

Characteristics of repaglinide and its mechanism of action on insulin secretion in patients with newly diagnosed type-2 diabetes mellitus

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Abstract

This study aims to compare the effect of repaglinide and metformin among Chinese patients with newly diagnosed diabetes, and explore the possible mechanisms by which repaglinide alters insulin secretion.

Sixty subjects with glycated hemoglobin (HbA1c) < 10.0% were randomly selected to receive repaglinide or metformin monotherapy for 15 weeks. Blood glucose levels, glycemic variability, β -cell function, and first-phase insulin secretion were compared between these 2 groups at baseline and at 15 weeks. Mouse insulinoma (MIN-6) cells were divided into 3 groups: low glucose, high glucose, and repaglinide 50 nm groups. Cells and cell culture mediums were collected at different timepoints. The expression of pericentrin (PCNT), F-actin, and insulin were tested with immunofluorescence and enzyme-linked immunosorbent assay.

All glycemic parameters and variability indexes significantly decreased from baseline to 15 weeks, while no significant difference was found between these 2 groups at baseline or at 15 weeks. Furthermore, there was no significant difference found in fasting insulin and postprandial insulin at baseline and at 15 weeks, while homeostasis model assessment β significantly increased. The first-phase glucose and insulin secretion of the intravenous glucose tolerance test improved in both groups, especially in the repaglinide group. Insulin, PCNT, and F-actin expression in MIN-6 cells decreased after 15 minutes of stimulation with repaglinide, while no difference was observed at 2, 6, and 12 hours. The insulin levels of the cell medium in the repaglinide group remained significantly higher at all timepoints.

This study manifests that repaglinide has a noninferiority effect on the glycemic parameters of Chinese patients with newly diagnosed diabetes, when compared with metformin. The PCNT-F-actin pathway plays an important role in the repaglinide regulation process of on-demand insulin secretion.

Abbreviations: BSA = bovine serum albumin, DMEM = Dulbecco modified Eagle medium, FINS = fasting insulin level, FPG = fasting plasma glucose, GLP-1 = glucagon-like peptide-1, HG = high glucose, HOMA- β = homeostasis model assessment β , IVGTT = intravenous glucose tolerance tests, LG = low glucose, MAGE = mean amplitude of glycemic excursions, PCNT = pericentrin, PPG = postprandial plasma glucose, SURs = sulfonylurea receptors, Sus = sulfonylureas, T2DM = type-2 diabetes mellitus, 2-hour INS = postprandial insulin level, UKPDS = United Kingdom Prospective Diabetes Study.

Keywords: diabetes, F-actin, first-phase insulin secretion, pericentrin, repaglinide

1. Introduction

With rapid economic growth and subsequent lifestyle changes, diabetes has become a worldwide epidemic, and its prevalence in

China has reached 10.9%.^[1] A diagnosis of diabetes relates closely with increased morbidity and mortality. Meanwhile the recent findings highlighted the indication to control blood glucose levels as early as midlife for prevention of late-life dementia.^[2] In fact, diabetes has been found to be well-controlled in <40% of cases, which is suboptimal.^[3] Type-2 diabetes mellitus (T2DM) is a heterogeneous metabolic disorder that incorporates insulin resistance and defective insulin secretion. It has been well-accepted that a reduction in β -cell function occurs prior to the diagnosis of T2DM, as indicated by the United Kingdom Prospective Diabetes Study (UKPDS).^[4] The impairment of early phase insulin secretion has been considered to be an early marker of T2DM.

A typical Chinese diet is primarily composed of carbohydrates. Hence, newly diagnosed patients are usually characterized by higher postprandial blood glucose levels.^[3] The restoration of early phase insulin secretion is therefore important for slowing disease progression, especially among Chinese patients.

Repaglinide, one of the nonsulfonylurea insulin secretagogues, belongs to the meglitinide class, which has a benzoic acid structure. Sulfonylurea receptors (SURs) are a subunit of the ATP-sensitive K⁺ channel that repaglinide may close, thereby inducing subsequent Ca²⁺ influx through voltage-gated Ca²⁺

Editor: Inyang Nora Osemene.

This study was supported by National Natural Science Foundation of China (no: 81373458).

The authors have no conflicts of interest to disclose.

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Medicine (2018) 97:38(e12476)

Received: 10 April 2018 / Accepted: 27 August 2018

<http://dx.doi.org/10.1097/MD.0000000000012476>

channels, followed by the exocytosis of Ca^{2+} -dependent insulin granules.^[5] The mechanism of repaglinide is similar to sulfonylureas. However, repaglinide exhibits distinct pharmacologic properties in terms of structure, binding profile, duration of action, and mechanisms of excretion.^[6] Several studies have demonstrated that Ca^{2+} elevation activates multiple pathways for the disruption of the cytoskeleton, which may lead to the attenuation of insulin granule mobility. Sulfonylureas (Sus) bind tightly to SURs and exhibit delayed onset and prolonged hypoglycemic effects. Glinides bind to a site at the β -cell membrane that is distinct from the binding site of sulfonylureas. Compared to sulfonylureas, glinides display anti-hyperglycemic activity that is more rapid and shorter.^[7] Furthermore, repaglinide has been considered to reduce postprandial glucose levels by enhancing the early phase of insulin secretion and increasing the total amount of insulin secreted.^[8]

Metformin has been suggested to be the drug of first choice after failure of lifestyle modification in obese patients with T2DM. Chinese Diabetes Society guidelines also suggest metformin as the first-line treatment for overweight/obese patients.^[4,9] The main mechanism of metformin is improving insulin resistance. It is also a relatively safe oral glucose-lowering drug with a low associated risk of hypoglycemia.

