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# Relationships between endogenous CYP3A markers and plasma amlodipine exposure and metabolism in early postpartum and non-peripartum women with hypertension

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ABSTRACT

*Objective:* This study aimed to evaluate the relationship between endogenous CYP3A markers and plasma amlodipine (AML) exposure and metabolism parameters in early postpartum and non-peripartum women.
*Methods:* Twenty-four AML-treated early postpartum women with hypertensive disorders of pregnancy and 30 non-peripartum women with essential hypertension were enrolled. Blood samples for determination of CYP3A markers including total cholesterol-adjusted 4β-hydroxycholesterol (4β-OHC/TC), 25-hydroxyvitamin D (25-OHD), and AML and its metabolites in plasma were collected at 24 h after the AML treatment.
*Results:* The plasma 4β-OHC/TC in postpartum women was higher than that in non-peripartum women, while the plasma 25-OHD was lower. The postpartum women had a lower plasma AML concentration and its metabolic ratio was higher. The plasma 4β-OHC/TC decreased as the number of days post-delivery increased. The plasma AML concentration increased as the number of days post-delivery increased, while the metabolic ratio of AML declined slightly. Tendency toward negative correlations between the plasma 4β-OHC/TC but not 25-OHD, and AML concentration were observed in both postpartum and non-peripartum women. In both groups, the plasma 4β-OHC/TC was correlated with the metabolic ratio of AML.
*Conclusions:* The early postpartum women had higher plasma 4β-OHC and AML metabolism. The plasma 4β-OHC had positive relationships with amlodipine metabolism in both women groups. AML metabolism and plasma 4β-OHC may be useful as CYP3A markers in early postpartum and non-peripartum women.

## 1. Introduction

Cytochrome P450 (CYP) 3A is an important hepatic enzyme which is involved in approximately half of all enzyme-related drug metabolism [1]. Since the alteration of CYP3A activity affects drug exposure in CYP3A substrates, the measurement of CYP3A activity in clinical settings is potentially useful for predicting therapeutic outcomes or adverse effects. Patient factors including drug-drug interactions, genetic polymorphisms, and gender are responsible for inter-individual variability in CYP3A activity. In addition, recent studies revealed the presence of intra-individual variability in CYP3A activity under patient conditions such as pregnancy, inflammation, and obesity [2–4].

The impact of pregnancy on CYP activity differs according to gestational period and CYP isoform [2]. CYP3A activity increases in all trimesters of pregnancy, and the induction is regulated by the secretion of pregnancy-related hormones such as placental growth hormone [5,6]. The combination of cortisol and placental growth hormone was found to strongly induce the expression of CYP3A4 and 3A5 mRNA in human hepatocytes [7]. Female hormones including estrogen and progesterone upregulate CYP3A4 mRNA expression by activation of estrogen receptors and pregnane X receptors (PXR) [8]. To date, little information has been published on the quantitative determination of CYP3A activity in peripartum women.

Several exogenous and endogenous CYP3A markers have been employed as practical tools to assess CYP3A-mediated drug clearance. Midazolam clearance is commonly used as an exogenous marker for the

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assessment of CYP3A activity [9]. The oral midazolam clearance was higher during pregnancy than postpartum and had returned near baseline by 6–10 weeks postpartum [10]. As for endogenous markers, plasma 4β-hydroxycholesterol (4β-OHC) and urinary 6β-hydroxycortisol are major markers used for determining CYP3A activity [9,11,12]. Plasma 4β-OHC is formed from cholesterol through CYP3A4 and 3A5 metabolism and has a long elimination half-life [11]. Plasma 25-hydroxyvitamin D (25-OHD) is a major circulating form of vitamin D and is considered to be an indicator of vitamin D status [13]. Determination of plasma 25-OHD is potentially useful for assessing CYP3A4 activity because expression of the CYP3A4 gene in intestine is regulated by nuclear vitamin D receptors in addition to PXR [14]. The characteristics of CYP3A markers such as plasma 4β-OHC and 25-OHD remain unclear in peripartum women.

Amlodipine (AML) is an anti-hypertensive drug used for the treatment of hypertensive disorders of pregnancy (HDP). The main route of AML elimination is dehydrogenation by hepatic CYP3A4 to dehydroamlodipine (DH-AML), and excretion in urine largely as *O*-des[2-aminoethyl]-*O*-carboxymethyl DH-AML (CM-DH-AML) [15]. In a previous study, early postpartum women had a higher serum level of 4β-OHC and a negative correlation with plasma AML [16]. These findings suggest the possibility that plasma AML may be useful as a probe for CYP3A4 activity. However, the associations between AML pharmacokinetics and other CYP3A markers including plasma 25-OHD remain to be clarified in peripartum women.

