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Nitric oxide and peroxynitrite platelet levels in women with small-for-gestational-age fetuses

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Objective The placenta produces reactive oxygen species (ROS) including nitric oxide (NO) and peroxynitrite ($ONOO^-$) that have pronounced effects on placental function. Excessive ROS production may occur in pathological pregnancies, such as those complicated by small-for-gestational-age (SGA) fetuses.

Design The aim of the present work was to study NO and $ONOO^-$ levels in platelets of pregnant women with SGA fetuses compared with a control group.

Setting and population The study was performed on 30 pregnant women with SGA fetuses (SGA group) and on 30 healthy pregnant women (appropriate-for-gestational-age [AGA] group) matched for maternal and gestational age. All women included in this study were in the third trimester of pregnancy.

Methods Platelets were isolated by differential centrifugation. NO metabolites, after enzymatic conversion followed by the Griess reaction, were measured as nitrite by spectrophotometric detection. Peroxynitrite ($ONOO^-$) levels were evaluated using the fluorescence probe 2,7-dichlorofluorescein diacetate (DCFDA).

Main outcome measures The following determinations were made: platelet nitric oxide and peroxynitrite levels in the SGA group and controls; inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS) and nitrotyrosine (N-Tyr) expression in the same groups.

Results Our results show that both platelet NO and $ONOO^-$ levels were significantly higher in the SGA group than in the controls.

Conclusion Increased platelets levels of nitric oxide and peroxynitrite might play a role in the pathophysiology of intrauterine growth restriction. Further investigations are in progress to clarify if these molecules are pathogenetic factors, an epiphenomenon or a pathophysiological marker.

Keywords Nitric oxide, peroxynitrite, platelets, pregnancy, SGA.

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Introduction

Pregnancy results in a state of oxidative stress arising from increased placental mitochondrial activity and the production of reactive oxygen species (ROS), mainly superoxide anion (O_2^-). The placenta also produces other ROS including nitric oxide (NO), carbon monoxide and peroxynitrite ($ONOO^-$) that have pronounced effects on placental function, including trophoblast proliferation, differentiation and vascular reactivity. Excessive production of ROS may occur at certain windows in placental development and pathological pregnancies, such as those complicated by pre-eclampsia and/or intrauterine fetal growth restriction (FGR) (which is more common in small-for-gestational-age [SGA] fetuses), overpowering antioxidant defences and producing a deleterious outcome.[1]

NO, produced by trophoblast during normal pregnancy, plays an important role in maintaining uterine quiescence by a paracrine effect and preventing premature birth;[2] moreover, this molecule, produced in adequate concentration, leads to vasodilation in the fetoplacental circulation to improve oxygen and nutritional supply to the fetus.

NO is a gaseous free radical produced intracellularly by nitric oxide synthases (NOSs) during the enzymatic conversion of L-arginine to L-citrulline.[3,4] Having an unpaired electron, NO is a highly reactive free radical that damages proteins, carbohydrates, nucleotides and lipids, and, together with other inflammatory mediators, results in cell and tissue damage, low-grade, sterile inflammation and cellular adhesions.[5] NO potently relaxes arterial and venous smooth muscle and, less strongly, inhibits platelets aggregation and

platelet adhesion. NO donors, acting as vasodilating agents, are therefore a possible therapeutic approach for safeguarding early embryo development.[5]

NO is an important mediator of physiological processes in a variety of tissues[4–9] but can also have a cytotoxic role in the presence of O_2^-.[10] In fact, it is able to combine with superoxide to generate $ONOO^-$ (a nitrogen free radical, reported to produce peroxidative damage). Normally, the free radical is metabolised by the antioxidant enzyme superoxide dismutase to hydrogen peroxide (H_2O_2), which is then metabolised by catalase to H_2O and O_2. Increasing the amount of O_2^- increases $ONOO^-$ and decreases the available NO. Placental oxidant–antioxidant imbalance and consequent peroxynitrite production could play an important role in the aetiology of intrauterine growth restriction (IUGR).[11]

