Reduced GLP-1 Secretion at 30 Minutes After a 75-g Oral Glucose Load Is Observed in Gestational Diabetes Mellitus: A Prospective Cohort Study

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Glucagon-like peptide 1 (GLP-1) levels may be reduced in type 2 diabetes, but whether a similar impairment exists in gestational diabetes mellitus (GDM) has not been established. We studied this in a prospective cohort study of pregnant women (*n* = 144) during oral glucose tolerance test (OGTT). GLP-1, glucose, and insulin were sampled at 30-min intervals during a 2-h 75-g OGTT, and indices of insulin secretion and sensitivity were calculated. In a nested case-control study, women with GDM (*n* = 19) had 12% lower total GLP-1 secretion area under the curve (AUC) compared with control subjects matched for age, ethnicity, and gestational age (*n* = 19), selected from within the lowest quartile of glucose$_{120\ min}$ values in our cohort. GDM had lower GLP-1 response in the first 30 min (19% lower GLP-1$_{30\ min}$ and 17% lower AUC$_{0–30\ min}$) after adjustment for possible confounders. Their glucose levels began to diverge at 30 min of the OGTT with increasing insulin levels, and by 120 min, their insulin levels were three times higher. In a secondary cohort of 57 women that included "high-normal" glucose$_{120\ min}$ values, low GLP-1 AUC$_{0–30\ min}$ was independently associated with lower indices of insulin secretion and sensitivity. In conclusion, we have observed that women with GDM have lower GLP-1 response at 30 min of an OGTT and hyperglycemia at 120 min despite significant hyperinsulinemia.

Gestational diabetes mellitus (GDM) is defined as diabetes first recognized during pregnancy and is associated with an alarmingly higher risk of type 2 diabetes and cardiovascular disease in the postpartum years (1). Approximately 5–30% of pregnancies globally are affected by GDM, depending on the diagnostic criteria used (2,3), but its pathogenesis has not been fully elucidated.

Glucagon-like peptide 1 (GLP-1) is one of two incretin hormones secreted by the L cells of the intestines in response to food, particularly glucose and triacylglycerol (4,5). Its primary function is to potentiate glucose-induced insulin secretion by pancreatic β-cells and, together with glucose-independent insulinotropic polypeptide (GIP), accounts for approximately two-thirds of the insulin response after an oral glucose load (6).

The incretin effect, defined as the amplification of insulin secretion with oral compared with intravenous glucose, is reduced in type 2 diabetes, which may be due to decreased secretion of the incretin hormones or reduced responsiveness of the pancreatic β-cells to them (7,8). Impairments in GLP-1 secretion may actually occur early in the disease process, as shown in adults and adolescents with insulin resistance and obesity (9,10). In addition, lower GIP levels and hyperglucagonemia have been demonstrated in type 2 diabetes, impaired glucose tolerance, and obesity (11,12).

The precise role of GLP-1 in pregnancies affected by GDM is still unclear. A search of the medical literature revealed five studies that measured GLP-1 levels in GDM

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(13–17). One showed a nonsignificant decrease in overall stimulated GLP-1 secretion between GDM and control subjects (13), and two did not show such a difference (14,16). Two measured only fasting GLP-1 (15,17). All were retrospective studies after a diagnosis of GDM was made, and hence, there is a need for prospective, adequately powered studies to validate the findings.

The primary aim of our study was to determine whether there is a difference in GLP-1 response as measured by total area under the curve (AUC_{total}) during a 2-h oral glucose tolerance test (OGTT) between GDM and control subjects at the time of diagnosis. Secondary aims were to examine the time course of GLP-1 during the OGTT and its relationship with indices of insulin secretion and sensitivity.

RESEARCH DESIGN AND METHODS

Study Design

A prospective cohort study, with a nested case-control component, was conducted in pregnant women between 2014 and 2016 in two hospitals in the West Midlands, U.K. The National Institute for Health and Care Excellence (NICE) 2015 selective screening criteria were used to screen high-risk women for GDM at 26–28 weeks' gestation (18). Exclusion criteria were pre-GDM (type 1 or type 2 diabetes) and multiple gestations. Informed written consent was obtained from all participants. The National Research Ethics Committee (South Birmingham) approved the study.

