Changes in the Urinary Excretion of the Metabolites of the Tryptophan-Niacin Pathway during Pregnancy in Japanese Women and Rats

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Summary NAD is biosynthesized from tryptophan. Therefore, experimental studies including tryptophan metabolism studies could provide insight into niacin nutrition in pregnancy. Our aim was to determine the change in niacin metabolism during pregnancy by a systemic investigation of how pregnancy alters the tryptophan-niacin metabolism in Japanese women and rats. For the human study, spot urine samples were collected from a total of 434 pregnant Japanese women who were at 5–40 wk of gestation, 50 women at 4–6 wk postpartum, and 10 nonpregnant women as the controls. For the animal study, pregnant rats were fed with a niacin-free diet, and daily urine samples were collected from day 6 of gestation to day 6 postpartum. The intermediates and metabolites of the tryptophan-niacin pathway in the urine samples were measured. The urinary excretions of niacin metabolites in humans and rats increased from mid pregnancy in a time-dependent manner, reached a peak of 2–3-fold during late pregnancy, and declined to control levels after childbirth.

Key Words pregnancy, tryptophan, niacin, human, rat, Japanese

NAD and NADP are biosynthesized from tryptophan in the liver, and this tryptophan-niacin pathway plays an important role in the supply of pyridine nucleotides. Figure 1 shows a schematic diagram of the tryptophan-niacin pathway. Numerous factors such as nutritional factors (1, 2), chemicals (3, 4), experimental models of disease (5, 6), hormones (7, 8) and stress (9) affect the efficiency of this conversion from tryptophan to niacin. These factors change in the activities of pathway enzymes including tryptophan 2, 3-dioxygenase (TDO), \(\alpha\)-amino-\(\beta\)-carboxymuconate-\(\epsilon\)-semialdehyde decarboxylase (ACMSD) and quinolinate phosphoribosyltransferase (QPRT). The increased excretion of certain metabolites of niacin during human pregnancy has been reported from the 1940s (10), and Wertz et al. reported that the conversion of tryptophan to niacin was more efficient in the pregnant than in the non-pregnant state (11). However, the administration of estrone and progesterone into male and female rats resulted in a decrease in conversion of tryptophan to niacin (12, 13). Although Brown et al. showed that the urinary excretion of some tryptophan metabolites in pregnant women loaded with tryptophan increased more than in the control women (14), no evidence has been found as to how pregnancy alters the tryptophan-niacin metabolism.

Excess intermediates and metabolites in the tryptophan-niacin pathway are soon excreted to the urine, and the amount of these substances reflects the changes in the activities of pathway enzymes. Therefore, measuring these substances in the urine can elucidate the tryptophan-niacin metabolism, and we have reported the effects of numerous factors on the tryptophan-niacin metabolism (2–4, 6, 8, 9, 12, 13). To determine how pregnancy alters the tryptophan-niacin metabolism, we investigated the urinary excretions of metabolites on the tryptophan-niacin pathway from spot urine samples in Japanese pregnant women. To confirm the change of tryptophan-niacin metabolism as a result of pregnancy, we assessed the daily urinary excretions of intermediates and metabolites on the tryptophan-niacin pathway in pregnant rats.

SUBJECTS AND METHODS

Subjects. We recruited volunteers for this study at our obstetrical clinic at Jin-no Ladies Clinic at Hikone, Japan, and selected a total of 434 pregnant women who were at 5–40 wk of gestation. 50 women at 4–6 wk postpartum, and 10 non-pregnant women as the controls. All subjects were non-smokers, Japanese, from the middle-upper socioeconomic class, and residing in the town of Hikone or surrounding areas. All were judged to be healthy through medical history screening and urinalysis. We guided them to take some defined life style including diet, and no intake of drugs nor vitamin supplements for at least 7 d prior to urine sampling.
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Fig. 1. Schematic diagram of the tryptophan-niacin pathway. Enzymes are underlined. AnA, anthranilic acid; KA, kynurenic acid; 3-HK, 3-hydroxykynurenine; XA, xanthurenic acid; 3-HA, 3-hydroxyanthranilic acid; AMS, α-amino-β-carboxymuconate-ε-semialdehyde; ACMS, α-aminomuconate-ε-semialdehyde; QA, quinolinate; NaMN, nicotinic acid mononucleotide; NMN, nicotinamide mononucleotide; MNA, N1-methylnicotinamide; 2-Py, N1-methyl-2-pyridone-5-carboxamide; 4-Py, N1-methyl-4-pyridone-3-carboxamide; TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; 3-HAO, 3-hydroxyanthranilic acid 2,3-dioxygenase; ACMSD, α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase; QPRT, quinolinate phosphoribosyltransferase.

