Title

Maternal Circulating Adipokine Profile and Insulin Resistance in Women at High Risk of Developing Gestational Diabetes Mellitus

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Abstract

Background: Cytokines produced by adipose and placental tissues (adipokines) have been implicated in the development of gestational diabetes mellitus (GDM). There is, however, limited research regarding the relationship between advancing pregnancy, maternal adipokine profile, insulin resistance and the development of GDM. Furthermore, no studies have investigated these parameters in women with a history of GDM who are at the highest risk of recurrence. This study examined the circulating concentrations of a number of adipokines associated with insulin resistance at two points in pregnancy, and determined whether they were altered in women who developed GDM.

Methods: Non-diabetic women with a history of GDM in a previous pregnancy (n=123) had blood drawn at 14 and 28 weeks of pregnancy for GDM diagnosis, together with assessment of a range of adipokine concentrations by multiplex assay (fatty acid-binding protein 4 [FABP4], leptin, chemerin, adiponectin and resistin).

Results: With advancing pregnancy, maternal adiponectin concentrations decreased, while leptin and resistin levels increased ($p < 0.05$). In women who developed GDM at 28 weeks of pregnancy (42%), fasting and postprandial glucose levels were already significantly elevated by 14 weeks ($p < 0.05$), while adiponectin concentrations were lower ($p<0.05$). Adiponectin remained lower at the time of GDM diagnosis ($p < 0.05$), while the other adipokines were similar between groups at each timepoint.

Conclusion: Maternal glucose and adipokine profile is altered early in pregnancy in women with a history of GDM who subsequently develop recurrent disease.

Keywords: pregnancy, glucose, adiponectin

1. Introduction

Gestational diabetes mellitus (GDM) affects up to 28% of pregnancies worldwide [1] and the prevalence is increasing [2]. This is of great concern given the serious health consequences of the condition for the woman and her offspring [3]. These include acute complications with pregnancy, labour and delivery, but also extend to the years that follow, with increased risk of type 2 diabetes and metabolic syndrome in later life. Furthermore, women with a history of GDM have a high rate of recurrence, with 48% of subsequent pregnancies affected [4]. Accordingly, early identification of the risk of GDM is vital to ensure appropriate management to minimise adverse effects.

The risk of GDM is increased in overweight and obese women [5]. This risk may be related, at least in part, to immunomodulatory factors (adipokines) released from adipose tissue, which have been shown to increase systemic inflammation and contribute to insulin resistance [6]. Many of these adipokines are produced in altered amounts in pregnancy and may be implicated in the development of glucose intolerance and GDM. For instance, the concentrations of leptin adiponectin and visfatin in early pregnancy have been reported to be predictive of GDM later in pregnancy [7-9]. Other novel adipokines including fatty acid-binding protein 4 (FABP4), chemerin and resistin have also been implicated in the development of GDM [10-12]. However, the literature regarding the roles and biological significance of many of these adipokines is inconsistent, prospective studies are lacking, and most research is limited to studies with small sample sizes focused on one or two markers in isolation, rather than the consideration of multiple adipokines simultaneously. More specifically, no research to date has examined the behaviour of these adipokines in women with a history of GDM who are at the highest risk of GDM recurrence.

Given the lack of research regarding the relationship between advancing gestation, maternal adipokine profile, insulin resistance and the development of GDM, the aims of the present investigation were to examine the maternal circulating concentrations of the key adipokines associated with insulin resistance pre- and post-GDM diagnosis, and to determine whether maternal concentrations of these factors reflect early signs of insulin resistance, or are altered in early pregnancy in women who subsequently develop GDM. These issues were addressed in women with a history of GDM in a previous pregnancy given their high risk of recurrence. Studying the patterns of change in these biomarkers may assist in establishing a clinical profile for identifying women at high risk of GDM recurrence.

