Adipokines and macrophage markers during pregnancy –possible role for sCD163 in prediction and progression of gestational diabetes mellitus

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Abstract

Aims: The risk of gestational diabetes mellitus (GDM) is increased in overweight and obese women potentially involving secreted mediators from adipose tissue. Our main aim was to evaluate if circulating adipokines and monocyte/macrophage markers were dysregulated in GDM and the influence body mass and indices of glucose metabolism had on this association. We further explored if early detection of these markers improved prediction of GDM and if they remained modified during long-term follow-up.

Materials and Methods: Population-based prospective cohort study in 273 pregnant women with markers measured four times during pregnancy and at 5 years follow-up.

Results: sCD163 was higher (25% at 14-16 weeks, $p<0.001$) and adiponectin lower (-17% at 14-16 weeks, $p<0.01$) early in pregnancy and at 5 years follow-up in GDM women, independent of BMI and other GDM risk factors. Leptin, adiponectin and chemerin were robustly associated with glucose metabolism throughout pregnancy while sCD163 was inversely associated with β-cell function early in pregnancy in women with increased BMI. Finally, the markers at 14-16 weeks displayed modest discriminatory properties with regard to prediction of GDM (AUC<0.7). Using a combination of fasting glucose and sCD163, 53% of GDM could be identified when 25% of the population scored positive suggesting some merit in a multimarker approach.

Conclusions: sCD163 and adiponectin were dysregulated in GDM, independent of body mass. None of the adipokines or monocyte/macrophage activation markers displayed clinically useful properties alone for early detection of GDM. Activation of monocytes/macrophages may be an important event in the early development of GDM.

Abbreviations

AUC	Area Under the Curve
CD163	Cluster of Differentiation 163
FPG	Fasting Plasma Glucose
GDM	Gestational Diabetes Mellitus
IL1Ra	Interleukin 1 Receptor antagonist
ISSI-2	Insulin Secretion-Sensitivity Index
NPV	Negative Predictive Value
PPV	Positive Predictive Value
T2DM	Type 2 Diabetes Mellitus

Introduction

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy, affects 4-18 % of pregnant women [1]. The risk of GDM is increased in overweight and obese women potentially involving secreted mediators from adipose tissue [2]. Thus, crosstalk between adipose tissue and other insulin targets tissues are mediated by a number of molecules secreted from adipocytes which may contribute to regulation of maternal energy metabolism and insulin resistance [3-6]. Several studies have demonstrated associations between circulating levels of adipokines early in pregnancy and risk for GDM, also after controlling for body mass index (BMI) [7, 8], indicating that the metabolic activity and/or distribution of adipose tissue may link adipokines to the development of GDM.

In addition to a central role for adipokines generated by adipocytes and cells of the stromal vascular function in linking obesity, low-grade inflammation and insulin resistance, infiltration of "classical" inflammatory cells into adipose tissue may also be central [9-11]. The predominant leukocyte-derived cells in adipose tissue are macrophages which have overlapping functions with adipocytes, and positively correlate with adipocyte size, BMI, and expression of pro-inflammatory cytokines [12].

While numerous studies have evaluated adipokine levels in GDM, few have systematically evaluated the temporal course of multiple adipokines and/or relevant markers of monocyte/macrophage activation in relation to the development of GDM. The STORK study was a prospective cohort study following 1031 Norwegian healthy pregnant women [13]. Participants were invited to participate in a follow-up 5 years after pregnancy to evaluate indices of glucose metabolism and cardiovascular risk. 300 women participated in the follow-up and we have previously evaluated glucose metabolism[14], body composition [15], CV risk [16] and a range of inflammatory biomarkers [17-19] in this cohort. Using this cohort, we measured maternal serum levels of some adipokines (adipsin, adiponectin, chemerin, interleukin 1 receptor antagonist (IL1Ra), at least partly regarded as an adipokine [20], leptin, resistin, clusterin) and the monocyte/macrophage activation markers soluble (s) CD163 and sCD14 four times during pregnancy and at 5 years follow-up in 273 women (i.e. excluding

women with preeclampsia and hypertension without GDM) and evaluated the association with the development of GDM diagnosed with the IADPSG 2010 criteria. Specifically, we aimed to evaluate i) if the temporal profile of these markers was different in uncomplicated pregnancy vs. GDM ii) if dysregulated levels of these markers was explained by differences in BMI iii) associations with different indices of glucose metabolism iv) if levels of these markers early in pregnancy could predict the development of GDM and finally, v) if dysregulated levels of these markers was sustained during long-term follow-up.

