

# Maternal serum adiponectin multimers in gestational diabetes

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## Abstract

**Objective:** Adiponectin, an adipokine with profound insulin-sensitizing effect, consists of heterogeneous species of multimers. These oligomeric complexes circulate as low-molecular-weight (LMW) trimers, medium-molecular-weight (MMW) hexamers and high-molecular-weight (HMW) isoforms and can exert differential biological effects. The aims of this study were to determine whether there is a change in circulating adiponectin multimers in the presence of gestational diabetes mellitus (GDM), overweight/obesity or with a treatment with sulfonylurea or insulin in patients with GDM.

**Study design:** This cross-sectional study included women with: 1) normal pregnancy (n=149); and 2) patients with GDM (n=72). Thirty-three patients with GDM were managed with diet alone. Among the others 39 diabetic patients, 17 were treated with Glyburide and 22 with insulin. The study population was further strati-

fied by first trimester body mass index (BMI) (normal weight <25 kg/m<sup>2</sup> vs. overweight/obese ≥25 kg/m<sup>2</sup>). Serum adiponectin multimers (total, HMW, MMW and LMW) concentrations were determined by ELISA.

**Results:** 1) The median maternal serum of total, HMW, MMW and LMW were lower in patients with GDM than in those with normal pregnancies (P<0.001 for all comparisons); 2) patients with GDM had a lower HMW/total adiponectin ratio and a higher MMW/total and LMW/total adiponectin ratio than those with a normal pregnancy (P<0.001 for all comparisons); and 3) among GDM patients, there were no differences in the concentrations and relative distribution of adiponectin multimers between those who were managed with diet, and those who were treated with pharmacological agents.

**Conclusion:** 1) GDM is characterized by a distinctive pattern of concentrations and relative distribution of adiponectin multimers akin to Type 2 diabetes mellitus; 2) dysregulation of adiponectin multimeres can provide a mechanistic basis for the association between adiposity and GDM.

**Keywords:** Adipokines; body mass index (BMI); diabetes; gestational diabetes; high-molecular-weight (HMW) adiponectin; low-molecular-weight (LMW) adiponectin; medium-molecular-weight (MMW) adiponectin; obesity; overweight; pregnancy.

## Introduction

Gestational diabetes mellitus (GDM), defined as a carbohydrate intolerance of varying severity with onset or first recognition during pregnancy [6, 42, 91, 102], affects 1–10% of all pregnancies [4, 5, 12, 24, 33, 67]. Several features of GDM make this common complication of pregnancy of special importance: 1) it is associated with an increased rate of short-term complications for the mother (e.g., cesarean section, and preeclampsia) [54, 70, 119, 129], the fetus (e.g., macrosomia, perinatal mortality) [122, 127], and the neonate (e.g., brachial plexus injury, hypoglycemia) [54, 55, 108, 120, 122, 127, 129, 148]; 2) weight reduction, exercise [19, 56], oral hypoglycemic agents and insulin [28, 68, 69, 121, 140] improve metabolic indices and reduce the rate of maternal, fetal and neonatal complications; 3) GDM is associated with long-term morbidity and mortality for the mothers (Type 2 diabetes mellitus, cardiovascular dis-

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ease) [2, 14, 20, 21, 32, 45, 60, 63, 109, 124] and their infants (childhood obesity, metabolic syndrome) [7, 17, 30, 110]; and 4) the prevalence of this metabolic complication is constantly increasing [36, 143].

Recently, adipose tissue has emerged as an important endocrine organ via the production of adipokines. Furthermore, dysregulation of adipokine production and/or secretion has been implicated in Type 2 DM. Indeed, polymorphisms of several adipokines such as adiponectin [38, 44, 53, 71, 90, 96, 131], resistin [89, 104], visfatin [153], and leptin receptor [123] are associated with insulin resistance and Type 2 DM. Moreover, compared to non-diabetic individuals, patients with Type 2 DM have higher circulating concentrations of resistin [41, 88, 151], TNF- $\alpha$  [31, 92], RBP-4 [25, 150] and CRP [37, 111], as well as lower concentrations of adiponectin [51, 61, 112, 145]. These findings laid the groundwork for the hypothesis that perturbation of adipokine homeostasis has also a role in the pathophysiology of gestational diabetes.

Adiponectin, an adipokine produced exclusively and abundantly by adipocytes, is the most abundant gene (*AMP1*) product of adipose tissue [52, 74, 94, 125], and circulates at high concentrations in the plasma [59]. Unlike other adipokines, adiponectin concentrations are negatively correlated with adiposity [8, 52], suggesting that adipose tissue exerts a negative feedback on adiponectin production and/or secretion. Adiponectin has insulin sensitizing [13, 26, 40, 75, 98, 135, 149] and anti-inflammatory [126] properties, thus providing a mechanistic molecular basis for the association between an excess fat depot and obesity-related complication including Type 2 DM. Adiponectin is secreted from adipocytes and circulates in the 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) [9, 16, 105, 138, 139, 144]. A growing body of evidence suggests that the different isoforms exert differential biological effects [9, 16, 64, 105, 106, 144]. Specifically, the anti-diabetogenic properties of adiponectin have been attributed to the HMW isoforms [11, 39, 47, 58, 64, 95, 106, 133, 136, 144].