Blood Sampling and Laboratory Analysis

On the day of the OGTT, participants were studied after a minimum of a 10-h overnight fast. Plasma and serum samples were taken 0, 30, 60, 90, and 120 min of the OGTT. Analysis of serum glucose was done by a hexokinase enzymatic method and insulin by human insulin ELISA kit (Abcam, Cambridge, U.K.). Plasma samples were stored at −80°C until the end of the study, when they were transferred on dry ice to the University of Copenhagen for analysis of GLP-1. GLP-1 measurements were done by radioimmunoassay, as previously described (19).

Determination of GDM and Control Groups

Women were diagnosed with GDM according to the NICE 2015 criteria: fasting plasma glucose ($glucose_{0\ min}$) ≥5.6 mmol/L or 2-h plasma glucose ($glucose_{120\ min}$) ≥7.8 mmol/L (18). The control group for the primary outcome (normal glucose tolerance [NGT] group) was selected from those in the lowest quartile of $glucose_{120\ min}$ values in the cohort, matched for age, ethnicity, and gestational age of OGTT to the GDM case subjects. For the secondary outcome of assessing the relationship between GLP-1, insulin, and glucose as continuous variables, the analyses were expanded to include additional participants who had the highest quartile of $glucose_{120\ min}$ values among the non-GDM subjects (known hereafter as the NGT2 subgroup).

Statistical Analysis

Based on a previous study (13), the primary effect size was determined to be 25% lower GLP-1 AUC_{total} in women who develop GDM compared with control subjects. To detect

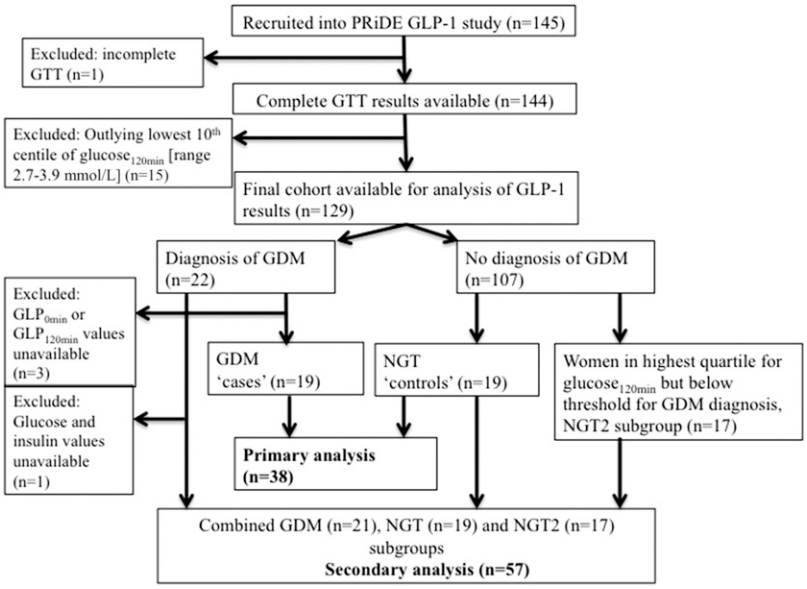

Figure 1—Flow diagram illustrating the selection of GDM case subjects and NGT control subjects for the primary analysis (n = 38) and additional subjects for the secondary analyses of the study (n = 57). The NGT control group includes women selected from the lowest $glucose_{120\ min}$ quartile, matched in age, ethnicity, and BMI to the GDM case subjects. The NGT2 subgroup includes additional women who had $glucose_{120\ min}$ in the highest quartile but below the threshold for GDM diagnosis. GTT, glucose tolerance test. PRiDE, Micronutrients in Pregnancy as a Risk Factor for Diabetes and Effects on Mother and Baby study.

