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Growth-differentiation-factor 15 levels in obese and healthy pregnancies: Relation to insulin resistance and insulin secretory function

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Abstract

Objective/Aim: Growth-differentiation-factor 15 (GDF15) has been suggested to improve or protect beta cell function. During pregnancy, beta cell numbers and function increase to overcome the natural rise in insulin resistance during gestation. In this study, we longitudinally measured serum GDF15 levels during and after pregnancy in women of normal weight (NW) and in women with obesity (OB) and explored associations between GDF15 and changes in beta cell function by homeostatic model assessment (HOMA).

Methods: The cohort participants were 38 NW (BMI 22.3 ± 1.7) and 35 OB (BMI 35.8 ± 4.2). Blood was sampled and body composition measured at each trimester (T1, T2, and T3) and at 6, 12 and 18 months postpartum. Fasting glucose, insulin and GDF15 were measured, and HOMA for insulin resistance (HOMA-IR) and beta cell function (HOMA-B) determined.

Results: GDF15 levels increased significantly each trimester and were ~200-fold higher at T3 than in the nonpregnant postpartum state. GDF15 was higher in NW than OB during pregnancy, but was reversed after pregnancy with a significant interaction effect. GDF15 correlated inversely with BMI and fat-free mass at T3. Low GDF15 was associated with lower incidence of nausea and with carrying a male foetus. The pregnancy induced increase in GDF15 associated with increased HOMA-B in OB and with reduced fasting glucose in all women.

Conclusion: Large gestational upregulation of GDF15 levels may help increase insulin secretory function to overcome pregnancy-induced insulin resistance.

KEYWORDS
beta cell function, body composition, GDF15, HOMA, obesity, pregnancy

1 | INTRODUCTION

Pregnancy is a natural state of insulin resistance, which progressively increases to ensure adequate glucose transport to the growing foetus.[1] In women with normal glucose tolerance, beta cells proliferate and their function is enhanced to increase insulin secretion and thereby compensate for the increased insulin resistance.[2] When insulin resistance becomes too great or beta cell function does not respond adequately, glucose tolerance is impaired, leading to gestational diabetes, which has important implications for pregnancy outcome and the long-term health of mother and child.[3,4]

Obesity is a major driver of insulin resistance, and both pre-gestational obesity and large increases in maternal fat mass during pregnancy are risk factors for gestational diabetes. The mechanisms of insulin resistance during pregnancy are complex and are believed to be caused partly by hormones from the placenta and partly by other obesity- and pregnancy-related factors that are not fully understood.[5,6]

Growth-differentiation-factor 15 (GDF15), a member of the transforming growth factor-beta family, is a cytokine that was first discovered to be involved in inflammation and stress pathways; it was later found to be an appetite suppressant and a potential weight loss therapeutic.[7-9] GDF15 has also been linked to glucose metabolism, although the mechanism is not clear. Circulating GDF15 has been positively related to beta cell function in patients with severe obesity[10] and also rescues compromised beta cells in human islets.[11] In cross-sectional studies, increased levels of GDF15 have been noted in subjects with obesity and diabetes.[12-14]

Maternal GDF15 levels have been reported to increase across gestation, probably due to placental expression,[15,16] and high levels of GDF15 have been linked to pregnancy-related nausea and hyperemesis.[17,18] However, it is not known how GDF-15 levels increase during pregnancy in normoglycaemic women that differ in body mass index (BMI) and whether these levels are linked to changes in insulin resistance and insulin secretory function.

In this study, we measured GDF15 levels during and after pregnancy in women of normal weight (NW) and those with obesity (OB) and sought to determine whether increasing levels of GDF15 are associated with improvements in beta cell function and glucose metabolism. We hypothesized that a rise in GDF15 levels during pregnancy might be differentially regulated according to maternal BMI and that GDF15 is linked to the increasing beta cell function needed to maintain normal glucose levels during pregnancy-induced insulin resistance.

2 | METHODS

2.1 | Ethical approval

The study was approved by the Regional Ethical Review Board in Gothenburg (Dnr 402-08). All women received oral and written information about the study and gave informed written consent before enrolment.

