

Research paper

Maternal serum placental growth hormone, insulin-like growth factors and their binding proteins at 20 weeks' gestation in pregnancies complicated by gestational diabetes mellitus

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ABSTRACT

OBJECTIVE: To investigate whether maternal serum concentrations of placental growth hormone (GH-V), insulin-like growth factor (IGF) 1 and 2, and IGF binding proteins (IGFBP) 1 and 3 were altered in pregnancies complicated by gestational diabetes mellitus (GDM). **METHOD:** In a nested case-control study, GDM cases (n=28) and matched controls (n=28) were selected from the Screening for Pregnancy Endpoints (SCOPE) biobank in Auckland, New Zealand. Maternal serum hormone concentrations at 20 weeks of gestation were determined by enzyme-linked immunosorbent assay (ELISA). **RESULTS:** There was no significant difference in maternal serum GH-V concentration in the GDM group compared to the control group (1.64 ± 0.11 ng/ml vs. 1.38 ± 0.10 ng/ml, $p=0.079$). However, GDM cases who delivered large for gestational age (LGA) babies had significantly higher serum GH-V concentrations compared to non-diabetic control cases. Maternal IGF-1 concentrations in GDM pregnancies were significantly higher than in controls (275.7 ± 11.5 ng/ml vs. 218.5 ± 11.1 ng/ml, $p < 0.001$). Maternal IGFBP-1 concentrations were significantly lower in GDM pregnancies than in controls (41.04 ± 3.42 ng/ml vs. 67.58 ± 6.17 ng/ml, $p < 0.001$). There were no significant differences in serum IGF-2 and IGFBP-3 concentrations between groups. **CONCLUSION:** Concentrations of IGF-1 and IGFBP-1 in maternal serum were altered in GDM pregnancies compared to controls, suggesting that the IGF axis plays a role in the development of this condition. GH-V may be associated with macrosomia as increased maternal GH-V was observed in GDM cases who delivered LGA babies.

Key words: Binding proteins, Gestational diabetes mellitus, Insulin-like growth factor, Placental growth hormone, Pregnancy

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INTRODUCTION

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy, represents a failure to maintain normal glucose tolerance during the metabolic stress of pregnancy.¹ It affects 5.8-12.9% of all pregnancies worldwide² and is associated with multiple gestational complications, including macrosomia, dystocia, still-birth, neonatal hypoglycaemia and respiratory distress.³ Both the foetus and the mother have increased risk for metabolic disorders and diabetes in later life.⁴

Placental growth hormone variant (GH-V) is the product of the *GH-V* gene specifically expressed in the syncytiotrophoblast layer of the human placenta.⁵ GH-V differs from pituitary growth hormone (GH-N) by 13 amino acids and shares similar physiological effects with GH-N.^{6,7} GH-N is mainly secreted in a pulsatile fashion from the pituitary, while GH-V is secreted from the placenta in a non-pulsatile manner during human pregnancy.⁸ GH-V is thought to play a key role in maternal adaptation to pregnancy as it stimulates foetal growth, gluconeogenesis, lipolysis, and anabolism thereby increasing substrate supply for the foetoplacental unit.^{6,9} We and others have demonstrated that transgenic expression or administration of exogenous GH-V causes insulin resistance in mice.¹⁰⁻¹² A positive association between maternal GH-V and foetal growth has been found in previous studies.¹³⁻¹⁵ Increased maternal GH-V concentrations were also observed in large for gestational age (LGA) pregnancies.¹⁶ Further, it is thought that GH-V increases maternal concentrations of other important growth factors, such as insulin-like growth factor (IGF)-1, during pregnancy.¹⁷⁻¹⁹ IGFs and their binding proteins (IGFBP) affect maternal metabolism and act as endocrine signals to enhance placental function and foetal growth.^{20,21} The structural homology between IGFs and insulin and the hypoglycaemic activity regulated by IGFBPs suggest that IGFs and their binding proteins have an intrinsic role in glucose metabolism and homeostasis.²² Limited studies implicate GH-V as a potential biomarker of GDM.^{14,23} Dysregulations of IGFs and their binding proteins were also observed in diabetic pregnancies.²⁴⁻²⁶

The aim of this study was to determine whether maternal serum concentrations of GH-V, IGF-1 and

2, IGFBP-1 and 3 at 20 weeks of gestation were altered in pregnancies complicated by GDM. We also hypothesized that these hormone were correlated with birth weight and maternal glycaemic status.

