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ORIGINAL

Bihormonal dysregulation of insulin and glucagon contributes to glucose intolerance development at one year post-delivery in women with gestational diabetes: a prospective cohort study using an early postpartum 75-g glucose tolerance test

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Abstract. Gestational diabetes mellitus (GDM) is known to be a significant risk factor for the future development of type 2 diabetes. Here, we investigated whether a precise evaluation of β- and α-cell functions helps to identify women at high risk of developing glucose intolerance after GDM. Fifty-six women with GDM underwent a 75-g oral glucose tolerance test (OGTT) at early (6–12 weeks) postpartum. We measured their concentrations of glucose, insulin, proinsulin and glucagon at fasting and 30, 60 and 120 min. At 1-year post-delivery, we classified the women into a normal glucose tolerance (NGT) group or an impaired glucose tolerance (IGT)/diabetes mellitus (DM) group. Forty-three of the 56 women completed the study. At 1-year post-delivery, 17 women had developed IGT/DM and 26 women showed NGT. In the early-postpartum OGTTs, the IGT/DM group showed a lower insulinogenic index, a less glucagon suppression evaluated by the change from fasting to 30 min (ΔGlucagon 30 min), and a higher glucagon-to-insulin ratio at 30 min compared to the NGT group. There were no significant between-group differences in proinsulin levels or proinsulin-to-insulin ratios. Insulinogenic index <0.6 and ΔGlucagon 30 min >0 pg/mL were identified as predictors for the development of IGT/DM after GDM, independent of age, body mass index, and lactation intensity. These results suggest that the bihormonal disorder of insulin and glucagon causes the postpartum development of glucose intolerance. The measurement of plasma insulin and glucagon during the initial OGTT at early postpartum period can help to make optimal decisions regarding the postpartum management of women with GDM.

Key words: Gestational diabetes, Glucagon, Insulin, Insulinogenic index, Hyperglucagonemia

GESTATIONAL DIABETES MELLITUS (GDM) is known to be a significant risk factor for the future development of type 2 diabetes mellitus (T2DM) [1, 2]. In a meta-analysis, women with GDM had as much as a 7.4-fold higher relative risk of developing T2DM in the future compared to women who had a normoglycemic pregnancy [3]. After the adoption of the World Health Organization (WHO) guideline in 2013 [4] based on the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria in 2010 [5], even women with milder glucose intolerance have been diagnosed as having GDM. The prevalence of GDM has been increasing worldwide. In Japan, it was estimated that up to 12% of all pregnant women develop GDM as defined

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Abbreviations: BMI: body mass index; ELISA: enzyme-linked immunosorbent assay; GDM: gestational diabetes mellitus; HOMA-IR: the homeostasis model assessment for insulin resistance; HOMA-β: the homeostasis model assessment for β-cell function; IADPSG: International Association of Diabetes and Pregnancy Study Group; IGT: impaired glucose tolerance; ISSI-2: the insulin secretion/sensitivity index-2; OGTT: oral glucose tolerance test; T2DM: type 2 diabetes mellitus; WHO: World Health Organization

by the new WHO-2013 criteria [6]. It is therefore important to identify women who are at high risk of developing T2DM in the future among the increased numbers of patients with GDM diagnosed based on the new criteria.

Clinical factors that increase the risk of developing T2DM after GDM have been reported, including higher age, higher body mass index (BMI), family history of T2DM, and the requirement of insulin treatment during pregnancy [7]. Postpartum weight change, physical activity, and breastfeeding also affect the incidence of T2DM [8, 9]. Not surprisingly, higher glucose levels at fasting, 1-hr, and 2-hr of an oral glucose tolerance test (OGTT) performed during either pregnancy or at early (*i.e.*, 4–12 weeks) postpartum were associated with the future development of T2DM [10-15]. Substantial investigations have attempted to use measurements of serum insulin (or C-peptide) levels in the OGTT to predict future T2DM after GDM. Both insulin sensitivity measured by the homeostasis model assessment for insulin resistance (HOMA-IR) [16] and the insulin secretory capacity measured by the insulinogenic index or the oral disposition index (which were evaluated using gestational and postpartum OGTTs) [10-13, 16-19] are known as useful predictors of the development of T2DM.

Recent studies revealed that T2DM involves not only insulin action deficiency but also a dysregulation of glucagon secretion from pancreatic α-cells [20]. When a healthy human consumes glucose, the secretion of glucagon is immediately suppressed [21]. In contrast, a paradoxical increase in glucagon secretion after glucose consumption occurs in patients with T2DM [22] and also in those with GDM [23]. Increased levels of serum proinsulin, a precursor of insulin peptide, and an elevated proinsulin-to-insulin ratio were reported as indicators of β-cell exhaustion in patients with T2DM [24, 25] and even in individuals with prediabetes [26]. An increased proinsulin-to-insulin ratio was strongly associated with an imminent development of T2DM in healthy middle-aged women [27]. These findings suggest that measurements of diabetes-related hormones in addition to insulin may improve the predictability of future T2DM in patients with GDM. It has not been determined whether the abnormalities in these hormones including proinsulin and glucagon are associated with the future development of T2DM after GDM.

In this study, we sought to identify whether the measurements of proinsulin and glucagon, in addition to glucose and insulin, by an early-postpartum OGTT can predict glucose intolerance at 1 year post-delivery in women with GDM.

Materials and Methods

Patients

Our subjects were Japanese singleton pregnant women who had been diagnosed with GDM based on the WHO-2013 criteria [4]. Patients were excluded if they were diagnosed with overt diabetes in pregnancy or if they needed anti-diabetic medications including insulin after delivery. Patients whose conditions were complicated with other diseases (including thyroid disease, collagen disease, renal disorder, liver disease, cardiovascular disease, and respiratory disease), a history of gastrointestinal surgery or pancreatomy, alcohol or drug abuse, and malignancy were excluded. Patients treated with a diabetogenic medication such as a steroid were also excluded.

