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Brief Report

Specific PERK inhibitors enhanced glucose-stimulated insulin secretion in a mouse model of type 2 diabetes

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ABSTRACT

Background: We have reported that partial PERK attenuation using PERK inhibitors (PI) enhanced glucose-stimulated insulin secretion (GSIS) from pancreatic islets and mice through induction of ER chaperone BIP. Therefore, we investigated if PI would have the same effects in a diabetic condition as well.
Methods: GSK2606414 was treated to mouse islets under 20-mM glucose and 0.5-mM palmitate to examine GSIS. To generate a mouse model of type 2 diabetes mellitus (DM), male C57BL/6J mice were fed with high-fat diet and injected with streptozotocin. Several doses (6–16 mg/kg/day) of GSK2656157 and glimepiride were administrated to the mice for 8 weeks, and metabolic phenotypes were evaluated such as body weight, blood glucose levels, insulin secretion and sensitivity, and then changes in the pancreas were measured.
Results: High-glucose and palmitate treatment significantly increased PERK phosphorylation in the isolated islets. Suppression of GSIS and glucose-stimulated Ca^{2+} transit was also observed. PI at 40 nM which decreased PERK phosphorylation by 40% significantly recovered the GSIS and cytosolic calcium. In the mice where significant weight gain and prominent hyperglycemia were induced, PI at 10 mg/kg/day significantly enhanced GSIS and reduced blood glucose levels compared to the vehicle. The effects were similar to those by 10 mg/kg/day of glimepiride. Administration of PI did not induce changes in beta cell mass or pancreatic insulin contents, however, high dose PI decreased pancreatic weight.
Conclusion: PI at low dose significantly enhanced GSIS in vitro and in vivo under metabolic stress and improved hyperglycemia in the mice mimicking type 2 DM, suggesting a potential as a new therapeutic approach for type 2 DM.

1. Introductions

PERK (pancreatic endoplasmic reticulum kinase) has a critical implication on pancreatic beta cells and diabetes mellitus (DM) [1], in terms of proliferation and differentiation during development, while insulin trafficking and cell survival during adults. PERK ablation by administration of PERK inhibitors (PI) to mice resulted in pancreatic atrophy and islet degeneration, leading insulin insufficiency and hyperglycemia [2,3].

In contrast, heterozygous *Perk* deletion in mice increased pancreatic insulin contents and insulin secretion, improving hyperglycemia [4,5]. We also reported that partial attenuation of PERK activity enhanced glucose-stimulated insulin secretion (GSIS) through induction of BIP, an ER chaperone [6]. In the study, treatment of PI at low dose which decreased PERK phosphorylation by 30%, enhanced GSIS from mouse and human islets. Enhanced GSIS by PI was associated with calcium regulation in the cytosol and ER, along with islet insulin contents. Administration of low-dose PI to mice enhanced GSIS, too.

From the previous findings, we speculated that PERK attenuation could be applied in the treatment of DM, and examined PI in a mouse model of type 2 DM, compared to a sulfonylurea an insulin secretagogue.

2. Methods

2.1. PERK Inhibitors and a Sulfonylurea

GSK2606414 and GSK2656157 (Selleck, Houston, TX, USA) were used for in vitro and in vivo experiments, respectively [6]. A

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2nd-generation sulfonylurea, glimepiride was provided by Yuhan Corp. (Seoul, Korea).

2.2. Antibodies

Anti-ACTIN was Sigma-Aldrich A5441 (1:5000) and the others were from Cell Signaling: anti-p-PERK (Thr980) (#3179, 1:1000), anti-PERK (#3192, 1:1000), anti-p-EIF2A (Ser51) (#9721, 1:1000), anti-EIF2A (#9722, 1:1000), anti-BIP (#3177, 1:1000), anti-CASPASE3 (#9661, #9662, 1:1000), and anti-INSULIN (#3014, 1:3000).

2.3. Cell Culture

Islets were isolated from C57BL/6J mice, and cultured in RPMI medium with 10% fetal bovine serum (Welgene, Gyeongsan-si, Korea) overnight, then intact islets were incubated in the same medium with 20-mM glucose and 0.5-mM palmitate for 24 h. Palmitate was conjugated with bovine serum albumin (1:3 molar ratio) (all from Sigma-Aldrich). The islets were treated with vehicle or GSK2606414 for 24 h. In vitro experiments were performed with islets of similar diameter around 100 μm [6].