2. Methods

In our previously reported randomised controlled trial (NCT01283854), women with a history of GDM in a prior pregnancy were randomised (with stratification by maternal age and body mass index [BMI]) between 12 – 14 weeks of pregnancy to a 14 week supervised home-based exercise intervention or to a control group. Full details of the trial, exercise intervention and primary outcomes were described previously [13]. Briefly, the exercise intervention involved three sessions per week of supervised stationary cycling until 28 weeks of pregnancy. Exclusion criteria were pre-existing diabetes, cardiac disease, multiple pregnancy or a medical condition that restricted exercise participation. The study was approved by Women and Newborn Health Service Ethics Committee and all women provided written informed consent.

The primary outcome of the trial was a diagnosis of GDM based on the criteria adopted in Western Australia at the time of the study (fasting venous blood glucose ≥ 5.5 mmol/L [99 mg/dl] and/or a 2 h OGTT glucose ≥ 8.0 mmol/L [144 mg/dl]). A range of other outcome

measures were assessed at recruitment at 12-14 weeks of pregnancy (pre-intervention) and at 28 weeks of pregnancy (post-intervention). These included a 75 g oral glucose tolerance test (OGTT) performed in the fasted state from which fasting glucose and insulin concentrations, together with glucose tolerance and insulin sensitivity could be assessed. In addition to the main outcomes reported in the trial, blood serum collected in the fasted state at the OGTT was stored for a random subset of participants (n = 123), allowing for the subsequent analysis of a range of factors associated with insulin resistance including FABP4, leptin, chemerin, adiponectin and resistin. These additional analyses conducted for the present investigation were performed using commercially available multiplex assay kits (R&D systems, Bio-Techne, Minneapolis, MN, USA) using the Luminex MAGPIX system (Luminex Corporation, Austin, TX, USA) as per manufacturer's instructions. The assessment of adipokines using multiplex technology has been well validated [14,15]. The intra-assay coefficient of variation was 6.9%, 2.4%, 2.6%, 2.2% and 2.6% for FABP4, leptin, chemerin, adiponectin and resistin respectively. All samples were analysed in two batches with an inter-assay coefficient of variation of 13%, 8%, 16%, 13% and 3% for FABP4, leptin, chemerin, adiponectin and resistin respectively. The limits of detection of the FABP4, leptin, chemerin, adiponectin and resistin assays were 0.77, 0.25, 0.25, 0.5 and 0.01 ng/ml. For FABP, 24% of samples were below the limits of detection. These values were replaced with the value of the limit of detection divided by the square root of 2.

2.1 Statistical analyses

Continuous data were summarised using means and standard deviation (maternal age, BMI and blood glucose), or medians and interquartile ranges (maternal insulin and adipokines), depending on data normality. Women randomised to the exercise and control groups in the original trial were well matched for age, BMI and baseline glucose tolerance, with no

difference between groups. There was no effect of the exercise intervention on the recurrence of GDM [13], nor did it alter the circulating concentrations of the cohort of adipokines measured here based on repeated measures analysis of variance. Therefore, subsequent data were examined independent of group allocation. The effect of advancing pregnancy (14 versus 28 weeks) on maternal adipokine profile was assessed using Wilcoxon signed ranks tests based on data normality. The metabolic profile of women who developed GDM was compared with women who did not develop GDM at both 14 and 28 weeks of pregnancy using independent samples t-tests (blood glucose) or Mann-Whitney U tests (insulin, adipokines) depending on data normality. In addition, relationships between maternal adipokine concentrations and other indicators of insulin resistance (glucose and insulin area under the curve [AUC] in response to the 75 g OGTT, together with the homeostatic model of assessment (HOMA) [16] and BMI, were evaluated using bivariate correlations (Spearmans rho). All data were analysed using SPSS software (version 23 SPSS Inc, Chicago, Ill), with significance accepted as $p < 0.05$.

3. Results

A subset of 123 women out of the 172 participants who were randomised in the original trial had serum available for adipokine analysis at 14 and 28 weeks of pregnancy and were included in the present investigation. The characteristics of these women upon study entry (12 – 14 weeks of pregnancy) are shown in Table 1. All women were confirmed as non-diabetic based on a normal OGTT at baseline and 50% were considered overweight or obese at this time (BMI $\geq$ 25.0 kg/m^2).