Data regarding circulating maternal adiponectin multimers concentrations in human pregnancy are limited [23, 35, 77, 103, 115, 116, 132]. Indeed, only one study has reported the concentration of HMW adiponectin in patients with GDM. To date there are no reports concerning the concentrations and relative distribution of MMW or LMW. Moreover, there are no data regarding the relationship between overweight/obesity and adiponectin multimers in patients with GDM. Thus, the aims of this study were to determine whether: 1) there is a change in adiponectin multimers concentrations in the presence of GDM or overweight/obesity; and 2) treatment with a sulfonylurea (Glyburide) or insulin is associated with a change in the concentrations of adiponectin and its multimers.

## Materials and methods

### Study groups and inclusion criteria

A cross-sectional study was conducted including patients in the following groups: 1) normal pregnant women ( $n=149$ ); and 2) patients with GDM ( $n=72$ ). The study population was further stratified by first trimester body mass index (BMI) (normal weight 18.5–24.9 kg/m<sup>2</sup> vs. overweight/obese  $\geq 25$  kg/m<sup>2</sup>).

Maternal serum and clinical data were obtained from our bank of biological samples and clinical databases. Many of these samples have been previously employed to study the biology of inflammation, angiogenesis regulation, and growth factor concentrations in normal pregnant women and in 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 (Santiago, Chile), Wayne State University (Detroit, MI, 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, and delivery at term of a healthy neonate with a birthweight above the 10<sup>th</sup> percentile for gestational age.

All patients were screened for GDM with a 1-h, 50-g oral glucose challenge test. Pregnant women with plasma glucose concentrations  $\geq 135$  mg/dL at 1 h underwent a 100-g oral glucose tolerance test (OGTT). Women with two or more abnormal plasma glucose values were given a diagnosis of GDM [2, 22]. Patients with gestational diabetes were excluded from the study if they had pregestational diabetes mellitus, collagen vascular diseases, inflammatory bowel disease and chronic inflammatory conditions, or if they were using corticosteroids.

### Maternal assessment and treatment of patients with gestational diabetes

All women diagnosed with GDM were given nutritional instructions for three meals and four snacks daily and individualized dietary advice from a qualified dietitian. Compliance with the dietary regimen was evaluated weekly during visits to the high-risk clinic. The diets were designed to provide 25 kcal/kg of body weight for obese women and 35 kcal/kg for lean women, with 40–45% of the calories from carbohydrates [2, 3].

A nurse educator instructed the women in how to measure blood glucose with a glucometer. The patients were asked to measure capillary blood glucose four times daily: after an overnight fast, and 2 h after each meal [68]. The goals of treatment were a fasting blood glucose concentration of 60–90 mg/dL and a postprandial blood glucose concentration of  $<120$  mg/dL [50]. If the blood glucose values of the patient treated with diet did not meet the goals for a two-week period the patient received a starting dose of 2.5 mg Glyburide orally in the morning. A physician evaluated the blood glucose values at each visit, and increased the Glyburide dose as needed to meet these goals. The dose of Glyburide was increased the following week

by 2.5 mg and thereafter by 5 mg weekly up to a total of 20 mg daily when necessary [66]. If the blood glucose values of a woman treated with the maximal dose of Glyburide did not meet the goals for a two-week period, the treatment was switched to insulin therapy. The starting dose for insulin was 0.7 U/kg of actual body weight at admission, given subcutaneously three times daily and increased weekly as necessary [68].

The BMI was calculated according to the formula: weight (kg)/height (m<sup>2</sup>). Normal weight women were defined as those with BMI of 18.5–24.9 kg/m<sup>2</sup> according to the definitions of the World Health Organization (WHO) [1]. Pregnant women were classified by their first trimester BMI into two groups: normal weight and overweight/obese (BMI  $\geq$  25 kg/m<sup>2</sup>).

Maternal blood samples were collected with a vacutainer directly 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 re-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 the 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 data distribution was tested using the Shapiro–Wilk or Kolmogorov–Smirnov tests. Since serum multimeric adiponectin isoforms concentrations were not normally distributed, Mann–Whitney *U*-test were used for comparisons of continuous variables. Kruskal–Wallis test was used for the comparison of normal pregnant women patients with diabetic patients with and without pharmacologic treatment, as well as for the comparison between normal weight and overweight pregnant women with and without GDM. Comparison of proportions was performed by  $\chi^2$  and Fisher's exact tests. Multiple linear regression analysis was used to determine which factors were significantly and independently associated with maternal serum adiponectin isoforms as well as their relative distribution (after log transformation). The following parameters were included in the model: maternal age, parity, gestational age at sampling, gestational age at delivery, birth weight, first trimester BMI, difference in BMI between first trimester and blood sampling, and the presence of GDM. A *P*-Value < 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

**Table 1** Clinical and demographic characteristics of the study population.

	Normal pregnancy (n = 149)	Gestational diabetes mellitus (n = 72)	P-value
Maternal age (years)	25.0 (21.0–31.0)	27.0 (23.0–33.0)	0.1
Ethnic origin			
African American	58 (80.6%)	110 (73.8%)	0.1
Hispanic	8 (11.1)	29 (19.5%)	0.2
Caucasian	4 (5.5%)	8 (5.3%)	0.9
Others	2 (2.7%)	2 (1.3%)	0.4
First trimester BMI (kg/m <sup>2</sup> )	24.0 (22.0–26.2)	30.6 (24.5–36.6)	<0.001
BMI at blood sampling (kg/m <sup>2</sup> )	30.0 (27.7–33.3)	35.3 (29.8–41.5)	<0.001
BMI difference between blood sampling and the first trimester	5.6 (4.4–7.2)	4.5 (3.0–6.1)	0.3
Gestational age at sampling (weeks)	37.6 (32.8–40.2)	37.6 (33.4–39.0)	0.1
Gestational age at delivery (weeks)	39.8 (39.0–40.2)	38.2 (36.8–39.2)	0.07
Birth weight (g)	3285 (2862–3687)	3440 (3210–3670)	0.018

Values are expressed as median (interquartile range); BMI, body mass index.

