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GRK2 Levels in Umbilical Arteries of Pregnancies Complicated by Gestational Hypertension and Preeclampsia

Raffaele Napolitano[1], Alfonso Campanile[2], Laura Sarno[1], Antonio Anastasio[2], Giuseppe M. Maruotti[1], Maddalena Morlando[1], Bruno Trimarco[2], Pasquale Martinelli[1] and Guido Iaccarino[2,3]

BACKGROUND
G-Protein coupled receptor kinase 2 (GRK2) represents a regulator of cell function in different cardiovascular conditions, including high blood pressure. The relationship between elevated GRK2 levels and impaired vasorelaxant responses is causative of hypertension through the increase in vascular resistances. The aim of this study is to ascertain if this feature is present in the fetal placental vasculature of pregnancies complicated by hypertensive disorders.

METHODS
We have assessed GRK2 levels in the umbilical arteries (UA) of 21 preeclamptic or gestational hypertensive and 23 normotensive women at time of delivery.

RESULTS
GRK2 levels were increased in the hypertensive group (0.83 ± 0.14 vs. 0.48 ± 0.06 densitometry units; $P < 0.05$). GRK2 levels were in correlation and in linear regression with systolic, diastolic, and mean arterial pressure ($P < 0.05$, $r^2 = 0.12$, $r^2 = 0.11$, $r^2 = 0.12$). Correlations did not reach a significant value for other clinical parameters such as gestational age at birth, umbilical artery pulsatility index, maternal proteinuria, and neonatal birth weight. Out of the 21 hypertensive women, 7 who developed a preeclampsia associated with early preterm delivery (before 34 weeks) had a significantly lower GRK2 levels compared to the remaining 14 (0.51 ± 0.12 vs. 1.08 ± 0.20 densitometry units, $P < 0.05$).

CONCLUSIONS
We conclude that elevated GRK2 levels in the umbilical vasculature is correlated to elevated blood pressure levels, with a likely compensatory rather than causative role since the lack of protective effect of elevated GRK2 levels may negatively affect the outcome of the hypertensive state.

Keywords: *blood pressure; preeclampsia; pregnancy; hypertension; hypertension in pregnancy; protein kinases; G proteins; vasculature.*

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Hypertension is reported to complicate about 7–13% of all pregnancies and it represents the most common medical disorder of pregnancy.[1] Preeclampsia (PE) affects 2–5% of pregnancies in developed countries[2] and is a major cause of maternal and perinatal morbidity and mortality, accounting a 0.7 maternal deaths per 100,000 live births.[3] This syndrome is thought to be a consequence of abnormalities in the maternal vessels supplying the placenta, leading to poor placental perfusion and release of factors causing widespread endothelial dysfunction with multiorgan system clinical features.[4] The clinical findings of PE can therefore manifest, as either a maternal syndrome (hypertension and proteinuria with or without other multisystem abnormalities), or fetal syndrome as fetal growth restriction (FGR).[5] This latter, though, is not exclusively observed in gestational hypertension (GH) or PE, and can be a complication of normotensive pregnancies.

Although the causes of PE are unknown, the origin of the condition is recognized as lying in the placenta. Shallow endovascular cytotrophoblast invasion in the spiral arteries and endothelial cell dysfunction are two key features in the pathogenesis of PE.[6] Placental ischemia is thought to develop as a result of this abnormal cytotrophoblastic invasion; this has been proposed as leading to release of placental factors and imbalance of angiogenic factors, causing the widespread endothelial dysfunction.[4,7–9]

G-Protein coupled receptor (GPCR) kinase 2 (GRK2) represents an important regulator of cell functions by means of phosphorylative events on activated GPCR leading to desensitization.[10] GRK2 was first identified for its ability to phosphorylate and mediate desensitization of βAR, and its transgenic overexpression causes βAR desensitization and impaired βAR mediated increase in cardiac contractility. Recently, its ability to recognize and phosphorylate many more substrates has been

[1]Department of Obstetrics and Gynecology, Prenatal Diagnosis and High Risk Pregnancy Unit, University of Naples, Federico II, Naples, Italy; [2]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, University of Naples, Federico II, Naples, Italy; [3]School of Medicine, University of Salerno, Salerno, Italy. Correspondence: Guido Iaccarino (giaccarino@unisa.it)

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described, leading to a scenario of interactions within the cell that appears to extend its role beyond receptor desensitization.