Study design

This was a single-center, prospective cohort study conducted at Nagasaki University Hospital from April 2016 to October 2019. The study is registered with the University Hospital Medical Information Network (UMIN) Clinical Trial Registry, no. UMIN000034337. The study protocol is shown in Fig. 1.

We obtained written informed consent from 59 women with GDM during their pregnancies to be enrolled in the study. Of the 59 participants, 56 women underwent a 75-g OGTT at 6–12 weeks postpartum (defined as the early-postpartum OGTT) to determine their glucose tolerance and secretory responses of insulin, proinsulin, and glucagon. We asked participants about their breastfeeding practice when performing the early-postpartum OGTT. We classified the women into five lactation intensity categories: (1) exclusively lactation (no formula), (2) mostly lactation (roughly 80% of total feeding), (3) mixed lactation (roughly 50% of total feeding), (4) mostly formula (roughly 20% of total feeding), and (5) exclusively formula (no lactation). All of the women were advised to consume 30 kcal/kg/day of their ideal body weight, and the women classified in the lactation-intensity categories 1 or 2 (defined as high-intensity breastfeeding) were instructed to consume 350 kcal/day in addition to the above-described calories.

Each of the women underwent another 75-g OGTT at 1 year post-delivery. We divided them into two groups based on their glucose tolerance: (1) the normal glucose tolerance (NGT) group defined by a fasting plasma glucose (FPG) value <110 mg/dL and a 2-hr plasma glucose (2-hr PG) value <140 mg/dL, or (2) the impaired glucose tolerance (IGT)/diabetes mellitus (DM) group defined by an FPG value ≥110 mg/dL and/or a 2-hr PG value ≥140 mg/dL.

We compared the secretory responses of hormones

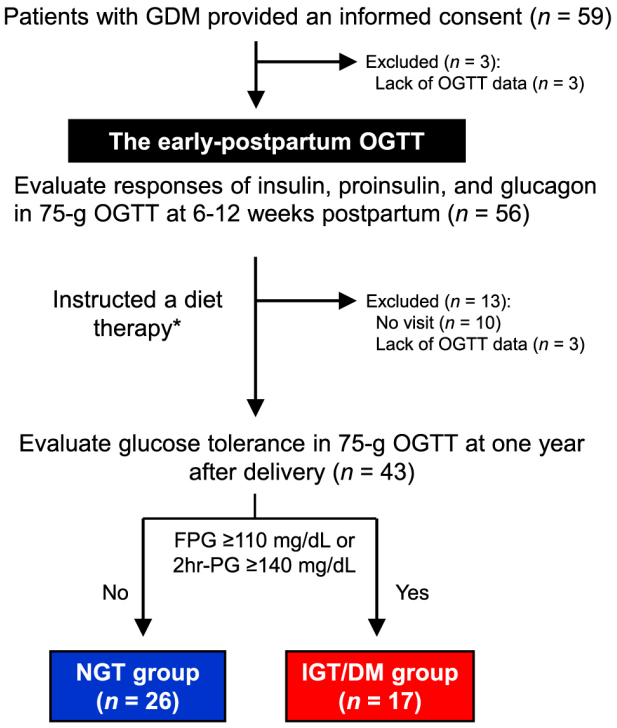

Fig. 1 Study design. Flow chart of the participants who completed the study (n = 43) and the 16 patients excluded from the study. The 43 women with recent GDM were classified into an NGT group and an IGT/DM group based on their glucose tolerance at 1 year after delivery. *All participants were advised to consume a diet ensuring 30 kcal/kg/day of their ideal body weight. The women with high-intensity breastfeeding (exclusively or mostly lactation) were instructed to consume 350 kcal/day in addition to these calories.

including insulin, proinsulin, and glucagon in the early-postpartum OGTT between the NGT and IGT/DM groups. We determined whether the secretory responses of these hormones in the early-postpartum period were associated with development of IGT/DM at 1 year post-delivery.

The study was approved by the ethical committee of Nagasaki University Hospital (approval no. 14032483) and was carried out in accordance with the Declaration of Helsinki.

Laboratory measurements

As described in Fig. 1, OGTTs were carried out twice in the morning after overnight fasting at the early (6–12 weeks) postpartum period and at 1 year post-delivery using a 75-g glucose formulation, the Trelan-G75 (AY Pharma, Tokyo, Japan). In the early-postpartum OGTT, the levels of serum insulin (μU/mL) and proinsulin (pmol/L) and the plasma glucagon (pg/mL) in addition to plasma glucose (mg/dL) were measured at fasting (0 min) and at 30, 60 and 120 min after the ingestion of the glucose formulation. In the 1-year post-delivery OGTT, we determined only the plasma glucose levels to evaluate the glucose tolerance of the subjects.

The levels of plasma glucose and serum insulin were measured by the hexokinase ultraviolet method using a JCA-BioMajesty6070 analyzer (JEOL, Tokyo, Japan) and an ECLusys kit using a Roche Modular Analytics E170 assay (Roche, Basel, Switzerland), respectively. Serum proinsulin levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) with 95% cross-reactivity against des 31–32 proinsulin but with less cross-reactivities against insulin (<0.03%) and C-peptide (<0.006%) (Mercodia, Uppsala, Sweden). Blood sampling for plasma glucagon was performed using BD P800 tubes (BD, Franklin Lakes, NJ, USA). Plasma glucagon was measured using a sandwich ELISA kit (Mercodia) which has much less cross-reactivity against other proglucagon fragments compared to those afforded by the conventional radioimmunoassay [28]. The blood samples were stored at –80°C, and each hormone was measured after each participant completed the study.