This study aimed to investigate the relationships between endogenous CYP3A markers including plasma 4β-OHC and 25-OHD, and plasma AML exposure and metabolism parameters in early postpartum and non-peripartum women with hypertension.

## 2. Materials and methods

### 2.1. Patients and study design

This study was an observation study conducted at Hamamatsu University Hospital. Twenty-four early postpartum women treated with AML besylate tablets (Sawai Pharmaceutical Co., Ltd., Osaka, Japan) for HDP after delivery and 30 non-peripartum women treated with AML besylate tablets for essential hypertension were enrolled. All patients received oral AML once daily in the morning for at least 5 days. Patients with liver dysfunction (total bilirubin > 2.0 mg/dL), renal dysfunction (estimated glomerular filtration rate < 50 mL/min/1.73 $m^2$), active inflammation (C-reactive protein > 3.0 mg/dL), receiving concomitant strong CYP3A modifiers such as rifampicin, carbamazepine, and triazole antifungals [17], vitamin D supplementation, or poor medication adherence based on a pharmacist interview and medical records were excluded. Blood samples for determination of AML and two of its metabolites, 4β-OHC and 25-OHD, were simultaneously drawn into tubes containing EDTA-2Na at 24 h on the 5th day or later after starting the AML treatment. This study is registered in the University Hospital Medical Information Network (UMIN000033745).

### 2.2. Determination of 4β-OHC and cholesterol in human plasma

Saponified plasma 4β-OHC was determined using an LC–MS/MS [16]. The calibration curve of 4β-OHC in human plasma was linear over the concentration range of 5–200 ng/mL. The intra- and inter-day accuracies and imprecisions of 4β-OHC in human plasma were 104.2–114.5% and 94.5–106.8%, and 3.0–9.3% and 1.7–13.6%, respectively. The lower limit of quantification (LLOQ) for 4β-OHC in human plasma was 5 ng/mL. Total cholesterol (TC) in human plasma was determined using an enzymatic colorimetric kit (Wako Cholesterol E, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma 4β-OHC concentrations were adjusted by the plasma level of TC as plasma 4β-OHC/TC.

### 2.3. Determination of 25-OHD in human plasma

The concentration of 25-OHD in human plasma was determined by a commercial method using an enzyme-linked immunosorbent assay kit (25(OH)-Vitamin D direct day ELISA, Immundiagnostik, Bensheim, Germany). Plasma concentrations of 25-OHD were determined quantitatively using a spectrophotometric method, based on the measurement of color intensity.

### 2.4. Determination of AML and its metabolites in human plasma

The plasma concentrations of AML and its two metabolites were simultaneously determined using an LC–MS/MS [18]. The LLOQs of AML, DH-AML, and CM-DH-AML were 0.5, 1, and 0.5 ng/mL, respectively. The intra- and inter-day accuracies of AML, DH-AML, and CM-DHAML were 97.5–111.2% and 97.0–103.5%, 97.9–109.0% and 98.1–103.0%, and 99.4–111.0% and 95.4–102.8%, respectively. The intra- and inter-day imprecisions of AML, DH-AML, and CM-DH-AML were 2.7–3.9% and 1.0–8.8%, 1.9–7.7% and 5.0–8.8%, and 3.5–9.0% and 3.5–10.8%, respectively.

### 2.5. Evaluation of plasma AML exposure and metabolism parameters

Plasma exposures of AML, DH-AML, and CM-DH-AML were assessed by the absolute plasma concentrations normalized with dose and body weight of each patient. AML metabolism was calculated from the plasma concentration ratio of the metabolites to parent drug as metabolic ratio. The plasma metabolic ratios of AML to DH-AML (DH-AML/AML), AML to CM-DH-AML (CM-DH-AML/AML), DH-AML to CM-DH-AML (CM-DH-AML/DH-AML), and AML to summation of the two metabolites ((DH-AML + CM-DH-AML)/AML) were evaluated as the parameters of AML metabolism.

