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ORIGINAL ARTICLE

Maternal, umbilical arterial and umbilical venous 25-hydroxyvitamin D and adipocytokine concentrations in pregnancies with and without gestational diabetes

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Summary

Objective Gestational diabetes mellitus (GDM) has been associated with inflammation as well as Vitamin D insufficiency. While Vitamin D has anti-inflammatory properties, relationships between Vitamin D and inflammatory markers remain unexplored in GDM. Therefore, this case-–control study investigated adipocytokine and Vitamin D [25(OH)D] concentrations and correlations in GDM and control women, as well as their neonates.

Design/Participants/Measurements seventy-three women participated: 36 GDM and 37 controls. Maternal samples were drawn at 31 weeks. Umbilical arterial and venous samples were collected at birth. 25(OH)D and adipocytokine concentrations were compared for GDM *vs* control maternal, umbilical arterial and venous samples. Correlations were explored between biochemical results, maternal and neonatal demographics.

Results Compared with age- and weight-matched control participants, GDM women had significantly lower concentrations of 25(OH)D (77·3 ± 24·3 *vs* 93·2 ± 19·2 nm/l; $P = 0{\cdot}009$); adiponectin (17·5 ± 11·8 *vs* 34·1 ± 20·3 μg/ml, $P < 0{\cdot}001$); resistin (25·4 ± 9·1 *vs* 31·9 ± 12·1 ng/ml, $P = 0{\cdot}045$); and plasminogen activator inhibitor-1 (PAI-1) 13·9 ± 10·0 *vs* 21·0 ± 12·6 ng/ml, $P = 0{\cdot}038$), while delivering 1 week earlier (38·2 ± 1·2 *vs* 39·5 ± 0·9 weeks, $P < 0{\cdot}001$). GDM maternal 25(OH)D concentrations positively correlated with PAI-1, IL-8 and TNF-α concentrations. Umbilical 25(OH)D concentrations were not significantly different in GDM *vs* control offspring, whereas adiponectin, resistin and PAI-1 concentrations were significantly lower in GDM offspring.

Conclusions GDM women had lower 25(OH)D concentrations than controls, while neonatal umbilical concentrations of 25 (OH)D did not differ. GDM maternal and GDM offspring had lower adiponectin, resistin and PAI-1 concentrations compared with controls. Results suggest that both GDM women and their offspring demonstrate abnormal adipocytokine patterns.

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Introduction

Metabolic changes associated with gestational diabetes mellitus (GDM) include altered insulin secretion and insulin sensitivity.[1] Women with GDM have also been reported to have lower levels of Vitamin D[2,3] as well as adipocytokine levels consistent with chronic inflammation.[4,5] Noting that Vitamin D has been shown to have anti-inflammatory properties,[6,7] this study evaluated Vitamin D [25(OH)D] and adipocytokine concentrations and correlations in GDM and control pregnancies.

Adipocytokines are proteins released mainly from adipose tissue[8] and while having a multiplicity of complicated interactions, they can be broadly categorized into metabolic regulators such as adiponectin, resistin and leptin; angiogenic proteins such as PAI-1; and inflammatory mediators such as IL-6, IL-8, TNF-α and MCP-1. Their intricate endocrine, paracrine and autocrine actions appear to include important roles in mediating insulin resistance, inflammation and immunity,[9–11] functions that could be of interest in the pathogenesis of GDM. The most consistently reported adipocytokine pattern associated with GDM is that of lower adiponectin concentrations,[4,12–14] coherent with observations in other nonpregnant insulin resistant states.[15,16]

Definitions of Vitamin D insufficiency and deficiency are evolving and consensus statements for populations suggest that 25(OH)D levels <30 nm/l are associated with vitamin D deficiency [based on the risk for osteomalacia and rickets], whereas 25(OH)D levels in the range of 30–50 nm/l may be inadequate for bone and overall health.[17] However, additionally, Vitamin

D insufficiency in adults has been suggested as influencing the risk for type 1, type 2 and gestational diabetes,[2,3,18,19] and may have anti-inflammatory effects.[6,7,19] To date, 25(OH)D concentrations and associations in offspring of GDM pregnancies have not been described. Further, profiles and correlates of infant adipocytokine concentrations in GDM pregnancies remain unclear with previous adipocytokine studies reporting various outcomes: lower adiponectin concentrations in umbilical bloods[12,13]; unchanged concentrations of resistin[13,20] and leptin[21] in GDM offspring; and unchanged interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α concentrations in GDM neonates.[22]

