CYP2B6*6* variant is associated with a reduced rate of BUP hydroxylation, as observed in men and nonpregnant women (Benowitz et al., 2013). Of note, in the mid-pregnancy group, the AUC_{ss} 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 that is present exclusively in individuals of African descent, with an allele frequency of 4–7% (Zanger and Klein, 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 PK of BUP and its metabolites. Nevertheless, it appears that decreased activity of CYP2C19 due to pregnancy, along with loss-of-function variants of *CYP2C19*, could contribute to higher steady-state exposure to TB and EB during pregnancy. The TB and EB metabolites of BUP have an inhibitory effect on the CYP2D6 enzyme (Parkinson et al., 2010), which is upregulated during pregnancy (Ke et al., 2013; Ryu et al., 2016). Therefore, possible drug–drug interactions of BUP and CYP2D6 substrates cannot be discounted in pregnancy, particularly in instances when dose adjustment of CYP2D6-metabolized medications is considered (Ryu et al., 2016).

In summary, we reported the effect of pregnancy on the pharmacologic profile of BUP, as well as the impact of *CYP2B6* and *CYP2C19* functional polymorphisms on BUP biodisposition during mid- and late pregnancy. It appears that the pregnancy-induced increase in CYP2B6-catalyzed BUP 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, although maternal exposure to BUP could be slightly decreased in pregnancy, the exposure to its pharmacologically active metabolite OHBUP appears similar to that of the nonpregnant state. The predicted metabolic phenotypes of *CYP2B6*6* and variant alleles of *CYP2C19* in pregnancy are similar to those in the nonpregnant state. The association of the *CYP2B6*6* variant with quit rates among pregnant smokers treated with BUP for smoking cessation remains to be investigated.

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Authorship Contributions

Participated in research design: Rytting, Abdel-Rahman, Oncken, Clark, Ahmed, Hankins, Nanovskaya.

Conducted experiments: Fokina, Xu, West.

Contributed new reagents or analytic 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.

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Address correspondence to: Dr. Tatiana N. Nanovskaya, Maternal-Fetal Pharmacology and Biodevelopment Laboratories, Department of Obstetrics and Gynecology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0587. E-mail: tnnanovs@utmb.edu

Pharmacokinetics of bupropion and its pharmacologically active metabolites in pregnancy

Supplemental data files

Valentina M Fokina, BS; Meixiang Xu, PhD; Erik Rytting, PhD; Sherif Z Abdel-Rahman, PhD; Holly West, DHEd; Cheryl Oncken, MD; Shannon M Clark, MD; Mahmoud S Ahmed, PhD; Gary DV Hankins, MD; and Tatiana N Nanovskaya, PhD

Department of Pharmacology & Toxicology, University of Texas Medical Branch, Galveston, Texas (V.M.F.);

Maternal-Fetal Pharmacology & Bio-development Laboratories, Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Texas (M.X., E.R., S.Z.A., M.S.A., T.N.N.);

Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Texas (H.W., S.M.C., G.DV.H.);

University of Connecticut Health Center, Farmington, Connecticut (C.O.)

Supplemental materials and methods

Section 1. Quantitative determination of bupropion, hydroxybupropion (OHBUP), threohydrobupropion (TB) and erythrohydrobupropion (EB) in plasma and urine

1. Chemicals

Chemicals were purchased from the following companies: bupropion (BUP), triprolidine hydrochloride, phenacetin, and β-Glucuronidase from *Helix pomatia*, ammonium acetate, from Sigma-Aldrich (St. Louis, MO); OHBUP, TB, EB, and the deuterium labeled internal standards BUP-$_{d9}$, OHBUP-$_{d6}$, EB-$_{d9}$ and TB-$_{d9}$, from Toronto Research Chemicals Inc. (North York, Canada); liquid chromatography-mass spectometry (LC/MS)-grade methanol, LC/MS-grade acetonitrile, methylene chloride, formic acid, acetic acid, trichloroacetic acid (TCA), potassium phosphate mono- and dibasic from Fisher Scientific (Fair Lawn, NJ).