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

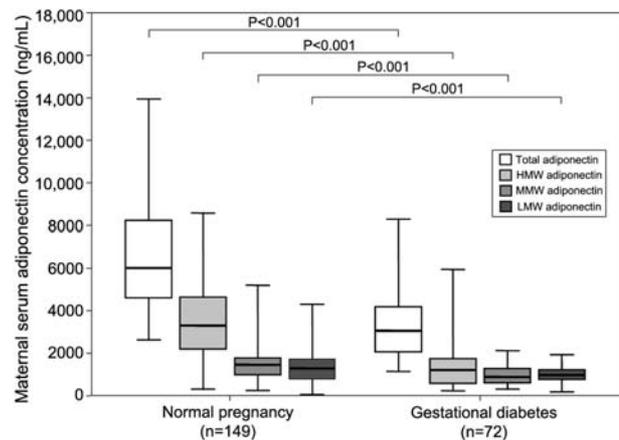
	Normal pregnancy (n = 94)		Overweight/obese (n = 55)		Gestational diabetes mellitus		P-value
	Normal weight (n = 24)	Overweight/obese (n = 48)	Normal weight (n = 24)	Overweight/obese (n = 48)	Normal weight (n = 24)	Overweight/obese (n = 48)	
Maternal age (years)	25.0 (20.7–30.0)	28.0 (22.0–33.0)	27.0 (20.5–32.2)	27.0 (23.0–33.7)	27.0 (20.5–32.2)	27.0 (23.0–33.7)	0.7
First trimester BMI (kg/m <sup>2</sup> )	22.5 (21.3–23.7)	27.0 (25.8–29.2)	23.7 (20.5–24.7)	33.2 (30.6–41.9)	23.7 (20.5–24.7)	33.2 (30.6–41.9)	<0.01
BMI at blood sampling (kg/m <sup>2</sup> )	28.4 (26.7–30.1)	33.3 (30.7–35.0)	28.9 (25.2–30.5)	40.3 (35.0–45.5)	28.9 (25.2–30.5)	40.3 (35.0–45.5)	<0.01
BMI difference between sampling and the first trimester	5.8 (4.7–7.4)	5.1 (3.6–6.5)	5.1 (3.7–6.4)	4.1 (2.7–5.9)	5.1 (3.7–6.4)	4.1 (2.7–5.9)	0.6
Gestational age at sampling (weeks)	38.1 (32.7–40.4)	33.7 (32.7–40.1)	37.1 (32.8–39.1)	37.6 (34.0–38.9)	37.1 (32.8–39.1)	37.6 (34.0–38.9)	0.9
Gestational age at delivery (weeks)	39.9 (39.0–40.2)	39.7 (38.8–40.7)	37.3 (36.7–39.2)	38.3 (36.6–39.1)	37.3 (36.7–39.2)	38.3 (36.6–39.1)	0.3
Birth weight (g)	3145 (2458–3772)	3335 (2875–3595)	3405 (3197–3690)	3500 (3230–3670)	3405 (3197–3690)	3500 (3230–3670)	0.5

GDM. Among patients with GDM, 33/72 were treated with diet alone. Among the other 39 diabetic patients, 17 were treated with Glyburide and 22 with insulin. As expected, patients with GDM had a higher first and third trimester BMI and larger neonates. There were no significant differences in maternal age, gestational age at blood sampling, gestational age at delivery and the difference in BMI between patients with a normal pregnancy and those with GDM (Table 1). Table 2 displays the demographic and clinical characteristics of the study population according to BMI. There were no significant differences in gestational age at blood sampling, gestational age at delivery and neonatal birthweight between normal weight and overweight/obese women in both groups (Table 2).

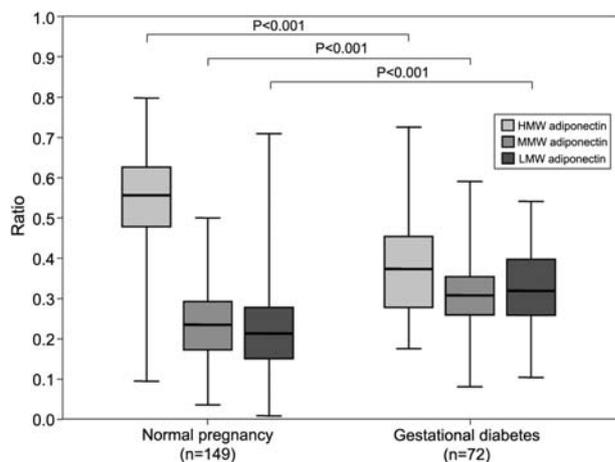
#### Adiponectin multimers concentrations and their relative distribution in GDM vs. normal pregnancy (non-GDM)