### 2.6. Statistical analysis

All statistics were analyzed with IBM SPSS statistics ver. 22 (IBM Japan Ltd, Tokyo). The patient characteristics were compared using the Mann-Whitney *U* test. Differences in the plasma CYP3A markers between early postpartum and non-peripartum women were evaluated by the Mann-Whitney *U* test. The differences in plasma exposure and metabolism parameters of AML between the two groups were analyzed using the Mann-Whitney *U* test. Correlations between the CYP3A markers and plasma AML concentration or plasma CM-DH-AML/AML were evaluated using Pearson's test. The values are expressed as the median and interquartile range (IQR) unless otherwise stated, and a *P* value of less than or equal to 0.05 for a two-sided test was considered statistically significant.

## 3. Results

### 3.1. Patient characteristics

Table 1 shows the patient characteristics in this study population. Serum albumin level in the postpartum women was significantly lower than that in the non-peripartum women. No difference in the estimated glomerular filtration rate was observed between the two groups, although a significant difference within the normal range was observed in the total bilirubin level. Systolic and diastolic blood pressures in the early postpartum women were significantly higher than those in the non-peripartum women.

### 3.2. Plasma 4β-OHC and 25-OHD

The median plasma concentrations of 4β-OHC and TC in the early postpartum women were 259 ng/mL and 279 mg/dL, and 44 ng/mL and 183 mg/dL in non-peripartum women, respectively (Fig. 1). The plasma

**Table 1**
Patient characteristics.

(A) Clinical laboratory and physiological data

| | Postpartum women n = 24 | | Non-peripartum women n = 30 | | *P* value |
|---|---|---|---|---|---|
| Age, years (range) | 35 | (23–44) | 74 | (32–87) | < 0.001 |
| Body weight, kg | 60.7 | (48.4–94.5) | 51.8 | (33.4–84.0) | 0.004 |
| Total protein, g/dL | 6.1 | (4.6–7.4) | 6.5 | (4.9–8.0) | 0.158 |
| Serum albumin, g/dL | 2.7 | (1.9–4.0) | 3.6 | (2.7–4.3) | < 0.001 |
| Serum creatinine, mg/dL | 0.64 | (0.43–1.0) | 0.58 | (0.45–1.1) | 0.408 |
| eGFR, mL/min/1.73 $m^2$ | 85 | (51–130) | 76 | (38–127) | 0.141 |
| Aspartate aminotransferase, IU/L | 21 | (10–61) | 20 | (10–171) | 0.695 |
| Alanine aminotransferase, IU/L | 12 | (5–51) | 17 | (5–173) | 0.012 |
| Total bilirubin, mg/dL | 0.5 | (0.4–1.3) | 0.8 | (0.3–2.0) | 0.009 |
| C-reactive protein, mg/dL | 0.68 | (0.09–2.8) | 0.23 | (0.02–2.9) | 0.077 |
| Systolic blood pressure, mmHg | 135 | (108–156) | 117 | (103–152) | < 0.001 |
| Diastolic blood pressure, mmHg | 89 | (74–99) | 72 | (44–89) | < 0.001 |

Patient data except for age represented median and interquartile range in parentheses.
eGFR, estimated glomerular filtration rate

(B) Maternal characteristics (n = 24)

| | Median (interquartile range) | |
|---|---|---|
| Blood sampling point after delivery, days | 13 | (10–14) |
| Gestational period, weeks and days | 36 and 3 | (34 and 5–37 and 4) |
| Vaginal delivery/Caesarean section | 5/19 | |
| Primipara/Multipara | 15/9 | |

(C) Major disease names in non-peripartum women (n = 30)

| Disease names | n |
|---|---|
| Angina pectoris | 6 |
| Rheumatoid arthritis | 4 |
| Deep vein thrombosis | 3 |
| Herpes zoster | 3 |
| Others | 14 |

concentration of 4β-OHC in the early postpartum women was approximately 6-fold higher than that in non-peripartum women ($P < 0.001$). The median plasma 4β-OHC/TC in early postpartum and non-peripartum women were $90.5 \times 10^{-6}$ and $24.1 \times 10^{-6}$, respectively, and a significant difference was observed ($P < 0.001$). The median plasma concentrations of 25-OHD in the early postpartum women and non-peripartum women were 12.9 and 16.9 ng/mL, respectively, and a significant difference was observed ($P = 0.032$). No

