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ORIGINAL ARTICLE

In vivo arginine production and nitric oxide synthesis in pregnant Indian women with normal and low body mass indices

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Background/Objectives: Nitric oxide (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 be a contributor to pregnancy-induced hypertension or preeclampsia. Because maternal weight is associated with blood pressure and risk of preeclampsia during pregnancy, it may also influence arginine and/or NO production. The purpose of this study was to determine the *in vivo* arginine production and NO synthesis rate in pregnant women with normal ($n=10$) and low ($n=10$) body mass indices (BMIs).
Subjects/Methods: Arginine flux and NO synthesis rate were measured in the postabsorptive state with constant infusions of $^{15}N_2$-arginine and $^{13}C,^{2}H_4$-citrulline. Plasma concentrations of arginine and NO metabolites were also measured. Kinetic parameters were correlated to maternal variables, gestational age, birth weight and blood pressure.
Results: Endogenous arginine flux was significantly faster in the low-BMI compared with normal-BMI women in the first trimester (63.1 ± 3.4 vs 50.2 ± 2.0 μmol/kg per h, $P<0.01$), but not in the second. Plasma NO concentration was higher (44.7 ± 5.3 vs 30.4 ± 1.9 μmol/l, $P=0.03$) and its rate of synthesis trended faster in the low-BMI compared with normal-BMI group in the second trimester. Maternal weight and BMI were negatively correlated with arginine flux in both trimesters and NO synthesis in the second trimester.
Conclusions: These findings suggest, but do not prove, that maternal BMI may be a factor in the ability to produce NO during pregnancy and may be one way by which BMI influences blood pressure during pregnancy.
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Keywords: arginine production; nitric oxide synthesis; maternal weight; maternal body mass index

Introduction

A physiological adaptation of pregnancy is expansion of the maternal vascular compartment. It starts with vasodilatation as peripheral resistance falls by 6 weeks and reaches a minimum in mid-pregnancy (Walters *et al.*, 1966; Mashini *et al.*, 1987; Robson *et al.*, 1989). What initiates sustained vasodilatation and thus blood volume expansion is poorly understood. Increase in the production of prostaglandins, particularly prostacyclin, may be one factor contributing to the fall in peripheral vascular resistance (Poston *et al.*, 1995; Carbillon *et al.*, 2000). In addition, nitric oxide (NO), which is produced by the endothelium when arginine is converted to NO and citrulline by the enzyme nitric oxide synthase (NOS), is a vasodilator that downregulates vascular responsiveness to vasoconstrictors, such as angiotensin, in pregnancy (Walters *et al.*, 1966; Mashini *et al.*, 1987; Robson *et al.*, 1989). In an earlier study, we showed that both arginine production and NO synthesis rose steeply in early

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pregnancy in normal healthy women, providing additional evidence that NO may have a function in vascular expansion during pregnancy (Goodrum *et al*., 2003).

Inability to adequately increase arginine production and/or NO synthesis may lead to pregnancy-induced hypertension. However, human studies that have used circulating or urinary concentrations of NO metabolites (nitrite and nitrate, NOx) as indices of NO production have produced conflicting results. Using this approach, NO production was found to be higher, lower or unchanged in preeclamptic women compared with normotensive women (Maul *et al*., 2003). Maternal weight may be one factor affecting NO production during pregnancy. A study of the influence of maternal pre-pregnancy body mass index (BMI) on blood pressure found that mean systolic blood pressure (SBP) was positively associated with maternal pre-pregnancy BMI, and lean women (BMI $<20\,kg/m^2$) had the lowest mean SBP in each trimester (Miller *et al*., 2007). In epidemiological studies, low BMI has been associated with decreased risk of preeclampsia (Sebire *et al*., 2001; Bodnar *et al*., 2005; Bhattacharya *et al*., 2007), and Bodnar *et al*. (2007) found a clear relationship between pre-pregnancy BMI and the risk of preeclampsia. In developing countries, such as India, underweight pregnant women are more prevalent than in developed countries and although low maternal BMI has been associated with adverse outcomes in the fetus, including low birth weight (LBW) and intrauterine growth retardation (Ehrenberg *et al*., 2003; Sahu *et al*., 2007), it appears to be protective against preeclampsia. The lower blood pressure and lower risk of preeclampsia seen in women with low BMI may be due to an increased arginine production facilitating a faster NO synthesis. In this study, we tested the hypothesis that arginine flux and NO synthesis will be faster in Indian pregnant women with low BMI compared with those with normal BMI during early pregnancy and mid-gestation. Using a stable isotope tracer technique, we measured arginine flux and NO synthesis in two groups of women with normal (BMI >18.5 and $\leqslant 25\,kg/m^2$) and low (BMI $\leqslant 18.5\,kg/m^2$) BMI at the end of the first and second trimesters of pregnancy.