Materials and Methods

The STORK study, a prospective longitudinal cohort study in which 1031 low-risk women of Scandinavian heritage were followed throughout their pregnancy and gave birth at Oslo University Hospital Rikshospitalet between 2002 and 2008 [13]. Each pregnant woman had four study-related antenatal visits at weeks 14-16, 22-24, 30-32, and 36-38. BMI was calculated at each visit. A 75g OGTT was performed on all women at 14-16 and 30-32 weeks of gestation. A follow-up OGTT was performed 5-years postpartum in 300 women[15]. We included only the women that had participated both during pregnancy and follow-up in this particular study. Women with preeclampsia, preterm birth and hypertension without GDM were excluded, and this study ended up with 273 participants (Figure 1). Written informed consent was obtained from all study participants. All clinical investigations were conducted in accordance with the principles enshrined in the Declaration of Helsinki. The study was approved by the Regional Committee for Medical Research Ethics of Southern Norway in Oslo, Norway.

Measurements of glucose and insulin from OGTT

All 75g OGTTs were performed in the morning after an overnight fast and glucose levels measured as previously reported [15]. Briefly, glucose was measured in serum samples collected at antenatal visits at 14 -16 and 30–32 weeks and frozen until analysis, using the hexokinase method at an accredited clinical chemistry laboratory at Oslo University Hospital (Cobas 6000 from Roche). For the 5-year

follow-up visit, fasting glucose measurements were collected using an Accu-check Sensor glucometer. Insulin levels were assayed in duplicate (RIA, DPC, Los Angeles, CA, USA) as previously reported [15].

Diagnosis of GDM and pre-diabetes

GDM was diagnosed using the IADPSG 2010 criteria (fasting plasma glucose (FPG) 5.1–6.9 mmol/L, 1h plasma glucose ≥10.0 mmol/L or 2h plasma glucose 8.5–11.0 mmol/L) following a 75 g oral glucose load at gestational week 30-32. Pre-diabetes was diagnosed at the 5-year follow-up visit using the following criteria: FPG 5.6–6.9 mmol/L or 2h plasma glucose 7.8–11.0 mmol/L after 75 g OGTT [21]. As previously reported 6.7 % of the women at 5 years were found to be pre-diabetic[15]. Of these 18.4 % were previously diagnosed with GDM and 4.5 % with non-GDM.

Insulin sensitivity was measured with the Matsuda index $10000/\sqrt{}$ of (fasting glucose (mmol/L)×fasting insulin (mU/L)×(mean glucose (mmol/L)×mean insulin (mU/L)) during 75g OGTT. This index is a measure of whole body insulin sensitivity that has been validated against the euglycemic-hyperinsulinemic clamp [22]. β-cell function was assessed with the insulin secretion-sensitivity index (ISSI-2) (area under the curve insulin$(mU/L)_{0-120}$/glucose$(mmol/L)_{0-120}$×Matsuda), validated against the disposition index from the intravenous glucose tolerance test [23]. HOMA-IR was calculated as fasting insulin (mU/L)×fasting glucose (mmol/L)/22.5, as described by Matthews *et al.* [24].

Blood sampling and measurement of adipokines and macrophage markers

Peripheral venous blood was drawn into pyrogen-free tubes with EDTA as anticoagulant. The tubes were immediately immersed in melting ice and centrifuged within 30 minutes at 2,000g for 20 minutes to obtain platelet-poor plasma. All samples were stored at -80°C and thawed <3 times. Adipokines (adipsin (DY1824), adiponectin (DY1065), chemerin (DY2324), leptin (DY398), resistin (DY1359), clusterin (DY5874)) and monocyte/macrophage markers (sCD163 (DY1607), sCD14 (DY383)) were measured in duplicate using commercially available antibodies (R&D Systems, Minneapolis, MN, USA) while CRP (DY1707) was measured as previously reported[19], also with commercially available antibodies (R&D Systems, Minneapolis, MN, USA), and IL-1Ra (900K574) was measured with

antibodies from PeproTech (Rocky Hill, NJ, USA), in a 384 format using the combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT, USA) dispenser/washer (EL406). Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (Synergy H1 Hybrid, Biotek, Vinooski, VT, USA). Leptin and adiponectin analysis has been published before [17]. Intra- and inter-assay coefficients of variation were <10% for all assays.