NO production in platelets is due to the presence of a constitutive isoform of the synthase enzyme.[12] Platelets are the first line of defence against loss of vascular integrity[13] and are involved in the maternal placental vascular damage in pathological pregnancies, such as those leading to IUGR.[14] Interaction of platelet membrane receptors with such injury-related factors as collagen microfibrils induces adhesion to other platelets and to the vessel wall.[13] It is well known that NO has a vasodilating effect on the vessels and an antiaggregation effect on platelets, thus increasing blood flow. Moreover, increased NO levels might be a compensatory response to improve blood flow in the placenta and/or might play a role in limiting platelet adhesion and aggregation.[15]

Abnormal development of the placental vascular tree is the primary step in a cascade of fetal compromises leading to IUGR.[16] The degree of placental vasculopathy determines the severity of fetal disease, which may be extended to many fetal organ systems with implications for wellbeing and neonatal outcomes.

The aim of the present work was to study nitric oxide and peroxynitrite levels in platelets from pregnant women with SGA fetuses compared with a control group. Moreover, the iNOS, eNOS and nitrotyrosine (N-Tyr) expression in the same groups were also observed. IUGR is a functional diagnosis with a wide range of diagnostic criteria. Although not all babies that are SGA are growth restricted, the likelihood of them having IUGR increases as the centile birthweight falls. Selection of a group of low centile birthweight babies can therefore be used as a proxy for the study of IUGR.

Subjects and methods

The study was performed on 30 pregnant women with SGA babies detected by clinical and ultrasound assessment and on 30 healthy pregnant women matched for maternal and gestational age admitted consecutively between May 2003 and October 2005 to wards of the Department of Obstetrics and Gynecology of the Polytechnic University of Marche, Ancona (Italy). Subjects in the control group were asked to participate at their first appointment in the fetal maternal unit. Informed consent was given by all women enrolled in the study.

The study was performed in accordance with the principles contained in the Declaration of Helsinki as revised in 2001, and the study was approved by the Bioethical Committee of the Polytechnic University of Marche.

A maternal venous blood sample was drawn at 33 weeks of gestation after the diagnosis of SGA and prior to any surgical intervention, after overnight fasting, and after at least 24 hours of nitrite-free diet to eliminate any impact on nitric oxide content. Nitric oxide levels and peroxynitrite production were determined on platelets obtained from venous samples. Moreover, iNOS, eNOS and nitrotyrosine (N-Tyr) expression in the same groups were also observed.

Specific exclusion criteria for the study were: conception by assisted reproduction, gestational diabetes, abnormal placental site, history of previous hypertension, proteinuria, renal disease, cardiac disease, diabetes mellitus and chromosomal or other fetal anomalies. Women in the SGA group did not have pre-eclampsia by the International Society for the Study of Hypertension Pregnancy (ISSHP) definition at the sampling date.[17]

According to our departmental protocol, gestational age was determined by reference to the last menstrual period and crown–rump length measurements between 8 and 12 weeks of gestation and confirmed by an early second-trimester ultrasonographic examination.

SGA was defined on the basis of a decrease in fetal growth and serial antenatal ultrasound biometry predicting a fetal abdominal circumference below the 10th centile for gestational age (±reduced amniotic fluid volume);[14] final inclusion in the SGA group was based on the delivery of an infant with a birthweight below the 5th percentile for gestation and sex.[18]

To investigate the placental vascular function, all the women were studied, before blood sampling, by Doppler velocimetry of the uterine and umbilical arteries. Flow velocity waveforms were imaged with a commercially available instrument (Aloka SSD 1700®; High Technology Inc., Walpole, MA, USA) using a 5-MHz convex probe and a 100-Hz filter. With the woman in a semirecumbent position, the uterine artery was identified on a longitudinal scan lateral to the uterus, which also showed the bifurcation of the common iliac artery; the recording was made where the uterine and the external iliac artery crossed, as detected by colour flow Doppler. The resistance index (RI) was calculated as the difference between the highest systolic velocity (A) and the lowest diastolic velocity (B) divided by the highest systolic velocity (A–B/A). Waveform analysis was performed both automatically, using the instrument, and manually. The average RI of both uterine arteries was calculated for each woman and considered abnormal when greater than 0.58 (mean + 2 SDs).[19,20] The umbilical artery was analysed by pulsatility index (PI) = ([peak systolic velocity – maximum diastolic velocity]/mean maximum velocity),[21]

calculated automatically with the software included in the ultrasound system. An average of three waveforms were analysed. Care was taken to include only measurements obtained at an angle between 0° and 30° during fetal apnoea.