2.2 | Subjects

Pregnant NW (BMI 18.5-24.9 kg/m^2) and OB (BMI ≥ 30 kg/m^2), aged 20-45 years, were recruited from six antenatal health units in the Gothenburg area as part of the Pregnancy Obesity Nutrition and Child Health (PONCH) study, as described.[19] Briefly, all women within the required BMI ranges at the six selected antenatal health clinics were informed about the study. If interested, the women were contacted by the study dietitian and called in for study visits at the Sahlgrenska Hospital. Exclusion criteria were non-European descent, diabetes mellitus (type 1, type 2, or gestational) or other chronic diseases, pregnancy-related complications, use of tobacco or neuroleptic drugs, and vegetarianism or veganism. The women came to the hospital for study visits once each trimester (weeks 8–12, 24–26 and 35–37; T1, T2 and T3, respectively) during pregnancy and at 6, 12 and 18 months (± 10 days) after pregnancy. Gestational age at study visits was on average 81 ± 8 days at T1, 175 ± 8 days at T2 and 253 ± 7 days at T3 and did not differ between NW and OB ($P > .4$).
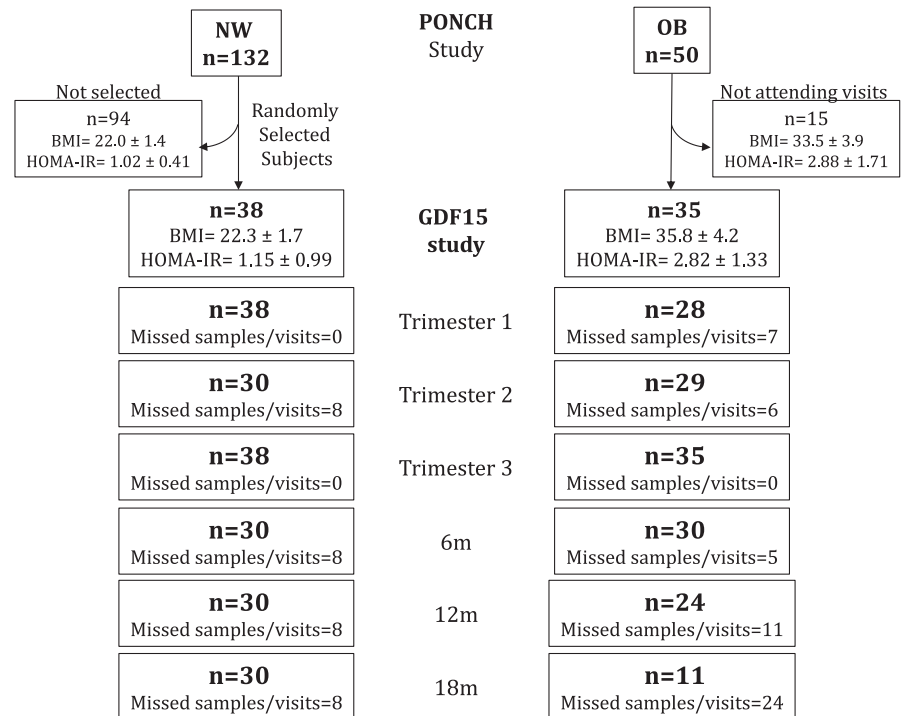
GDF15 was measured in a subset of the total 132 NW and 50 OB that enlisted in PONCH. For OB, the 35 women with most complete data were selected. For NW, 38 women were randomly selected. A few subjects were lacking in T2 samples. This resulted in differences in the numbers for visits according to Figure 1. The reasons given for postpartum drop-outs in the OB group were lack of time, moving to a different part of Sweden, or a new pregnancy.

2.3 | Study visits

All visits during and after pregnancy took place in the morning after an overnight fast and included collection of anthropometric and body composition measurements, blood samples and completion of life-style questionnaires, as described elsewhere in detail.[19] As part of the PONCH study, the pregnant women were randomized into dietary intervention or control subgroups[20]; the intervention subgroup received dietary counselling to increase adherence with Nordic Nutrition Recommendations. In the current study, none of the outcome measures differed between women in the dietary intervention and those in control subgroups, and subjects in the dietary subgroups were evenly distributed in the NW and OB groups. Therefore, data for the intervention/control subgroups were combined in all analyses.

Body composition was measured by ADP with the Bod Pod Gold Standard system (Bod Pod 2007 A, Life Measurement) and software versions 4.2.1 and 5.2.0. Adjustments were made for increased hydration of fat-free mass (FFM) during pregnancy in measurements made in T2 and T3 as described.[19] The coefficient of variation (CV) for body composition measurements on our equipment was 2.4%. As part of the life-style questionnaires, the women were asked whether they had experienced nausea during pregnancy. This question was missing for part of the study resulting in a total of 54 women from the present study answering the question.

FIGURE 1 Flow chart of GDF15 study population. The population for the present study was derived from the Pregnancy Obesity Nutrition and Child Health (PONCH) study. NW, women of normal weight; OB, women with obesity

2.4 | Biochemical analysis

Blood glucose and insulin were analysed with a Cobas Modular system (Roche Diagnostics) at the Clinical Chemistry Laboratory, Sahlgrenska University Hospital (accredited in accordance with the International Standard ISO 15189:2007). HOMA-IR was calculated as (fasting glucose × fasting insulin)/22.5 and HOMA-B as (20 × fasting insulin) / (fasting glucose − 3.5).[21] GDF15 was analysed with an ELISA (Human GDF-15 Quantikine Elisa Kit, R&D Systems); serum samples during and after pregnancy were diluted 1:64 and 1:4, respectively. The intra- and inter-assay CVs for GDF15 measurements were 1.7% and 7%, respectively.

