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Decreasing resistance in the maternal uterine and peripheral arterial system is apparently unrelated to plasma and urinary levels of nitrite/nitrate and cyclic-guanosinmonophosohate during the course of normal pregnancies

Barbara Schiessl[1], Christian J. Strasburger[2], Martin Bidlingmaier[2], Michael Spannagl[3], Bernhard Ugele[4], and Franz Kainer[1]

[1]Department of Obstetrics and Gynaecology, Prenatal Medicine Unit, [2]Department of Internal Medicine, Neuroendocrine Unit, [3]Department of Internal Medicine, Angiology, [4]Department of Obstetrics and Gynecology, Clinical Chemistry, Ludwig-Maximilians-University, Munich, Germany

1 Introduction

Physiologic pregnancy is associated with an increase in cardiac output due to an increase in circulating intravascular volume and an increase in heart rate. Paralleling this phenomenon in normotensive women a steady decrease in systolic and diastolic arterial blood pressure (BP) is described as a result of decreased peripheral vascular resistance. Nitric oxide (NO) is a potent vasodilator and is thought to cause a majority of the vasodilation during the course of normal pregnancy [1, 8, 14]. NO is synthesized by the enzyme nitric oxide synthase (NOS) from the substrate L-arginin. The enzyme is localized in the vascular endothelium, in platelets and placental tissue and exists in a constitutive (calcium-dependent) and as an inducible (calcium-independent) form [11].

According to classical embryology intervillous circulation is established early in the first trimester starting with the first trophoblast invasion. These gradual processes finish with the second trophoblast invasion with direct opening of the spiral arteries in the intervillous space [9]. The transformation is characterized by a gradual loss of the normal musculoelastic structure of the arterial wall [10]. This phenomenon is reflected in the disappearance of the early diastolic notch and in a decrease in resistance index and pulsatility index of the uterine arteries until 24 weeks of gestation. Uterine artery Doppler examination can identify impaired trophoblast invasion in the second trimester of pregnancy when a high resistance and an early diastolic notch persists after 24 weeks of gestation [7].

NO is released from placental tissue into the maternal circulation. Several investigations have been undertaken to describe the amount of NO-production and the level of NO-metabolites in the maternal circulation. Seligman reported that serum concentrations of nitrite/nitrate were significantly increased in pregnant compared with non-pregnant women [13]. Begum analyzed urinary nitrate/nitrite levels which tended to be higher in the second and third trimester than in the first trimester of pregnancy. These levels as well as the level of cGMP tended to be lower in the puerperial period. No correlation could be found between NO-metabolites and maternal blood pressure. Urinary nitrite/nitrate was significantly lower in patients with preeclampsia [2]. Davidge also reports on a decrease of urinary nitrite/nitrate levels in the presence of unchanged plasma levels in preeclamptic women compared to normal pregnancies [5]. In contrast to these reports Di Irio and colleagues could not demonstrate a change in nitrite/nitrate plasma concentrations or urinary excretion in patients with preeclampsia [6]. Curtis demonstrated no difference between the plasma Nitrate/Nitrite concentrations of normal or preeclamptic pregnant women and non-pregnant women [4].

Few investigations have been undertaken to examine the relation of Doppler sonografic parameters and NO-production. Beinder et al. found elevated blood flow resistance in the uterine arteries in patients with preeclampsia and a reduced NOS-activity in the placental bed [3]. We have previously demonstrated a significant decrease of the PI monitored at the maternal cubital artery with a significant correlation to the PI of the uterine arteries during the course of normal pregnancies [12]. To test the hypothesis that changes in the maternal vascular peripheral and uterine arterial resistance are mediated by the NO-system located in the uteroplacental tissue we conducted a prospective longitudinal trial: Plasma and urinary concentrations of nitrite/nitrate as the stable end-products of NO and of its second messenger cGMP were determined and correlated to the PI of the maternal cubital, placental and non-placental uterine artery.

2 Methods

49 patients were investigated in a prospective study for repeated Doppler sonografic evaluation and determination of serum and urinary levels of nitrate/nitrite and cGMP. They were admitted to the trial between 10 and 16 weeks of gestation. Inclusion criteria were: singleton pregnancy, normal maternal blood pressure, and absence of maternal proteinuria.