2. Quantitative determination of bupropion, OHBUP, TB and EB in plasma

2.1. Instrumental and analytical conditions

The analysis of BUP and its metabolites in plasma were conducted simultaneously using an Agilent high performance liquid chromatography (HPLC) 1200 series system coupled with an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) controlled by Analyst 1.5 Software (MDS INC. and Applera Corporation, USA). The detailed description of HPLC system and analytical conditions were reported previously by Wang et al., (2012).

2.2. Preparation of stock and working standard solutions

The following serial dilution of working standard solutions were prepared in 30% methanol: BUP, 2.5-2500 ng/mL; OHBUP, 20-20 X 10^3 ng/mL; TB, 12-1200 ng/mL; and

EB, 10-10 X 10^3 ng/mL. The stock solutions for internal standards (IS) were prepared in 30% methanol at the final concentrations of 80 ng/mL for each of BUP-$_{d9}$, OHBUP-$_{d6}$, EB-$_{d9}$ and TB-$_{d9}$. The solutions were stored at 4°C.

2.3. Calibration standards and quality control samples

Calibration standards for plasma samples were prepared by adding 5 µl of working standard solution into a 50 µl blank plasma. The calibration standards were prepared in the following concentrations: BUP, 0.5-250 ng/mL; OHBUP, 2-2000 ng/mL; TB, 1.2-1200 ng/mL; and EB, 1-1000 ng/mL. 10 µl of IS solution was added to each of the calibration sample. Quality control (QC) samples were prepared at low, medium and high concentrations as well as lower limits of quantification (LLOQ).

2.4. Preparation of plasma samples

10 µl of IS solution was added to a plasma sample of 50 µl, vortexed for 30 sec, then 90 µl of 5%, w/v of TCA (final 3%, w/v) and 1000 µl of acetonitrile were added, vortexed for 30 sec. Each sample was then centrifuged for 10 min at 12000 x g. The supernatant was transferred to another tube and dried at 40°C under a stream of nitrogen. The residues were reconstituted in 150 µl of initial mobile phase and 10 µl was injected into the HPLC system for analysis by LC-MS/MS.

2.5. Validation of LC-MS/MS method

The method was partially validated following the US Food and Drug administration guideline (FDA, 2001) for matrix effect, extraction recovery, precision and accuracy as described previously (Wang et al., 2012). The matrix effect for bupropion and its major metabolites ranged from 94.2% and 114.5% with relative standard deviation (RSD) being ≤ 4.6%; extraction recovery for these analytes was between 91.1% and 112.5% with RSD

≤ 4.3%. The calibration curves were fit using weighed (*$1/y^2$*) least-squares linear regression analysis. The linearity was achieved for the following ranged of the drug and its metabolites: bupropion, 0.5-250 ng/mL; OHBUP, 2-2000 ng/mL; TB, 2.8-1200 ng/mL; EB, 2-1000 ng/mL. The LLOQ and lower limits of detection (LOD) were: bupropion, 0.5 ng/mL and 0.125 ng/mL; OHBUP, 2 ng/mL and 0.5 ng/mL; TB, 2.8 ng/mL and 0.5 ng/mL; EB, 2 ng/mL and 0.5 ng/ml. The intra-day accuracy for LLOQ, low, middle and high concentrations ranged from 92.4% and 100.1%, with RSD being ≤ 6.93%, while inter-day accuracy ranged from 94.7% to 101.7%, with RSD being ≤ 5.76%.

