

Maternal serum adiponectin multimers in preeclampsia

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Abstract

Objective: Obesity, insulin resistance, and dyslipidemia are associated with preeclampsia. Recently, “adipose tissue failure”, characterized by dysregulation of adipokine production, has been implicated in the pathophysiology of these metabolic complications. Adiponectin, an insulin-sensitizing, anti-atherogenic, anti-inflammatory and angiogenic adipokine, circulates in oligomeric complexes including: low-molecular-weight (LMW) trimers, medium-molecular-weight (MMW) hexamers and high-molecular-weight (HMW) isoforms. These multimers exert differential biological effects, and HMW to total adiponectin ratio (S_n) has been reported to be a specific marker of adiponectin activity. The aim of this study was to determine whether preeclampsia is associated with changes in circulating adiponectin multimers.

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Study design: This cross-sectional study included women with: 1) normal pregnancy ($n=225$); and 2) patients with mild preeclampsia ($n=111$). The study population was further stratified by first trimester BMI (normal weight <25 kg/m² vs. overweight/obese ≥ 25 kg/m²). Serum adiponectin multimers (total, HMW, MMW and LMW) concentrations were determined by ELISA. Non-parametric statistics were used for analysis.

Results: 1) The median maternal HMW and LMW adiponectin concentrations were lower in patients with preeclampsia than in those with normal pregnancies ($P<0.001$ and $P=0.01$, respectively); 2) patients with preeclampsia had a lower HMW/total adiponectin ratio ($P<0.001$) and higher MMW/total adiponectin and LMW/total adiponectin ratios than those with a normal pregnancy ($P<0.001$ and $P=0.009$, respectively); 3) the presence of preeclampsia was independently associated with lower maternal serum HMW adiponectin concentrations ($P=0.001$) and with a low HMW/total adiponectin ratio ($P<0.001$) after correction for maternal age, maternal BMI, the difference in BMI between the third and the first trimester, and gestational age at sampling; and 4) overweight/obese pregnant women had a lower median total and HMW adiponectin concentration than normal weight pregnant women among women with normal pregnancies, but not among those with preeclampsia.

Conclusion: 1) Preeclampsia is associated with a lower median concentration of the HMW adiponectin isoform, the most active form of this adipokine, and a low HMW/total adiponectin ratio, a specific marker of adiponectin biologic activity; 2) in contrast to normal pregnancy, preeclampsia is not associated with decreased circulating adiponectin multimers in overweight/obese individuals suggesting altered regulation of this adipokine in preeclampsia; 3) collectively, these findings suggest that preeclampsia is characterized by alterations in adiponectin multimers and their relative distribution implying a role for adiponectin multimers in the mechanism of disease in preeclampsia.

Keywords: Adipokines; BMI; high-molecular-weight (HMW) adiponectin; low-molecular-weight (LMW) adiponectin; medium-molecular-weight (MMW) adiponectin; obesity; overweight; pregnancy.

Introduction

Preeclampsia, one of the “great obstetrical syndromes” [139], is a leading cause of maternal and perinatal morbidity and mortality [35, 89, 150, 153]. Consistent with

its syndromic nature, preeclampsia has been associated with an anti-angiogenic state [18, 23–25, 36, 39, 40, 49, 50, 78, 80, 85, 86, 90, 93, 98, 106, 140, 166, 181], endothelial cell dysfunction [28, 60, 77, 114, 129, 133, 147, 161], an exaggerated intravascular pro-inflammatory response [11, 22, 37, 47, 49, 53, 132, 143, 163] and a predominantly T helper (Th1)-biased immune response [30, 81, 108, 144, 158, 170]. In addition, preeclampsia has been associated with metabolic complications such as obesity, insulin resistance and dyslipidemia. This is of special importance, since the rising prevalence of metabolic complications is glaringly evident in women of reproductive age who are increasingly plagued by obesity [20] and because these metabolic alterations can be subjected to therapeutic manipulation by lifestyle modifications, medications or surgery.

A growing body of evidence strongly supports the association between preeclampsia and common metabolic complications: 1) obesity is an independent risk factor for preeclampsia [12, 20, 29, 38, 138, 152, 153, 174]; 2) patients with insulin resistance are more likely to develop preeclampsia [46, 64, 67, 69, 95, 141, 157, 176]; 3) preeclampsia is also associated with hypertriglyceridemia [51, 171], hypercholesterolemia [162], increased concentrations of free fatty acids [91] and reduced high density lipoprotein (HDL) concentrations [171]; and 4) women who had preeclampsia have an increased risk for metabolic syndrome-related morbidity [27, 48, 54, 154] and mortality [65, 68, 156] later in life. Despite the compelling evidence for the association between obesity-related complications and preeclampsia, the mechanism by which excess adipose tissue exerts its deleterious effect and predisposes pregnant women to develop preeclampsia remains unknown.

Adiponectin is the most abundant gene (*AMP1*) product of adipose tissue [62, 94, 110, 146] and it circulates at high concentrations in plasma [6, 62, 180]. Paradoxically, circulating concentrations of this adipokine are lower in obese than in non-obese subjects [8, 62], suggesting that an excess of adipose tissue can exert a negative feedback on adiponectin production or secretion. Adiponectin has been implicated in the pathophysiology of insulin resistance [14, 45, 55, 61, 175, 179], atherosclerosis [121, 123], hypertension [66, 72], dyslipidemia [97], and angiogenesis [124, 151]. These unique properties have prompted many investigators to explore the putative role of adiponectin in the physiologic metabolic adaptation during human pregnancy [21, 102, 107, 117], gestational diabetes mellitus (GDM) [73, 131, 177] and preeclampsia [31, 33, 58, 59, 70, 92, 113, 116, 130].

