ARTICLE

Deteriorating beta cell function is the dominant determinant of progression from normal glucose tolerance to prediabetes/diabetes in young women following pregnancy

Ravi Retnakaran[1,2,3] · Chang Ye[1] · Caroline K. Kramer[1,2,3] · Anthony J. Hanley[1,2,4] · Philip W. Connelly[2,5,6] · Mathew Sermer[7] · Bernard Zinman[1,2,3]

Abstract

Aims/hypothesis Excess adiposity, insulin resistance and beta cell dysfunction each contribute to the development of prediabetes (impaired glucose tolerance and/or impaired fasting glucose)/diabetes but their comparative impact in relation to one another remains uncertain. We thus ranked their contributions to incident dysglycaemia over the first 5 years postpartum in women reflecting the full spectrum of gestational glucose tolerance (spanning normoglycaemia to gestational diabetes) and hence a range of future diabetic risk.

Methods In this study, 302 women with normal glucose tolerance (NGT) on OGTT at 3 months postpartum underwent repeat OGTT at 1 year, 3 years and 5 years, enabling serial assessment of glucose tolerance, insulin sensitivity/resistance (Matsuda index, HOMA-IR) and beta cell function (insulin secretion-sensitivity index-2 [ISSI-2], insulinogenic index [IGI]/HOMA-IR). Determinants of prediabetes/diabetes were ranked by change in concordance index (CCI) of Cox proportional hazard regression models.

Results Over 5 years of follow-up, 89 women progressed from NGT to prediabetes/diabetes (progressors). At 3 months postpartum, though all women were normoglycaemic, future progressors had higher fasting glucose ($p=0.03$) and 2 h glucose ($p<0.0001$) than non-progressors, coupled with higher BMI ($p=0.001$), greater insulin resistance (both Matsuda index and HOMA-IR, $p\leq0.02$) and poorer beta cell function (both ISSI-2 and IGI/HOMA-IR, $p\leq0.006$). Unlike their peers, progressors exhibited deteriorating beta cell function from 1 year to 5 years (both $p<0.0001$). On regression analyses, the dominant determinants of progression to prediabetes/diabetes were time-varying ISSI-2 (change in CCI 25.2%) and IGI/HOMA-IR (13.0%), in contrast to time-varying Matsuda index (2.9%) and HOMA-IR (0.5%). Neither time-varying BMI nor waist were significant predictors after adjustment for beta cell function and insulin sensitivity/resistance.

Conclusion/interpretation Declining beta cell function is the dominant determinant of incident prediabetes/diabetes in young women following pregnancy.

Keywords Beta cell dysfunction · Insulin resistance · Overweight · Prediabetes · Women's health

Abbreviations

CCI	Concordance index
GCT	Glucose challenge test
GDM	Gestational diabetes mellitus
GIGT	Gestational impaired glucose tolerance
IFG	Impaired fasting glucose tolerance
IGI	Insulinogenic index
IGT	Impaired glucose tolerance
ISSI-2	Insulin secretion-sensitivity index-2
NGT	Normal glucose tolerance

✉ Ravi Retnakaran
Ravi.Retnakaran@sinaihealth.ca

1 Leadership Sinai Centre for Diabetes, Mount Sinai Hospital, Toronto, ON, Canada

2 Division of Endocrinology, University of Toronto, Toronto, ON, Canada

3 Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

4 Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada

5 Keenan Research Centre for Biomedical Science of St Michael's Hospital, Toronto, ON, Canada

6 Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

7 Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada

Research in context

What is already known about this subject?

- Excess adiposity, insulin resistance and beta cell dysfunction each contribute to the development of prediabetes/diabetes but their comparative impact in relation to one another remains uncertain, particularly early in the natural history

What is the key question?

- What is the ranking of the respective contributions of adiposity, insulin resistance and beta cell dysfunction to the progression from normal glucose tolerance to incident prediabetes/diabetes?

What are the new findings?

- Even while normoglycaemic, women who are going to progress from normal glucose tolerance to incident prediabetes/diabetes in the future exhibit higher fasting and 2 h post-challenge glucose values than their peers

- Notably, those who progress to prediabetes/diabetes experience deterioration of beta cell function in the intervening years

- Declining beta cell function over time is the dominant determinant of incident prediabetes/diabetes, far outstripping the impact of concomitant changes in adiposity and insulin resistance on quantitative ranking of their pathophysiological contributions

How might this impact on clinical practice in the foreseeable future?

- Deteriorating beta cell function is the central pathophysiological feature to be targeted to reduce progression from normal glucose tolerance to prediabetes/diabetes in young women

Introduction

Excessive weight gain, insulin resistance and beta cell dysfunction are all associated with the development of type 2 diabetes [1–12]. However, their comparative pathogenic impact in relation to one another remains uncertain [1–12], particularly early in the natural history when at-risk individuals first progress from normal glucose tolerance (NGT) to dysglycaemia. Moreover, a practical limitation in addressing this question is the challenge of identifying a normoglycaemic population that is likely to exhibit sufficient rates of progression to prediabetes (impaired glucose tolerance and/or impaired fasting glucose) or diabetes within the limited window of surveillance of a research study.

In this context, we reasoned that the prognostic insight offered by glucose tolerance in pregnancy could provide an opportunity for identifying such a population for study. Indeed, it is known that any degree of dysglycaemia in pregnancy identifies an elevated likelihood of future type 2 diabetes, with the highest such risk seen in women who had gestational diabetes (GDM) [13–18]. Accordingly, longitudinal evaluation of women who had recent gestational dysglycaemia that subsequently reverted to NGT after delivery could provide a model for studying early events in the progression to prediabetes/diabetes. Thus, we sought to rank the contributions made by adiposity, insulin resistance and beta cell dysfunction to the development of incident prediabetes/diabetes through prospective serial metabolic characterisation over 5 years of a cohort of women reflecting the full spectrum of gestational glucose tolerance (ranging from normoglycaemia to mild dysglycaemia to GDM) who all had NGT at 3 months after delivery.

