

Maternal and Cord Blood Adiponectin Multimeric Forms in Gestational Diabetes Mellitus

A prospective analysis

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OBJECTIVE—To analyze the relationship between maternal adiponectin (mAdiponectin) and cord blood adiponectin (cbAdiponectin) multimeric forms (high molecular weight [HMW], medium molecular weight [MMW], and low molecular weight [LMW]) in a cohort of gestational diabetes mellitus (GDM) and normal glucose-tolerant (NGT) pregnant women.

RESEARCH DESIGN AND METHODS—A total of 212 women with a singleton pregnancy, 132 with NGT and 80 with GDM, and their offspring were studied. Maternal blood was obtained in the early third trimester and cord blood was obtained at delivery. Total adiponectin and the multimeric forms of adiponectin were determined in cord blood and maternal serum. Spearman rank correlation and stepwise linear correlation analysis were used to assess the relationship between cbAdiponectin levels and clinical and analytical parameters.

RESULTS—No differences in cbAdiponectin concentration or its multimeric forms were observed in the offspring of diabetic mothers compared with NGT mothers. The HMW-to-total adiponectin ratio was higher in cord blood than in maternal serum, whereas the MMW- and LMW-to-total adiponectin ratio was lower. Cord blood total and HMW adiponectin levels were positively correlated with birth weight and the ponderal index (PI), whereas cord blood MMW adiponectin was negatively correlated with the PI. In addition, cbAdiponectin and its multimeric forms were correlated with mAdiponectin concentrations. In the multivariate analysis, maternal multimeric forms of adiponectin emerged as independent predictors of cbAdiponectin, its multimers, and their distribution.

CONCLUSIONS—cbAdiponectin concentrations are independently related to mAdiponectin levels and unrelated to the diagnosis of GDM. Maternal multimeric forms of adiponectin are independent predictors of the concentrations of cbAdiponectin and its multimeric forms at delivery.

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Adiponectin is one of the most abundant adipose tissue-specific proteins that is supposed to play a role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues (1). After post-translational modifications,

adiponectin is secreted into the bloodstream in three different multimeric forms: a trimeric or low molecular weight form (LMW-Ad), a hexameric or medium molecular weight form (MMW-Ad), and a high molecular weight form (HMW-Ad)

(2,3). The amount and distribution of these molecular forms can determine the activity of adiponectin in different tissue (2–4).

The last half of pregnancy is characterized by an insulin-resistant state, and an inverse relationship between adiponectin levels and insulin resistance has been reported (5). Lower adiponectin levels have been observed in gestational diabetes mellitus (GDM) and in obese pregnant women when compared with uncomplicated pregnancies (5–8). Considering the fundamental role of adiponectin in insulin metabolism and the importance of glucose and insulin in fetal growth, maternal adiponectin (mAdiponectin) may play a regulatory role in fetal growth. Unfortunately, the results published to date have yielded inconsistent results (5,9–11). Adiponectin is also present during fetal life and it is detectable in cord serum at as early as 24 weeks of pregnancy (12). Its levels increase markedly during the gestation progresses until delivery, and adiponectin levels in neonates are several fold higher than those reported in adults (13). These findings suggest a role for fetal adiponectin in the modulation of intrauterine growth. However, the role of adiponectin during fetal life is not well known.

A crosstalk between mAdiponectin and fetal metabolism has been proposed. Nevertheless, studies have failed to find a relationship between mAdiponectin and fetal adiponectin (11,13–15). Most of the clinical studies published to date have evaluated total cord blood adiponectin (cbAdiponectin) in the peripartum period, so it has not been possible to establish a long-term modulation of fetal adiponectin production by mAdiponectin levels. We hypothesize that mAdiponectin could influence fetal adiponectin levels during the third trimester of pregnancy, given that the multimeric forms are a key element of this regulation. For this purpose, we have measured mAdiponectin levels in the late second trimester or early third trimester and cbAdiponectin levels at delivery, analyzing

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the relationship between mAdiponectin and cbAdiponectin levels, its multimeric adiponectin distribution, and its relationship with clinical and metabolic features.