Table 1—Participant characteristics at baseline and during OGTT

Variables	GDM (n = 19)	NGT (n = 19)	P value
Gestation at baseline (weeks)	12^{+5} (6^{+4}, 15^{+5})	12^{+3} (7^{+0}, 16^{+3})	NS
Age (years)	29.7 ± 5.3	28.4 ± 4.7	NS
Baseline weight (kg)	99.5 ± 17.9	81.2 ± 16.6	0.003
Baseline BMI (kg/m^2)	37.3 ± 7.5	29.1 ± 5.3	0.001
Waist circumference (cm)	114.7 ± 14.9	96.0 ± 12.9	<0.001
Current smokers	4 (21.1)	1 (5.9)	NS
Ethnicity			
European	17 (89.5)	14 (82.4)	NS
South Asian	2 (10.5)	2 (11.8)	
Afro-Caribbean	0	1 (5.9)	
History of GDM in a previous pregnancy	4 (21)	3 (16)	NS
Family history of diabetes	8 (42)	10 (53)	NS
Gestation of OGTT (weeks)	26^{+6}(15^{+6}, 30^{+2})	26^{+6}(15^{+6}, 29^{+5})	NS
Fasting glucose (mmol/L)	5.6 ± 1.03; 5.3 (4.5, 8.5)	4.7 ± 0.27; 4.7 (4.2, 5.1)	0.002
2-h glucose (mmol/L)	9.3 ± 1.79; 9.0 (5.4, 12.7)	4.8 ± 0.34; 4.8 (4.1, 5.5)	<0.001
Fasting insulin (pmol/L)	50.0 ± 23.5	67.2 ± 51.2	NS
2-h insulin (pmol/L)	490.0 ± 235.3	150.7 ± 124.1	<0.001
Insulin secretion			
HOMA2-B	72.3 ± 19.4	144.7 ± 53.5	<0.001
Insulin sensitivity (fasting values)			
HOMA-IR	2.18 ± 1.40	2.33 ± 1.77	NS
Insulin sensitivity (OGTT values)			
$ISI_{Stumvoll}$	0.03 ± 0.03	0.10 ± 0.019	<0.001
OGIS	349.0 ± 81.7	472.3 ± 58.3	<0.001

Continuous variables are mean ± SD or median (range) and were compared with the t test. Categorical variables are n (%) and were compared with the χ^2 test.

this difference with 80% power at 5% significance (two-tailed), the estimated sample size was 20 case subjects. Because the detection rate of GDM in our cohort was ~15%, we planned to recruit 150 women into the study.

The AUC for GLP-1 was determined using the trapezoidal method, and the incremental AUC was calculated as the AUC above baseline. Surrogate markers of indices of insulin secretion and sensitivity were used in the absence of hyperglycemic or hyperinsulinemic-euglycemic clamps, namely HOMA2-B (20) and HOMA- insulin resistance (IR) ($insulin_{0\ min} \times glucose_{0\ min}/135$). Insulin sensitivity was additionally measured using the Insulin Sensitivity Index (ISI) Stumvoll ($0.226 - 0.0032 \times BMI - 0.0000645 \times insulin_{120\ min} - 0.0037 \times glucose_{90\ min}$) and the oral glucose sensitivity index (OGIS), which uses $glucose_{0\ min}$, $glucose_{90\ min}$, $glucose_{120\ min}$, $insulin_{90\ min}$, and $insulin_{120\ min}$ values as well as participant height and weight (21–23). The $ISI_{Stumvoll}$ and OGIS correlate more highly with the gold standard clamp studies (r = 0.79 and r = 0.70, respectively) than HOMA-IR, because they incorporate late-phase glucose and insulin levels from the OGTT (21,23,24).

Statistical analysis was performed using SPSS 22.0 software (25). Comparison of GLP-1, glucose, and insulin parameters between GDM and control subjects was done by ANCOVA with post hoc Bonferroni adjustment for multiple comparisons. All of the analyses included the covariates of age, BMI, ethnicity, smoking, and gestational week of OGTT.

RESULTS

Characteristics of Study Population and OGTT Results

GDM developed in 22 of 144 women who completed the study. Three of these women were excluded from the primary analysis because their $GLP\text{-}1_{0\ min}$ or $GLP\text{-}1_{120\ min}$ values, which are required for calculation of GLP-1 AUC_{total}, were unavailable, resulting in 19 GDM case subjects and matched NGT control subjects (Fig. 1). An additional 17 participants (i.e., the NGT2 subgroup) were included for the secondary analyses. The maternal characteristics of the 38 women included in the primary analysis and 57 women included in the secondary analysis are presented in Table 1 and Supplementary Table 1, respectively.