Methods

This study was performed at our institution in the setting of a prospective observational cohort programme that is characterising the relationship between maternal glucose tolerance in pregnancy and subsequent metabolic function in the years thereafter. The population studied is reflective of the obstetrical population seen at our institution as evidenced by age and self-reported ethnicity. The study protocol has been described in detail previously [19, 20]. Specifically, all pregnant women at our centre are screened for GDM by a 50 g glucose challenge test (GCT) at 24–28 weeks' gestation, followed by an OGTT in those in whom the GCT is abnormal (plasma glucose ≥7.8 mmol/l at 1 h post-challenge). For this study, participants were recruited either before or after the GCT, with the recruitment of women after an abnormal GCT serving to enrich the study population for women with varying degrees of dysglycaemia in pregnancy (i.e. those with an abnormal GCT are more likely to have an abnormal subsequent OGTT). For this study, all participants completed a

3 h 100 g OGTT irrespective of the outcome of their GCT. As previously described [15, 20], this recruitment strategy was specifically designed to generate a cohort of women comprising the full spectrum of glucose tolerance in pregnancy (from normal [normal GCT and normal OGTT] to mild dysglycaemia [abnormal GCT with normal OGTT] to gestational impaired glucose tolerance [GIGT] to GDM) and hence reflecting a broad range of future risk of prediabetes/diabetes [13–18]. The study protocol has been approved by the Mount Sinai Hospital Research Ethics Board and all women provided written informed consent for participation. For the current analysis, the study population was limited to women who had NGT on 2 h 75 g OGTT at 3 months postpartum.

Serial metabolic characterisation in the years after pregnancy Study participants underwent serial metabolic characterisation at our clinical investigation unit at 3 months, 1 year, 3 years and 5 years postpartum. At each study visit, participants presented in the morning after overnight fast and underwent a 2 h 75 g OGTT. As previously described [19, 20], during each OGTT, venous blood samples were drawn for measurement of glucose and specific insulin at fasting and at 30, 60 and 120 min post-challenge. These measurements enabled assessment of glucose tolerance status, insulin sensitivity/resistance and beta cell function.

Glucose tolerance status (NGT, prediabetes, diabetes) was defined according to Diabetes Canada clinical practice guidelines [21]. Prediabetes refers to impaired fasting glucose tolerance (IFG), impaired glucose tolerance (IGT) or combined IFG and IGT [21]. Whole-body insulin sensitivity was assessed with the Matsuda index [22] and insulin resistance (primarily hepatic) was measured with HOMA-IR [23]. Beta cell compensation was assessed by insulin secretion-sensitivity index-2 (ISSI-2), which is an OGTT-based measure that is analogous to the disposition index obtained from the IVGTT against which it has been directly validated [24–26]. A second measure of beta cell compensation was provided by the insulinogenic index (IGI)/HOMA-IR [19].

Statistical analyses All analyses were performed with Statistical Analysis System 9.4 (SAS Institute, Cary NC, USA) and R 4.2.1 (https://cran.r-project.org). The study population was stratified into the following two groups: (1) progressors, defined as women who developed prediabetes or diabetes at any of 1 year, 3 years or 5 years postpartum; and (2) non-progressors, who maintained NGT at all visits. Baseline characteristics of these groups at 3 months postpartum were compared by ANOVA for normally distributed continuous variables and Wilcoxon Rank-Sum non-parametric test for those that were skewed (Table 1). Kolmogorov–Smirnov test and Q–Q plot were used to assess the normality of the data. Categorical variables were compared with χ^2 test. Mixed

models were constructed to compare the trajectories of cardiometabolic risk factors in the two groups over follow-up from 1 year to 5 years postpartum (electronic supplementary material [ESM] Fig. 1).

To identify and rank the predictors of progression, we constructed Cox proportional hazard models with length of follow-up as the time-scale (calculated from the date of the 3 months postpartum visit to either the date of first occurrence of prediabetes/diabetes for progressors or the date of the 5 year postpartum visit for non-progressors). We first identified clinical predictors at baseline (Table 2) with the following three-step procedure:

Step 1: constructing an unadjusted model and obtaining the unadjusted HR (95% CI) for each clinical predictor at baseline.

Step 2: constructing an adjusted model and obtaining the adjusted HR (95% CI) for each clinical predictor at baseline. Covariates in each model were the following clinical risk factors for diabetes: age; ethnicity; family history of diabetes; BMI at 3 months; duration of breastfeeding; and preceding GDM.

Step 3: calculating and comparing the concordance index (CCI) for each model with the CONCORDANCE package in R. CCI can be interpreted as the fraction of all pairs of participants whose predicted survival times are correctly ordered among all participants that can actually be ordered [27]. In other words, it is the probability of concordance between the predicted and the observed survival time. The larger the CCI, the greater the contribution of the predictor. A core model adjusting for age, ethnicity, family history of diabetes, BMI at 3 months, duration of breastfeeding and preceding GDM was constructed and its CCI was calculated. To test the importance of each clinical predictor, a reduced model was constructed by removing the tested predictor from the core model. The percentage change in CCI between the core model and reduced model was computed to quantify the impact of each predictor and enable the ranking thereof.

Next, we applied the same three-step procedure to compare pathophysiological predictors at 3 months postpartum (i.e. in step 3 we added each of the tested predictors to the same core model and calculated the percentage change in CCI between this model and the core model) (Table 2). We also performed sensitivity analyses in which binary GDM status (GDM or non-GDM) was replaced by four-level gestational glucose tolerance status (GDM, GIGT, abnormal GCT with NGT on the antepartum OGTT, or normal GCT with antepartum NGT) (ESM Table 1).