There was no effect of advancing gestation on maternal circulating FABP4 or chemerin concentrations, with concentrations remaining similar at 14 and 28 weeks of pregnancy ($p >$

0.05; Table 2). However, there were statistically significant alterations in the concentrations of leptin ($p = 0.001$), resistin ($p = 0.003$) and adiponectin ($p < 0.001$) from 14 to 28 weeks of pregnancy (Table 2). Specifically, the concentration of adiponectin decreased, while leptin and resistin increased with advancing gestation, although the magnitude of these changes was small.

From the subset of 123 women included in these secondary analyses, 52 (42%) were diagnosed with GDM based on the 28 week OGTT. When the women who developed GDM were compared with the women who avoided GDM recurrence, women who developed GDM were significantly older (34.4 ± 4.0 ± 32.8 ± 3.9 yr; $p = 0.036$), but did not have significant differences in BMI (26.9 ± 5.9 versus 25.5 ± 5.1 kg/m^2) or ethnicity (81% versus 86% Caucasian ethnicity; $p > 0.05$). Comparing the metabolic profile of these women developing GDM with their non-GDM counterparts, significant differences in the circulating concentrations of glucose (Table 3) and insulin (Table 3) were already evident by 14 weeks of pregnancy. More specifically, women who went on to develop GDM had significantly higher blood glucose concentrations both in the fasted state and 2 h following the OGTT in early pregnancy ($p < 0.05$). Insulin concentrations were also elevated 2 h after the OGTT in these women by 14 weeks of pregnancy (Table 3). At 28 weeks of pregnancy, these differences had increased in magnitude. There was no difference in glycated haemoglobin between groups at 14 or 28 weeks of pregnancy (GDM recurrence 5.4 ± 0.3%; No GDM 5.3 ± 0.3%; $p > 0.05$).

With respect to maternal adipokine profiles, women who went on to develop GDM had lower circulating adiponectin concentrations in early pregnancy compared with women who avoided GDM recurrence ($p = 0.02$; Table 4). There were no differences in any of the other

adipokines measured in early pregnancy between women who developed GDM and those who did not. At the time of GDM diagnosis (28 weeks of pregnancy), adiponectin concentrations remained lower in women developing GDM compared with those who avoided recurrence (Table 4). There were no significant differences in the circulating concentrations of FABP4, leptin, chemerin or resistin between groups at 28 weeks of pregnancy (Table 4).

The relationships between each adipokine and indicators of insulin resistance are shown in Supplementary Table 1, with key relationships highlighted in Figure 1. We identified an inverse relationship between adiponectin and all of the indictors of insulin resistance examined here (glucose and insulin AUC in response to the 75 g OGTT and HOMA) at both 14 and 28 weeks of pregnancy. In contrast, a positive relationship was noted for leptin at 14 and 28 weeks of pregnancy. There were no significant correlations between resistin concentrations and any of the measured indicators of insulin resistance. For the remaining adipokines, some significant correlations were observed with specific indicators of insulin resistance at various times in pregnancy. There was a positive correlation between FABP4 and insulin AUC in early pregnancy and HOMA at 28 weeks and chemerin concentrations were positively correlated with insulin AUC and HOMA at 14 weeks of pregnancy; however, these relationships were no longer significant at 28 weeks of pregnancy. With respect to the relationship between each adipokine and BMI (Supplementary Table 1), significant correlations were noted at 14 weeks of pregnancy (inverse correlation for adiponectin; positive correlations for other adipokines); however, at 28 weeks of pregnancy adiponectin, chemerin and resistin concentrations were no longer significantly correlated to BMI.

4. Discussion

A number of adipokines have been implicated in the progressive state of insulin resistance associated with pregnancy. However, no previous research has examined a large cohort of these adipokines and their relationship to insulin resistance with advancing pregnancy within a single sample of women using a longitudinal study design. Furthermore, this study is the first to simultaneously determine whether maternal concentrations of these factors are altered in early pregnancy in women who subsequently develop GDM. We identified alterations in a number of adipokines as pregnancy advanced from 14 to 28 weeks, some of which were associated with indications of insulin resistance. When comparing women who went on to develop GDM with those that did not, we found differences in the circulating concentrations of glucose and insulin, together with adiponectin, as early as 14 weeks of pregnancy.