After informed consent, spot urine samples were collected at 9:00 a.m.-12:00 p.m. or 3:00 p.m.-5:00 p.m. from the subjects. After collecting urine, 9mL of urine was immediately added to 1mL of 1M HCl in tubes, and stored at -25°C until needed.

Chemicals. Vitamin-free milk casein, sucrose, L-methionine, nicotinamide (Nam) and OA were purchased from wako Pure Chemical Industries (Osaka, Japan). Kynurenic acid (KA), xanthurenic acid (XA) and N1-methylnicotinamide (MNA) chloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N1-methyl-2-pyridone-5-carboxamide (2-Py) and N1-methyl-4-pyridone-3-carboxamide (4-Py) were respectively synthesized by the methods of Pullman and Colowick (15) and of Shibata et al. (16). Creatinine was from Calbiochem (La Jolla, CA). Gelatinized cornstarch and corn oil were respectively purchased from Nichiden Kagaku (Tokyo, Japan) and Ajinomoto (Tokyo, Japan). The mineral and vitamin mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan), and all of the other chemicals used were of the highest purity available from commercial sources.

Animal and diet. The animal room was maintained at a temperature of around 22°C and at about 60% humidity with a 12-h light/12-h dark cycle. Food intake was measured daily at around 10:00 a.m., and food and water were renewed daily. Body weight was not measured because the handling of pregnant rats sometimes increases abortion rates.

The care and treatment of the experimental animals conformed to The University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals. Male and female rats of the Wistar strain (11 wk old, a total of 9 pairs) were obtained from Clea Japan (Tokyo, Japan) and immediately placed and housed in individual wire-bottomed cages for 7 d for accommodation to their new environment. They were fed with a niacin-free 20% casein diet ad libitum through the experiment. The diet consisted of 20% casein, 0.2% L-methionine, 45.9% gelatinized cornstarch, 22.9% sucrose, 5% corn oil, 5% mineral mixture (AIN-93 mineral mixture), and 1% vitamin mixture (niacin-free AIN-93 vitamin mixture). Each female rat was then mated with one male rat for 8 d. After mating, the female rats were placed alone, and daily urine samples were collected with 1 mL of 1 m HCl from day 6 of gestation to day 6 postpartum, and stored at -25°C until needed. The delivery day was designated as day 22 of gestation. The urine samples on the day of delivery could not be measured because maternal materials contaminated the urine samples.

Analysis. Urinary creatinine concentrations were determined according to the Jaffe reaction (17). The contents of Nam, 2-Py, and 4-Py in the urine samples were simultaneously measured by the HPLC method of Shibata et al. (16), while the content of MNA in the urine samples was measured by the HPLC method of Shibata (18). The contents of KA (19), XA (20), 3-HA (20), and AnA (21) in the urine samples were measured by HPLC methods.

Statistical analysis. Statistical analyses were performed with StatView 5.0 for Macintosh (SAS Institute, Cary, NC, USA). The differences between groups in the humans were determined by ANOVA. If significance was indicated, post hoc testing using Fisher's multiple range tests was used to determine where the significance occurred. For comparison between groups in rats, a paired ANOVA with post hoc testing using Tukey's multiple comparison tests was used. A difference of p<0.05 was considered statistically significant. Values are expressed as means±SE.

RESULTS

Humans

Changes in the urinary excretion of upper metabolites on the tryptophan-niacin pathway in humans. The urinary outputs of upper metabolites on the tryptophan-niacin pathway, AnA, KA and 3-HA, are expressed as µmol/mol creatinine, since spot urine samples were collected from the subjects. The AnA output to urine remained unchanged during pregnancy (Fig. 2A). KA decreased to 60% from week 9 of gestation (Fig. 2B). 3-HA gradually increased from week 15 of development, and reached a peak of 2.8-fold at week 35 (Fig. 2C).
The urinary outputs of QA, MNA, 2-Py, and 4-Py are also expressed as µmol/mol creatinine. The urinary outputs of QA and MNA gradually increased from about week 20, and reached peaks at about week 35 to 2.7-fold and 4.1-fold, respectively (Figs. 3A and 3B). Pregnancy did not affect the urinary excretions of 2-Py or 4-Py (Figs. 3C and 3D).

Nam is catabolized to MNA, 2-Py or 4-Py, and these three compounds are excreted as Nam metabolites into the urine in humans. To assess the changes in niacin metabolism, the sum of MNA, 2-Py and 4-Py was calculated. This sum gradually increased from about week 20, reaching a peak of 2.3-fold at week 33, and declining to control levels postpartum (Fig. 4).