Pericentrin (PCNT), a component of the pericentriolar material (PCM), is a highly conserved protein throughout the animal up to human. PCNT has important functions including regulation the structure and function of centrosome, spindle organization, and microtubule nucleation in cell cycle progression and signaling processes.^[10] Certain kinds of diseases including premature diabetes were related to PCNT mutation or disruption.^[11,12] Recently, emerging studies suggest that PCNT is involved in the progression of the secretion of insulin in beta cells.^[13] A previous study conducted by the investigators revealed that PCNT plays an important role in insulin secretion regulation effect through F-actin. Decreased PCNT expression lead to F-actin depolymerization, which may cause hypersecretion of insulin.^[14] The present study sought to address whether repaglinide could induce the restoration of early phase insulin secretion noninferiority with metformin. Furthermore, the present study investigates the changes of PCNT and F-actin in the first and second phases, and determines whether these play a role in the mechanism by which repaglinide acts like a fast secretagogue. Moreover, the present study aims to compare the effect of repaglinide and metformin among Chinese patients with newly diagnosed diabetes and explores the possible mechanisms of PCNT and F-actin in the effect of repaglinide on insulin secretion using mouse insulinoma (MIN-6) cells.

2. Methods

2.1. Ethical approval

The present study was approved by the Medical Ethics Committee of the Chinese PLA General Hospital, and a written informed consent was obtained from each subject before any study procedure was performed. This trial is registered at chictr.org.cn (ChiCTR-ONRC-11001647). Please refer to the previous research reference.^[4]

2.2. Study subjects

Chinese, drug-naive patients, who were within 20 to 90 years old, newly diagnosed with T2DM and have an HbA1c level of

<10.0%, were randomized (2:1) to receive either repaglinide (n = 40, M/F 21/19) or metformin (n = 20, M/F 16/4) monotherapy. None of these subjects had a history of coronary heart disease, abnormal renal function, active liver disease, unstable angina, alcohol or drug abuse, chronic or severe metabolic acidosis, or chronic gastrointestinal disease.

2.3. Patient study protocol

The dose of metformin was 500 mg tds. The administration of repaglinide was adjusted according to blood glucose levels during the 1st week. During this phase, the doses of all patients were optimized. Then, the patients were reviewed once/week for the first 1 month, and once/month for the next 2 months. All patients arrived at 8 AM after fasting overnight (10–12 hours), and an intravenous catheter was inserted for blood sampling. Blood samples were collected for the measurement of the levels of fasting blood glucose, insulin, HbA1c, and lipids. Body weight, height, and blood pressure were also measured. Then, all patients were given a standard meal (total energy content: 2625 kJ) with 34% fat, 14% protein, and 52% carbohydrates. Postprandial blood samples and insulin levels were collected at 2 hours after breakfast. Intravenous glucose tolerance tests (IVGTT) were performed on all patients at baseline and at week 15. All participants received both dietary and exercise advice. They were asked to follow a recommended controlled-energy diet (25–35 kcal/kg per day) and to undertake aerobic activity for at least 30 minutes on 5 occasions per week. Plasma glucose levels were measured using a glucose-oxidase-based approach, and insulin concentration was determined using a RIA kit (American Diagnostic Products Corporation, Hauppauge, NY), according to the manufacturer's manual.

2.4. Evaluation indexes

The primary endpoint was the change in HbA1c. Secondary endpoints included the evaluations of β -cell function and glycemic variability.

β -cell function was evaluated using the following 4 methods:

1. Fasting insulin level (FINS) and postprandial insulin level (2-hour INS)
2. Curves of the IVGTT: the glucose and insulin curves in IVGTT, including the 1st phase (0–15 minutes), second phase (30–120 minutes), and total IVGTT (0–120 minutes)
3. AUCs: area under the glucose and insulin curve in IVGTT, including the 1st phase AUCs (0–15 minutes), second phase AUCs (30–120 minutes), and total IVGTT AUCs (0–120 minutes)
4. Homa beta cell function index ($\text{HOMA-}\beta$) = $\text{FINS} \times 20 / (\text{GLU } 0-3.5) (\%)$.^[15]

Glycemic variability was evaluated using a continuous glucose monitoring system (MiniMed, Medtronic, Inc, Northridge, CA) at both baseline and at week 15. A 48-hour recording was performed using the continuous glucose monitoring system (CGMS) device to quantify the mean amplitude of glycemic excursions (MAGEs) and standard deviation of blood glucose in each patient at base line and at week 15 (16).

2.5. MIN6 cell culture

The MIN6 cells were maintained in Dulbecco modified Eagle medium (DMEM; 1 g/L of glucose; Gibco, Grand Island, NY)

supplemented with 15% FBS. Cells were incubated at 37°C in a 5% CO₂-humidified incubator. When cells reached 70% to 80% confluence, the culture medium was sucked out and changed to low sugar DMEM (1 g/L of glucose; Gibco), high sugar DMEM (4.5 g/L of glucose; Gibco), and 50 nM medium prepared from high sugar DMEM (containing 4.5 g/L of glucose; Gibco) + repaglinide (Sigma-Aldrich, St. Louis, MO). After 15 minutes of stimulation, cells were collected for trichrome immunofluorescence staining (PCNT, INS, and F-actin), and the cell culture was collected to test for insulin concentration.