Increased levels of GRK2 are a hallmark of different cardiovascular conditions, including high blood pressure. In particular, GRK2 is increased in both experimental and human hypertension.[11] Vascular smooth muscle cells restricted overexpression of GRK2 in transgenic mice causes the development of hypertension and hypertension induced cardiac hypertrophy.[12]

The close relationship between elevated GRK2 levels and impaired vasorelaxant responses is therefore thought to be causative in hypertension through the increase in vascular resistances. Interestingly, this association is also present in other conditions characterized by increased blood pressure such as portal hypertension.[13] It is therefore possible to suppose that this feature is also present in the vasculature of the placenta of pregnancies complicated with hypertensive disorders.

To follow this lead, we have assessed GRK2 levels in the vasculature of umbilical arteries (UA) of preeclamptic or gestational hypertensive women.

METHODS

Design and study population. In a 2-year observation period (January 2008–December 2009) we enrolled 23 normotensive pregnant women with no history of GH and 21 women with pregnancies complicated by hypertension, of which 13 patients with PE and 8 with GH, consecutively admitted at the High Risk Pregnancy Unit, Department of Obstetrics and Gynecology of the University of Naples Federico II. We also enrolled five women with FGR, in absence of any increased blood pressure, since in this group, FGR is obviously not related to blood pressure. All patients provided written informed consent prior the inclusion in the study. The study was approved by the University ethical committee and it conforms with the principles outlined in the Declaration of Helsinki.

GH was defined as the occurrence of a diastolic blood pressure ≥90 mm Hg on at least two occasions 4 h apart, developing after 20 weeks of gestation in previously normotensive women in the absence of significant proteinuria. PE was defined as the occurrence of GH with proteinuria of 300 mg or more in 24 h or two readings of at least 2+ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection is available. Superimposed PE on chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease), was defined by the presence of significant proteinuria (as defined above) after 20 weeks of gestation.[4] The diagnosis of FGR was made according to the following criteria: ultrasound measurement of the fetal abdominal circumference below the 10th centile and UA pulsatility index above the 95th centile.[14] The systolic and diastolic pressure values reported were collected at the time of the caesarean section.

As an outcome for PE we used the gestational age at birth. We divided the PE group in those with an early delivery (Early PE) when gestational age at birth was <34 weeks or a late delivery (Late PE), when gestational age at birth was >34 weeks.[15,16]

UA sampling and GRK2 evaluation. After elective or emergency Caesarean section, UA segments of 2–3 cm were collected by the dissection of Wharton's jelly, frozen and stored at −80 °C until the day of the assay. On a following day, samples were dissolved in radio immunoprecipitation assay-sodium dodecyl sulfate buffer (50 mmol/l Tris-HCl (pH 7,5), 150 mmol/l NaCL, 1% NP-40, 0,25% deoxycholate, 9,4 mg/50 ml sodium orthovanadate, 20% sodium dodecyl sulfate) to obtain the overall extract.[17] Samples were analyzed by western blot (WB). Proteins were electrophoresed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and transferred to nitrocellulose. GRK2 was detected with a specific primary antibody 1:1,000 (Santa Cruz Biotechnology, Santa Cruz, CA) followed by incubation with an anti-mouse HRP-conjugated secondary antibody 1:2,000 (Santa Cruz Biotechnology) as appropriate, and standard chemiluminescence (Reinassance, NEN). Densitometry analysis of digitalized autoradiographies was performed using a dedicated software (Image QuaNT Molecular Diagnostic; GE Healthcare Europe, Milan, Italy). GRK2 expression was corrected by actin expression (corrected densitometry units).