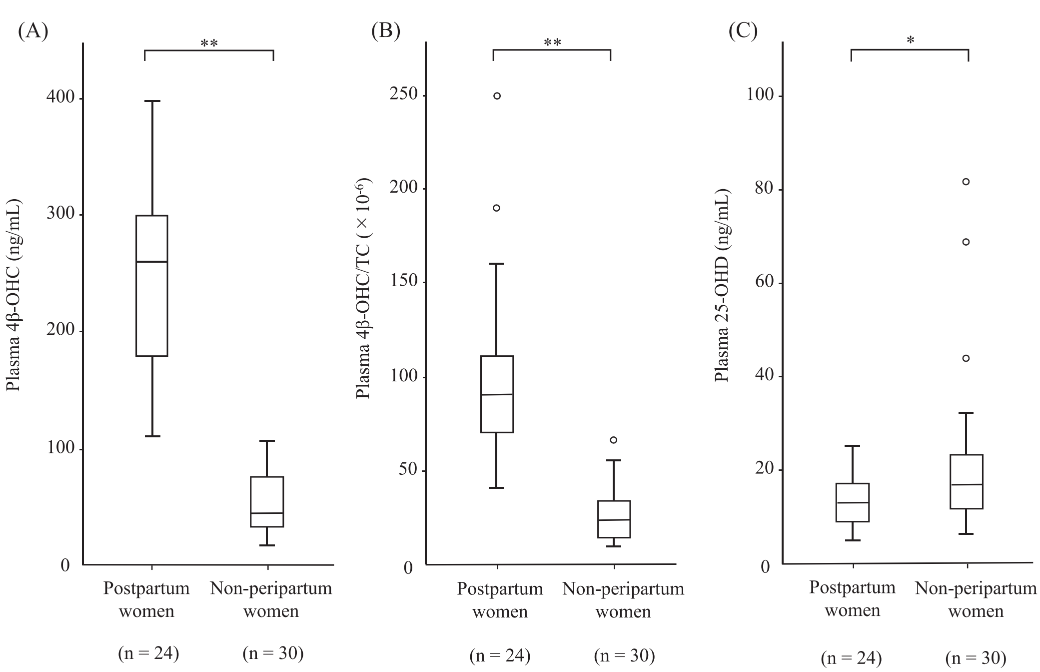


**Fig. 1.** Endogenous CYP3A markers in postpartum and non-peripartum women. (A) Plasma 4β-hydroxycholesterol (4β-OHC) concentration, (B) total cholesterol-adjusted plasma 4β-OHC (4β-OHC/TC), and (C) plasma 25-hydroxyvitamin D (25-OHD) concentration. Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend within 1.5 times the length of the inner quartiles. The differences between the early postpartum and non-peripartum women were analyzed using the Mann-Whitney *U* test. $^{*}P \le 0.05$, $^{**}P \le 0.01$, different from the non-peripartum women.

**Table 2**
Plasma amlodipine exposure and metabolism in early postpartum women and non-peripartum women.

| | Postpartum women n = 24 | | Non-peripartum women n = 30 | | *P* value |
|---|---|---|---|---|---|
| AML dose, mg/kg | 0.095 | (0.079–0.125) | 0.095 | (0.069–0.116) | 0.548 |
| Plasma AML, ng/mL per mg/kg | 112 | (84.6–139) | 155 | (101–187) | 0.042 |
| Plasma DH-AML, ng/mL per mg/kg | 32.4 | (23.0–46.9) | 41.1 | (23.4–58.1) | 0.408 |
| Plasma CM-DH-AML, ng/mL per mg/kg | 112 | (63–147) | 89 | (60–122) | 0.394 |
| Plasma DH-AML/AML | 0.330 | (0.248–0.401) | 0.261 | (0.182–0.369) | 0.192 |
| Plasma CM-DH-AML/AML | 0.862 | (0.703–1.108) | 0.658 | (0.450–0.914) | 0.026 |
| Plasma CM-DH-AML/DH-AML | 3.01 | (2.39–3.47) | 2.29 | (1.56–3.70) | 0.194 |
| Plasma (DH-AML + CM-DH-AML)/AML | 1.17 | (0.99–1.45) | 0.91 | (0.65–1.22) | 0.028 |

Data represent median and interquartile range in parentheses.
AML, amlodipine; DH-AML, dehydroamlodipine; CM-DH-AML, *O*-des[2-aminoethyl]-*O*-carboxymethyl dehydroamlodipine; DH-AML/AML, metabolic ratio of AML to DH-AML; CM-DH-AML/AML, metabolic ratio of AML to CM-DH-AML; CM-DH-AML/DH-AML, metabolic ratio of DH-AML to CM-DH-AML; (DH-AML + CM-DH-AML)/AML, metabolic ratio of AML to summation of DH-AML and CM-DH-AML.

correlation was observed between the plasma 4β-OHC/TC and 25-OHD concentrations in early postpartum ($R^2$ = 0.052, $P$ = 0.279) and non-peripartum women ($R^2$ = 0.017, $P$ = 0.496).

