Platelets in Preeclamptic Pregnancies Fail to Exhibit the Decrease in Mitochondrial Oxygen Consumption Rate Seen in Normal Pregnancies

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Background

- In normal pregnancy, placental mitochondria produce excessive reactive oxygen species[1]. An imbalance of antioxidant capacity can result in oxidative damage[1]. Preeclampsia, a leading cause of maternal and fetal/neonatal morbidity and mortality[2-4], is characterized by abnormal placentation (imbalance in pro- and anti-angiogenic factors), an inflammatory response with accompanying immunologic dysfunction, placental and maternal systemic endothelial dysfunction, and altered coagulation[5].
- Though platelets have few mitochondria per cell, established techniques for the isolation of a large number of platelets provide a sample with a relatively high mitochondrial content for respirometric analysis. Therefore, platelets are a sensitive indicator of changes in mitochondrial function[5,6], consistent with their deficiency in mitochondrial turnover. In preeclamptic pregnancy, platelets are reported to exhibit increased oxidative protein carbonyl modifications and decreased antioxidant catalase enzyme activity compared with platelets in normal pregnancy[7].

Objective

- Cellular oxygen consumption and lactate production rates have been measured in both placental and myometrial cells to study obstetrics-related disease states such as preeclampsia. Platelet metabolic alterations indicate systemic bioenergetic changes that can be useful as disease biomarkers. We tested the hypothesis that platelet mitochondria display functional alterations in preeclampsia.

Methods

Table 1 Demographic and clinical characteristics of study patients

Characteristics	Non-pregnant (n=19)		Healthy pregnant (n=20)		Preeclamptic pregnant (n=20)		P-value
	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	
Age (years)	28.50 (± 2.48)	22–32	27.25 (± 4.36)	21–35	27.05 (± 6.13)	19–39	0.775
Race/ethnicity							
NHW	14		2		6		
EI	2		0		0		
AA	1		17		11		
AS	2		0		1		
HS	1		0		2		
AF	0		1		0		
Gravidity	0	0	3.15 (± 2.83)	1–14	2.80 (± 2.48)	1–11	0.680
Parity	0	0	1.15 (± 0.99)	0–3	0.55 (± 0.95)	0–4	0.057
EGA (weeks)			$36^{1/7}$(± 4.6)		$32^{1/7}$(± 3.56)		0.005

Abbreviations: AA, African American; AF, African; AS, Asian; EGA, estimated gestational age; EI, East Indian; HS, Hispanic; NHW, non-Hispanic white. Data are presented as mean ± S.D. One-way ANOVA was used to determine intergroup differences for age. Gravidity, parity, and EGA gestational age were examined by t test for direct comparison between Healthy pregnant and Preeclamptic pregnant.

- **Platelet isolation:** Whole blood was spun at 500×*g* to separate the platelet-rich plasma, then spun at 1600×*g* to collect platelet pellets.
- **Cell counts:** Size restrictions were set to measure particles between 1.8 and 3.9 µm.
- **Platelet attachment for bioenergetic studies:** Platelets were diluted in assay measurement buffer to yield 60 million cells/well. Platelets were seeded in a 200-µl volume onto plates pre-coated with the cell adhesive. Bioenergetic measurements were initiated for all samples within 180–240 min after venipuncture.
- **Platelet bioenergetic measurements:** Following three OCR (basal oxygen consumption rate) measurements, oligomycin was injected and two OCR measurements were acquired. To induce maximal respiration, the proton ionophore 2,4-DNP was injected and two OCR measurements were performed. A second injection of 2,4-DNP was made to ensure that mitochondria were fully uncoupled. After an additional two measurements, antimycin A was injected to assess antimycin A-insensitive non-mitochondrial oxygen consumption. Three measurements were acquired and then the experiment was terminated.
- **Statistical analysis:** The pre-experimental statistical plan considered results from three groups of 20 patients using ANOVA enough to detect a 15% difference in means and a 15% difference in S.D. with a power = 0.8; α = 0.05. All results were reported as mean +/− S.D. Rates of mitochondrial respiration were compared by one-way ANOVA. Direct comparisons between two groups were compared by *t*-test with multiple linear regression analyses to control for demographic and clinical characteristics; *P*-values <0.05 were considered significant.

Results

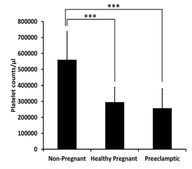

Figure 1. Platelet counts in platelet rich plasma
Platelet number per microliter was assessed in platelet rich plasma isolated from whole blood collected from non-pregnant (n=19), healthy pregnant (n=20), and preeclamptic pregnant (n=20) women. Data are presented as mean ± S.D. ***P<0.001.

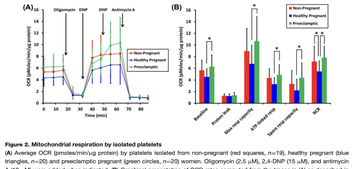

Figure 2. Mitochondrial respiration by isolated platelets
(**A**) Average OCR (pmoles/min/µg protein) by platelets isolated from non-pregnant (red squares, n=19), healthy pregnant (blue triangles, n=20) and preeclamptic pregnant (green circles, n=20) women. Oligomycin (2.5 µM), 2,4-DNP (15 µM), and antimycin A (10 µM) were added when indicated. (**B**) Graphical presentation of OCR rates computed from the traces in (A) as described in the "Materials and methods" section. Red lines or bars (non-pregnant), blue lines or bars (Healthy pregnant) and green lines or bars (Preeclamptic pregnant). Data are presented as mean ± S.D. *P<0.05.