Adiponectin circulates in human plasma in distinct forms: 1) low-molecular-weight (LMW) trimers; 2) medium-molecular-weight (MMW) hexamers; and 3) high-molecular-weight (HMW) oligomers (12–18 subunits) [8, 126, 146, 168, 169, 172]. The significance of the various adiponectin multimers stems from the fact

that each can exert differential biological effects [15, 75, 126, 169, 172]. Furthermore, several studies have highlighted the importance of the adiponectin sensitivity index (the ratio of HMW to total adiponectin- S_n) [127] as a sensitive marker of its biological activity [9, 15, 44, 56, 71, 75, 109, 111, 127, 165]. Collectively these findings suggest that the physiological activity of adiponectin is primarily determined by the relative distribution of its isoforms.

Data regarding circulating maternal adiponectin multimers concentrations in human pregnancy are scarce [21, 42, 122, 134, 135, 160]. Indeed, only two studies reported the relative distribution of adiponectin isoforms in patients with preeclampsia [42, 160], of which only one [160] included all three isoforms in the analysis. Thus, the aim of this study was to determine whether there are changes in adiponectin multimers in normal and overweight/obese patients with preeclampsia.

Materials and methods

Study groups and inclusion criteria

A cross-sectional study was conducted including patients in the following groups: 1) normal pregnant women ($n=225$); and 2) patients with mild preeclampsia ($n=111$). The study population was further stratified by first trimester body mass index (BMI: normal weight 18.5–24.9 kg/m² vs. overweight/obese ≥ 25 kg/m²). Samples were taken immediately after the diagnosis of preeclampsia.

Samples and data were retrieved from our bank of biological samples and clinical databases. Many of these samples have previously been employed to study the biology of inflammation, hemostasis, angiogenesis regulation, and growth factor concentrations in normal pregnant women and those with pregnancy complications.

Written informed consent was obtained from all participants after approval by the Institutional Review Boards of the Sotero del Rio Hospital (Chile), Wayne State University (Detroit, Michigan, USA) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD; Bethesda, MD, USA).

Clinical definitions

The inclusion criteria for normal pregnant women were: singleton gestation, no prior diabetes mellitus, no maternal or fetal complications during pregnancy, normal plasma glucose concentrations in the first trimester, normal oral glucose challenge test [3, 19], and delivery at term of a healthy neonate with a birth-weight above the 10th percentile for gestational age [7].

Preeclampsia was defined as the presence of hypertension (systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg on at least two occasions, 4 h to 1 week apart) and proteinuria (≥ 300 mg in a 24-h urine collection or at least one dipstick measurement $\geq 2+$) [1].

The BMI was calculated according to the formula: weight (kg)/height (m²). Normal weight women were defined as those with a BMI of 18.5–24.9 kg/m² according to the definition of the

World Health Organization (WHO) [2]. Pregnant women were classified by their first trimester BMI into two groups: normal weight and overweight/obese (BMI ≥ 25 kg/m²).

Sample collection and quantitative determination of multimeric forms of adiponectin in maternal serum

Maternal blood samples were collected with a vacutainer into tubes. Samples were centrifuged and the sera were stored at -80°C until analysis. Sensitive enzyme-linked immunoassays were used to determine the concentrations of adiponectin multimeric forms in maternal serum immunoassays were purchased from ALPCO Diagnostics (Salem, NH, USA). The assays were run according to the manufacturer's recommendations. To detect HMW adiponectin, serum samples were pre-treated with a specific protease that selectively digested MMW and LMW adiponectin. We were also able to determine the combined HMW and MMW adiponectin concentrations by pre-treating the samples with a protease that specifically digested LMW adiponectin. Maternal serum samples were assayed directly to determine total adiponectin concentrations. Briefly, untreated and pre-treated maternal serum samples were incubated in duplicate wells of the micro titer plates, which had been pre-coated with a monoclonal antibody specific for adiponectin. During this incubation any adiponectin present in the standards and untreated or pre-treated maternal serum samples was bound by the immobilized antibodies. After repeated washing and aspiration to remove all unbound substances, an enzyme-linked polyclonal antibody specific for adiponectin was added to the wells. Unbound materials were removed with repeated washing and a substrate solution was added to the wells and color developed in proportion to the amount of adiponectin bound in the initial step. The color development was stopped with the addition of an acid solution and the intensity of color was read using a programmable spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA, USA). The concentration of adiponectin in untreated and treated maternal serum samples was determined by interpolation from individual standard curves composed of human adiponectin. Total, HMW, and HMW-MMW adiponectin concentrations were derived directly from the assay plates. MMW adiponectin concentrations were obtained by subtracting HMW adiponectin value from the combined HMW-MMW value. Finally, the LMW adiponectin value was computed by subtracting HMW and MMW adiponectin values from the total adiponectin values. The calculated inter- and intra-assay coefficients of variation for adiponectin multimers immunoassays in our laboratory were 2.2% and 4.2%, respectively. The sensitivity was calculated to be 0.04 ng/mL.

