Arginine Flux, but Not Nitric Oxide Synthesis, Decreases in Adolescent Girls Compared with Adult Women during Pregnancy\textsuperscript{1,2}

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Abstract

NO has been proposed as a mediator of vascular expansion during pregnancy. Inability to increase NO synthesis and/or production of its precursor, arginine, may contribute to pregnancy-induced hypertension. Adolescents have a higher incidence of gestational hypertension. It is not known whether pregnant adolescents can produce sufficient arginine to meet overall demands. Our objective was to measure and compare the arginine flux and NO synthesis rates of pregnant adolescents and adult women. Arginine, citrulline, and NO kinetics were measured by i.v. infusions of $^{15}$N$_2$-arginine and $^2$H$_2$-citrulline in 8 adolescents and 8 adult women in the fasted state at the end of the first and the beginning of the 3rd trimesters of pregnancy. Arginine flux decreased ($P < 0.05$) from trimester 1 to 3 in the adolescents but not in the adult women. NO synthesis rate did not change significantly in either group from trimester 1 to 3. In trimester 3, there was a positive association ($r = 0.55; P = 0.02$) between arginine flux and participants’ age, indicating that flux was slower in the younger participants. These findings suggest that after a brief period of food deprivation, the pregnant adolescent cannot maintain arginine production like her adult counterpart in late pregnancy. This inability to maintain arginine production seems to be related to her younger age. It does not, however, affect her ability to synthesize NO in late pregnancy.


Introduction

Adolescent pregnancy in Jamaica, as elsewhere, is a major health concern, because it is associated with increased risks for adverse complications to the mother and child (1,2). These include higher rates of neonatal malformations, short for gestational age, low and very low birth weight, smaller head circumferences, prematurity delivery, and neonatal mortality (1,2). In addition, pregnant teenagers have an increased risk for pregnancy-induced hypertension, preeclampsia, and eclampsia (3,4). At present, the pathogenesis of pregnancy-induced hypertension remains unknown.

During normal pregnancy, although there are marked increases in maternal blood volume and cardiac output, blood pressure actually falls as peripheral vascular resistance decreases by 6 wk, reaching a minimum at mid-pregnancy. Although what initiates vasodilation and thus, blood volume expansion, is poorly understood, there is evidence that prostaglandins, such as prostacyclin and prostaglandin E, and NO may be involved (5–8). NO, a vasodilator made by the endothelium when arginine is converted to citrulline by the enzyme NO synthase, has been shown to downregulate the vascular response to vasoconstrictors like angiotensin during pregnancy (7,8). In an earlier study, we showed that both arginine production and NO synthesis rose steeply at mid-pregnancy in normal healthy women, suggesting that increasing arginine supply and its conversion to NO may be important in facilitating vascular expansion during pregnancy (9). In support of this argument, inhibition of NO synthase by infusion of N\textsuperscript{G}-monomethyl-$l$-arginine produced a greater reduction in blood flow in the hands of pregnant women compared with nonpregnant women, implicating increased NO synthesis in the decrease of vascular resistance during human pregnancy (10). Further, decreased plasma nitrite (a proxy for NO) concentrations in patients with preeclampsia (11) suggest that inadequate NO synthesis may contribute to pregnancy-induced hypertension. Therefore, the inability to increase arginine production and NO synthesis may explain the higher incidence of gestational hypertension and preeclampsia reported in pregnant adolescents (3,4).

In fasting humans, the major source of arginine is whole-body protein breakdown, as de novo arginine synthesis constitutes only 5–15% of flux (12). Based on the report that whole-body protein breakdown increases to a greater extent in pregnant women...
whose BMI exceeds 25 kg/m² (13) and that pregnant adolescent girls have been shown to have less lean body mass than adult women (14), it is a distinct possibility that pregnant adolescents will have slower endogenous arginine production rates, which may affect their ability to synthesize NO. We proposed that the pregnant adolescent will not be able to increase arginine production and its conversion to NO to the same extent as her adult counterpart as pregnancy progresses to late gestation. To test this hypothesis, arginine flux and NO synthesis were measured in pregnant adolescents and adult women at the end of the first and beginning of the 3rd trimester of pregnancy. This study was part of a larger study of glucose and amino acid metabolism in pregnancy, and the data reported here were obtained from the same participants studied in 2 earlier reports (15,16).