Therefore, it was hypothesized that GDM women and their offspring would manifest Vitamin D deficiency as well as adipocytokine profiles consistent with inflammation, and that 25(OH)D and inflammatory cytokine concentrations would be negatively correlated. Hence, this study had two aims: (i) to document whether differing 25(OH)D and adipocytokine concentrations were present in women with GDM as well as their neonates when compared to a weight-matched pregnant control group; (ii) to investigate for correlations between maternal and neonatal 25(OH)D and adipocytokine concentrations.

Subjects and methods

This was a case–control study of pregnant women with and without GDM who were recruited from a tertiary care Endocrine Pregnancy and Obstetrics clinics in London, Ontario, which is a Canadian city with a latitude of 49°N. All physician-led obstetrical care for the referral area is provided through these clinics.

All women had GDM proven or ruled out by contemporary criteria [23] aiming for maternal screening between 24 and 28 weeks. Criteria were as follows: GDM was diagnosed if the 1 h post-50 g glucose screen test result was >10·2 mm/l; GDM was ruled out if the 1 h post-50 g challenge was <7·8 mm/l; women proceeded to a 75 gr 2-h-fasted OGTT when the results of the 50 g test were 7·8–10·2 mm/l. GDM was diagnosed from the 75 gr OGTT if 2 or more glucose values were out of range: normal results being fasting: <5·3 mm/l; 1 h <10·6 mm/l; 2 h <8·9 mm/l.

The study was completed between 2008 and 2011 with maternal recruitment paused during the winter months of November–March to minimize sunlight influences. No woman required insulin at the time of blood sampling. All women were fair-skinned. Women were excluded if they were taking antihypertensive medication, any form of glucocorticoid medication or kept all limbs modestly clothed. No woman with any known or suspected active infection was included in the study to minimize potential effects on adipocytokine concentrations. Women with GDM were offered participation at first presentation to the Endocrine and Pregnancy clinic, which occurred at approximately 31 weeks gestation, and before receiving standard GDM dietary advice. The maternal blood sampling time was chosen to minimize the risk for GDM women receiving advice on Vitamin D intake that would not have occurred in control women; but also to avoid potential confounding of adipocytokines by the onset pre-eclampsia or the possible inflammatory changes associated with delivery. Control pregnant women were also recruited and had blood sampling similarly taken in the early third trimester. Two women with GDM and two control women smoked during pregnancy.

Study design and data collection were approved by the The University of Western Ontario Research Ethics Board for Health Sciences Research involving Human Subjects (#14011E).

After obtaining informed consent, nonfasting maternal blood was collected to measure concentrations of 25(OH)D, calcium, phosphate, glucose (BG), alkaline phosphatase, parathyroid hormone (PTH), C-reactive protein (CRP) and adipocytokines [adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6), interleukin-8 (IL-8), leptin, tumour necrosis factor-alpha (TNF-α) and monocyte chemotactic protein (MCP-1)]. Bloods were promptly centrifuged with serum [25(OH)D] and plasma [remainder of samples] frozen at −70 °C for batch analysis.

On the day of the maternal blood sampling, oral intakes of Vitamin D and calcium were documented by 2-day food and beverage intake records, medication and vitamin supplement history. The diet intake record was checked for completeness by the research co-ordinator and subsequently analysed using The ESHA Food Processor SQL, which included the Canadian Nutrient File (version 10.5; ESHA Research, Salem, OR, USA). No recommendations concerning Vitamin D supplementation were made to either GDM or control women by any study staff.

After delivery, placental blood samples (from both umbilical arterial and umbilical venous circulations) were taken by delivery room staff for analysis of CRP, 25(OH)D, calcium, BG, alkaline phosphatase, PTH and adipocytokine concentrations, as outlined above. Note that umbilical arterial blood flows from foetus to placenta; umbilical venous blood flows from placenta to foetus. Bloods were promptly centrifuged with serum and plasma frozen at −70 °C for batch analysis.