Statistical analysis

Normality of the data was tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Since serum multimeric adiponectin isoforms concentrations were not normally distributed, Kruskal-Wallis tests with post-hoc analysis by Mann-Whitney *U*-tests were used for comparisons of continuous variables. Comparison of proportions was performed by χ^2 or Fisher's exact tests. Multiple linear regression analysis was performed to determine which factors were significantly and independently associated with maternal serum adiponectin isoforms concentrations as well as their relative distribution; due to skewed distribution logarithmic (log) transformation was employed in the latter analysis. The following parameters were included in the

model: maternal age, maternal BMI (normal weight vs. overweight/obese), the difference in BMI between the third and the first trimesters, gestational age at sampling, and the presence of preeclampsia. A $P < 0.05$ was considered statistically significant. Analysis was performed with SPSS, version 14 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 displays the demographic and clinical characteristics of women with a normal pregnancy and those with preeclampsia. Patients with preeclampsia had a higher first and third trimester BMI and delivered smaller neonates. There were no significant differences in maternal age, parity, gestational age at blood sampling, gestational age at delivery and the third-to-first trimester BMI difference between patients with a normal pregnancy and those with preeclampsia (Table 1). Table 2 displays the demographic and clinical characteristics of the study population according to BMI. In both groups (normal pregnancy and preeclampsia) normal weight pregnant women were younger than overweight/obese patients. There were no significant differences in parity, gestational age at blood sampling, gestational age at delivery and neonatal birth-weight between normal weight and overweight/obese women in both groups (Table 2).

Adiponectin multimers concentrations and their relative distribution in preeclampsia vs. normal pregnancy

The median maternal serum concentration of total adiponectin was lower in patients with preeclampsia than those with a normal pregnancy (median: 5035 ng/mL, interquartile range [IQR] 3329–6979 vs. 6415 ng/mL, IQR 4651–8630; $P < 0.001$, Figure 1). Similarly, patients with preeclampsia had a lower median serum concentration of HMW (2348 ng/mL, IQR 1259–3763 vs. 3619 ng/mL, IQR 2252–5142; $P < 0.001$, Figure 1) and LMW adiponectin (1084 ng/mL, IQR 812–1493 vs. 1304 ng/mL, IQR 919–1798; $P = 0.01$, Figure 1) than those with a normal pregnancy. The median maternal serum concentration of MMW adiponectin did not differ between patients with preeclampsia and women with a normal pregnancy (1386 ng/mL, IQR 892–1789 vs. 1427 ng/mL, IQR 1010–1902; $P = 0.7$, Figure 1).

The median maternal HMW/total adiponectin ratio was lower in patients with preeclampsia than in those with a normal pregnancy (0.46, IQR 0.36–0.55 vs. 0.55, IQR 0.47–0.62; $P < 0.001$, Figure 2). In contrast, patients with preeclampsia had a higher median MMW/total adiponectin ratio (0.29, IQR 0.22–0.35 vs. 0.22, IQR 0.16–0.28; $P < 0.001$, Figure 2) as well as a higher LMW/total adiponectin ratio (0.25, IQR 0.17–0.31 vs. 0.21, IQR 0.15–0.28; $P = 0.009$, Figure 2) than those with a normal pregnancy.

Table 1 Clinical and demographic characteristics of the study population.

	Normal pregnancy (n = 225)	Preeclampsia (n = 111)	P-value
Maternal age (years)	32.8 (29.4–39.2)	36.8 (32.4–38.3)	NS
Parity	1 (0–2)	2 (1–4)	NS
First trimester BMI (kg/m ²)	23.5 (21.6–26.7)	29.1 (24.5–35.8)	<0.001
Third trimester BMI (kg/m ²)	27.3 (25.0–32.4)	30.5 (24.5–42.7)	<0.001
BMI difference between the third and first trimesters	5.6 (4.3–7.1)	5.8 (4.3–7.1)	NS
Gestational age at blood sampling (weeks)	32.8 (29.4–39.2)	36.3 (32.4–38.3)	NS
Gestational age at delivery (weeks)	39.9 (39.0–40.4)	37.0 (33.4–38.3)	NS
Birth weight (g)	3460 (3220–3670)	2517 (1840–3142)	<0.001

Values are expressed as median (interquartile range); NS = not significant; BMI = body mass index.

Table 2 Clinical and demographic characteristics of the study population according to body mass index.

	Normal pregnancy		P-value	Preeclampsia		P-value
	Normal weight (n = 141)	Overweight/obese (n = 84)		Normal weight (n = 48)	Overweight/obese (n = 63)	
Maternal age (years)	25.0 (21.0–31.0)	29.0 (24.0–33.0)	<0.01	21.0 (18.5–27.0)	26.0 (22.0–31.0)	<0.01
Parity	1 (0–2)	1 (1–2)	NS	2 (1–3)	2 (1–4)	NS
First trimester BMI (kg/m ²)	22.2 (20.5–23.3)	27.2 (25.9–29.5)	<0.01	22.8 (21.5–24.4)	32.4 (27.8–39.7)	<0.01
Third trimester BMI (kg/m ²)	28.0 (26.3–29.6)	33.2 (30.4–35.4)	<0.01	28.8 (26.7–30.7)	38.9 (34.2–45.9)	<0.01
BMI difference	5.9 (4.8–7.3)	4.9 (3.5–5.8)	<0.01	6.0 (4.7–7.2)	5.3 (4.0–7.7)	NS
Gestational age at blood sampling (weeks)	33.0 (29.4–39.4)	32.8 (29.3–38.6)	NS	33.2 (32.0–38.9)	37.0 (33.5–38.2)	NS
Gestational age at delivery (weeks)	39.9 (39.2–40.3)	39.9 (38.9–40.7)	NS	36.0 (32.7–39.0)	37.1 (34.0–38.2)	NS
Birth weight (g)	3420 (3227–3660)	3510 (3220–3690)	NS	2125 (1772–3095)	2625 (1895–3155)	NS