MATERIALS AND METHODS

Ethical approval was obtained from New Zealand Health and Disability Ethics Committees (AKX/02/00/364/AM03) and all women provided written informed consent. Between November 2004 and October 2007, 2,032 nulliparous women with singleton pregnancies were recruited to the Screening for Pregnancy Endpoints (SCOPE) study in Auckland, New Zealand. The inclusion criteria has been described previously.²⁷

Participants were interviewed and examined by a SCOPE research midwife at 15 and 20 weeks of gestation. At the first visit, detailed clinical and demographic data were collected and entered into an internet accessed, central database with a complete audit trail (MedSciNet, Stockholm, Sweden). Maternal serum samples were collected at 20 weeks and stored at -80°C for subsequent analyses. The specimens did not undergo any freeze/thaw cycles prior to these analyses. Birth weight was recorded using electronic scales at the time of birth. Customized birth weight centile adjusted for mother's height and weight at 15 weeks' visit, ethnicity, sex and gestation at delivery, was calculated to allow comparison of relative foetal growth across a range of gestational ages. Small for gestational age (SGA) and LGA were defined as birth weight <10th and >90th customized birth weight centiles, respectively. All participants underwent the glucose screening (50 g polycose) from 15 to 28 weeks depending on risk factors. Participants who had abnormal glucose screening results or who were at high risk of developing GDM were advised to perform a two-hour 75 g oral glucose tolerance test (OGTT). GDM for the purposes of this case control study was diagnosed by International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria based on Hyperglycemia and Adverse Pregnancy Outcome (HAPO) data (fasting plasma glucose ≥ 5.1 mmol/L or 1 h glucose ≥ 10.0 mmol/L or 2 h glucose ≥ 8.5 mmol/L).²⁸

In this nested case-control study, 28 GDM cases

were selected from the New Zealand SCOPE cohort and matched by ethnicity, age (± 3 years), and body mass index (BMI) (± 3 kg/m²) to 28 controls.

Materials

Recombinant human GH-V (22 kDa) was purchased from Protein Laboratories Rehovot (Rehovot, Israel) and was reconstituted in 0.4% NaHCO₃ pH 9.²⁹ Human GH-V monoclonal antibodies 78.8E8 (E8; MCA5827G) and 78.7C12 (7C12; MCA5828G) were obtained from Bio-Rad AbD Serotec (NC, US). E8 does not cross-react with GH-N or prolactin; 7C12 shows some cross-reactivity with GH-N (5%) as per the manufacturer's documentation. Antibody 7C12 was biotinylated using a LYNX Rapid Biotin Antibody Conjugation Kit (Bio-Rad AbD Serotec) according to the manufacturer's instructions.

