



## Relationship between adiponectin and nitrite in healthy and preeclampsia pregnancies



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### ABSTRACT

**Background:** Controversial results have been reported regarding plasma adiponectin levels in preeclampsia (PE) compared to healthy pregnancies (HP). Adiponectin activates eNOS, increasing the levels of the vasodilator nitric oxide (NO). PE reduces the levels of nitrite (an NO marker) and induces higher levels of ADMA (an endogenous eNOS inhibitor) compared to HP. No previous study has examined whether a positive correlation exists between adiponectin and nitrite in HP and PE and how ADMA may interfere with this correlation.

**Methods:** We measured plasma nitrite concentrations using an ozone-based chemiluminescence assay, and plasma ADMA and adiponectin levels using enzyme immunoassays in 117 pregnant women (70 healthy and 47 preeclamptic).

**Results:** We found higher adiponectin levels ( $23.6 \pm 13.0$  vs.  $17.8 \pm 5.6$   $\mu\text{g/ml}$ ;  $P < 0.05$ ) and lower plasma nitrite levels ( $104.5 \pm 84.3$  vs.  $177.2 \pm 151.3$  nM;  $P < 0.05$ ) in PE compared to HP. We found a significant positive correlation between these markers in HP ( $r = 0.3$ ;  $P < 0.05$ ), but no correlation in PE. However, when we grouped PE women regarding ADMA levels (low and high levels), a strong positive correlation was found in the group with lower ADMA levels ( $r = 0.67$ ;  $P < 0.05$ ), suggesting that high ADMA concentrations may interfere with the physiological activation of eNOS by adiponectin in PE.

**Conclusions:** Our findings showed higher levels of adiponectin and lower nitrite levels in PE compared to HP, and these levels were positively correlated in HP and in PE presenting lower concentrations of ADMA.

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### 1. Introduction

Preeclampsia (PE) is a syndrome of pregnancy characterized by hypertension and proteinuria after 20 weeks of gestation which affects 3–5% of all pregnancies. The development of PE results in maternal and fetal complications, including renal failure, cerebral edema, liver failure, low birth weight, prematurity, and death [1]. Although the pathophysiology of preeclampsia is not completely understood, several lines of evidence point to a failure of cytotrophoblast invasion and the absence of dilatation of uterine arteries leading to a high-resistance uteroplacental circulation, thus decreasing placental perfusion and causing hypoxia, which in turn induces the release of factors causing systemic endothelial dysfunction into the maternal circulation [2].

Some authors have suggested the involvement of adiponectin in PE pathology due to its participation in endothelial nitric oxide synthase (eNOS) activation and consequently in nitric oxide (NO) synthesis,

which plays an essential role in the reduction of blood pressure during healthy pregnancy [3]. Additionally, lower NO bioavailability has been observed in preeclamptic women compared to healthy pregnant women [4]. Abnormal circulating adiponectin levels have been detected in many cardiovascular conditions [5–7], including PE [8,9]. However, the results are controversial, with some studies demonstrating higher levels of adiponectin in PE compared to healthy pregnant (HP) women [8,10], and others showing decreased levels in PE women [11,12].

In the present study, we compared the circulating adiponectin concentrations in healthy pregnant women to those found in preeclamptic women. Moreover, based on the fact that adiponectin activates endogenous NO formation, we hypothesized that nitrite levels are positively associated with plasma adiponectin concentrations in pregnant women.

### 2. Materials and methods

#### 2.1. Subjects

Approval for use of human subjects was obtained from the Institutional Review Board at the Faculty of Medicine of Ribeirao Preto,

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University of Sao Paulo, Brazil, and informed consent was obtained from all participants. A total of 117 pregnant women were enrolled in the Department of Obstetrics and Gynecology, University Hospital of the Faculty of Medicine of Ribeirao Preto. Of these, 70 were HP women with uncomplicated pregnancies and 47 had PE. Hypertensive disorders were defined according to the guidelines of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (NHBPEP) [13].