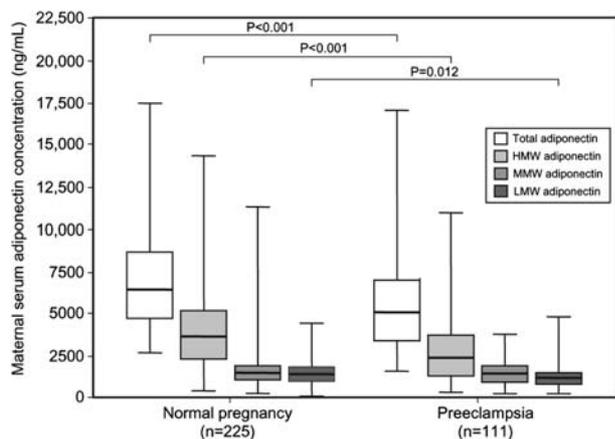


Figure 1 Comparison of the median serum total, HMW, MMW and LMW adiponectin concentrations between pregnant women with normal pregnancies and those with preeclampsia. The median maternal serum concentration of total adiponectin was lower in patients with preeclampsia than in those with a normal pregnancy. Similarly, patients with preeclampsia had lower serum concentrations of HMW and LMW adiponectin than those with a normal pregnancy. The median maternal serum concentration of MMW adiponectin did not differ between patients with preeclampsia and those with a normal pregnancy ($P = 0.7$).

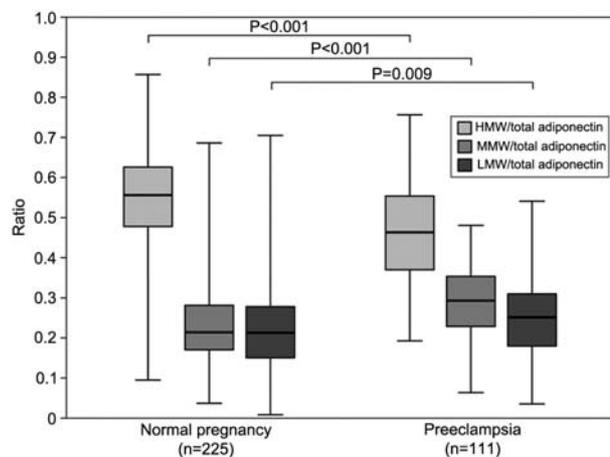


Figure 2 Comparison of HMW/total adiponectin, MMW/total adiponectin and LMW/total adiponectin ratios between pregnant women with normal pregnancies and those with preeclampsia. The median maternal HMW/total adiponectin ratio was lower in patients with preeclampsia than in those with a normal pregnancy. In contrast, patients with preeclampsia had a higher median MMW/total adiponectin ratio as well as a higher LMW/total adiponectin ratio than those with a normal pregnancy.

Adiponectin multimers concentrations and their relative distribution in preeclampsia: normal weight vs. overweight/obesity

Among patients with preeclampsia, women with normal weight and those with overweight/obesity did not differ in the median serum concentration of total adiponectin (4010 ng/mL, IQR 2968–6748 vs. 5228 ng/mL, IQR 3378–6810; $P=0.3$, Figure 3A), HMW adiponectin (1434 ng/mL, IQR 1130–3659 vs. 2369 ng/mL, IQR 1402–3763; $P=0.2$, Figure 3B), MMW adiponectin (1176 ng/mL, IQR 826–1691 vs. 1349 ng/mL, IQR 992–1930; $P=0.2$, Figure 3C) and LMW adiponectin (1031 ng/mL, IQR 787–1484 vs. 1096 ng/mL, IQR 777–1493; $P=0.98$, Figure 3D).

Similarly to women with a normal pregnancy, patients with preeclampsia with normal weight and those with

overweight/obesity had a comparable median ratios of HMW/total adiponectin (0.40, IQR 0.32–0.53 vs. 0.46, IQR 0.38–0.55; $P=0.1$, Figure 4A), MMW/total adiponectin (0.29, IQR 0.22–0.37 vs. 0.28, IQR 0.21–0.35; $P=0.9$, Figure 4B), and LMW/total adiponectin (0.25, IQR 0.21–0.39 vs. 0.15, IQR 0.15–0.30; $P=0.2$, Figure 4C).

Adiponectin multimers concentrations and their relative distribution in normal weight pregnant women: normal pregnancy vs. preeclampsia

Among patients with a normal weight, women with a normal pregnancy had a higher median serum concentrations of total (7025 ng/mL, IQR 5235–3763 vs. 4010 ng/mL, IQR 2968–6748; $P<0.001$, Figure 3A), and HMW adiponectin (3968 ng/mL, IQR 2535–5487 vs. 2348 ng/mL, IQR 1259–3763; $P<0.001$, Figure 3B) than those