2.5 | Statistical analysis

The number of subjects selected for the study was based on power calculations of GDF15 differences between NW or OB in previous studies in nonpregnant subjects[13,14]; a minimum of 11 subjects needed to be included per group for a power of 0.80 and alpha = 0.05 with an estimated effect size of 1.26 using two-sided two-sample t test. Differences in ordered categorical variables between the NW and OB groups were assessed with chi-square or Fisher´s exact test; between-group differences for background continuous variables were assessed with a t test, and within-group comparisons for continuous variables were assessed with a paired t test. To best take advantage of the longitudinal design, mixed models for repeated measures (MMRM) were used for the main outcomes. To assess GDF15 differences between NW and OB, an MMRM adjusted for maternal age and gestational age for each time point was used. A lognormal distribution of GDF15 was used in order to fulfil the modelling assumptions. An MMRM was also used for associations

between changes in GDF15 and BMI, glucose, insulin, HOMA-IR and HOMA-B. All variables were modelled using lognormal distribution in order to fulfil the modelling assumptions. The association of between change in GDF15 and the change in outcome variable is presented as mean difference changed in outcome variable per one unit change of GDF15 in logarithmic scale, and as a relative difference after transformation to the original scale.

Analyses were adjusted for mother's age at T1, time-updated gestational age at T2 and T3, time-updated BMI at T2 and T3, T1 value for the outcome, and any significant interactions between adjustment variables and visit. Unstructured (general) covariance matrix was found most optimal based on lowest Akaike's Information Criterion. Bonferroni-Holm adjustment was performed for all analyses considering association between change in GDF15 and all six outcome variables. Associations between GDF15 and body composition variables and glucose changes at relevant time points were analysed with Pearson's correlation or linear regression models, adjusted for gestational age BMI and maternal age. Categorical variables were expressed as number or percentage, and continuous variables as mean ± SD. All tests were two-tailed; $p < .05$ was considered statistically significant, after application of multiple adjustments specified above.

3 | RESULTS

3.1 | Subject characteristics and glucose metabolism

Age, parity, education, foetal sex and birth weight did not differ between the NW and OB groups (Table 1). BMI, fat mass (FM) and fat-free mass (FFM) were lower in NW than OB during and after

TABLE 1 Maternal characteristics

	NW		OB		p (NW vs OB)
	n	Mean ± SD[a]	*n*	Mean ± SD[a]	
Age (years)	38	31.3 ± 3.4	35	31.4 ± 4.0	.92
Nulliparous (%)	38	45%	35	51%	.64
Male/female foetus (n)	38	20/18	35	20/15	.44
Birth weight of child (kg)	38	3.78 ± 0.39	34	3.86 ± 0.50	.46
Nausea during pregnancy (%)	32	53%	24	58%	.79
Pregnancy					
BMI (kg/m^2), T1	38	22.3 ± 1.7	35	35.8 ± 4.2	<.001
Fat mass (kg), T1	38	16.7 ± 4.5	28	47.2 ± 11.1	<.001
Fat-free mass (kg), T1	38	45.1 ± 4.4	28	54.0 ± 6.3	<.001
Glucose (mmol/L), T1	35	4.69 ± 0.39	27	4.88 ± 0.41	.065
HOMA-IR, T1	34	1.15 ± 0.99	27	2.82 ± 1.33	<.001
HOMA-B (%), T1	34	92 ± 40	27	197 ± 99	<.001
Fat mass gain (kg), T1 to T3	37	4.0 ± 2.9	28	1.1 ± 4.0	.001
Fat-free mass gain (kg), T1 to T3	37	6.7 ± 1.8	28	6.5 ± 2.8	.68
Glucose change, (mmol/L), T1 to T3	34	-0.23 ± 0.43	27	-0.09 ± 0.37	.19
HOMA-IR change, T1 to T3	34	0.43 ± 0.97	27	1.98 ± 1.52	<.001
HOMA-B change, (%), T1 to T3	34	93 ± 77	27	189 ± 203	.01
After pregnancy					
BMI (kg/m^2), 6 months	30	22.1 ± 1.9	30	34.8 ± 4.8	<.001
BMI (kg/m^2), 18 months	30	22.0 ± 1.9	11	33.1 ± 5.0	<.001
Fat mass (kg), 6 months	30	16.9 ± 5.0	30	46.9 ± 12.7	<.001
Fat mass (kg), 18 months	30	15.5 ± 5.4	11	43.2 ± 13.1	<.001
Fat-free mass (kg), 6 months	30	44.8 ± 3.7	30	52.4 ± 6.3	<.001
Fat-free mass (kg), 18 months	30	45.5 ± 4.5	11	48.4 ± 4.4	.076
Glucose (mmol/L), 6 months	30	4.95 ± 0.29	30	5.22 ± 0.45	.007
Glucose (mmol/L), 18 months	30	4.97 ± 0.30	11	5.19 ± 0.41	.061
HOMA-IR, 6 months	30	0.96 ± 0.36	30	2.86 ± 1.73	<.001
HOMA-IR, 18 months	30	1.35 ± 0.56	11	2.79 ± 1.38	<.001
HOMA-B (%), 6 months	30	60 ± 22	30	147 ± 71	<.001
HOMA-B (%), 18 months	30	86 ± 44	11	141 ± 50	.001