### 3.3. Plasma exposure and metabolism parameters of AML

Table 2 shows the plasma AML exposure and metabolism in early postpartum and non-peripartum women. The plasma AML concentration in early postpartum women was lower than that in non-peripartum women ($P$ = 0.042). There were no differences in the plasma concentrations of DH-AML and CM-DH-AML between the two groups. With respect to the metabolic ratios, plasma CM-DH-AML/AML and (CM-DH-AML + DH-AML)/AML in the early postpartum women were 31% and 29% higher than in the non-peripartum women, respectively ($P$ = 0.026 and $P$ = 0.028). No differences were observed in plasma DH-AML/AML and CM-DH-AML/DH-AML between the two groups.

### 3.4. Time-dependent changes after delivery

A negative correlation was observed between the plasma 4β-OHC/TC and the number of days that had elapsed after delivery ($r$ = −0.439, $P$ = 0.032) (Fig. 2). A slightly positive correlation was observed between plasma 25-OHD concentration and the number of days that had elapsed after delivery ($r$ = 0.395, $P$ = 0.056). The plasma AML concentration was significantly correlated with the number of days that had elapsed after delivery ($r$ = 0.451, $P$ = 0.027). The plasma CM-DH-AML/AML also showed a slightly negative correlation with the number of days that had elapsed after delivery ($r$ = −0.378, $P$ = 0.069).