2.6. Trichrome immunofluorescence staining

Cells were grown in glass-bottom culture dishes at 37°C with 5% CO₂, according to the indicated timepoints. Then, cells were washed in phosphate buffered saline (PBS), fixed/permeabilized on ice for 30 minutes in fixation solution (2% paraformaldehyde in PBS), washed 4 times in PBS, and blocked for 15 minutes with 1% bovine serum albumin (BSA) in PBS. Next, the fixed cells were incubated at 4°C overnight with primary antibodies against PCNT (Abcam, Cambridge, MA), insulin (Abcam), and F-actin (Abcam), which were diluted at 1:400, 1:200, or 1:100 in PBS with 1% BSA. Then, cells were incubated for 1 hour in PBS containing 1% BSA and the appropriate secondary antibodies (bs-0295G, goat anti-rabbit IgG/Cy3, 1:100 dilution; bs-0358G, goat anti-guinea pig IgG/fluorescein isothiocyanate (FITC) 1:50 dilution; bs-0368G, goat anti-mouse IgM/Alexa flour 647, 1:100 dilution; Bioss, Beijing, China). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (Abcam) and the slides were analyzed by microscopic examination (Olympus U-RFL-T, Tokyo, Japan). Confocal imaging was performed using a confocal microscope (Radiance 2000; BioRad, California) with a 60× chromatic aberration free+infinity (CFI) plan Apo objective and a filter optimized for mCherry fluorescence.

2.7. Statistical analysis

All measured parameters were determined as mean ± standard deviation values. The number of subjects was indicated by n. The normality of the data was tested using SPSS 10.0 software (SPSS Inc, Chicago, IL). Independent samples *t* test was used to compare each parameter between the 2 patient groups. A *P*-value <.05 was considered statistically significant.

3. Results

3.1. Patients

A total of 71 subjects were screened, and 60 participants were randomly assigned to receive either repaglinide (n=40) or metformin (n=20). The data of 1 patient in the repaglinide group, who dropped out at the last visit, were excluded from the study. Demographic and clinical characteristics, including age (46.4 ± 10.6 vs 49.7 ± 10.0), diabetes duration (months, 0.8 ± 1.3 vs 0.4 ± 0.4), and body mass index (26.2 ± 3.5 vs 25.1 ± 3.1) were well-balanced between the repaglinide and metformin groups.

3.2. Evaluation of HbA1c

At baseline, there was no statistically significant difference in mean HbA1c between the repaglinide group and metformin group (8.18 ± 1.8% vs 8.12 ± 1.8%). The mean changes in HbA1c during the study period were -1.8 ± 1.5% in the repaglinide group (*P* <.01) and -1.6 ± 1.5% in the metformin

group (*P* <.01). No significant difference was found in HbA1c between these 2 groups at 15 weeks. At baseline, 22.5% of patients in the repaglinide group and 35% of patients in the metformin group had an HbA1c of <7.0%. At the end of the trial, 87.2% of patients in the repaglinide group and 90% of patients in the metformin group achieved an HbA1c of <7.0%. The distribution of HbA1c considerably changed from baseline to 15 weeks (*P* <.01). The proportion of patients with an HbA1c of <6.5% and 6.5% to 7% increased, while patients with an HbA1c of 7.0% to 8.0% and >8.0% decreased (Fig. 1A).

3.3. Evaluation of glucose levels

The fasting plasma glucose (FPG) level was slightly higher in the metformin group at baseline (8.40 ± 1.91 mmol/L in the repaglinide group vs 9.18 ± 2.60 mmol/L in the metformin group). However, no significant difference could be found. There was also no difference in 2-hour postprandial plasma glucose (PPG) at baseline (14.40 ± 3.60 mmol/L in the repaglinide group vs 14.20 ± 4.30 mmol/L in the metformin group). The mean changes in FPG and PPG from baseline were -1.7 ± 1.7 and -3.8 ± 3.1 mmol/L in the repaglinide group (both, *P* <.01) and -2.1 ± 1.7 and -3.8 ± 3.6 mmol/L in the metformin group (both, *P* <.01), respectively. No significant difference was found in the above glycemic parameters at 15 weeks between these 2 groups (Fig. 1B).

3.4. Evaluation of glycemic variability

The MAGE at baseline was 4.8 ± 2.1 mmol/L in the repaglinide group and 4.4 ± 1.3 mmol/L in the metformin group. At 15 weeks, the changes were -1.4 ± 2.0 mmol/L in the repaglinide group vs -1.4 ± 1.6 mmol/L in the metformin group (both, *P* <.01). Mean blood glucose and Min and Max glucose levels also exhibited significant decreases from baseline (9.09 ± 2.47 mmol/L, 6.14 ± 1.80 mmol/L, and 13.09 ± 3.85 mmol/L in the repaglinide group; 9.20 ± 0.98 mmol/L, 6.30 ± 1.83 mmol/L, and 13.70 ± 3.76 mmol/L in the metformin group) to 15 weeks (6.88 ± 2.47 mmol/L, 4.63 ± 0.87 mmol/L, and 10.01 ± 2.12 mmol/L in the repaglinide group; 6.90 ± 1.48 mmol/L, 5.10 ± 1.22 mmol/L, and 9.54 ± 2.34 mmol/L in the metformin group) in both treatment groups (all, *P* <.01). For each of the above glycemic variability parameters from baseline to 15 weeks, there were no significant differences between the 2 treatment groups (Fig. 1C).

3.5. β-Cell function significantly improved in both the repaglinide group and metformin group

The FINS and 2-hour INS at baseline were 8.06 ± 4.71 U/L and 39.19 ± 22.03 U/L in the repaglinide group, respectively, and 8.81 ± 6.44 U/L and 34.82 ± 33.94 U/L in the metformin group, respectively. At 15 weeks, the FINS and 2-hour INS at baseline were 9.45 ± 6.91 U/L and 49.78 ± 33.57 U/L in the repaglinide group, respectively, and 7.71 ± 4.71 U/L and 33.46 ± 17.87 U/L in the metformin group, respectively. No significant difference was found in these 2 indexes between the 2 groups at baseline or at 15 weeks. There were also no differences between baseline and 15 weeks in these 2 groups (Fig. 2A). The same results were also found in fasting C-peptide and postprandial C-peptide (Fig. 2B).

The HOMA-β levels at baseline were 35.80 ± 30.82% in the repaglinide group and 37.63 ± 27.69% in the metformin group.

