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Prenatal lignan exposures, pregnancy urine estrogen profiles and birth outcomes

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ABSTRACT

During pregnancy, human exposure to endogenous estrogens and xenoestrogens (such as lignans) may comprehensively impact the gestational maintenance and fetal growth. We measured the concentrations of 5 lignans and the profile of 13 estrogen metabolites (EMs) in the urine samples of 328 pregnant women and examined their associations with birth outcomes. We found significantly positive associations between gestational age and urinary matairesinol (MAT), enterodiol (END) and enterolactone (ENL), as well as 16-hydroxylation pathway EMs. There were consistently positive relationships between END and the 16-hydroxylation pathway EMs. The positive relationships of MAT, END and ENL exposures with the length of gestation were mainly in the low exposure strata of the levels of these EMs. This study reveals that MAT, END and ENL as well as 16-hydroxylation pathway EMs are associated with birth outcomes, and that there are interactive relationships between lignans and 16-hydroxylation pathway EMs with birth outcomes.

1. Introduction

Birth outcomes such as gestational age and birth weight have been routinely used for the evaluation of fetal development in both laboratory and population studies (Suzuki et al., 2010; Tang et al., 2013). Moreover, human birth outcomes have been applied to predict occurrence of diseases, such as cardiovascular disease, in the later life (Lau and Rogers, 2004; Suzuki et al., 2010). Therefore, there is a great need to study those factors that can impact birth outcomes.

It is known that the endogenous estrogens in pregnancy play very important roles in the maintenance of gestation and fetal growth, yet the effect of specific estrogen metabolites (EMs) on birth outcomes remains unclear. The metabolism of two primary estrogens, estrone (E1) and estradiol (E2), occurs through three major competitive pathways (Figure S1). The first pathway leads to the 2-hydroxy derivatives (2-OHE1, 2-OHE2) and 2-methoxy derivatives (2-MeOE1, 2-MeOE2) of these compounds, as well as 2-hydroxyestrone-3-methyl ether (3-MeOE1); the second one leads to the 4-hydroxy (4-OHE1) and 4-methoxy derivatives (4-MeOE1, 4-MeOE2); the third one leads to the 16α-hydroxy derivatives (16α-OHE1) as well as estriol (E3), 16-epiestriol (16-epiE3) and 17-epiestriol (17-epiE3) (Xu et al., 2007). As for these EMs, it has been shown in laboratory studies that they exhibit differences in biological activity that may differentially impact human health (Sturgeon et al., 2010). Therefore, it is warranted that studies with a broad panel of EM profiles are carried out to comprehensively evaluate possible impacts of these EMs on birth outcomes.

Aside from endogenous estrogens, there are also plant-derived

Abbreviations: BMI, body mass index; CI, confidence intervals; CR, creatinine; EM, estrogen metabolite; E1, estrone; E2, estradiol; E3, estriol; END, enterodiol; ENL, enterolactone; GMs, geometric means; LODs, the limits of detection; MAT, matairesinol; PNR, pinoresinol; QC, Quality control; SEC, secoisolariciresinol; UPLC–MS/MS, liquid chromatography tandem mass spectrometry.

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xenoestrogens called "phytoestrogens" that are not generated from the endocrine system but can be acquired through eating phytoestrogenic plants. Because their structures are similar to that of estradiol (E2), these phytoestrogens have the ability to interact with estrogen receptors to cause estrogenic or/and antiestrogenic effects (Kunisue et al., 2010) and may also impact the birth outcomes.

Lignans are one class of "phytoestrogens" (Adlercreutz, 2007). The plant lignans such as secoisolariciresinol (SEC), matairesinol (MAT) and pinoresinol (PNR) are widely occurring in the whole-grain cereals, beans, flaxseed, sesame seed, vegetables, berries, and some other fruits (Milder et al., 2005; Penalvo et al., 2005a, 2005b; Thompson et al., 2006). There are also two enterolignans (mammalian lignans), enterodiol (END) and enterolactone (ENL), which are formed from the plant lignans in the large bowel of animals and humans by intestinal microbiota (Adlercreutz, 2007; Wang, 2002). During pregnancy, the developing fetus may be exposed to lignans that originate from the maternal diet, and indeed these compounds can be detected in the amniotic fluid and umbilical cord blood (Engel et al., 2006; Luoto et al., 2010). Thus, concern should be raised as to whether prenatal exposure to these agents may affect fetus growth, reproduction and development. However, currently, there is very limited evidence in epidemiological and animal studies on birth outcomes in association with prenatal exposure to lignans.

As mentioned above, it should be noted that, due to their highly related biological activity, the lignans may impact on estrogen metabolism, and they can further affect birth outcomes. Additionally, many studies also demonstrated that certain beneficial effects of lignans may be dependent on the endogenous levels of estrogens (Adlercreutz, 2007; Dai et al., 2003; Wang, 2002). Therefore, estrogen levels may also affect the relation between prenatal lignan exposure and birth outcomes. In this regard, there is an urgency to comprehensively understand the relationships among lignan exposures, estrogen levels and birth outcomes.