3. Quantitative determination of bupropion, OHBUP, TB and EB in urine

3.1. Instrumental and analytical conditions

The analysis of bupropion in urine was performed separately from analysis of urinary OHBUP, TB and EB using same LC-MS instrumental conditions. The Waters HPLC system coupled with a Waters EMD 1000 single quadrupole mass spectrometer (Waters, Milford, MA) was used for both analysis. The HPLC system consisted of Waters 1525 binary HPLC pump and a 717 plus autosampler controlled by Empower™2 chromatography Data Software (Waters, Milford, MA) (Wang et al., 2010). The separation of analytes was conducted using Waters Symmetry C_{18} column (150 mm × 4.6 mm, 5 μm) connected to a Phenomenex C_{18} guard column (4 mm × 3.0 mm) by isocratic elution of the mobile phase at a rate of 1.0 mL/min. For the analysis of OHBUP, TB and EB, the mobile phase consisted of 40% methanol and 60% of 10mM ammonium acetate buffer with 0.02% (v/v) of acetic acid (Wang et al., 2010). For the analysis of bupropion, the mobile phase consisted of 25% methanol and 75% of 0.04% formic acid aqueous solution (v/v). In both analyses, the 10% of post column eluent flow was directed to the mass spectrometer.

The mass spectrometer (Waters EMD 1000 single-quadrupole; Milford MA) was supplied with an electrospray ion source (ESI) operated in positive mode. The MS parameters were: capillary voltage, 2.2 kV; cone voltage, 40 V; source temperature, 95°C; desolvation temperature, 350 °C; desolvation gas flow rate, 450 L/h; cone gas flow rate, 100 L/h (Wang et al., 2010). The analytes were monitored by selective ion monitoring (SIM) at *m*/*z* 184 for bupropion, m/z 179 for triprolidine hydrochloride (IS for bupropion); *m*/*z* 238 for OHBUP, *m*/*z* 168 for TB and EB, and *m*/*z* 180 for phenacetin (IS for OHBUP, TB and EB analysis).

3.2. Preparation of stock and working standard solutions

All stock solutions were prepared in 30 % methanol and stored at 4°C. For analysis of OHBUP, TB and EB, the working standard solutions were prepared in the following ranges: OHBUP, 50-20 X 10^4 ng/mL; TB, 50-10 X 10^4 ng/mL; EB, 10-20 X 10^4 ng/mL. The IS, phenacetin, was prepared at the final concentration of 1 µg/ml. The stock solutions for bupropion ranged from 2.5 to 20 X 10^3 ng/mL. For detection and quantification of BUP on urine samples, the stock solutions of BUP ranged from 2.5 to 20 X 10^3 ng/mL. The IS, triprolidine hydrochloride, was prepared at the final concentration of 1 µg/ml.

3.3. Quantitative determination of free- and conjugated OHBUP, TB and EB in urine samples

3.3.1. Calibration standards and quality control samples

Calibration curves for OHBUP, TB and EB standards were constructed as follows: 10 µl of standard working solution was combined with 10 µl of blank urine, and the final concentrations of the analytes were as follows: OHBUP, 50-10 X 10^4 ng/mL; TB, 50-10 X 10^4 ng/mL; EB, 10-2 X 10^4 ng/mL. 10 µl of IS (phenacetin) working solution was added to each sample. QC samples were prepared for each of the curves at high, middle, low concentrations and LLOQ.

3.3.2. Preparation of urine samples for OHBUP, TB and EB analysis

To quantify OHBUP, TB and EB in urine, the samples were processed in the presence or absence of β-glucuronidase.

For analysis of non-conjugated OHBUP, TB and EB, the samples were processed as follows: 10 µl of IS was added to 10 µl of urine and mixed with 90 µl of 0.1 M phosphate-buffered saline, pH=5. Then, 40 µl of 40%, w/v of TCA (final TCA 11 % (w/v) and 750 µl of acetonitrile was added, sample vortexed for 30 sec and centrifuged for 10 min at 12,000 x g. The supernatant fro each sample was transferred to another tube and dried at 40°C under a stream of nitrogen. The dried residues were reconstituted in the 200 µl of initial mobile phase; an aliquot of 50 µl of each sample was injected into HPLC system for MS analysis.

The concentrations of the glucuronidated OHBUP, TB and EB in the urine was computed as difference from the non-conjugated (free) and the total drug. To analyze the total OHBUP, TB and EB, the samples were treated with β-glucuronidase, the procedure was a follows: 10 µl of IS was added to 10 µl of each urine sample mixed with 90 µl of 0.1 M phosphate-buffered saline, pH=5, containing 200 units of β-glucuronidase; the samples then were incubated at 37°C for 16 hrs. The reaction was terminated by adding 40 µl of 40%, w/v of TCA to each tube, and the samples were processed as described above.