Assessments of the secretion/sensitivity of hormones

The areas under the curve (AUCs) of insulin, proinsulin, and glucagon from 0 to 120 min (indicated as AUC Insulin 0–120 min, AUC Proinsulin 0–120 min, and AUC Glucagon 0–120 min, respectively) were calculated using the trapezoidal rule. Insulin secretion was assessed by the homeostasis model assessment for β-cell function (HOMA-β) [29], the insulinogenic index (the increase in insulin from 0 to 30 min divided by the increase in glucose from 0 to 30 min) [30]. Insulin sensitivity was estimated by HOMA-IR [29] and the Matsuda index [10,000/√(fasting glucose × fasting insulin × mean glucose × mean insulin)] [31]. The β-cell function was assessed by the disposition index (the Matsuda index × the insulinogenic index) [32] and the insulin secretion/sensitivity index-2 (ISSI-2) (the Matsuda index × AUC Insulin 0–120 min/AUC Glucose 0–120 min) [33].

To assess the glucagon secretory responses after the glucose load, we determined the changes in the levels of plasma glucagon from baseline (0 min) to each time point (30, 60 and 120 min) during the OGTT. These changes are indicated as ΔGlucagon 30 min, ΔGlucagon 60 min, and ΔGlucagon 120 min, as described [21]. We determined the relative changes in glucagon to those in glucose from fasting to 30 min during the OGTT which imitates the calculation of the insulinogenic index, shown as ΔGlucagon 30 min/ΔGlucose30min. We also determined the ratio of the plasma glucagon levels at 30 min to those at fasting (0 min). The molar ratio of proinsulin to insulin was calculated as an indicator of β-cell dysfunction as described [24-26, 34]. The molar ratio of

glucagon to insulin was also calculated as described [35].

Statistical analysis

The values are presented as the mean ± standard deviation (S.D.) in cases of normal distribution or otherwise as the medians plus 25th and 75th percentiles. We used Student's *t*-test, the Wilcoxon rank sum test, and the Chi-square test to test differences in clinical characteristics between the NGT and IGT/DM groups. A repeated measures analysis of variance was used to test the differences in the values of glucose, insulin, proinsulin, and glucagon at each time-point during the OGTT between the NGT and IGT/DM groups. Logistic regression analyses of the results obtained from the early-postpartum OGTT were used to predict the risk of developing IGT/DM at 1 year post-delivery. In the multivariate analyses, the risk factors were calculated after adjustment for age, BMI, and lactation intensity at the time-point of the early-postpartum OGTT. The adjustment of lactation intensity was performed using continuous variables depending on each woman's lactation-intensity category; *i.e.*, categories 1 (exclusively lactation), 2 (mostly lactation), 3 (mixed lactation), 4 (mostly formula), and 5 (exclusively formula) were converted into 1, 0.8, 0.5, 0.2, and 0, respectively. The statistical analyses were carried out using JMP pro ver. 15 software (SAS, Cary, NC). *P*-values <0.05 were considered significant.

Results

Over one-third of the women with GDM had developed glucose intolerance at 1 year post-delivery

As shown in Fig. 1, 56 of the 59 patients with GDM who provided an informed consent underwent the early-postpartum OGTT at 6–12 weeks post-delivery. All of the enrolled women were advised to consume a diet that was based on their lactation intensity category, as described in Study design. Three of these 56 women were withdrawn from the study because of a lack of hormone data in the early-postpartum OGTT. Ten women were withdrawn because they did not visit our hospital to undergo an OGTT at 1 year post-delivery. Accordingly, a final total of 43 women with recent GDM completed the study.

In the OGTT at 1 year post-delivery, 17 (39.5%) of the 43 women had developed glucose intolerance (two with DM and 15 with IGT), defined as the IGT/DM group. The other 26 (60.5%) women showed a normal glycemic result at 1 year postpartum, defined as the NGT group.

Comparisons of ante- and postpartum characteristics between the IGT/DM and NGT groups

As shown in Table 1, all of the antenatal characteristics including maternal age, parental history of T2DM, pre-gravid weight and BMI, glycemic results of the OGTT during pregnancy, and the rate of insulin treatment during pregnancy were not significantly different between the IGT/DM and NGT groups. At the early (6–12 weeks) postpartum period, there were no significant between-group differences in body weight or BMI. The number of postpartum weeks at which the OGTT was performed was not significantly different between the groups.

The percentage of showing IGT in the early-postpartum OGTT was significantly higher in the IGT/DM group than the NGT group (64.7 *vs.* 11.5, $p < 0.001$). Six (20.7%) of the 29 women who showed a normal glycemic profile at the early postpartum period had developed IGT or DM at 1 year post-delivery, while three (21.4%) of the 14 women who showed IGT during the early postpartum period recovered to NGT during the year post-delivery. The IGT/DM group showed a tendency of less breastfeeding compared to the NGT group, but no significant between-group differences were observed in any of lactation-intensity categories.

The clinical characteristics at 1 year post-delivery are summarized in Table 1. There were no significant differences in body weight, BMI, weight gain after delivery, or the rate of women continuing breastfeeding between the groups.

Differences in the glycemic and hormonal responses during the early-postpartum OGTT between the IGT/DM and NGT groups

The IGT/DM group showed significantly higher glucose levels at 30, 60 and 120 min post-glucose load during the early-postpartum OGTT compared to those in the NGT group (Fig. 2A). The IGT/DM group showed a delayed peak of insulin secretion; however, there were no significant between-group differences in the concentrations of serum insulin at each time-point of the OGTT (Fig. 2B). The levels of serum proinsulin at each time-point of the OGTT and its secretory patterns in the IGT/DM group were quite similar to those in the NGT group (Fig. 2C). The IGT/DM group did not show an evident suppression of glucagon at 30 min after the glucose load, whereas a prompt decline of glucagon was observed in the NGT group. The levels of plasma glucagon (pg/mL) at 30 min during the OGTT were significantly higher in the IGT/DM group compared to the NGT group (14.7 ± 7.9 *vs.* 8.8 ± 6.8, $p = 0.011$) (Fig. 2D).