Note: HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B; homeostatic model assessment for beta cell function; NW, women of normal weight; OB, women with obesity; T1, trimester 1; T3, trimester 3.

[a]Values are mean ± SD except for parity, foetal sex and nausea.

pregnancy; at 18 months after pregnancy, the difference in FFM had disappeared. During pregnancy, total body weight (p = .016) and FM (p = .001) increased more in NW, but the gain in FFM was similar in the two groups. There was no difference between NW and OB in self-reported nausea during pregnancy. There was, however, a significant difference in nausea between women carrying a female or male foetus (nausea reported in 72% of women carrying a female foetus and 42% in women carrying male a foetus, p = .03).

HOMA-IR and HOMA-B were lower in NW than OB at all time points during and after pregnancy (Table 1). In NW during pregnancy, blood glucose levels decreased whereas HOMA-IR and HOMA-B increased (p = .04, p = .014 and p < .001 for changes between T1

and T3 in glucose, HOMA-IR and HOMA-B, respectively). In OB, HOMA-IR and HOMA-B increased significantly during pregnancy, but the change in blood glucose was not significant (p = .20, p < .001 and p < .001 for changes between T1 and T3 in glucose, HOMA-IR and HOMA-B, respectively). After pregnancy in both groups, glucose levels increased whereas HOMA-IR and HOMA-B decreased (p < 0.001 for all changes between T3 and 6 months in glucose, HOMA-IR and HOMA-B in both NW and OB).

NW women selected for the present study did not differ from non-selected NW women in the overarching PONCH study in terms of BMI, body composition or homeostatic model assessment of insulin resistance (HOMA-IR), p = .3–0.8 (Figure 1). Women in PONCH

are randomized into two intervention arms. Intervention arms were evenly distributed within each BMI group (19/19 in NW and 19/16 in OB), and none of the outcomes in the present study differed between the arms for either NW or OB (for differences between intervention groups in T1-T3 outcomes p = .95 for BMI, p = .70 for glucose, p = .32 for HOMA-IR and p = .21 for HOMA-B in NW, and p = .25 for BMI, p = .61 for glucose, p = 70 for HOMA-IR and p = .98 for HOMA-B in OB).

3.2 | GDF15 levels during and after pregnancy

In NW, serum GDF15 increased 40-fold in T1 to 200-fold in T3 compared with nonpregnant values (ie after pregnancy), as shown in Figure 2A. OB showed a similar trend although the increases were

lower than in NW. After the initial postpartum decrease, GDF15 did not significantly change between 6, 12 and 18 months in either group. GDF15 tended to be higher in OB than in NW after pregnancy (p = .1–0.2), with a significant interaction over time for difference between NW and OB.