Subjects and methods

The study was conducted at the obstetrics ward of St John's Medical College Hospital, Bangalore, India. The experimental protocols were reviewed and approved by the institutional ethical review board of St John's Medical College Hospital and by the institutional review board for Human Subject Research of Baylor College of Medicine and affiliated hospitals. A written consent was obtained from each subject at enrollment.

Subjects

Subjects were recruited from pregnant women who were below 13 weeks of gestation and registered for antenatal screening at the Department of Obstetrics and Gynecology, St John's Medical College Hospital. Women with multiple pregnancies, those with the clinical diagnosis of chronic illness, such as diabetes mellitus, hypertension, heart disease, thyroid disease, epilepsy, and those who tested positive for hepatitis B surface antigen, HIV or syphilis were excluded. Only subjects from the same dwelling area and similar socioeconomic background were invited to join the study. After a low-BMI (BMI $\leqslant 18.5\,kg/m^2$) woman was identified, a corresponding subject, matched closely for age and parity, and with a normal BMI (BMI >18.5 and $\leqslant 25\,kg/m^2$) was identified and invited to join the study. A total of 20 subjects, 10 with low BMI and 10 with normal BMI, were enrolled for the study after screening (Clinical guidelines,1998). At recruitment, routine antenatal tests were carried out.

Sociodemographic and anthropometric data

At the baseline visit information on age, obstetric history and socioeconomic status was obtained from each subject. Gestational age was assessed by ultrasonographic measurements carried out within 2 weeks of the initial visit. A digital balance (Soehnle, Germany) was used to record the weights of all women to the nearest 100 g, whereas height was measured using a stadiometer to the nearest 1 cm. Blood pressure was measured with a digital sphygmomanometer (HEM-757CAN; OMRON, Burlington, Canada).

Tracer infusion protocol

All subjects were studied in the postabsorptive state on two occasions, at the end of the first trimester (12 ± 1 weeks of gestation) and the second trimester (24 ± 1 weeks of gestation). Subjects were admitted to the obstetrics ward in the evening and ate dinner, which was based on their habitual diet, at 1900 hours. After 6 h, an intravenous catheter (Jelco, 22G; Medex Medical Ltd, Lancashire, UK) was inserted into the antecubital vein of one arm for the infusion of isotopes whereas another catheter was inserted in the antiflow direction into the dorsal vein of the contralateral hand for drawing blood samples. The hand was heated to arterialize venous blood samples.

Sterile solutions of $^{15}N_2$-guanidino-arginine and $^{13}C,^2H_4$-citrulline (Cambridge Isotope Laboratories, Woburn, MA, USA) were prepared in 9 g/l NaCl. After a 5 ml baseline blood sample was collected, a primed, continuous infusion of $^{15}N_2$-arginine (prime $= 6\,\mu mol/kg$, infusion $= 6\,\mu mol/kg$ per h) and $^{13}C,^2H_4$-citrulline (prime $= 1\,\mu mol/kg$, infusion $= 1\,\mu$ mol/kg per h) was started and maintained for 6 h. In addition, the citrulline pool was primed with ^{15}N-citrulline (prime $= 0.16\,\mu mol/kg$). Three additional blood samples were drawn at 15-min intervals during the last 45 min of the $^{15}N_2$-arginine and $^{13}C,^2H_4$-citrulline infusions. At the end of the infusions, the subjects were given breakfast and sent home after a general medical checkup.