To evaluate the impact of changes over time in adiposity, insulin sensitivity/resistance and beta cell function, we next applied the same three-step approach in constructing Cox

Table 1 Clinical and metabolic characteristics at 3 months postpartum of those who maintained NGT by 5 years postpartum (non-progressors) and those who progressed to prediabetes/diabetes within that time (progressors)

Characteristic	Non-progressors (n=213)	Progressors (n=89)	p value
Age, years	35.2±4.4	35.7±4.3	0.34
Ethnicity, n (%)			0.14
White	159 (74.6)	57 (64.0)	
Asian	24 (11.3)	12 (13.5)	
Other	30 (14.1)	20 (22.5)	
Family history of diabetes, n (%)	120 (56.3)	60 (67.4)	0.07
Parity, n (%)			0.31
One	116 (54.5)	40 (44.9)	
Two	82 (38.5)	42 (47.2)	
Greater than two	15 (7.0)	7 (7.9)	
GDM in recent pregnancy, n (%)	53 (24.9)	42 (47.2)	0.0001
BMI, kg/m^2	25.8±4.5	27.8±5.6	0.001
Waist circumference, cm	90±12	91±13	0.34
Breastfeeding, months	3 (3–3)	3 (3–3)	0.59
OGTT, mmol/l			
Fasting glucose	4.5±0.3	4.6±0.5	0.03
2 h glucose	5.6±1.1	6.2±1.0	<0.0001
Insulin sensitivity/resistance			
Matsuda index	11.0 (7.1–15.3)	7.8 (5.5–13.4)	0.001
HOMA-IR	0.8 (0.6–1.5)	1.2 (0.7–1.8)	0.02
Beta cell function			
ISSI-2	863 ±288	763 ±284	0.006
IGI/HOMA-IR	11.1 (7.5–17.5)	8.6 (5.4–11.8)	<0.0001

Continuous data are presented as mean ± SD (if normal distribution) or median followed by IQR in parentheses (if skewed distribution); categorical variables are presented as n (%)

proportional hazard models with their time-varying measures over the 5 years of follow-up and thereby ranked their respective contributions to incident prediabetes/diabetes (Table 3). Furthermore, we constructed fully adjusted models with combinations of time-varying measures of adiposity (BMI, waist), insulin sensitivity/resistance (Matsuda index, HOMA-IR) and beta cell function (ISSI-2, IGI/HOMA-IR) to identify the dominant predictor(s) when considering all three pathophysiological elements concurrently (Table 4). The resultant four models were all adjusted for age, ethnicity, family history of diabetes mellitus, and duration of breastfeeding, along with the following time-varying measures: Model I, waist, Matsuda index and IGI/HOMA-IR; Model II, BMI, Matsuda index and IGI/HOMA-IR; Model III, waist, HOMA-IR and ISSI-2; and Model IV, BMI, HOMA-IR and ISSI-2 (Table 4).

Finally, as shown in Fig. 1, we assessed time to progression from NGT to prediabetes/diabetes in relation to baseline measures of the key clinical factor (glucose levels on OGTT at 3 months) and the dominant pathophysiological factor (beta cell function) after stratifying the study population into tertiles based on baseline fasting glucose, 2 h glucose, ISSI-2 and IGI/HOMA-IR, respectively. The logrank test was used to compare survival curves.

Results

The 302 women comprising the study population were stratified as follows: (1) those who maintained NGT over the 5 years follow-up (non-progressors, n=213); and (2) those who progressed to prediabetes/diabetes within that time (progressors, n=89). The latter group consisted of 77 women who progressed to prediabetes (76 with IGT and one with combined IFG and IGT) and 12 who developed diabetes. Table 1 shows the clinical characteristics of the future progressors and non-progressors at 3 months postpartum (baseline), when all of the women had NGT. There were no significant differences between the groups in age, ethnicity, family history of diabetes or duration of breastfeeding. As anticipated, there was a higher prevalence of GDM in the recent pregnancy in future progressors (p=0.0001). Progressors also had higher BMI (p=0.001), while waist circumference did not differ between the groups at 3 months after delivery. Though all glucose measurements were in the normal range, progressors had higher fasting glucose (mean ± SD 4.6±0.5 vs 4.5±0.3 mmol/l, p=0.03) and higher 2 h glucose (mean ± SD 6.2±1.0 vs 5.6±1.1 mmol/l, p<0.0001) than non-progressors. Moreover, progressors had greater

Table 2 Clinical and pathophysiological predictors at 3 months postpartum of progression to prediabetes/diabetes

Predictor	Unadjusted model HR (95% CI)	p value	Adjusted model[a] HR (95% CI)	p value	Model CCI[b]	Rank by CCI	Change in CCI	Change in CCI (%)
Clinical predictors								
Age	1.06 (1.01, 1.12)	0.02	1.05 (1.00, 1.11)	0.05	0.662	3	−0.016	2.4
Ethnicity					0.665	5	−0.013	1.9
Asian vs White	1.52 (0.81, 2.83)	0.19	1.62 (0.85, 3.10)	0.15				
Other vs White	1.60 (0.96, 2.68)	0.07	1.57 (0.91, 2.71)	0.10				
Family history of diabetes	1.64 (1.05, 2.55)	0.03	1.42 (0.89, 2.27)	0.14	0.677	7	−0.001	0.1
BMI	1.06 (1.02, 1.10)	0.002	1.06 (1.03, 1.10)	0.0009	0.653	2	−0.025	3.7
Breastfeeding								
≤2 months vs >2 months	0.86 (0.51, 1.45)	0.58	0.84 (0.49, 1.44)	0.84	0.674	6	−0.004	0.6
OGTT								
Fasting glucose	1.90 (1.10, 3.27)	0.02	1.11 (0.61, 2.05)	0.73	0.679	7	0.001	0.1
2 h glucose	1.60 (1.30, 1.99)	<0.0001	1.59 (1.69, 1.99)	<0.0001	0.708	1	0.03	4.4
GDM	2.24 (1.47, 3.41)	0.0002	2.28 (1.48, 3.51)	0.0002	0.663	4	−0.015	2.2
Pathophysiological predictors								
Insulin sensitivity/resistance								
Matsuda index	0.94 (0.90, 0.98)	0.003	0.98 (0.93, 1.02)	0.31	0.682	3	0.004	0.6
HOMA-IR	1.52 (1.09, 1.91)	0.0003	1.32 (0.95, 1.82)	0.10	0.678	4	0	0.0
Beta cell function								
ISSI-2	0.998 (0.998, 0.999)	0.0005	0.999 (0.998, 1.000)	0.01	0.697	2	0.019	2.8
IGI/HOMA-IR	0.935 (0.902, 0.969)	0.0003	0.940 (0.905, 0.977)	0.002	0.709	1	0.031	4.6

HRs for continuous variables are presented per unit change

[a] Adjusted models are adjusted for age, ethnicity, family history of diabetes, BMI at 3 months, breastfeeding and GDM

[b] CCI is 0.678 for core model consisting of age, ethnicity, family history of diabetes, BMI, duration of breastfeeding and GDM. When testing the importance of each clinical predictor, the reduced model was constructed by removing the tested predictor from the core model

insulin resistance (Matsuda index, $p=0.001$; HOMA-IR, $p=0.02$) and poorer beta cell function (ISSI-2, $p=0.006$; IGI/HOMA-IR, $p<0.0001$).