GLP-1, Glucose, and Insulin Profiles: GDM and NGT

Women with GDM had 12% lower GLP-1 AUC_{total} (2,034 vs. 2,321 pmol/L × min; adjusted P = 0.046) (Fig. 2*A*) and 19% lower GLP-1 at 30 min (16.0 ± 3.90 vs. 19.8 ± 4.52

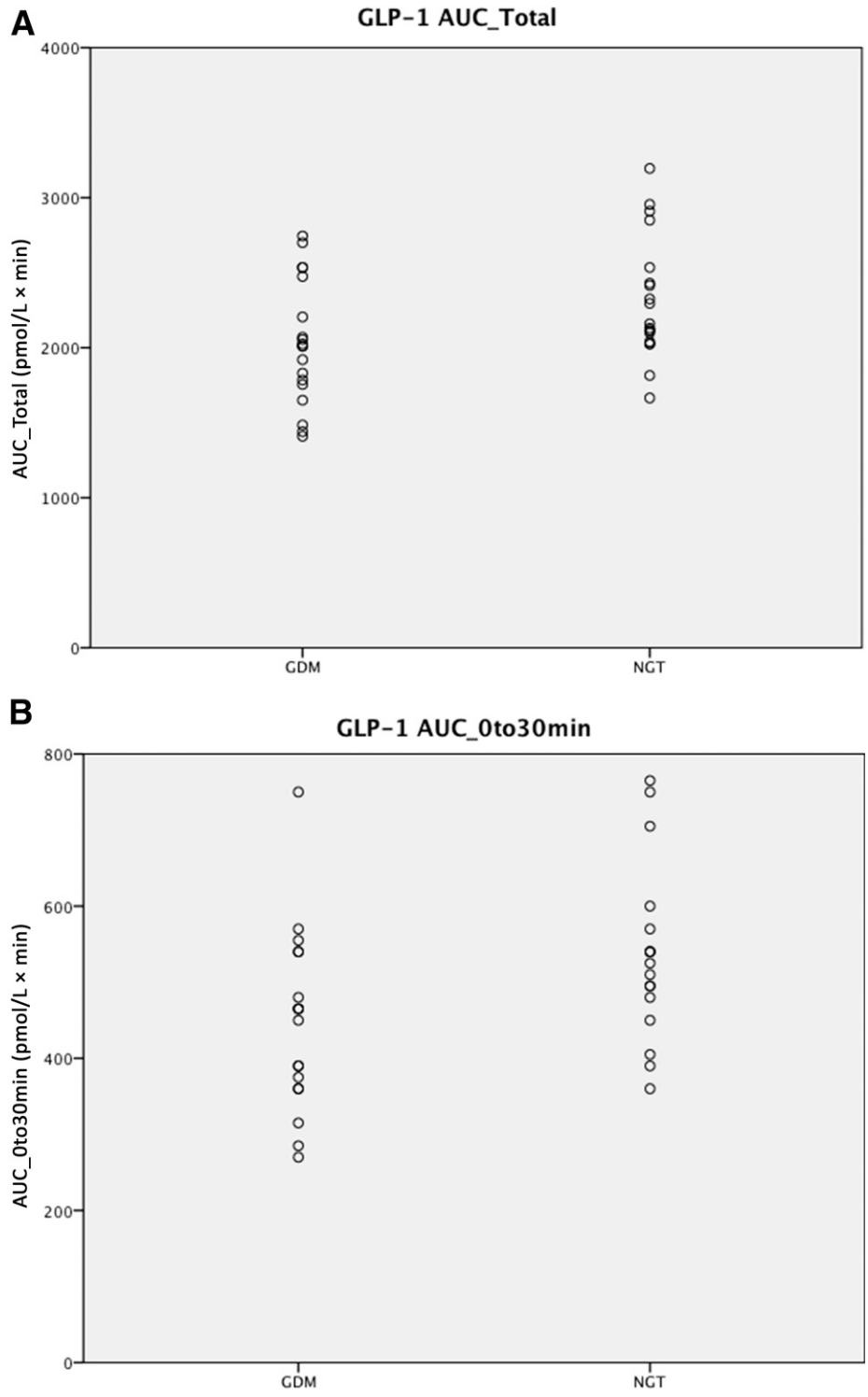

Figure 2—Univariate scatter plots of two parameters of GLP-1 response during an OGTT in pregnant women diagnosed with GDM and control subjects with NGT, selected from the lowest quartile of glucose$_{120\ min}$ values. The parameters are AUC$_{total}$ (mean 2,034 vs. 2,321 pmol/L × min; adjusted P = 0.046) (*A*) and AUC$_{0–30\ min}$ of the OGTT (mean 446 vs. 536 pmol/L × min; adjusted P = 0.041) (*B*).

pmol/L, adjusted P = 0.042) (Fig. 3*A*) compared with NGT. The early-phase GLP-1 response, measured as AUC$_{0–30\ min}$, was 17% lower in the former group (446 vs. 536 pmol/L × min; adjusted P = 0.041) (Fig. 2*B*).