Both EMs and lignans mentioned above are mainly excreted into human urine (Harrison et al., 1999; Xu et al., 2007). The total level (conjugated and free) of these chemicals measured in human urine can reflect the individual internal exposure level and has been widely used in exploring relationships between exposure to such compounds and diseases as well as laboratory abnormalities (Dai et al., 2003; Fuhrman et al., 2013; Kim et al., 2014; Kunisue et al., 2010; Sturgeon et al., 2010). In this study, we first evaluated the association of urinary levels of lignans and EM profiles in pregnant women with birth outcomes, and next investigated the correlation between lignan exposures and EM levels, as well as the effect of different estrogen levels on the relationship between lignan exposures and birth outcomes.

2. Methods

2.1. Study participants

From September 2010 to November 2011, pregnant women were recruited from the affiliated hospitals of Nanjing Medical University (NMU Birth Cohort). Eligible women with singleton pregnancy were ≥18 years of age at enrollment, and reported no assisted reproduction, pre-gestational or gestational diabetes, chronic or pregnancy-associated hypertension and HIV infection or AIDS (Tang et al., 2013). Of 345 eligible women, 339 (response rate: 98.3%) consented to take part in this study. Among these women, we further excluded 11 of them whose infants were very premature births (delivery at <32 completed gestational weeks or birth weight <1500 g), or born with genetic abnormalities or malformations (Tang et al., 2013). Finally, 328 women were enrolled in the analysis. All participants claimed that their life styles and environments had not changed for several months leading up to sample collection. Written informed consent was obtained from each participating woman, and this study was approved by the Institutional Review Board of Nanjing Medical University.

2.2. Maternal interviews

A questionnaire was administered to each participant shortly after delivery, and maternal information was collected as follows: demographic and socioeconomic information (age, height, end-of-pregnancy weight, education level, and household income), dietary habits, smoking or alcohol use during pregnancy, occupational history and reproduction status. Maternal body mass index (BMI) in late pregnancy was calculated as end-of-pregnancy weight (kg) divided by the height (meter) squared. Other relevant information such as previous pregnancies, medical conditions, current pregnancy complications and self-reported last menstrual period (LMP) were also obtained by interview and confirmed by medical records.

2.3. Outcome measures

Information on newborns was obtained from hospital delivery logs and medical records, including gestational age at birth, infant sex, birth weight, crown–heel length, Apgar scores and presence of apparent congenital malformations. Data on clinical estimate of gestational age (ultrasound) were also collected. Birth weight and crown–heel length of newborns were measured at birth. Gestational age was estimated based on the onset of LMP; if an LMP was unreliable or if there was a significant discordance between the clinical estimate and LMP (>2 weeks), the first clinical estimation of gestational age was used (Eskenazi et al., 2004; Qin et al., 2007). Low birth weight was defined as < 2500 g. Preterm delivery was defined as birth at less than 37 completed weeks of gestation.

2.4. Measurements of lignan exposures and EM levels

Urine samples were collected from each subject during hospital admission for delivery, and were frozen at −20 °C until analysis. Urinary concentrations of lignans (SEC, PNR, MAT, END and ENL) and EMs (E1, E2, E3, 16α-OHE1, 16-epiE3, 17-epiE3, 2-MeOE1, 3-MeOE1, 4-MeOE1, 2-MeOE2, 2-OHE1, 4-OHE1 and 2-OHE2) were measured using the modified methods of Yu et al. and Xu et al., respectively (Xu et al., 2007; Yu et al., 2009). The metabolic pathway of estrogen profiles and chemical structure of lignans are shown in Figure S1 and S2.

Briefly, 1 ml aliquot of urine was divided into 2 equal portions, both of which were incubated with β -glucuronidase/sulfatase (Sigma–Aldrich) at 37 °C overnight, and then the hydrolyzed compounds in urine were extracted with a liquid–liquid extraction technique. The samples were dried, and dissolved in methanol for lignan analysis, while the other samples for EM analysis were further derivatized. All the solutions were eventually measured using ultra high performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS, Waters, USA). The limits of detection (LODs) of lignans were 0.18 μg/L (SEC), 0.12 μg/L (PNR), 0.25 μg/L (MAT), 0.04 μg/L (END) and 0.08 μg/L (ENL). And for the 13 EMs studied, their LODs were in the range of 1–10 pg/ml. The intra- and inter-day precisions for lignans were between 3.2% and 13.4%, and those for EMs were between 2.3% and 13.8%. The recoveries for lignans were between 82.8% and 106.3%, and for EMs, between 85.4% and 107.6%. Quality control (QC) samples prepared from spiked pooled urine were analyzed along with standards, blanks and unknown samples. Urinary creatinine (CR) concentrations were analyzed using an automated chemistry analyzer (7020