As shown in ESM Fig. 1, the differences between the groups in BMI, Matsuda index, HOMA-IR, ISSI-2 and IGI/HOMA-IR persisted in their respective trajectories from 1 year to 5 years postpartum (all $p<0.0001$), coupled with concordant divergence of waist circumference during that time ($p=0.004$). Though waist circumference appeared to decrease after 3 months in non-progressors, there was no group–time interaction for waist (ESM Fig. 1b, $p=0.60$). Between 1 year and 5 years, insulin sensitivity/resistance worsened in both groups, though to a greater extent in progressors (ESM Fig. 1c, d). More strikingly, however, progressors experienced deterioration of beta cell function during this time, in stark contrast to the relative stability thereof in non-progressors (as evident with both ISSI-2 and IGI/HOMA-IR; ESM Fig. 1e, f).

Having identified that there were modest differences between future progressors and non-progressor even when all were normoglycaemic at 3 months postpartum, we next constructed Cox proportional hazard regression models to identify and rank predictors of progression at baseline (i.e. 3 months). On adjusted analyses of baseline clinical predictors (Table 2), the following four factors emerged as significant independent determinants of future dysglycaemia: 2 h glucose on OGTT ($p<0.0001$); recent GDM ($p=0.0002$); BMI ($p=0.0009$); and age ($p=0.05$). Based on their impact on the CCI of the models, these predictors were ranked as follows in descending order of importance: 2 h glucose (change in CCI 4.4%); BMI (3.7%); age (2.4%); and recent GDM (2.2%). On sensitivity analyses (ESM Table 1), in which the classification of glucose tolerance in the preceding pregnancy was changed from binary GDM status (GDM or non-GDM) to a more granular four-level stratification (GDM, GIGT, abnormal GCT NGT, or normal GCT NGT), findings were similar in that significant independent predictors of dysglycaemia were 2 h glucose on the OGTT ($p<0.0001$), BMI ($p=0.002$), and each degree of glucose intolerance in

Table 3 Models of progression to prediabetes/diabetes with time-varying pathophysiological predictors across the first 5 years postpartum

Time-varying predictor	Unadjusted model		Adjusted model[a]		Model CCI[b]	Rank by CCI	Change in CCI	Change in CCI (%)
	HR (95% CI)	p value	HR (95% CI)	p value				
BMI	1.06 (1.03, 1.10)	0.0006	--	--	--	--	--	--
Waist	1.02 (1.00, 1.03)	0.009	0.99 (0.97,1.02)	0.66	0.665	5	0.001	0.2
Insulin sensitivity/ resistance								
Matsuda index	0.86 (0.81, 0.92)	<0.0001	0.89 (0.83, 0.96)	0.001	0.683	3	0.019	2.9
HOMA-IR	1.31 (1.17, 1.46)	<0.0001	1.19 (1.03, 1.38)	0.02	0.667	4	0.003	0.5
Beta cell function:								
ISSI-2	0.994 (0.993, 0.996)	<0.0001	0.994 (0.993, 0.996)	<0.0001	0.831	1	0.167	25.2
IGI/HOMA-IR	0.863 (0.820, 0.907)	<0.0001	0.865 (0.820, 0.913)	<0.0001	0.750	2	0.086	13.0

[a]Time-varying predictors adjusted for age, ethnicity, family history of diabetes, BMI at 3 months and duration of breastfeeding at 3 months

[b]CCI is 0.664 for core model consisting of age, ethnicity, family history of diabetes, BMI at 3 months and duration of breastfeeding

pregnancy (GDM, p=0.0002; GIGT, p=0.04; abnormal GCT NGT, p=0.02). Gestational glucose tolerance status was the highest ranked predictor (change in CCI 5.3%), followed by 2 h glucose on the OGTT at 3 months (3.7%) (ESM Table 1).

We next constructed pathophysiological models that evaluated baseline measures of insulin sensitivity/resistance and beta cell function in conjunction with clinical risk factors for diabetes. On these adjusted analyses (Table 2), lower beta cell function predicted future prediabetes/diabetes (ISSI-2, p=0.01; IGI/HOMA-IR, p=0.002) whereas insulin sensitivity/resistance was not independently associated with this outcome. Indeed, ranking of these pathophysiological

factors prioritised beta cell function (IGI/HOMA-IR, change in CCI 4.6%; ISSI-2, 2.8%) over insulin sensitivity/resistance (Matsuda index, 0.6%; HOMA-IR, 0.0%) in predicting future prediabetes/diabetes. These findings were unchanged on sensitivity analyses with four-level gestational glucose tolerance status, with IGI/HOMA-IR (change in CCI: 3.4%) and ISSI-2 (2.1%) again emerging as the dominant pathophysiological predictors at baseline (in contrast to Matsuda index and HOMA-IR [both 0.1%], ESM Table 1).

