Preeclampsia Alters Insulin Signaling Pathway Protein Expression in the High-Altitude Placenta

Maggie Stalker, Colleen Julian (PhD)
Division of Biomedical Informatics and Personalized Medicine, University of Colorado Anschutz Medical Campus

Picture taken in La Paz, Bolivia elevation 11,942 feet

Introduction

Preeclampsia (PE) is a multisystem vascular disorder originating in the placenta that is responsible for over 70,000 maternal deaths and 50,000 fetal deaths worldwide annually.[1] In Bolivia, a low to middle income country in Latin America, maternal and infant mortality rates are third highest in the Western Hemisphere.[2] This high rate is in part due to the fact that 2/3 of Bolivia's population (~35 million) reside at high altitude (≥ 2500 m) where incidence of PE, fetal growth restriction, and neonatal respiratory distress are three-fold greater than at low altitude.[3-4]

Despite the public health concern and increased funding to identify therapeutic targets and molecular markers, the precise etiology of and treatment for preeclampsia remain elusive. There has been an increasing body of literature supporting the involvement of insulin signaling pathway dysfunction in PE patients.[5] Insulin resistance has been found in placentas from PE, fetal growth restriction, and gestational diabetes pregnancies.[5-7] Additionally, impaired insulin signaling has been reported to induce vasoconstriction, perhaps contributing to the vascular dysfunction that is a hallmark of PE.[8]

Normally, binding of insulin to the insulin receptor (IR) leads to its activation, yielding phosphorylation of the insulin receptor substrate (IRS) proteins.[9] Activation of IRS creates a docking point for phosphatidylinositol 3-kinase (PI3K), and with its binding, leads to the activation of protein kinase B (Akt).[9] Once activated, Akt phosphorylates proteins involved with glucose uptake and metabolism. For example, Akt inhibits Glycogen Synthase 3 (GSK3), leading to glycogen synthesis.[10] However, in insulin resistance, activation of PI3K causes a decrease in Akt activity, leading to a reduction of glycogen synthesis, as well as decreased nitric oxide production, causing vasoconstriction.[11]

Insufficient oxygen tension, or hypoxia, has been shown to affect the insulin signaling pathway and to be involved in the pathophysiology of PE. Placental expression of markers of hypoxia, such as erythropoietin receptor (EpoR) have been shown to be increased in high-altitude or PE pregnancy.[12] Hypoxia has been shown to create a state of insulin resistance through hypoxia-inducible factor-1 alpha and could be implicated in the development of PE via insulin signaling dysfunction.[13] The PI3K/Akt pathway has also been involved in hypoxia-ischemia alongside insulin resistance, suggesting a possible link between hypoxia, the Akt pathway and insulin resistance as a potential mechanism for the development of PE.[14]

Hypothesis

- The insulin signaling pathway is impaired in preeclampsia placentas versus controls, specifically the IRS/Akt pathway protein expression is enhanced in PE.
- Placental protein expression involved with the insulin signaling pathway will be correlated with the hypoxia marker EpoR.

Aims

1. To establish whether placental insulin signaling was altered in PE cases at high altitude.
2. To determine whether PI3K/Akt protein expression was enhanced in PE placenta, and to establish the relationship between protein expression and placental hypoxia.

Methods

Patients were enrolled following written informed consent under COMIRB No. 18-0210 as well as the Bolivian equivalent operated by the Caja Nacional de Salud and its Hospital Materno-Infantil. Patients receiving prenatal care and delivering at Hospital Materno-Infantil in La Paz, Bolivia were included for study. PE was defined using American College of Obstetrics and Gynecology (ACOG): new-onset hypertension accompanied by proteinuria, thrombocytopenia, impaired liver function, renal insufficiency, cerebral or visual disturbances, or pulmonary edema.[15] Inclusion criteria were maternal age between 18-45 years, no significant health history (diabetes, chronic hypertension, collagen disease, cardiopulmonary disease), no moderate-severe anemia, singleton pregnancy, and permanent residence at high altitude. PE cases and controls were matched within three weeks for gestational age at delivery and infant sex.

At enrollment, information regarding maternal age, ethnicity, reproductive history, health history, history of placental complications, residential history, education level, and marital status was obtained from medical records or by questionnaire. Placental villous biopsies were obtained within 15 minutes after delivery of the infant. Prenatal visit information, including maternal blood pressure, laboratory values, and pregnancy complications were obtained from medical records. Newborn and delivery complications, birth weight, gestational age at delivery, infant sex, length, head circumference, and Apgar scores were recorded from hospital records.