GH-V ELISA procedure

We have previously described the development and validation of an in-house enzyme-linked immunosorbent assay (ELISA) for the measurement of GH-V in serum.³⁰ In brief, microtiter plates were coated with antibody E8 diluted in phosphate buffer (0.1M Sodium Carbonate, pH 9.5) at a concentration of 2 μ g/ml by overnight incubation at 4°C. Coated plates were washed three times with wash buffer (PBS-T; 10 mM phosphate buffer pH 7.4, 150 mM NaCl, 0.05% Tween 20). Blocking was achieved by 1 hour incubation at room temperature with Ultrablock (Bio-Rad AbD Serotec). Standards were prepared from GH-V solution with a range from 0.078 to 5 ng/ml. Standards and 1:2 diluted serum samples were incubated for 2 hours at room temperature, then washed three times. All serum samples were measured in duplicate. 8 μ g/ml biotinylated antibody C12 was added and incubated for 1 hour. After being washed three times, 200 ng/ml horseradish peroxidase conjugated streptavidin (Bio-Rad AbD Serotec) was added and incubated for 30 minutes. The microtiter plates were washed four times. End-point detection was processed by using 3, 3', 5, 5'-Tetramethylbenzidine (TMB) Substrate Reagent Set (BD Biosciences) and stop solution (2N H₂SO₄). Absorbance was read at 450 nm and 590 nm within 30 minutes of stopping reaction. Serum samples were spiked with GH-V and the average recovery rate was 106%. Coefficients of variation (CV) of intra-assay and inter-assay were 4.8% and 6.8%, respectively.

Serum analysis

Serum total IGF-1, total IGF-2, IGFBP-1, and IGFBP-3 were assayed with human-specific ELISA as per the manufacturer's instructions (Mediagnost, Germany).

Statistical analysis

Concentrations of GH-V, IGF-2, and IGFBP-1 were positively skewed. Data were log-transformed to improve the approximation of normal distribution and linearize relationships where appropriate. Data are expressed as means \pm S.E.M unless stated otherwise. Group means were compared using a Student's *t* test. Categorical variables were compared using chi-square or Fisher's exact test. Pearson's coefficient was used to determine correlations between variables, presented as *r* values. Multivariate linear regression analysis was adopted to determine the association of maternal hormone concentrations with changes in glucose levels. All analyses were conducted using IBM SPSS Statistics 21. A *p*-value of <0.05 was accepted as statistically significant.

RESULTS

The demographic and clinical details are shown in Table 1.

Concentrations of maternal GH-related hormones

There was no significant difference in maternal serum GH-V concentration in the GDM group at 20 weeks of gestation when compared with the control group (1.64 ± 0.11 ng/ml vs. 1.38 ± 0.10 ng/ml, *p*=0.079) (Figure 1A). However, GDM cases who delivered LGA babies (*n*=7) had significantly higher serum GH-V concentrations compared to non-diabetic controls (*n*=28) (1.93 ± 0.21 ng/ml vs. 1.38 ± 0.10 ng/ml, *p*=0.02). Maternal serum IGF-1 concentrations in GDM pregnancies were significantly higher than in the control group (275.7 ± 11.5 ng/ml vs. 218.5 ± 11.1 ng/ml, *p*<0.001) (Figure 1B). Maternal serum IGFBP-1 concentrations were significantly lower in GDM pregnancies than in controls (41.04 ± 3.42 ng/ml vs. 67.58 ± 6.17 ng/ml, *p*<0.001) (Figure 1D). There was no significant difference in serum IGF-2 or IGFBP-3 concentrations between groups (Figure 1C and E).

Table 1. Demographic and clinical findings

	GDM n=28	Control n=28	P value
Maternal characteristics			
Maternal age (yrs)	31.4 (0.9)	31.3 (0.8)	-
Socioeconomic index*	47 (3)	48 (3)	ns
Caucasian, n (%)	18 (64.3)	18 (64.3)	-
Family history of diabetes	10 (35.7)	6 (21.4)	ns
Smoked at 15 wks, n (%)	3 (10.7)	5 (17.9)	ns
Any alcohol intake at 15 wks, n (%)	2 (7.1)	2 (7.1)	ns
BMI at 15 wks (kg/m ²)	27.2 (0.9)	26.2 (0.8)	-
Gestational age at delivery (wks)	38.2 (0.4)	39.6 (0.2)	0.0054
Infant characteristics			
Birth weight (g)	3449 (101)	3460 (85)	ns
SGA at birth	2 (7.1)	0 (0)	ns
LGA at birth	7 (25)	4 (14.3)	ns
Male, n (%)	16 (57.1)	15 (53.6)	ns

Results expressed as mean (SEM) or n (%); ns, not significant.