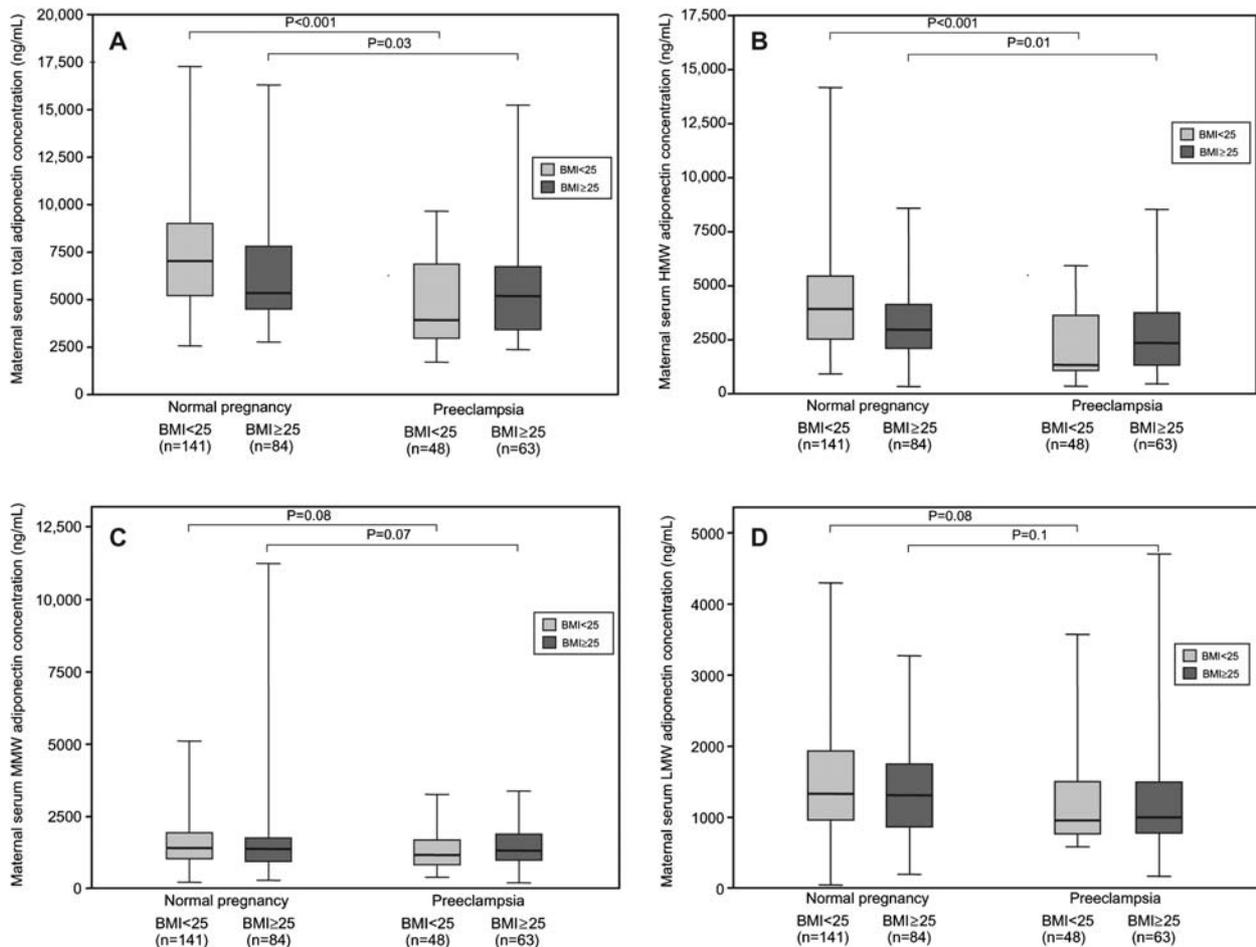
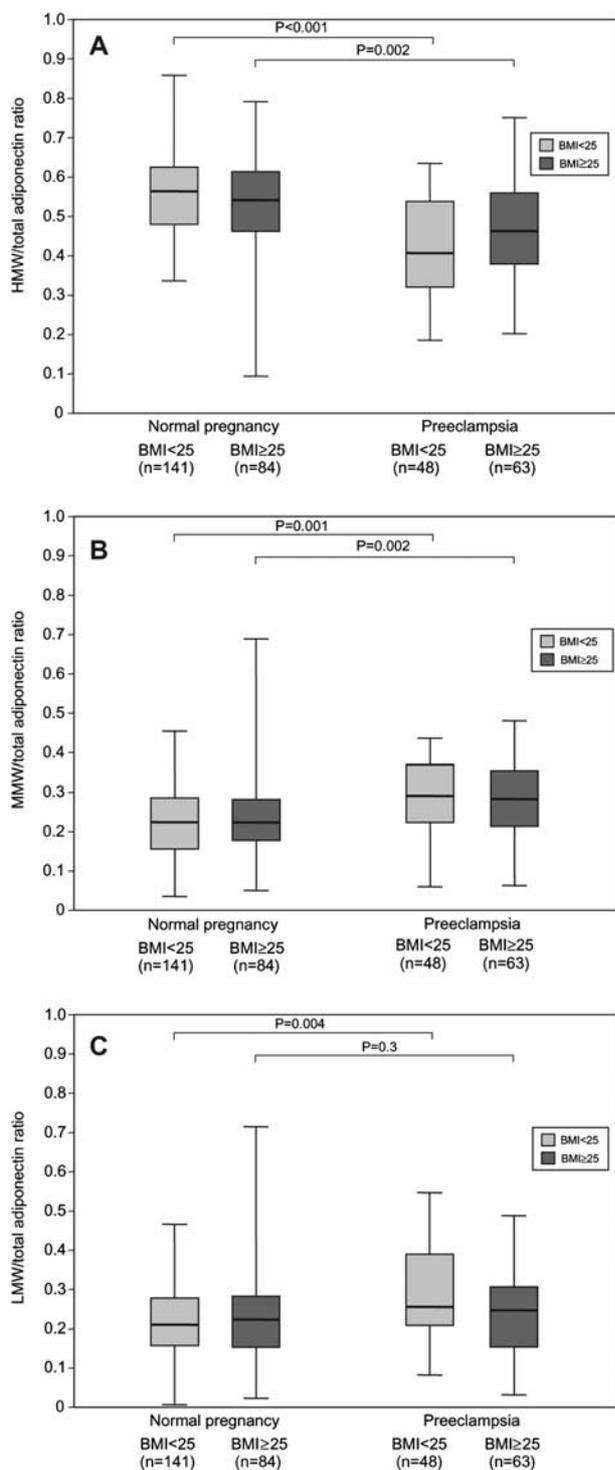


Figure 3 Maternal serum total adiponectin (A), HMW adiponectin (B), MMW adiponectin (C) and LMW adiponectin (D) concentrations in women with a normal pregnancy and patients with preeclampsia according to first trimester BMI (normal weight vs. overweight/obese).