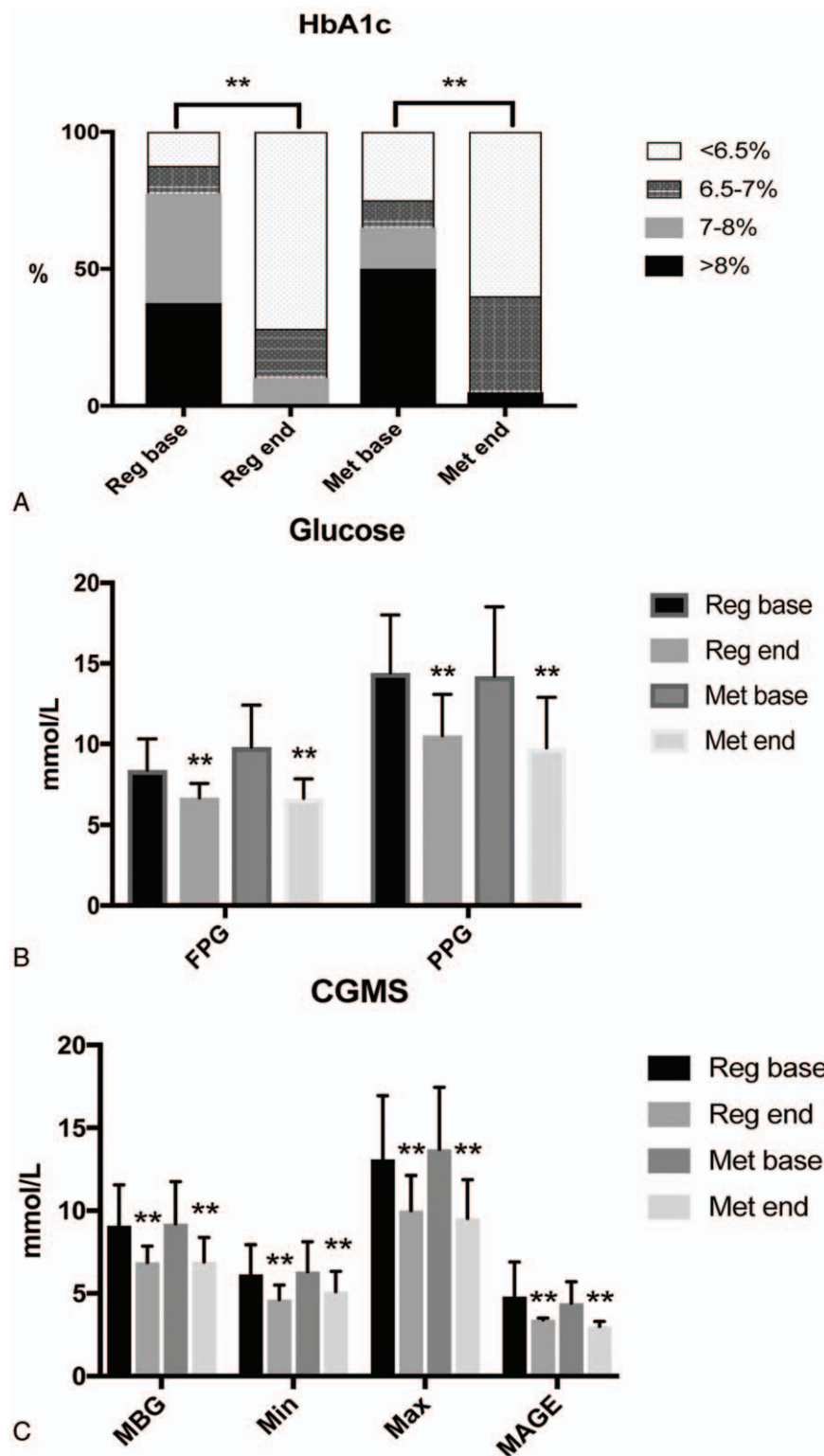


Figure 1. Glucose levels and glycemic variability at baseline and 15 weeks for the 2 groups. (A) The distribution of HbA1c changed obviously from baseline to 15 weeks for both groups. (B) fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) levels decreased significant from baseline to 15 weeks for both groups. (C) Glycemic variability parameters improved markedly from baseline to 15 weeks in both groups. MAGE = mean amplitude of glycemic excursions, MBG = mean blood glucose.

No significant difference was found between these 2 groups at baseline. HOMA-β levels increased by 44.44% in the repaglinide group and 63.91% in the metformin group at 15 weeks. Furthermore, at 15 weeks, HOMA-β levels were $51.71 \pm 27.41\%$

in the repaglinide group and $61.68 \pm 44.50\%$ in metformin group, respectively (both $P < .01$, from baseline to 15 weeks). No significant difference was found between these 2 groups at 15 weeks (Fig. 2C).