Preeclampsia was defined as increased blood pressure ( $\geq 140$  mm Hg systolic or  $\geq 90$  mm Hg diastolic on the occasion of 2 or more measurements at least 6 h apart) with significant proteinuria ( $\geq 0.3$  g/24 h) in a woman after 20 weeks of gestation. No women with pre-existing hypertension, with or without superimposed PE, were included in the present study. Exclusion criteria were: twin or multiple pregnancies, pre-pregnancy body mass index (BMI) over  $30 \text{ kg/m}^2$  or any evidence of previous medical diseases. Intrauterine growth restriction (IUGR) was considered to be present if the infant birth weight was the below the 10th percentile of a normal reference curve reported for the Brazilian population [14].

At the time of clinic attendance, maternal venous blood samples were collected into standard Vacutainer tubes (Becton–Dickinson) containing heparin (to measure plasma nitrite concentrations) and EDTA (to measure plasma ADMA and adiponectin concentrations). The tubes were immediately centrifuged at room temperature and plasma samples were stored at  $-70^\circ\text{C}$  until the time for biomarker measurements.

## 2.2. Assessment of biomarkers

Plasma concentrations of ADMA were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (DLD Diagnostika GmbH, Hamburg, Germany) according to manufacturer's instructions. Plasma adiponectin concentrations were measured using commercially available ELISA kits (Human Adiponectin EZHADP-61K – Millipore, St. Charles, MO), according to the manufacturer's instructions.

Nitrite concentration was measured by injecting  $200 \mu\text{l}$  of plasma samples into a solution of acidified triiodide and purging with nitrogen in-line with a gas-phase chemiluminescence NO analyzer (Sievers Model 280 NO Analyzer). Approximately  $8 \text{ ml}$  of triiodide solution ( $2.0 \text{ g}$  of potassium iodide and  $1.3 \text{ g}$  of iodine dissolved in  $40 \text{ ml}$  of water with  $140 \text{ ml}$  of acetic acid) were placed in the purge vessel into which plasma samples were injected. The triiodide solution reduced nitrites to NO gas, which was detected by the NO analyzer [4].

## 2.3. Statistical analysis

Clinical features and biomarker data are reported as mean  $\pm$  SD. The groups assessed were compared by the Student *t*-test. Biomarkers were correlated by the Pearson test. The level of significance was set at  $P < 0.05$ .

## 3. Results

Clinical data were collected from the HP and PE groups (Table 1). We found no statistically significant differences in age, % primigravida or BMI between groups PE (all  $P > 0.05$ ). However, PE women had a significantly higher percentage of IUGR and higher systolic and diastolic blood pressure levels than HP. Lower birth weights and gestational ages at delivery were found in PE compared to HP. Although a significant difference was found regarding gestational age sampling, none of the parameters measured was influenced by this variation. As previously reported [4], plasma nitrite levels were significantly reduced in PE compared to HP, while plasma adiponectin levels were 32.6% higher in PE compared to HP ( $P < 0.05$ ; Table 1). In addition, we performed correlation analysis between adiponectin

**Table 1**  
Demographic characteristics of study participants.

Parameters	Healthy pregnancy	Preeclampsia
<i>n</i>	70	47
Age (y)	25.1 $\pm$ 5.8	27.1 $\pm$ 7.7
Primigravida (%)	44.3	42.5
SBP (mm Hg)	114.5 $\pm$ 10.1	143.3 $\pm$ 16.7*
DBP (mm Hg)	74.0 $\pm$ 8.5	89.5 $\pm$ 14.1*
GA sampling	36.8 $\pm$ 2.6	34.2 $\pm$ 4.0*
GAD (weeks)	39.7 $\pm$ 1.4	35.7 $\pm$ 3.7*
Pre-pregnancy BMI ( $\text{kg/m}^2$ )	22.8 $\pm$ 3.3	23.8 $\pm$ 4.0
Newborn weight (g)	3341.0 $\pm$ 596.0	2411.2 $\pm$ 889.7*
IUGR (%)	6.1	28.2
24-h-Pr (mg/24 h)	ND	1418.7 $\pm$ 1379.4
Creatinine (mg/dl)	ND	0.68 $\pm$ 0.14
Plasma nitrite (nM)	177.2 $\pm$ 151.3	104.5 $\pm$ 84.3*
Adiponectin ( $\mu\text{g/ml}$ )	17.8 $\pm$ 5.6	23.6 $\pm$ 13.0*

SBP, systolic blood pressure; DBP, diastolic blood pressure; GA, gestational age; GAD, gestational age at delivery; BMI, body mass index; IUGR, intrauterine growth restriction; 24-h-Pr, 24-h proteinuria; ND: not determined. Values are the mean  $\pm$  S.D.