The RI can also be useful in umbilical artery measurements, although the PI is more reliable in settings with low diastolic flow, such as IUGR. All Doppler examinations were performed by the same research assistant (S.R.G.).

Platelet isolation

Peripheral venous blood was drawn, after overnight fasting, and immediately mixed with the anticoagulant Citrate Dextrose (ACD) (36 ml citric acid, 5 mM potassium chloride, 90 mM NaCl, 5 mM glucose, 10 mM ethylenediamine tetraacetic acid [EDTA], pH 6.8). During platelet isolation, appropriate steps were taken to ensure that there was no white blood cell contamination. Platelets were isolated by differential centrifugation according to the method of Rao.[22] This involves a preliminary centrifugation step (200 × *g* for 10 minutes) to obtain platelet-rich plasma (PRP). The platelets were then washed three times in the ACD buffer and centrifuged as above. The PRP was centrifuged again at 200 × *g* for 10 minutes to remove any residual red cells and finally centrifuged at 400 × *g* for 20 minutes to isolate the platelets. The platelet pellet was washed twice in phosphate-buffered saline (PBS) (containing 135 mM NaCl, 5 mM potassium chloride, 10 mM EDTA, 8 mM Na_2PO_4, 2mM NaH_2PO_4 H_2O, pH 7.2) and immediately used for the experiments.

NO determination

NO was measured as nitrite–nitrate release in supernatants of lysed platelets, as described by Camilletti *et al.*[23] Briefly, platelets were suspended in NO buffer (25 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES], 140 mmol/l NaCl, 5.4 mmol/l potassium chloride, 1 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, pH 7.4) in the presence of 1.44 mmol/l NADPH and 20 mu of nitrite reductase and incubated for 1 hour at 37°C to convert all available nitrate to nitrite by the enzyme. The reaction was stopped by freeze–thawing the samples, which were then sonicated and centrifuged at 1500 × *g* for 15 minutes. NO concentration, which is related to nitrite and nitrate levels, was determined using a spectrophotometric assay based on the formation of a coloured azo dye product when a Griess reagent (1% sulfanilamide, 0.1% naphthalenediamine dihydrochloride and 2.5% H_3PO_4) was mixed in equal volumes with the sample according to the method of Chen and Mehta[24] and modified method of Camilletti *et al.*[23] The chromophore absorption was then read at 543 nm, and nitrite concentration was determined with sodium nitrite in water as standard. NO level was expressed in nmol NO produced/mg protein. Protein concentration was determined by Bradford BioRad protein assay using serum albumin as a standard to normalise the NO concentration data.[25]

Preparation of DCFDA-free base

DCFDA-free base was prepared daily by mixing 0.05 ml of 10 mM/l DCFDA with 2 ml of 0.01 N NaOH at room temperature for 30 minutes. The mixture was neutralised with 18.0 ml of 25 mmol/l PBS, pH 7.4. This solution was maintained on ice in the dark until use.[26]

Peroxynitrite determination

NO is able to combine, in a rate limiting reaction catalysed by iNOS, with superoxide anions to generate peroxynitrite, a nitrogen free radical that produces relevant peroxidative damage.

Peroxynitrite levels were evaluated using the fluorescence probe 2,7-dichlorofluorescein diacetate (DCFDA) as previously described.[27] Briefly, cells were incubated for 15 minutes with 5 μM DCFDA-free base at 37°C. Then, the DCFDA-treated samples were incubated with or without addition of 100 mM L-arginine and 100 mM N^G-monomethyl-L-arginine (L-NMMA) for 15 minutes at 37°C in the dark. After washing in PBS, pH 7.4, cells were broken by sonication. The mixture was then centrifuged at 1000 rpm for 5 minutes, and the fluorescence was measured in the supernatant in a Perkin-Elmer LS-50B spectrofluorometer, at an excitation wavelength of 475 nm and emission wavelength of 520 nm. $ONOO^-$ production was expressed in arbitrary units/mg protein.