We evaluated indexes of the hormones obtained from the early-postpartum OGTT (Table 2). The insulin sensitivity evaluated using the HOMA-IR and Matsuda index tended to be lower but not significantly so in the

Table 1 Comparison of ante- and postpartum characteristics between the IGT/DM and NGT groups

	IGT/DM group (n = 17)	NGT group (n = 26)	p-value
Antepartum			
Age, yrs	35.1 ± 5.6	32.8 ± 4.9	0.19
Parental history of T2DM, *n* (%)	6 (35.3)	8 (30.8)	0.75
Height, cm	157 ± 5	158 ± 6	0.24
Pre-gravid body weight, kg	56.6 ± 9.6	55.5 ± 10.3	0.74
Pre-gravid BMI, kg/m^2	22.9 ± 3.4	21.9 ± 3.3	0.26
75-g OGTT during pregnancy, mg/dL			
Fasting glucose	82 ± 12	79 ± 8	0.27
1-hr glucose	185 ± 27	174 ± 25	0.082
2-hr glucose	167 ± 35	155 ± 22	0.11
Insulin therapy during pregnancy, *n* (%)	8 (47.1)	8 (30.8)	0.18
Early postpartum			
Postpartum weeks at 75-g OGTT	8.3 ± 0.8	9.1 ± 2.1	0.095
Glucose tolerance, *n* (%)			
NGT	6 (35.3)	23 (88.5)	<0.001
IGT	11 (64.7)	3 (11.5)	<0.001
Body weight, kg	56.8 ± 9.5	55.3 ± 9.9	0.64
BMI, kg/m^2	23.1 ± 3.7	21.8 ± 3.1	0.19
Lactation intensity, *n* (%)			
Exclusive lactation	4 (23.5)	13 (50.0)	0.082
Mostly lactation	1 (5.9)	3 (11.5)	0.53
Mixed lactation	8 (47.0)	5 (19.2)	0.052
Mostly formula	3 (17.6)	1 (3.8)	0.13
Exclusive formula	1 (5.9)	4 (15.7)	0.34
One year post-delivery			
Postpartum months at 75-g OGTT	12.1 ± 1.6	12.5 ± 1.0	0.24
Glucose levels in OGTT, mg/dL			
Fasting	98 ± 11	92 ± 6	0.021
2-hr	166 ± 20	110 ± 14	<0.001
Body weight, kg	55.7 ± 9.0	55.1 ± 10.7	0.84
Weight gain after delivery, kg	–1.1 ± 5.4	–0.2 ± 3.4	0.52
BMI, kg/m^2	22.6 ± 3.5	21.7 ± 3.5	0.42
Continued breastfeeding, *n* (%)	6 (35.3)	14 (53.8)	0.45

The data are mean ± S.D. unless otherwise indicated. BMI, body mass index; DM, diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus

IGT/DM group compared to the NGT group. The insulin secretory capacity expressed by the insulinogenic index was significantly decreased in the IGT/DM group compared to the NGT group (median, 0.46 *vs.* 0.76; p = 0.014). The β-cell function assessed by the disposition index and ISSI-2 in the IGT/DM group was inferior to that of the NGT group.

The glucose-stimulated early response of glucagon evaluated by the changes in glucagon concentrations from fasting to 30 min (ΔGlucagon 30 min, pg/mL) was significantly different between the IGT/DM and NGT groups (–1.4 ± 8.3 *vs.* –6.7 ± 6.4, p = 0.019). The ratio of the plasma glucagon levels at 30 min to those at baseline (0 min), shown as Glucagon 30 min/Glucagon 0 min, was significantly higher in the IGT/DM group than the NGT group (1.2 ± 0.7 *vs.* 0.6 ± 0.4, p = 0.003). The

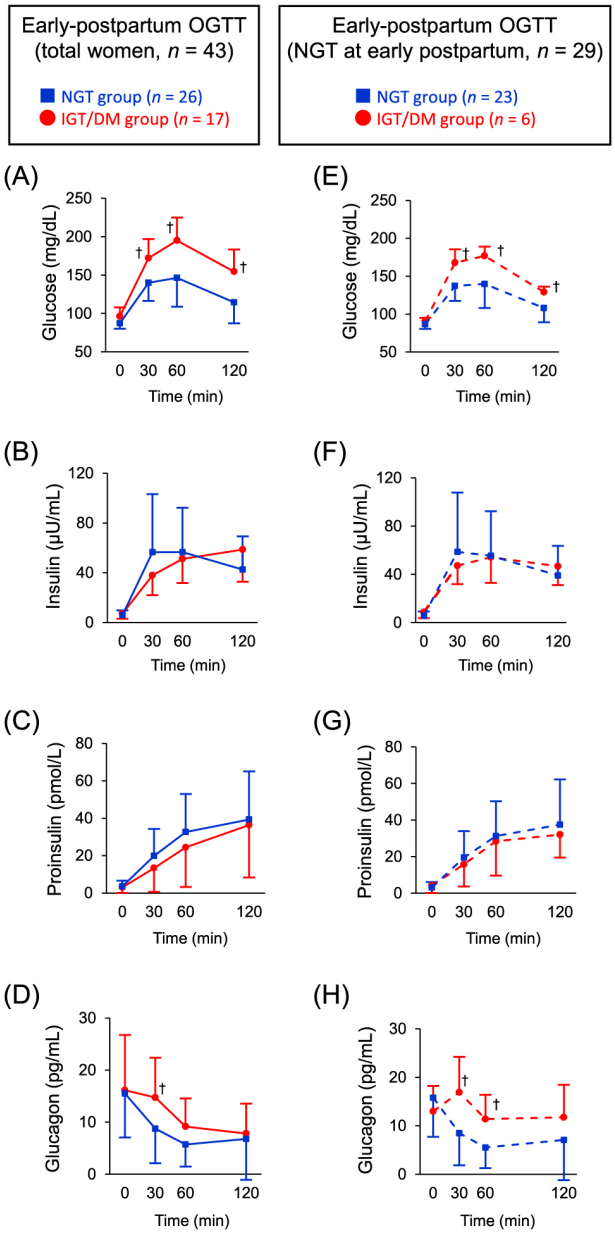

Fig. 2 Values of glucose (A), insulin (B), proinsulin (C) and glucagon (D) of the early-postpartum OGTT in the total 43 women with recent GDM. Values of glucose (E), insulin (F), proinsulin (G) and glucagon (H) of the early-postpartum OGTT in the 29 women who showed a normal glycemic result in the OGTT. *Blue squares*: the NGT group. *Red circles*: the IGT/DM group. *Bar lines*: S.D. † $p < 0.05$ for the IGT/DM group *vs.* the NGT group.