Statistical analysis

Data are expressed as mean (SD) when normally distributed and median (25^{th}, 75^{th} percentile) when skewed. Comparison between women with GDM and non-GDM were performed using *t*-test or Mann–Whitney's *U* depending on distribution, and χ^2 test for categorical variables. Temporal changes in markers were assessed with repeated measures ANOVA and if the group effect was significant, multivariate linear regression analyses were carried out on log transformed variables (if skewed) at each visit adjusting first with BMI and then age, CRP, diabetes in family and parity. Associations between protein levels and indices of glucose metabolism were assessed using multivariate regression including BMI as a covariate. If an interaction between protein level and BMI was detected, the association was explored and the interaction term reported, otherwise univariate associations are reported.

Prediction of GDM by marker level at 14-16 weeks was evaluated by receiver operating characteristic (ROC) analysis. Logistic regression identified the strongest predictors of GDM. BMI, age, CRP, diabetes in family, parity and fasting glucose were forced in step 1 and the protein biomarkers were included by backward stepwise addition in step 2. Accuracy was represented using the terms sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) as described previously[14]. P-values are two-sided and $p<0.05$ was considered statistically significant.

Results

Table 1 shows the characteristics of the study population at 14-16 weeks, 30-32 weeks of pregnancy and at the time of the 5-year follow-up visit, respectively, stratified into those women who did and did

not have GDM in the index pregnancy using IADPSG 2010 diagnostic criteria [25]. In general, women who developed GDM were older and had higher BMI, CRP and glucose measures.

Maternal adipokine and monocyte/macrophage marker profiles during pregnancy (Figure 2)

Circulating chemerin, IL-1Ra, leptin, resistin, sCD14 and sCD163 levels increased while clusterin and adiponectin decreased during pregnancy ($p<0.001$). Levels of adipsin decreased initially with a subsequent increase, giving levels similar at 14-16 and 36-38 weeks. An interaction between the temporal course (i.e. time) and GDM group was observed for sCD163 ($p=0.073$), leptin ($p<0.001$) and resistin ($p=0.023$). The difference between GDM and non-GDM as a whole group were significant for sCD163 ($p=0.002$), IL1Ra ($p=0.042$), leptin ($p=0.031$) and adiponectin ($p=0.001$) (Figure 2).

We next investigated markers that were different with regard to GDM status at individual time-points. In general, all markers correlated more strongly with BMI compared to weight gain and BMI was used for adjustment purposes. After adjusting for BMI at each visit, sCD163 remained elevated and adiponectin lower at visit 14-16 and 22-24 weeks, and these differences persisted following further adjustment for age, CRP (to adjust for general systemic inflammation), diabetes in family and parity. The association between IL-1Ra and leptin and GDM was markedly attenuated and not significant following adjustment for BMI.

Maternal adipokines and monocyte/macrophage activation markers and associations with BMI, CRP and glucose measures during pregnancy

As seen in Figure 3, sCD163, IL1Ra, leptin, adiponectin and chemerin was associated with BMI at both 14-16 and 30-32 weeks. Adipsin was associated with BMI only at 14-16 weeks. Further, IL1Ra, leptin, and chemerin correlated positively with CRP at 14-16 and 30-32 weeks. sCD163 was positively correlated with CRP at 14-16 weeks and adiponectin was inversely associated with CRP at 30-32 weeks.

We next investigated which of the markers that was associated with glucose measures, in univariate and multivariate analysis adjusted for BMI (Figure 3). At 14-16 weeks of gestation, sCD163

was modestly inversely associated with insulin sensitivity. For β-cell function (ISSI-2) at 14-16 weeks, an interaction between BMI and sCD163 was observed (Figure 3A) indicating an inverse association between sCD163 and β-cell function only in individuals with high BMI. Further, sCD163 was inversely associated with insulin sensitivity and positively with insulin resistance at 30-32 weeks, also following adjustment for BMI. IL-1Ra was inversely associated with β-cell function in BMI adjusted analysis at both time-points. Leptin and chemerin was inversely correlated with insulin sensitivity and positively associated with insulin resistance, and although attenuated, remained significantly associated after adjusting for BMI. An opposite pattern was observed for adiponectin, which also correlated positively with β-cell function at both time-points.