β-adrenergic receptors (β-AR) radioligand binding. Since increased GRK2 associates with β-AR downregulation and desensitization, we assessed β-AR density exposed on uterine arteries. Membrane fractions obtained as previously described[18] were used for β-AR radioligand binding studies with the nonselective β-AR antagonist ligand [^{125}I]-Cyanopindolol. Maximal binding was measured using a saturating amount of [^{125}I]-Cyanopindolol on 25 μg membrane protein. Binding was allowed to occur for 1 h at 37 °C, as described previously.[18] Inclusion of 10 μmol/l alprenolol defined nonspecific binding. This analysis was performed for 7 patients with complicated pregnancies and 11 patients with normal pregnancies.

Statistical analysis. Statistical analysis was performed using SPSS 13.0 (SPSS, Chicago, IL). Values are given as the mean ± s.e. Chi-square test was performed for categorical variables while an ANOVA test was used for comparison of means values of the three groups. For the continues variables a Pearson's correlation and a linear regression analysis were considered. All the analyses were performed using a two-sided model, considering a normal distribution as appropriate. The *P* value less than 0.05 was considered statistically significant.

Fetus–maternal outcome parameters considered for the analysis were: gestational age at delivery, proteinuria, birth weight, and UA pulsatility index.

RESULTS

Clinical characteristics

All the women were white. Out of 21 women with hypertensive pregnancies, 3 of them had a chronic hypertension. Overall, 11 women developed PE (7 with an associated FGR), 7 women developed GH (4 with an associated FGR). Clinical characteristics of normotensive and complicated pregnancies are depicted in **Tables 1** and **2**. The two groups were comparable for anamnestic and physical characteristics. Women with

hypertension in pregnancy had a significant obstetric/clinical history associated with the conditions. All patients were not in labour at the time of recruitment and at delivery and all patients delivered by elective or emergency caesarean section.

GRK2 levels in normotensive and hypertensive pregnancies

GRK2 levels in the hypertensive group were higher when compared either with the normotensive group (0.83 ± 0.14 vs. 0.48 ± 0.06 densitometry units; $P < 0.05$) or with the FGR group (0.83 ± 0.14 vs. 0.48 ± 0.06 vs. 0.39 ± 0.16 densitometry units; $P < 0.05$; Figure 1). FGR pregnancies did not differ in GRKs levels with normotensive group.

As a functional correlation of increased GRK2 levels, we also observed β-AR downregulation, as shown by lower density of β-AR in the hypertensive group ($N = 11$) when compared with the normotensive group ($N = 7$) (7.73 ± 1.36 vs. 20.96 ± 7.57 pmol/μg, $P < 0.05$; Figure 2).

In the study population, GRK2 levels were in correlation ($P < 0.05$, Pearson's test) and in linear regression with systolic, diastolic, and mean arterial pressure ($P < 0.05$) (Figure 3).

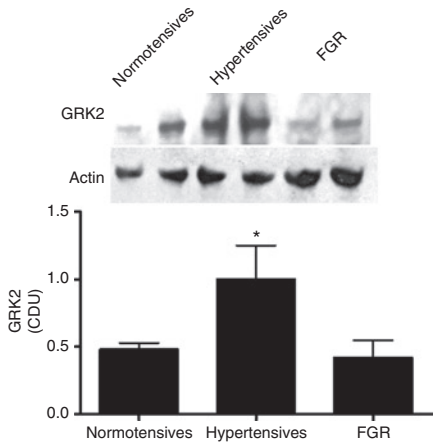

Figure 1 | Densitometry analysis of WB from UA of different groups. GRK2 data were corrected by those of actin and expressed as densitometry units. Data are expressed as means ± s.e. of GRK2 levels in each group. (*$P < 0.05$ vs. normotensive). CDU, corrected densitometry units; FGR, fetal growth restriction; GRK2, G-protein coupled receptor kinase 2; UA, umbilical arteries; WB, western blot.