Among patients with preeclampsia, women of normal weight and those with overweight/obesity did not differ in the median serum concentration of total, HMW, MMW, and LMW adiponectin. Among patients with a normal weight, the median serum concentrations of total (A) and HMW (B) adiponectin were higher in women with a normal pregnancy than in those with preeclampsia. Among patients with overweight/obesity, the median serum concentrations of total (A) and HMW (B) adiponectin were higher in women with a normal pregnancy than in those with preeclampsia.



with preeclampsia. There was no significant difference in the median maternal serum concentrations of MMW (1446 ng/mL, IQR 1010–1970 vs. 1176 ng/mL, IQR 826–1691; $P = 0.08$, Figure 3C) and LMW adiponectin (1321 ng/mL, IQR 964–1924 vs. 1031 ng/mL, IQR 787–1484; $P = 0.08$, Figure 3D) between women with a normal pregnancy and those with preeclampsia.

Figure 4 Comparison of HMW/total adiponectin (A) MMW/total adiponectin (B) and LMW/total adiponectin (C) ratios between pregnant women with a normal pregnancy and those with preeclampsia according to first trimester BMI (normal weight vs. overweight/obese).

Among patients with preeclampsia, patients with normal weight and those with overweight/obesity had a comparable median ratio of HMW/total, MMW/total, and LMW/total adiponectin. Pregnant women with a normal pregnancy had a higher HMW/total adiponectin ratio than those with preeclampsia (A). MMW/total ratio (B) and LMW/total adiponectin ratio (C) were lower in pregnant women with a normal pregnancy than in those with preeclampsia. Overweight/obese pregnant women with a normal pregnancy had a higher HMW/total adiponectin ratio than those with preeclampsia (A). MMW/total adiponectin ratio was lower in overweight/obese pregnant women with a normal pregnancy than in those with preeclampsia (B).

Among normal weight women, those with a normal pregnancy had a higher HMW/total adiponectin ratio than those with preeclampsia (0.56, IQR 0.48–0.63 vs. 0.40, IQR 0.32–0.53; $P < 0.001$, Figure 4A). In contrast, MMW/total (0.22, IQR 0.16–0.28 vs. 0.29, IQR 0.22–0.37; $P = 0.001$, Figure 4B) and LMW/total adiponectin ratios (0.20, IQR 0.15–0.28 vs. 0.25, IQR 0.21–0.39; $P = 0.004$, Figure 4C) were lower in women with a normal pregnancy than in those with preeclampsia.

Adiponectin multimers concentrations and their relative distribution in overweight/obese women: normal pregnancy vs. preeclampsia

Among patients with overweight/obesity, women with a normal pregnancy had a higher median serum concentration of total (5370 ng/mL, IQR 4464–5142 vs. 5228 ng/mL, IQR 3378–6810; $P = 0.03$, Figure 3A) and HMW adiponectin (3015 ng/mL, IQR 2118–4134 vs. 2369 ng/mL, IQR 1402–3763; $P = 0.01$, Figure 3B) than those with preeclampsia. There was no significant difference in the median maternal serum concentrations of MMW adiponectin (1403 ng/mL, IQR 973–1762 vs. 1349 ng/mL, IQR 992–1930; $P = 0.7$, Figure 3C) and LMW adiponectin (1286 ng/mL, IQR 850–1741 vs. 1096 ng/mL, IQR 777–1493; $P = 0.1$, Figure 3D) between women with a normal pregnancy and those with preeclampsia.

Overweight/obese women with a normal pregnancy had a higher HMW/total adiponectin ratio than those with preeclampsia (0.54, IQR 0.46–0.61 vs. 0.46, IQR 0.38–0.55; $P = 0.002$, Figure 4A). In contrast, MMW/total adiponectin ratio was lower in overweight/obese women with a normal pregnancy than in those with preeclampsia (0.22, IQR 0.17–0.28 vs. 0.28, IQR 0.21–0.35; $P = 0.002$, Figure 4B). The median LMW/total adiponectin ratio did not differ significantly between the two groups (0.22, IQR 0.15–0.28 vs. 0.15, IQR 0.15–0.30; $P = 0.3$, Figure 4C).

Multivariable logistic regression analysis was used to examine the contribution of the presence of preeclampsia on the serum concentration of adiponectin isoforms, while adjusting for maternal age, maternal BMI (normal

weight vs. overweight/obese), the difference in BMI between the third and the first trimester and gestational age at sampling. The final regression model suggested that the presence of preeclampsia was independently associated with decreased maternal serum HMW adiponectin concentrations ($P=0.001$) and HMW/total adiponectin ratio ($P<0.001$), and with increased MMW/total adiponectin ratio ($P=0.007$) and LMW/total adiponectin ratio ($P=0.006$).

Discussion

Principal findings of the study

1) The median maternal HMW and LMW adiponectin concentrations were significantly lower in patients with preeclampsia than in those with normal pregnancies; 2) patients with preeclampsia had a significantly lower HMW/total adiponectin ratio and a higher MMW/total and LMW/total adiponectin ratio than those with a normal pregnancy; 3) among patients with preeclampsia, neither maternal serum concentrations of adiponectin multimers, nor their relative distribution differ between normal weight and overweight/obese patients; and 4) the presence of preeclampsia was independently associated with low maternal serum HMW adiponectin concentration ($P=0.001$) and HMW/total adiponectin ratio ($P<0.001$).