With respect to the effect of advancing pregnancy on maternal adipokine profile, we observed an increase in the concentration of leptin and resistin, at the same time as decreases in adiponectin in our sample of women with a history of GDM in a previous pregnancy. In contrast, we saw no significant alterations in FABP4 or chemerin between 14 and 28 weeks of pregnancy. These findings are consistent, at least in part, with previous studies reporting increases in resistin and leptin, and decreasing adiponectin with advancing gestation [17-19], although most previous research has focused on comparisons between early and late pregnancy, rather than changes leading up to the time of GDM manifestation. Furthermore, little is known regarding changes in other adipokines such as chemerin and FABP4, as previous research has tended to be limited by small sample sizes or the utilisation of a cross sectional study design.

Regarding the issue of whether maternal concentrations of these biomarkers are altered in early pregnancy in women who subsequently develop GDM, our findings both support and

contrast with previous reports in the literature. For instance, our observation of lower adiponectin at 14 weeks of pregnancy in women who subsequently develop GDM is consistent with previous observations that adiponectin in early pregnancy is predictive of an increased risk of GDM as gestation advances [9]. On the other hand, previous research has also reported alterations in leptin concentrations in early pregnancy [8], observations not supported by our data. Likewise, we saw no differences in the concentrations of other adipokines measured here at 14 weeks of pregnancy, suggesting potential limited use for identifying women at increased risk of GDM recurrence in the first trimester. Interestingly, the circulating concentrations of fasting and postprandial glucose and insulin were already higher at 14 weeks of pregnancy (although still well below diagnostic thresholds) in women who subsequently developed GDM. Whether these observations are specific to women with a history of GDM in a previous pregnancy is unclear. Regardless, when considering this elevation in glucose concentration in early pregnancy, in combination with our previous finding that 16% of women consenting to our original trial had plasma glucose levels consistent with the GDM diagnosis criteria by 14 weeks of pregnancy [13], optimal screening for diabetes in women with a history of the condition may need to commence earlier than is currently practiced.

Despite limited differences in the early pregnancy adipokine profile of women who went on to develop GDM compared with those that did not, additional differences emerged by the time of diagnosis. Our finding of lower adiponectin concentrations in women diagnosed with GDM is consistent with the literature and its beneficial role in relation to glucose regulation [17]. In contrast, there were no significant alterations in chemerin or FABP4 between groups, despite being reported by others to be altered in women with GDM [10-12].

The adipokines studied here were chosen based on previous research implicating a potential role in the development of insulin resistance in pregnancy. Our findings lend some support for this notion, with significant correlations between leptin and adiponectin with all of the markers of insulin resistance examined here at 14 weeks of pregnancy and most or all markers examined at 28 weeks of pregnancy. Meanwhile, FABP4 and chemerin concentrations correlated with some specific indicators of insulin resistance at various times in pregnancy. Interestingly, some of these relationships that were significant in early pregnancy were no longer evident at 28 weeks of pregnancy; this may simply reflect random changes in significance due to limited sample numbers or small effect sizes. We did not observe any significant relationships between resistin and any of the indicators of insulin resistance examined here at 14 or 28 weeks of pregnancy.

There are a number of reasons for discrepancies between the observations from our study and others. First, it should be noted that prospective data availability is limited, and others have also reported inconsistent findings between studies [20]. Furthermore, our study focused specifically on women with a history of GDM and it is possible that these women have an altered metabolic profile. It is also important to consider variation in the specific stages of pregnancy examined between studies. Indeed, it is likely that further differences may emerge later in pregnancy given the expected progressive increase in insulin resistance until delivery. For instance, resistin has been reported to increase in GDM pregnancies from 28-38 weeks of pregnancy [21]. Also of note, there was no significant difference in BMI between women who were diagnosed with GDM and those who avoided recurrence in our cohort. This may reflect, at least in part, the inadequacy of BMI to assess adiposity, especially with advancing pregnancy [22]. In support of this notion, all measured adipokines correlated with BMI at 14 weeks of pregnancy (before the expected large gain in fluid volume and fetal weight), but

many of these relationships appeared weaker or were no longer significant at 28 weeks of pregnancy (i.e. adiponectin, resistin). It is possible that some of the adipokines studied here are released in altered amounts in pregnancy independent of adiposity [23].