RESEARCH DESIGN AND METHODS

This is a prospective case-control study conducted at the Joan XXIII University Hospital. The study protocol was approved by the Research Ethics Board of the center and all subjects gave written informed consent. Three hundred and seventy-seven pregnant Caucasian women were recruited at the time of antepartum screening for GDM. All of the women who participated underwent a 3-h, 100-g oral glucose tolerance test (OGTT) and were monitored from the time of inclusion to delivery. Serum and plasma samples were kept in a GDM biobank collection. According to the OGTT, women with two or more values above the threshold proposed by the National Diabetes Data Group (16) were considered to have GDM, and women who had all the values below the threshold were classified in the normal glucose-tolerant (NGT) group. In this study we included 212 women that fulfilled the following criteria at the end of pregnancy: 1) a singleton pregnancy, 2) accurate gestational age confirmed by an ultrasound examination before 20 weeks of gestation, 3) the absence of fetal anomalies identified at birth, 4) cord blood sample obtained at delivery, and 5) normal glucose tolerance or GDM diagnosed before 30 weeks of pregnancy. Women with only one value above the threshold after OGTT were excluded from the study. Women with pre-existing diabetes, inflammatory or chronic diseases, or current use of drugs known to affect carbohydrate metabolism were also excluded.

GDM women were given an individualized diet with 40% carbohydrates and they were instructed to self-monitor blood glucose six times a day (fasting and 1-h postprandial). Insulin therapy was recommended when fasting glucose levels were repeatedly >95 mg/dL and/or 1-h postprandial values were repeatedly >140 mg/dL. According to these criteria, 51 women were treated only with diet, and 29 women required the addition of insulin.

Clinical and demographic data

Upon inclusion, demographic and historical information was collected by an interviewer-administered questionnaire that included patient demographics, personal medical and obstetrical history, and information regarding the current pregnancy,

with special attention to risk factors for GDM. Maternal anthropometric measurements of height and weight were obtained using a medical scale. At the end of pregnancy, data concerning weight gain, gestational age at delivery, and neonatal anthropometry were also collected. BMI was calculated using the formula $BMI = \text{weight (kg)}/\text{height (m)}^2$. Increase in BMI was calculated by the formula $BMI \text{ gain} = \text{final BMI} - \text{pregravid BMI}$. Neonatal length and weight were determined using a measuring board and a calibrated scale. The ponderal index (PI) was calculated using the formula $PI = \text{birth weight (g)}/[\text{body length (cm)}^3] \times 100$.

Laboratory measurements

The 100-g OGTT was performed in the morning after overnight fast. Venous blood samples were drawn at baseline and 60, 120, and 180 min after ingestion of a standard 100-g glucose load. Glucose levels were determined in an ADVIA 2400 (Siemens AG, Munich, Germany) autoanalyzer using the standard enzyme methods. Fasting plasma insulin and C-peptide were determined by immunoassay in an ADVIA Centaur System (Siemens AG). This assay shows a cross-reactivity of <0.1% to intact human proinsulin and the primary circulating split form des 31,32 proinsulin. Homeostasis model assessment of insulin resistance (HOMA-IR) was determined according to the following equation: $\text{fasting plasma glucose (mmol/L)} \times \text{fasting plasma insulin } (\mu\text{U/mL})/22.5$ (17).

Serum adiponectin levels were determined using a human ELISA kit (Multimeric Adiponectin ELISA Kit; Bühlmann, Schönenbuch, Switzerland). The intra- and interassay coefficient of variations (CVs) were <15%, and assay sensitivity was 0.08 ng/mL. We calculated the ratio of HMW-to-total adiponectin levels (S_A) for mAdiponectin and cbAdiponectin concentrations, mS_A and cbS_A , respectively. In addition, the ratio of MMW- and LMW-to-total adiponectin levels was calculated for maternal ($mMMW/\text{Total}$ and $mLMW/\text{Total}$, respectively) and cord blood ($cbMMW/\text{Total}$ and $cbLMW/\text{Total}$) determinations.