Materials and Methods

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving humans were approved by the Ethics Committee of the University of the West Indies and by the Institutional Review Board for Human Subject Research of Baylor College of Medicine and Affiliated Hospitals. Written informed consent was obtained from each pregnant woman and adolescent at recruitment.

Pregnant adolescents and adult women who were <13 wk of gestation and registered at the antenatal clinic of the University Hospital of the West Indies were invited to join this prospective study and were enrolled consecutively. Women with chronic illnesses, such as diabetes mellitus, hypertension, heart disease, or genetic abnormality such as sickle cell disease, or women with multiple gestations were excluded. Eight adolescents and 8 adult women with normal BMI (>18.5 and ≤25 kg/m²) were enrolled into the study. Maternal weight was measured to the nearest 0.01 kg using a Tanita digital scale (CMS Weighing Equipment) and height to the nearest 0.1 cm using a stadiometer (CMS Weighing Equipment). Maternal weight measurement was repeated at 28 and 36 wk of gestation.

Once written informed consent was obtained, a questionnaire, which provided information on demographics; socioeconomic status; use of substances such as cigarettes, alcohol, marijuana, and cocaine; and menstrual details, was administered to all the pregnant women and adolescents. A socioeconomic score was calculated using education and occupation of the mother and father, household possessions, and a crowding index (calculated as the number of habitable rooms in the dwelling divided by the number of people living in that dwelling). A higher score denoted a better socioeconomic status. Gestational age was determined by the last menstrual period and confirmed by an ultrasound measurement performed at the time of the first experimental study. Maternal weight gain from the first to the 3rd trimester (12–36 wk of gestation) was calculated. Birth weight was measured to the nearest 0.01 kg using a Tanita digital scale (CMS Weighing Equipment), crown-heel length was measured to the nearest 0.1 cm using a Harpenden infantometer (CMS Weighing Equipment), and head circumference was measured with a fiberglass tape measure.

Tracer infusion protocol. All of the pregnant women and adolescents were studied on 2 occasions after fasting for 8 h: at the end of the first trimester (12.8 ± 0.39 wk of gestation) and the beginning of the 3rd trimester (27.8 ± 0.4 wk of gestation). They were admitted to the obstetrics ward in the evening and had their last meal at 2200 h. Eight hours later, an i.v. catheter (Sescure, 18 G, Morningside Pharmaceuticals) was inserted into the antecubital vein of one arm for the infusion of isotopes while another catheter was inserted in an antiflow direction into the dorsal vein of the contralateral hand for drawing blood samples. This cannula was kept patent with intermittent small infusions of heparinized saline.

Sterile solutions of 15N2-arginine, 15N-citrulline, and 5,5 2H2-citrulline (Cambridge Isotope Laboratories) were prepared in 9 g/L NaCl. After a baseline 5-mL blood sample was collected, a primed, continuous infusion of 15N2-arginine (prime = 10 μmol/kg, infusion = 10 μmol·kg⁻¹·h⁻¹) and 5,5 2H2-citrulline (prime = 1 μmol/kg, infusion = 1 μmol·kg⁻¹·h⁻¹) was started and maintained for 6 h. In addition, the citrulline pool was primed with 15N-citrulline (prime = 0.16 μmol/kg). More blood samples were collected at 3, 4, 5, and 6 h of the infusion. At the end of the infusion, the catheters were removed and the women and adolescents were given lunch and discharged.