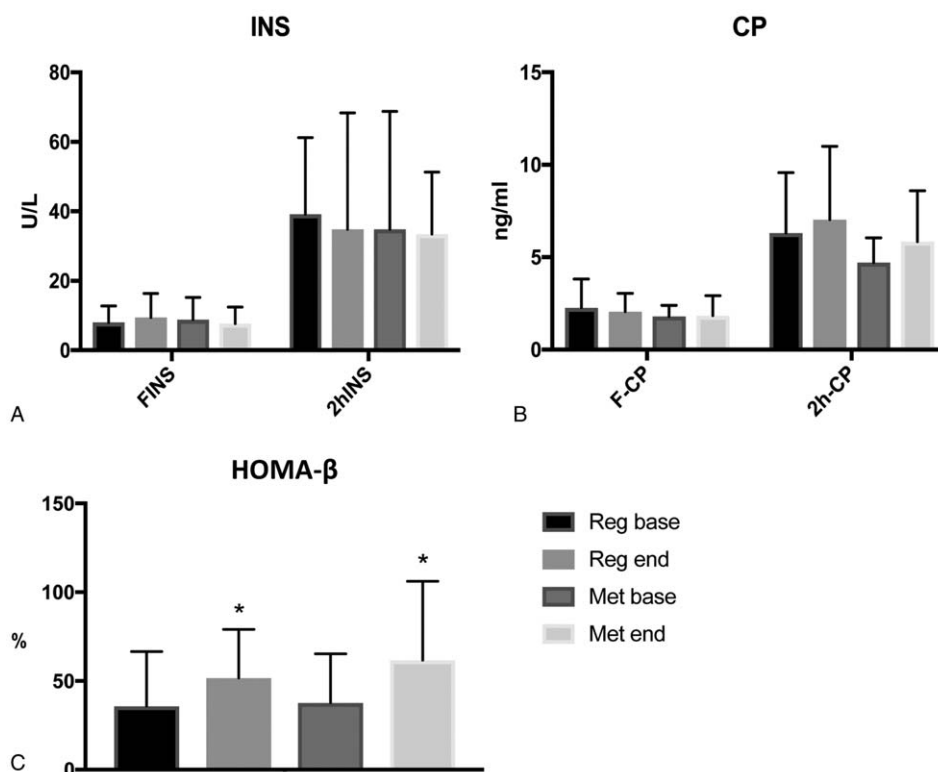


Figure 2. β -Cell function at baseline and 15 weeks in the 2 groups. (A) The fasting insulin level (FINS) and 2 hours INS at baseline and 15 weeks. (B) The F-CP and 2-hour CP at baseline and 15 weeks. (C) The homa beta cell function index (HOMA- β) level increased from baseline to 15 weeks in both groups.

3.6. The 1st-phase insulin secretion of the repaglinide group increased significantly more than that of the metformin group

In the IVGTT, glucose levels at 0, 2, 3, 4, 5, 7, and 10 minutes for the metformin group at baseline were slightly higher than those for the repaglinide group. At 15 weeks, the glucose levels of IVGTT in these 2 groups all improved ($P < .05$ or $P < .01$, Fig. 3A), and there were no differences between the repaglinide group and metformin group. Similarly, the AUC_{GLU} of IVGTT (0–10 minutes) at baseline were 181.70 ± 10.95 mmol/L per minute in the repaglinide group and 188.90 ± 11.18 mmol/L per minute in the metformin group ($P < .05$). The AUC considerably decreased after treatment, and the difference between baseline and 15 weeks was statistically significant (both, $P < .01$). However, there was no difference between these 2 groups at 15 weeks (Fig. 3B).

The insulin levels of the metformin group at 0, 2, 3, 4, 5, 7, and 10 minutes at baseline were also slightly higher than those of the repaglinide group. After treatment, these insulin levels increased in the repaglinide group, and the difference between baseline and 15 weeks was statistically significant. Furthermore, these insulin levels also increased in the metformin group, but there was no difference between levels at baseline and at 15 weeks (Fig. 3C). The AUC_{INS} of IVGTT (0–10 minutes) at baseline were 95.96 ± 20.23 U/L/min in the repaglinide group and 121.10 ± 27.70 U/L/min in the metformin group ($P < .01$). The AUC increased considerably after treatment, and exhibited a difference from baseline to 15 weeks in the repaglinide group ($P < .01$). However, no difference could be found in the metformin group from baseline to 15 weeks (Fig. 3D).

3.7. Repaglinide regulates insulin secretion through the pericentrin-F-actin pathway

Trichrome immunofluorescence staining was used in MIN-6 cells for the present study. After 15 minutes of stimulation with repaglinide, the insulin expression of MIN-6 cells considerably decreased in the repaglinide group, when compared with LG and HG controls (Fig. 4A). At different time intervals, the insulin expression of MIN-6 cells maintained a smooth decrease without significant differences from 2 hours to 12 hours ($P > .05$). This pattern was also detected in PCNT and F-actin expression in MIN-6 cells at 15 minutes, 2 hours, 6 hours, and 12 hours. An apparent decrease occurred at 15 minutes, while no difference was observed at 2, 6, and 12 hours (Fig. 4B).

The insulin levels of the cell culture were also tested. These insulin levels increased with time in all groups. These levels were higher in the high glucose (HG) groups than in the low glucose (LG) groups. In addition to the 15-minute and 6-hour timepoints, significant differences could be found between the HG and LG groups ($P < .05$). The repaglinide group has the highest levels at all timepoints. Furthermore, significant differences were observed at all timepoints between the repaglinide group and HG or LG group ($P < .05$ and $P < .01$, Fig. 4C).

4. Discussion

Abnormalities in the insulin secretory pattern have been demonstrated in the presence of diabetes, as well as in nondiabetic glucose intolerance.^[16] Pancreatic β -cell dysfunction is a key determinant of the development and progression of T2DM. This pathophysiologic defect causes a progressive