Table 1 | Anamnestic and physical characteristics of study population

Characteristics mean (s.e.)/*n* (%)	PE/GH	Early PE	Late PE/GH	FGR	Normotensive	*P* values
Women	21	7	14	5	23	
Maternal age (years)	33 (1)	30 (3)	32 (3)	28 (3)	32 (1)	NS
Weight (kg)	66 (3)	68 (7)	65 (4)	56 (2.7)	63 (2)	NS
Height (m)	1.6 (0)	1.6 (0)	1.6 (0)	1.6 (0)	1.6 (0)	NS
BMI (kg/m^2)	26 (6)	26 (7)	24 (5)	21 (1)	24 (1)	NS
Nulliparous	18 (86)	7 (100)	11 (79)	5 (100)	20 (87)	NS
Smokers	0 (0)	0 (0)	0 (0)	1(20)	1 (4)	NS
Preexisting clinical conditions[a]	4 (19)	1 (14)	3 (21)	0 (0)	0 (0)	NS
Previous complicated pregnancies[b]	2(9)	2 (29)	0 (0)	0 (0)	2 (9)	NS

BMI, body mass index; Early PE, PE with delivery occurred <34 weeks; FGR, fetal growth restriction; GH, gestational hypertension; Late PE, PE with delivery occurred >34 weeks; PE, preeclampsia.
[a]Chronic hypertension, kidney diseases, thrombophilia. [b]Intrauterine fetal death, preeclampsia, gestational hypertension, HELLP syndrome, abruptio placentae, preterm delivery.

Table 2 | Clinical details and outcome of study population

Characteristics mean (s.e.)/*n* (%)	PE/GH	Early PE	Late PE/GH	FGR	Normotensive
Women	21	7	14	5	23
Systolic blood pressure (mm Hg)	149 (4)*,**	149 (8)*,**	149 (4)*,**	115 (4)	115 (3)
Diastolic blood pressure (mm Hg)	94 (2)*,**	91 (4)*,**	96 (2)*,**	72 (8)	74 (2)
Proteinuria (mg/24 h)	1,356 (480)*,**	1,771 (1223)*,**	1,148 (420)*,**	160 (40)	110 (4)
Gestational age at delivery (weeks)	34 (1)*	31 (1)*	36 (1)*	33 (4)*	39 (0)
Birth weight (g)	1,833 (199)	1,104 (147)*	2,198 (235)*	1,402 (296)*	3,010 (91)
Obstetric complications[a]	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)

Early PE, PE with delivery occurred <34 weeks; FGR, fetal growth restriction; GH, gestational hypertension; Late PE, PE with delivery occurred >34 weeks; PE, preeclampsia.
[a]abruptio placentae ($n = 1$).
*$P < 0.05$ vs. normotensive group, **$P < 0.05$ vs. FGR.

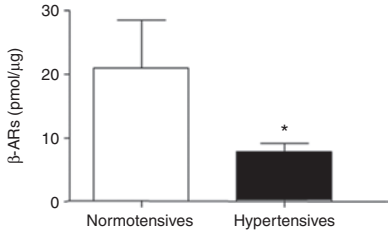

Figure 2 | β-AR density. Data are expressed as means ± s.e. of β-AR densitometry units (*$P < 0.05$). β-AR, β-adrenergic receptors.