Adipokines and monocyte/macrophage markers at 14-16 weeks for prediction of GDM

We next assessed the discriminatory properties of the adipokines from the 14-16 week OGTT to predict GDM at 30-32 weeks using ROC analysis. As shown in Figure 4A, the accuracy varied but was in general poor or modest for all markers (i.e., AUC 0.5-0.7). The best performance was achieved for IL-1Ra and sCD163. Multivariable regression (Figure 4B) revealed the low levels of adiponectin (34% reduced risk per SD increase in log adiponectin) and high levels of IL-1RA (57% increased risk per SD change in log IL-1Ra) and sCD163 (103% increased risk per SD change in log sCD163) were associated with GDM in adjusted analysis. Diagnostic test characteristics were further analyzed for sCD163 (Figure 4C). Sensitivity was poor at higher percentiles of sCD163 and only approximated 80% at around the 50 percentile (i.e. half of the population is characterized as positive). Specificity rapidly declined and was 78% at the 25^{th} percentile and 58% at the 50^{th} percentile. Figure 4D shows the sensitivity of fasting glucose, sCD163 and their combination across different percentiles and shows that a more sensitive prediction of GDM is achieved when combing these to. Thus, when 25% of the population display increased glucose and sCD163, 53% of GDM cases can be identified.

Adipokines 5 years after pregnancy

After 5 years post-partum adiponectin, leptin, sCD163, chemerin, IL1Ra were different between previous GDM and non-GDM. When adjusting for BMI, age, CRP, diabetes in family and parity we found adiponectin, adipsin and sCD163 still different between the groups (Table 2).

Discussion

Our prospective cohort study evaluating a range of circulating adipokines and monocyte/macrophage activation markers during normal and GDM pregnancies revealed i) sCD163 was higher and adiponectin lower early in pregnancy and at 5 years follow-up in GDM women, independent of BMI and other GDM risk factors. ii) leptin, adiponectin and chemerin were robustly associated with indices of glucose metabolism throughout pregnancy while sCD163 was inversely associated with β-cell function early in pregnancy in women with increased BMI. iii) sCD163 at 14-16 weeks in combination with glucose levels enhanced prediction of GDM. Further evaluation of sCD163 as a risk marker and interactions between BMI, macrophage activation and β-cell function early in pregnancy of GDM women is warranted.

In our study, weight gain was less pronounced in GDM pregnancies, and the difference in BMI compared to non-GDM women was smaller approaching term. Thus, the largest difference in markers between these groups was observed early in pregnancy. In particular adiponectin was low and sCD163 elevated in GDM, and although this association was attenuated in BMI adjusted analysis, it persisted indicating both BMI dependent and independent associations with GDM for these proteins. Thus, adiponectin remained associated with integrated measures of glucose metabolism both early and late in pregnancy supporting its prominent role in glucose regulation [26, 27]. As GDM women are at increased risk of developing T2DM, our finding that both adiponectin and sCD163 were dysregulated at 5-years follow-up, supports these proteins as risk factors for T2DM [27, 28].

Soluble CD163 has been identified as a macrophage specific risk predictor for developing T2DM [28] and marker of obesity-related insulin resistance [29, 30]. Our data support a previous smaller study showing increased sCD163 in 18 GDM women compared to 20 BMI matched controls at 39-40 weeks of gestation [31]. The increased sCD163 in late pregnancy, as opposed to early changes in our