**Rats**

Changes in the urinary excretion of upper metabolites on the tryptophan-niacin pathway in rats. The urinary out-
puts of AnA, KA, XA and 3-HA are expressed as nmol/day, since the exact tryptophan-niacin metabolism can be assessed using daily urine samples. The AnA output to the urine did not change during pregnancy (Fig. 5A), a similar result to that found in humans. KA, XA and 3-HA gradually increased from day 11 or 12 of gestation (mid pregnancy) and reached peaks of 2-fold at day 13 or 14 (Figs. 5B, 5C and 5D). These excretions declined to control levels postpartum.

Changes in the urinary excretion of lower metabolites on the tryptophan-niacin pathway in rats. The urinary outputs of QA, Nam, MNA, 2-Py, and 4-Py are also expressed as nmol/d. QA increased from day 12 of gestation to day 18, Nam increased at day 14 and 15, MNA increased from day 16 of gestation to day 3 postpartum, and 2-Py and 4-Py increased from day 13 of gestation to day 21 or 19, respectively (Fig. 6). The peak in increasing QA was on day 13 of gestation with a 2.3-fold increase, MNA peaked at day 21 of gestation and day 1 postpartum with an 8.4-fold increase, 2-Py was at day 14 with a 3.0-fold increase, and 4-Py was on day 15 with a 2.6-fold increase.

The sum of Nam, MNA, 2-Py and 4-Py was calculated, since these are all excreted as Nam metabolites into urine in rats. This sum gradually increased from day 13 of gestation to day 1 postpartum, reaching a peak of a 2.7-fold increase on day 16, and decreasing to control levels from day 2 postpartum (Fig. 7).

Changes in the conversion ratio of tryptophan-niacin in rats. The conversion ratio of tryptophan-niacin was significantly changed with pregnancy as shown in Fig. 8.

Correlations of the urinary excretion of nicotinamide metabolites with the intermediates in the tryptophan-niacin pathway in rats. To elucidate the change in the tryptophan-niacin metabolism during pregnancy, correlations of the urinary excretion of the sum of Nam, MNA, 2-Py and 4-Py with 3-HA or QA, the intermediates in the tryptophan-niacin pathway in rats were calculated.

Fig. 4. Changes in the sum of urinary excretion of nicotinamide metabolites in Japanese women during pregnancy. Values are means±SE, n=5–50. Significantly different from non-pregnant women; *p<0.05; ‡p<0.01.

Fig. 5. Changes in the urinary excretion of AnA (A), KA (B), XA (C) and 3-HA (D) in rats during pregnancy and postpartum. Values are means±SE, n=9. Significantly different from day 6 of gestation; *p<0.05; ‡p<0.01.
During the whole of the period analyzed from day 6 of gestation to day 6 postpartum, the sum was significantly correlated with 3-HA and QA (correlation \( r=0.337; p<0.0001 \) and \( r=0.614; p<0.001 \), respectively). The data were divided into three periods: mid pregnancy ranged from day 6 of gestation to day 15, late pregnancy from day 16 to 21, and postpartum from day 1 to 6 postpartum. 3-HA was significantly correlated with the sum in mid pregnancy \( (r=0.633, p<0.0001) \) but not in late pregnancy \( (r=0.015, p=0.916) \) nor postpartum \( (r=0.093, p=0.505) \). QA was significantly correlated with the sum in all three periods, mid pregnancy \( (r=0.675, p<0.0001) \), late pregnancy \( (r=0.613, p<0.0001) \) and postpartum \( (r=0.511, p<0.0001) \).

**DISCUSSION**

There is a report that the deaths by pellagra in women was approximately 2-fold excess those in men (22). This report implies that female hormones are
associated with a factor in the etiology of pellagra. In fact, Shibata et al. (12, 13) reported that the administration of estrone and progesterone into male and female rats resulted in a decrease in conversion of tryptophan to niacin. However, Wertz et al. (11) reported the conversion of tryptophan to niacin was more efficient in the pregnant than in the nonpregnant state. There are two papers which referred to the result of Wertz et al. (11); Kamimura et al. (23) and Suzuki et al. (24) clarified that a high tryptophan-degrading activity was detected in the human placenta and early concepti of mice, respectively. However, the changes of systemic metabolism of tryptophan-niacin in the pregnant state have not yet been elucidated.