Statistics

All statistical analysis was performed using SPSS 13.0 software (SPSS, Chicago, IL). Normally distributed data were expressed as the mean \pm SD, whereas variables with a skewed distribution were represented as the median (interquartile range). Categorical variables were reported

by number (percentages). Student *t* test analysis was used to compare the mean value of normally distributed continuous variables. For variables with skewed distributions, we used the Mann-Whitney *U* test. To analyze the differences in nominal variables between groups, we performed the χ^2 test. Spearman rank correlation coefficient was used to analyze the bivariate correlation between adiponectin levels and clinical or metabolic parameters. To evaluate the effect of clinical and metabolic variables on the relationship observed between mAdiponectin and cbAdiponectin, we conducted partial correlations. Finally, stepwise multiple regression analysis including mAdiponectin levels and metabolic and clinical variables that could be associated with cbAdiponectin was performed to determine whether mAdiponectin or its isoforms were independently associated with umbilical adiponectin. $P < 0.05$ was considered significant.

RESULTS

Maternal outcome

The main clinical and metabolic characteristics of the population included in the study are presented in Table 1. The increase in BMI at the end of pregnancy was significantly greater in the NGT group ($P < 0.001$), whereas no difference in prepregnancy BMI had been observed between groups. A history of GDM in a previous pregnancy was more frequent in the GDM than in the NGT group, whereas no differences were observed in previous delivery of a macrosomic infant or Caesarean section. Fasting insulin (fInsulin) levels and HOMA-IR index were higher in the GDM group.

Adiponectin and multimeric forms

Total mAdiponectin (mTotal-Ad) and HMW mAdiponectin (mHMW-Ad) levels were higher in the NGT group compared with the GDM group, whereas no differences were observed in the remaining multimeric forms or in their distribution (see Table 1). In the GDM group, the women treated with insulin showed lower mS_A values and mHMW-Ad concentrations compared with the group treated only with diet (0.563 ± 0.091 vs. 0.485 ± 0.118 , $P = 0.002$, and 2.93 ± 1.28 vs. 2.22 ± 1.55 $\mu\text{g/mL}$, $P = 0.032$, respectively). No other differences were observed between the GDM patients treated only with diet compared with those treated with insulin.