study, could be related to a higher macrophage infiltration in obese subjects. Indeed, adipose tissue explants from these women produced more sCD163 compared to their BMI matched controls [31]. In addition, they detected increased staining of CD163 in placental tissue, presumably from placental macrophages as well as increased secretion from placental explants suggesting the placenta as an alternative source of increased sCD163 in GDM, although this would have less impact in early pregnancy. The non-significant increase in sCD163 in another study of 66 GDM and 71 control women at 27 weeks of gestation [32] could partly be related to the relative late sampling. Furthermore, at 14-16 weeks high sCD163 was associated with poorer β-cell function, but only in individuals with high BMI as also observed in T2DM [29, 30]. As circulating sCD163 levels have been shown to correlate with CD163 expression, macrophage content and insulin sensitivity in adipose tissue [33, 34], we speculate that early activation of monocytes/macrophages and infiltration in adipose tissue may be an important event in the early development of GDM, at least in a subgroup of women with increased body mass. The association between sCD163 and insulin resistance at 30-32 weeks, as also observed in the above GDM studies [31, 32], may suggest a role for sCD163 and monocyte/macrophage activations in the normal adaption of insulin resistance in later pregnancy. Thus, our findings may reflect that macrophages exert distinct effects depending on the presence of adiposity, hyperglycemia and inflammation. In patients with T2DM and diabetic mice, increased sCD163 and a decreased percentage of CD163+ monocytes preceded the development of diabetic complications while shedding of sCD163 in response to inflammatory stimuli also may attenuate immune activation [35, 36]. Our finding that sCD14, which also reflects monocyte activation, was not associated with GDM supports that the increased sCD163 in GDM to a larger degree reflects macrophage activation, although these markers may represent distinct but overlapping biological pathways or subtypes of macrophages.

The discriminatory properties of markers at 14-16 weeks as early predictors of GDM were poor for most and modest for leptin, IL-1Ra and sCD163. However, multivariable regression including conventional risk factors, identified sCD163 as an independent predictor of GDM with a Wald-score similar to glucose, only surpassed by age. Further analysis of the diagnostic properties of sCD163 at 14-16 weeks in predicting GDM using different cut-offs revealed that a specificity of 80% was

achieved at the 75th percentile of sCD163 levels. At this level sensitivity was around 45%. Thus, evaluated as a stand-alone marker, sCD163 is not viable as an early screening biomarker to rule out women less likely to develop GDM. In combination with fasting glucose however (i.e. high glucose and high sCD163), we did find that more than half of the GDM cases could be identified when 25% of the population would score positive (i.e. high sCD163 and/or high glucose). As several studies have shown that calorie restriction reduces sCD163 in parallel to improvement in insulin resistance [29, 33], it would be interesting to monitor sCD163 in existing dietary intervention studies in GDM.

In contrast to several studies presenting elevated levels in chemerin between GDM and non-GDM [37], also in early pregnancy [38], and presented in a recent meta-analysis[39] we found similar levels in these groups. However, chemerin was correlated with BMI as well as insulin resistance/sensitivity in both early and late pregnancy, supporting a role for this protein in normal glucose homeostasis. In support of our lack of differences in several adipokines, a recent study evaluating a FABP4, leptin, chemerin, adiponectin and resistin at 14 and 28 weeks of pregnancy in a high-risk GDM population (i.e. all with previous GDM) detected only lower levels of adiponectin [40]. However, other studies have shown elevated levels of FABP4 in GDM[41, 42] and in a systematic review they reported elevated levels of leptin in GDM and inconclusive result for resistin[26] between GDM and non-GDM. Few studies have investigated circulating clusterin, sCD14 and adipsin in GDM women and our results could therefore not be compared to other studies. Discrepancies with previous studies could include timing of blood sample collection, different diagnostic criteria, population characteristics, sample size, and diverse assay method, to mention some. The strength of this study, however, includes the prospective design including four time-points in pregnancy and a well described cohort.

Limitations to our study include a modest number of GDM women, without a history of diabetes mellitus or GDM limiting the interpretation of our results to concern normal pregnancies. Third, with regard to insulin resistance, central visceral fat as best evaluated by computed tomography, is strongly associated with insulin resistance and type 2 diabetes but not usable during pregnancy. Finally, the diagnosis of the GDM was performed later than the standard protocol, at 30-32 weeks and

not at 24-28 weeks as recommended, although the prevalence of GDM suggests that this is an average risk cohort when it comes to GDM risk.