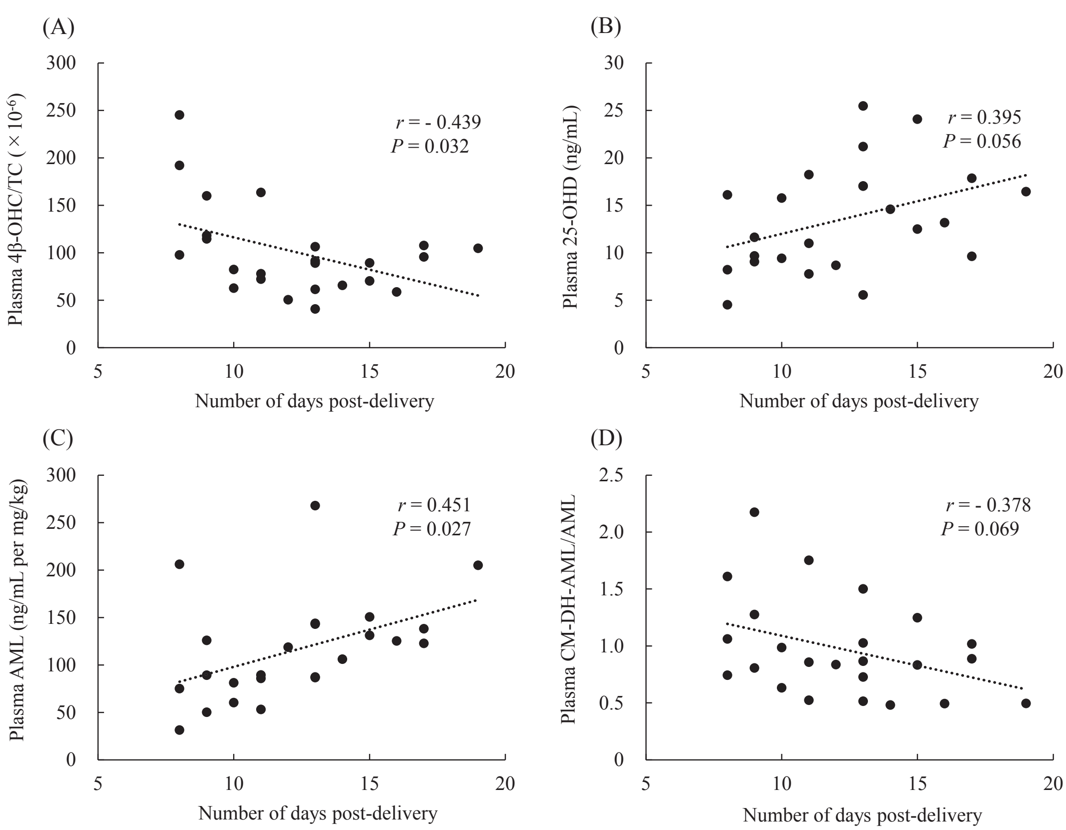


**Fig. 2.** Time-dependent changes of CYP3A markers and amlodipine (AML) disposition parameters after delivery. (A) Total cholesterol-adjusted plasma 4β-hydroxycholesterol (4β-OHC/TC), (B) plasma 25-hydroxyvitamin D (25-OHD) concentration, (C) plasma AML concentration, and (D) plasma metabolic ratio of AML to *O*-des[2-aminoethyl]-*O*-carboxymethyl dehydroamlodipine (CM-DH-AML/AML). The correlations were evaluated using Pearson's test.

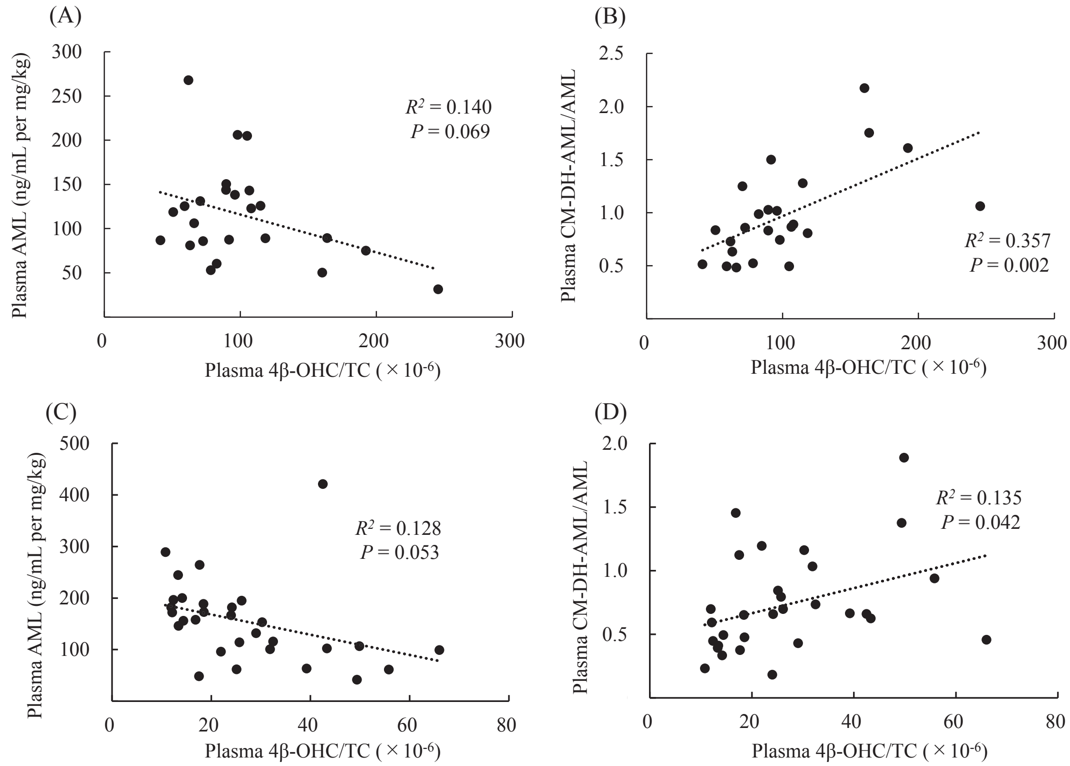


**Fig. 3.** Correlations between plasma 4β-hydroxycholesterol (4β-OHC) and amlodipine (AML) disposition parameters. (A) Plasma AML concentration and (B) plasma metabolic ratio of AML to *O*-des[2-aminoethyl]-*O*-carboxymethyl dehydroamlodipine (CM-DH-AML/AML) in postpartum women, and (C) plasma AML concentration and (D) plasma CM-DH-AML/AML in non-peripartum women. Plasma 4β-OHC concentration was adjusted by serum level of total cholesterol as plasma 4β-OHC/TC. The correlations were evaluated using Pearson's test.