In line with the differences in GDF15 levels between the two groups during pregnancy, GDF15 levels correlated negatively with body weight and BMI at T2 (R = −0.385, p = .003 and R = −0.336, p = .009, respectively) and T3 (R = −0.344, p = .003 and R = −0.289, p = .013, respectively). In analyses of body composition, GDF15 correlated negatively with FFM (but not FM) in NW at both T1 (R = −0.433, p = .007) and T3 (R = −0.336, p = .042). However, after adjustment for maternal BMI, only the negative correlation between GDF15 and FFM at T3 remained significant (Figure 2B). When analysing the BMI groups separately, GDF15
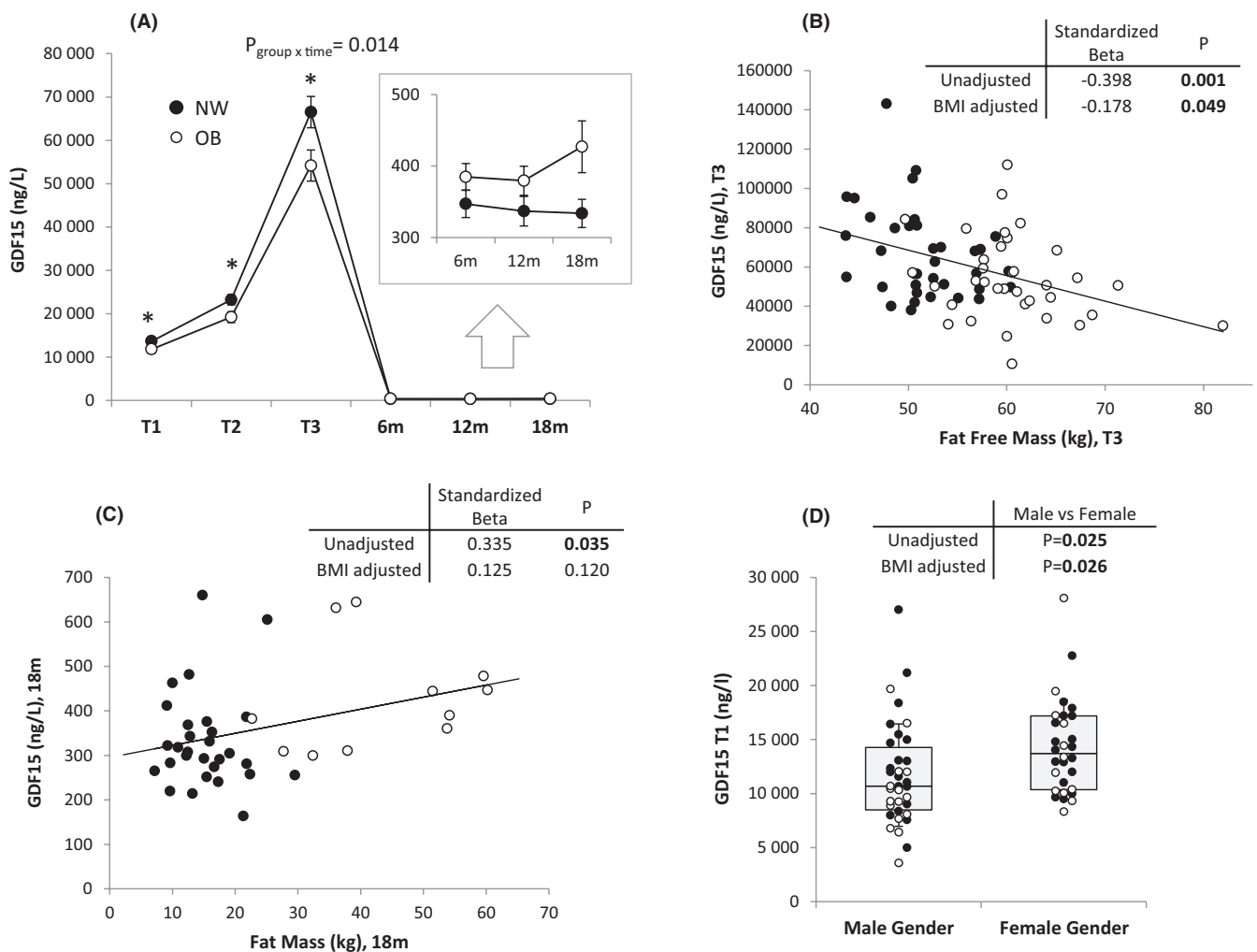

FIGURE 2 A, GDF15 serum levels during and after pregnancy in women of normal weight or with obesity. Box included: GDF15 vales after pregnancy shown in greater detail. *p < .05 for differences between NW and OB groups. p < .001 for all increases between trimesters and for all differences between pregnancy and post-pregnancy values for both groups. Values are mean ± SEM. B, Correlation between GDF15 levels and fat-free mass at T3. C, Correlation between GDF15 levels and fat mass at 18 months after pregnancy. D, Maternal GDF serum levels in T1 depending on foetal sex. NW (closed circles), women of normal weight; OB (open circles), women with obesity; T1, trimester 1; T2, trimester 2; T3, trimester 3; 6 m, 6 months after delivery; 12 m, 12 months after delivery; 18 m, 18 months after delivery

correlated negatively with FFM (but not FM) in NW at both T1 and T3 (R = −0.433, p = .007 at T1 and R = −0.336, p = .042 at T3). In the OB group, GDF15 during pregnancy did not correlate significantly with weight, BMI or body composition, although the correlation between GDF15 and FFM at T2 narrowly missed significance (p = .051). After pregnancy, GDF15 correlated positively with FM at 18 months, but not after adjustment for BMI (Figure 2C).

GDF15 levels were not consistently associated with changes in weight or body composition, apart from a negative association between early GDF15 change (T1 to T2) and gestational FM gain (T1 to T3) in NW (R = −0.382, p = .037).

In analysis by foetal sex, GDF15 at T1 was significantly higher in women carrying a female foetus than in those carrying a male foetus (Figure 2D). The difference remained significant after adjustment for maternal BMI. When analysing the relationship between GDF15 and nausea, there was a trend for lower GDF15 at T1 in women not reporting nausea compared to those that did (12 046 ± 4298 ng/L vs 13 893 ± 5081 ng/L, respectively, p = .12). Dividing GDF15 at T1 into quarters, the women with the lowest quarter of GDF15 reported lower incidence of nausea compared to the rest (33% vs 64%, respectively, p = .04). This difference remained significant after BMI adjustment (p = .04), but was non-significant after adjustment for foetal sex (p = .16).