In summary, we identified early alterations in adiponectin and sCD163 in GDM pregnancies. In early pregnancy, high sCD163 was associated with poor β-cell function but was associated with insulin resistance later in pregnancy. Our finding may suggest a complex role for sCD163+macrophages and indicate that early activation of monocytes/macrophages may be an important event in the early development of GDM, but could also have a role in the normal adaption of insulin resistance. Further evaluation of sCD163 as a risk marker and interactions between body mass, macrophage activation and β-cell function early in pregnancy of GDM women is warranted.

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Declaration of interest

No conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Table 1. Characteristics of the study population of GDM (IADPSG criteria) and control women during the index pregnancy

Variable		14-16 weeks	30-32 weeks	5 year FU
N=	Controls	225	225	225
	GDM	48	48	48
Age (years)	Controls	32 (4)		37 (4)
	GDM	34 (4)*		39 (4)*
Height (cm)	Controls	169 (6)		169 (6)
	GDM	169 (6)		169 (6)
Gestational weight gain (kg) V1-V4	Controls	10.1 (8.0,12.6)		
	GDM	9.6 (8.3, 12.0)		
BMI (kg/m^2)	Controls	23.4 (21.4, 25.5)	26.1 (23.8, 28.3)	22.6 (20.8, 24.5)
	GDM	26.0 (23.5, 28.5)**	28.4 (26.8, 29.8)**	24.8 (22.6, 27.9)*
CRP (ng/mL)	Controls	1.56 (0.88, 2.41)	1.37 (0.82, 2.16)	0.38 (0.19, 0.71)
	GDM	2.01 (1.18, 3.15)*	1.92 (1.03, 2.66)*	0.44 (0.25, 1.10)
Primipara n (%)	Controls	113 (50.9)		25 (11.1)
	GDM	19 (39.6)		5 (10.4)
Currently smoking n (%)	Controls	7 (3.8)		35 (19.8)
	GDM	1 (2.9)		12 (36.4)*
Previous smoker n (%)	Controls	39 (17.9)		48 (25.3)
	GDM	13 (27.7)		15 (41.7)*
Family history diabetes n (%)	Controls	69 (30.7)		
	GDM	18 (37.5)		
Systolic blood pressure (mmHg)	Controls	110 (100, 117)*	110 (105, 120)	110 (100, 120)
	GDM	110 (110, 120)	110 (105, 120)	110 (100, 130)
Diastolic blood pressure (mmHg)	Controls	65 (60, 70)	70 (60, 70)	70 (60, 74)
	GDM	70 (65, 70)*	70 (60, 75)	70 (65, 75)
Matsuda-index (insulin sensitivity)	Controls	221 (158, 314)	124 (87, 183)	277 (209, 383)
	GDM	152 (102, 198)**	63 (45, 91)**	189 (138, 298)**
ISSI-2 (insulin resistance)	Controls	0.71 (0.46, 1.04)	0.97 (0.67, 1.50)	0.64 (0.43, 0.94)
	GDM	1.0 (0.81, 1.67)**	2.01 (1.38, 2.73)**	1.04 (0.69, 1.31)**
HOMA-IR (β-cell function)	Controls	1195 (927, 1586)	1003 (781, 1282)	1050 (848, 1386)
	GDM	811 (615, 1078)**	549 (449, 653)**	760 (592, 1137)**

Data given as mean(SD) when normal distributed and median (25^{th}, 75^{th}) when skewed distributed. $p<0.05$ *, $p<0.001$** between GDM and controls.

Table 2. Levels of adipokines and macrophage markers at 5 years follow-up between previous GDM and non-GDM

	Controls	Previous GDM	Uni	BMI[¶]	Multi[$]
sCD163 (µg/mL)	0.49 (0.37, 0.65)	0.61 (0.46, 0.74)	6.8[b]	4.7[a]	4.1[a]
IL1Ra (pg/mL)	45.1 (27.5, 72.2)	62.8 (41.8, 124.0)	7.6[b]	2.8	3.8
Leptin (ng/mL)	24.5 (15.3, 40.7)	33.0 (24.9, 49.0)	9.1[b]	0.1	0.2
Adiponectin (ng/mL)	8.01 (6.22, 10.81)	6.17 (5.30, 7.91)	11.8[b]	5.1[a]	6.2[a]
Chemerin (ng/mL)	173 (150, 217)	191 (158, 221)	3.9[a]	0.6	0.4
Clusterin (ng/mL)	457 (424, 486)	463 (432, 484)	0.1	0.1	0.1
Resistin (ng/mL)	28.1 (21.3, 34.8)	28.8 (22.5, 35.0)	0.6	0.4	0.3
Adipsin (µg/mL)	1.80 (1.59, 2.08)	1.78 (1.52, 1.99)	3.0	4.3[a]	4.7[a]
sCD14 (µg/mL)	4.57 (4.23, 4.86)	4.63 (4.32, 4.84)	0.4	0.2	0.3