Maternal and infant demographics were collected, including age, prepregnancy weight and body mass index (BMI), maternal current weight, infant birth weight, infant gestational age, Apgar scores, infant or pregnancy complications and duration of hospital stay.

Assays

Glucose, calcium, PTH, alkaline phosphatase and CRP were measured on the Cobas Modular analyser using reagents from Roche (Roche Diagnostics, Laval, QC, Canada). Twenty-five hydroxyvitamin D was measured by an isotopic radioimmunoassay method (DiaSorin Canada Inc., Mississauga, Ontario, Canada). Quality assurance for 25(OH)D measurements is monitored 3 times/year through mandatory laboratory participation in the Quality Management Program, Laboratory Services, Ontario Ministry of Health.

Adipocytokines were quantified in plasma using multiplexed immunoassay kits according to manufacturers' instruction (Milliplex; Millipore Corp, Billeria, MA, USA). A Bio-Plex™ 200

(Bio-Rad Laboratories, Hercules, CA, USA) readout system was used, which utilizes Luminex® xMAP™ fluorescent bead-based technology (Luminex Corp., Austin, TX, USA). Concentrations were automatically calculated from standard curves using Bio-Plex Manager software (v.4.1.1; Bio-Rad). Adiponectin measured was the high molecular weight form; the manufacturer's normal range for this method is 1·16–34·0 pg/ml. Interassay and intra-assay coefficients of variation were as follows: adiponectin 5·3/6·2; resistin 12·0/13·5; PAI-1 6·0/7·5; IL-6 9·7/7·9; Il-8 12·0/9·8; leptin 6·5/4·6; TNA-α 9·2/7·3; MCP-1 3·3/4·7.

Statistical methods

Sample size was calculated assuming a mean 25(OH)D level of 100 nm/l in controls, with a standard deviation of 35. Therefore, 24 subjects per group (GDM and controls) would be needed to detect a clinically important reduction of 30 nm/l from the expected control group mean, at a two-sided 5% level of significance with 80% power.

Pregnant GDM and control women were compared in terms of maternal demographic, maternal blood measurements, infant arterial umbilical measurements and infant venous umbilical measurements using unpaired *t*-tests. Where variances were found not to be equal, either a logarithmic transformation was applied or a Wilcoxon two-sample test was used as deemed appropriate.

Analysis of covariance was used to compare pregnant GDM and control women in terms of maternal 25(OH)D and adipocytokines, adjusting for the confounding effect of maternal age and pre-prepregnacy BMI. Similarly, comparisons involving cord blood results [25(OH)D and adipocytokines] were made using analysis of covariance adjusting for maternal BMI, weight and glucose as well as infant weight and gestational age.

Spearman rank correlations were used to evaluate associations between 25(OH)D concentrations and other maternal and infant outcomes. Data were analysed using SAS 9.2 (software, Cary, NC, USA).

Probability values <0·05 were considered to be statistically significant. Results are presented as mean ± standard deviation (SD).

Results

Maternal demographics and chemistry

Seventy-three women participated: 36 with GDM and 37 controls. GDM and control participants did not differ for week of gestation, weight, pre-pregnancy weight, BMI, or maternal 25(OH)D intake. GDM women had significantly higher BG concentrations than controls and significantly lower 25(OH)D concentrations. No woman had a serum 25(OH)D concentration under 30 nm/l, 5 GDM women had 25(OH)D concentrations 30-50 nm/l and all other participants (GDM and control) had 25(OH)D concentrations >50 nm/l. Calcium, PTH, phosphate and alkaline phosphatase concentrations were not significantly different. Mean CRP concentrations were consistent with pregnancy as they were higher for both control and GDM women than the upper limit of laboratory normal (<5 mg/l) and did not differ between GDM and control women. Adiponectin, resistin and PAI-1 concentrations were significantly lower in GDM women, whereas IL-6, IL-8, leptin, TNF-α and MCP-1 concentrations did not differ (Table 1).