Western blotting

Washed platelets were lysed in RIPA lysis buffer containing 1× PBS, 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 10 mg/ml phenyl methylsulphonyl-fluoride (PMSF), aprotinin, 100 mM sodium orthovanadate and 4% protease inhibitor cocktails by microcentrifugation at 10 000 × *g* for 10 minutes at 4°C. The supernatants were collected and treated with an equal volume of sample application buffer (125 mmol/l Tris-HCl, pH 6.8, 2% SDS, 5% glycerol, 0.003% bromophenol blue, 1% β-mercaptoethanol). The mixture was boiled for 5 minutes; 15 μl of each sample was applied to each well of an 8% SDS polyacrylamide gel and electrophoresed for 1 hour at 130 V together with a set of molecular weight markers (Broad Range; Sigma Chemical Co., St Louis, MO, USA). The resolved protein bands were then transferred onto polyvinylidene fluoride (PVDF) membranes at 100 V for 60 minutes using a transfer buffer of 25 mmol/l Tris base, 192 mmol/l glycine and 20% methanol. The blots were blocked overnight at 4°C with blocking buffer (5% nonfat milk in 10 mmol/l Tris, pH 7.5, 100 mmol/l NaCl, 0.1% Tween 20). The blocking buffer was decanted and blots were incubated for 1 hour at room temperature with primary antibodies: rabbit antiendothelial nitric oxide synthase (eNOS, 1:1000; Chemicon, CA, USA), rabbit anti-inducible nitric oxide synthase (iNOS, 1:1000; Chemicon) and rabbit antityrosine (Tyr, 1:2000; Chemicon) diluted in blocking buffer. Positive controls were included in all experiments as provided by the manufacturer

to confirm antibody specificity. As an internal control, blots were reprobed with an anti-β-actin antibody (Sigma Chemical Co.).

Blots were then washed using TTBS washing buffer (10 mmol/l Tris, pH 7.5, 100 mmol/l NaCl, 0.1% Tween 20) and incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (1:5000; Sigma Chemical Co.) for 1 hour at room temperature following washes in TTBS. Peroxidase activity was revealed using 3,3′-diaminobenzidine (Sigma Chemical Co.) as a substrate.

Densitometry was performed using software AMERSHAM Image Master 1D. All densitometric data are expressed as mean densities, defined as the sum of the grey values of all pixels in a selection divided by the number of pixels.

Statistical analysis

All experiments were carried out at least in duplicate and were usually repeated three times. Results are expressed as mean ± SD. Student's *t* test was used to analyse the results. Differences were considered significant with $P < 0.05$.

Results

Subject characteristics are shown in Table 1. Figure 1 shows that both platelets NO and $ONOO^-$ levels were higher in the SGA group than in the controls. Platelet NO concentration, measured as nitrite–nitrate release into the medium, was significantly higher ($P < 0.001$) in the SGA group than in the controls (28.33 ± 2.21 nmol/mg protein in the SGA group versus 18.84 ± 3.26 nmol/mg protein in controls). $ONOO^-$ levels were significantly higher ($P < 0.001$) in the SGA group than in the controls (3.29 ± 0.25 arbitrary units/mg protein in the SGA group versus 2.74 ± 0.40 arbitrary units/mg protein in controls) (Figure 2). Moreover, a positive correlation was found between NO levels and umbilical artery PI in the SGA group ($r = 0.79$, $P < 0.001$) (Figure 3), while no correlation was observed between $ONOO^-$ and umbilical artery PI in the SGA group ($r = 0.02$, $P = 0.90$) (Figure 4).

There was no statistical correlation between platelet NO or ONOO levels and gestational age, birthweight or placental weight. Western blot analysis using anti-iNOS and anti-eNOS monoclonal antibodies demonstrated that both isoforms were detectable in platelet lysates. Densitometric analysis of bands indicated that iNOS protein levels were significantly higher in the SGA group compared with controls (0.173 ± 0.005 in the SGA group, 0.081 ± 0.016 in controls; $P < 0.001$) (Figure 5).

The eNOS antibody reacted with a 140-kDa protein in the cell lysates, corresponding to eNOS. Densitometric analysis of bands showed slightly but not significantly higher eNOS protein levels in the SGA group samples compared with the control group (0.091 ± 0.005 in SGA group, 0.087 ± 0.009 in controls; not significant).