Adiponectin multimers: distinct structures, diverse actions

Adipocytes secrete adiponectin as multimeric complexes including: LMW trimers, MMW hexamers, and HMW oligomers (12–18 monomers) [126, 128, 168, 172, 173]. Of note, these multimeric forms cannot interchange with each other after secretion into the circulation [126]. This is pertinent, since distinct adiponectin isoforms have different biological actions. This evidence includes: 1) LMW adiponectin has an anti-inflammatory effect *in vitro* – monocytes and macrophages exposure to LMW adiponectin results in increased secretion of IL-10 [115] and a decreased release of IL-6 [149]; 2) HMW and MMW adiponectin isoforms have pro-inflammatory properties *in vitro* – exposure of monocytes to HMW and MMW adiponectin results in increased secretion of IL-6 [4] and IL-8 [142] chemokines; 3) only the HMW adiponectin isoform protects endothelial cells against apoptosis [75]; interestingly, the HMW, but not the LMW isoform, inhibits prostate cancer cell growth [17]; and 4) the different adiponectin multimers exert their action via activation of dissimilar signalling pathways, whereas LMW adiponectin activates AMP-activated protein kinase (AMPK) [169], and HMW as well as MMW activate NF- κ B [57, 169].

Taken together, these reports support the notion that different adiponectin multimers have unique effects in the regulation of the metabolic and inflammatory pathways. These findings underline the significance of the relative

distribution of adiponectin multimers as a method for the modulation of the diverse actions of this adipokine.

Alterations in adiponectin concentrations during gestation are associated with obesity-related pregnancy complications

The observations regarding the importance of various adipokines such as leptin [59, 74, 105, 107], C-reactive protein [107], tumor necrosis factor (TNF)- α [74, 107, 148], resistin [31, 58, 59, 82, 118], visfatin [26, 43, 52, 79, 88, 104] and specifically adiponectin [10, 31, 34, 99–103, 116, 117, 130, 131, 135, 167] in the regulation of metabolism and the inflammatory response, have been corroborated in human gestation.

Evidence in support of a role for adiponectin in normal gestation, preeclampsia and other pregnancy complications includes: 1) first trimester circulating adiponectin concentrations are significantly lower in pregnant women destined to develop preeclampsia than in those who will subsequently have gestational hypertension or a normal pregnancy [34]; 2) patients with previous preeclampsia have a lower adiponectin concentration (7–8 years after the index pregnancy) than those with a normal pregnancy [48]; 3) overweight pregnant patients have a lower adiponectin concentration than normal weight pregnant women [117]; and 4) pregnancy is associated not only with alterations in adiponectin concentrations [100, 102, 103, 155] but also with changes in the relative distribution of its multimers [21, 99, 134, 135].

Variation in circulating maternal concentration of adiponectin in patients with preeclampsia – evidence for the syndromic nature of preeclampsia?

The evidence concerning maternal adiponectin concentrations in the presence of preeclampsia is inconsistent: higher [41, 58, 59, 70, 92, 112, 113, 116, 130, 159], lower [31, 33, 34, 63, 125], and similar adiponectin concentrations [119, 120, 145, 160] have been reported in patients with preeclampsia compared to normal pregnant women. This alleged inconsistency may be due to differences in the study population characteristics, definitions of preeclampsia (mild vs. severe, early vs. late onset), sample size or methods by which adiponectin concentrations were determined. However, although it is possible to explain some of the discrepancies by differences in study design, the inconsistency of the results may reflect the syndromic nature of preeclampsia.

Altered function of adipose tissue is a feature of preeclampsia

The findings that the median maternal serum concentrations of HMW and LMW adiponectin are lower in patients with preeclampsia than in those with a normal pregnancy are novel. *Prima facie*, it seems that the decreased concentration of these isoforms in patients with preeclampsia

sia merely reflects the reduction in total adiponectin concentrations in those patients. However, the lack of a significant change in MMW adiponectin, as well as the remarkable decrease in HMW adiponectin, suggest that additional mechanism(s) are involved in these changes. Moreover, the lower HMW/total adiponectin ratio and the higher MMW/total and LMW/total adiponectin ratios, reported herein for the first time in patients with preeclampsia, suggest that the regulation of adiponectin production and/or secretion is altered in the presence of preeclampsia. Of interest, the various adiponectin isoforms cannot interchange with each other after secretion [126]. Thus, the altered regulation of adiponectin multimeric complexes occurs at the tissue level, specifically in the adipocytes. Collectively, the results of this study characterize altered function of adipose tissue as a feature of preeclampsia.

While there is ample literature regarding the changes in total adiponectin concentrations in patients with preeclampsia, data concerning the relative distribution of the different multimeric complexes of this hormone are limited. Indeed, only two studies reported the relative distribution of adiponectin isoforms in patients with preeclampsia [42, 160]. Takemura et al. [160] described similar total adiponectin concentrations, but a higher maternal HMW adiponectin concentrations and an elevated HMW/total adiponectin ratio in lean patients with preeclampsia ($n=14$) compared to lean normal pregnant women ($n=14$). Similarly, Fasshauer et al. [42] reported higher maternal serum HMW adiponectin concentrations in patients with preeclampsia ($n=16$) compared to lean normal pregnant women ($n=20$). The findings of the present study are in apparent contrast with the previous two reports. Several explanations for the alleged disparity in these findings can be hypothesized including differences in study population (specifically ethnicity, age) and clinical characteristics (i.e., higher prevalence of overweight/obesity in the present study), inclusion criteria for the control group and in the methods by which the adiponectin multimers were determined.

Why is preeclampsia associated with dysregulation of adiponectin multimeric complexes?