Table 1—Clinical and metabolic characteristics of the population studied

	Whole group	NGT (n = 132)	GDM (n = 80)	P
Maternal characteristics				
Age (years)	31.54 ± 4.99	31.33 ± 4.86	31.88 ± 5.19	NS
Gestational age (weeks)	27 (26.25–29.00)	27.46 ± 1.40	27.51 ± 1.41	NS
Parity (n)	1.00 (0.00–2.00)	1.00 (0.00–1.00)	1.00 (0.00–2.00)	NS
Prepregnancy BMI (kg/m ²)	25.10 ± 5.06	24.83 ± 5.13	25.56 ± 4.94	NS
ΔBMI total (kg/m ²)	4.37 ± 2.12	4.79 ± 2.06	3.67 ± 2.05	<0.001
Tobacco use n (%)	36 (17.0)	21 (15.9)	15 (18.75)	NS
Previous GDM n (%)*	18 (15.52)	4 (5.8)	14 (29.8)	0.001
Previous macrosomia n (%)*	14 (9.07)	9 (13.0)	5 (10.6)	NS
Previous Caesarean section n (%)*	19 (16.38)	9 (13.0)	10 (21.8)	NS
Glucose (mg/dL)	82.69 ± 9.59	80.69 ± 7.23	85.99 ± 11.89	<0.001
Glucose 60' (mg/dL)	175.85 ± 38.48	154.07 ± 23.46	211.79 ± 30.71	<0.001
Glucose 120' (mg/dL)	143.26 ± 37.68	119.81 ± 21.71	181.96 ± 23.98	<0.001
Glucose 180' (mg/dL)	109.47 ± 34.34	95.42 ± 26.72	132.65 ± 33.04	<0.001
A1C (%)	—	—	4.86 ± 0.39	
mTotal-Ad (μg/mL)†	5.44 ± 2.32	5.80 ± 2.37	4.85 ± 2.11	<0.01
mHMW-Ad (μg/mL)	28.01 (1.73–3.92)	3.27 ± 1.72	2.45 ± 1.42	<0.01
mMMW-Ad (μg/mL)	1.19 ± 0.55	1.24 ± 0.56	1.12 ± 0.53	NS
mLMW-Ad (μg/mL)	1.11 (0.73–1.58)	1.29 ± 0.84	1.12 ± 0.76	NS
mS _A	0.539 ± 0.114	0.5440 ± 0.115	0.533 ± 0.108	NS
mMMW/Total	0.237 ± 0.095	0.230 ± 0.091	0.248 ± 0.101	NS
mLMW/Total	0.228 ± 0.115	0.226 ± 0.112	0.2316 ± 0.122	NS
fInsulin (mUI/L)	8.76 (6.11–14.15)	7.73 (5.83–13.28)	10.03 (7.01–15.12)	0.03
HOMA-IR	1.77 (1.19–2.94)	1.50 (1.12–2.74)	2.08 (1.42–3.53)	0.010
Gestational age at delivery (weeks)	39 (38–40)	39.37 ± 1.57	39.00 ± 1.43	NS
Fetal characteristics				
Birth weight (g)	3,271.48 ± 484.10	3,275.91 ± 485.50	3,255.67 ± 493.87	NS
Ponderal index (kg/m ³)	2.70 ± 0.24	2.70 ± 0.24	2.69 ± 0.25	NS
cbTotal-Ad (μg/mL)	17.34 ± 6.44	17.58 ± 6.31	16.94 ± 6.70	NS
cbHMW-Ad (μg/mL)	12.35 ± 5.13	12.50 ± 5.04	12.03 ± 5.31	NS
cbMMW-Ad (μg/mL)	3.19 ± 1.77	3.25 ± 1.78	3.09 ± 1.73	NS
cbLMW-Ad (μg/mL)	2.06 (1.086–3.294)	2.42 ± 1.71	2.354 ± 1.94	NS
cbS _A	0.702 ± 0.112	0.7045 ± 0.117	0.700 ± 0.104	NS
cbMMW/Total	0.191 ± 0.086	0.194 ± 0.104	0.195 ± 0.096	NS
cbLMW/Total	0.133 ± 0.098	0.1337 ± 0.094	0.130 ± 0.105	NS
Cord blood insulin (mU/mL)	4.47 (2.65–8.03)	4.34 (2.47–6.89)	5.36 (3.12–10.72)	0.010

A1C, glycosylated hemoglobin; NS, not significant. *Only women with a previous pregnancy included in the analysis. †mAdiponectin was measured early in the third trimester.

Fetal outcome

One hundred and five of the neonates were male and one hundred and seven were female. No differences were observed in the distribution between NGT and GDM groups. Mean birth weight (BW) and PI were similar in both groups (Table 1).

No differences were observed in total cbAdiponectin, the multimeric forms or cbS_A, cbMMW/Total, and cbLMW/Total between NGT and GDM patients. Only cord blood insulin concentration was higher in infants of GDM mothers (Table 1). No differences in total cbAdiponectin levels and cbAdiponectin distribution were observed between the GDM patients treated only with diet and those treated with insulin.

Relationship between mAdiponectin and cbAdiponectin levels

Total adiponectin levels and each of its isoforms were higher in cord blood than in the mother ($P < 0.001$ for all, see Table 1). Also, the S_A was higher in cord blood, but MMW-Ad- and LMW-Ad-to-total adiponectin ratios were lower than in maternal serum ($P < 0.001$ for all, see Table 1). Similar results were observed when we analyzed the NGT and the GDM groups separately. Hence, the bivariate and multivariate analyses were performed with the whole group.