### *3.5. Relationships between CYP3A markers and plasma AML exposure and metabolism parameters*

Plasma AML concentrations had a slightly negative correlation with plasma 4β-OHC/TC in postpartum women ($R^2$ = 0.140, $P$ = 0.069) (Fig. 3). In women with essential hypertension, the plasma AML concentration was slightly negatively correlated with the plasma 4β-OHC/TC ($R^2$ = 0.128, $P$ = 0.053). Significant correlations were observed between the plasma 4β-OHC/TC and CM-DH-AML/AML in early postpartum women ($R^2$ = 0.357, $P$ = 0.002) and in non-peripartum women ($R^2$ = 0.135, $P$ = 0.042). There were no correlations between the plasma concentrations of 25-OHD and AML in early postpartum women ($R^2$ = 0.045, $P$ = 0.333) and in non-peripartum women ($R^2$ = 0.073, $P$ = 0.149) (Fig. S1). A significant correlation was observed between the plasma 25-OHD concentration and plasma CM-DH-AML/AML in non-peripartum women ($R^2$ = 0.304, $P$ = 0.001) but not in early postpartum women ($R^2$ = 0.002, $P$ = 0.846).

## 4. Discussion

This study investigated the CYP3A activity profile using endogenous CYP3A markers and plasma AML exposure and metabolism parameters in early postpartum women and then compared them to that in non-peripartum women. Our findings suggest that the parameters of plasma AML metabolism are useful as CYP3A markers likewise plasma 4β-OHC in early postpartum and non-peripartum women. To the best of our knowledge, this is the first investigation that characterized plasma AML exposure and metabolism using CYP3A markers including plasma 4β-OHC and 25-OHD during non- and peripartum periods.

The early postpartum and non-peripartum women in our study had median plasma 4β-OHC concentrations of 259 and 44 ng/mL, respectively. Bodin et al. reported that the mean plasma 4β-OHC concentrations in female healthy volunteers and in women treated with carbamazepine were 30 and 240 ng/mL, respectively [19]. The plasma 4β-OHC concentration in non-peripartum women in the present study was similar to that in healthy women, while the plasma 4β-OHC concentration in postpartum women was comparable to that in patients treated with a CYP3A inducer. Kim et al. reported that plasma 4β-OHC in pregnant women in their third trimester was 2.3 times higher than that in non-peripartum women [20]. The serum 4β-OHC in women with HDP was 1.6 times higher than that in normotensive women in their third trimester [12]. In the present study, a significant difference in plasma 4β-OHC concentration was observed after correction with the TC level. The TC level in pregnant women was found to increase as pregnancy progressed towards the third trimester [21]. Our data showed that early postpartum women have higher CYP3A activity and suggest the plasma 4β-OHC/TC is one marker that may be used to assess CYP3A activity.

Although the plasma 25-OHD level in early postpartum women was lower than that in non-peripartum women, the plasma 25-OHD in this study population ranged from 10 to 20 ng/mL, which represents a moderate deficiency of vitamin D. Vitamin D is a modulator of CYP3A4 in intestine, and its supplementation decreased the plasma concentrations of atorvastatin, a substrate for CYP3A4 [22]. Our results indicated that vitamin D status in early postpartum women did not contribute to the induction of CYP3A activity based on plasma 4β-OHC. Pregnant women without vitamin D supplementation had lower plasma 25-OHD than nonpregnant women of childbearing age [23]. The lower plasma 25-OHD level in early postpartum women may reflect its consumption with fetal development and delivery, or induced CYP3A may decrease the plasma 25-OHD level [24]. Plasma 25-OHD has no relation to the plasma 4β-OHC/TC in this study population, indicating that vitamin D status is independent of plasma 4β-OHC concentration.

A lower plasma concentration of AML was observed in early postpartum women than in non-pregnant women in our study. Unadkat et al. reported that women at 14–28 weeks of gestation had lower plasma exposure of indinavir, a CYP3A4 substrate, than those at 12 weeks after delivery [25]. The pharmacokinetic changes of AML observed in this study were similar to those in earlier reports of drugs metabolized by CYP3A4 during the peripartum period [25–27]. AML metabolism assessed by plasma CM-DH-AML/AML was higher in the early postpartum women than in the non-pregnant women. Although plasma DH-AML/AML is the metabolic process directly catalyzed by CYP3A4, it was less reflective of the induction of CYP3A activity than plasma CM-DH-AML/AML. This is probably attributable to the feature of DH-AML as an intermediate, and DH-AML is rapidly converted to CM-DH-AML in the metabolic pathway [28].