Fetal intrauterine growth retardation, maternal pregnancy induced hypertension or proteinuria, pathologic pregnancy outcome, maternal heart rate beyond 60 or above 100 beats per minute were exclusion criteria. None of the women had evidence of any active or potential infective process, such as urinary tract infection or chorioamnionitis.

Doppler sonografic examination, urine and serum sampling was repeated every four to six weeks until delivery and once within the first seven days postpartum. Ultrasound and Doppler sonografic examination during pregnancy included evaluation of biometric data of the fetus, estimation of the amniotic fluid index, localization of the placenta and vitality of the fetus. Doppler sonografic investigations were performed on the left and right uterine artery – but were characterized by the localization of the placenta (placental and non-placental uterine artery). The maternal right cubital artery was chosen as an easily accessible peripheral arterial vessel. Doppler sonografic investigation was performed on a Logic 400 machine (GE, Kranzbühler, Solingen, Germany) with a 3,5 MHz device. From the analysis of the Doppler spectra the parameters maximum systolic velocity (Vmax), maximum enddiastolic velocity (Vmin) and mean velocity (Vmean) were derived. From these parameters the pulsatility index (PI) was calculated: PI = [Vmax – Vmin/ Vmean]. All examinations were conducted by the same experienced investigator.

At each interval maternal blood and urine samples were collected and, in addition, blood and urine samples were collected on admission to the delivery room. The blood samples underwent centrifugation and both plasma and urinary probes were stored at –21 °C in aliquots until determination. Plasma NO-concentrations were determined using the Greiss reaction by measuring combined oxidation products of NO, plasma nitrite [NO_2^-] and nitrate [NO_3^-] after reduction with nitrate reductase in a Colorimetric Assay (Cayman Inc., USA). Concentrations of the second messenger cGMP serum and urinary samples were determined with a J^{125}-Radio-Immuno-Assay (ibl Inc., Germany).

The Stat View Program (Abacus Concepts, Inc., Berkeley, CA, 1992–98) was used for statistical analysis (Descriptive statistics, Correlation matrix, Fisher's r to z transformation, Spearman rank correlation coefficient, Kruskal-Wallis-test, unpaired t-test, Wilcoxon signed rank test, Mann Whitney U-test). A p-value beyond 0.05 was considered to be significant.

The study had the approval of the local university's ethical committee (No.158/00) and was funded by the Ludwig-Maximilians-University Project for FöFoLe (No. 151). Each patient gave written informed consent.

The follow up during the protocol was in accordance with the procedures in our department and if any pathological findings occurred further diagnostic approach and treatment was offered to the patient.

3 Results

The mean age of the pregnant patients was 31,1 years (yrs) (SD 4,49), the mean week of deliv-

ery was 40,4 (SD 1,06) and the mean birth weight of the newborns was 3353 grams (SD 363). The mean pH-value measured at the umbilical artery was 7,316 (SD 0,09), the median APGAR score was 9 at five minutes (range 8–10) and 10 (range 8–10) at ten minutes.

During the course of pregnancy the PI of the placental and non-placental uterine artery, as well of the cubital right artery, decreases continuously with a significant inverse correlation to the week of gestation ($p < 0{,}001$ Cubital artery; $p < 0{,}0001$ Uterine arteries). The PI of both the placental uterine artery and the non-placental uterine artery is significantly correlated with the PI of the cubital artery ($p < 0{,}0001$ each). The placental and non-placental uterine artery also show a significant correlation ($p < 0{,}0001$). Postpartum meas-

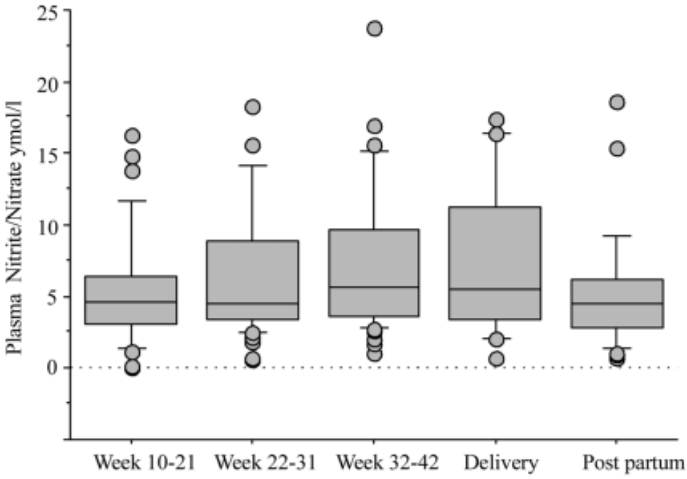

Figure 1. Plasma nitrite/nitrate concentration during pregnancy intervals, on admission to delivery and post partum.