The same technique revealed the presence of nitrotyrosine that was more pronounced in cell lysates from the SGA group than in those from the controls (Figure 6) (0.165 ± 0.025 in SGA group, 0.094 ± 0.014 in controls; $P < 0.001$)

Discussion

The aetiopathogenetic basis of IUGR suggests early pregnancy placental damage as a *primum movens* (primary cause), although the impaired growth itself is not established before the middle of the second trimester. The degree of placental dysfunction determines the severity of the IUGR, with implications for wellbeing and neonatal outcomes. This damage leads to the onset of chronic hypoxia, which inhibits metabolitic release of factors such as cyclic guanosine monophosphate (cGMP) that can function as vasodilators.

Recent studies have shown that in FGR, the umbilical vein endothelial cells do not or cannot respond to chronic hypoxia by increasing cGMP, which may lead to fetoplacental vasoconstriction.[28]

Table 1. Maternal and neonatal characteristics. Data are presented as mean ± SD

	SGA ($n = 30$)	Controls ($n = 30$)	*P* value
Maternal age (years)	33.1 ± 0.3	32.0 ± 0.2	NS
Number of primigravidas	12	10	NS
Gestational age at sample (weeks)	33.1 ± 0.5	33.4 ± 1.1	NS
Umbilical artery PI	1.41 ± 1.1	0.98 ± 1.8	NS
Mean of uterine artery RI	0.52 ± 0.2	0.73 ± 0.2	<0.001
Gestational age at delivery (weeks)	36.2 ±2.7	39.3 ± 1.4	NS
Birthweight (g)	2041.0 ± 581.7	3380.4 ± 421.2	<0.001
Centile birthweight	4.1 ± 1.1	51.9 ± 8.2	<0.001
Placental weight (g)	458.2 ± 141.9	658.9 ± 138.1	NS
Late pregnancy pre-eclampsia, *n* (%)	12 (40)	—	

NS, not significant.

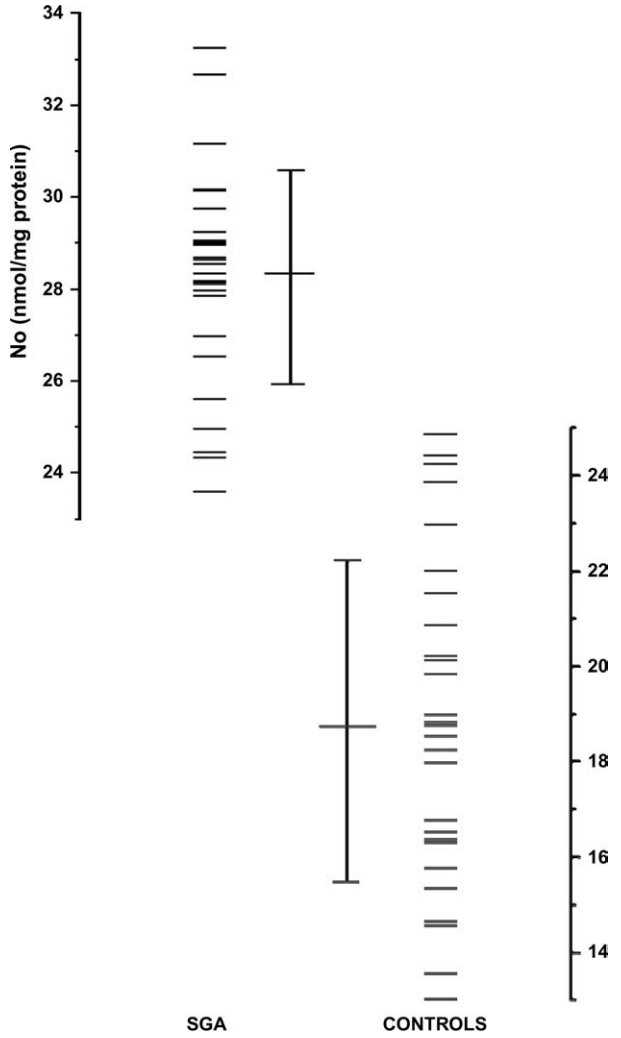


Figure 1. NO levels (nmol/mg protein) in platelets obtained from healthy pregnant women (controls) and from women with SGA fetuses. (The bars on one side of the plots indicate means ± SD, $P < 0.001$).