The median maternal serum concentration of total adiponectin was lower in patients with GDM than those with a normal pregnancy (median: 3022 ng/mL, interquartile range (IQR) 2102–4204 vs. 6019 ng/mL, IQR 4596–8256;  $P < 0.001$ , Figure 1). Similarly, patients with GDM had a lower median serum concentration of HMW (1203 ng/mL, IQR 593–1800 vs. 3297 ng/mL, IQR 2187–4657;  $P < 0.001$ , Figure 1), MMW (881 ng/mL, IQR 639–1259 vs. 1394 ng/mL, IQR 993–1839;  $P < 0.001$ , Figure 1), and LMW adiponectin (968 ng/mL, IQR 782–1218 vs. 1236 ng/mL, IQR 818–1724;  $P < 0.001$ , Figure 1) than those with a normal pregnancy.

The median maternal HMW/total adiponectin ratio was lower in patients with GDM than those with a normal pregnancy (0.37, IQR 0.27–0.45 vs. 0.54, IQR 0.47–0.62;



**Figure 1** Comparison of the median serum total, HMW, MMW and LMW adiponectin concentrations between pregnant women with normal pregnancies and those with GDM. The median maternal serum concentration of total, HMW, MMW and LMW were significantly lower in patients with GDM than in those with normal pregnancies ( $P < 0.001$  for all comparisons).



**Figure 2** Comparison of HMW/total adiponectin, MMW/total adiponectin, and LMW/total adiponectin ratios between pregnant women with normal pregnancies and those with GDM.

The median maternal HMW/total adiponectin ratio was lower in patients with GDM than in those with a normal pregnancy. In contrast, patients with GDM had a higher median MMW/total adiponectin and LMW/total adiponectin ratios than those with a normal pregnancy.

$P < 0.001$ , Figure 2). In contrast, patients with GDM had a higher median MMW/total adiponectin ratio (0.30, IQR 0.26–0.35 vs. 0.23, IQR 0.16–0.29;  $P < 0.001$ , Figure 2), and a higher LMW/total adiponectin ratio (0.32, IQR 0.25–0.39 vs. 0.21, IQR 0.15–0.28;  $P < 0.001$ , Figure 2) than those with a normal pregnancy.

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

Among patients with GDM, women with normal weight and those with overweight/obesity did not differ in the median serum concentration of total adiponectin (3731 ng/mL, IQR 2218–5809 vs. 2848 ng/mL, IQR 2070–4092, respectively;  $P = 0.13$ , Figure 3A), HMW adiponectin (1315 ng/mL, IQR 679–3239 vs. 1168 ng/mL, IQR 570–1645, respectively;  $P = 0.12$ , Figure 3B), MMW adiponectin (1071 ng/mL, IQR 650–1271 vs. 858 ng/mL, IQR 625–1218, respectively;  $P = 0.21$ , Figure 3C), and LMW adiponectin (1001 ng/mL, IQR 746–1291 vs. 951 ng/mL, IQR 785–1218, respectively;  $P = 0.97$ , Figure 3D).

Similar to women with a normal pregnancy, among patients with GDM, patients with normal weight and those with overweight/obesity had a comparable median ratio of HMW/total adiponectin (0.39, IQR 0.29–0.52 vs. 0.36, IQR 0.27–0.43;  $P = 0.1$ , Figure 4A), MMW/total adiponectin (0.30, IQR 0.21–0.36 vs. 0.30, IQR 0.27–0.35, respectively;  $P = 0.5$ , Figure 4B), and LMW/total adiponectin (0.29, IQR 0.17–0.38 vs. 0.33, IQR 0.28–0.33;  $P = 0.2$ , Figure 4C).

#### Adiponectin multimers concentrations and their relative distribution in normal weight pregnant women: normal pregnancy (non-GDM) vs. GDM

Among patients with a normal weight, women with a normal pregnancy had a higher median serum concentration of total (6987 ng/mL, IQR 5051–8713 vs. 3731 ng/mL, IQR 2218–5809;  $P < 0.001$ , Figure 3A), HMW (3916 ng/mL, IQR 2536–5169 vs. 1315 ng/mL, IQR 679–3239;  $P < 0.001$ , Figure 3B), MMW (1446 ng/mL, IQR 1021–1914 vs. 1071 ng/mL, IQR 650–1271;  $P = 0.005$ , Figure 3C) and LMW adiponectin (1236 ng/mL, IQR 916–2052 vs. 1001 ng/mL, IQR 746–1291;  $P = 0.01$ , Figure 3D) than those with GDM.

Among patients with a normal weight, women with a normal pregnancy had a higher HMW/total adiponectin ratio than those with GDM (0.55, IQR 0.49–0.63 vs. 0.39, IQR 0.29–0.52;  $P < 0.001$ , Figure 4A). In contrast, MMW/total ratio (0.23, IQR 0.16–0.28 vs. 0.30, IQR 0.21–0.36;  $P = 0.007$ , Figure 4B) and LMW/total adiponectin ratio (0.21, IQR 0.15–0.28 vs. 0.29, IQR 0.17–0.38;  $P = 0.013$ , Figure 4C) were lower in women with a normal pregnancy than those with GDM.