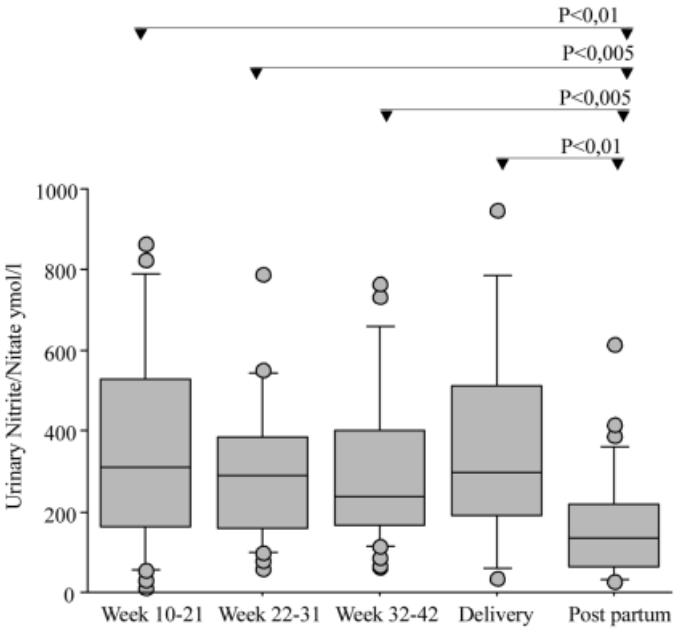

Figure 2. Urinary nitrite/nitrate concentration during pregnancy intervals, on admission to delivery and post partum (Kruskal-Walis test $p < 0{,}01$; Wilcoxon signed rank test).

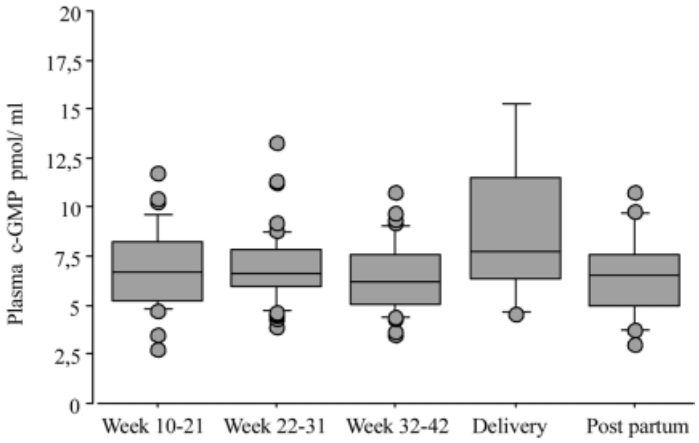

Figure 3. Plasma cGMP concentration during pregnancy intervals, on admission to delivery and post partum.

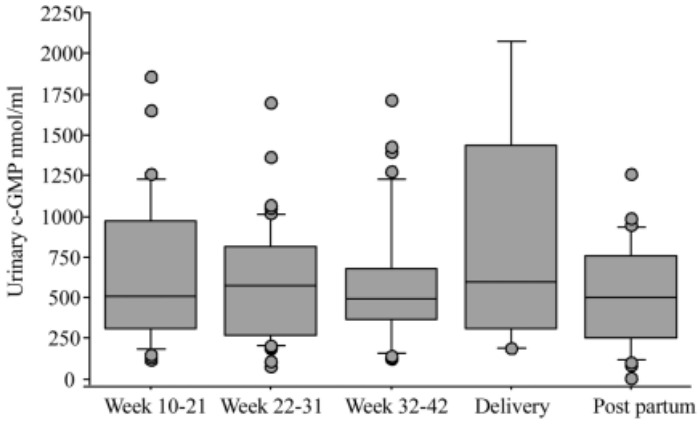

Figure 4. Urinary cGMP concentration during pregnancy intervals, on admission to delivery and post partum.

urements of the PI in the cubital artery ($p < 0{,}005$) as well as the PI of the uterine arteries show a significant re-increase within the first seven days after delivery ($p < 0{,}001$). (Correlation matrix, Fisher's r to z transformation, Spearman rank correlation coefficient).