Analysis of blood samples

The blood samples were drawn into prechilled tubes containing sodium fluoride and potassium oxalate, centrifuged at 4 °C, and the plasma was removed and stored at −80 °C. First, plasma arginine and citrulline were converted to their 5-(dimethylamino)-1-napthalene sulfonamide (DANS) derivatives. Briefly, 0.05 ml of 0.1 M sodium tetraborate buffer was added to 0.1 ml plasma and mixed by vortex. Then, 0.1 ml of 20 mM DANS-Cl reagent was added, mixed by vortex and allowed to stand for 30 min. Proteins were precipitated by adding 1.0 ml of chilled (0 °C) acetonitrile, mixing by vortex and allowing to stand on ice for 30 min. After centrifugation in a high-speed Eppendorf microcentrifuge at 10 000 r.p.m. for 10 min, the supernatant was removed and dried. The plasma arginine and citrulline isotopic enrichments were measured by tandem liquid chromatography–mass spectrometry using electrospray ionization and selected reaction monitoring at *m/z* 408–410 at 34 eV for arginine and *m/z* 409–414 at 14 eV for citrulline.

Plasma arginine concentrations were measured by standard ion-exchange chromatography. Plasma concentrations of NO metabolites nitrite and nitrate (NOx) were measured by *in vitro* isotope dilution as previously described by us (Villalpando *et al*, 2006). Briefly, 0.5 ml of the baseline plasma sample was spiked with a known quantity of $Na^{15}NO_3$, the internal standard and the nitrate reduced to nitrite. The isotopic enrichment was then determined by negative chemical ionization gas chromatography–mass spectrometry by selectively monitoring ions as *m/z* ratios of 46–47.

Calculations

Total arginine and citrulline flux (or rate of appearance, Q_{Arg} and Q_{Cit}) were calculated by the steady-state equation:

$$\text{Flux}\,(\mu\text{mol/kg per min}) = (IE_{Inf}/IE_{Pl})\times i$$

where IE_{Inf} and IE_{Pl} are the isotopic enrichments of the tracer in the infusate and in plasma at isotopic steady state (M + 2 isotopomer for arginine and M + 5 isotopomer for citrulline) and i is the rate of infusion of $^{15}N_2$-arginine or $^{13}C,^{2}H_4$-citrulline in μmol/kg per h.

Endogenous Q_{Arg} was obtained by subtracting the rate of infusion of labeled arginine. Whole-body arginine flux was calculated as the product of endogenous flux and body weight.

NO synthesis rate was calculated from the rate of conversion of arginine to citrulline by the NO synthesis reaction as previously described:

$$\text{NO synthesis}\,(\mu\text{mol/kg per h}) = Q_{Arg\rightarrow Cit} = Q_{Cit}\times IE_{Cit}/IE_{Arg} \times [Q_{Arg}/I_{Arg} + Q_{Arg}]$$

where Q_{Arg} and Q_{Cit} are the fluxes of arginine and citrulline, IE_{Cit} is the plasma enrichment of the M + 1 isotopomer of citrulline (that is, ureido-^{15}N-citrulline derived from $^{15}N_2$-arginine), IE_{Arg} is the plasma enrichment of the M + 2 isotopomer of arginine and I_{Arg} is the rate of infusion of $^{15}N_2$-arginine.

Whole-body NO synthesis was calculated as the product of NO synthesis and body weight.

Statistical analysis

Data are expressed as mean ± s.e.m. Differences in subject characteristics between the low- and normal-BMI groups were assessed by unpaired *t*-test. The metabolic variables and blood pressure were analyzed by mixed-model (repeated measure two-factor) ANOVA. This model included the groups (low and normal BMI) and trimesters (first and second). *Post hoc* comparisons were performed using Bonferroni's test. Correlations between measured kinetic variables and subject or baby characteristics were performed for each group using Pearson's correlation. The data from the two groups were combined and the analysis repeated for all subjects after a regression analysis showed that the slopes of the groups were not different. Tests were considered statistically significant if $P<0.05$. Data analyses were performed with GraphPad Prism version 4 software (GraphPad Software, San Diego, CA, USA).