*Socioeconomic index calculated using the New Zealand Socioeconomic Index guide (1996).

Correlation analysis

In the GDM group, maternal IGF-1 concentration was positively related to the changes in GH-V and IGFBP-3 but negatively related to the IGFBP-1 concentrations (Table 2). Maternal fasting glucose concentration in OGTT was positively related to IGF-1 ($r = 0.38$, $p = 0.046$) and negatively related to IGFBP-1 ($r = -0.465$, $p = 0.013$), but these correlations were not seen with 1 hour and 2 hour OGTT measurements. In a multivariate analysis, which included maternal age, ethnicity, socioeconomic status, family history of diabetes, smoking and drinking habits, and maternal BMI as explanatory variables, this model eliminated the significant associations of IGF-1 and IGFBP-1 with fasting glucose, as fasting glucose was strongly associated with BMI ($p < 0.001$) (Table 3). There was no correlation between maternal GH-V, IGF-1, IGFBP-1 or IGFBP-3, and customized birth weight centiles in the GDM group.

In the control group, maternal IGF-1 concentration was positively associated with GH-V and IGFBP-3

Table 2. Pearson's correlation coefficients between GH-related hormones

Parameter	r	P value
GDM group		
IGF-1 vs. GH-V	0.417	0.03
IGF-2	-0.155	0.432
IGFBP-1	-0.564	0.002
IGFBP-3	0.418	0.027
Control group		
IGF-1 vs. GH-V	0.511	0.006
IGF-2	-0.238	0.222
IGFBP-1	-0.348	0.069
IGFBP-3	0.499	0.007

(Table 2). In addition, maternal IGF-1 had a negative relationship with customized birth weight centiles ($r = -0.395$, $p = 0.038$).

DISCUSSION

In this nested case-control study of GDM pregnancies, we found that maternal serum concentration of GH-V at 20 weeks of gestation was not altered in pregnancies complicated by GDM, although maternal GH-V was increased in GDM cases who delivered LGA babies. In addition, we found that maternal IGF-1 concentrations were significantly higher in GDM pregnancies compared to controls and that IGFBP-1 concentrations were significantly lower in GDM pregnancies compared to controls.

Human pregnancy is characterized by a series of metabolic changes that induce a physiologic form of insulin resistance. GDM develops when insulin secretion is inadequate to compensate for this insulin resistance. The rationale for this study was that GH-V is thought to be the primary regulator of IGF-I in normal and abnormal human pregnancies and to be a potential candidate to mediate the insulin resistance of pregnancy.^{10,17} Previously we treated both pregnant and non-pregnant female mice with recombinant GH-V and found that GH-V reduced maternal insulin sensitivity in a dose-dependent manner.^{11,12} We therefore hypothesized that the maternal serum GH-V would also be altered in GDM pregnancies. However, a direct diabetogenic effect