\*  $P < 0.05$  vs. healthy pregnant group.

and BMI and found a negative correlation in the HP group ( $r = -0.40$ ;  $P = 0.001$ ), but not in the PE group ( $r = -0.08$ ;  $P = 0.61$ ).

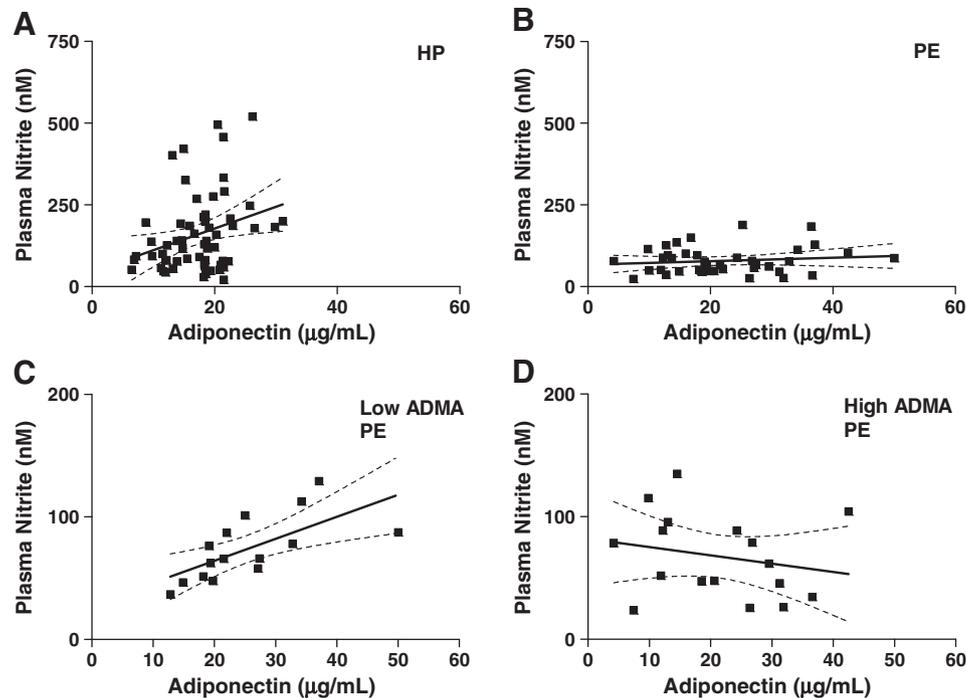
To determine whether any association existed between the circulating adiponectin and nitrite levels (NO marker); we performed correlation analysis separately in HP and PE women. We observed a significant positive correlation between adiponectin and nitrite levels only in the HP group ( $r = 0.3$ ;  $P = 0.02$ ; Fig. 1A), but not in the PE group ( $r = 0.12$ ;  $P = 0.42$ ; Fig. 1B). Since adiponectin activates eNOS, we assume that the endogenous eNOS inhibitor (ADMA), which is elevated in PE [15], may interfere with a possible correlation between adiponectin and nitrite levels in the PE group. Thus, we measured ADMA in a set of PE women ( $n = 34$ ) and sub-grouped those presenting the 50% lowest (L group,  $n = 17$ ) and 50% highest (H group,  $n = 17$ ) ADMA levels. Next, we calculated the correlation between nitrite and adiponectin in each group. Interestingly, we found a positive correlation between adiponectin and nitrite levels in the L group ( $r = 0.67$ ;  $P = 0.006$ , Fig. 1C), whereas no correlation was detected in the H group ( $r = -0.22$ ,  $P = 0.39$ , Fig. 1D).