Results

The low-BMI group had significantly lower weight and BMI at the time of recruitment into the study (Table 1). Between the groups, the increase in body weight (normal = 3.26 ± 0.62 kg (from 49.1 ± 1.3 to 52.3 ± 1.4); low = 3.83 ± 1.34 kg (from 42.3 ± 0.9 to 46.1 ± 0.76) and BMI (normal = 1.53 ± 0.28 kg/m^2, low = 1.82 ± 0.39 kg/m^2)) from trimester

Table 1 Characteristics at recruitment and pregnancy outcomes of normal- and low-BMI pregnant women

	Normal BMI	*Low BMI*
Age (years)	24.5 ± 5.0	21.4 ± 3.0
Primiparous	6	7
Weight (kg)	49.1 ± 3.9	42.2 ± 2.9[a]
Height (cm)	151.4 ± 3.4	153.3 ± 5.1
BMI (kg/m^2)	21.46 ± 1.73	17.93 ± 0.69[a]
Fat mass (kg)	14.4 ± 2.6	9.2 ± 1.8[a]
Fat mass (%)	29.2 ± 3.12	21.8 ± 3.10[a]
Fat-free Mass (kg)	34.7 ± 1.7	32.9 ± 1.5[a]
Fat-free mass (%)	71 ± 3.7	77.8 ± 2.9[a]
Hb (g/100 ml)	12.34 ± 0.86	11.85 ± 1.72
Physical activity level	1.50 ± 0.13	1.50 ± 0.19
Gestational age at birth (weeks)	37.0 ± 3.1	38.1 ± 1.5
Baby birth weight (g)	2354 ± 491	2639 ± 326
Low birth weight (<2.5 kg)	6	3

Abbreviations: BMI, body mass index; Hb, hemoglobin.
Values are mean ± s.d., $n = 10$ per group; low BMI, BMI ⩽ 18.5; normal BMI, BMI > 18.5 < 25.
[a]$P<0.001$ by Unpaired *t*-test.

Table 2 Maternal arginine, citrulline and NO kinetics and blood pressures at the end of first and second trimesters

Plasma variable	*First trimester*		*Second trimester*	
	Normal BMI	*Low BMI*	*Normal BMI*	*LOW BMI*
Arginine concentration (μmol/l)	58.5 ± 2.9	54.5 ± 5.2	48.7 ± 2.3	54.7 ± 4.9
Arginine R_a				
(μmol/kg per h)[a,b]	50.2 ± 2.0	63.1 ± 3.4	45 ± 2.4	50.3 ± 2.3
(μmol/h)	2443 ± 69	2674 ± 160	2342 ± 119	2317 ± 117
(μmol kg/FFM per h)[b]	70.7 ± 2.5	81.3 ± 4.8	67.5 ± 3.3	70.3 ± 3.2
Citrulline R_a				
(μmol/kg per h)[b]	6.62 ± 0.46	7.69 ± 0.35	5.9 ± 0.3	5.8 ± 0.3
(μmol/h)[b]	324 ± 25	325 ± 14	304 ± 17	266 ± 10.5
(μmol kg/FFM per h)[b]	9.8 ± 0.78	10.6 ± 0.48	8.8 ± 0.44	7.9 ± 0.3
NOx concentration (μmol/l)[a]	38.9 ± 4.1	41.0 ± 4.2	30.0 ± 1.9	44.2 ± 5.3
NO synthesis				
(μmol/kg per h)	0.184 ± 0.029	0.249 ± 0.048	0.143 ± 0.021	0.233 ± 0.034
(μmol/h)	9 ± 1.5	10.3 ± 1.9	7.3 ± 1.0	10.6 ± 1.4
(μmol kg/FFM per h)	0.259 ± 0.041	0.319 ± 0.060	0.212 ± 0.030	0.325 ± 0.045
% Arg R_a to NO	0.38 ± 0.07	0.40 ± 0.08	0.31 ± 0.04	0.47 ± 0.07
SBP (mm Hg)	105.5 ± 8.5	100.1 ± 9.1	104.0 ± 9.7	101.9 ± 7.4
DBP (mm Hg)[a]	68.2 ± 6.4	60.1 ± 5.0	65.8 ± 7.5	60.4 ± 5.0

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FFM, fat-free mass; R_a, endogenous rate of appearance (production); SBP, systolic blood pressure.
Values except SBP and DBP are mean ± s.e., SBP and DBP are mean ± $n = 10$ per group; low BMI, BMI ⩽ 18.5; normal BMI, BMI > 18.5 < 25.
[a]Significant main effect of BMI (low BMI compared with normal BMI, $P < 0.005$).
[b]Significant main effect of trimester (trimester 1 compared with trimester 2, $P < 0.01$).