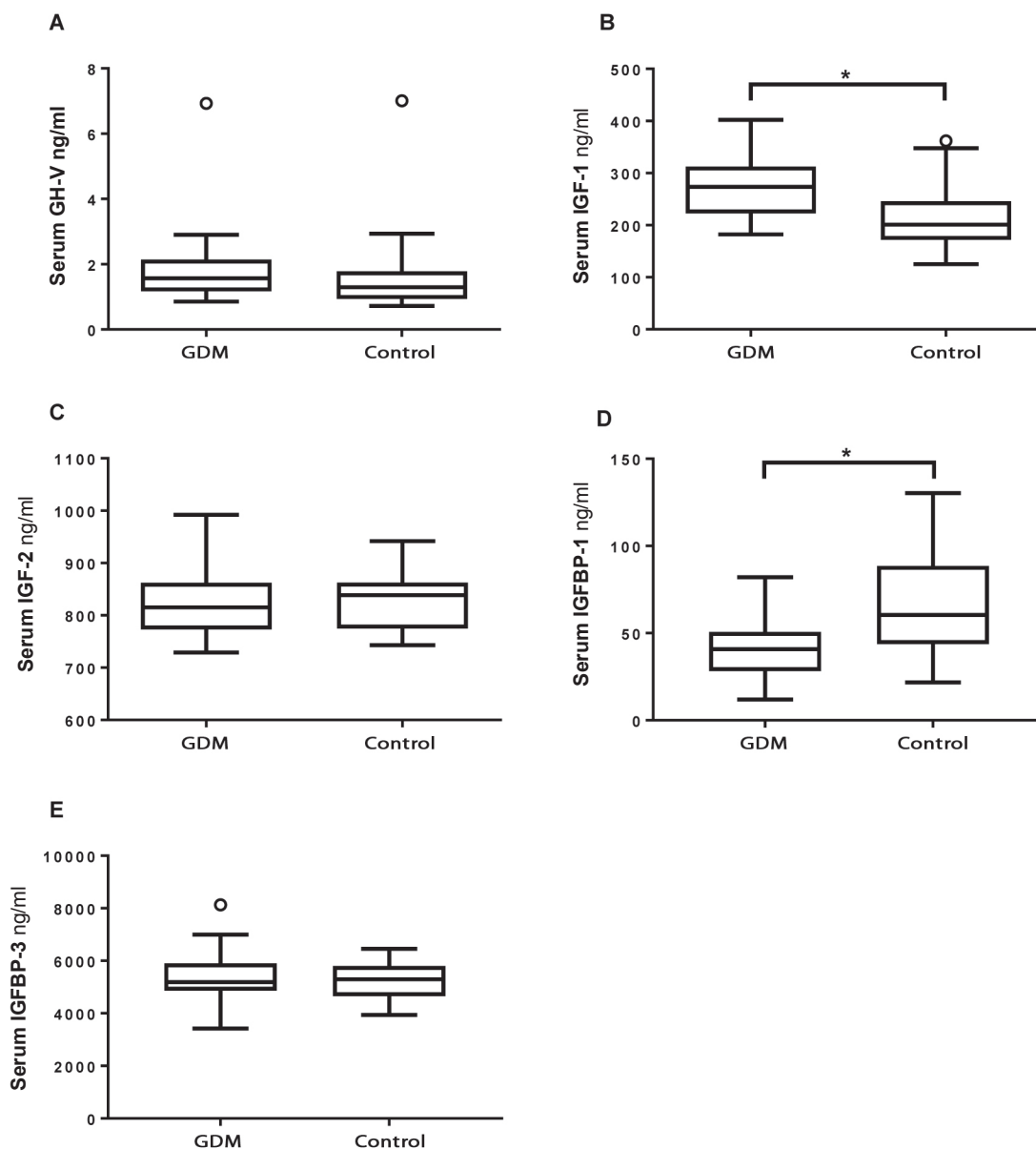


Figure 1. Serum GH-V, IGF-1, IGF-2, IGFBP-1 and IGFBP-3 concentrations. Data are shown as Tukey box-whisker plots (median, 25th centile, 75th centile and range). Outliers are presented as hollow symbols. * $p < 0.05$.

of GH-V at 20 weeks of gestation was not supported by the present findings.

Limited studies suggest a possible regulatory effect of glucose levels on GH-V secretion. Patel et al observed a dose-dependent inhibition of GH-V secretion by glucose in human placental explants and in trophoblast cultures.³¹ Bjorklund et al described an increase in GH-V during a hyperinsulinaemic

hypoglycaemic clamp in pregnant Type 1 diabetes patients.³² No studies to date have demonstrated higher levels of GH-V in diabetic pregnancies. McIntyre et al found that maternal GH-V concentrations were positively correlated with maternal glycaemia in women with established Type 1 and Type 2 diabetes, particularly in the postprandial state.¹⁴ However, the study by McIntyre et al and two other studies conducted by Higgins et al, and Verhaeghe et al failed