Laboratory analysis. Blood was drawn in prechilled tubes containing sodium fluoride and potassium oxalate and centrifuged at 4°C and the plasma removed and stored at −70°C for later analysis. First, plasma arginine and citrulline were converted to their 5-dimethylamino)-1-naphthalene sulphonamide derivatives. The plasma arginine and citrulline isotopic enrichments were measured on a triple quadrupole mass spectrometer (TSQ Quantum Ultra, Thermo Fisher Scientific) by tandem liquid chromatography-MS using electrospray ionization and selected reaction monitoring at m/z 409 and 410 (product ion m/z 392) at 14eV for 15N-citrulline and m/z 409–411 (product ions m/z 392 and 394) for 2H2-citrulline. The isotopic enrichment of arginine was measured by monitoring m/z 408–410 (product ion m/z 397) at 34 eV. The isotopic enrichment obtained for arginine in this analysis includes contribution from the infused 15N2-arginine plus some 2H2-arginine derived from the infused of 5,5 2H2-citrulline. To correct for the contribution of 2H2-citrulline to the M+2 arginine enrichment, we also monitored ions m/z 408 (product ion m/z 391) and 410 (product ion m/z 393) to measure the enrichment of the 5,5 2H2-arginine derived from the infused 2H2-citrulline tracer. By monitoring these product ions, the contribution of the 2 ureido nitrogens to arginine enrichment is excluded. The correct M +2 arginine enrichment derived from the infused 15N2-arginine tracer was then calculated by subtracting the 5,5 2H2-arginine enrichment from the arginine enrichment obtained for the whole arginine molecule (i.e. m/z 410, product ion m/z 170).

Plasma arginine and citrulline concentrations were measured by standardized ion-exchange chromatography. Plasma concentrations of the NO metabolites nitrite and nitrate (NOx) were measured by in vitro isotope dilution, as we previously described (17). Briefly, 0.5 mL of the baseline plasma sample was spiked with a known quantity of Na15NO3, the internal standard, and the nitrate was reduced to nitrite. The isotopic enrichment was then determined by negative chemical ionization GC-MS by selectively monitoring ions as m/z ratios of 46–47.

Calculations. Arginine and citrulline fluxes (or rates of production, QArg and QCit) were calculated by the steady-state equation:

\[ Q(\mu mol·kg⁻¹·h⁻¹) = \left(\frac{IE_{\text{inf}}}{IE_{\text{pl}}}\right) \cdot \left(\frac{\text{Flux}}{C_0}\right) \]

where Q is the flux of arginine or citrulline, IEinf and IEpl are the isotopic enrichments of the tracer in the infusate and in plasma at isotopic steady state (M+2 isotopeometer), and i is the rate of infusion of 15N2-arginine or 5,5 2H2-citrulline in μmol·kg⁻¹·h⁻¹.

Endogenous QArg was obtained by subtracting the rate of infusion of labeled arginine.

The NO synthesis rate was calculated from the rate of conversion of arginine to citrulline via the NO synthesis reaction, as previously described (18):

\[ \text{NO Synthesis}(\mu mol·kg⁻¹·h⁻¹) = \frac{Q_{\text{Cit}} - Q_{\text{Arg}}}{IE_{\text{Cit}} \cdot Q_{\text{Arg}} \cdot IE_{\text{Arg}}} = \frac{Q_{\text{Cit}} - Q_{\text{Arg}}}{IE_{\text{Arg}} \cdot Q_{\text{Cit}} \cdot (IE_{\text{Arg}} + Q_{\text{Arg}})} \]

where QArg and QCit are the fluxes of arginine and citrulline, IECit is the plasma enrichment of the M+1 isopomer of citrulline (i.e. ureido-15N-citrulline derived from 15N2-arginine), IEArg is the plasma enrichment of the M+2 isopomer of arginine, and QArg is the rate of infusion of 15N2-arginine.

Statistical analysis. Data are expressed as mean ± SE. Differences in characteristics between the adolescent and the adult woman were assessed by unpaired t test. The metabolic variables and blood pressure measurements were analyzed by repeated-measures 2-factor ANOVA. This model included the participants (adolescent and adult women) and trimesters (first and 3rd). Post hoc comparisons were performed using Bonferroni’s test. In addition, whole body arginine flux was compared...
between trimester 1 and 3 within each group using the paired t test. Correlations between measured kinetic variables and participant and baby characteristics were performed using Pearson correlation. Tests were considered significant if \( P < 0.05 \). Data analyses were performed with GraphPad Prism version 4 (GraphPad Software).