1 to 2 was not different. There were no differences in age, parity, height and hemoglobin concentration between groups. There were no significant differences in gestational age at birth, and one mother of each group had a premature delivery (35th and 36th week in the normal- and low-BMI group, respectively), followed by death of the baby. Because this occurred well after the trimester 2 experiment, their arginine and NO kinetics data were included in the analysis. Similarly, there were no significant differences in birth weight, although the low-BMI mothers gave birth to babies that were on average ~300 g heavier. The low-BMI mothers also gave birth to only three LBW babies compared with six LBW babies in the normal-BMI group (Table 1).

There was no significant group by trimester interaction for any of the kinetic measurements or for plasma arginine and NO concentrations (Table 2). There were significant main effects of BMI and trimester for arginine flux when expressed per unit of body weight. Flux was significantly faster ($P < 0.01$) in the low-BMI group compared with the normal-BMI group in trimester 1 and there was a significant reduction in arginine flux from trimester 1 to 2 in the low-BMI group ($P < 0.01$). When the data were expressed per unit fat-free mass (FFM), however, only the effect of trimester was significant. When expressed per whole body, this reduction in arginine flux in the low-BMI group from trimester 1 to 2 did not achieve statistical significance. There were no differences in the percent of arginine flux converted to NO between groups in either trimester.

There was a significant main effect of trimester for citrulline flux regardless of how the data were expressed, as citrulline flux decreased significantly ($P < 0.001$) in the low-BMI group from trimester 1 to 2.

For plasma concentration of NO metabolites (NOx) there was a significant main effect of BMI as concentration was significantly higher ($P < 0.05$) in the low-BMI group at trimester 2. NOx concentration did not change from trimester 1 to 2 in the low-BMI group, but there was a trend toward a lower concentration in the normal-BMI group. The rate of NO synthesis did not differ between groups and trimesters, but in trimester 2 it was ~50–60% greater in the low-BMI group compared with the normal-BMI group regardless of how the data were expressed.

Systolic blood pressure (SBP) was not different between the low-BMI and normal-BMI groups in either trimester, and it did not change significantly from trimester 1 to 2 (Table 2). With respect to diastolic blood pressure (DBP), there was a significant main effect of BMI regardless of trimester, as DBP was significantly lower ($P < 0.05$) in the low-BMI group compared with the normal-BMI group.

The pooled data of all subjects ($n = 20$) were used to look for correlations among arginine kinetic variables, rate of NO synthesis, blood pressure, maternal variables, gestational age and birth weight. In trimester 1, there were significant negative correlations between arginine flux and maternal weight and BMI (Table 3). BMI also correlated positively with SBP and DBP. Birth weight correlated (positively) with only one kinetic parameter, citrulline flux.

In trimester 2, there were significant negative correlations between maternal weight and BMI and rate of NO synthesis as well as between BMI and arginine flux. BMI also correlated

Table 3 Correlations between different parameters in all ($n=20$) women

Parameters	*Pearson's correlation r*	P-*value*
Trimester 1		
Fat-free mass vs arginine R_a	−0.40	0.081
BMI vs arginine R_a	−0.59	0.005
DBP vs BMI	0.65	0.002
DBP vs fat-free mass	0.54	0.01
SBP vs BMI	0.53	0.02
SBP vs fat-free mass	0.50	0.02
Birth weight vs citrulline flux	0.44	0.05
Trimester 2		
BMI vs arginine R_a	−0.43	0.05
BMI vs nitric oxide R_a	−0.45	0.04
Fat-free mass vs nitric oxide R_a	−0.52	0.02
DBP vs BMI	0.59	0.006
DBP vs fat-free mass	0.54	0.02
Gestational age vs NO flux	0.4	0.08
Gestational age vs arginine flux	0.38	0.09

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; R_a, endogenous rate of appearance; SBP, systolic blood pressure.

positively with SBP and DBP. There were also weak correlations between gestational age and arginine and NO fluxes.