The concentrations of plasma nitrite/nitrate ($p = 0{,}08$), plasma cGMP ($p = 0{,}80$), urinary nitrite/nitrate ($p = 0{,}15$) and urinary cGMP ($p = 0{,}92$) do not correlate with advancing gestational age. (Correlation matrix, Fisher's r to z transformation, Spearman rank correlation coefficient).

For the following intervals of pregnancy the mean value was calculated: 10 to 21, 22 to 31, and 32 to 42 weeks of gestation, admission to delivery and postpartum.

By analyzing these intervals no significance can be calculated for the plasma nitrite/nitrate ($p = 0{,}301$), plasma cGMP ($p = 0{,}793$) and urinary cGMP ($p = 0{,}897$) (Kruskal-Wallis-test) (figure 1, 3, 4).

Postpartum measured concentrations of urinary Nitrite/Nitrate are significantly lower than the calculated intervals of pregnancy and admission to the delivery room. (Kruskal-Walis-test $p = 0{,}0075$, Wilcoxon signed rank test) (figure 2).

No correlation can be shown between the PI of the cubital and uterine arteries and the plasma and urinary concentrations of nitrite/nitrate and cGMP during the course of pregnancy. (Correlation matrix, Fisher's r to z transformation, Spearman rank correlation coefficient) (table 1).

Table I. Correlation between pulsatility index (PI) and concentration of nitrite/nitrate and plasma and urinary cyclic guanosinmonophosphate (cGMP)

	Placental artery	Nonplacental artery	Cubital artery
Plasma nitrite/nitrate	P = 0,7	P = 0,56	P = 0,35
Plasma cGMP	P = 0,25	P = 0,19	P = 0,93
Urinary nitrite/nitrate	P = 0,68	P = 0,37	P = 0,76
Urinary cGMP	P = 0,90	P = 0,52	P = 0,51

4 Discussion

In the present study we analyzed the maternal cubital artery as an easily accessible and representative peripheral maternal arterial vessel and the uterine arteries by Doppler sonografic evaluation. Paralleling each Doppler sonografic investigation blood and urine samples were taken. We focused on the change in uterine and peripheral resistance in comparison to the concentrations of metabolites of NO (nitrite/nitrate) and its second messenger cGMP during the normal course of pregnancy and postpartum.

In contrast to the decreasing local uterine and decreasing peripheral arterial resistance, we cannot demonstrate an increase in the circulating and renal excreted metabolites and the second messenger (cGMP) of NO. Several investigators postulate an increased production of NO during pregnancy. Conrad reports on an increasing excretion of cGMP with advancing gestational age in rats [14]. Our tests do not affirm these findings in human pregnancy. If there is an increased NO production that is released into the maternal and feto-placental circulation, there are stable peripheral concentrations of nitrite/nitrate and cGMP.

In contrast to these stable concentrations we measured a decreasing peripheral arterial resistance in the uterine and peripheral maternal arterial system with significant inversed correlation to advancing gestational age.

We found a significant difference in the concentrations of urinary nitrite/nitrate comparing the postpartum samples to those taken during the course of pregnancy. This may be a false positive result because of the low case number. On the other hand this may be linked to a decreasing turnover of NO in maternal circulation postpartum. The plasma nitrite/nitrate does not show a significant change nor do the concentrations of plasma and urinary cGMP.

Our findings support the reports of Lyall and Robson that the cytotrophoblast derived NO is unlikely to contribute to the spiral artery dilation [10]. That NO-production is increased only in certain compartments [e.g. placenta, uterus or maternal platelets] cannot be excluded, and helps to explain the confusing data in the literature. Even though we cannot measure peripheral plasma and urinary rising concentrations of nitrite/nitrate and cGMP during pregnancy we postulate peripheral acting mediators that contribute to the decreasing arterial resistance in physiologic pregnancy.