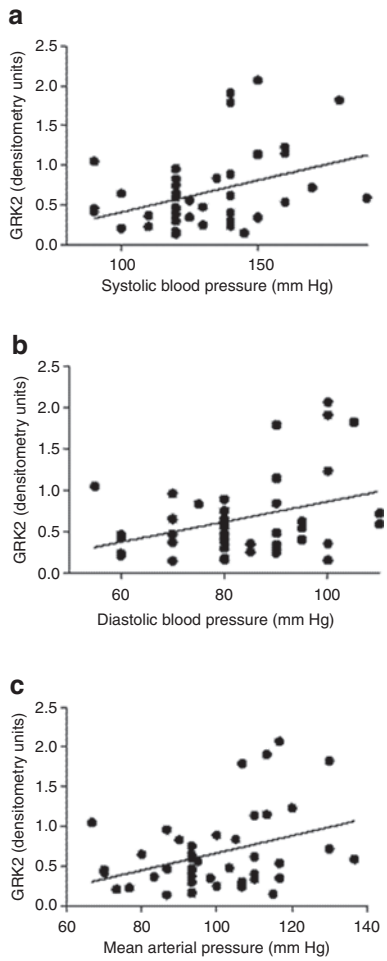

Figure 3 | Linear regression analysis of parameters of blood pressure and GRK2 expression. (**a**) Linear regression analysis of systolic blood pressure and GRK2 expression ($r^2 = 0.12$, $P < 0.05$); (**b**) Linear regression analysis of diastolic blood pressure and GRK2 expression ($r^2 = 0.11$, $P < 0.05$). (**c**) Linear regression analysis of mean arterial pressure and GRK2 expression ($r^2 = 0.12$, $P < 0.05$). GRK2, G-protein coupled receptor kinase 2.

Interestingly, correlations did not reach a significant value for other clinical parameters such as gestational age at delivery, UA pulsatility index, proteinuria, and birth weight.

On the contrary, in the hypertensive group, GRK2 levels predicted Early PE (7 women, 33%). Indeed, GRK2 levels in this subgroup were lower when compared with women with Late PE or GH ($n = 14$) (0.51 ± 0.12 vs. 1.08 ± 0.20 densitometry units, $P < 0.05$) (**Figure 4**).

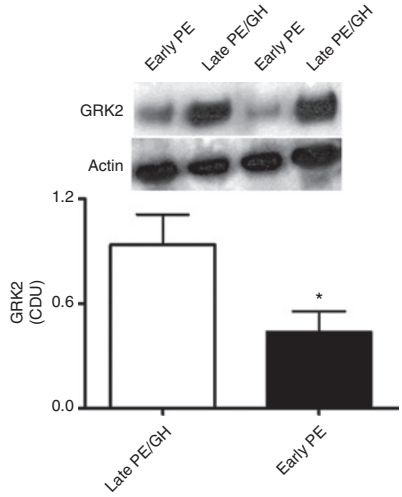

Figure 4 | GRK2 levels of women with Early PE and Late PE or GH. Data are expressed as means ± s.e. densitometry units (*$P < 0.05$). CDU, corrected densitometry units; Early PE, PE with delivery occurred <34 weeks; GRK2, G-protein coupled receptor kinase 2; GH, gestational hypertension; Late PE, PE with delivery occurred >34 weeks; PE, preeclampsia.

DISCUSSION

This study is the first report to describe a difference in the GRK2 levels in UA of pregnant women affected by hypertensive disorders. Our results are the first of this kind, also because the expression of the kinase in the umbilical vasculature was never demonstrated before. In fact, it was previously described that the myometrium expresses GRK2, and that this levels are increased during physiological pregnancy.,[19–21] To the best of our knowledge, our study is the first to associate increased GRK2 levels in the UA and GH.