Discussion

The purpose of this study was to determine whether there were differences in the rate of production of arginine and its conversion to NO in pregnant women with low BMI compared with those with normal BMI. Our results indicate that arginine flux was faster in pregnant women with low BMI compared with those with normal BMI at the end of trimester 1, but not at the end of trimester 2. NO synthesis was not different between the groups and did not change significantly from trimester 1 to 2, though on average it was 50% faster in the low-BMI group compared with the normal-BMI group in trimester 2. This latter finding, plus the fact that NO synthesis correlated negatively with maternal weight and BMI whereas blood pressure positively correlated with BMI, suggests but do not prove that BMI may be influencing blood pressure through NO synthesis during pregnancy.

In a previous study of healthy normal weight pregnant women, we reported increased whole-body NO production during mid-pregnancy (Goodrum *et al*., 2003), supporting the findings of animal and *in vitro* studies that NO may be one factor involved in expansion of the vascular compartment (Sladek *et al*., 1997). If true, then the finding that pregnant women with low BMI have lower blood pressure (Miller *et al*., 2007) and a decreased risk of preeclampsia (Sebire *et al*., 2001; Bodnar *et al*., 2005, 2007; Bhattacharya *et al*., 2007) suggests that women with low BMI may have faster NO synthesis than women with normal and high BMI. At the end of trimester 2, though NO synthesis was not statistically different between the groups, the low-BMI group was synthesizing weight-specific NO at a rate that was ~50% faster than the normal-BMI group (Table 2). Furthermore, the total amount of NO made by the low-BMI group was 45% greater than the amount made by the normal-BMI group, though the low-BMI group weighed 12% less and had 5% less FFM than the normal-BMI group. This can only be explained by a faster rate of NO synthesis by the women with low BMI compared with those with normal BMI at the end of trimester 2. Not surprisingly, plasma NOx concentration was also higher in the women with low BMI at the end of trimester 2. A closer examination of the data reveals that NO synthesis and concentration trended lower from trimester 1 to 2 in the women with normal BMI. This trend, however, was not present in the women with low BMI. As a matter of fact at the whole-body level, the total amount of NO produced by the low-BMI group at trimester 2 was almost identical to the amount produced at trimester 1 (10.6 ± 1.4 vs 10.3 ± 1.9 μmol/h), whereas in the normal-BMI group, total NO produced decreased by 19% from trimester 1 to 2, from 9 ± 1.5 to 7.3 ± 1 μmol/h, though this change was not significant.

The lower blood pressure of the low-BMI group compared with the normal-BMI group plus the negative correlation between maternal BMI and NO synthesis and the positive correlation between BMI and blood pressures at trimester 2 suggest that BMI may influence blood pressure through NO synthesis. This may not represent a problem in women with normal BMI who may still be producing sufficient NO to facilitate maintenance of vascular expansion. This negative correlation between maternal BMI and NO synthesis, however, suggests higher BMIs may lead to decreased production of NO, and therefore may make overweight and obese women more susceptible to pregnancy-induced hypertension. This may be the reason for the higher incidence of hypertension in obese pregnant women (Baeten *et al*., 2001), although this needs to be investigated in further studies. It has been proposed that reduced availability of NO could increase the release of NO-inhibited endothelin-1 leading to vasoconstriction (Davidge 1998). Although it is generally accepted that deficient placentation and poor uteroplacental circulation underlie preeclampsia, several other factors including higher concentrations of the vasoconstrictor thromboxane, systemic inflammation and oxidative stress may be contributing to its pathogenesis (Walsh *et al*., 1993; Redman *et al*., 1999). The present finding of an association between BMI, NO synthesis and blood pressure offers one more possible explanation for the lower incidence of preeclampsia among pregnant women with low BMI (Bodnar *et al*., 2005, 2007); that is, women with low BMI may be able to produce sufficient quantities of NO to counter the effects of the increased vasoconstrictors associated with preeclampsia. This may not be the case in women with BMIs $>25\,kg/m^2$. In support of this argument, it has been reported that compared with placebo, oral supplementation with arginine for 3 weeks in women with preeclampsia lowered blood

pressure and raised plasma citrulline levels, the latter being indicative of increased NO synthesis (Rytlewski *et al*., 2005). An alternative explanation for the persistently high NO production of the low-BMI group is that it is a compensatory response because these women may be facing a persistent constraint (such as increased vasoconstrictors) in intravascular expansion that is not present in the normal-BMI group. It has been shown, for example, that human placental NOS activity and NO production are significantly increased in preeclampsia and eclampsia than those of normal pregnancy, suggesting a physiological adaptive response to overcome the increased placental vascular resistance (Shaamash *et al*., 2001).