relative change in glucagon to those in glucose from fasting to 30 min, shown as ΔGlucagon 30 min/ΔGlucose 30 min, was also significantly different between the IGT/DM and NGT groups ($p = 0.022$). The late-phase responses of glucagon indicated by ΔGlucagon 60 min and ΔGlucagon 120 min were comparable between the groups (Table 2).

The AUCs of insulin, proinsulin, and glucagon from fasting to 120 min during the OGTT were not significantly different between the groups. The molar ratios of proinsulin-to-insulin at all of the time-points and the AUCs during the OGTT were comparable between the groups. The glucagon-to-insulin ratios at 30 and 60 min were significantly higher in the IGT/DM group than the NGT group (Table 2).

Next, we studied the hormonal responses in only women who showed a normal glycemic result at early postpartum ($n = 29$). As shown in Fig. 2E, the women who developed IGT/DM 1 year post-delivery (the IGT/DM group, $n = 6$) showed higher glucose levels at 30, 60 and 120 min during the early-postpartum OGTT compared to the women who maintained NGT until 1 year post-delivery (the NGT group, $n = 23$). The levels of insulin and proinsulin were not different between the IGT/DM and NGT groups (Fig. 2F, G). The IGT/DM groups showed a paradoxical increase in plasma glucagon levels after glucose load, while the NGT group immediately suppressed the glucagon secretion (Fig. 2H). These glycemic and hormonal responses of the initial group of women who showed a normal glycemic result at early postpartum ($n = 29$) closely resembled those in the total ($n = 43$) women regardless of their glucose tolerance at early postpartum. Likewise, the significant differences in the indexes of the disposition index, ISSI-2, ΔGlucagon 30 min, ΔGlucagon 30 min/ΔGlucose 30 min and Glucagon 30 min/Glucagon 0 min between the IGT/DM and NGT groups were preserved even when analyzing only the normal glycemic women at early postpartum ($n = 29$). The insulinogenic index did not show a significant difference but tended to be lower in the IGT/DM group compared to the NGT group when analyzing only the normal glycemic women at early postpartum (Supplemental Table 1).

Predictive factors from the early-postpartum OGTT results for the development of IGT/DM at 1 year post-delivery

The insulinogenic index is a reliable and widely recognized index of insulin secretory capacity from β-cells. The ΔGlucagon 30 min is not a fully recognized parameter, but it is a simple and easily comprehensible index that can be used to evaluate the early-phase secretory response of glucagon to glucose ingestion. Considering that both the insulinogenic index and ΔGlucagon 30 min in the early-postpartum OGTT results in the present series of women were significantly different between the IGT/DM and NGT groups (Table 2), we hypothesized that these indexes might serve as predictive factors for the development of IGT/DM at 1 year post-delivery.

We performed logistic regression analyses of the factors obtained from the early-postpartum OGTT results to

Table 2 Comparison of the indexes from the early-postpartum OGTT between the IGT/DM and NGT groups

	IGT/DM group (n = 17)	NGT group (n = 26)	p-value
HOMA-IR	1.7 ± 1.0	1.3 ± 1.0	0.29
Matsuda index	4.5 (3.6–7.1)	7.1 (4.8–9.9)	0.072
HOMA-β	78.9 ± 46.7	87.1 ± 42.4	0.56
Insulinogenic index	0.46 (0.35 ± 0.58)	0.76 (0.35 ± 1.32)	0.014
Disposition index	2.3 (1.4 ± 2.9)	4.5 (3.1 ± 6.6)	0.001
ISSI-2	1.3 (1.1 ± 1.7)	2.1 (1.7 ± 2.5)	<0.001
ΔGlucagon 30 min, pg/mL	–1.4 ± 8.3	–6.7 ± 6.4	0.019
ΔGlucagon 60 min, pg/mL	–7.0 ± 9.0	–9.8 ± 6.8	0.21
ΔGlucagon 120 min, pg/mL	–8.3 ± 10.9	–8.7 ± 9.1	0.85
ΔGlucagon 30 min/ΔGlucose 30 min, $\times 10^2$	–3.1 ± 1.2	–13.4 ± 1.3	0.022
Glucagon 30 min/Glucagon 0 min	1.2 ± 0.7	0.6 ± 0.4	0.003
AUC Insulin 0–120 min, μU/mL	5,394 ± 1,866	5,614 ± 3,215	0.80
AUC Proinsulin 0–120 min, pmol/L	2,634 ± 2,080	3,294 ± 2,032	0.32
AUC Glucagon 0–120 min, pg/mL	1,331 ± 704	956 ± 659	0.090
Molar ratio of proinsulin-to-insulin, $\times 10^{-2}$			
Fasting (0 min)	7.5 ± 7.2	10.0 ± 1.6	0.63
30 min	3.7 ± 3.0	5.5 ± 3.7	0.12
60 min	6.9 ± 5.0	9.1 ± 4.4	0.15
120 min	9.5 (4.5 ± 16.0)	12.0 (8.6 ± 18.0)	0.086
AUC 0–120 min	7.4 ± 4.8	9.1 ± 4.7	0.259
Molar ratio of glucagon-to-insulin, $\times 10^{-2}$			
Fasting (0 min)	9.2 (5.3 ± 18.0)	11.3 (7.5 ± 16.0)	0.57
30 min	1.3 (0.9 ± 2.4)	0.7 (0.3 ± 1.2)	0.004
60 min	0.8 (0.4 ± 1.1)	0.4 (0.2 ± 0.8)	0.030
120 min	0.6 (0.3 ± 0.8)	0.4 (0.2 ± 1.2)	0.89
AUC 0–120 min	1.2 ± 0.7	0.9 ± 0.9	0.19