To investigate the changes in the systemic metabolism of tryptophan-niacin during pregnancy, we selected pregnant Japanese women at 5–40 wk of gestation, postpartum women at 4–6 wk, and non-pregnant women as the controls. Our results clearly showed that the urinary excretion of 3-HA, QA and niacin metabolites increased from mid pregnancy to delivery (Figs. 2C, 3A and 4). In short, pregnancy enhanced tryptophan and niacin metabolism in Japanese women. These subjects took some defined diets for at least 7 d, and their niacin metabolites were derived from tryptophan, nicotine, nicotinic acid and other pyridine nucleotide precursors. To investigate the precise changes in tryptophan-niacin metabolism during pregnancy, we used pregnant rats fed with niacin-free 20% casein diets in which niacin metabolites are derived only from tryptophan. Our results also clearly showed that the tryptophan-niacin metabolism was enhanced from mid pregnancy to delivery in rats (Fig. 7), similar to the results found in Japanese women, and consistent with previous reports (11, 22). Therefore, we conclude that additional niacin supplement is not needed during pregnancy.

The discrepancy of the changes of the urinary excretion of tryptophan-niacin metabolites between the results of the administration of female hormones (12, 13) and the pregnant state (11, 25 and the present data) would be attributed to existence of the placenta, which contains a high tryptophan-degrading enzyme (23, 24).

The tryptophan-niacin pathway consists of the kynurenine pathway and the NAD pathway (Fig. 1). The kynurenine pathway is initiated by tryptophan oxidase by TDO in the liver or indoleamine 2,3-dioxigenase (IDO) in other tissues including the placenta. α-Amino-β-carboxymuconate-ε-semialdehyde (ACMS) is converted by ACMSD to α-amino-muconate-ε-semialdehyde (AMS) that eventually leads to acetyl-CoA through the glutarate pathway; otherwise the nonenzymatic cyclization of ACMS results in the formation of QA, from which NAD is synthesized through the NAD pathway. Since tryptophan degradation and the ACMS metabolism are the rate-limiting points in the tryptophan-niacin pathway, 3-HA formation is predominantly affected by tryptophan degradation, and QA is by the ACMS metabolism. The urinary excretion of niacin metabolites was significantly correlated with 3-HA in mid pregnancy (r=0.633), and with QA throughout pregnancy (r=0.675) and in postpartum (r=0.511). These results suggest that the changes in the tryptophan-niacin metabolism in mid pregnancy are affected by changes in tryptophan degradation, and those in late pregnancy and postpartum are by those of the ACMS metabolism.

The expression of IDO is ubiquitous in many tissues, and its expression level is higher in the placenta, intestine and lung (23, 26). Placental IDO activity and mRNA expression are positively regulated by cytokines such as interferon-γ (27). Munn et al. showed that the administration of 1-methyltryptophan, an inhibitor of IDO, to pregnant mice induced a rapid abortion of allo-geneic concepti due to T-cells (28). They propose a role of IDO in the placenta during pregnancy in suppressing the fetal alloantigen-induced activation of maternal T cells, preventing them from elaborating further immune responses, which are lethal to the developing fetus (29). Plasma tryptophan concentration decreases during pregnancy in women, and plasma concentrations of immune activation markers neopterin and 55 kD soluble tumor necrosis factor receptor (sTNFR55) are inversely correlated to the plasma tryptophan, suggesting an involvement of IDO activation in tryptophan degradation during pregnancy (30). A high tryptophan-degrading activity is detected in early concepti in mice, whereas IDO protein and its mRNA are not expressed during early gestation but appear 2–3 d later to the peak of activity (24). The early tryptophan-degrading activity in murine concepti is due to the gene expression of TDO (24). These findings cannot determine whether the enhancement of the tryptophan-niacin metabolism from mid pregnancy to delivery is due to the increase in IDO activity in the placenta or not. TDO is specifically expressed in the liver (31), and the liver TDO activity in pregnant rats increases from the 12th day of pregnancy to the 20th day relative to non-pregnant rats (32). Although the precise reason for this enhancement of tryptophan degradation in the tryptophan-niacin metabolism is unclear, it is plausible that it results from changes in activity of IDO or TDO.

In conclusion, acceleration in urinary excretions of niacin metabolites occurs both in Japanese women and in rats during pregnancy. Changed urinary excretions of niacin metabolites reflect mainly the increased tryptophan-niacin metabolism, and are parallel with those of 3-HA in mid pregnancy and with QA throughout pregnancy. We conclude that the changes in the urinary excretion of the metabolites of the tryptophan-niacin pathway is due to changes in the IDO and/or TDO and ACMSD activities during pregnancy.

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