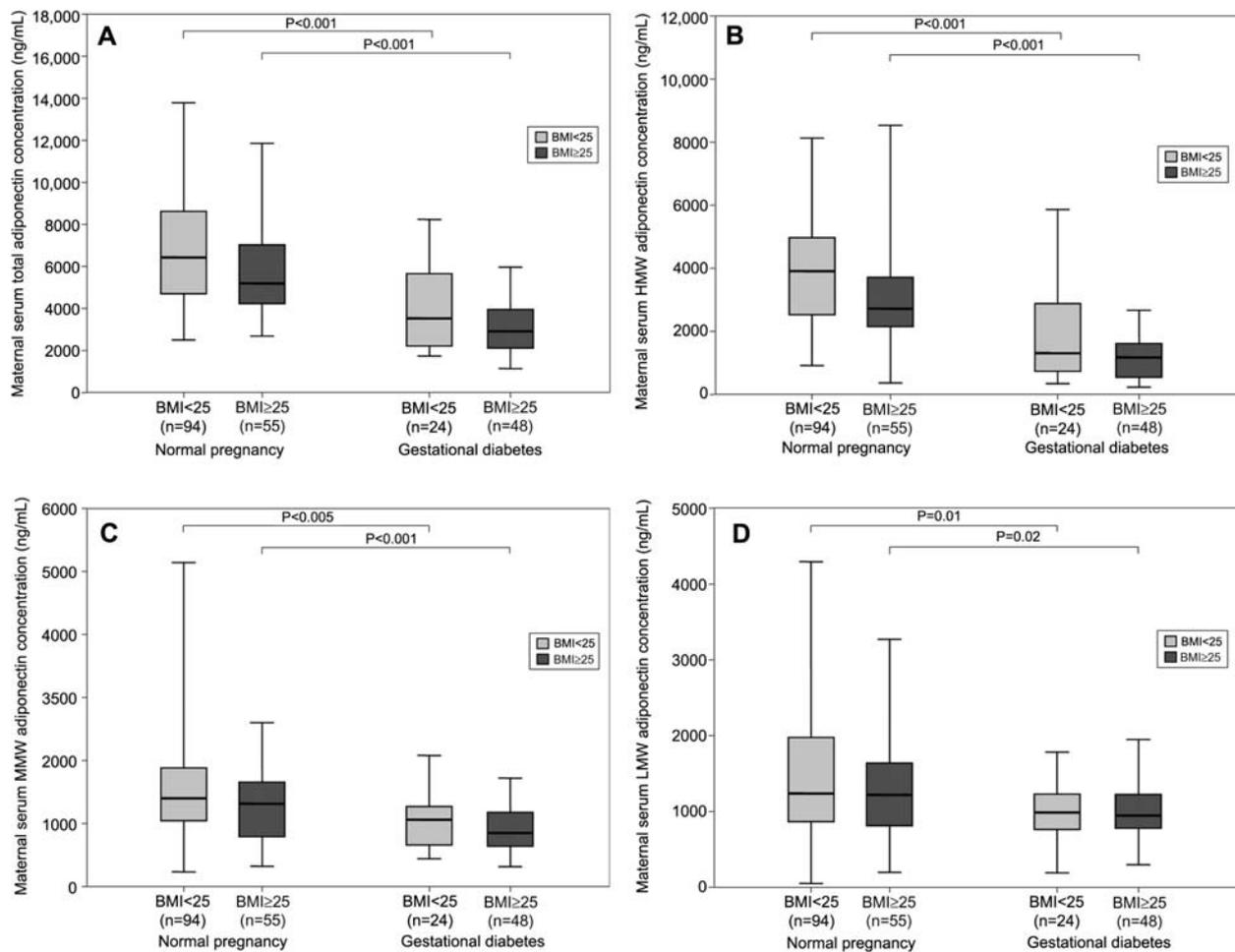
#### Adiponectin multimers concentrations and their relative distribution in overweight/obese pregnant women: normal pregnancy (non-GDM) vs. GDM

Among patients with overweight/obesity, women with a normal pregnancy had a higher median serum concentration of total (5207 ng/mL, IQR 4291–7095 vs. 2848 ng/mL, IQR 2070–4092;  $P < 0.001$ , Figure 3A), HMW (2733 ng/mL, IQR 2118–3733 vs. 1168 ng/mL, IQR 570–1645;  $P < 0.001$ , Figure 3B), MMW (1330 ng/mL, IQR 788–1683 vs. 858 ng/mL, IQR 625–1218;  $P < 0.001$ , Figure 3C), and LMW adiponectin (1237 ng/mL, IQR 813–1649 vs. 951 ng/mL, IQR 785–1218;  $P = 0.02$ , Figure 3D) than those with GDM.

Among patients with overweight/obesity, women with a normal pregnancy had a higher HMW/total adiponectin ratio than those with GDM (0.54, IQR 0.45–0.61 vs. 0.36, IQR 0.27–0.43;  $P < 0.001$ , Figure 4A). In contrast, MMW/total ratio (0.23, IQR 0.16–0.29 vs. 0.30, IQR 0.27–0.35;  $P < 0.001$ , Figure 4B) and LMW/total adiponectin ratio (0.21, IQR 0.15–0.28 vs. 0.29, IQR 0.17–0.38;  $P < 0.001$ , Figure 4C) were lower in overweight/obese women with a normal pregnancy than those with GDM.