The decrease in HMW and LMW adiponectin and the significant dysregulation of its multimeric complexes in patients with preeclampsia reported herein are novel findings. The cross-sectional nature of the present study does not allow us to discern a cause-effect relationship between the changes in maternal serum adiponectin multimers and preeclampsia. However, several explanations for these intriguing results can be hypothesized:

1. Metabolic alterations in preeclampsia and functional implications of adiponectin multimeric abundance – HMW adiponectin is considered to be the most active form of adiponectin multimers. A growing body of evi-

dence suggests that HMW adiponectin confers a protective metabolic effect: 1) the circulating concentration of HMW adiponectin has a superior correlation with several components of the metabolic syndrome (e.g., insulin resistance, dyslipidemia) over total adiponectin concentrations [83]; 2) increases in the HMW/total adiponectin ratio, but not in the total adiponectin concentration, correlate with improved insulin sensitivity in patients with Type 2 diabetes mellitus (T2DM) treated with the thiazolidinediones (an insulin-sensitizing drug) [127]; and 3) a selective reduction in HMW adiponectin accounts for the decreased concentrations of total adiponectin in patients with T2DM [13] or obesity [75].

In the present study, both HMW and LMW adiponectin were lower in patients with preeclampsia than in those with a normal pregnancy. However, while the reduction in the absolute concentrations of HMW adiponectin resulted in a low HMW/total adiponectin ratio in preeclampsia, the opposite was true for LMW/total ratio. Thus, the decrease in total adiponectin concentration should be attributed primarily to reduced HMW adiponectin concentrations, suggesting that HMW adiponectin plays a role in the metabolic impairments associated with preeclampsia. Alternatively, the decreased concentrations and relative distribution of these multimeric complexes may be a reaction aimed to induce insulin resistance in order to allow decreased glucose utilization by maternal peripheral tissues to ensure increased availability of nutrients to the fetus. This may be of special importance in the presence of placental dysfunction, one of the hallmarks of preeclampsia.

2. Intravascular pro-inflammatory response, endothelial cell dysfunction and the changes in adiponectin isoforms – adiponectin has a potent anti-inflammatory action and an important regulatory effect on the integrity of the vasculature system. Preeclampsia is characterized by an exaggerated intravascular pro-inflammatory response [11, 22, 37, 47, 49, 53, 132, 143, 163] as well as by endothelial cell dysfunction [16, 28, 77, 114, 129, 133, 136, 137, 147, 161]. Hence, the decrease in adiponectin multimers in the presence of preeclampsia may point to a regulatory role for adiponectin in the pro-inflammatory response and endothelial cell dysfunction of preeclampsia. Noteworthy is that the concentration of LMW adiponectin, which has anti-inflammatory properties [149, 178], is reduced in patients with preeclampsia.
3. Adiponectin as a pro-angiogenic agent – adiponectin stimulates new blood vessel growth *in vitro* [124]. In accordance with this finding, adiponectin administration is required for angiogenesis in response to tissue ischemia in adiponectin knock-out mice [151]. Recently, a perturbation of angiogenesis has been implicated in the pathophysiology of preeclampsia.

Indeed, both longitudinal and cross-sectional studies have characterized preeclampsia as an “anti-angiogenic state” [5, 18, 23–25, 32, 36, 39, 40, 49, 50, 76, 78, 80, 84–87, 90, 93, 98, 140, 164, 166]. In addition, to its direct effect on angiogenesis, a recent study demonstrated a positive correlation between soluble Endoglin (sEng) and circulating adiponectin concentrations in patients with preeclampsia, but not in pregnant women with a normal pregnancy [96]. Similarly, a positive correlation between adiponectin and Soluble fms-like Tyrosine kinase 1 (sFlt-1) as well as a negative correlation with Placental Growth Factor (PlGF) in pregnant women with and without preeclampsia have been demonstrated [159]. Thus, low adiponectin concentrations may aggravate the endothelial dysfunction caused by alterations in angiogenesis in patients with preeclampsia.

In conclusion, it is tempting to postulate that the decrease in adiponectin multimers concentrations and the changes in their relative distribution results in a failure of this adipokine to exert its metabolic, inflammatory and angiogenic protective effects. This, in turn, may lead to the development or aggravation of the underlying mechanisms of disease implicated in preeclampsia.

Disparity between adiponectin multimers in normal and overweight/obese patients – evidence for altered regulation of adiponectin multimers production in preeclampsia

The results of the present study indicate that while the expected lower concentrations of total adiponectin and its multimers among women with normal pregnancies were documented in overweight/obese compared with normal weight patients with normal pregnancy [8, 99, 117], the decrement was blunted in overweight/obese patients with preeclampsia who had comparable concentrations of all adiponectin multimers to normal weight patients with preeclampsia. Interestingly, among both normal and overweight/obese patients, those with preeclampsia had lower concentrations of HMW, LMW and a low HMW/total adiponectin ratio, suggesting that the alterations in maternal circulating adiponectin should be attributed primarily to the presence of preeclampsia.

Adiponectin is an exceptional adipokine since it is down-regulated in the presence of obesity. The results of this report underline preeclampsia as a condition in which the strong link between hypoadiponectinemia and obesity is disrupted. This finding further supports the notion of altered regulation of adiponectin multimers production by adipose tissue in preeclampsia.

In conclusion, adiponectin has been implicated in the pathophysiology of endothelial cell dysfunction, insulin resistance, inflammation, and anti-angiogenesis, all well recognized features of preeclampsia. Alterations in the relative distribution of adiponectin multimers are the

result of regulation at the adipose tissue level and appear to determine the physiological effects of adiponectin. Comparing circulating adiponectin isoforms concentrations between normal weight and overweight/obese women with a normal pregnancy and those with preeclampsia reveals a distinct pattern of concentration and relative distribution of adiponectin multimers in patients with preeclampsia. Collectively the results of the present study suggest that dysregulation of adiponectin multimeric complexes are associated with preeclampsia and can provide a mechanistic molecular basis for the association between metabolic impairments, altered inflammatory response and preeclampsia.

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