Bivariate correlation analysis mAdiponectin with metabolic and clinical variables. mTotal-Ad and mHMW-Ad levels were negatively correlated with

prepregnancy BMI ($r = -0.183$, $P < 0.01$, for both), fInsulin ($r = -0.340$, $P < 0.001$; and $r = -0.376$, $P < 0.001$, respectively) and HOMA-IR ($r = -0.325$, $P < 0.001$; and $r = 0.367$, $P < 0.001$, respectively). mS_A was also negatively correlated with fasting glucose ($r = -0.186$, $P < 0.01$), fInsulin ($r = -0.284$, $P < 0.001$), HOMA-IR ($r = -0.295$, $P < 0.001$), PI ($r = -0.200$, $P < 0.01$), and cord blood insulin concentrations ($r = -0.140$, $P < 0.05$). Maternal MMW-Ad (mMMW-Ad) was positively correlated with gain in BMI ($r = 0.165$, $P < 0.05$) and PI ($r = 0.193$, $P < 0.01$), whereas the mMMW/Total was correlated with fInsulin ($r = 0.262$, $P < 0.001$), HOMA-IR ($r = 0.245$, $P < 0.001$) and PI ($r = 0.210$, $P < 0.01$). mLMW/Total concentrations

were only marginally related with fasting glucose levels ($r = 0.158, P < 0.05$).

cbAdiponectin with metabolic and clinical variables. Cord blood total adiponectin (cbTotal-Ad) and cord blood HMW (cbHMW) were related with the gain in BMI ($r = 0.250, P < 0.01$; and $r = 0.163, P < 0.05$, respectively), BW ($r = 0.154, P < 0.05$; and $r = 0.165, P < 0.05$), and PI ($r = 0.228, P = 0.001$; and $r = 0.221, P = 0.001$). Cord blood MMW adiponectin (cbMMW-Ad) was negatively correlated with PI ($r = -0.185, P < 0.01$) and, finally, cord blood LMW adiponectin (cbLMW-Ad) was weakly associated with fasting glucose levels ($r = 0.184, P < 0.05$). A scatterplot figure of the relationship between cbTotal-Ad and PI is available as Supplementary Fig. 1.

mAdiponectin and cbAdiponectin bivariate correlations. The most significant correlations observed between mAdiponectin and cbAdiponectin and their multimeric forms are presented in Table 2. These associations persisted after controlling for possible confounding factors such as gestational age, maternal prepregnancy BMI, PI, HOMA-IR, and the diagnosis of GDM (Table 2).

In this subset of correlations, when GDM and NGT were studied separately, we observed that the correlation coefficients between MMW-Ad and LMW-Ad were similar in both groups, but there were some differences in the strength of correlations between total adiponectin and HMW-Ad adiponectin. The correlation coefficient for total adiponectin was higher in the GDM group ($r = 0.335, P < 0.01$, vs. $r = 0.193, P < 0.05$), and for the HMW isoform it was only significant in the GDM group ($r = 0.294, P < 0.01$).

Multivariate regression analysis

To analyze the relationship between cord blood and mAdiponectin levels, a stepwise multiple linear regression analysis was performed with dependent variable

cbTotal-Ad concentrations. Variables considered for selection in the model included mTotal-Ad, PI, maternal age, gestational age at delivery, GDM, neonatal sex, cord blood insulin, HOMA-IR, prepregnancy BMI, and gain in BMI. The final regression model identified PI and mTotal-Ad as positive independent determinants and maternal age as a negative determinant of cbTotal-Ad levels (Table 3).

We repeated this multiple regression analysis with the introduction of the maternal multimeric forms of adiponectin and the exclusion of total adiponectin in order to determine which of these forms was responsible for the association. mHMW-Ad, PI, and LMW-Ad were positive, independent predictors of cbTotal-Ad (see Table 3).