**Results**

The data on maternal characteristics and pregnancy outcomes has been reported in 2 previous publications (15,16) and will be described only briefly in this paper for convenience to the reader. There was no report of substance abuse during pregnancy among the 16 participants, and socioeconomic scores did not differ between the adults and adolescents. Both the adult women and adolescent girls had body mass indices within the normal range, 22.6 ± 0.4 and 20.8 ± 0.3 kg/m², respectively, indicating that they were well nourished at the trimester 1 study. Maternal weight and BMI at the trimester 1 study, however, were lower \( (P < 0.05) \) in the adolescents compared with their adult counterparts, indicating that the adolescents had less lean body mass than the adults. Weight gain from wk 12 to 36 of gestation was greater in the adolescents compared with adults \( (P < 0.01) \). Among the 16 participants in the study, there was 1 fetal loss in the adolescent group. Although there was no significant difference in gestational age between the groups, the adolescents had 2 premature deliveries and the adults had none. The mean birth weights of the groups were not significantly different and each group had 1 low-birth weight infant. Similarly, placental weight, newborn head circumference, and crown-heel length did not differ, although the latter tended to be shorter in the adolescent group \( (P = 0.10) \).

For arginine flux, there was an interaction between age group and time of pregnancy \( (P < 0.01) \), because flux decreased by 24% from trimester 1 to 3 in the adolescent group but increased by ~3% in the adult group (Table 1). Similarly, the age group × time interaction tended to affect the plasma arginine concentration \( (P = 0.07) \), which increased by ~14% in the adults \( (P = 0.02) \) but did not change in the adolescents from trimester 1 to 3. Citrulline flux and concentration did not differ between the groups at any time.

The NO synthesis rate did not change significantly in either group from trimester 1 to 3. The plasma NOx concentration was affected by time of pregnancy and increased from trimester 1 to 3 in the adolescents \( (r = 0.58; P = 0.01) \) and trimester 3 \( (r = 0.58; P = 0.01) \) and with the NO synthesis rate \( (r = 0.51; P = 0.04) \) in trimester 3. Trimester 3 arginine flux was associated with the participants’ age \( (r = 0.51; P = 0.04) \) and NO synthesis rate \( (r = 0.61; P = 0.01) \).

**Discussion**

To test the hypothesis that the pregnant adolescent cannot increase arginine production and its conversion to NO to the same extent as her adult counterpart as pregnancy progresses to late gestation, arginine, citrulline, and NO kinetics were measured in adolescent girls and adult women after an overnight fast at the end of the first and the beginning of the 3rd trimesters of pregnancy. As pregnancy progressed from trimester 1 to 3, there was a decrease in weight-specific arginine flux in the adolescents, whereas there was a modest increase in the adults. There were no significant differences in citrulline flux and NO synthesis rate between the groups at any time. The plasma NOx concentration also increased significantly from the end of trimester 1 to the beginning of trimester 3 in both groups. At the beginning of trimester 3, there was a positive correlation between arginine flux and participants’ age, indicating that flux was slower in the younger participants. These findings suggest that after a brief period of food deprivation, the pregnant adolescent cannot maintain arginine production like her adult counterpart at the beginning of trimester 3. This inability to maintain arginine production seems to be related to her younger age. It did not, however, affect her ability to synthesize NO in trimester 3.

To our knowledge, this is the first study of arginine, citrulline, and NO kinetics in pregnant adolescents and it shows for the first time that whereas overnight-fasted, pregnant, adolescent girls produce arginine at the same rate as their adult counterparts at the end of the first trimester, they produce it at a 24% slower rate at the beginning of the 3rd trimester. This finding is similar to the 39% reduction in glycine flux observed over the same time period in these same participants (15). In this study of well-nourished adolescents, the slower arginine flux after a normal overnight fast did not seem to have a negative effect on maternal and fetal tissue deposition, because they gained more