The strengths of this study include the relatively large sample size, prospective design, and in-depth analysis of a number of biomarkers associated with insulin resistance at two time-points in pregnancy. However, it must be acknowledged that the results presented here are descriptive in nature, and that a greater sample size would be needed in order to further explore the relationships between adipokines, advancing gestation and GDM risk using more advanced statistical methods and predictive models. It should also be acknowledged that numerous other biomarkers exist besides the cohort of adipokines measured here, such as specific free fatty acids and amino acids [24], and these have been associated with pregnancy-induced insulin resistance. Furthermore, the sample of women studied here was from our previously conducted randomised controlled trial of a 14 week supervised home-based exercise program [13]. Although the intervention had no effect on the recurrence of GDM or the circulating concentrations of the adipokines measured in this cohort, justifying our decision to examine the data independent of group allocation, the study was not purely observational in nature. Indeed, the lack of effect of the exercise intervention on maternal adipokine profile contrasts with the observations of Clapp and Kiess [25] who reported an attenuation in leptin concentrations in women who continued regular weight bearing exercise during pregnancy compared with control women and a group of women who stopped exercise mid-pregnancy. To our knowledge, no previous research has examined the effect of maternal exercise on the other adipokines measured here. The lack of effect of exercise in the present cohort may be related, at least in part, to the specific characteristics of the study volunteers, particularly in relation to their background physical activity levels upon study

entry which were relatively high. Alternatively, it may be that regular exercise commenced at 14 weeks of pregnancy is insufficient to alter the pro-inflammatory state associated with pregnancy in women with a history of GDM. Despite the lack of benefit seen here in relation to maternal adipokine profile, it is important to note that exercise during pregnancy has many other well-established benefits including improving maternal cardiovascular fitness, psychological well-being and assisting with the management of postprandial glucose control in women diagnosed with GDM [13,26,27].

In summary, we have identified alterations in the circulating concentrations of a number of adipokines as pregnancy advances from 14 to 28 weeks gestation, some of which are associated with indicators of insulin resistance. In addition, we have found differences in the circulating concentrations of blood glucose, insulin, and adiponectin concentrations as early as 14 weeks of pregnancy in women who go on to develop GDM, compared with those who avoid GDM recurrence. These findings suggest some prognostic utility of maternal adipokine profile and glucose tolerance testing in early pregnancy for women at high risk of GDM recurrence. Future research is needed to further understand the role of these adipokines in the progression of insulin resistance and the development of GDM, as well as to identify interventions to effectively modify these biomarkers.

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Conflicts of interest: None to declare.

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Table 1. Participant characteristics at 12-14 weeks of pregnancy [n = 123; mean ± SD; median (interquartile range)].

Age (yr)	33.5 ± 4.0
BMI (kg/m^2)	26.1 ± 5.5
Caucasian Ethnicity (%)	84
Fasting glucose (mM)	4.3 ± 0.4
Fasting insulin (mU/L)	5.0 (3.0 – 7.0)

Table 2. Effect of advancing gestational age (from 14 to 28 weeks) on maternal serum adipokine profile (median and interquartile range).

	14 weeks of pregnancy	28 weeks of pregnancy
FABP4 (ng/mL)	2.02 (1.09 – 3.09)	2.32 (1.55 – 3.37)
Leptin (ng/mL)	10.4 (6.2 - 17.9)	13.6 (7.0 - 20.9)*
Chemerin (ng/mL)	3.13 (2.53 – 3.79)	3.26 (2.63 – 4.08)
Adiponectin (ng/mL)	17.6 (13.3 - 23.7)	14.1 (11.3 - 19.0)*
Resistin (pg/mL)	690 (540 - 1,002)	823 (594 - 1,220)*

*Indicates significant difference between time points based on Wilcoxon signed ranks tests ($p < 0.05$).