Placental biopsies were obtained and processed per established protocols.[16] Placental samples were processed within 10 minutes of placental delivery. Protein was extracted from frozen placental tissue with total protein concentration determined by bicinchoninic acid analysis (Thermo Fisher Scientific) using 1:10 dilution of each sample. Placenta samples were then calculated to a 1 mg/ml final concentration in 0.1x sample buffer (ProteinSimple) to ensure even loading. IRS/Akt pathway protein expression in placental villous tissue homogenates was assessed by determining total Akt and pAKT (Thr308) protein expression and key upstream regulators and downstream targets, using the Western capillary electrophoresis method (ProteinSimple). Data were analyzed with Compass software (ProteinSimple).

Demographic and clinical data were collected and stored using REDCap database. Demographic characteristics were analyzed by one-way ANOVA or Chi-square test, as appropriate. Data obtained from Western electrophoresis were extracted and quantified using Biotechne's Compass software. Placental protein expression was compared between PE vs controls using independent Student's t-tests or Mann-Whitney non-parametric statistics as appropriate. Pearson correlations and linear regression models were used to test the relationship between placental protein expression and indices of maternal/fetal hypoxia. P-value <.05 was considered significant.

Results

Table 1. Maternal, Newborn and Delivery Characteristics

A. Maternal Characteristics			
Variable	Normotensive	Preeclampsia	P-value
Maternal age, yrs	32.4 ± 5.8 (36)	33.3 ± 5.3 (29)	NS
Altitude of birth, m	3391 ± 881 (36)	3644 ± 221 (29)	0.10
Residence > 2500 m, yrs	29.8 ± 8.2 (35)	31.9 ± 6.8 (29)	NS
Married or stable union, %	97 [86,100] (36)	79 [61, 90] (29)	<0.05
Ancestry, % Aymara or Quechua	53 [37, 68] (36)	52 [34, 69] (29)	NS
Height, m	1.56 ± 0.08 (35)	1.55 ± 0.07 (25)	NS
Weight pre-pregnancy, kg	58.5 ± 9.4 (33)	64.5 ± 10.3 (36)	<0.05
Smoker (prior to pregnancy), % yes	19 [10, 35] (36)	21 [10, 38] (29)	NS
Obstetric and Health History			
Parity, number	1.0 ± 0.8 (36)	0.8 ± 0.8 (29)	NS
Primiparous, % yes	28 [16, 44] (36)	41 [26, 59] (29)	NS
Initial prenatal visit, week	18.7 ± 9.1 (31)	16.2 ± 9.2 (28)	NS
Prenatal visits, number	5.8 ± 1.7 (28)	4.7 ± 2.0 (24)	<0.05
Preeclampsia (prior), % yes	12 [4, 30] (25)	29 [13, 53 (17)	NS
B. Newborn and Delivery Characteristics			
Variable	Normotensive	Preeclampsia	P-value
Gestational age, weeks	38.0 ± 2.0 (33)	35.6 ± 2.6 (26)	< 0.001
Infant sex, % male	29/32 (52.8)	16/27 (55.2)	NS
Birth weight, g	3147 ± 521 (36)	2171 ± 694 (27)	< 0.001
Birth weight, g (GA adjusted)	2913 ± 69 (33)	2460 ± 78 (26)	<0.001
Birth weight, < 5th percentile, % yes	3 (1/31) (31)	32 (8/25) (25)	<0.01
Birth weight <10th percentile, % yes	6 (2/31) (31)	48 (12/25)	<0.001
Body length, cm	47.6 ± 2.7 (35)	43.5 ± 4.2 (26)	<0.001
Head circumference, cm	34.4 ± 2.0 (35)	32.0 ± 3.7 (25)	<0.01
Chest circumference, cm	33.6 ± 1.5 (28)	29.3 ± 3.2 (15)	<0.001
Apgar, 5 min	8.9 ± 0.3 (36)	8.7 ± 0.7 (28)	0.08
Placental length, cm	19.4 ± 2.5 (35)	17.5 ± 3.1 (28)	< 0.01
Placental width, cm	16.5 ± 2.3 (36)	14.6 ± 3.3 (28)	< 0.01