<table>
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<th>Variables</th>
<th>First trimester</th>
<th>Third trimester</th>
<th>Interaction</th>
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<tr>
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<td>Adult</td>
<td>Adolescent</td>
<td>Adult</td>
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<td>Arginine flux, ( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</td>
<td>120 ± 7.8</td>
<td>122 ± 4.8</td>
<td>123 ± 13*</td>
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<td>Arginine, ( \mu \text{mol} / \text{L} )</td>
<td>93 ± 16</td>
<td>106 ± 16</td>
<td>120 ± 16*</td>
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<td>Citrulline flux, ( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</td>
<td>3.57 ± 0.11</td>
<td>4.1 ± 0.26</td>
<td>4.1 ± 0.4</td>
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<td>Citrulline, ( \mu \text{mol} / \text{L} )</td>
<td>37.4 ± 2.2</td>
<td>42 ± 5.8</td>
<td>47.5 ± 3.2</td>
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<td>NO synthesis, ( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</td>
<td>0.180 ± 0.02</td>
<td>0.197 ± 0.03</td>
<td>0.215 ± 0.04</td>
</tr>
<tr>
<td>NOx, ( \mu \text{mol} / \text{L} )</td>
<td>11.99 ± 1.56</td>
<td>12.49 ± 1.62</td>
<td>12.04 ± 1.57*</td>
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1 Values are mean ± SE, \( n = 8 \). *Different from trimester 1, \( P < 0.05 \).
2 Trimester 1 study was performed at 12.8 ± 0.4 wk of gestation.
3 Trimester 3 study was performed at 27.8 ± 0.4 wk of gestation.
weight from the end of the first trimester to the beginning of the 3rd trimester than their adult counterparts. In a previous study, we showed that this greater weight gain by pregnant adolescents was comprised primarily of lean tissue (14). There was also no marked negative effect on pregnancy outcome, because there were no significant differences in babies’ birthweight and length between the 2 groups. Nevertheless, it is worth noting that both babies’ birthweight and length of the adolescent group were 8.6 and 6% less, respectively, than the mean values of the adult group.

Although our data do not provide an explanation for the slower arginine production by the adolescents in the 3rd trimester, the strong association between maternal age and arginine flux is intriguing. Because the endogenous flux of a dispensable amino acid consists of its release from whole-body protein breakdown plus its de novo synthesis, the slower arginine flux of the adolescents could have been due to a decrease in either one or both mechanisms. Evidence from studies in pregnant, normal-weight, healthy adult women suggests that the extra amino acids required for increased maternal protein synthesis are provided by an overall decrease in amino acid catabolism (13,19,20). It is therefore possible that the slower arginine production by the adolescents in the 3rd trimester is due to their inability to downregulate the rate of amino acid oxidation to conserve nitrogen for the de novo synthesis of dispensable amino acids such as arginine.

On the other hand, based on our findings from a previous study of healthy, normal-weight, pregnant adult American women (9), it is possible that the decrease in arginine production in the adolescent group at the beginning of trimester 3 represents a normal metabolic adaptation during pregnancy. In our earlier study of healthy, normal-weight, pregnant adult American women, we reported higher rates of arginine production and NO synthesis at mid-pregnancy (18–20 wk) compared with values in late trimester 3 (36–39 wk) and at 8–10 wk postpartum (9). Although in the current study no measurements were made in late trimester 3, arginine production at the beginning of trimester 3 (wk 28) was significantly lower in the adolescent group, which is similar to our finding in the adult American women. In addition, the values for arginine production in both the adults (120 μmol·kg⁻¹·h⁻¹) and adolescents (122 μmol·kg⁻¹·h⁻¹) at the end of trimester 1 (12.8 wk) are similar to the value reported at mid-pregnancy (18–20 wk; 108 μmol·kg⁻¹·h⁻¹) for the American women. Taken together, these findings suggest that during pregnancy, arginine production increases in trimester 1 and this higher rate is maintained through mid-pregnancy before it starts falling toward nonpregnant values in trimester 3. It is conceivable that a similar decrease in arginine production was not observed in the adult women in the current study because it occurred later in trimester 3, i.e. after wk 28 of pregnancy, when we made the second set of measurements.

Although arginine flux correlated positively with NO synthesis at the start of trimester 3 (r = 0.62; P = 0.01), NO synthesis did not differ between the groups at this time. That is, despite having a slower arginine flux at the start of trimester 3, the adolescents were still able to synthesize NO at the same rate as their adult counterparts. This implies that the relatively smaller amount of arginine being produced by the adolescents at the start of trimester 3 is still sufficient to support an adequate NO synthesis rate. Finally, it is interesting that maternal weight gain, which was greater in the adolescent group, correlated positively with both NO synthesis and NOx concentrations in trimester 3, suggesting that arginine supply was adequate to maintain both lean tissue deposition and NO synthesis.

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Literature Cited