## 4. Discussion

This is the first study to demonstrate a positive association between circulating adiponectin concentrations and nitrite levels in HP. Moreover, we observed increased levels of adiponectin in PE compared to HP, and reduced nitrite levels in the PE group.

Several studies have measured adiponectin levels in PE expecting reduced values compared to HP since this molecule activates eNOS. However, literature findings are controversial [8,10–12] moreover, no study was performed in the Brazilian population. Ramsay et al. [8] were the first to demonstrate high levels of adiponectin in PE, and they suggested that this increase was due to the increased release mediated by excessive lipolysis in adipocytes or a physiological response to minimize the excess accumulated fat tissue in PE. However, other authors [16] have suggested that elevated adiponectin levels may represent a response caused by an imbalance of angiogenic factors derived from the placenta in patients with PE. On the other hand, Sullivan et al. [17] suggested that the higher levels of adiponectin do not indicate an important link between PE and endothelial dysfunction, suggesting the study of adiponectin isoforms to amplify our knowledge. However, studies addressing the evaluation of adiponectin isoforms are also controversial [18,19].

Besides the importance of comparing the adiponectin levels between the HP and PE groups, our results also reflect a biological effect on endothelial function. Therefore, the current study aimed to evaluate this process through the correlation of adiponectin concentration with plasma levels of nitrite, and we found a contribution of NO



**Fig. 1.** Correlation between plasma adiponectin and nitrite levels in healthy pregnant women (A), all preeclampsia pregnant women (B), and in pregnant women with preeclampsia according to the lowest (L group, C) and highest (H group, D) ADMA levels.

synthesis via adiponectin in HP. Similarly to obesity, human pregnancy is characterized by increased body weight including fat deposits and consequently elevations in the concentrations of adipokines [20], in particular adiponectin. The latter, in turn, through interaction with specific receptors promotes activation of eNOS and thus of NO. Plasma nitrite levels represent approximately 70% of eNOS [21], and inhibition of NO synthase activity was associated with corresponding decreases in circulating nitrite concentrations [22]. We observed a significant decrease of nitrite levels in PE compared to HP, supporting previous findings [4]. Interestingly, when we sub-grouped PE women into subjects with lower or higher levels of ADMA, the endogenous NOS inhibitor, we found a positive correlation between adiponectin and nitrite, suggesting that higher ADMA concentrations may interfere with the physiological activation of eNOS by adiponectin. The biological mechanism behind this finding is still unknown, limiting an in-depth discussion of our work. However, these results can direct other clinical and basic studies, besides demonstrating once again the importance of ADMA in the physiology of PE, as reported in several studies [15,23–26].

In conclusion, our findings show higher levels of adiponectin and lower nitrite levels in PE compared to HP, and these levels are positively correlated in HP and in PE presenting lower concentration of ADMA. Furthermore, these results have important implications for future studies on adiponectin, NO and ADMA biology and therapeutic perspectives for PE.

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### References

[1] Sibai BM. Intergenerational factors. A missing link for preeclampsia, fetal growth restriction, and cardiovascular disease? *Hypertension* 2008;7:7.