Table 1. Maternal characteristics and biochemistry

Maternal characteristics and biochemistry	Control	GDM	*P*
N	37	36	
Age (yrs)	30·2 ± 4·1	31·6 ± 5·0	0·19
Weeks gestation	31·4 ± 3·6	31·6 ± 2·9	0·84
Current weight (kg)	85·8 ± 21·3	89·1 ± 16·0	0·47
Prepregnancy weight (kg)	74·4 ± 18·9	77·7 ± 17·4	0·44
Prepregnancy BMI (kg/m^2)	27·2 ± 7·2	28·7 ± 5·5	0·34
Maternal vitamin D intake (μg/day)	14·4 ± 6·4	15·8 ± 2·9	0·44‡
25(OH)D (nm/l) [range]	93·2 ± 19·2 [55–135]	77·3 ± 24·3 [33–128]	0·009
PTH (pm/l)	4·33 ± 16·57	1·78 ± 1·10	0·732†
Calcium (mm/l)	2·20 ± 0·13	2·20 ± 0·09	0·898
Phosphate (mm/l)	1·06 ± 0·18	1·04 ± 0·17	0·519
Glucose (mm/l)	4·68 ± 0·89	5·46 ± 1·29	0·008
Alkaline phosphatase (*μ*/l)	82·2 ± 27·1	89·5 ± 18·8	0·234
CRP (mg/l)	6·03 ± 4·99	6·00 ± 5·36	0·983
Adiponectin (μg/ml)	34·1 ± 20·3	17·5 ± 11·8	<0·001†
Resistin (ng/ml)	31·9 ± 12·1	25·4 ± 9·1	0·045
PAI-1 (ng/ml)	21·0 ± 12·6	13·9 ± 10·0	0·038
IL-6 (pg/ml)	1·93 ± 1·32	1·76 ± 1·00	0·627
IL-8 (pg/ml)	2·39 ± 0·98	2·25 ± 1·92	0·185†
Leptin (ng/ml)	41·2 ± 33·7	40·1 ± 26·4	0·899
TNF-α (pg/ml)	4·99 ± 2·08	5·83 ± 2·46	0·196
MCP-1 (pg/ml)	115·6 ± 52·8	115·9 ± 81·1	0·688†

Results presented as mean ± SD; †log-transformed; ‡Wilcoxon two-sample.

After adjusting results for maternal age and pre-pregnancy BMI, significant differences remained between control and GDM concentrations of 25(OH)D ($P = 0·01$), adiponectin ($P = 0·002$, log-transformed) and resistin ($P = 0·045$), although PAI-1 results no longer reached significance ($P = 0·064$).

Neonatal outcomes and chemistry

Control infants delivered at 39·5 ± 0·9 weeks, GDM infants delivered at 38·2 ± 1·2 weeks ($P < 0·001$). Rates of induced labour and Caesarian deliveries were similar between the two groups (Table 2). No significant differences were found between GDM and controls for infant weights, Apgar scores, duration of labour, placental weight and duration of postpartum hospital stay.

No infant had a detectable umbilical blood CRP concentration (data not shown). Despite the suggestion of a trend to lower 25(OH)D in the GDM infant arterial samples, concentrations of 25(OH)D from GDM and control infants did not significantly differ in either umbilical arterial or umbilical venous samples. Adiponectin, resistin and PAI-1 concentrations were significantly lower in GDM than in control neonates in both umbilical arterial and venous samples (Table 2).

When neonatal results were adjusted for maternal age, pre-pregnancy BMI, maternal glucose and infant weight, significant