3.3.3. Validation of LC-MS method for quantitative determination of OHBUP, TB and EB in urine

The method was partially validated following the US Food and Drug administration guideline (FDA, 2001) for specificity, matrix effect, extraction recovery, precision, inter- and intra-day accuracy and stability (room temperature, 4 hours at 37°C, three freeze-thaw cycles) as described previously (Wang et al., 2012). The selectivity was achieved by comparing the SIM chromatograms of six different blank urine samples (from individuals not exposed to bupropion). The matrix effect for OHBUP, TB and EB ranged from 85.8% to 94.2%, with the relative standard deviation being (RSD) being ≤9.1%. The matrix effect for corresponding IS was 87.9 with RSD=11.7%. The extraction recovery of OHBUP, TB and EB ranged from 72.5% to 101.6%, with RSD being ≤ 15.2%; the extraction recovery for IS was 103.2% with RSD=12.6%. The calibration curves were fit using weighed ($1/y^2$) least-squares linear regression analysis. The constructed calibration curves were constructed, and the linearity was achieved within the following ranges: OHBUP, 50-20 X 10^4 ng/mL; TB, 50-10 X 10^4 ng/mL; EB, 10-20 X 10^4 ng/mL. The LLOQ and LOD were: OHBUP, 50 ng/mL and 20 ng/mL; TB, 50 ng/mL and 20 ng/mL; EB, 20 ng/mL and 10 ng/mL. The inter-day accuracy for low, middle and high concentrations were 93.7% - 110.3% with RSD ≤ 8.5%, while for LLOQ it was 90.1% - 99.8%, with RSD ≤ 15.7%. The accuracy after three freeze-thaw cycles ranged from 89.0% to 119.3%, with RSD being ≤ 5.9%. The room temperature stability test resulted in the accuracy ranging from 87.7% to 108.3%, with RSD being ≤ 8.3%, while the 37°C stability test resulted in the accuracy ranging from 89.9% to 104.2%, with RSD being ≤ 7.4%.

3.4. Quantitative determination of bupropion in urine

3.4.1. Calibration standards and quality control samples

Calibration curves for bupropion standards were prepared as follows: 10 µl of standard working solutions were combined with 100 µl of blank urine; bupropion final concentrations ranged from 0.25 to 2 X 10^3 ng/mL. 10 µl of working solution of corresponding IS, triprolidine hydrochloride, was added to each sample. QC samples were prepared at high, middle, low concentrations and LLOQ.

3.4.2. Preparation of urine samples

10 µl of IS was spiked with 100 of a urine sample, then sample 40 µl of 40% w/v TCA was added (final 11%, w/v), sample vortexed. Each sample was extracted with 1000 µl of methylene chloride by vortexing for 10 min, than sample was centrifuged at 12000 x g for 10 min, organic layer transferred to a tube. Extraction was repeated, organic layers combined and evaporated to dryness at 40°C under a stream of nitrogen. The residues were reconstituted in 200 µl of initial mobile phase containing 8% w/v of TCA, and 50 µl was injected into the HPLC system for analysis on the MS.

3.4.3. LC/MS method validation

The method was partially validated similar to as described above. The selectivity was achieved by comparing the SIM chromatograms of six different blank urine samples to that of QC sample. The matrix effect for QCs of low, medium and high concentrations of bupropion ranged from 96.9% to 103.4%, with the RSD ≤4.1%. The matrix effect for IS was 83.2 with RSD=7.1%. The extraction recovery of bupropion ranged from 96.5% to 101.0%, with RSD ≤ 7.2%; the extraction recovery for IS was 118.9% with RSD=6.2%. The calibration curves were constructed using weighed ($1/y^2$) least-squares linear regression analysis and exhibited linearity within the bupropion concentrations ranging rom 4 ng/mL to 800 ng/mL. The LLOQ and LOD were 0.5 ng/mL and 4 ng/mL, respectively. The inter-day accuracy for bupropion concentrations ranged from 93.7 to 10.3.3, with RSD ≤ 7.7. For LLOQ, the inter-day accuracy was 93.7%, with RSD=7.7%. The QCs were tested for stability, the results are as follows: after three freeze-thaw cycles, the accuracy of bupropion concentration ranged from 101.1% to 103.1%, with RSD being ≤ 5%; room temperature stability, accuracy ranged from 99.9% to 102 %, with RSD ≤ 3.6%.