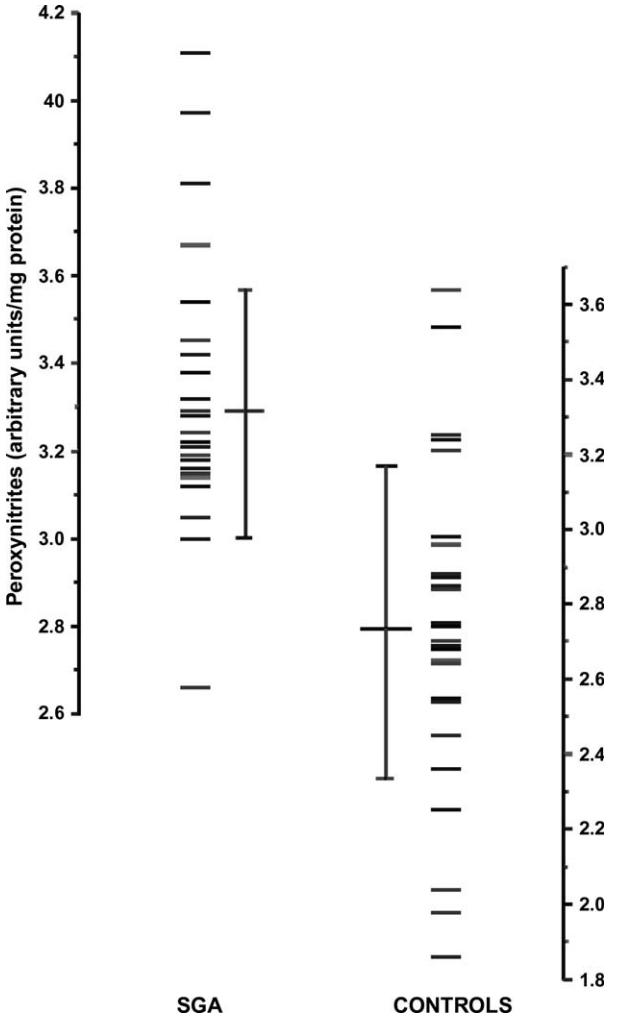


Figure 2. Peroxynitrite levels (arbitrary units/mg protein) in platelets obtained from healthy pregnant women (controls) and from women with SGA fetuses. (The bars on one side of the plots indicate means ± SD, $P < 0.001$).

NO, as a locally potent vasodilator, seems to be involved in the establishment and maintenance of the fetoplacental circulation, and it helps regulate perfusion by counter balancing the effects of other vasoactive mediators.[29] Many biochemical observations have demonstrated that nitric oxide is involved in the vascular angiogenic activity of the fetoplacental unit.[30] NO is known to be an unstable molecule rapidly oxidised to nitrite and nitrate in the presence of oxygen.[31] Under conditions of excess production, NO and/or derivates, such as the potent pro-oxidant peroxynitrite, are involved in the many pathological mechanisms. In fact, peroxynitrite may damage cells in various ways, including nitrosylation of protein tyrosine residues, inhibition of mitochondrial functions, lipid peroxidation, DNA strand breaking and reaction with protein sulphydryl groups to form nitrosothiol derivates.[32]

Placental NOS activity has been reported to be reduced in placentas from pregnancies with abnormal umbilical artery flow velocity waveforms;[33] furthermore, infusion of a nitric oxide donor improved uterine artery diastolic blood flow in high-risk pregnancies.[34] Numerous investigations on the NO system have been carried out in obstetric pathologies and during normal pregnancy course. No changes was observed for urinary nitrite/nitrate and cGMP, as well as for serum nitrite/nitrate and cGMP with advanced gestational age.[35] Reduced expression of iNOS and eNOS has been reported in placental tissue of pre-eclamptic and SGA pregnancies. Moreover, significant different uterine and peripheral vascular impedance in pre-eclamptic and SGA pregnancies compared with normal pregnancy have been described.[36,37]

In our research, NO levels were increased in platelets of women with SGA fetuses during the third trimester compared