Table 3. Multiple linear regression results after adjusting for potential confounders (maternal age, ethnicity, socioeconomic status, family history of diabetes, smoking and drinking habits, and maternal BMI). Model 1: association between fasting glucose level and IGF-1; Model 2: association between fasting glucose level and IGFBP-1. Fasting glucose was the dependent variable. Socioeconomic status was represented by socioeconomic index. Categorical variables were coded by dummy variables (ethnicity: Caucasian = 1/non-Caucasian = 0; family history of diabetes: yes = 1/no = 0; smoking and drinking habits: yes = 1/no = 0)

Variables	Coefficient Beta	Std. Error	t	P value
Model 1 (R ² = 0.718)				
Maternal age	-0.017	0.026	-0.654	0.521
Ethnicity	-0.659	0.354	-1.860	0.078
Socioeconomic status	0.010	0.008	1.338	0.197
Family history of diabetes	0.152	0.355	0.428	0.673
Smoking	1.029	0.408	2.524	0.021
Drinking	0.481	0.570	0.844	0.409
Maternal BMI	0.101	0.024	4.203	<0.001
IGF-1	0.003	0.002	1.452	0.163
Model 2 (R ² = 0.688)				
Maternal age	-0.012	0.027	-0.452	0.657
Ethnicity	-0.773	0.364	-2.122	0.047
Socioeconomic status	0.009	0.008	1.120	0.277
Family history of diabetes	-0.047	0.354	-0.132	0.897
Smoking	1.052	0.439	2.398	0.027
Drinking	0.337	0.594	0.567	0.578
Maternal BMI	0.107	0.032	3.384	0.003
IGFBP-1	-0.003	0.011	-0.266	0.793

to show differences between GH-V concentrations in women with normal glucose tolerance and diabetic patients.^{33,34} Further, Fuglsang et al demonstrated that the increase in insulin requirements during pregnancy in Type 1 diabetes was not related to GH-V levels.^{23,35} In our study, GH-V concentration at 20 weeks' gestation was not altered in GDM pregnancies. Although blood glucose levels are regulated primarily by adjustments in insulin concentrations, accumulating evidence indicates a complementary role of IGF-1 through its insulin-like activity.³⁶ A family of six IGFBPs has been characterized to prolong IGF half-life in the circulation and regulate IGF-1 bioactivity.³⁷ Their relationships are complex. IGFBPs not only regulate IGFs bioavailability but also have biological activity that is independent of IGF.³⁸ Moreover, the hypothesis has been made that cleavage of the IGFBPs into fragments with lower affinity to IGFs allows for increased IGF receptor activation.³⁹ As much as 99% of IGF-1 in the circulation is bound

to IGFBP-3 with an acid-labile subunit to form a 150 kDa ternary complex. However, the exact role of IGFBP-3 in glucose metabolism, either a protective effect or enhancing effect on insulin resistance, is still unclear.⁴⁰⁻⁴³ Although it is less abundant than IGFBP-3, IGFBP-1 has been proposed as playing an important role in glucose homeostasis as a dynamic regulator of IGF bioactivity.⁴⁴⁻⁴⁷ It has been shown that conditions characterized by insulin resistance are associated with decreased IGFBP-1 levels.^{48,49} In GDM patients, increased IGF-1 and decreased IGFBP-1 concentrations were observed in maternal serum at mid-late pregnancy (24 weeks onwards).⁵⁰⁻⁵² Qiu et al also reported that free IGF-1 and IGFBP-1 at 13 weeks had an inverse association with the subsequent GDM risk.⁵³ Our study provides further evidence for the change of the IGFs and IGFBPs at 20 weeks of gestation in GDM pregnancies.

The findings of our study indicate that the maternal serum concentration of placental growth hormone

at 20 weeks' gestation is unlikely to be useful in the early prediction of GDM. However, only a single sampling time-point (20 weeks) was applied in this study and the sample sizes were relatively small. Maternal levels of GH-V increase dramatically during mid-late pregnancy.⁵⁴ Large-scale prospective studies to investigate the change of GH-V during pregnancy complicated by GDM would provide valuable insights and support our findings.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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