Section 2. *CYP2B6* and *CYP2C19* genotyping

Genomic DNA was isolated from whole blood or buffy coat using the Puregene Blood Core Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol. DNA concentration and quality were determined with a DeNovix DS-11 FX Spectrophotometer (DeNovix, Wilmington, Delaware, USA). Five non-synonymous SNPs of CYP2B6 defining 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) were identified following the PCR-RFLP methods (Lang et al., 2001) and allele discrimination assays utilizing TaqMan probes (Thermo Fisher Scientific Inc., Waltham, MA). Specifically, for 64C>T genotyping, the forward 5'- ACATTCACTTGCTCACCT and reverse 5'- GTAAATACCACTTGACCA-3' primers for PCR-RFLP and the TaqMan C__2818162_20 assay were used. For 1459C>T the forward 5'-TGAGAATCAGTGGAAGCCATAGA-3' and reverse 5'-TAATTTTCGATAATCTCACTCCTGC-3' primers for PCR-RFLP and the TaqMan C__30634242_40 assay were used. For 516G>T, the forward 5'-GGTCTGCCCATCTATAAAC-3' and reverse 5'-CTGATTCTTCACATGTCTGCG-

3'primers for PCR-RFLP and the TaqMan C__7817765_60 assay were used. To determine **3*(777C>A) and **4*(785A>G) alleles, new primers were designed: the forward, 5'- GGGCACACAGGCAAGTTTAC-3', and the reverse, 5'-CTACACATCCAACCGCGT-3', and the PCR-RFLP procedures were performed as described previously (Lang et al., 2001). These primers were also used for direct sequencing of select DNA samples. In addition, a High Resolution Melt (HRM) assay with the forward 5'- GGCACACAGGCAAGTTTACA-3' and reverse 5'-AGCAGGTAGGTGTCGATGAG-3' primers was developed to determine the *CYP2B6*3*(777C>A) and **4*(785A>G) SNPs. Sequences were assembled into one contig and both SNPs were determined using a DNA Baser sequence assembler version 3.1 (Heracle BioSoft S.R.L, Pitesti, România).

For identification of *CYP2C19 *2* (681G>A; rs4244285), *CYP2C19 *3* (636G>A; rs4986893) and *CYP2C19 *17* (-806C>T; rs12248560) TaqMan based assays were used as described by Zhu et al. (2014). Genotyping reactions were performed with 5 µl of TaqMan GTXpress master mix and 5 µl of water containing 10 ng of DNA and 0.5 µl of 20x TaqMan probes (CYP2C19*2: C__25986767_70; CYP2C19*3: C__27861809_10; and CYP2C19*17: C____469857_10; Thermo Fisher).