3.3 | Associations between GDF15 expression and beta cell function during and after pregnancy

Associations between changes in GDF15 from T1 to T2 and T3, respectively, and pregnancy-induced corresponding changes in BMI, glucose, insulin resistance and beta cell function are shown in Table 2. In this mixed model for repeated measures (adjusted for maternal age, gestational age and BMI at T2 and T3 visits and T1 value for the outcome), an increase in GDF15 from T1-T3 was associated with a decrease in fasting glucose. When looking at different time points in glucose during pregnancy, fasting blood glucose decreased significantly late in pregnancy (p = .008, T2 to T3). This decrease between T2 and T3 was inversely associated with both early changes in GDF15 (T1 to T2) and late (T2 to T3), visualized in Figure 3.

There was also a significant association between the increase in GDF15 and HOMA-B, although the character of the association differed in the NW and OB groups (interaction p = .002 between groups). In OB, the HOMA-B increase doubled in value with a one unit increase in log GDF15, while in NW there was no significant association.

Since GDF15 levels did not change significantly between time points after pregnancy (Figure 2A), associations between measures of glucose metabolism and changes in GDF15 after pregnancy were not deemed relevant. Only associations for absolute values at 6, 12 and 18 months were investigated. GDF15 did not correlate with

TABLE 2 Mixed model for repeated measures of associations between GDF15 and BMI and glucose metabolism variables

Outcome	Time	n	Difference per 1 unit increase in log scale of change in GDF15		p^a	p^b
			Relative difference (95% CI) [original scale]	Mean difference (95% CI) [log scale]		
BMI	T1-T2	121	1.00 (0.97–1.03)	-0.00 (−0.03–0.03)	.97	.21
	T1-T3		1.02 (0.99–1.05)	0.02 (−0.01–0.05)	.24	
Glucose	T1-T2	109	0.99 (0.92–1.05)	-0.02 (−0.08–0.05)	.64	.033
	T1-T3		0.92 (0.88–0.97)	-0.08 (−0.13−−0.03)	.0015*	
Insulin	T1-T2	119	1.05 (0.85–1.31)	0.05 (−0.17–0.27)	.63	.59
	T1-T3		0.98 (0.79–1.23)	-0.02 (−0.24–0.20)	.89	
HOMA-IR	T1-T2	107	1.06 (0.81–1.39)	0.06 (−0.21–0.33)	.66	.14
	T1-T3		0.84 (0.65–1.09)	-0.17 (−0.43–0.09)	.20	
HOMA-B NW[c]	T1-T2	57	0.80 (0.57–1.13)	-0.22 (−0.57–0.12)	.20	.60
	T1-T3		0.90 (0.62–1.31)	-0.11 (−0.48–0.27)	.58	
HOMA-B OB[c]	T1-T2	50	1.25 (0.80–1.96)	0.22 (−0.23–0.67)	.31	.029
	T1-T3		1.99 (1.48–2.66)	0.69 (0.39–0.98)	<.0001*	

Note: All variables were modelled using lognormal distribution in order to fulfil the modelling assumptions. Analyses were adjusted for mother's age at T1, time-updated gestational age at T2 and T3, time-updated BMI at T2 and T3, baseline value for the outcome, and any significant interactions between adjustment variables and visit. T1, trimester 1; T2, trimester 2; T3, trimester 3; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, homeostatic model assessment for beta cell function.

[a]Bonferroni-Holm adjustment was performed for all analyses considering association between change in GDF15 and all six outcome variables. p-values marked with '*' are considered significant after Bonferroni-Holm adjustment.

[b]p-value for interaction between change in GDF15 and visit.