The higher glucose levels of GDM were prominent from 30 min onward of the OGTT but the insulin levels began to diverge at 90 min and peaked at 120 min (Fig. 3*B* and *C*).

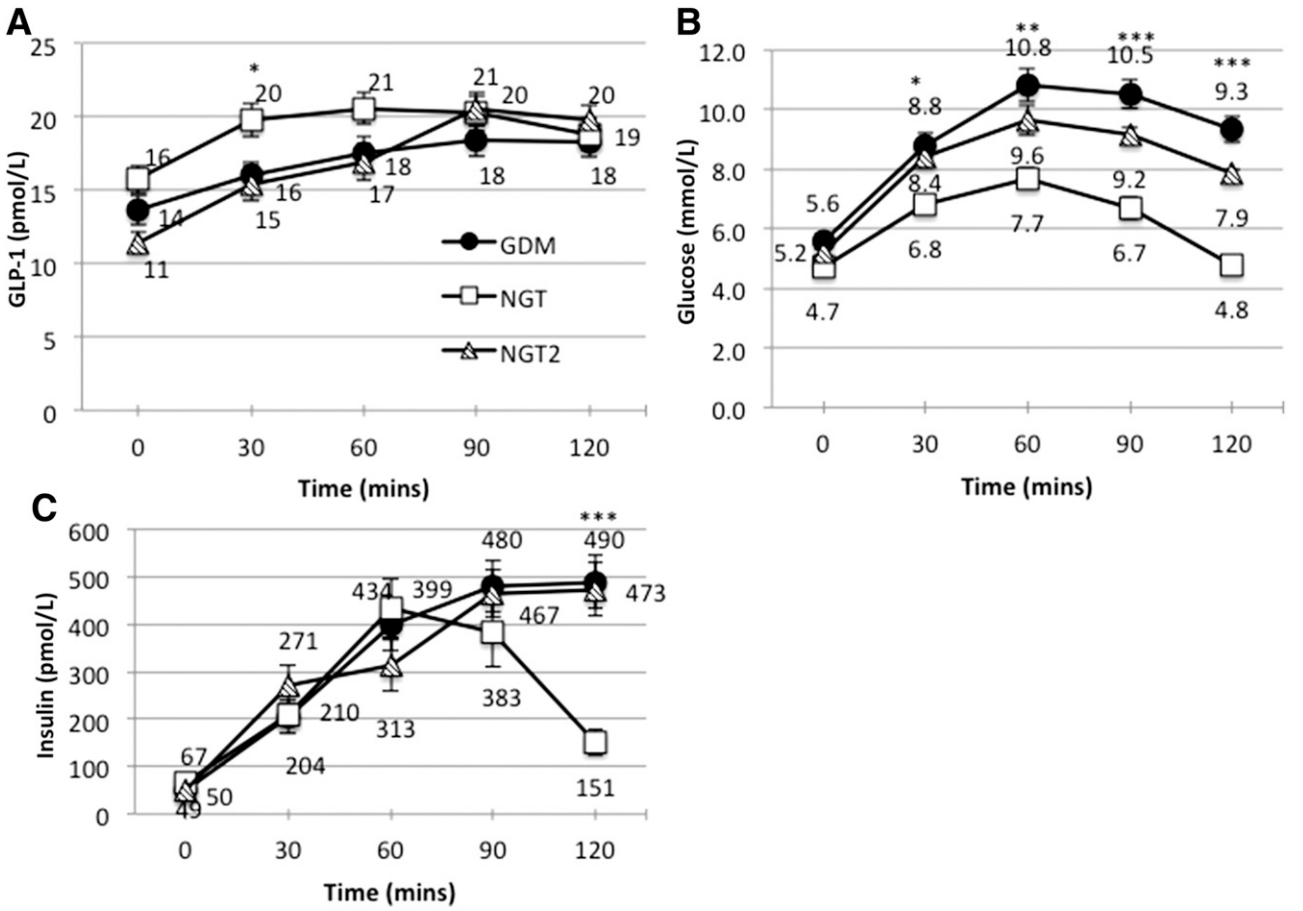

Figure 3—Line charts of GLP-1 (*A*), glucose (*B*), and insulin (*C*) concentrations during OGTT in women diagnosed with GDM (black circles); control subjects with NGT, selected from the lowest quartile of glucose$_{120\ min}$ values (white squares); and NGT2 control subjects, selected from the highest quartile of glucose$_{120\ min}$ values (striped triangles). The points represent the mean ± SEM concentrations of the respective parameters measured at 30-min intervals during a 2-h 75-g OGTT. ANCOVA was used to compare the means of each parameter between the GDM and NGT groups after adjustment for age, BMI, ethnicity, and smoking. Adjusted $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Relationship Between GLP-1, Glucose, and Insulin Parameters: GDM, NGT, and NGT2 Subgroups