2.4. Animal Study

At 4 weeks of age, male C57BL/6J mice (Jackson laboratory) housed under standardized conditions (12-h dark/12-h light cycle) with water and food ad libitum in specific pathogen-free conditions were allocated into seven groups. One group was maintained on a normal diet (Purina, Seongnam-si, Korea) as a control, and the others were given a high-fat diet (HFD) (Envigo, Madison, WI, USA) throughout the study, which contained 60% fat, 18% protein, and 22% carbohydrate. In each cage, a maximum of 5 mice of various treatment groups were housed. After 4 weeks of HFD, mice were injected with 50 mg/kg streptozotocin (STZ) (Sigma-Aldrich) intraperitoneally for 3 successive days. Two weeks after STZ injection, GSK2656157 (3, 5, and 8 mg/kg), glimepiride (5 and 8 mg/kg) or equal volume of vehicle started twice a day by oral gavage. The doses of GSK2656157 were determined as before [6], and those of glimepiride were determined empirically. Body weights and fed blood

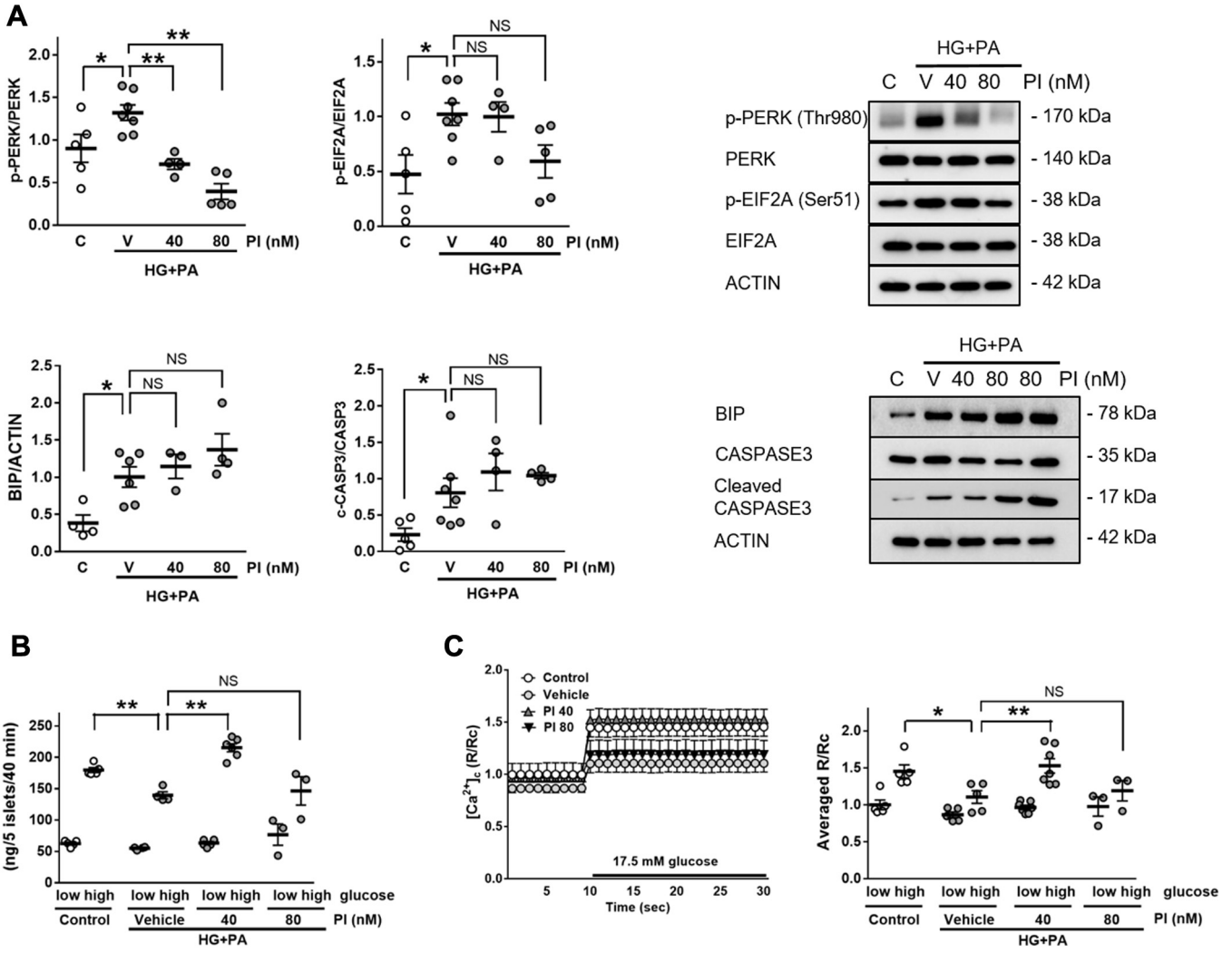

Fig. 1. Low-dose PI treatment recovered GSIS in the islets under metabolic stress by high-glucose and palmitate (HG + PA). Mouse islets were stimulated with 20-mM glucose and 0.5-mM palmitate for 24 h with and without GSK2606414. Control group islets were incubated in RPMI medium containing 10-mM glucose. Numbers of experiments were 3–7. (A) Phosphorylation of PERK and EIF2A, BIP expression, and CASPASE3 cleavage with representative pictures of western blotting. (B) GSIS (17.5-mM glucose for 40 min). (C) Glucose-stimulated calcium transit. Cytosolic calcium concentrations (R) were expressed as relative ratios to the control before stimulation (Rc). For statistical analyses, the R/Rc measures were averaged as in the right panel. For (A), one-way ANOVA with Bonferroni's multiple comparison test was performed. For (B) and (C), 2-way repeated-measures ANOVA and Bonferroni posttests were performed. *P < 0.05 and **P < 0.01 compared among indications. C, control; c-CASPASE3, cleaved CASPASE3; GSIS, glucose-stimulated insulin secretion; HG + PA, high-glucose and palmitate treatment; PI, PERK inhibitor; V, vehicle.