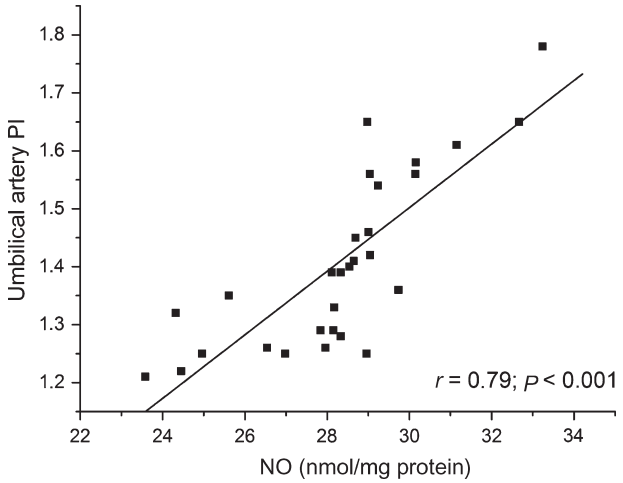


Figure 3. Correlation analysis between platelets nitric oxide levels and umbilical artery PI in women with SGA fetuses.

with controls; this result suggests a compensatory mechanism, inherent to pregnancy, that contributes to NO formation. In fact, our previous studies indicate that low levels of NO during the early second trimester could represent an impaired stimulus to vascular formation and endothelial regulation, resulting in placental disease and subsequent FGR.[29] However, in the present work, the trend of NO levels in women with SGA fetuses during the third trimester showed an increase compared with controls. We hypothesised that the opposite trends of NO production in the second[29] compared with the third trimester might be due to chronic hypoxia (typical of IUGR), inducing oxidative stress. This period might be considered short related to the length of pregnancy, but long enough to allow the establishment of vasoconstriction, responsible for the fetuses becoming SGA.

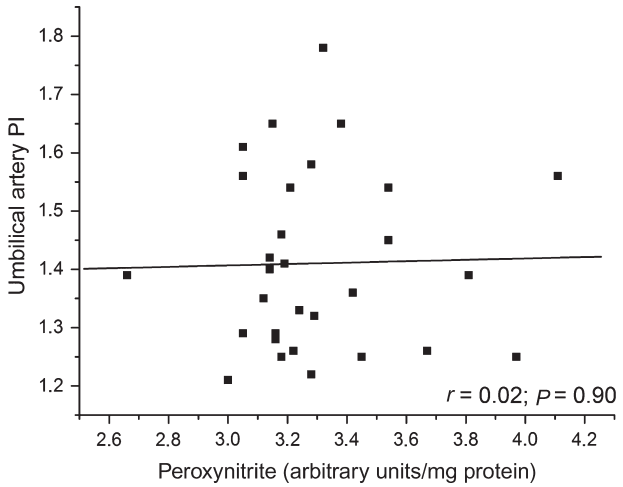
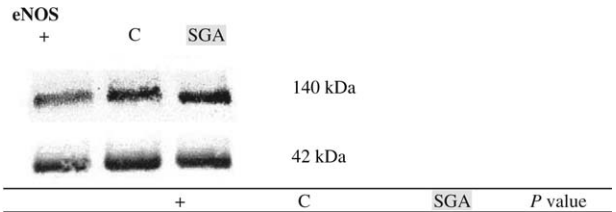
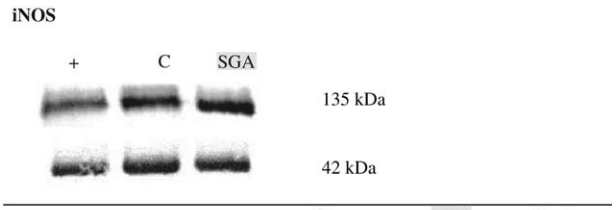


Figure 4. Correlation analysis between platelets peroxynitrite levels and umbilical artery PI in women with SGA fetuses.