#### Adiponectin multimers concentrations and their relative distribution in patients with GDM: the effect of pharmacologic treatment

Patients with GDM who were managed with diet and those treated with a pharmacologic agent (Glyburide or insulin) had comparable concentrations of total adiponectin (2811 ng/mL, IQR 2077–3874 vs. 3454 ng/mL, IQR 2130–4613, respectively;  $P = 0.2$ ). Similarly, median



**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 those with GDM according to first trimester BMI (normal weight vs. overweight/obese). Among patients with a normal pregnancy, the median serum concentration of total and HMW adiponectin were higher in women with normal weight than in those with overweight/obesity. Among patients with GDM, women with normal weight and those with overweight/obesity did not differ in the median serum concentrations of total, HMW, MMW and LMW adiponectin. Among patients with overweight/obesity, women with a normal pregnancy had higher median serum concentrations of total, HMW, MMW and LMW adiponectin ( $P=0.020$ ) than those with GDM.

maternal serum concentrations of HMW, MMW and LMW adiponectin did not differ significantly between the two groups. The relative distribution of adiponectin isoforms was also comparable between the two groups. Similarly, there were no differences in the concentrations and relative distribution of adiponectin multimers between patients who were treated with Glyburide and those who were treated with insulin.

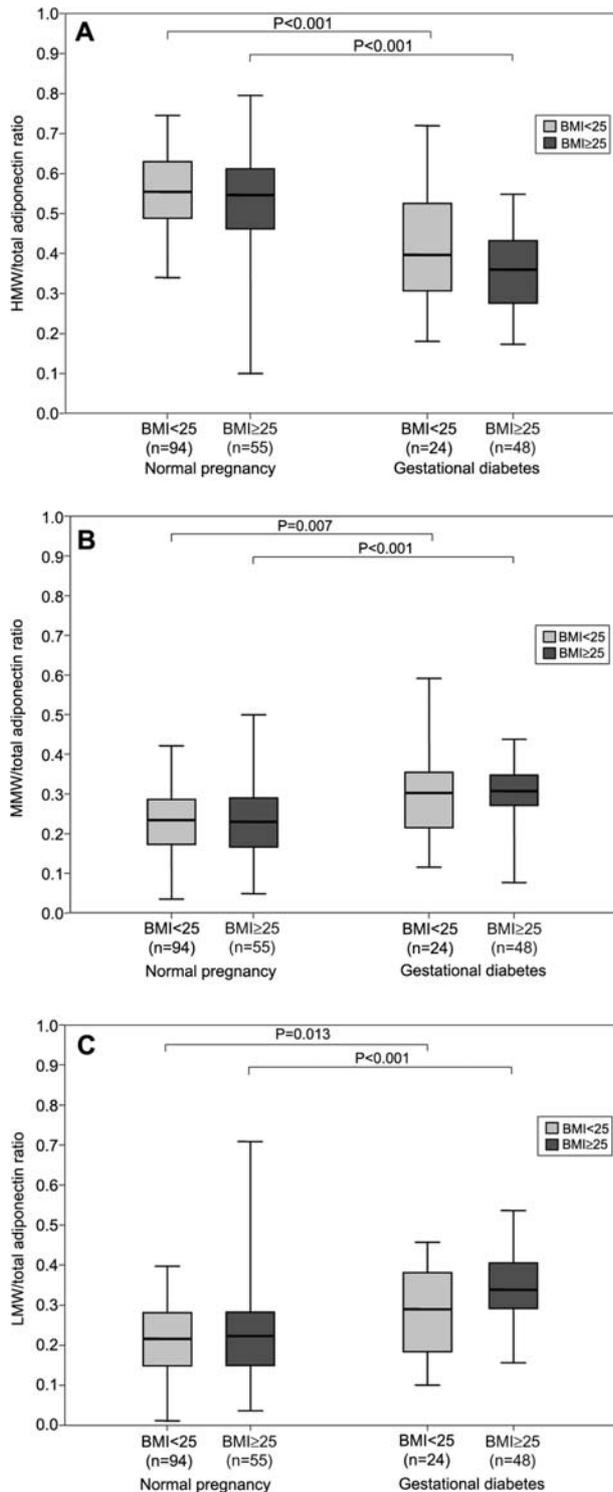
Multiple regression analysis was employed to examine the contribution of the presence of GDM on the serum concentration of adiponectin isoforms, while adjusting for maternal age, maternal BMI (normal weight vs. overweight/obese), the difference in BMI between the third and the first trimester, gestational age at blood sampling, and birthweight. The final regression model suggested that the presence of GDM and first trimester BMI were independently associated with decreased maternal

serum total adiponectin ( $P < 0.001$  and  $0.031$ , respectively), and HMW adiponectin concentrations ( $P < 0.001$  and  $0.009$ , respectively), as well as with decreased HMW/total adiponectin ratio ( $P < 0.001$  and  $0.02$ , respectively).