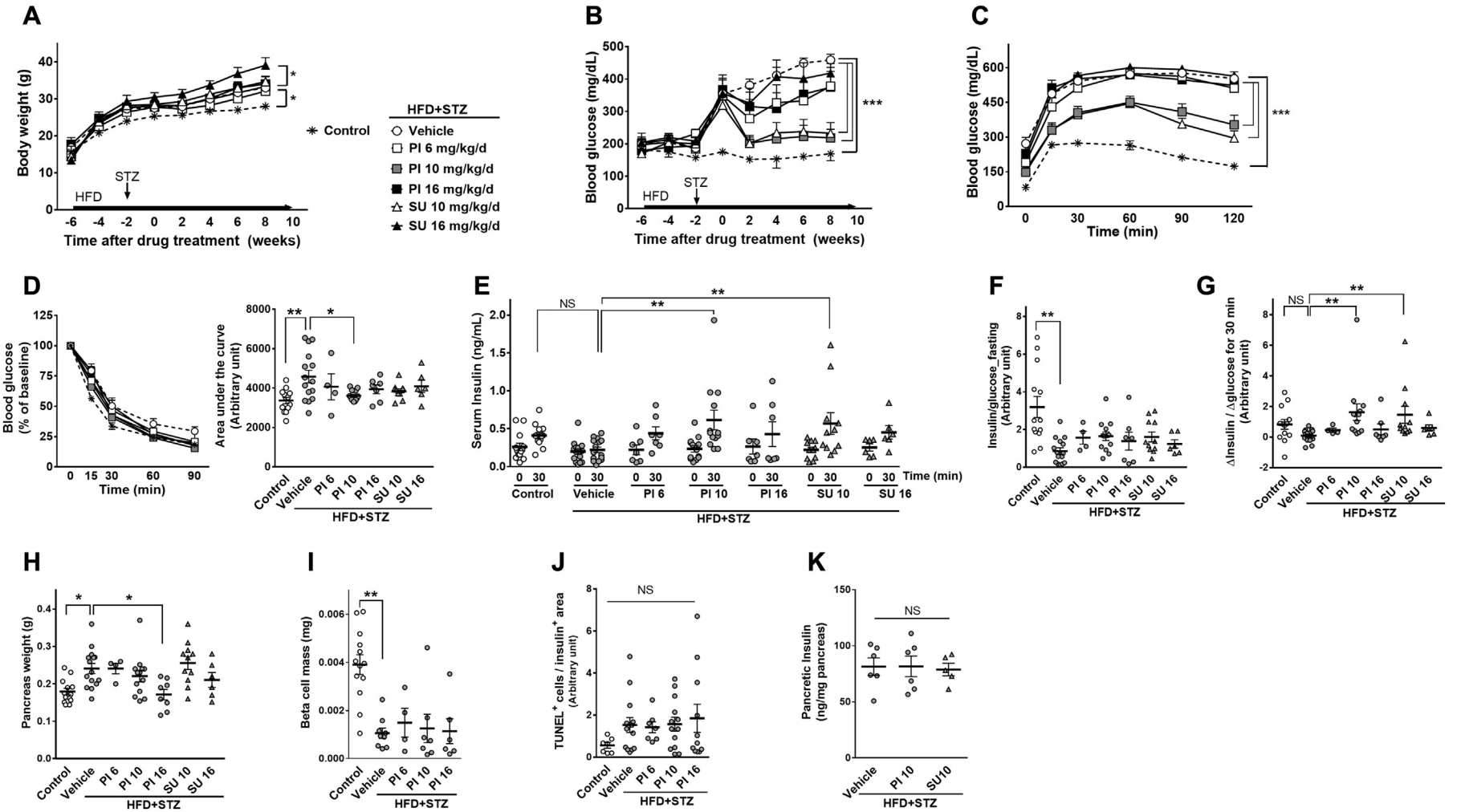

Fig. 2. Oral administration of low-dose PI enhanced GSIS and improved hyperglycemia in a mouse model of type 2 DM. HFD for 6 weeks combined with STZ injection induced obese diabetic mice. GSK2656157 at 6–16 mg/kg/day (n = 4–15) or glimepiride at 10–16 mg/kg/day (n = 6–11) were administrated via oral gavage for 8 weeks and the effects were compared to those of vehicle (n = 15). Control mice (n = 13) were on a normal chow and received vehicle administration. (A) Body weights. (B) Fed blood glucose levels. (C) Intraperitoneal glucose tolerance test using 1 g/kg of glucose injection. (D) Insulin tolerance test using 0.5 U/kg of regular insulin injection, presented as % change from each baseline. Area under the curves were calculated as in the Rt. panel. (E) Serum insulin levels before and 30 min after glucose loading during the intraperitoneal glucose tolerance test. (F) Fasting insulin to glucose ratio. (G) Insulinogenic index during 30 min after glucose loading, calculated by the ratio of insulin change to glucose change. (H) Pancreas weight. (I) Beta cell mass estimated by multiplying pancreas weight and relative beta cell ratio from point counting after immunohistochemical staining for insulin. (J) Ratio of TUNEL$^+$ beta cell numbers to insulin$^+$ area. (K) Pancreatic insulin contents. For (A)–(E), 2-way repeated-measures ANOVA and Bonferroni posttests were performed. For the others, One-way ANOVA with Bonferroni posttests or the Kruskal-Wallis test with Dunn's multiple comparison test were applied. *P < 0.05, **P < 0.01, and ***P < 0.001 compared to the vehicle group. HFD, high-fat diet; HFD + STZ, HFD and STZ injection; PI, PERK inhibitor; STZ, streptozotocin; SU, sulfonylurea.

glucose levels were monitored every 2 weeks. After 8-week treatment, intraperitoneal glucose tolerance test and insulin tolerance test were performed as described [6]. Then the mice were euthanized by cervical dislocation to extract pancreas. All animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee of Seoul National University Hospital (SNU-150327-3-2).