Having identified beta cell function as the predominant pathophysiological determinant of future dysglycaemia at baseline (3 months postpartum) when all of the women were

Table 4 Fully adjusted models of progression to prediabetes/diabetes with time-varying pathophysiological predictors across the first 5 years postpartum

Model	Model I (CCI=0.753)[a]		Model II (CCI=0.760)[b]		Model III (CCI=0.838)[c]		Model IV (CCI=0.839)[d]	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Time-varying BMI			1.02 (0.974, 1.057)	0.49			1.04 (0.997, 1.082)	0.07
Time-varying waist	0.992 (0.975, 1.010)	0.40			1.004 (0.985, 1.022)	0.71		
Time-varying Matsuda index	0.920 (0.854, 0.992)	0.03	0.95 (0.888, 1.020)	0.16				
Time-varying IGI/HOMA-IR	0.865 (0.818, 0.916)	<0.0001	0.87 (0.823, 0.921)	<0.0001				
Time-varying HOMA-R					0.866 (0.722, 1.039)	0.12	0.80 (0.658, 0.961)	0.02
Time-varying ISSI-2					0.994 (0.992, 0.995)	<0.0001	0.99 (0.992, 0.995)	<0.0001

[a]Model I adjusted for age, ethnicity, family history of diabetes, breastfeeding, time-varying waist, time-varying Matsuda index, and time-varying IGI/HOMA-IR

[b]Model II adjusted for age, ethnicity, family history of diabetes, breastfeeding, time-varying BMI, time-varying Matsuda index, and time-varying IGI/HOMA-IR

[c]Model III adjusted for age, ethnicity, family history of diabetes, breastfeeding, time-varying waist, time-varying HOMA-IR, and time-varying ISSI-2

[d]Model IV adjusted for age, ethnicity, family history of diabetes, breastfeeding, time-varying BMI, time-varying HOMA-IR, and time-varying ISSI-2
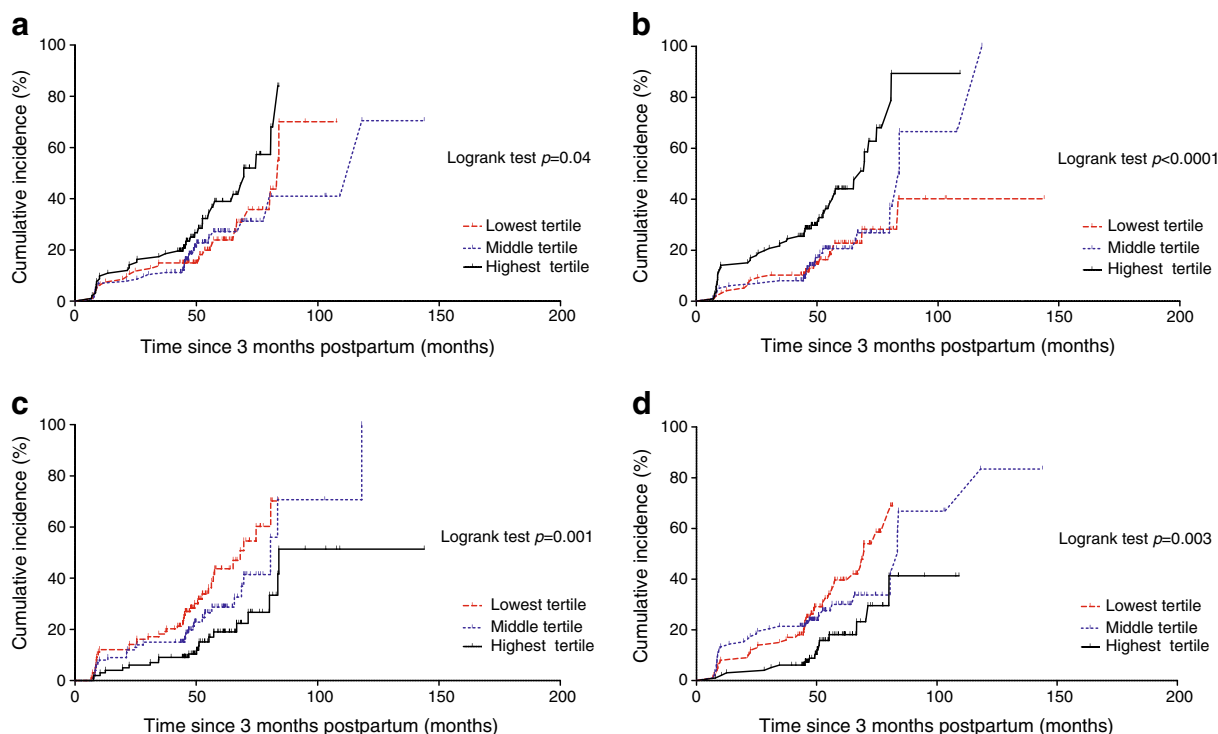
Fig. 1 Cumulative incidence of prediabetes/diabetes in study population stratified into tertiles at 3 months postpartum as follows: fasting glucose (**a**); 2 h glucose (**b**); ISSI-2 (**c**); and IGI/HOMA-IR (**d**)

normoglycaemic, we next constructed models with time-varying measures in order to evaluate changes over time in adiposity, insulin sensitivity/resistance and beta cell function, and thereby rank their respective contributions to incident prediabetes/diabetes over the ensuing 5 years (Table 3). On these adjusted analyses, lesser beta cell function across the 5 years (time-varying ISSI-2 and IGI/HOMA-IR, both $p<0.0001$) and poorer insulin sensitivity (Matsuda index, $p=0.001$; HOMA-IR, $p=0.02$) both emerged as significant independent predictors of incident dysglycaemia. Importantly, the dominant determinants of progression to prediabetes/diabetes were time-varying ISSI-2 (change in CCI, 25.2%) and IGI/HOMA-IR (13.0%), in contrast to the much more modest impact of time-varying Matsuda index (2.9%) and HOMA-IR (0.5%).

These findings were further reinforced upon sequential construction of fully adjusted models that concurrently tested combinations of time-varying measures of adiposity (BMI, waist), insulin sensitivity/resistance (Matsuda index, HOMA-IR) and beta cell function (ISSI-2, IGI/HOMA-IR), respectively. While the combinations of measures evaluated (Table 4) yielded progressive improvement of the models (as evidenced by the increasing C statistic from Model I to Model IV), it is notable that the measure of beta cell function (whether time-varying ISSI-2 or IGI/HOMA-IR) consistently emerged as being inversely associated with incident prediabetes/diabetes in each model (all $p<0.0001$). Conversely, time-varying adiposity

(BMI or waist) was not significantly associated in any of the models and insulin sensitivity/resistance yielded equivocal findings (i.e. Matsuda index reaching nominal significance in Model I but not II, with HOMA-IR similarly meeting nominal significance in Model IV but not III).