	+	C	SGA	*P* value
eNOS	0.081 ± 0.004	0.087 ± 0.009	0.091 ± 0.005	NS
β-actin	0.074 ± 0.005	0.075 ± 0.005	0.074 ± 0.005	NS

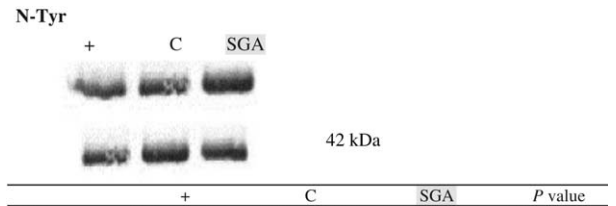


	+	C	SGA	*P* value
iNOS	0.064 ± 0.003	0.081 ± 0.016	0.173 ± 0.005	0.001
β-actin	0.079 ± 0.008	0.080 ± 0.009	0.081 ± 0.007	NS

Figure 5. Western blot analysis of eNOS and iNOS protein expression, together with the internal control β-actin, in both control (C) and intrauterine fetal growth restriction (SGA). + indicate positive control. NS, not significant.

Oxidative stress represents one of the most important factors inducing iNOS expression and the consequent increase in NO levels that augment oxidative damage. However, NO synthesised from eNOS, as a compensatory mechanism, might lead to an improvement in fetal condition, balancing the vasoconstriction effects.

In the present study, this compensatory mechanism was not found; in fact, NO produced by iNOS might probably lead to an increase in peroxynitrite production, which may increase the oxidative damage, responsible for long-term vascular injuries. Peroxynitrite is formed in biological systems when superoxide and NO are produced at near equimolar ratio, and although not a free radical by chemical nature, peroxynitrite is a powerful oxidant exhibiting a wide array of tissue damaging effects, ranging from lipid peroxidation to other tissue damage. Moreover, $ONOO^-$ may be considered



	+	C	SGA	*P* value
N-Tyr	0.064 ± 0.04	0.094 ± 0.014	0.165 ± 0.025	0.001
β-actin	0.071 ± 0.007	0.072 ± 0.008	0.070 ± 0.007	NS

Figure 6. Western blot analysis of nitrotyrosine protein (N-Tyr) expression, together with the internal control β-actin, in both control (C) and intrauterine fetal growth restriction (SGA). + indicate positive control. NS, not significant.

a marker of oxidative stress, and its action involves a variety of biomolecules including tyrosine. Nitrotyrosine is not formed by the action of hydrogen peroxide, superoxide or hydroxyl radical;[38] therefore, its appearance is presumably indicative of the action of peroxynitrite in the vascular endothelial cells of the placenta. Our investigation has highlighted an increase in platelet NO levels in the SGA group compared with controls. Moreover, $ONOO^-$ levels were significantly higher in the SGA group than in the controls. In fact, SGA platelets expressed higher iNOS than controls, while eNOS expression was not significantly modified in both the SGA group and controls. The eNOS, which produces small amounts of NO, is beneficial, while activation of the iNOS, which produces much more NO, causes injury, its toxicity being greatly enhanced by generation of peroxynitrite. Moreover, platelets from the SGA group showed a higher content of nitrotyrosine, a marker of nitrated tyrosines in proteins, compared with controls. Detection of nitrotyrosine at inflammatory sites has been hypothesised to be a biochemical marker for peroxynitrite formation.[38]

Our results also show that NO levels are directly correlated with umbilical artery PI, which in turn is correlated with umbilical blood flow, so NO increase, produced by eNOS, is considered as a compensatory mechanism to improve fetal condition. However, high levels of NO, synthesised by iNOS, lead to peroxynitrite production, which is not significantly correlated with umbilical artery PI and leads to a wide array of tissue damage.

It can be hypothesised that pathological conditions such as IUGR induce an increase in iNOS expression, leading to higher production of NO levels. This overproduction of NO produces a shift in metabolite formation. Among these metabolites, peroxynitrite and other free radicals are mainly responsible for the oxidative damage that affects the cell membrane in many tissues.

In conclusion, the data presented in this research suggest that alterations in the NO pathway may not only play a role in the physiological changes of advancing gestation but also contribute to the pathophysiology of IUGR. In our series, 40% of women with SGA fetuses developed pre-eclampsia later in pregnancy; therefore, increased platelet levels of nitric oxide and peroxynitrite might also play a role in the pathophysiology of pre-eclampsia. Further investigations are in progress to clarify if NO is a pathogenic factor, an epiphenomenon or a pathophysiological marker. ■

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