The data are mean ± S.D. in cases of normal distribution, or otherwise as a median plus interquartile range. AUC, area under the curve; HOMA-β, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; ISSI-2, insulin secretion/sensitivity index-2

predict the development of IGT/DM at 1 year post-delivery (Table 3). In univariate analyses, the 1-year postpartum development of IGT/DM was significantly associated with the insulinogenic index (odds ratio [OR] 0.04, p = 0.001) and ΔGlucagon 30 min (OR 1.11, p = 0.019) and with the FPG (OR 1.12, p = 0.003) and 2-hr PG (OR 1.06, p < 0.001) values. In multivariate regression models, the insulinogenic index was identified as an independent risk factor of the 1-year postpartum development of IGT/DM when adjusted for age, BMI, lactation intensity, and FPG (OR 0.01, p = 0.001) (Model 1), but not when adjusted for 2-hr PG instead of FPG (Model 2). ΔGlucagon 30 min did not remain as an independent variable after the adjustment (Models 1 and 2).

From the clinical point of view, the optimal thresholds of these secretory indexes should be determined. We used cut-off values of 0.6 unit for the insulinogenic index and 0 pg/mL for ΔGlucagon 30 min, which were determined using receiver operating characteristic analyses. An insulinogenic index value <0.6 and a ΔGlucagon 30 min value >0 pg/mL were significantly associated with the 1-year postpartum development of IGT/DM in univariate analyses (OR 5.20, p = 0.013 and OR 4.89, p = 0.024, respectively). These associations remained significant after adjusting for age, BMI, lactation intensity, and FPG (OR 10.2, p = 0.010 for the insulinogenic index <0.6 and OR 6.83, p = 0.046 for ΔGlucagon 30 min >0 pg/mL) (Model 3). ΔGlucagon 30 min >0 pg/mL remained as a significant risk factor after the adjustment for age, BMI, lactation intensity, and 2-hr PG (OR 9.84, p = 0.049) (Model 4).

The combination of the insulinogenic index <0.6 and

Table 3 Logistic regression analyses of risk factors obtained from the early-postpartum OGTT to predict development of IGT/DM one year after delivery

Predictive factors from the early-postpartum OGTT	Univariate		Multivariate*			
			Model 1		Model 2	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
FPG, mg/dL	1.12 (1.04–1.25)	0.003	1.14 (1.01–1.37)	0.024	N.A.	
2-hr PG, mg/dL	1.06 (1.03–1.11)	<0.001	N.A.		1.06 (1.02–1.13)	0.001
Insulinogenic index	0.04 (0.00–0.44)	0.001	0.01 (0.00–0.29)	0.001	0.02 (0.00–1.05)	0.068
ΔGlucagon 30 min, pg/mL	1.11 (1.02–1.25)	0.019	1.07 (0.95–1.23)	0.24	1.07 (0.95–1.23)	0.28

Predictive factors from the early-postpartum OGTT	Univariate		Multivariate*			
			Model 3		Model 4	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
FPG, mg/dL	1.12 (1.04–1.25)	0.003	1.13 (1.03–1.32)	0.010	N.A.	
2-hr PG, mg/dL	1.06 (1.03–1.11)	<0.001	N.A.		1.07 (1.03–1.14)	<0.001
Insulinogenic index <0.6	5.20 (1.32–20.4)	0.013	10.2 (1.68–104)	0.010	5.66 (0.53–104)	0.16
ΔGlucagon 30 min >0 pg/mL	4.89 (1.17–20.4)	0.024	6.83 (1.04–68.7)	0.046	9.84 (1.01–231)	0.049
Insulinogenic index <0.6 and ΔGlucagon 30 min >0 pg/mL	4.99 (0.93–38.6)	0.061	N.A.		N.A.	

Predictive factors from the early-postpartum OGTT	Univariate		Multivariate*			
			Model 5		Model 6	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
FPG, mg/dL	1.12 (1.04–1.25)	0.003	1.13 (1.02–1.29)	0.007	N.A.	
2-hr PG, mg/dL	1.06 (1.03–1.11)	<0.001	N.A.		1.05 (1.02–1.10)	0.003
Molar ratio of glucagon-to-insulin						
Fasting (0 min)	0.29 (0.00–26.0)	0.59	N.A.		N.A.	
30 min	4.34 (1.16–4.49)	0.029	1.64 (1.48–2.95)	0.001	4.09 (0.00–7.62)	0.053
60 min	2.61 (1.30–6.23)	0.26	N.A.		N.A.	
120 min	6.47 (5.04–17.7)	0.22	N.A.		N.A.	

Predictive factors from the early-postpartum OGTT	Univariate		Multivariate*			
			Model 7		Model 8	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
FPG, mg/dL	1.12 (1.04–1.25)	0.003	1.13 (1.01–1.36)	0.029	N.A.	
2-hr PG, mg/dL	1.06 (1.03–1.11)	<0.001	N.A.		1.06 (1.02–1.13)	0.001
Insulinogenic index	0.04 (0.00–0.44)	0.001	0.01 (0.00–0.38)	0.003	0.02 (0.00–1.08)	0.090
Glucagon 30 min/Glucagon 0 min	4.29 (1.30–19.6)	0.014	3.61 (0.68–40.9)	0.14	4.39 (0.66–90.3)	0.13

* Adjusted for the age, BMI and lactation intensity categories at performing the early-postpartum OGTT in all the models of multivariate regression analyses. FPG, fasting plasma glucose; 2-hr PG, plasma glucose at 2 hours during the OGTT; OR, odds ratio; CI, confidence interval; N.A., not applicable