To further illustrate their relative effects on progression to dysglycaemia, we considered the comparative impact of a 5% decrease in time-varying ISSI-2, a 5% increase in time-varying BMI and a 5% rise in HOMA-IR, respectively, in Model IV. This sensitivity analysis revealed that a 5% decline in ISSI-2 would yield a 30% higher hazard for progression to prediabetes/diabetes, whereas 5% increases in BMI and HOMA-IR would change this hazard by 2–5% (data not shown). Thus, at both baseline (Table 2) and across follow-up to 5 years (Tables 3, 4), beta cell dysfunction was the dominant determinant of progression from NGT to prediabetes/diabetes, far exceeding the impact of adiposity and insulin sensitivity/resistance.

Finally, we assessed the time to progression from NGT to prediabetes/diabetes in relation to baseline measures of the key clinical factor (glucose levels on the OGTT at 3 months) and the dominant pathophysiological factor (beta cell function). As shown in Fig. 1a, the cumulative incidence of prediabetes/diabetes was greatest in the highest tertile of fasting glucose at 3 months postpartum (logrank test $p=0.04$). As would be expected based on the ranking in Table 2, tertiles of 2 h glucose on the OGTT at 3 months yielded a far more striking separation

of incident dysglycaemia over time (logrank test $p<0.0001$) (Fig. 1b). Similarly, the cumulative incidence of prediabetes/diabetes progressively increased from the highest to middle to lowest tertile of beta cell function at 3 months postpartum, whether measured by ISSI-2 (Fig. 1c; logrank test $p=0.001$) or IGI/HOMA-IR (Fig. 1d; logrank test $p=0.003$), again consistent with the ranking of baseline pathophysiological predictors.

Discussion

In this study comparing women who progressed from NGT to prediabetes/diabetes over 5 years and those who did not, three key findings emerge. First, even while clinically normoglycaemic at baseline, future progressors had higher fasting and post-challenge glucose than their peers (consistent with earlier studies [28–31]), coupled with higher BMI, greater insulin resistance and poorer beta cell function. Second, while the differences in BMI and insulin resistance persisted over the ensuing 5 years, it is notable that progressors experienced deterioration of beta cell function during this time, in contrast to those that maintained NGT. Third, and most importantly, the ranking of predictors on Cox proportional hazard regression analyses identified measures of beta cell function across the duration of follow-up (time-varying ISSI-2 and IGI/HOMA-IR) as the most important determinants of incident dysglycaemia, far outstripping the impact of time-varying measures of insulin sensitivity/resistance (Matsuda index, HOMA-IR) and adiposity (BMI, waist). It thus emerges that declining beta cell function is the dominant determinant of progression from NGT to prediabetes/diabetes in young women.

Previous studies have consistently identified excessive adiposity, insulin resistance and beta cell dysfunction as pathophysiological factors for type 2 diabetes. However, since all three factors frequently co-exist in individuals who develop diabetes, dissecting their relative contributions to the pathogenesis of the disease has been challenging, with conflicting findings reported in this regard. Specifically, studies have variously highlighted either insulin resistance [2, 8] or insulin secretory insufficiency [3, 5, 7] as the dominant determinant, while other reports have implicated both features as similarly important [1, 6, 9, 10]. Several factors are likely contributing to this discordance between studies. First, to enhance the logistical feasibility of longitudinal assessment, some studies determined glucose tolerance status by fasting glucose measurement [9] or focused on fasting indices of insulin sensitivity and insulin secretion [2, 10], which may lack the capacity of dynamic (post-challenge) testing for identifying differences in beta cell function. Second, there have been methodological differences in how the prioritisation of potential pathological factors was determined. For example, some studies have compared predictors by the magnitude/significance of their risk ratios [6, 9, 11] while others have stratified participants into those in whom metabolic dysfunction

was primarily attributed to insulin action, insulin secretion or both [1, 2, 5]. Third, the inter-relationships between adiposity, insulin sensitivity and beta cell function likely change over time as the pathogenic process evolves such that the time course of assessment may influence findings [7, 10, 11].

In light of these considerations, we reasoned that prospective serial GTTs in a young at-risk population at a time when individuals are still normoglycaemic could provide the capacity to disentangle the relative contributions of these pathological processes early in their natural history. Women with postpartum NGT following recent glucose intolerance in pregnancy thus presented a model that served these exacting requirements. Although we cannot assume generalisability of this model to other populations, there are three important factors that support the clinical relevance of this model. First, in 2021, the IDF reported that maternal hyperglycaemia now affects one in every six pregnancies around the world [32]. Second, women who have hyperglycaemia in pregnancy have an elevated future risk of type 2 diabetes [13–18]. Third, women of childbearing age comprise the segment of the Canadian population that has experienced the greatest rise in the prevalence of diabetes in recent decades [33]. Thus, though generalisability cannot be assumed beyond postpartum women, these three factors suggest that enhanced understanding of the pathophysiological determinants of progression from postpartum NGT to incident dysglycaemia holds relevance for the growing burden of diabetes in women.

In this setting, it is notable that, while adiposity, insulin sensitivity and beta cell function all differed between progressors and non-progressors, only beta cell function exhibited a differential deterioration over time in the former group. This observation is consistent with other reports demonstrating declining beta cell function prior to the development of diabetes [7, 10, 11]. Importantly, the current study further extends this literature through its quantification and objective ranking of the impact of predictors based on the change in CCI of Cox proportional hazard regression models. This approach not only identified beta cell dysfunction, both at baseline (Table 2) and across the duration of follow-up (Table 3), as the predominant determinant of progression from NGT to prediabetes/diabetes, but also provided a relative quantification of the magnitude by which it exceeds insulin resistance and adiposity in this regard. This hierarchy was further confirmed by the fully adjusted models wherein beta cell function across follow-up (whether measured by time-varying ISSI-2 or IGI/HOMA-IR) consistently predicted progression, in contrast to insulin sensitivity/resistance and adiposity (Table 4). Moreover, the comparative impact of 5% changes in these measures on the risk of incident dysglycaemia again illustrated the concept that declining beta cell function is a far more influential determinant than either adiposity or insulin resistance. Taken together, these data are consistent with a pathophysiological model wherein

the diabetogenic effects of weight gain and insulin resistance may be ultimately mediated by their implications for the secretory demands placed on the beta cells.