Secondary analyses to determine the associations between GLP-1, glucose, and insulin were done by including the NGT2 group. This represents a group with intermediate glucose values at all time points, although interestingly, their insulin levels at 90 and 120 min were similar to GDM (Fig. 3*B* and *C*). These women showed a higher incremental GLP-1 rise overall and in the first 30 min of the OGTT compared with the GDM group (incremental AUC_{total}: 398 ± 455, 685 ± 455, and 427 ± 433 pmol/L × min; incremental $AUC_{0–30\ min}$: 34 ± 46, 61 ± 56, and 56 ± 62 pmol/L × min in GDM, NGT2, and NGT, respectively; $P = 0.04$ GDM vs. NGT2 for incremental AUC_{total}) (Fig. 3*A*).

To investigate the relationship between GLP-1 and glucose and insulin, multiple linear regression models were fitted looking at predictors of glucose and insulin at the five time points in the secondary analysis of the cohort (n = 57) (Supplementary Table 2). None of the GLP-1 parameters were associated with glucose or insulin levels in the first 60 min of the OGTT (data not shown). A temporal relationship between lower GLP-1 $AUC_{0–30\ min}$ levels and hyperglycemia was observed. GLP-1 $AUC_{0–30\ min}$ was an independent negative predictor of glucose and insulin at 90 and 120 min in separate models. However, when glucose values were added to the insulin regression model, the GLP-1 parameter lost significance.

Indices of Insulin Secretion and Sensitivity

We next investigated the influence of GLP-1 parameters on OGTT-derived indices of insulin sensitivity and secretion in our cohort. Women with GDM had lower insulin secretion as determined by HOMA2-B than NGT controls ($P < 0.001$) (Table 1) as well as significantly lower $ISI_{Stumvoll}$ and OGIS values than NGT ($P < 0.001$ for both) and lower $ISI_{Stumvoll}$ than the NGT2 subgroup (0.03 ± 0.03 vs. 0.06 ± 0.04, $P = 0.036$). Combining all the patients (n = 57) in multiple linear regression analyses, GLP-1 $AUC_{0–30\ min}$ was an independent positive predictor of insulin secretion as measured by HOMA2-B. Similarly, GLP-$1_{30\ min}$, GLP-1 $AUC_{0–30\ min}$, and GLP-1 AUC_{total} were also positively associated with OGIS, a marker of insulin sensitivity, and GLP-$1_{30\ min}$ with $ISI_{Stumvoll}$ (Supplementary Table 3).

DISCUSSION

Our study reveals three key findings: *1*) overall GLP-1 response is reduced in women with GDM compared with a control group selected from the lowest quartile of $glucose_{120\ min}$ values during an OGTT; *2*) impairment in GLP-1 secretion occurs in the first 30 min in GDM (as shown by lower $GLP\text{-}1_{30\ min}$ and $AUC_{0–30\ min}$ levels), and *3*) the lower GLP-1 levels at 30 min may contribute to impaired glucose metabolism in pregnancy, regardless of GDM status.

GLP-1 Profile in GDM Pregnancy

Our primary outcome result of 12% lower GLP-1 AUC_{total} concentrations in GDM pregnancies was statistically significant after adjustment for possible confounders. Among other studies that reported AUC_{total} for GLP-1 response in GDM, Bonde et al. (13) found a nonsignificant decrease of 25% in GDM but used a liquid meal test and sampled GLP-1 over 4 h. Two other studies found no difference in the total response of GLP-1 in GDM during a 3-h 100-g and 2-h 75-g OGTT, respectively (14,16). The study by Cypryk et al. (14) had similar sampling times and GLP-1 assay as our study, but possible reasons for the variance in their results could be a smaller sample size (n = 13), and differences in baseline BMI, which was higher in our study.