[c]Two separate models were performed, per NW and OB group, due to significant interaction between change in GDF15 and change in HOMA-B at time point T3 (p = .0018).
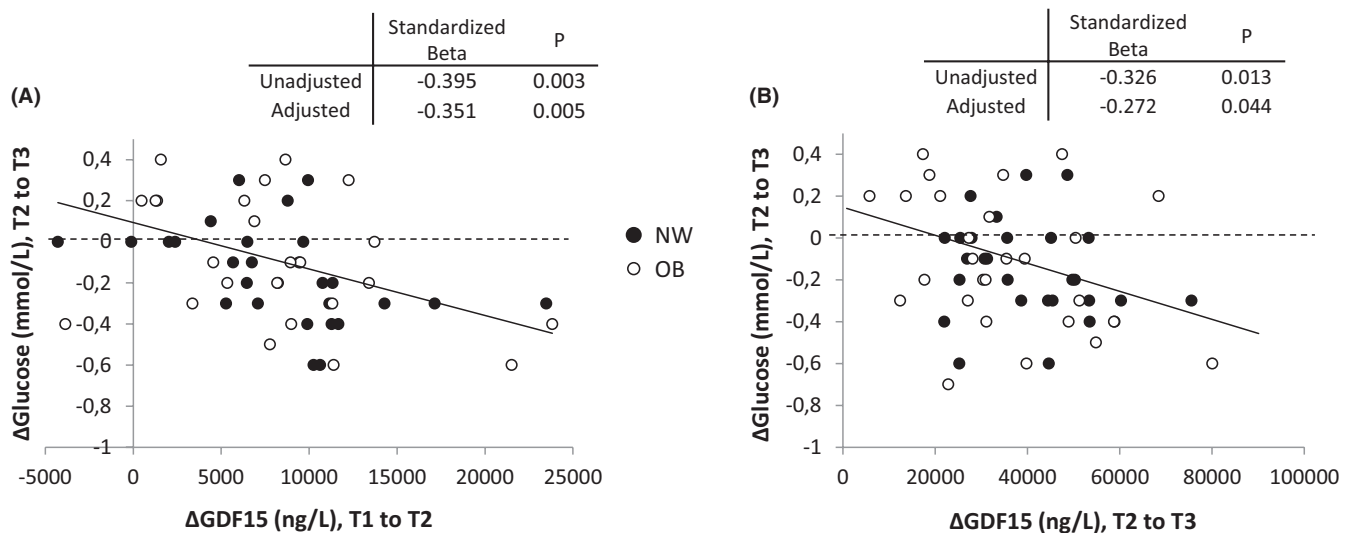
	Standardized Beta	P
Unadjusted	-0.395	0.003
Adjusted	-0.351	0.005

(A)

	Standardized Beta	P
Unadjusted	-0.326	0.013
Adjusted	-0.272	0.044

(B)

FIGURE 3 Associations between changes in fasting glucose and changes in GDF15 during pregnancy. A, Changes in fasting glucose between T2 and T3 as a function of changes in GDF15 between T1 and T2. B, Changes in fasting glucose between T2 and T3 as a function of changes in GDF15 between T2 and T3. The adjusted model includes maternal BMI and age at T1 as covariates. NW, women of normal weight; OB, women with obesity; T1, trimester 1; T2, trimester 2; T3, trimester 3

HOMA-IR or HOMA-B at 6 or 12 months. At 18 months, however, GDF15 was significantly associated with HOMA-B (beta = 0.301, p = .039) and HOMA-IR (beta = 0.304, p = .009) among all women after adjustment for maternal age and BMI. In subgroup analyses, GDF15 associated significantly with HOMA-B in NW (beta = 0.424, p = .031) and with HOMA-IR in OB (beta = 0.587, p = .048).

4 | DISCUSSION

This is the first study to both longitudinally measure GDF15 during and after pregnancy and to compare these levels in NW and OB women. For the first time in normoglycaemic women, we also linked GDF15 levels during pregnancy with beta cell function and glucose levels. Circulating GDF15 increased throughout pregnancy, and GDF15 levels in late pregnancy were up to 200-fold higher than in the nonpregnant state. The increases were higher in NW than OB, and the levels correlated inversely with FFM. Further, the increasing GDF15 levels during pregnancy were associated with increased beta cell function in the OB group and lower glucose levels in all women.

Diabetes and obesity are considered to be conditions of inflammation and metabolic stress and are associated with high GDF15 levels.[12-14] However, the link between GDF15 and glucose metabolism is not clear from previous studies. Pregnancy is a naturally occurring state of increasing insulin resistance and insulin secretory capacity, which peak in late pregnancy[6] and is therefore a good model for studying longitudinal factors that might affect beta cell function. We found that in normoglycaemic women, increasing GDF15 levels during pregnancy were associated with increased HOMA-B and decreased fasting glucose levels, without effecting insulin resistance. Previous cross-sectional studies have shown

higher serum levels of GDF15 in patients with insulin resistance or type 2 diabetes,[13,14,22] and a study investigating GDF15 in Chinese pregnant women linked high GDF15 to gestational diabetes.[23] Other studies show that GDF15 positively affects beta cells, as judged from its ability to rescue beta cell function in compromised human islets and to reduce the incidence of diabetes in nonobese diabetic mice.[11] In addition, GDF15 was positively associated with beta cell function in a cross-sectional study of obese subjects.[10] Intriguingly, in a longitudinal study, GDF15 levels predicted impairment of glucose metabolism in obese subjects,[24] suggesting that the increased concentrations of GDF15 seen in obesity were insufficient to improve beta cell function. Our results imply that insufficient increase in GDF15 early in pregnancy might predict higher glucose levels during the last trimester, which is when gestational diabetes is most often diagnosed.[25]