Table 3. Maternal plasma glucose (mean ± SE) and insulin concentrations (median and interquartile range) in the fasted state and 2 h following a 75 g oral glucose tolerance test (OGTT) in women who develop GDM (n = 52) and women who avoid GDM recurrence (No GDM; n = 71) at 14 and 28 weeks of pregnancy.

	14 weeks of pregnancy		28 weeks of pregnancy	
	No GDM	GDM	No GDM	GDM
Glucose (mM)				
Fasting	4.26 ± 0.34	4.41 ± 0.37*	4.33 ± 0.42	4.58 ± 0.49*
2 h OGTT	5.53 ± 1.24	6.49 ± 0.97*	6.61 ± 0.87	9.12 ± 1.11*
Insulin (mU/L)				
Fasting	4.5 (3.0-7.0)	5.5 (3.0-8.0)	7.0 (5.0-9.0)	8.0 (5.8-11.3)
2 h OGTT	28.0 (17.0-37.3)	46.5(27.3-61.8)*	46 (36-61.3)	77.5 (52.0-110.5)*

*Indicates significant difference between groups based on independent samples t-test or Mann-Whitney U test ($p < 0.05$).

Table 4. Maternal adipokine profiles of women who developed GDM and women who avoided GDM recurrence (no GDM) at 14 and 28 weeks of pregnancy (median and interquartile range).

	14 weeks of pregnancy		28 weeks of pregnancy	
	No GDM	GDM	No GDM	GDM
FABP4 (ng/mL)	2.03 (1.11-2.90)	1.94 (1.02-3.51)	2.18 (1.55-2.88)	2.46 (1.64-4.15)
Leptin (ng/mL)	8.97 (4.85-16.07)	11.48 (6.95-19.63)	13.63 (6.33-20.45)	13.71 (7.85-22.29)
Chemerin (ng/mL)	3.13 (2.45-3.69)	3.13 (2.62-4.00)	3.11 (2.51-4.10)	3.54 (2.81-4.05)
Adiponectin (ng/mL)	18.4 (14.2 -25.2)	15.0 (11.7-20.4)*	16.6 (12.3-21.8)	12.5 (10.7-15.2)*
Resistin (pg/mL)	697 (564-900)	667 (501-1,196)	806 (590-1,152)	896 (599-1,338)

*Indicates significant difference between groups based on Mann-Whitney U test ($p < 0.05$).

Figure Legend

Figure 1. Relationships between circulating maternal adipokine concentrations and insulin resistance based on the homeostatic model assessment (HOMA) at 14 weeks (●) and 28 weeks (○) of pregnancy. *Indicates significant correlation based on Spearman's rho ($p < 0.05$).

Supplementary Table 1. Correlations between circulating maternal adipokine concentrations, indicators of insulin resistance and body mass index

Adipokine	Stage of pregnancy	75 g OGTT glucose AUC	75 g OGTT insulin AUC	HOMA	BMI
FABP4	14 weeks	0.090	**0.214***	0.184	**0.403***
	28 weeks	0.080	0.167	**0.261***	**0.415***
Leptin	14 weeks	**0.241***	**0.584***	**0.689***	**0.720***
	28 weeks	0.109	**0.432***	**0.664***	**0.706***
Chemerin	14 weeks	0.156	**0.208***	**0.197***	**0.207***
	28 weeks	0.136	0.119	0.152	0.168
Adiponectin	14 weeks	**-0.210***	**-0.433***	**-0.354***	**-0.297***
	28 weeks	**-0.192***	**-0.394***	**-0.306***	-0.127
Resistin	14 weeks	0.114	0.084	0.102	**0.188***
	28 weeks	0.078	0.099	0.063	0.167

*Indicates significant correlation based on Spearman's rho ($p < 0.05$).

OGTT = oral glucose tolerance test; AUC = area under the curve; HOMA = homeostatic model assessment; BMI = body mass index; FABP4 = fatty acid binding protein 4.