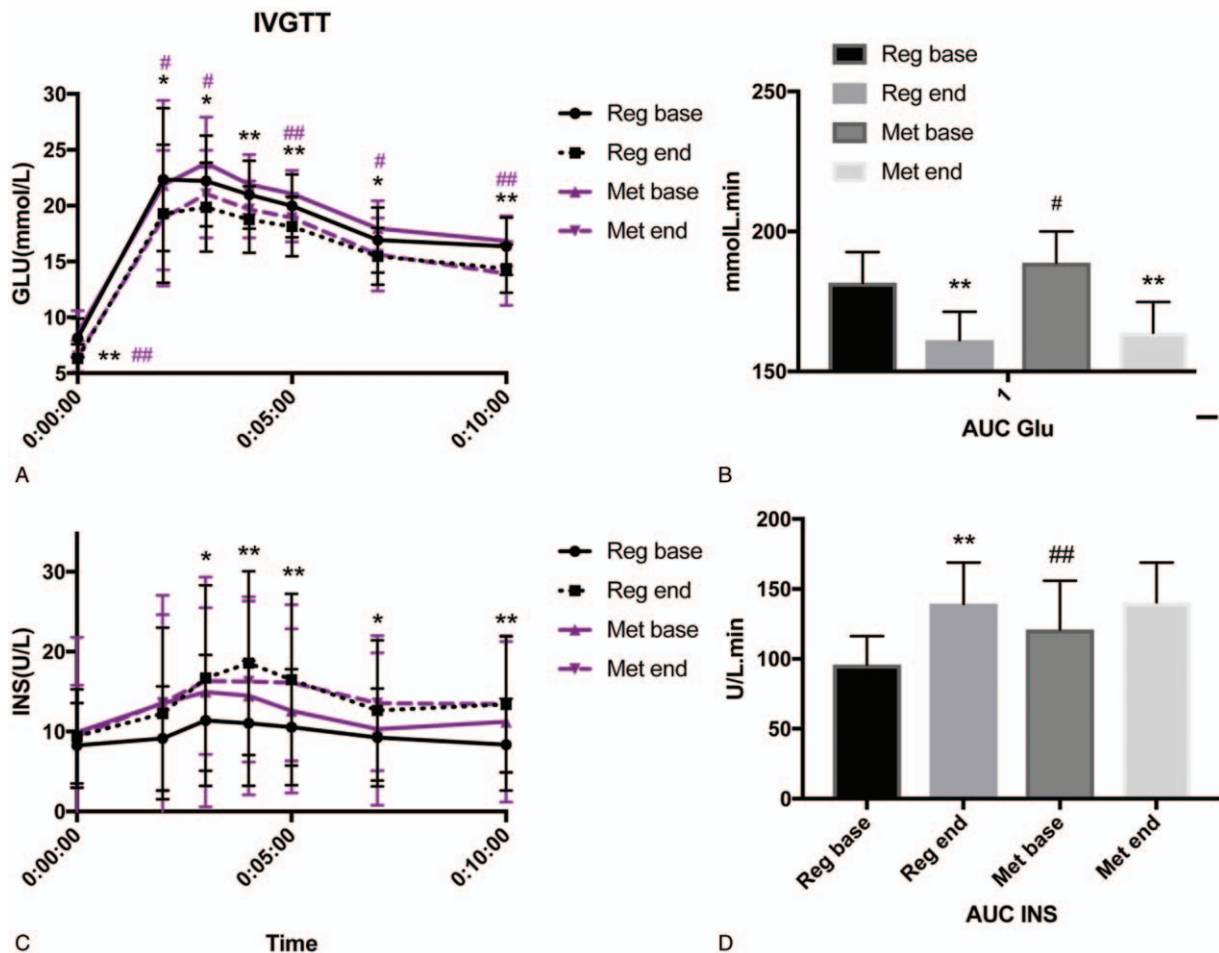


Figure 3. Intravenous glucose tolerance tests (IVGTTs) 0 to 10 minutes curves and area under curves (AUCs) for the repaglinide group and the metformin group. (A) The glucose levels of 0, 2, 3, 4, 5, 7, and 10 minutes of IVGTT at baseline and 15 weeks. A significant difference was seen between baseline and 15 weeks ($P < .05$ or $P < .01$). (B) The AUC_{GLU} 0 to 10 minutes decreased after treatment with a significant difference between baseline and 15 weeks (both $P < .01$). (C) The insulin levels of 0, 2, 3, 4, 5, 7, and 10 minutes of IVGTT at baseline and 15 weeks. There were significant differences between baseline and 15 weeks ($P < .05$ or $P < .01$). (D) The AUC_{INS} of IVGTT 0 to 10 minutes at baseline and 15 weeks. The AUC_{INS} 0 to 10 minutes increased considerably after treatment and showed a difference from baseline to 15 weeks in the repaglinide group ($P < .01$).

increase in blood glucose levels, with the deterioration of glycemic control.^[17] Evidence from laboratory studies has suggested that glycemic variability may more likely be the result than chronic sustained hyperglycemia by impairment of pancreatic β cells.^[18]

The present study revealed that the metformin group had higher glucose and insulin levels at baseline. However, after 15 weeks of treatment, the glucose levels and insulin levels in the metformin group and repaglinide group were comparable. No significant difference was found in baseline and 15-week fasting and 2-hour postprandial insulin and C-peptide levels between the metformin group and repaglinide group. Furthermore, the glucose variation also improved from baseline to 15 weeks without any difference between these 2 groups.

The HOMA- β significantly improved in both groups, especially in the metformin group. This result is of great significance, because repaglinide has been considered to increase insulin secretion, making it more suitable for controlling postprandial glucose levels, while metformin acts more like an insulin sensitizer. This poses the question, why did these 2 groups have comparable glucose and insulin levels after 15 weeks of treatment, instead of allowing repaglinide to stimulate more

insulin secretion and consequently lower glucose levels? The answer may be due to the fact that these 2 medications could both induce insulin secretion on-demand, but have different mechanisms of action. Metformin may improve the insulin sensitivity of peripheral organs, which could relieve the burden on β -cells and provide protection.^[19] Repaglinide mainly regulates the 1st phase of insulin secretion. Hence, the fasting and 2-hour postprandial tests and the HOMA- β calculated based on these could not reflect the actual insulin secretion effect.