ΔGlucagon 30 min >0 pg/mL was not significant value for the prediction of IGT/DM at 1-year postpartum in a univariate analysis (OR 4.99, $p = 0.061$). The molar ratio of glucagon-to-insulin at 30 min during the early-postpartum OGTT was a significant value to predict the development of IGT/DM 1 year after delivery in a univariate analysis (OR 4.34, $p = 0.029$), and the bihormonal value was remained as a significant factor after a certain adjustment in Model 5 (OR 1.64, $p = 0.001$) but not in Model 6 of multivariate analyses (OR 4.09, $p = 0.053$). The ratio of Glucagon 30 min/Glucagon 0 min was a significant value for prediction of 1-year postpartum IGT/DM in a univariate analysis (OR 4.29, $p = 0.014$) but not in multivariate analyses (Model 7 and 8).

Discussion

We investigated hormonal responses to a glucose challenge during the early postpartum period in Japanese women with recent GDM based on the WHO-2013 criteria. The results of our analyses demonstrated that in addition to impaired acute-phase insulin secretion, the insufficient early-phase glucagon suppression in response to glucose stimulation observed during the early (6–12 weeks) postpartum period was another significant risk factor for the development of glucose intolerance at 1 year after childbirth. In the early-postpartum OGTT, it was useful to evaluate the insulinogenic index and ΔGlucagon 30 min to predict glucose intolerance at 1 year post-delivery, whereas the measurements of proinsulin did not contribute to the improvement of the predictability.

Women with a history of GDM have a greatly increased risk of developing T2DM later in life [1-3, 7]. Clinical practice guidelines recommend that women with GDM should undergo a 75-g OGTT at the early postpartum period [1, 2]. An early postpartum OGTT provided the best discrimination of individuals at high and low risk of developing T2DM 5–7 years after GDM compared to other clinical variables including age, parity, BMI, and fasting glucose values [36]. A study from Korea showed that the 2-hr PG in an OGTT performed at 1 year post-delivery had a unique and significant association with the subsequent development of T2DM [13]. To provide an efficient strategy to predict the progression to T2DM after GDM, it is desirable to identify women who develop glucose intolerance (*i.e.*, prediabetes) at 1 year after childbirth by an initial OGTT performed during the early postpartum period.

It was reported that people in East Asia including Japan tend to develop both T2DM and GDM with a lesser degree of obesity compared to people in other regions [37-39], due to a lesser β-cell capacity to secret insulin. In a Japanese population, impaired insulin secretion had a greater impact on the incidence of T2DM compared to insulin resistance [40]. Likewise, a lower insulin secretory capacity (lower value in the insulinogenic index) rather than higher insulin resistance was associated with the subsequent development of T2DM after GDM in the women in the Korean study [13]. Four genetic variants were recently nominated among the 45 known T2DM-associated genetic variants for a significant association with postpartum glucose intolerance in Japanese women with GDM [41]. Of the four genetic variants, three were identified as the genes responsible for insulin secretion. Therefore, impaired insulin secretion is an indisputable factor of postpartum glucose intolerance in Japanese women with GDM.

The dynamics of glucose-stimulated proinsulin, a precursor of insulin peptide, has been reported as a useful biomarker to detect β-cell dysfunction at an early stage [25, 26]. The hyperproinsulinemia caused by the increase in a premature release of proinsulin can reflect an increased demand for insulin. An elevated proinsulin-to-insulin ratio was reported as an indicator of impaired acute insulin response in prediabetic patients [42]. However, another study indicated that an elevated fasting proinsulin-to-insulin ratio could not predict later impairment of glucose tolerance in Caucasian women with previous GDM [43]. We did not detect any significant differences in the serum proinsulin level or proinsulin-to-insulin ratio in the early-postpartum OGTT between the NGT and IGT/DM groups (Fig. 2C, Table 2). Nor did we observe validity for the measurements of circulating proinsulin levels to predict a 1-year postpartum development of glucose intolerance after GDM.

It was reported that the insulin-resistant subjects showed a decreased proinsulin-to-insulin ratio because of reduced insulin clearance (reflecting hepatic insulin resistance) rather than enhanced proinsulin processing in β-cells [44]. In our present study, the hepatic insulin resistance evaluated by the HOMA-IR was higher (but not significantly so) in the IGT/DM group compared to the NGT group (Table 2). The slight difference in insulin resistance between the groups might have affected the values of proinsulin-to-insulin ratio.

Compared to the plentiful investigations of β-cell impairment, few studies have evaluated α-cell dysfunction for the pathophysiology of glucose intolerance in patients with GDM. We have demonstrated that an insufficient suppression of early-phase glucagon secretion in response to glucose ingestion was significantly associated with the requirement of insulin treatment during pregnancy in patients with GDM [23]. Most of those patients showed an amelioration of the glucagon abnormality concurrently with a recovery of glucose tolerance in the early postpartum period [23]. The glucagon secretory abnormality may emerge as early as the stage at which insulin action cannot compensate for increased insulin resistance caused by diabetogenic situations such as pregnancy or obesity.

In the present study, the glucagon abnormality in the early-postpartum OGTT was an independent risk factor for the subsequent development of glucose intolerance in women with recent GDM (Table 3). Most women with GDM return to seemingly normal glucose tolerance within several weeks after delivery by recovering from the increased insulin resistance caused by pregnancy. It is important to be able to identify women with stronger diabetogenicity among women with recent GDM by administering an OGTT in the early postpartum period.

To date, a gold standard of the indexes that evaluate glucagon abnormality has not been established. Individuals with diabetes show an inappropriate increase in glucagon during the early phase (around 30 min) after an oral glucose load [45], which is called "paradoxical hyperglucagonemia". This is the most distinguishable difference in glucagon secretions between individuals with and without diabetes. Herein, we observed that the positive change in the levels of plasma glucagon from fasting to 30 min during the OGTT (shown as ΔGlucagon 30 min >0 pg/mL) was useful to predict the development of IGT/DM at 1 year post-delivery (Table 3). The index of ΔGlucagon 30 min >0 pg/mL indicates paradoxical hyperglucagonemia.