A strength of this study is the serial assessment of glucose tolerance, insulin sensitivity and beta cell function on four occasions from 3 months to 5 years postpartum in a cohort of normoglycaemic women reflecting a broad range of future diabetes risk. Conversely, a limitation is that gestational weight gain was not assessed. Additionally, in those with incident dysglycaemia, we did not do a second confirmatory OGTT in the days/weeks thereafter. Further, C-peptide was not assessed. Another limitation is that the study design involved OGTT-based indices of insulin sensitivity and beta cell function, rather than direct measurements from clamp studies. However, these indices (Matsuda index, HOMA-IR, ISSI-2, IGI/HOMA-IR) are validated measures that have been widely used in previous studies [8, 19, 20, 22–26, 34]. Moreover, their concomitant measurement at the time of the serial OGTTs that enabled ongoing surveillance of glucose tolerance status yielded an efficiency in study design that supported participant acceptance of the protocol.

The findings from this study hold implications for both clinical practice and research. In practice, it is recommended that women with GDM undergo postpartum assessment of glucose tolerance by OGTT rather than by measurement of fasting glucose alone [35]. This concept is further supported by the demonstration herein that, even when normoglycaemic, the 2 h glucose value clearly surpasses the fasting glucose as a predictor of incident prediabetes/diabetes in the first 5 years postpartum (Table 2, Fig. 1 and ESM Table 1). Moreover, it is notable that the 2 h glucose measurement at 3 months postpartum also ranked higher than traditional clinical risk factors for diabetes (i.e. age, ethnicity, family history and current BMI), thus supporting its consideration when determining future surveillance protocols for women with recent GDM. From a research perspective, our findings highlight declining beta cell function as the central pathophysiological defect that ideally should be targeted when designing interventions aimed at the prevention of diabetes in women with recent glucose intolerance in pregnancy. In this context, we are currently conducting a randomised placebo-controlled trial of the sodium–glucose cotransporter -2 (SGLT2) inhibitor empagliflozin for the preservation of beta cell function (primary outcome) in women with recent GDM (ClinicalTrials.gov registration no. NCT03215069).

In conclusion, women who progressed from NGT to incident prediabetes/diabetes over the first 5 years postpartum had higher fasting and 2 h post-challenge glucose values than their peers at baseline, even though all were in the normal range. At baseline and across follow-up, they also had higher BMI, greater insulin resistance and poorer beta cell function. The latter difference was further amplified by the deterioration of beta cell function over time in those that progressed to dysglycaemia. Indeed, on Cox proportional hazard regression analyses, beta cell function across the duration of follow-up was the dominant determinant of incident dysglycaemia, far outstripping the impact of concomitant changes in adiposity and insulin resistance. Declining beta cell function thus emerges as the central pathophysiological determinant of progression from NGT to prediabetes/diabetes in young women.

References

1. Yoshinari M, Hirakawa Y, Hata J et al (2021) Comparison of the contributions of impaired beta cell function and insulin resistance to the development of type 2 diabetes in a Japanese community: the Hisayama Study. Diabetologia 64(8):1775–1784. https://doi.org/10.1007/s00125-021-05459-7

2. Wang T, Lu J, Shi L et al (2020) Association of insulin resistance and β-cell dysfunction with incident diabetes among adults in China: a nationwide, population-based, prospective cohort study. Lancet Diabetes Endocrinol 8(2):115–124. https://doi.org/10.1016/S2213-8587(19)30425-5

3. Ohn JH, Kwak SH, Cho YM et al (2016) 10-year trajectory of β-cell function and insulin sensitivity in the development of type 2 diabetes: a community-based prospective cohort study. Lancet Diabetes Endocrinol 4(1):27–34. https://doi.org/10.1016/S2213-8587(15)00336-8

4. Lai KZH, Semnani-Azad Z, Retnakaran R, Harris SB, Hanley AJ (2021) Changes in adiposity mediate the associations of diet quality with insulin sensitivity and beta-cell function. Nutr Metab Cardiovasc Dis 31(11):3054–3063. https://doi.org/10.1016/j.numecd.2021.07.025

5. Morimoto A, Tatsumi Y, Deura et al (2013) Impact of impaired insulin secretion and insulin resistance on the incidence of type 2 diabetes mellitus in a Japanese population: the Saku study. Diabetologia 56(8):1671–1679. https://doi.org/10.1007/s00125-013-2932-y

6. Lorenzo C, Wagenknecht LE, D'Agostino RB Jr, Rewers MJ, Karter AJ, Haffner SM (2010) Insulin resistance, beta-cell

dysfunction, and conversion to type 2 diabetes in a multiethnic population: the Insulin Resistance Atherosclerosis Study. Diabetes Care 33(1):67–72. https://doi.org/10.2337/dc09-1115

7. Xiang AH, Kjos SL, Takayanagi M, Trigo E, Buchanan TA (2010) Detailed physiological characterization of the development of type 2 diabetes in Hispanic women with prior gestational diabetes mellitus. Diabetes 59(10):2625–30. https://doi.org/10.2337/db10-0521

8. Stancáková A, Javorský M, Kuulasmaa T, Haffner SM, Kuusisto J, Laakso M (2009) Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6,414 Finnish men. Diabetes 58(5):1212–1221. https://doi.org/10.2337/db08-1607

9. Hanley AJ, Wagenknecht LE, Norris JM et al (2009) Insulin resistance, beta cell dysfunction and visceral adiposity as predictors of incident diabetes: the Insulin Resistance Atherosclerosis Study (IRAS) Family study. Diabetologia 52(10):2079–86. https://doi.org/10.1007/s00125-009-1464-y

10. Tabák AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR (2009) Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. Lancet 373(9682):2215–21. https://doi.org/10.1016/S0140-6736(09)60619-X