To further analyze the relationship between maternal and cord blood multimeric forms of adiponectin, we repeated the last multiple regression model with dependent variables cbHMW-Ad, cbMMW-Ad, and cbLMW-Ad, respectively, in place of cbTotal-Ad (Table 3). mMMW-Ad emerged as a positive, independent predictor for both dependent variables cbHMW-Ad and cbMMW-Ad, whereas mHMW-Ad was also a negative, independent predictor of cMMW-Ad. mLMW-Ad was a positive predictor of cbLMW-Ad, and PI was a positive, independent predictor of cbHMW-Ad, the dominant multimeric form in cord blood.

As multimeric form distribution was also different in cord blood compared with maternal serum, we also explored the independent relationship between the ratios of each multimer in cord blood with maternal and neonatal parameters. Stepwise multiple linear regression analyses were performed with dependent variable cbS_A , cbMMW/Total, and cbLMW/Total ratios in each model. For cbS_A as a dependent variable, mLMW/Total emerged as a negative, independent variable ($B = -0.273$ [95% CI -0.412 to -0.134]; $P < 0.001$) and gestational week at delivery as a positive, independent variable ($B = 0.014$

[0.004–0.024; $P = 0.006$]). On the other hand, when cbMMW/Total was introduced in the model as a dependent variable, the mMMW/Total ratio was positively associated ($B = 0.224$ [0.098–0.351; $P = 0.001$]), whereas gestational week at delivery ($B = -0.010$ [-0.017 to 0.002 ; $P = 0.013$]) was negatively related. Finally, when cbLMW/Total was introduced in the model as a dependent variable, mLMW-Ad ($B = 0.031$ [0.013–0.048; $P = 0.001$]) and the mLMW/Total ratio ($B = 0.181$ [0.013–0.319; $P = 0.010$]) were the two variables associated with it.

CONCLUSIONS—In this study, we have shown similar concentrations of total adiponectin and its multimeric forms in cord blood of offspring of GDM and NGT mothers as well as similar multimeric form distribution. We also report that the differences in adiponectin levels observed between GDM and NGT pregnant women are restricted to the mHMW-Ad and are present despite similar BMI. Also, our data provide a comprehensive examination regarding the relationship of adiponectin and circulating multimeric forms in cord blood with maternal levels and some clinical and metabolic parameters. Here, we report that umbilical and mAdiponectin concentrations differ not only in total adiponectin and multimeric form levels but also in the distribution of multimeric forms. Likewise, we describe a close relationship between maternal and umbilical adiponectin levels.

Recently, Mazaki-Tovi et al. (6,8) analyzed adiponectin levels and its multimeric forms during pregnancy, but little is known about the distribution of the multimeric forms of adiponectin in cord blood. In our study, similar total adiponectin levels were observed between offspring of GDM and NGT women, as has been reported (18). We also failed to find any difference between both groups in any of the multimeric forms or in their distribution. In contrast, other authors

Table 2—Spearman and adjusted correlation coefficients between mAdiponectin and cbAdiponectin and its multimeric forms

	cbTotal-Ad		cbHMW-Ad		cbMMW-Ad		cbLMW-Ad	
	Unadjusted	Adjusted§	Unadjusted	Adjusted§	Unadjusted	Adjusted§	Unadjusted	Adjusted§
mTotal	0.268‡	0.252†	0.225†	0.230†	—	—	0.271‡	0.294‡
mHMW	0.221†	0.188*	0.225†	0.210†	—	—	0.146*	0.155*
mMMW	0.298‡	0.282‡	0.280‡	0.266‡	0.251‡	0.219†	—	—
mLMW	0.179*	0.154*	—	—	—	—	0.452‡	0.474†

§Partial correlation coefficients adjusted for gestational age, prepregnancy BMI, PI, HOMA-IR, and GDM. cbMMW-Ad was correlated in the unadjusted analysis with mMMW-Ad, $r = 0.251‡$, but not with mTotal-Ad. cbMMW-Ad was correlated in the adjusted analysis with mMMW-Ad, $r = 0.219†$, but not with mTotal-Ad. cbLMW-Ad was correlated in the unadjusted analysis with mLMW-Ad, $r = 0.452‡$, but not with mMMW-Ad. cbLMW-Ad was correlated in the adjusted analysis with mLMW-Ad, $r = 0.474†$, but not with mMMW-Ad. ‡ $P < 0.001$. † $P < 0.01$. * $P < 0.05$.