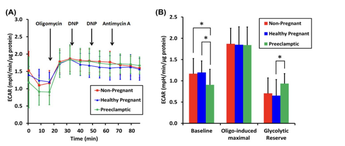

Figure 3. ECARs by isolated platelets
(**A**) Average ECAR (mpH/min/µg protein) by platelets isolated from non-pregnant (red squares, n=19), healthy pregnant (blue triangles, n=20), and preeclamptic pregnant (green circles, n=20) women. The measurements were acquired simultaneously with OCRs and platelets received the same drug injections as in Figure 2A. (**B**) Graphical presentation of ECARs computed from the traces in (A) as described in the "Results" section. Red bars (non-pregnant), blue bars (Healthy pregnant), and green bars (Preeclamptic pregnant). Data are presented as mean ± S.D. *P<0.05.

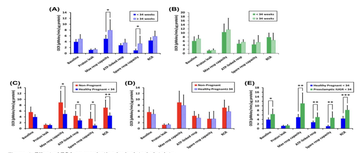

Figure 4. Effect of EGA on respiration by isolated platelets
(**A**) Average OCRs (pmoles/min/µg protein) for the experiment described by Figure 2 were compared for platelets isolated from healthy pregnant women ≥34 weeks EGA (n=8, solid blue bars) with those cases ≤34 weeks EGA (n=12, striped blue bars). (**B**) The same comparison as in (A) was made for platelets from preeclamptic pregnant ≥34 weeks EGA (n=16, solid green bar) and preeclamptic pregnant ≤34 weeks EGA (n=4, striped green bars). (**C**) Average OCRs by isolated platelets from non-pregnant women (red bars) and healthy pregnant women ≥34 weeks EGA (blue bars) were compared. (**D**) Average OCRs by isolated platelets from non-pregnant women (red bars) and ≤34 weeks EGA (blue bars) were compared. (**E**) Average OCRs between healthy pregnant women ≥34 weeks EGA (n=8, blue bars) and preeclamptic pregnant ≤34 weeks EGA that also had IUGR (n=10, green bars) were compared. Data are presented as mean ± S.D. *P<0.05, **P<0.01, ***P<0.001.

Table 2 Multivariate regression analysis of associations between OCR and clinical parameters

	Coefficients[1]			
	Unstandardized coefficients			
Model	B	S.E.M.	t	P-value
Baseline				
(Constant)	4.213	0.689	6.110	<0.001
Pregnancy group	2.004	0.625	3.205	0.003
EGA	0.956	0.609	1.570	0.125
Parity	−0.358	0.574	−0.624	0.537
Maximal respiratory capacity				
(Constant)	5.387	1.496	3.601	<0.001
Pregnancy group	4.808	1.357	3.544	0.001
EGA group	2.241	1.321	1.697	0.098
Parity	0.0278	1.245	0.0224	0.982
ATP-linked respiration				
(Constant)	3.101	0.563	5.507	<0.001
Pregnancy group	1.793	0.511	3.511	0.001
EGA group	0.775	0.497	1.558	0.128
Parity	−0.396	0.469	−0.845	0.404
Spare respiratory capacity				
(Constant)	1.174	0.896	1.311	0.198
Pregnancy group	2.804	0.812	3.451	0.001
EGA group	0.775	0.791	1.626	0.113
Parity	0.386	0.746	0.517	0.608
RCR				
(Constant)	5.139	0.764	6.730	<0.001
Pregnancy group	2.607	0.692	3.765	<0.001
EGA group	0.783	0.674	1.161	0.253
Parity	−0.227	0.635	−0.357	0.723

[1]Dependent variable: OCR.

Discussion

- Our results support the use of platelet OCR and ECAR (extracellular acidification rate) measurements as tools to better understand the potential differences in systemic energy metabolism between non-preeclamptic pregnancy and preeclamptic pregnancy with and without IUGR.
- We made three striking observations: (i) there is a decline in platelet mitochondrial respiratory function associated with third-trimester non-preeclamptic pregnancy; (ii) this decline is entirely absent from platelets harvested from third-trimester pregnancies afflicted with preeclampsia; and (iii) platelets from preeclamptic pregnancies display decreased ECAR, consistent with the possibility of decreased glycolysis and increased oxidative phosphorylation.
- It remains to be determined whether the reversal of the pregnancy-associated platelet respiratory suppression is relatively unique to preeclampsia or is a feature of multiple disease states. It also remains to be seen whether the pregnancy-linked change in platelet mitochondrial function is adaptive and/or whether loss of this mechanism in preeclampsia contributes to the disease.

Conclusion

- Given that mitochondrial bioenergetics abnormalities have been observed in the placenta and myometrial cells harvested at delivery of preeclamptic pregnancies, we test the hypothesis that preeclampsia is accompanied by dysfunction of mitochondrial energy metabolism (cellular oxygen consumption and lactate production rates) in circulating maternal platelets during pregnancy.
- The present study found a decline in platelet mitochondrial respiratory function associated with third-trimester normal pregnancy prior to 34 weeks' EGA, a decline absent from platelets harvested in the third-trimester pregnancy afflicted with preeclampsia, and a decrease in ECAR in preeclamptic pregnancy consistent with decreased glycolysis and increased oxidative phosphorylation.
- Platelet bioenergetics in preeclampsia are altered compared with those of normal pregnancy suggesting the effects of preeclampsia on systemic mitochondrial energy metabolism as well as future studies to confirm circulating platelet bioenergetics as a tool to monitor preeclampsia-associated changes in systemic mitochondrial bioenergetics during pregnancy.

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