Table 2. Delivery outcomes and biochemistry

Delivery outcomes	Control	GDM	*P*
Infant weight (g)	3457·8 ± 455·2	3384·6 ± 504·2	0·547
Gestational age (weeks)	39·5 ± 0·9	38·2 ± 1·2	<0·001
Apgar 1	8·2 ± 1·7	8·0 ± 2·1	0·749
Apgar 2	8·8 ± 0·5	8·9 ± 0·6	0·689
Labour duration (h)	8·1 ± 5·8	7·6 ± 4·0	0·891†
Placental weight (g)	677·2 ± 169·7	746·0 ± 197·6	0·159
Postpartum stay (h)	46·8 ± 15·5	45·5 ± 25·4	0·353‡
Sex – male (%)	19 (59)	19 (61)	0·877
Induced labour (%)	17 (53)	20 (64)	0·359
Caesarian section (%)	8 (22)	6 (16)	0·51
Infant arterial umbilical chemistry	**Control**	**GDM**	***P***
25(OH)D (nm/l)	65·6 ± 17·6	58·0 ± 20·8	0·195
Calcium (mm/l)	2·54 ± 0·21	2·46 ± 0·35	0·420
Glucose (mm/l)	3·67 ± 0·81	3·44 ± 1·50	0·227‡
Adiponectin (μg/ml)	100·0 ± 52·2	57·0 ± 31·7	0·006
Resistin (ng/ml)	222·4 ± 456·5	57·1 ± 34·5	0·030‡
PAI-1 (ng/ml)	21·5 ± 22·7	11·2 ± 6·6	0·049†
IL-6 (pg/ml)	37·8 ± 105·7	16·9 ± 22·3	0·779†
IL-8 (pg/ml)	20·7 ± 23·0	11·8 ± 5·8	0·784‡
Leptin (ng/ml)	44·7 ± 46·4	46·1 ± 37·9	0·910
TNF-α (pg/ml)	10·7 ± 2·1	11·7 ± 3·0	0·209
MCP-1 (pg/ml)	690·6 ± 552·6	574·8 ± 275·4	0·608†
Infant venous umbilical chemistry	**Control**	**GDM**	***P***
25(OH)D (nm/l)	64·8 ± 11·5	66·3 ± 19·5	0·952‡
Calcium (mm/l)	2·62 ± 0·25	2·50 ± 0·18	0·086
Glucose (mm/l)	3·70 ± 1·24	3·96 ± 0·84	0·422
Adiponectin (μg/ml)	109·9 ± 49·5	64·0 ± 33·7	0·004
Resistin (ng/ml)	237·4 ± 529·2	47·5 ± 17·9	<0·001‡
PAI-1 (ng/ml)	15·5 ± 13·9	8·4 ± 8·2	0·009†
IL-6 (pg/ml)	38·4 ± 109·4	11·2 ± 12·4	0·871†
IL-8 (pg/ml)	15·7 ± 18·6	8·7 ± 5·0	0·464‡
Leptin (ng/ml)	49·9 ± 40·1	55·4 ± 48·6	0·675
TNF-α (pg/ml)	10·9 ± 2·5	11·8 ± 2·8	0·253
MCP-1 (pg/ml)	457·4 ± 289·7	425·0 ± 247·9	0·690

Quantitative results are mean ± SD; †log-transformed; ‡Wilcoxon two-sample.

differences remained between GDM and control infant cord arterial adiponectin ($P = 0{\cdot}005$), resistin ($P = 0{\cdot}028$, log-log-transformed) but not PAI-1 ($P = 0{\cdot}06$, log-transformed). Significant differences remained between GDM and control cord venous adiponectin ($P = 0{\cdot}006$), resistin ($P = 0{\cdot}14$, log-log-transformed) and PAI-1 ($P = 0{\cdot}015$ log-transformed).When infant gestational age was added to the analysis, no significant differences remained for cord arterial or cord venous results.

Correlations with maternal 25(OH)D concentrations

Correlations were explored between maternal blood 25(OH)D concentrations, maternal characteristics and biochemical results.

Table 3. Correlations with maternal serum 25(OH)D concentrations

	r	*P*
Correlations with maternal serum 25(OH)D concentrations:controls		
Maternal resistin	0·529	0·010
Correlations with maternal serum 25(OH)D concentrations:GDM		
Maternal PAI-1	0·578	0·008
Maternal IL-8	0·515	0·020
Maternal TNF-a	0·482	0·032

Acknowledging that the large volume of available comparisons could result in correlations due to chance alone, only the most robust results pertinent to the primary goals of the study are presented.

Table 3 lists correlations which achieved a Spearman $r > 0{\cdot}4$ or $r < -0{\cdot}4$ and $P < 0{\cdot}05$. Maternal control 25(OH)D concentrations were positively correlated with maternal resistin concentrations but not with any of the other adipocytokines, Vitamin D intake, pre-pregnancy weight/BMI and current pregnancy weight. Maternal GDM 25(OH)D concentrations were positively correlated with maternal PAI-1, IL-8 and TNF-α.

When maternal correlations were adjusted for age and pre-pregnancy BMI, a significant positive correlation remained between control maternal resistin and 25(OH)D concentrations, whereas the GDM correlations no longer reached significance.