Table 3—Stepwise multiple linear regression models of dependent variable cbTotal-Ad and its multimeric forms

Dependent variable	Variables included in the model	B (95% CI)	P
cbTotal-Ad (model adjusted $r^2 = 0.121$; $P < 0.001$)*	mTotal-Ad	0.743 (0.385–1.101)	<0.001
	PI	6.312 (2.802–9.823)	<0.001
	Age	−0.186 (−0.357 to −0.015)	0.033
cbTotal-Ad (model-adjusted $r^2 = 0.147$; $P < 0.001$)†	mMMW-Ad	3.397 (1.835–4.958)	<0.001
	PI	4.920 (1.316–8.524)	0.008
	mLMW-Ad	1.194 (0.156–2.231)	0.024
cbHMW-Ad (model-adjusted $r^2 = 0.100$; $P < 0.001$)†	mMMW-Ad	2.399 (1.100–3.688)	<0.001
	PI	3.615 (0.641–6.589)	0.017
cbMMW-Ad (model-adjusted $r^2 = 0.089$; $P < 0.001$)†	mMMW-Ad	1.038 (0.575–1.502)	<0.001
	mHMW-Ad	−0.225 (−0.379 to −0.070)	0.005
cbLMW-Ad (model-adjusted $r^2 = 0.236$; $P < 0.001$)†	mLMW-Ad	1.075 (0.788–1.362)	<0.001

*Model 1 covariates considered for selection: PI, maternal age, gestational age at delivery, GDM, neonatal sex, cord blood insulin, HOMA-IR, prepregnancy BMI, and gain in BMI. †Model 2 covariates considered for selection: PI, maternal age, gestational age at delivery, GDM, neonatal sex, cord blood insulin, HOMA-IR, prepregnancy BMI, gain in BMI, mHMW-Ad, mMMW-Ad, and mLMW-Ad.

(19,20) have found lower adiponectin levels in cord blood of offspring of GDM compared with control subjects, and this finding was unrelated to gestational age and BW. We have no explanation for this discrepancy, but as cbAdiponectin levels seem to be more closely related to adiposity than BW (18), differences in this parameter could be a possible explanation, and considering that we would remark that in our study, BW and PI were similar in both groups, probably due to the effect of treatment. These data suggest that maternal glucose tolerance status, at least when treated, does not play a relevant role in fetal adiponectin secretion.

We also confirm that cbAdiponectin levels are rather higher than in maternal serum, in agreement with previous observations (21), with the HMW-Ad being the predominant form and the MMW-Ad form being the second in abundance. Interestingly, we also describe a different distribution in the multimeric forms in cord blood than in the maternal serum. Thus, a higher proportion of the S_A with a lower ratio of the remaining isoforms was the profile depicted in cord blood in contrast with maternal distribution. This may be important concerning the biological effects of adiponectin, because it has become apparent that not only the absolute amount of adiponectin but also the distribution of the multimeric forms seem to play a role in its biological activity (2–4). In addition, it is interesting that as gestational age progresses, there is an increase in the cbS_A ratio while the $cbMMW/Total$ ratio decreases, probably indicating a change in the pattern

of adiponectin secretion by fetal tissues as the fetus grows. It is well known that when adiponectin isoforms appear in circulation, they do not interconvert (22), and at the end of pregnancy, there seems to be a decline of adiponectin secretion by nonadipose tissues (23), whereas there is an increase of adiponectin secretion from adipose tissue that produces predominantly HMW-Ad.