Therefore, the investigators further examined the 1st-phase insulin secretion in the 2 groups. As expected, it was detected that repaglinide could increase the 1st-phase insulin secretion. This finding is in accordance with 1 study that reported that repaglinide improved β -cell function and mimicked the normal postprandial early phase insulin secretion in patients with T2DM.^[6] This also agrees with other studies that show that repaglinide enhanced β -cell function more effectively, compared with traditional sulfonylureas.^[20] Other studies revealed that compounds that regulate insulin secretion, including glucose, sulfonylurea, glucagon-like peptide 1, and somatostatin, all appear to exert their actions primarily via the modulation of insulin secretory burst mass, rather than the regulation of burst

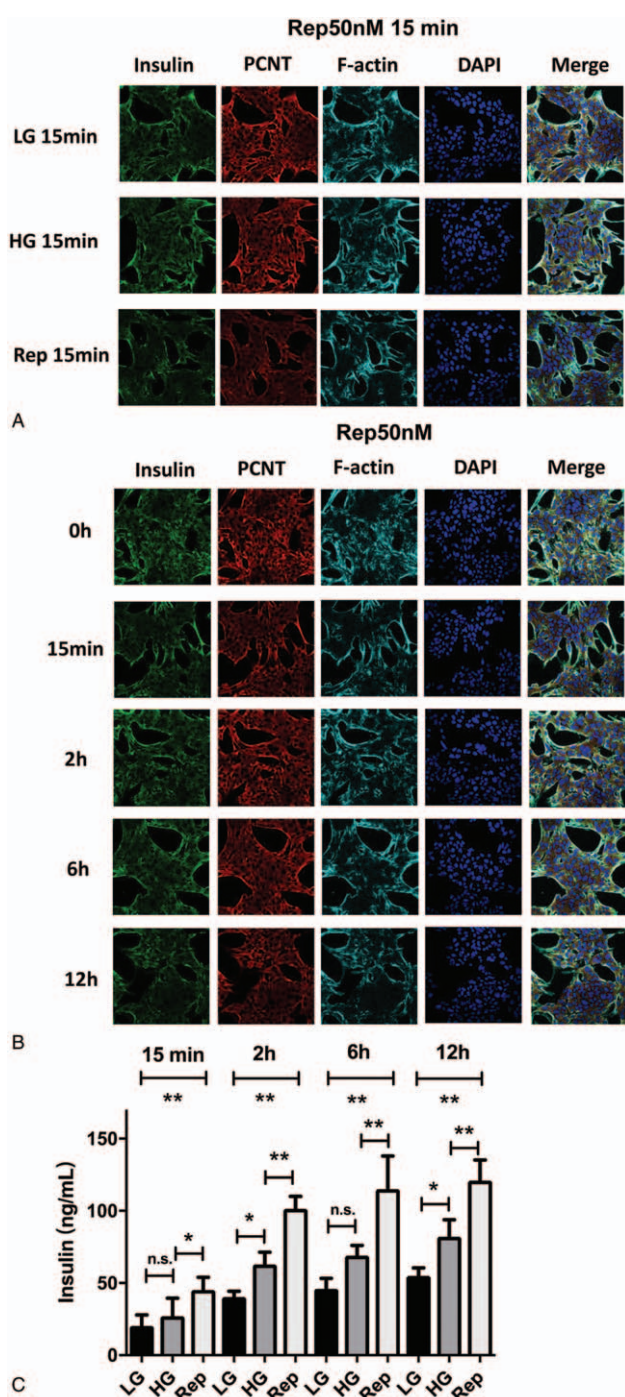


Figure 4. Trichrome immunofluorescence staining of mouse insulinoma (MIN-6) cells after repaglinide interference. (A) The insulin, F-actin, and pericentrin expression decreased considerably in the repaglinide group compared with the LG group. Each of the above expressions all decreased slightly in the HG group compared with the LG group without statistical significance. Pericentrin (PCNT) (red), insulin (green), F-actin (light blue), DAPI (dark blue). (B) The insulin, F-actin, and pericentrin expression decreased considerably at 15 minutes in the repaglinide group, while no more marked decreases occurred following detection. PCNT (red), insulin (green), F-actin (light blue), DAPI (dark blue). (C) The insulin level in the cell culture increased with time. The HG groups were higher than the LG groups and the repaglinide group was the highest. Significant differences could be found at all timepoints between the repaglinide group and the HG group and the repaglinide group and the LG group ($P < .05$ and $P < .01$).

frequency.^[21] Juhl et al reported that a single dose of repaglinide amplifies insulin secretory burst mass (and basal secretion) with no change in burst frequency.^[22] Repaglinide failed to stimulate exocytosis over 6 minutes when applied at a concentration of $0.1 \mu\text{mol/L}$. The same negative results were obtained with a 50-fold higher concentration of the compound.^[23]

The most interesting finding was that although repaglinide could increase the 1st-phase insulin secretion, no statistically significant difference could be found between these 2 groups in the end. Deeper MIN-6 cell research revealed that insulin level, PCNT level, and F-actin level decreased at 15 minutes after the intervention, but these did not decrease further after 12 hours, and these did not recover. Furthermore, insulin level, PCNT level and F-actin level exhibited positive relationships in MIN-6 cells. A previous study conducted by the investigators revealed that PCNT closely correlated with MIN-6 cells, and insulin secretion and F-actin might be the targets of PCNT regulation.^[14] The microfilament network of F-actin has been confirmed to be mainly distributed in the peripheral part of β -cells, preventing insulin secretion particles from being close to the cell membrane. Furthermore, the depolymerization and reassembly of actin filaments play important roles in the anchoring of vesicles in the cell membrane.^[21,24] The number of docked insulin granules was decreased after the 1st repaglinide stimulation, despite the later recovery period, suggesting that repaglinide may affect the intracellular motility of insulin granules and their recruitment to the plasma membrane. The investigators assumed that the decrease in PCNT and F-actin induced by repaglinide would affect the exocytotic process, which is probably involved in the regulation of insulin granule motility. This may also explain why repaglinide could not increase insulin secretion without limitations in the later phase. Regulating insulin secretion on demand could effectively prevent hypoglycemia, improve glucose variation, and subsequently protect the vessels.