Effect of Low Early-Phase GLP-1 Response

A novel finding of our study was the lower GLP-1 response in the first 30 min of the OGTT in GDM pregnancies. Lower GLP-1 and insulin responses at 30 min of an OGTT have been shown in women with a history of GDM despite normal glucose values at 5 years postpartum (26). The authors suggest that this may put them at higher risk of progression to type 2 diabetes. This was not replicated in another cohort, however (27). In addition, GLP-1 impairment in the first 30 min has been demonstrated in nonpregnant adults with prediabetes and type 2 diabetes, where it was shown to influence β-cell function (9).

We observed a correlation between lower GLP-1 levels at 30 min and late-phase high insulin levels during the OGTT. This negative relationship may seem paradoxical because one of the primary functions of GLP-1 is to stimulate insulin secretion by pancreatic β-cells. Although early-phase GLP-1 levels were predictive of late-phase hyperinsulinemia in our regression analyses, when glucose values (90 and 120 min) were introduced in the model, this relationship weakened. This suggests that the late-phase hyperinsulinemia is driven by hyperglycemia. Therefore, our hypothesis is that the early phase GLP-1 response is critical to ensure that pancreatic β-cells produce the appropriate amount of insulin to deal with a glucose load in a timely manner.

Although BMI has been shown to be negatively associated with GLP-1 in type 2 diabetes (19), there was no correlation between first trimester BMI and any of the GLP-1 parameters in our cohort, nor was it a significant covariate in the ANCOVA analyses of mean $GLP\text{-}1_{30\ min}$, AUC_{total}, and $AUC_{0–30\ min}$ between the three subgroups (data not shown). This lack of association is likely because women in our selectively screened, high-risk cohort were predominantly obese, and therefore, the effect of BMI on GLP-1 secretion may not be apparent.

Strengths and Weaknesses

This is the largest study, to our knowledge, investigating GLP-1 levels in GDM pregnancy using the widely available 75-g OGTT. Its significant advantage is that at the time of GLP-1 sampling, neither the pregnant woman nor the research team knew her GDM status, thereby minimizing selection bias.

However, there are some important limitations which cannot be ignored. Our findings cannot be extrapolated to a diverse population of women with GDM, such as women from ethnicities other than white Caucasian or those with predominantly fasting hyperglycemia (15 of 19 women in our cohort had isolated postprandial hyperglycemia, likely due to the NICE diagnostic criteria). Secondly, we have not analyzed GIP and glucagon from this cohort and, hence, cannot be certain that impairments in the related incretin hormones will not contribute to our observations. However, two previous studies that measured GIP during GDM (albeit with smaller number of patients) did not notice any difference in GDM (13,14). In addition, it has been demonstrated that the insulinotropic effect of GIP, rather than its secretion, is impaired in type 2 diabetes (28). Therefore, we do not believe that plasma GIP results from the OGTT will alter the conclusions. Finally, although our study demonstrated that GLP-1 secretion is lower in the early part of the OGTT in women with GDM, we cannot be sure that the trough does not occur before 30 min due to lack of sampling at earlier times.

In summary, we have shown that GDM is associated with a lower GLP-1 response in the first 30 min of an OGTT, which may independently contribute to late-phase hyperglycemia, hyperinsulinemia, and reduced insulin sensitivity.

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Author Contributions. N.S. designed the study, performed the clinical experiments, analyzed the data, and wrote the manuscript. C.B., I.G., and S.G. contributed to the clinical experiments and laboratory analyses. Y.W. assisted with the statistical calculations. B.K.T. contributed to the data analysis and reviewed the manuscript for intellectual content. J.J.H. performed the laboratory analysis and reviewed the manuscript for intellectual content. P.S. conceived the research question, designed the study, contributed to data analysis, and reviewed the manuscript for intellectual content. All authors read and approved the final version