In the three columns to the right, F-scores are shown. [¶] Adjusted for BMI, [$] adjusted for BMI, age, parity, diabetes in family, CRP. [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

Figure 1. Participants flow chart for this study.

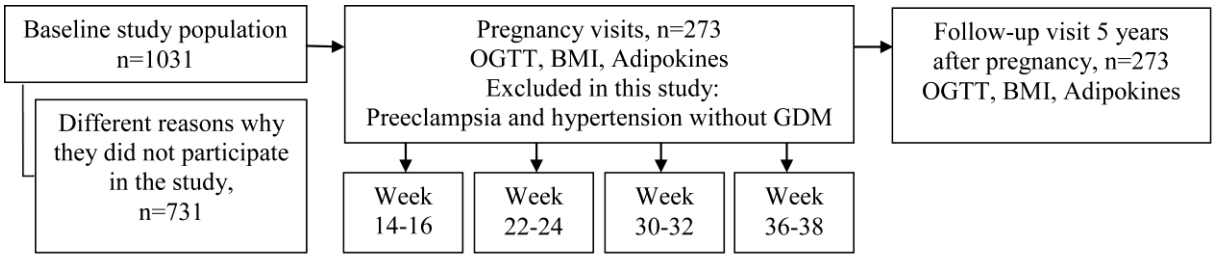

Figure 2. Levels of adipokines and monocyte/macrophage markers in non-GDM and GDM during pregnancy. A**.** The monocyte/macrophage activations markers sCD163 and IL-1Ra. B. The diabetic adipokines leptin and adiponectin and C. Other adipokines including chemerin, clusterin, resistin, adipsin and the monocyte/macrophage activation markers sCD14. Uni: univariate associations (i.e. unadjusted), BMI: adjusted for BMI, MULTI: multivariate associations (adjusted for BMI, age, parity, diabetes in family, CRP) are given at each visit and presented as F-scores. [a] p<0.05, [b] p<0.01, [c] p<0.001