Correlations with umbilical 25(OH)D and umbilical adipocytokine concentrations

No correlations were found between infant weight, placental weight, Apgar scores, labour duration, postpartum stay duration and any of the infant umbilical blood 25(OH)D concentrations (control venous or arterial, GDM venous or arterial). There were also no significant correlations found between umbilical arterial 25(OH)D concentrations and any of the adipocytokine concentrations in GDM offspring (data not shown).

Discussion

The first aim of this study was to explore whether 25(OH)D and adipocytokine patterns differed for GDM women and their offspring in comparison with controls. As in previous reports, it was found that study women with GDM had lower adiponectin concentrations than control women.[4,12–14] Because adiponectin concentrations are negatively associated with fat mass,[16] it is noteworthy that women with GDM in the present study had lower adiponectin concentrations despite being compared with control women matched for both pre-pregnancy BMI and current pregnancy weight. Further, considering lower concentrations of adiponectin are known to be associated with increased risks for type 2 diabetes,[24] lower adiponectin concentrations would not be unexpected in the study women with GDM.

However, despite lower adiponectin concentrations, which would be consistent with a pro-inflammatory state in GDM

participants, resistin and PAI-1 concentrations were found to be lower in this GDM cohort, while leptin concentrations were not significantly different. These findings were unexpected as resistin and PAI-1 have been positively associated with obesity, insulin resistance and inflammation.[25] However, definitive conclusions from previous studies of these cytokines in GDM remain elusive as increased, decreased or unchanged concentrations have all been reported.[12,26–28] It is possible that the observed variability in previous reports reflects differing gestational week of blood sampling or perhaps associated effects of labour on maternal results. Further, maternal adipocytokine results indicate that maternal age and pre-pregnancy BMI may influence PAI-1 outcomes.

Because leptin concentrations are also known to reflect fat mass,[29] the present lack of leptin differences may also result from the similar pre-pregnancy BMI and study weight of the study participants or perhaps be related to the relatively mild metabolic impairment of the study GDM participants.

Considering that obesity itself is associated with inflammation,[30] it is of interest that no differences were found between GDM and control women in concentrations of the inflammatory markers CRP, IL-6, Il-8, TNF-α or MCP-1. This result may also reflect the comparison against weight-matched controls, or again the relatively mild metabolic dysfunction of the GDM cohort.

Noting that umbilical arterial samples may more closely reflect foetal chemistry than umbilical venous samples, it is of unique interest that lower concentrations of adiponectin, resistin and PAI-1 were found in both arterial as well as venous umbilical blood of GDM neonates in comparison with control neonates and that concentrations did not correlate with birth weight. Further, after adjusting for the possible confounders maternal age, maternal pre-pregnancy BMI, glucose and infant weight, cord arterial adiponectin and resistin concentrations remained significantly different and cord venous adiponectin, resistin and PAI-1 also remained significantly different. With two known exceptions,[12,13] previous umbilical adipocytokine studies have reported on umbilical venous blood concentrations, concluding that adiponectin concentrations rise as pregnancy progresses,[31] are higher in pre-eclampsia[32] and lower in GDM offspring or macrosomic babies.[12,13,33] While the lower cord adiponectin concentrations would be consistent with inflammation, the lower (rather than higher) resistin and PAI-1 concentrations in umbilical GDM samples argue against a simple conclusion that GDM offspring are born manifesting a consistent inflammatory state. Until there is more clarity around the complex multiple roles for adipocytokines, it is perhaps most cautious to conclude that the present umbilical arterial findings suggest GDM offspring manifest abnormal profiles of adipocytokines as early as birth and that these adipocytokine concentrations may not only reflect maternal transfer or placental issues.

It must also be noted that control infants were approximately 1.5 weeks older in gestational age than the GDM infants. This finding could not be explained by any difference in rates of C section or induction. It is unclear what influence infant gestational age has upon 25(OH)D or adipocytokine concentrations, with most reports dealing with preterm or small for gestational age infants.[34] It has been concluded that infants born after 40 weeks have lower 25(OH)D concentrations than infants born at 37–39 weeks[35] and that cord adiponectin levels rise until 40 weeks.[31,34] In the present study, when infant gestational age was incorporated into the regression along with maternal age, pre-pregnancy BMI, weight, glucose and infant weight, the differences in cord adiponectin, resistin and PAI-1 were no longer significantly different between control and GDM offspring. Therefore, while it is possible that the 10-day difference in gestational age was an important modifier of foetal adipocytokine concentrations. However, a study with a wider range of weight and age results would be needed to more fully explore whether infant age is an independent modifier of adipocytokine concentrations.