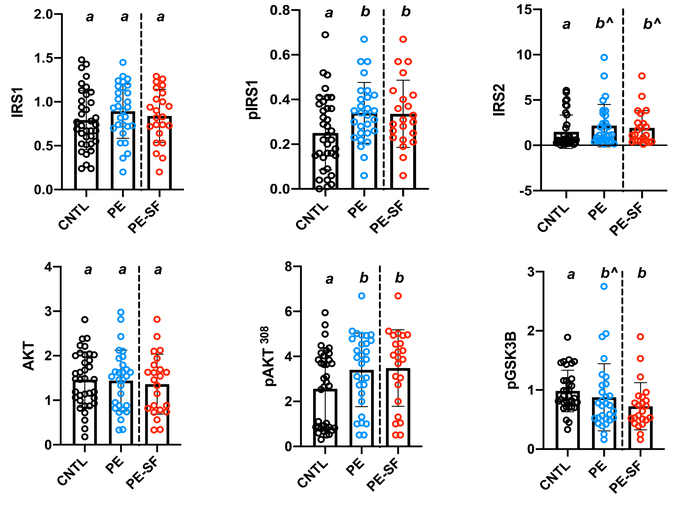

Figure 1. Placental expression of insulin signaling pathway proteins IRS1, pIRS1, IRS2, Akt, pAkt, and pGSK3B in preeclampsia, severe preeclampsia, and normotensive pregnancy (control) at high altitude.

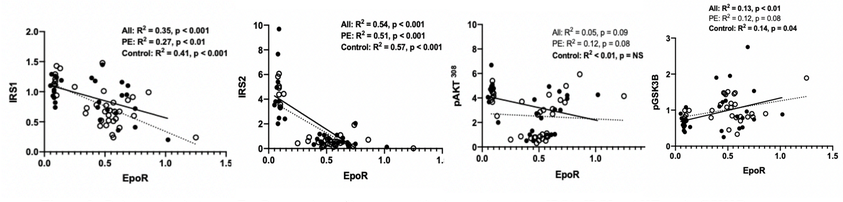

Figure 2 . Relationship between EpoR, a marker of hypoxia, with placental proteins IRS1, IRS2, pAKT, and pGSK3B.

Discussion

Our findings showed that placental insulin signaling was altered in PE cases compared to normotensive controls. Specifically, we found that pIRS1, IRS2, and pAKT protein expression levels were elevated in PE compared to controls. Additionally, placental IRS1, pIRS1, and IRS2 protein expression were inversely correlated with EpoR expression, an index of placental hypoxia, while pGSK3B and EpoR were positively associated. Here, we have shown that insulin signaling is impacted in PE pregnancy and that proteins involved in the insulin signaling pathway are associated with hypoxia, suggesting a link between insulin resistance, hypoxia, and the development of PE.

Our study results showing greater pIRS1 and a trend towards greater IRS2 in PE aligns with previous literature showing increased protein expression as a compensatory mechanism to attempt insulin sensitivity.[6,17-18] Despite concordance with this data, our results showed a greater activated Akt, while previous studies demonstrated a decrease in Akt activity.[19] Likewise a previous study of insulin resistance showed that the activation of GSK3B contributes to the induction of insulin resistance via the degradation of IRS1, but our results revealed lower pGSK3B, which insinuates a lack of insulin resistance.[20] One possible explanation is that Akt phosphorylation at both the Thr308 and Ser473 sites is required for full activity, and our results did not find a difference in Akt Ser473 phosphorylation between cases and controls; therefore, this might explain the observation that pGSK3B is not elevated in PE, despite higher pAKT Thr308 expression. Additionally, differences in our data could be due to hypoxia and its impact on the pathway, as previously described in the literature.[21-24]

The limitations of this study include that preeclampsia is a heterogeneous disease, and we accounted for numerous factors known to influence PE, but certainly, not all. Additionally, it is not routine in Bolivian prenatal care to measure proteinuria until latter stages of pregnancy, so therefore, we were unable to account for differential effects of early-onset versus late-onset PE. Lastly, unique genetic attributes or environmental exposures may have contributed to the differences between our high-altitude Bolivian cohort and prior human studies. Incorporating a sea-level Bolivian population would help us further isolate the effects of high-altitude on protein expression.

For future directions, placental metabolism protein expression will be studied to determine how dysfunction in metabolomics alters the adenosine monophosphate kinase (AMPK) and mammalian target of rapamycin (mTOR) pathway and leads to the development of PE at high altitude. Additionally, we hope to study placental protein expression in Bolivia at a sea-level population to control for genetic factors in our study.

Significance

PE is a leading cause of maternal and infant mortality and is of utmost public health concern. Understanding this disease and how hypoxia plays a role in insulin resistance would be beneficial to develop therapeutics or management procedures to reduce morbidity and mortality especially at high-altitude in under-resourced areas.

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Disclosures

The authors have no conflicts of interest to disclose.

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