Our results suggest that GRK2 might play a functional role of in the umbilical vasculature. First of all, the increased GRK2 are accompanied by the reduced number of β-AR on UA. A similar result is found in the vasculature of in essential hypertension,[22] where this finding has been causally related to the increase in vascular resistances. Indeed, it has been demonstrated that increased GRK2 levels depress vasodilatation through phosphorylative events on GPCRs, increase vascular resistance and therefore blood pressure.[12,23] Another possible causal mechanism derives from recent literature showing that increased GRK2 can inhibit AKT and endothelial nitric oxide synthase activity in sinusoidal hepatic capillaries causing increased vascular resistance in the liver and, as a consequence, portal hypertension.[13] In fact, a recent study demonstrated that the best independent predictor for maternal and fetal complications in a population of 526 high-risk primigravidas was total vascular resistance calculated at 24 weeks gestation.[24] Based on previous report, we felt that our findings allow to speculate that also in GH, increased levels of GRK2 are causative of elevated UA resistances.

To test this hypothesis, we assessed whether GRK2 and parameters of UA resistances (UA pulsatility index) correlate. Unexpectedly, we did not find this correlation, indicating that GRK2 cannot be causative of increases in UA resistances.

Therefore, if not causative, the increase in GRK2 in the UA can then be a consequence of increased blood pressure, to compensate for the excessive vascular tension. Support to this second hypothesis derives from the finding that, within the PE group, those patients with lower levels of GRK2 in the UA presented with a worse outcome, as they had to deliver at an earlier gestational age. In this light, the observed lower levels of expression of GRK2 in UA of Early PE women might be considered as the lack of a protective mechanism. Accordingly, effects of GRK2 on β-AR sensitivity and density can be considered protective rather than causative for hypertensive states.

Our data, therefore, suggest that the increase in GRK2 levels in the UA might play a protective role, rather than a causal one. If this is the case, the potential mechanism may be identified in the metabolic effect of the kinase. Indeed, we have recently demonstrated that GRK2 reduces glucose uptake[25] and adenosine triphosphate consumption, placing the cell in a low energy state that might favor cell survival in stress condition.[26]

The function of the β-AR has been investigated in pregnancy, but its significance in UA is controversial.[27–29] It has been argued that the adrenergic dysfunction in PE could be related to β-AR signaling.[29] Nevertheless, a supposed signaling alteration has not been further investigated. Increased GRK2 levels are an important signaling regulator which can favour cAMP independent, β-Arrestin dependent pathways in response to β-AR stimulation.[30] GRKs have been found to be also involved in the mechanism of myometrial uterine contractions in vitro and in experimental animal models.[20,21] These findings show that the myometrium in labour presents increased GRK2 localization on cell membrane, as a consequence of intense activation of myometrium GPCRs, such as the histamine receptor H1. Our data are original towards this previous finding since we studied UA, representing a different histological entity from the maternal myometrium. Also, in our group all the women were not in labour; considering these evidences, it is unlikely that the above mentioned GRK associated mechanism could be a confounding factor for our findings.

FGR, GH, and PE have common pathogenetic features.[31] In this study, FGR pregnancies do not present differences in GRK2 levels. This could be related to the exclusive association between GRK2 and elevated blood pressure levels, and therefore adds more to the protective role of GRK2 in PE.

Study limitation

The most important limitation of the study is the small sample size and the heterogeneity of the women recruited. A larger number is necessary to assess the maternal response to hypertension and developing of PE, as it would be testing GRK2 levels in maternal blood. In fact, it has been previously reported a correlation between lymphocytes GRK2 isolated from peripheral blood sampling and tissue GRK2.[32] Also, we have explored fetal rather than the maternal placental vasculature, which has otherwise been extensively reported in literature to be associated with incomplete spiral artery remodeling and inadequate blood supply in PE.[33]GRK2 regulation can help understanding the biology of the UA and the mechanisms of hypertension in pregnancy, which still remain to be clarified. It is necessary to relate this protein either to the endothelial dysfunction and the release of vasoconstrictive factors or to a subsequent fetal mechanism to contrast the increased placental arteries resistance.

GRK2 has not been investigated in the maternal lymphocytes of serum blood. Longitudinal observations along gestation would help to study it as tool for diagnostic screening or innovative therapeutic purposes.

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