In the present study, the lack of correlation between arginine flux and NO synthesis was surprising, because in our earlier study of healthy American women, we reported concomitant increases in arginine flux and NO production at mid-pregnancy (Goodrum *et al*., 2003). Even though both extracellular and intracellular arginine concentrations far exceed the saturation point of NOS, availability of arginine seems to affect NO synthesis (Bruckdorfer 2005). Although arginine flux was different in normal-BMI compared with low-BMI women in trimester 1, NO synthesis rates were not different. In trimester 2, NO synthesis seems higher in the low-BMI group compared with the normal-BMI group, even though arginine flux and plasma concentration were similar. In the low-BMI group, arginine flux decreased from trimester 1 to 2, but NO production did not change. These results demonstrate a lack of association between arginine production and the rate of NO synthesis. It is possible that changes in arginine production did not translate into differences in NO production, because NO synthesis represents only a small percentage of total arginine flux (less than 1% in both groups at both time points).

Despite the lack of correlation between arginine and NO fluxes, an interesting observation was that both had fairly good ($P = 0.08$; 0.09), though not statistically significant correlations with gestational age (Table 3), suggesting that they may be influencing pregnancy outcome possibly by promoting uterine quiescence longer (Shaamash *et al*., 2001). Although arginine and NO fluxes did not correlate with baby birth weight, it is interesting to note that the low-BMI group, with faster arginine and NO fluxes in the second trimester, gave birth to babies that were on average ~ 300 g heavier and this group had just three LBW babies compared with six in the normal-BMI group. Interestingly, trimester 1 citrulline flux, a fraction of which is derived from arginine by the NOS reaction, was also significantly correlated to baby birth weight. These observations suggest that increased NO may be improving delivery of nutrients to the fetus possibly through improved placental perfusion (Maul *et al*., 2003). These preliminary findings suggest the need for a larger study to determine whether there is a real association between arginine, citrulline and NO metabolism and pregnancy outcome in this population of pregnant women.

In the present study, NOx concentration in plasma was higher in the low-BMI group than in the normal-BMI group in the second trimester. This difference paralleled the difference in NO synthesis, but overall, NOx concentrations did not correlate with rates of NO synthesis. Many previous studies on pregnancy have used plasma NOx level and urinary NOx excretion as indirect measures of NO production (Maul *et al*., 2003). However, sources of NOx, in addition to production from NO synthesis (Helmke and Duncan, 2007), may include diet and the environment, and plasma concentrations will be affected by renal clearance (Villalpando *et al*., 2006). These influences on NOx levels may explain the differing findings of studies using these indirect approaches to investigate NO kinetics in pregnancy.

There are several limitations to this study. First, several results approached but did not reach statistical significance. This is likely the result of a type II error. This study was conducted in India, and these results may not be generalizable to Western populations. Because maternal undernutrition is a common problem in developing countries, this study compared pregnant women with low vs normal BMI, and did not include subjects with high BMI. Finally, this study was designed to investigate only one of several potential factors that may influence blood pressure during pregnancy. Nevertheless, this study provides baseline knowledge for further investigations into the relationship between BMI and arginine, citrulline and NO metabolism with pregnancy-related complications such as preeclampsia.

Conflict of interest

The authors declare no conflict of interest.

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References

Baeten JM, Bukusi EA, Lambe M (2001). Pregnancy complications and outcomes among overweight and obese nulliparous women. *Am J Public Health* **91**, 436–440.

Bhattacharya S, Campbell DM, Liston WA, Bhattacharya S (2007). Effect of body mass index on pregnancy outcomes in nulliparous women delivering singleton babies. *BMC Public Health* **7**, 168.