The progressive increase in adiponectin levels from week 24 of pregnancy until delivery would suggest a role of this adipokine in the regulation of fetal growth and fat deposition. It has been observed in experimental models that adiponectin is a potent stimulator of adipogenesis (24). Our results support this hypothesis, as we observe that cbTotal-Ad and cbHMW-Ad are positively related with PI and BW, whereas cbMMW-Ad is only inversely related to PI. One is tempted to hypothesize that HMW-Ad stimulates fetal growth and MMW-Ad inhibits it.

The association between mAdiponectin levels and fetal growth are less clear. A negative correlation with BW has been reported by some authors (9,14), but not others (11). We failed to find a relationship, but mS_A was inversely related to PI, whereas mMMW-Ad was positively related. These data would suggest an inverse effect of both the mS_A and mMMW-Ad on fetal growth. A low mS_A is associated with a higher insulin resistance state that could contribute to a metabolically altered environment that favors an increased fetoplacental availability of nutrients and fetal overgrowth.

Interestingly, we observed a relationship between mAdiponectin and umbilical adiponectin levels in the bivariate correlate that persisted after adjustment for factors known to be associated with mAdiponectin levels. In the GDM group, the mHMW isoform had stronger correlation coefficients with cord blood total and HMW adiponectin than the NGT group, so despite similar cbAdiponectin levels in both groups, we cannot rule out that the impaired glucose metabolism may have a role in the crosstalk between mAdiponectin and fetal adiponectin production. Furthermore, in the regression model, mAdiponectin multimers and PI were found to be independent predictors of cbAdiponectin levels. This finding contrasts with previous reports in which no relationship between mAdiponectin and umbilical adiponectin is observed (11,13–15). A possible explanation of these discrepancies may lie in the study design and the population evaluated. We assessed mAdiponectin levels in the early third trimester, before any therapeutic intervention had been started, which allowed us to evaluate a long-term effect on fetal growth and on cbAdiponectin concentrations. As far as we are aware, in other published studies, mAdiponectin levels were obtained in the peripartum period, just before (11,13,14) or after delivery (15). As fetal adiponectin levels increase and mAdiponectin concentration decreases as the pregnancy progresses, differences in the time of extraction could be a possible explanation for the discordant data. Thus, our data would suggest that mAdiponectin

early in the third trimester may be one of the factors implicated in the modulation of fetal adiponectin secretion, and that the multimeric forms and their distribution are the most implicated.

This analysis must be interpreted within the context of certain limitations. First, in our study, we have included only Caucasian women and so we cannot extrapolate to other ethnicities. Also, HOMA-IR has limitations as a measure for insulin resistance. However, we noted that the previously observed inverse associations between insulin resistance and mTotal-Ad and mHMW-Ad were also observed in this study. Nevertheless, this study represents one of the first investigations into the specific relationships of HMW-Ad, MMW-Ad, and LMW-Ad in umbilical serum with mAdiponectin levels and metabolic parameters and should lead to further studies.

In conclusion, we report that adiponectin multimeric distribution in cord blood is different than in adult life and is not different in the offspring of GDM and NGT mothers. We also describe that total adiponectin circulating levels and the multimeric forms of adiponectin in cord blood are independently related to PI and the multimeric forms of mAdiponectin, suggesting a crosstalk between mAdiponectin levels and fetal adiponectin production.

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M.B. held the outpatient clinic, researched data, and wrote and edited the manuscript. I.S. contributed to the study concept and design, recruited patients, discussed the study results, and reviewed the final manuscript. J.V. contributed to writing and reviewing the final manuscript. V.C.-M. conducted measurements and reviewed the manuscript. R.M.M. reviewed the manuscript. G.A. helped in the outpatient clinic and contributed to the manuscript. F.T. reviewed the manuscript. A.M. held

the outpatient clinic, contributed to the study concept and design, recruited patients, discussed the study results, and reviewed the final manuscript.

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