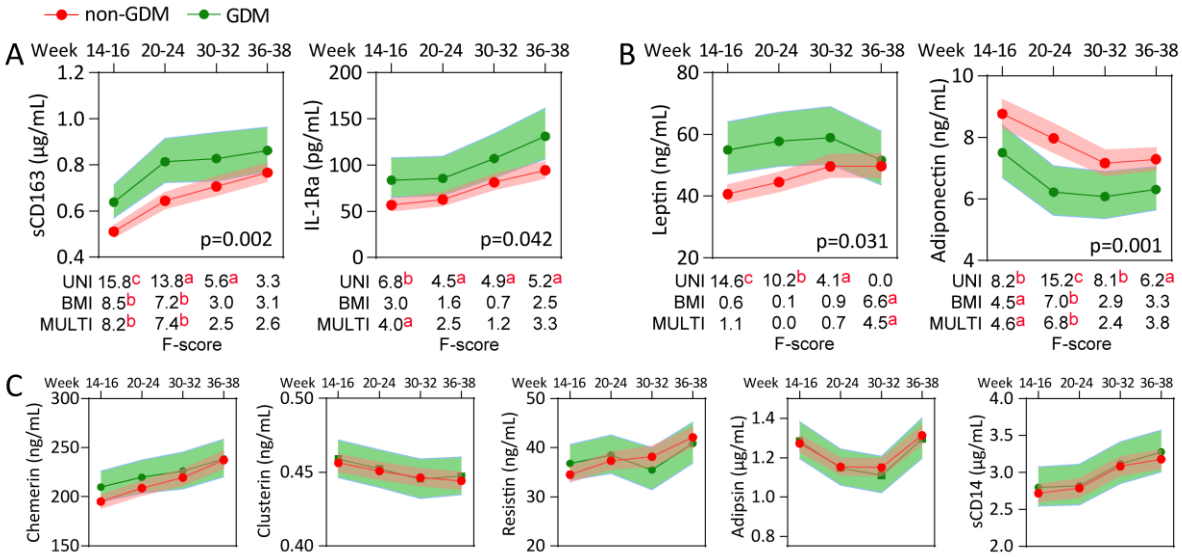

Figure 3. Associations between adipokines, monocyte/macrophage markers and BMI, CRP, and glucose measures at 14-16 and 30-32 weeks. A. The monocyte/macrophage activations marker sCD163, showing the interaction between sCD163 and BMI with BMI dicotomized at 25. B. The monocyte/macrophage activations marker sCD14 and the diabetic adipokines leptin, adiponectin and chemerin C. the adipokines clusterin, resistin, adipsin and the monocyte/macrophage activation markers sCD14. Univariate associations and multivariate associations adjusted for BMI are presented as regression coefficients and only significant associations are shown. In A, the interaction between sCD163 and BMI is shown with BMI dicotomized at 25.

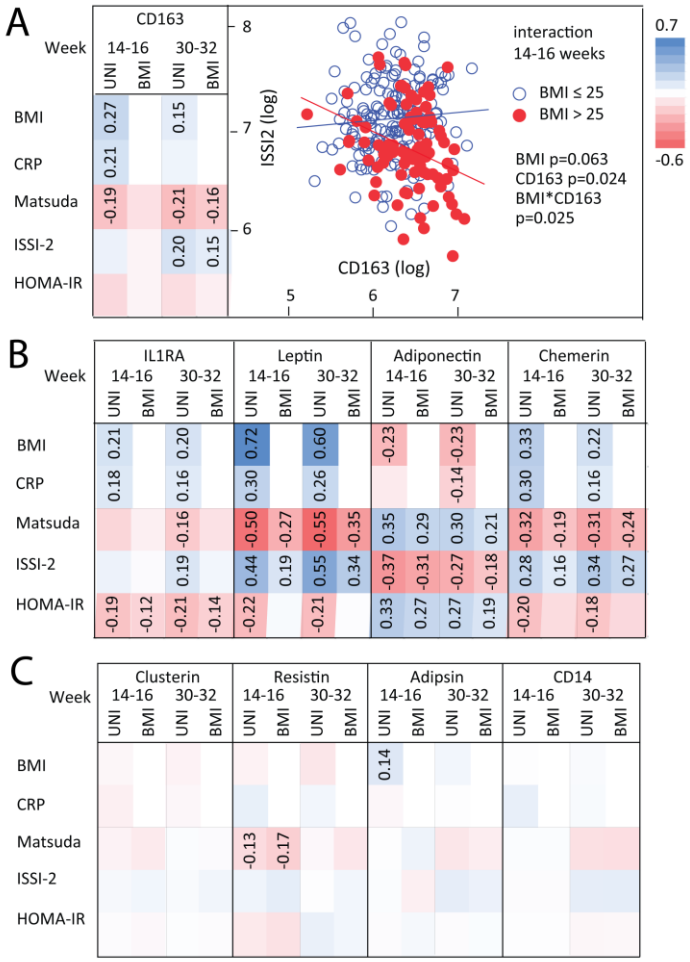

Figure 4. Adipokines and monocyte/macrophage markers at 14-16 weeks as predictors of GDM at 30-32 weeks. A**.** Receiver operating characteristic curves for predicting GDM by adipokines and macrophage markers at 14-16 weeks in pregnancy. Area with confidence intervals and p-values are shown to the right of the graph. B**.** Backward stepwise regression showing predictors (red circles) of GDM. BMI, age, family history of diabetes, parity and glucose were forced in the model in step 1 while adipokines and monocyte/macrophage markers were included by backward regression in step 2. C**.** Test characteristics of different cut-offs of sCD163 at 14- 16 weeks in pregnancy based on percentiles obtained from the total population for predicting GDM. D. Sensitivity of fasting glucose and sCD163 alone or in combination at 14-16 weeks in predicting GDM. The green line shows the sensitivity obtained at the 75th percentile, i.e. when 25% of the population is characterized as having a positive test.