11. Lyssenko V, Almgren P, Anevski D et al (2005) Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. Diabetes 54:166–174. https://doi.org/10.2337/diabetes.54.1.166

12. Weyer C, Tataranni PA, Bogardus C, Pratley RE (2001) Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. Diabetes Care 24:89–94. https://doi.org/10.2337/diacare.24.1.89

13. Bardugo A, Bendor CD, Rotem RS et al (2023) Glucose intolerance in pregnancy and risk of early-onset type 2 diabetes: a population-based cohort study. Lancet Diabetes Endocrinol 11(5):333–344. https://doi.org/10.1016/S2213-8587(23)00062-1

14. Vounzoulaki E, Khunti K, Abner SC, Tan BK, Davies MJ, Gillies CL (2020) Progression to type 2 diabetes in women with a known history of gestational diabetes: systematic review and meta-analysis. BMJ 369:m1361

15. Kramer CK, Swaminathan B, Hanley AJ et al (2014) Each degree of glucose intolerance in pregnancy predicts distinct trajectories of beta-cell function, insulin sensitivity and glycemia in the first 3 years postpartum. Diabetes Care 37:3262–3269. https://doi.org/10.2337/dc14-1529

16. Retnakaran R, Shah BR (2009) Abnormal screening glucose challenge test in pregnancy and future risk of diabetes in young women. Diabet Med 26(5):474–7. https://doi.org/10.1111/j.1464-5491.2009.02712.x

17. Retnakaran R, Qi Y, Sermer M, Connelly PW, Hanley AJ, Zinman B (2008) Glucose intolerance in pregnancy and future risk of pre-diabetes or diabetes. Diabetes Care 31:2026–31. https://doi.org/10.2337/dc08-0972

18. Vambergue A, Dognin C, Boulogne A, Réjou MC, Biausque S, Fontaine P (2008) Increasing incidence of abnormal glucose tolerance in women with prior abnormal glucose tolerance during pregnancy: DIAGEST 2 study. Diabet Med 25(1):58–64

19. Retnakaran R, Ye C, Hanley AJ, Connelly PW, Sermer M, Zinman B (2021) Subtypes of gestational diabetes and future risk of pre-diabetes or diabetes. EClinicalMedicine 40:101087

20. Kramer CK, Ye C, Hanley AJ et al (2015) Delayed timing of post-challenge peak blood glucose predicts declining beta cell function and worsening glucose tolerance over time: insight from the first year postpartum. Diabetologia 58(6):1354–62. https://doi.org/10.1007/s00125-015-3551-6

21. Diabetes Canada Clinical Practice Guidelines Expert Committee, Punthakee Z, Goldenberg R, Katz P (2018) Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Can J Diabetes 42(Suppl 1):S10–S15. https://doi.org/10.1016/j.jcjd.2017.10.003

22. Matsuda M, DeFronzo R (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22:1462–1470. https://doi.org/10.2337/diacare.22.9.1462

23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7):412–419. https://doi.org/10.1007/BF00280883

24. Retnakaran R, Shen S, Hanley AJ, Vuksan V, Hamilton JK, Zinman B (2008) Hyperbolic relationship between insulin secretion and sensitivity on oral glucose tolerance test. Obesity 16(8):1901–1907. https://doi.org/10.1038/oby.2008.307

25. Retnakaran R, Qi Y, Goran M, Hamilton J (2009) Evaluation of proposed oral disposition index measures in relation to the actual disposition index. Diabet Med 26:1198–1203. https://doi.org/10.1111/j.1464-5491.2009.02841.x

26. Santos JL, Yévenes I, Cataldo LR et al (2016) Development and assessment of the disposition index based on the oral glucose tolerance test in subjects with different glycaemic status. J Physiol Biochem 72(2):121–131. https://doi.org/10.1007/s13105-015-0458-0

27 Harrell FE Jr (2001) Regression modeling strategies, with applications to linear models, logistic regression, and survival analysis. Springer, New York, NY. USA

28. Færch K, Witte DR, Tabák AG, Perreault L, Herder C, Brunner EJ, Kivimäki M, Vistisen D (2013) Trajectories of cardiometabolic risk factors before diagnosis of three subtypes of type 2 diabetes: a post-hoc analysis of the longitudinal Whitehall II cohort study. Lancet Diabetes Endocrinol 1(1):43–51. https://doi.org/10.1016/S2213-8587(13)70008-1

29. Brambilla P, La Valle E, Falbo R, Limonta G, Signorini S, Cappellini F, Mocarelli P (2011) Normal fasting plasma glucose and risk of type 2 diabetes. Diabetes Care 34(6):1372–1374. https://doi.org/10.2337/dc10-2263

30. Nichols GA, Hillier TA, Brown JB (2008) Normal fasting plasma glucose and risk of type 2 diabetes diagnosis. Am J Med 121(6):519–524. https://doi.org/10.1016/j.amjmed.2008.02.026

31. Tirosh A, Shai I, Tekes-Manova D, Israeli E, Pereg D, Shochat T, Kochba I, Rudich A, Israeli Diabetes Research Group (2005) Normal fasting plasma glucose levels and type 2 diabetes in young men. N Engl J Med 353(14):1454–1462. https://doi.org/10.1056/NEJMoa050080

32. International Diabetes Federation. IDF Diabetes Atlas, 10th ed. Brussels, Belgium: International Diabetes Federation, 2021. https://www.diabetesatlas.org/en/

33. Lipscombe LL, Hux JE (2007) Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995–2005: a population-based study. Lancet 369(9563):750–6. https://doi.org/10.1016/S0140-6736(07)60361-4

34. Zinman B, Harris SB, Neuman J et al (2010) Low-dose combination therapy with rosiglitazone and metformin to prevent type 2 diabetes mellitus (CANOE trial): a double-blind randomized controlled study. Lancet 376(9735):103–11. https://doi.org/10.1016/S0140-6736(10)60746-5

35. American Diabetes Association (2023) Management of diabetes in pregnancy: standards of care in diabetes—2023. Diabetes Care 46(suppl 1):S254–S266