- [2] Molvarec A, Szarka A, Walentin S, Szucs E, Nagy B, Rigo Jr J. Circulating angiogenic factors determined by electrochemiluminescence immunoassay in relation to the clinical features and laboratory parameters in women with pre-eclampsia. *Hypertens Res* 2010;33:892–8.
- [3] Zhu W, Cheng KK, Vanhoutte PM, Lam KS, Xu A. Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention. *Clin Sci (Lond)* 2008;114:361–74.
- [4] Sandrim VC, Palei AC, Metzger IF, Gomes VA, Cavalli RC, Tanus-Santos JE. Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia. *Hypertension* 2008;52:402–7.
- [5] Ouchi N, Ohishi M, Kihara S, et al. Association of hypo adiponectinemia with impaired vasoreactivity. *Hypertension* 2003;42:231–4.
- [6] Arahamian TR, Sam F. Adiponectin in cardiovascular inflammation and obesity. *Int J Inflamm* 2011;376909.
- [7] Wang W, Xing W, Zhang H, et al. Reduced high molecular weight adiponectin is an independent risk factor of cardiovascular lesions in hypercholesterolemic patients. *Clin Endocrinol Apr* 2013;78(4):539–44 (Oxf).
- [8] Ramsay JE, Jamieson N, Greer IA, Sattar N. Paradoxical elevation in adiponectin concentrations in women with preeclampsia. *Hypertension* 2003;42:891–4.
- [9] Avci I, Ozerkan K, Uncu G. Serum adiponectin levels increase in lean preeclamptic women. *Prenat Diagn* 2010;30:91–2.
- [10] Naruse K, Yamasaki M, Umekage H, Sado T, Sakamoto Y, Morikawa H. Peripheral blood concentrations of adiponectin, an adipocyte-specific plasma protein, in normal pregnancy and preeclampsia. *J Reprod Immunol* 2005;65:65–75.
- [11] D'Anna R, Baviera G, Corrado F, et al. Adiponectin and insulin resistance in early- and late-onset pre-eclampsia. *BJOG* 2006;113:1264–9.
- [12] Herse F, Bai Y, Staff AC, Yong-Meid J, Dechend R, Zhou R. Circulating and uteroplacental adipocytokine concentrations in preeclampsia. *Reprod Sci* 2009;16:584–90.
- [13] Anonymous. Report of the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy. *Am J Obstet Gynecol* 2000;183:S1–S22.
- [14] Pedreira CE, Pinto FA, Pereira SP, Costa ES. Birth weight patterns by gestational age in Brazil. *An Acad Bras Cienc* 2011;83:619–25.
- [15] Sandrim VC, Palei AC, Metzger IF, Cavalli RC, Duarte G, Tanus-Santos JE. Interethnic differences in ADMA concentrations and negative association with nitric oxide formation in preeclampsia. *Clin Chim Acta* 2010;411:1457–60.
- [16] Suwaki N, Masuyama H, Nakatsukasa H, et al. Hypoadiponectinemia and circulating angiogenic factors in overweight patients complicated with pre-eclampsia. *Am J Obstet Gynecol* 2006;195:1687–92 [Epub 2006 Jun 1612].
- [17] Sullivan KJ, Kissoon N, Duckworth LJ, et al. Low exhaled nitric oxide and a polymorphism in the NOS1 gene is associated with acute chest syndrome. *Am J Respir Crit Care Med* 2001;164:2186–90.
- [18] Mazaki-Tovi S, Romero R, Vaisbuch E, et al. Maternal serum adiponectin multimers in preeclampsia. *J Perinat Med* 2009;37:349–63.
- [19] Takemura Y, Osuga Y, Koga K, et al. Selective increase in high molecular weight adiponectin concentration in serum of women with preeclampsia. *J Reprod Immunol* 2007;73:60–5.

- [20] Nien JK, Mazaki-Tovi S, Romero R, et al. Adiponectin in severe preeclampsia. *J Perinat Med* 2007;35:503–12.
- [21] Kleinbongard P, Dejam A, Lauer T, et al. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic Biol Med* 2003;35:790–6.
- [22] Lauer T, Preik M, Rassaf T, et al. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc Natl Acad Sci U S A* 2001;98:12814–9.
- [23] Pettersson A, Hedner T, Milsom I. Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. *Acta Obstet Gynecol Scand* 1998;77:808–13.
- [24] Fickling SA, Williams D, Vallance P, Nussey SS, Whitley GS. Plasma concentrations of endogenous inhibitor of nitric oxide synthesis in normal pregnancy and pre-eclampsia. *Lancet* 1993;342:242–3.
- [25] Holden DP, Fickling SA, Whitley GS, Nussey SS. Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. *Am J Obstet Gynecol* 1998;178:551–6.
- [26] Ellis J, Wennerholm UB, Bengtsson A, et al. Levels of dimethylarginines and cytokines in mild and severe preeclampsia. *Acta Obstet Gynecol Scand* 2001;80:602–8.