## Discussion

### Principal findings of the study

1) The median maternal serum of total, HMW, MMW and LMW were lower in patients with GDM than in those with a normal pregnancy; 2) patients with GDM had a lower Median HMW/total adiponectin ratio and a higher MMW/total and LMW/total adiponectin ratio than those with a normal pregnancy; 3) among patients with GDM, median maternal serum concentrations of adiponectin multimers



and their relative distribution did not differ between women with normal weight and those with overweight/obesity; 4) the presence of GDM was independently associated with low maternal serum of total and HMW adiponectin concentration as well as with decreased total adiponectin, HMW adiponectin and HMW/total adipo-

**Figure 4** Comparison of HMW/total adiponectin (A) MMW/total adiponectin (B) and LMW/total adiponectin (C) ratios between in women with a normal pregnancy and those with GDM according to first trimester BMI (normal weight vs. overweight/obese). Women with normal weight and those with overweight/obesity did not differ in the median ratio of HMW/total adiponectin, MMW/total adiponectin and LMW/total adiponectin. Similarly, among patients with GDM, these ratios were comparable between patients with normal weight and those with overweight/obesity. Overweight/obese women with a normal pregnancy had a higher HMW/total adiponectin ratio than those with GDM. In contrast, the MMW/total adiponectin and LMW/total adiponectin ratios were lower in overweight/obese pregnant women with a normal pregnancy than in those with GDM.

nectin ratio; and 5) among GDM patients, there were no differences in the concentrations and relative distribution of adiponectin multimers between patients who were managed with diet, and those who were treated with pharmacological agents (Glyburide or insulin).

#### The role of adiponectin in obesity-related complications of pregnancy

Recently, adipokines have been implicated in physiologic adaptation of normal and complication of pregnancy [65, 77–86, 99–101, 141]. Indeed, the unique properties of adiponectin have prompted many investigators to explore the role of maternal circulating adiponectin in the metabolic adaptations occurring during human pregnancy [23, 82, 87], as well as in obesity-related complications of pregnancy such as GDM [62, 114, 134, 147] and preeclampsia [27, 29, 48, 49, 57, 73, 97, 113]. Indeed, normal pregnancy is associated with alterations in maternal circulating adiponectin [80, 82, 84, 128] and with changes in the relative distribution of its isoforms [23, 77, 115, 116]. In addition, even in the presence of a normal pregnancy, overweight pregnant women have a lower adiponectin concentration than those with a normal weight [43]. Moreover, low concentrations of adiponectin in the first or early second trimester is an independent risk factor for the development of GDM. Indeed, maternal adiponectin concentration  $\leq 6.4$   $\mu\text{g/mL}$  at 13 weeks of gestation is associated with a 4.6-fold increased risk to develop GDM later in pregnancy [146], suggesting a causal relationship between low circulating adiponectin and GDM. Consistent with this finding, GDM is characterized by a low circulating concentrations of adiponectin [10, 62, 114, 117, 118, 134, 137, 147]. Collectively, a growing body of evidence points to a key role of adiponectin in the pathophysiology of both Type 2 DM and GDM.

#### An asymmetrical decrease in adiponectin multimers concentrations and alteration in their relative distribution are features of gestational diabetes

The findings of the present study characterize GDM as a condition in which there is a decrease in the maternal

serum concentrations of all adiponectin multimers. In addition, there is a concomitant decrease in the HMW/total adiponectin ratio and increases in MMW/total as well as LMW/total adiponectin ratios in patients with GDM. These findings are novel. Only one study has reported the concentrations of HMW adiponectin in patients with GDM [115]. The lower median maternal serum HMW concentration and HMW/total adiponectin ratio in patients with GDM than those with a normal pregnancy, reported herein, are in agreement with the findings of Retnakaran et al. [115]. The present report extends these observations by demonstrating that the maternal concentrations of all adiponectin isoforms are decreased and that MMW/total and LMW/total ratios are increased in patients with GDM. Despite the fact that the decrease in adiponectin multimers was across the board, only HMW/total adiponectin ratio was lower in these patients compared with normal pregnant women, indicating that the decrease in HMW adiponectin was far more significant than the reduction in the concentrations of MMW or LMW isoforms. Indeed, MMW/total adiponectin and LMW/total adiponectin ratios were higher in patients with GDM than in those with a normal pregnancy. In conclusion, the results of this study clearly reveal that the low concentration of adiponectin in patients with GDM should be attributed to an accentuated decrease in HMW adiponectin.

#### **Why is gestational diabetes associated with a disparate decrease of adiponectin multimeric complexes and their relative concentrations?**

The findings regarding the asymmetrical decrease in adiponectin multimers and their relative concentrations reported herein support the emerging concept that the anti-diabetic effect of adiponectin may be mediated by the HMW isoforms. The cross sectional nature of the present study limits our ability to infer a causal relationship between adiponectin and GDM; however, the robust association of low circulating total and HMW adiponectin with states of insulin resistance has raised the possibility that there may be a cause and effect relationship between adiponectin and both Type 2 DM and GDM. Several lines of evidence suggest a causal linkage between the two: 1) treatment of adipose like cell lines (3T3-L1 cells) with peroxisome proliferator-activated receptor- $\gamma$  agonist (Thiazolidinediones) results in increased adiponectin expression [76]; 2) *in vivo*, exposure of normal or obese mice to adiponectin increases insulin sensitivity [13, 40, 149]; 3) mutations in the adiponectin gene that result in failure to form HMW adiponectin (e.g., G84R and G90S) are associated with Type 2 DM and low adiponectin concentrations [144]. Similarly, polymorphisms in the adiponectin gene increase susceptibility to develop metabolic syndrome and Type 2 DM [38, 44, 46, 53, 71, 90, 96, 131, 142] and; 4) low adiponectin concentrations confer an increased risk for the development

of Type 2 DM even in the absence of any other biomarkers of impaired glucose metabolism [72, 130].