Supplemental data

Supplemental table 1. Demographics and bupropion dosing

subjects	Race/ ethnicity	Genotype		At the time of initial enrollment		Early pregnancy		Mid-pregnancy		Late pregnancy		Lactation		Post-/non-lactating	
						n=5		n=13		n=21		n=8		n=8	
		CYP2B6	*CYP2C19*	Age, years	Smoking	Weight kg	Dose, mg	Weight kg	Dose, mg	Weight kg	Dose, mg	Weight kg	Dose, mg	Weight kg	Dose, mg
1	wt/non-hisp	*1/*1	*1/*2	32	Yes	53.1	150*BID	53.9	150*BID	59.0	150*BID				
2	wt/non-hisp	*1/*1	*1/*1	24	Yes	76.2	150*BID			77.6	150*BID	68.0	150*BID	69.4	150*BID
3	wt/non-hisp	*1/*6	*1/*1	39	Yes	70.8	150*BID					76.2	150*BID		
4	wt/hisp	*1/*6	*1/*2	33	No			93.8	150*BID	105.2	150*BID			94.3	150*BID[a]
5	wt/non-hisp	*6/*6	*1/*17	38	Yes			97.1	150*BID	109.6	150*BID				
6	wt/hisp	*1/*1	*1/*2	38	Yes			90.2	150*BID[a]	90.9	150*BID			76.1	150*BID
7	wt/hisp	*1/*1	*1/*1	24	No			95.3	150*BID	99.1	150*BID				
8[b]	wt/hisp	*1/*1	*1/*1	23	No					85.8	150*BID	89.4	150*BID	88.9	150*BID
9	wt/hisp	*1/*9	*2/*17	20	No					82.6	150*BID			70.8	150*BID
10	wt/hisp	*1/*6	*1/*1	36	No					61.7	100*TID	59.9	100*TID		
11	wt/hisp	*1/*1	*1/*1	32	No					95.0	100*TID	89.8	100*TID		
12	wt/hisp	*6/*6	*1/*2	38	No					66.6	150*BID			60.8	150*BID
13	wt/non-hisp	ND	ND	23	No			93.0	150*QD	100	150*QD[a]			101.5	150*BID[a]
14	wt/hisp	ND	*1/*1	22	No	74.4	150*QD	77.1	150*QD			81.6	150*BID		
15	wt/non-hisp	*4/*4	*1/*1	21	Yes					94.8	150*QD			89.4	150*QD
16	wt/non-hisp	ND	ND	25	Yes					89.1	150*BID	85.5	150*BID		
17	wt/non-hisp	*1/*1	*1/*2	39	No			93.0	150*QD	94.8	150*QD [a]				
18	wt/non-hisp	*1/*1	*1/*1	27	No					66.7	150*QD	55.7	150*QD		
19	wt/non-hisp	*1/*6	*2/*2	22	Yes			63.1	150*BID[a]	74.4	150*BID[a]				
20	bl/hisp	*1/*6	*1/*1	40	No	98.1	150*BID								
21	wt/non-hisp	*1/*1	*1/*1	27	Yes					75.3	150*BID				
22	wt/non-hisp	*1/*6	*1/*1	25	Yes					95.6	150*BID				
23	wt/non-hisp	*1/*5	*1/*1	37	Yes			84.1	150*BID						
24	wt/hisp	*1/*6	*1/*1	21	No			63.7	100*TID						
25	wt/non-hisp	*1/*1	*1/*17	28	No					168.8	300*QD				
26	wt/non-hisp	*1/*1	*1/*1	32	No					117.7	300*QD[a]				
27	wt/non-hisp	*1/*1	*17/*17	22	Yes			50.3	150*BID						
28	bl/non-hisp	ND	*1/*17	30	Yes			135.2	150*BID[a]						

[a] Incomplete urine sample collection

[b] Same subject as subject #7, different pregnancy

ND, not determined; QD, once a day; BID, twice daily; TID, trice daily; wt, white; bl, black; hisp, Hispanic; non-hisp, non-Hispanic; early pregnancy, 10-14 weeks of gestation; mid-pregnancy, 22-26 weeks of gestation; late pregnancy, 34-38 weeks of gestation. Due to no effect of cigarette smoking on the pharmacokinetics of bupropion and its metabolites (Hsyi et al., 1997), subjects were enrolled in the study irrespective of their smoking status.

Supplemental table 2. Effect of *CYP2B6* genetic variability on the pharmacokinetic parameters and urinary excretion of bupropion and its metabolites in mid- and late pregnancy.