Bodnar LM, Catov JM, Klebanoff MA, Ness RB, Roberts JM (2007). Prepregnancy body mass index and the occurrence of severe hypertensive disorders of pregnancy. *Epidemiology* **18**, 234–239.

Bodnar LM, Ness RB, Markovic N, Roberts JM (2005). The risk of preeclampsia rises with increasing prepregnancy body mass index. *Ann Epidemiol* **15**, 475–482.

Bruckdorfer R (2005). The basics about nitric oxide. *Mol Aspects Med* **26**, 3–31.

Carbillon L, Uzan M, Uzan S (2000). Pregnancy, vascular tone, and maternal hemodynamics: a crucial adaptation. *Obstet Gynecol Surv* **55**, 574–581.

Davidge ST (1998). Oxidative stress and altered endothelial cell function in preeclampsia. *Semin Reprod Endocrinol* **16**, 65–73.

Ehrenberg HM, Dierker L, Milluzzi C, Mercer BM (2003). Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes. *Am J Obstet Gynecol* **189**, 1726–1730.

Goodrum LA, Saade GR, Belfort MA, Moise Jr KJ, Jahoor F (2003). Arginine flux and nitric oxide production during human pregnancy and postpartum. *J Soc Gynecol Investig* **10**, 400–405.

Helmke SM, Duncan MW (2007). Measurement of the NO metabolites, nitrite and nitrate, in human biological fluids by GC-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* **851**, 83–92.

Mashini IS, Albazzaz SJ, Fadel HE, Abdulla AM, Hadi HA, Harp R *et al.* (1987). Serial noninvasive evaluation of cardiovascular hemodynamics during pregnancy. *Am J Obstet Gynecol* **156**, 1208–1213.

Maul H, Longo M, Saade GR, Garfield RE (2003). Nitric oxide and its role during pregnancy: from ovulation to delivery. *Curr Pharm Des* **9**, 359–380.

Miller RS, Thompson ML, Williams MA (2007). Trimester-specific blood pressure levels in relation to maternal pre-pregnancy body mass index. *Paediatr Perinat Epidemiol* **21**, 487–494.

Poston L, McCarthy AL, Ritter JM (1995). Control of vascular resistance in the maternal and feto-placental arterial beds. *Pharmacol Ther* **65**, 215–239.

Redman CW, Sacks GP, Sargent IL (1999). Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol* **180**, 499–506.

Robson SC, Hunter S, Boys RJ, Dunlop W (1989). Serial study of factors influencing changes in cardiac output during human pregnancy. *Am J Physiol* **256**, H1060–H1065.

Rytlewski K, Olszanecki R, Korbut R, Zdebski Z (2005). Effects of prolonged oral supplementation with l-arginine on blood pressure and nitric oxide synthesis in preeclampsia. *Eur J Clin Invest* **35**, 32–37.

Sahu MT, Agarwal A, Das V, Pandey A (2007). Impact of maternal body mass index on obstetric outcome. *J Obstet Gynaecol Res* **33**, 655–659.

Sebire NJ, Jolly M, Harris J, Regan L, Robinson S (2001). Is maternal underweight really a risk factor for adverse pregnancy outcome? A population-based study in London. *BJOG* **108**, 61–66.

Shaamash AH, Elsonosy ED, Zakhari MM, Radwan SH, El-Dien HM (2001). Placental nitric oxide synthase (NOS) activity and nitric oxide (NO) production in normal pregnancy, pre-eclampsia and eclampsia. *Int J Gynaecol Obstet* **72**, 127–133.

Sladek SM, Magness RR, Conrad KP (1997). Nitric oxide and pregnancy. *Am J Physiol* **272**, R441–R463.

Villalpando S, Gopal J, Balasubramanyam A, Bandi VP, Guntupalli K, Jahoor F (2006). *In vivo* arginine production and intravascular nitric oxide synthesis in hypotensive sepsis. *Am J Clin Nutr* **84**, 197–203.

Walsh SW, Wang Y, Jesse R (1993). Peroxide induces vasoconstriction in the human placenta by stimulating thromboxane. *Am J Obstet Gynecol* **169**, 1007–1012.

Walters WA, MacGregor WG, Hills M (1966). Cardiac output at rest during pregnancy and the puerperium. *Clin Sci* **30**, 1–11.