In conclusion, the findings reported herein define dysregulation of adipose tissue as a feature of GDM. It is tempting to postulate that the decrease in adiponectin multimeric complexes concentrations and their relative distribution can lead to modulation of adiponectin effect on target tissues (i.e., muscles, liver, adipose tissue), thus providing a mechanistic basis to GDM.

#### **Gestational diabetes and overweight/obesity – an ominous metabolic combination**

The finding that adiponectin multimers and their relative concentrations are comparable between normal weight and overweight/obese patients with GDM is novel. The negative association between circulating adiponectin and excess adipose tissue accrual is well established [8, 77]. However, while overweight/obesity and weight gain usually mitigates adiponectin concentrations, we found no significant change in the concentrations of adiponectin multimers between normal weight and overweight/obese patients in the presence of GDM. Thus, it can be concluded that the effect of obesity on the quantitative and qualitative alterations in adiponectin multimers in obese pregnant women is overwhelmed by the presence of GDM. The fact that the median maternal serum concentrations of all adiponectin multimers were lower in overweight/obese patients with GDM than in overweight/obese individuals with a normal pregnancy further supports this hypothesis. Of interest, among overweight/obese subjects, the decrease in HMW adiponectin was disproportionate in patients with GDM as indicated by a lower median HMW/total adiponectin ratio in patients with GDM than that of those with a normal pregnancy and higher median MMW/total and LMW/total adiponectin ratios.

Previous reports demonstrate that higher order adiponectin multimers do not interconvert in the circulation. Thus, the quantitative and qualitative changes in adiponectin multimers reported herein must be attributed to altered production/secretion by adipocytes. Hence, it can be concluded that GDM is characterized by adipose tissue dysfunction as manifested by dysregulation of circulating adiponectin multimers. This finding may have clinical implications since manipulation of adiponectin concentrations and the relative distribution of its multimeric complexes opens new avenues for the treatment of Type 2 DM, as well as GDM.

#### **Diet, sulfonylurea and insulin and adiponectin multimers concentrations and their abundance**

There was no difference in the concentrations of adiponectin isoforms or their relative distribution between patients who were managed with diet and those who were treated with Glyburide or insulin. Similarly, patients

treated with Glyburide and those treated with insulin had comparable concentrations of adiponectin multimers. These findings are novel. Indeed, there are no reports concerning the effect of sulfonylurea exposure on adiponectin concentrations during pregnancy; thus, the present study is the first to compare circulating adiponectin and adiponectin multimers in pregnant women with and without sulfonylurea treatment. The literature regarding the effect of insulin on adiponectin is controversial. Insulin's inhibitory effect on adiponectin gene expression has been demonstrated *in vitro* [34]. In accordance with this finding, a 10–20% reduction in adiponectin concentrations was reported in several hyperinsulinemic-euglycemic clamp studies in lean humans [18, 93, 152]. In contrast, other investigators found no effect of insulin on circulating adiponectin [107]. Importantly, contradictory reports concerning the effect of insulin on adiponectin multimers have been published. Selective decreases in HMW [11] as well as no changes in circulating concentrations of this isoforms [15] were reported. A possible explanation for the lack of insulin effect on adiponectin concentrations is that the inhibitory effect of insulin on adiponectin is not dose depended. In other words, once insulin reaches a certain concentration it cannot exert any additional inhibitory effect on adiponectin [15]. Since hyperinsulinemia is a characteristic of GDM, supplementation with insulin may not be accompanied by a reduction in adiponectin concentrations as insulin has already exerted its maximal inhibitory effect.

Only one study, by Catalano et al. [23] determined the effect of insulin on adiponectin concentrations in a longitudinal study of lean pregnant women. Interestingly, the authors reported that insulin infusion decreased HMW adiponectin complexes in pregravid women but this suppressive effect of insulin was lost during pregnancy. The result of this study supports the report by Catalano et al. and extends it by demonstrating comparable maternal circulating adiponectin in patients with lean and overweight/obese patients with GDM. Congruent with this observation, we found no difference between patients who were treated with insulin and those who were managed with diet alone.

In conclusion, perturbation of adiponectin homeostasis and alterations in the relative distribution of its multimers are considered to be a marker of adipose tissue function since adiponectin is produced and modified exclusively by adipocytes. Evaluation of adiponectin isoforms in normal weight and overweight/obese patients with GDM reveals that this condition is characterized by a distinctive pattern of concentrations and a relative distribution of adiponectin multimers akin to Type 2 DM. Taken together, the results of the present study suggest that dysregulation of adiponectin multimers can provide a mechanistic basis for the association between adiposity and GDM. Moreover, the pattern of circulating adiponectin multimers concentrations and their relative abundance

is congruent for both GDM and Type 2 DM. This finding suggests that adiponectin dysregulation can account for the long-term complication of GDM. Further studies are needed in order to elucidate the intriguing relationships between adiponectin multimers, GDM and the subsequent development of Type 2 DM.

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