The insulinogenic index used in the present study is a widely recognized, simple, and useful index for evaluating the insulin secretory capacity. To evaluate dual functions of pancreatic β- and α-cells, it may be acceptable to use both the insulinogenic index and ΔGlucagon 30 min, which can be simply calculated from the values of glucose, insulin, and glucagon at both fasting (0 min) and 30 min during an OGTT.

It was recently shown that lactation has a protective effect against the development of glucose intolerance during the first year postpartum, by improving insulin resistance [46]. Our present findings demonstrated that the insulinogenic index and ΔGlucagon 30 min were significant factors for the development of IGT/DM at 1 year post-delivery, independent of the lactation intensity (Table 3). This is a novel and strong finding.

We also demonstrated that the development of glucose intolerance at 1-year post-delivery was associated with other indexes of insulin and glucagon including the molar ratio of Glucagon 30 min/Insulin30min, the value of ΔGlucagon 30 min/ΔGlucose30min, and the value of Glucagon 30 min/Glucagon 0 min. These results can emphasize the significance of the bihormonal dysregulation to develop glucose intolerance in women with recent GDM.

Our study has several limitations to address. First, the sample size was small (n = 43), and all of the participants were recruited from a single center. The mean BMI of the analyzed women was slightly smaller than the previous reports in Japan [47]. The participants may thus not be representative of the entire population of Japanese women who were diagnosed with GDM based on the WHO-2013 criteria. Second, the study was conducted with only Japanese women, who are considered to be a high-risk ethnic group for T2DM because of the genetic characteristic of less insulin secretion capacity [37-39]. Our results might not be universally applicable. Third, we could not determine predictive factors for developing glucose intolerance at 1 year post-delivery among women who had shown a normal glycemic result in early postpartum, because the number of those women was too small to conduct logistic regression analyses. Fourth, we did not evaluate the insulin and glucagon responses during OGTT at 1-year postpartum in the participants. We could not ascertain whether the bihormonal disorder in women of the IGT/DM group had remained until 1 year after delivery. Fifth, the follow-up rate of the participants who underwent the early-postpartum OGTT was 77% (43 of 56 women). This might have affected the results of the study.

In summary, the results of our analyses demonstrated that the subsequent development of glucose intolerance at 1 year after delivery was associated with dysregulated glucagon secretion in addition to impaired insulin secretion in women with GDM diagnosed based on the WHO-2013 criteria. The bihormonal disorder of glucagon and insulin contributed to the 1-year postpartum development of glucose intolerance after GDM, independent of the women's age, BMI, and lactation intensity. The measurement of plasma glucagon during the initial OGTT at the early postpartum period can help clinicians make optimal decisions regarding the postpartum management of women with GDM.

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Disclosure

The authors declare no conflict of interest.

Supplemental Table 1 Comparisons of the results of the early-postpartum OGTT between the IGT/DM group and the NGT group only in women who showed a normal glycemic result at early postpartum (n = 29)

	IGT/DM group (n = 6)	NGT group (n = 23)	p-value
HOMA-IR	2.0 ± 1.2	1.3 ± 0.8	0.48
Matsuda index	5.6 ± 2.9	7.8 ± 3.7	0.11
HOMA-β	107 ± 50.2	86.9 ± 44.2	0.32
Insulinogenic index	0.46 (0.43–0.57)	0.76 (0.39–1.34)	0.17
Disposition index	2.4 (1.6–3.2)	4.6 (2.3–6.6)	0.011
ISSI-2	1.4 ± 0.3	2.3 ± 0.8	0.006
ΔGlucagon 30 min, pg/mL	3.9 ± 7.6	–7.3 ± 6.4	0.007
ΔGlucagon 60 min, pg/mL	–1.6 ± 7.2	–10.3 ± 5.8	0.023
ΔGlucagon 120 min, pg/mL	–1.2 ± 8.5	–8.7 ± 8.7	0.084
ΔGlucagon 30 min/ΔGlucose 30 min, $\times 10^2$	3.8 ± 11.3	–13.8 ± 12.6	0.009
Glucagon 30 min/Glucagon 0 min	1.6 ± 0.9	0.6 ± 0.4	0.005
AUC Insulin 0–120 min, μU/mL	5,397 ± 1,718	5,518 ± 3,304	0.69
AUC Proinsulin 0–120 min, pmol/L	2,777 ± 1,523	3,163 ± 1,922	0.87
AUC Glucagon 0–120 min, pg/mL	1,332 ± 607	957 ± 678	0.027
Molar ratio of proinsulin-to-insulin, $\times 10^{-2}$			
Fasting (0 min)	10.0 ± 10.5	7.8 ± 5.9	0.50
30 min	4.8 ± 3.7	5.5 ± 3.7	0.70
60 min	8.5 ± 6.1	9.1 ± 4.4	0.92
120 min	11.8 ± 6.2	19.4 ± 2.6	0.46
AUC 0–120 min	8.3 ± 5.1	8.7 ± 3.9	0.84
Molar ratio of glucagon-to-insulin, $\times 10^{-2}$			
Fasting (0 min)	6.2 (3.9–7.6)	14.8 (9.0–16.1)	0.14
30 min	1.6 ± 0.9	0.9 ± 0.7	0.031
60 min	0.9 ± 0.4	0.5 ± 0.5	0.024
120 min	1.1 (0.6–1.6)	0.4 (0.2–1.3)	0.24
AUC 0–120 min	1.3 ± 0.6	0.8 ± 0.7	0.12

The data are mean ± S.D. in cases of normal distribution, or otherwise as a median plus interquartile range. AUC, area under the curve; HOMA-β, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; ISSI-2, insulin secretion/sensitivity index-2

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