Consistent with previous reports,[2,3] GDM participants were found to have lower serum 25(OH)D concentrations than control participants. However, the present study is among the first to report serum 25(OH)D concentrations in neonates of women with GDM: 25(OH)D concentrations in both umbilical arterial and umbilical venous bloods were found to be lower than in maternal blood, but did not differ significantly between GDM and control offspring.

The second aim of the study was to examine whether correlations could be found between 25(OH)D and adipocytokine concentrations in GDM women or in their offspring. While the only 25(OH)D correlation in control participants was a positive one with resistin, GDM maternal 25(OH)D concentrations were positively correlated with maternal PAI-1, IL-8 and TNF-α concentrations. This positive correlation between 25(OH)D concentrations and inflammatory adipocytokines was unexpected in light of the postulated anti-inflammatory associations of Vitamin D. Further, the correlations were noted despite maternal serum 25(OH)D concentrations that did not meet present (nonpregnant) population criteria for deficiency. While these positive relationships were unanticipated, not only does more need to be known about the roles for both Vitamin D and adipocytokines in pregnancy, but also interventional studies would be necessary in control as well as GDM pregnancies to clarify issues of correlation *vs* causation. Further, the regression results indicate that maternal age and prepregnancy BMI may influence results, particularly in women with GDM.

No consistent correlations were found between umbilical serum 25(OH)D concentrations and neonatal adipocytokine concentrations, nor did concentrations of the inflammatory cytokines PAI-1, IL-8 and TNF-α differ between GDM and control neonates.

The present study has some acknowledged limitations. A larger sample size may have allowed for significant differences to be found among the other adipocytokines. Different associations may have been found if maternal testing had been performed later in gestation. However, the decision to sample maternal bloods early in the third trimester was necessary to minimize confounding of maternal results by variables not under study control: lack of sunlight exposure due to bed rest, late pregnancy hospitalization, delivery type, delivery-associated medications and intervening pre-eclampsia.

The present study did not document infant girth or skin-fold thicknesses, maternal prepregnancy waist–hip ratios or maternal fat mass measures. Because contemporary clinical criteria were used to diagnose or rule out GDM, women did not undergo a study OGTT whereby insulin as well as glucose samples could be assessed. If the foregoing more intensive measures of adiposity and glucose tolerance had been available, then possibly more subtle associations between body habitus, insulin resistance and biochemical outcomes could have become evident. A population of GDM and control women, which had wider variability for 25 (OH)D concentrations, may have shown differences in offspring 25(OH)D levels as well as differing correlations. Finally, as this study measured maternal blood results at only one point in time, it is possible that more frequent 25(OH)D and adipocytokine sampling throughout pregnancy in a larger population would have differing outcomes and associations.

It is concluded that in comparison with control participants, women with GDM at 31 weeks gestation had significantly lower concentrations of adiponectin, resistin, PAI-1 and 25(OH)D; maternal serum 25(OH)D concentrations correlated positively rather than negatively with the maternal inflammatory adipocytokines PAI-1, Il-8 and TNF-α; umbilical adiponectin, resistin and PAI-1 concentrations were lower in GDM offspring while neonatal 25(OH)D concentrations did not differ from controls. While both GDM women and their neonates have adipocytokine patterns that differed from controls, the findings are not wholly consistent with insulin resistance and inflammation; and further, neonatal adipocytokine concentrations may be influenced by gestational age. These results may add to the discussions surrounding adipocytokine changes with pregnancy, as well as investigations into the timing of transmission of metabolic risk factors to offspring.

Duality/Conflict of interest

The authors declare that there is no duality of interest nor conflict of interest associated with this manuscript.

Contributions statement

RM conceived the study, reviewed the data and wrote the manuscript. KS reviewed and edited the manuscript and also provided cytokine assay quality assurance. BV reviewed and edited the manuscript. NC researched data and reviewed the manuscript. AT researched data. IG supervised AT, reviewed and edited the manuscript.

Financial disclosure

Nothing to declare.

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