	Mid-pregnancy (22-26 weeks)			Late pregnancy (34-38 weeks)		
***CYP2B6* genotypes**	**All[a]**	***1/*1**	***1/*6 and *6/*6**	**All**	***1/*1**	***1/*6 and *6/*6**
BUP all doses	**(n=10)**	**(n=5)**	**(n=4)**	**(n=19)**	**(n=11)**	**(n=6)**
CL/F_{ss} (L/h)	340 ± 344	458 ± 481	212 ± 31	300 ± 128	323 ± 121	275 ± 138
CL/F_{ss} (L/h/kg)	4.40 ± 3.83	5.96 ± 5.15	2.77 ± 0.69	3.50 ± 1.75	3.78 ± 2.04	3.18 ± 1.04
OHBUP/BUP M.R.	21.8 ± 25.6	32.8 ± 34.0	9.46 ± 4.44	23.3 ± 11.6	24.8 ± 10.2	20.0 ± 11.0
TB/BUP M.R.	7.22 ± 3.89	7.11 ± 4.10	7.55 ± 4.77	8.44 ± 4.05	8.00 ± 4.55	8.89 ± 3.19
EB/BUP M.R.	1.28 ± 0.66	1.36 ± 0.75	1.17 ± 0.72	1.23 ± 0.52	1.18 ± 0.60	1.28 ± 0.39
BUP SR dose 150 mg BID	**(n=8)**	**(n=4)**	**(n=3)**	**(n=12)**	**(n=6)**	**(n=5)**
AUC_{ss} BUP (ng*h/ml)	556 ± 228	414 ± 225	742 ± 114	545 ± 211	510 ± 207	617 ± 234
AUC_{ss} OHBUP (ng*h/ml)	8952 ± 3224	9188 ± 4672	8572 ± 1815	10873 ± 4144	10656 ± 4361	10118 ± 4031
AUC_{ss} TB (ng*h/ml)	4458 ± 3189	2553 ± 2084	7263 ± 3116	4053 ± 2625	2799 ± 1501	5435 ± 3361
AUC_{ss} EB (ng*h/ml)	754 ± 441	477 ± 340	1119 ± 393 *****	592 ± 359	432 ± 276	776 ± 421
% of BUP dose recovered as	**(n=6)**	**(n=3)**	**(n=2)**	**(n=11)**	**(n=6)**	**(n=4)**
BUP-free	0.45 ± 0.20	0.51 ± 0.06	0.50 ± 0.35	0.16 ± 0.16	0.14 ± 0.19	0.18 ± 0.14
OHBUP-free	1.54 ± 0.99	1.26 ± 0.92	1.43 ± 1.34	0.51 ± 0.35	0.39 ± 0.23	0.49 ± 0.20
OHBUP-glucuronide	12.8 ± 8.61	13.5 ± 12.4	8.95 ± 1.57	12.3 ± 5.75	13.7 ± 6.49	9.04 ± 3.65
TB-free	13.1 ± 9.71	7.04 ± 5.81	23.6 ± 8.08	5.61 ± 5.54	2.76 ± 2.03	8.23 ± 7.44
TB-glucuronide	3.87 ± 3.52	2.41 ± 1.88	2.81 ± 1.98	3.32 ± 2.38	2.97 ± 1.78	3.82 ± 3.60
EB-free	1.52 ± 1.37	0.73 ± 0.67	2.77 ± 1.87	0.45 ± 0.40	0.25 ± 0.17	0.60 ± 0.50
EB-glucuronide	0.87 ± 0.68	0.76 ± 0.64	1.41 ± 0.57	0.73 ± 0.52	0.67 ± 0.42	0.84 ± 0.77

BID, twice a day; AUC_{SS}, 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; SR, sustained release

Data presented as mean ± standard deviation

[a] Subjects with undetermined *CYP2B6* genotype were not included

**P* < .05

Supplemental table 3. Effect of *CYP2C19* genetic variability on the pharmacokinetic parameters and urinary excretion of bupropion and its metabolites in mid- and late pregnancy.