We found that postpartum GDF15 levels tended to be higher in OB than in NW (400 vs 350 ng/L). These levels are comparable to those in healthy nonpregnant subjects in previous studies.[10,13,14,26,27] In contrast, during pregnancy, the increases in GDF15 were greater in NW, than in OB. In agreement with a previous study (that did not include OB), GDF15 during pregnancy correlated inversely with BMI at the start of pregnancy.[17] The large increases in circulating GDF15 during pregnancy are believed to be of placental origin,[15] and differences in the placentas of NW and OB might be of importance for the release of GDF15. For example, macrophage M1 infiltration in the decidua parietalis during pregnancy is lower in OB than NW.[28] This difference was speculated to indicate a compensation mechanism for the pro-inflammatory state in OB to ensure healthy pregnancy outcomes. Presumably, placentas from women who differ in BMI also differ in expression of immune factors such as GDF-15 and other cytokines; such differences could influence immune reactions and metabolism during pregnancy. However, a conclusive

explanation for the difference in GDF15 levels between NW and OB in our study is lacking and needs to be clarified in future research.

We found an inverse correlation between GDF15 increase during pregnancy and FM gain in NW but did not see a relationship between GDF15 and gestational weight gain. Increases in GDF15 mediate adiposity resistance[29] and are linked to reductions in body weight[9,30,31] and in FM.[32] The effect of GDF15 on weight loss is thought to be associated with appetite reduction regulated by binding to the GDNF family receptor α-like (GFRAL) receptor in the hindbrain.[29,33] The effect of GDF15 on pregnancy-related weight gain has not previously been reported, but GDF15 has been linked to pregnancy-related nausea and hyperemesis gravidarum.[17,18] We confirmed these earlier findings by showing that women with the lowest quartile of GDF15 in T1 reported lower incidence of pregnancy-related nausea. Interestingly, hyperemesis may be more common in women of low BMI[34] and in those carrying a female foetus.[35,36] We did not see a difference in nausea between NW and OB, but we did see a higher incidence of nausea in women carrying female foetuses. We also found that high GDF15 levels were linked to carrying a female foetus. Ie GDF15 could potentially be the link between nausea and foetal sex; in support of this, we found that the difference in nausea between GDF15 quarters was abolished when adjusted for foetal sex. GDF15 inhibition has been suggested as a treatment for hyperemesis gravidarum.[17,18] However, if the link between GDF15 and beta cell function we found proves to be casual, manipulating GDF15 action might disturb glucose homeostasis. Our results also raise the question whether there is a link between pregnancy-related nausea and beta cell function, and whether GDF15 has a role in this. In severe hyperemesis, starvation might negatively affect glucose metabolism early in pregnancy, but by the second trimester there seems to be no difference in glucose tolerance.[37] Research on less severe emesis is lacking. We saw a tendency of lower T3 glucose in women reporting nausea (data not shown, $p = .09$), but this needs to be confirmed in other cohorts.

After a large postpartum reduction in circulating GDF15, there were no changes between 6 and 18 months after pregnancy; glucose metabolism followed a similar pattern. At 18 months after pregnancy, GDF15 was positively associated with HOMA-B for all women and NW and, in OB, with HOMA-IR. The latter finding is in agreement with cross-sectional studies linking insulin resistance and GDF15 in nonpregnant OB.[14,24] This association can be explained by the higher release of GDF15 in inflammatory conditions such as insulin resistance and obesity as discussed above. In future studies, it would be of interest to investigate how the postpartum changes in GDF15 levels might predict glucose intolerance long term, especially in women with gestational diabetes who have a 30% risk of developing type 2 diabetes within 5 years.[38]

The major strength of the current study is the longitudinal design comprising three time points during pregnancy and three time points after pregnancy, all involving identical study visits at a single centre. Our subjects were well characterized through blood sampling and use of a reference method to measure body composition. However, oral glucose tolerance testing in addition to HOMA calculations would have provided a more extensive evaluation of glucose metabolism and insulin release. As in all such studies, additional limitations include the difficulty of proving causation in relationships, recruitment bias and generalizability. With a focus on health and anthropometric measurements, recruited women are generally more inclined to keep healthy habits. However, relationships among the various factors measured should not be affected by this potential bias. A final limitation was the reduced statistical power resulting from the number of drop-outs in the OB group for the last visit at 18 months after pregnancy.

In conclusion, we found that the serum level of GDF15 increases throughout pregnancy, and does so to greater extent in NW than OB, and is associated with lowered glucose and increased insulin secretory function in normoglycaemic obese pregnancies. Adding to previous research in nonpregnant subjects, our findings suggest that GDF15 has a beneficial effect on beta cell function and may have implications for treatment to overcome insulin resistance. More mechanistic studies will be needed to confirm the effect of GDF15 on beta cells during pregnancy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

UAH, PS and AH involved in conception, data acquisition, data analysis and interpretation. UAH, LJ, CM and AH involved in drafting or revising article for intellectual content. All authors read and approved the final manuscript.

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