Abstract

Aims: The aim of the presented study was to clarify the relationship between the pulsatility index of the uterine arteries and the maternal cubital artery and peripheral concentrations of the metabolites of nitric oxide (NO) and its second messenger cyclic guanosinmonophosphate (cGMP) during the normal course of pregnancy and postpartum.
Methods: 49 uncomplicated pregnancies were investigated every 4–6 weeks until delivery, 29 of them were additionally investigated postpartum. Paralleling each Doppler sonografic investigation maternal blood and urine samples were taken. The measurements of nitrite/nitrate and cGMP were performed with a colorimetric and radio immuno assay.
We demonstrate a significant decrease of the PI of the uterine arteries and of the cubital artery with inverse correlation to advancing gestational age.
Results: The concentrations of nitrite/nitrate and cGMP remain stable during gestation and do not correlate to the PI of the uterine and cubital artery. Postpartum a re-

increase in the uterine and peripheral resistance can be shown. The concentrations of urinary cGMP and nitrite/nitrate as well as plasma cGMP remain unchanged, whereas plasma nitrite/nitrate decreases postpartum.

Conclusions: The status of NO biosyntheses in normal pregnancy remains controversial. We hypothesize further systemically acting mediators which contribute to the decreasing vascular resistance.

Keywords: Doppler sonography, nitric oxide, peripheral resistance, pregnancy, uterine resistance.

References

[1] Ayala DE, RC Hermida: Influence of parity and age on ambulatory monitored blood pressure during pregnancy. Hypertension 38 (2001) 753
[2] Begum S, M Yamasaki, M Mochizuki: Urinary levels of nitric oxide metabolites in normal pregnancy and preeclampsia. J Obstet Gynaecol Res 22 (1996) 551
[3] Beinder E, MG Mohaupt, D Schlembach, T Fischer, RB Sterzel, N Lang et al: Nitric oxide synthase activity and Doppler parameters in the fetoplacental and uteroplacental circulation in preeclampsia. Hypertens Pregnancy 18 (1999) 115
[4] Curtis NE, M.Gude, RG King, PJ Mariott: Nitric oxide metabolites in normal human pregnancy and preeclampsia. Hypertens Pregnancy 14 (1995) 339
[5] Davidge ST, CP Stranko, JM Roberts: Urine but not plasma nitric oxide metabolites are decreased in women with preeclampsia. Am J Obstet Gynecol 174 (1996) 1008
[6] Di Iorio R, E Marinoni, S Emiliani, B Villaccio, EV Cosmi: Nitric oxide in preeclampsia: lack of evidence for decreased production. Eur J Obstet Gynecol Reprod Biol 76 (1998) 65
[7] Hafner E, M Metzenbauer, B Dillinger-Paller, D Hoefinger, K Schuchter, H Sommer-Wagner et al: Correlation of first trimester placental volume and second trimester uterine artery Doppler flow. Placenta 22 (2001) 729
[8] Hermida RC, DE Ayala, M Iglesias: Predictable blood pressure variability in healthy and complicated pregnancies. Hypertension 38 (2001) 736
[9] Kurjak A, S Kupesic, T Hafner, M Kos, L Kostovic-Knezevic, D Grbesa: Conflicting data on intervillous circulation in early pregnancy. J Perinat Med 25 (1997) 225
[10] Lyall F, JN Bulmer, H Kelly, E Duffie, SC Robson: Human trophoblast invasion and spiral artery transformation: the role of nitric oxide. Am J Pathol 154 (1999) 1105
[11] Norris LA, JR Higgins, MR Darling, JJ Walshe, J Bonnar: Nitric oxide in the uteroplacental, fetoplacental, and peripheral circulations in preeclampsia. Obstet Gynecol 93 (1999) 958
[12] Schiessl B, CJ Strasburger, M Spannagl, F Kainer: Decreasing peripheral resistance during the course of normal pregnancy monitored at the cubital artery by Doppler sonography. Eur J Clin Invest 33 (2003) 346
[13] Seligman SP, JP Buyon, RM Clancy, BK Young, SB Abramson: The role of nitric oxide in the pathogenesis of preeclampsia. Am J Obstet Gynecol 171 (1994) 944
[14] Sladek SM, RR Magness, KP Conrad: Nitric oxide and pregnancy. Am J Physiol 272 (1997) R441

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B. Schiessl, MD
Department of Obstetrics and Gynecology
Prenatal Medicine Unit
Ludwig-Maximilians-University
Maistr. 11
80337 Munich
Germany
Tel.: +49 89 5160 4111
Fax: +49 89 5160 4913
e-mail: bsc@fk-i.med.uni-muenchen.de