	Mid-pregnancy (22-26 weeks)			**Late pregnancy (34-38 weeks)**		
***CYP2C19* genotypes**	**All[a]**	**EM and UM**	**PM and IM**	**All**	**EM and UM**	**PM and IM**
BUP all doses	**(n=12)**	**(n=7)**	**(n=5)**	**(n=19)**	**(n=12)**	**(n=7)**
CL/F_{ss} (L/h)	308 ± 321	210 ± 50	445 ± 489	300 ± 128	296 ± 127	305 ± 138
CL/F_{ss} (L/h/kg)	3.90 ± 3.66	2.66 ± 1.05	5.63 ± 5.35	3.50 ± 1.75	3.23 ± 1.36	3.96 ± 2.32
OHBUP/BUP M.R.	19.9 ± 23.6	12.6 ± 9.12	30.1 ± 34.5	23.3 ± 11.6	22.2 ± 11.7	22.2 ± 11.7
TB/BUP M.R.	7.12 ± 3.53	5.18 ± 2.50	9.83 ± 3.02 *	8.44 ± 4.05	6.58 ± 3.33	11.6 ± 3.16 *****
EB/BUP M.R.	1.24 ± 0.61	0.95 ± 0.56	1.64 ± 0.46	1.23 ± 0.52	1.03 ± 0.52	1.57 ± 0.34 *****
BUP SR dose 150 mg BID	**(n=8)**	**(n=4)**	**(n=4)**	**(n=12)**	**(n=6)**	**(n=6)**
AUC_{ss} BUP (ng*h/ml)	586 ± 232	628 ± 130	534 ± 338	545 ± 211	536 ± 211	554 ± 246
AUC_{ss} OHBUP (ng*h/ml)	8826 ± 3039	8546 ± 4041	9177 ± 1603	10873 ± 4144	10424 ± 4546	11322 ± 4101
AUC_{ss} TB (ng*h/ml)	4574 ± 3003	3537± 1668	5870 ± 4039	4053 ± 2625	2333 ± 1313	5773 ± 2517 *
AUC_{ss} EB (ng*h/ml)	749 ± 413	634 ± 281	893 ± 548	592 ± 359	403 ± 273	782 ± 350
% of BUP dose recovered as	**(n=6)**	**(n=4)**	**(n=2)**	**(n=11)**	**(n=6)**	**(n=5)**
BUP-free	0.45 ± 0.20	0.48 ± 0.22	0.41 ± 0.23	0.16 ± 0.16	0.14 ± 0.19	0.18 ± 0.13
OHBUP-free	1.54 ± 0.99	1.75 ± 1.09	1.12 ± 0.89	0.51 ± 0.35	0.35 ± 0.16	0.71 ± 0.42
OHBUP-glucuronide	12.8 ± 8.6	14.2 ± 10.6	10.0 ± 3.05	12.3 ± 5.75	11.0 ± 5.64	13.9 ± 6.11
TB-free	13.1 ± 9.71	11.8 ± 12.1	15.8 ± 3.04	5.61 ± 5.54	2.21 ± 1.41	9.69 ± 6.01
TB-glucuronide	3.87 ± 3.52	3.72 ± 4.53	4.18 ± 0.04	3.32 ± 2.38	1.80 ± 1.18	5.15 ± 2.17 *
EB-free	1.52 ± 1.37	1.55 ± 1.77	1.47 ± 0.03	0.45 ± 0.40	0.23 ± 0.14	0.72 ± 0.45
EB-glucuronide	0.87 ± 0.68	0.50 ± 0.47	1.60 ± 0.30	0.73 ± 0.52	0.58 ± 0.42	0.91 ± 0.63

BID, twice a day; AUC_{SS}, area under the curve at steady state; BUP, bupropion; OHBUP, hydroxybupropion; TB, threohydrobupropion; EB, erythrohydrobupropion; EM, extensive metabolizer phenotype; UM, ultra-rapid metabolizer phenotype; PM, poor metabolizer phenotype, IM, intermediate metabolizer phenotype; M.R., metabolic ratio, defined as the ratio of AUCs, corrected for molecular weight

Data presented as mean ± standard deviation.

[a] Subjects with undetermined *CYP2C19* genotype were not included; *P < .05