All of the parameters in this study were trending toward the normal values with the number of days that had elapsed after delivery. Tracy et al. reported that CYP3A activity was 30% higher at all trimesters of pregnancy than at 6 weeks after delivery [5]. Nylén et al. reported that the increased plasma 4β-OHC/TC observed at the time of delivery was not observed at four months postpartum [29]. The present study found that the CYP3A activity based on plasma 4β-OHC and AML metabolism was shifting toward normalization for 3 weeks after delivery, however, further investigations are needed to clarify the CYP3A marker profile over a longer term after delivery.

Plasma AML was slightly inversely correlated with plasma 4β-OHC in early postpartum women. Our previous research also showed a weak negative correlation between plasma AML and 4β-OHC/TC [16]. A positive correlation was observed between plasma CM-DH-AML/AML and 4β-OHC/TC in our study populations, indicating the usefulness of the AML metabolic ratio as a marker to assess the induction of hepatic CYP3A activity. Plasma 25-OHD showed no association with plasma AML exposure and metabolism in early postpartum women, while a positive correlation was observed between plasma 25-OHD and CM-DH-AML/AML in non-peripartum women. Among non-peripartum women, patients with higher plasma 25-OHD had higher AML metabolism. Our results suggest that the modulation of intestinal CYP3A by vitamin D affects AML metabolism under conditions with higher plasma 25-OHD, but not under conditions with slight vitamin D deficiency, such as early postpartum women.

A time-dependent reduction of CYP3A activity after delivery was observed in this study. This suggests the necessity for tapering the AML dose or lengthening dosage interval in HDP treatment. In addition, HDP symptoms are usually relieved along with the amount of time that has elapsed since delivery. Although no difference was observed in AML dose and dosage interval between the two groups, the blood pressures in early postpartum women were higher than that in non-peripartum women in the present study. Dihydropyridine calcium channel blockers are likely to have less efficacy in postpartum women [30]. The induction of CYP3A activity may be one of the reasons for the difficulty in managing HDP. Evaluation of CYP3A activity during the postpartum period using clinically adoptable CYP3A markers would be important for controlling HDP and obstetric complications.

The present study has a few limitations. First, the age of non-peripartum women differed from that of the early postpartum women. Hepatic and renal function in both groups were within the normal range. The total bilirubin level also showed a difference within the normal range between the two groups because elderly patients tend to have a higher total bilirubin level [31]. It was quite difficult to gather an age-matched non-peripartum population receiving AML in a clinical setting. Little influence of age differences on endogenous CYP3A markers was observed in non-peripartum women in this study (Table S1). Although the exclusion criteria in the present study reduce the possible influence of age differences on hepatic and renal function, further research is needed to assess the effect of aging on AML pharmacokinetics. Second, this study did not evaluate the influence of CYP3A5 genotype on CYP3A markers and plasma AML exposure and metabolism parameters. AML pharmacokinetics is not affected by CYP3A5 genotype, while plasma 4β-OHC is explained by CYP3A4 and CYP3A5 activity [28,32]. Further investigations including CYP3A5 genotype are needed to clarify the relationships between CYP3A markers and plasma AML exposure and metabolism parameters. Third, the CYP3A markers evaluated in this study, plasma 4β-OHC, 25-OHD, and AML, possess relatively longer half-lives in plasma. The plasma markers potentially did not reflect the fast change of CYP3A activity during the present study period. Although the CYP3A activity after delivery might have been overestimated, the study period was long enough to evaluate the association with plasma AML exposure and metabolism.

## 5. Conclusions

Early postpartum women had higher plasma 4β-OHC levels and AML metabolism than the non-peripartum women. The plasma 4β-OHC had positive relationships with amlodipine metabolism in both women groups. AML metabolism and plasma 4β-OHC may be useful as CYP3A markers in early postpartum and non-peripartum women.

## Declaration of Competing Interest

None.

## Acknowledgement

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## Author's contributions

Reina Taguchi and Takafumi Naito were involved in developing the study concept and design. Reina Taguchi, Takafumi Naito, and Naoko Kubono performed the research. All authors analyzed the data, contributed to interpretation and writing of the manuscript and approved the final manuscript for submission.

## Compliance with ethical standards

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine (16-018).

**Informed consent:** Eligible patients were provided with an explanation of the study protocol and the scientific aim of the study according to the patient information sheet. Informed consent was obtained from all individual participants included in the study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.07.002.

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