of the manuscript. N.S. and P.S. are the guarantors of this work, and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Daly B, Toulis KA, Thomas N, et al. Increased risk of ischemic heart disease, hypertension, and type 2 diabetes in women with previous gestational diabetes mellitus, a target group in general practice for preventive interventions: a population-based cohort study. PLoS Med 2018;15:e1002488
2. Duran A, Sáenz S, Torrejón MJ, et al. Introduction of IADPSG criteria for the screening and diagnosis of gestational diabetes mellitus results in improved pregnancy outcomes at a lower cost in a large cohort of pregnant women: the St. Carlos Gestational Diabetes Study. Diabetes Care 2014;37:2442–2450
3. Meek CL, Lewis HB, Patient C, Murphy HR, Simmons D. Diagnosis of gestational diabetes mellitus: falling through the net. Diabetologia 2015;58:2003–2012
4. Gribble FM, Williams L, Simpson AK, Reimann F. A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLUTag cell line. Diabetes 2003;52:1147–1154
5. Rocca AS, Brubaker PL. Stereospecific effects of fatty acids on proglucagon-derived peptide secretion in fetal rat intestinal cultures. Endocrinology 1995;136:5593–5599
6. Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. Am J Physiol Endocrinol Metab 2004;287:E199–E206
7. Nauck MA, Homberger E, Siegel EG, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 1986;63:492–498
8. Calanna S, Christensen M, Holst JJ, et al. Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. Diabetologia 2013;56:965–972
9. Færch K, Torekov SS, Vistisen D, et al. GLP-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and influenced by sex: the ADDITION-PRO study. Diabetes 2015;64:2513–2525
10. Manell H, Staaf J, Manukyan L, et al. Altered plasma levels of glucagon, GLP-1 and glicentin during OGTT in adolescents with obesity and type 2 diabetes. J Clin Endocrinol Metab 2016;101:1181–1189
11. Faerch K, Vaag A, Holst JJ, Glümer C, Pedersen O, Borch-Johnsen K. Impaired fasting glycaemia vs impaired glucose tolerance: similar impairment of pancreatic alpha and beta cell function but differential roles of incretin hormones and insulin action. Diabetologia 2008;51:853–861
12. Knop FK, Aaboe K, Vilsbøll T, et al. Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. Diabetes Obes Metab 2012;14:500–510
13. Bonde L, Vilsbøll T, Nielsen T, et al. Reduced postprandial GLP-1 responses in women with gestational diabetes mellitus. Diabetes Obes Metab 2013;15:713–720
14. Cypryk K, Vilsbøll T, Nadel I, Smyczyńska J, Holst JJ, Lewiński A. Normal secretion of the incretin hormones glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 during gestational diabetes mellitus. Gynecol Endocrinol 2007;23:58–62
15. Avila C, Garduno E, Chen J, et al. Fasting plasma active glucagon-like peptide-1 (GLP-1) in pregnancies with and without gestational diabetes (GDM). Am J Obstet Gynecol 2011;204(Suppl.):S106
16. Lencioni C, Resi V, Romero F, et al. Glucagon-like peptide-1 secretion in women with gestational diabetes mellitus during and after pregnancy. J Endocrinol Invest 2011;34:e287–e290
17. Reyes-López R, Pérez-Luque E, Malacara JM. Metabolic, hormonal characteristics and genetic variants of TCF7L2 associated with development of gestational diabetes mellitus in Mexican women. Diabetes Metab Res Rev 2014;30:701–706
18. National Institute for Health and Care Clinical Excellence. Diabetes in pregnancy: management from preconception to the postnatal period. NICE guideline (NG3). London, U.K., National Institute for Health and Care Excellence, 2015, p. 1–66
19. Toft-Nielsen M-B, Damholt MB, Madsbad S, et al. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. J Clin Endocrinol Metab 2001;86:3717–3723
20. The Oxford Centre for Diabetes, Endocrinology, and Metabolism. HOMA calculator [Internet]. Available from http://www.dtu.ox.ac.uk/homacalculator/. Accessed 15 August 2017
21. Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 2000;23:295–301
22. OGIS. Insulin sensitivity from the oral glucose test [Internet]. Available from http://webmet.pd.cnr.it/ogis/. Accessed 15 August 2017
23. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001;24:539–548
24. Otten J, Ahrén B, Olsson T. Surrogate measures of insulin sensitivity vs the hyperinsulinaemic-euglycaemic clamp: a meta-analysis. Diabetologia 2014;57:1781–1788
25. IBM Corp. *IBM SPSS Statistics for Windows, Version 22.0*. Armonk, NY, IBM Corp., 2013
26. Forbes S, Moonan M, Robinson S, et al. Impaired circulating glucagon-like peptide-1 response to oral glucose in women with previous gestational diabetes. Clin Endocrinol (Oxf) 2005;62:51–55
27. Meier JJ, Gallwitz B, Askenas M, et al. Secretion of incretin hormones and the insulinotropic effect of gastric inhibitory polypeptide in women with a history of gestational diabetes. Diabetologia 2005;48:1872–1881
28. Vilsbøll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. Diabetologia 2002;45:1111–1119