In conclusion, repaglinide does not continue to stimulate the secretion of insulin. This is likely to be one of the important reasons by which repaglinide secretes insulin on-demand. The PCNT-F-actin pathway also plays an important role in the process of the repaglinide regulation effect on insulin secretion.

The present study revealed that the PCNT-F-actin pathway may be one of the mechanisms in the effect of repaglinide on insulin secretion, but the target of molecular regulation in this pathway is not definite, and further research is needed.

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References

- [1] Wang L, Gao P, Zhang M, et al. Prevalence and Ethnic Pattern of Diabetes and Prediabetes in China in 2013. *JAMA* 2017;317:2515–23.
- [2] Tortelli R, Lozupone M, Guerra V, et al. Midlife metabolic profile and the risk of late-life cognitive decline. *J Alzheimers Dis* 2017;59:120–30.
- [3] Yang W, Lu J, Weng J, et al. Prevalence of diabetes among men and women in China. *N Engl J Med* 2010;362:1090–101.
- [4] Fang FS, Gong YP, Li CL, et al. Comparison of repaglinide and metformin monotherapy as an initial therapy in Chinese patients with newly diagnosed type 2 diabetes mellitus. *Eur J Endocrinol* 2014;170:901–8.
- [5] Quast U, Stephan D, Bieger S, et al. The impact of ATP-sensitive K⁺-channel subtype selectivity of insulin secretagogues for the coronary vasculature and the myocardium. *Diabetes* 2004;53:S156–64.
- [6] Guardadomendoza R, Prioletta A, Jiménezceja LM, et al. The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus. *Arch Med Sci* 2013;9:936–43.
- [7] Chen M, Hu C, Jia W. Pharmacogenomics of glinides. *Pharmacogenomics* 2015;16:45–60.
- [8] Zhang H, Bu P, Xie YH, et al. Effect of repaglinide and gliclazide on glycaemic control, early-phase insulin secretion and lipid profiles in. *Chin Med J (Engl)* 2011;124:172–6.
- [9] Ma J, Liu LY, Wu PH, et al. Comparison of metformin and repaglinide monotherapy in the treatment of new onset type 2 diabetes mellitus in China. *J Diabetes Res* 2014;2014:294017.
- [10] Zimmerman WC, Sillibourne J, Rosa J, et al. Mitosis-specific anchoring of gamma tubulin complexes by pericentrin controls spindle organization and mitotic entry. *Mol Biol Cell* 2004;15:3642–57.
- [11] Delaval B, Doxsey SJ. Pericentrin in cellular function and disease. *J Cell Biol* 2010;188:181–90.
- [12] Huang-Doran I, Bicknell LS, Finucane FM, et al. Genetic defects in human pericentrin are associated with severe insulin resistance and diabetes. *Diabetes* 2011;60:925–35.
- [13] Jurczyk A, Pino SC, O'Sullivanmurphy B, et al. A novel role for the centrosomal protein, pericentrin, in regulation of insulin secretory vesicle docking in mouse pancreatic cells. *PLoS One* 2010;5:e11812.
- [14] Zu Y, Gong Y, Wan L, et al. Pericentrin is related to abnormal β -cell insulin secretion through F-actin regulation in mice. *PLoS One* 2015;10:e0130458.
- [15] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modelling. *Diabetes Care* 2004;27:1487–95.
- [16] Fang FS, Cheng XL, Gong YP, et al. Association between glycemic indices and beta cell function in patients with newly diagnosed type 2 diabetes. *Curr Med Res Opin* 2014;30:1437–40.
- [17] Yang G, Li C, Gong Y, et al. Assessment of insulin resistance in subjects with normal glucose tolerance, hyperinsulinemia with normal blood glucose tolerance, impaired glucose tolerance, and newly diagnosed type 2 diabetes (Prediabetes Insulin Resistance Research). *J Diabetes Res* 2016;2016:9270768.
- [18] Yang G, Li C, Gong Y, et al. A prospective, randomized, open-label study comparing the efficacy and safety of preprandial and prandial insulin in combination with acarbose in elderly, insulin-requiring patients with type 2 diabetes mellitus. *Diabetes Technol Ther* 2013;15:513–9.
- [19] Ji L, Li H, Guo X, et al. Impact of baseline BMI on glycemic control and weight change with metformin monotherapy in Chinese type 2 diabetes patients: phase IV open-label trial. *PLoS One* 2013;8:e57222.
- [20] Li Y, Xu L, Shen J, et al. Effects of short-term therapy with different insulin secretagogues on glucose metabolism, lipid parameters and oxidative stress in newly diagnosed type 2 diabetes mellitus. *Diabetes Res Clin* 2010;88:42–7.
- [21] Quinault A, Gausseres B, Bailbe D, et al. Disrupted dynamics of F-actin and insulin granule fusion in INS-1 832/13 beta-cells exposed to glucotoxicity: partial restoration by glucagon-like peptide 1. *Biochim Biophys Acta* 2016;1862:1401–11.
- [22] Juhl CB, Porksen N, Hollingdal M, et al. Repaglinide acutely amplifies pulsatile insulin secretion by augmentation of burst mass with no effect on burst frequency. *Diabetes Care* 2000;23:675–81.
- [23] Fuhlerdorff J, Rorsman P, Kofod H, et al. Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. *Diabetes* 1998;47:345–51.
- [24] Yang SY, Lee JJ, Lee JH, et al. Secretagogin affects insulin secretion in pancreatic β -cells by regulating actin dynamics and focal adhesion. *Biochem J* 2016;473:1791–803.