2.5. Histology

Pancreas was weighed, fixed, and embedded in paraffin. TUNEL staining (Roche, Basel, Swiss) combined with immunohistochemical staining with anti-INSULIN was conducted on the paraffin sections. For relative beta cell ratio to estimate beta cell mass, point counting was applied [7]. Five thousands to 10,000 points were counted per mouse, and the average islet numbers from diabetic mice was 7.5 ± 0.6/section.

2.6. Pancreatic Insulin Contents

Pancreatic insulin was extracted, measured, and normalized by pancreas weight according to a previously published method [8].

2.7. Statistics

Data are expressed as the mean ± standard error of the mean. Statistical analyses were executed using Prism 5 software (GraphPad). Variables which change over time were analyzed by two-way repeated measures ANOVA with Bonferroni posttest. For the others, one-way ANOVA with Bonferroni posttest or the Kruskal-Wallis test with Dunn's multiple comparison test was applied. P values < 0.05 were considered statistically significant.

3. Results

Combined high-glucose and palmitate treatment (HG + PA) in vitro enhanced phosphorylation of PERK and EIF2A ($P < 0.05$, Figs. 1A and S1). Treatment of GSK2606414 (40 and 80 nM) decreased the phosphorylation of PERK dose-dependently, however, significant reduction in EIF2A phosphorylation was not accompanied. BIP levels and CASPASE3 cleavage were induced by HG + PA as expected, but there were no significant changes by addition of PI (Fig. 1A). Induction of *Bip* transcript by PI was not observed, either (Fig. S2). HG + PA decreased GSIS and glucose-stimulated calcium transit ($P < 0.01$), and 40-nM GSK2606414 significantly enhanced them ($P < 0.01$, Fig. 1B and C). These effects were not observed at 80 nM.

Next, HFD and STZ injection (HFD + STZ) in mice resulted in significant weight gain and hyperglycemia compared to the control mice ($P < 0.05$, Fig. 2A–C). Administration of GSK2656157 did not affect body weights, in contrast to the weight gain by glimepiride (16 mg/kg/day) (Fig. 2A). Fed glucose levels, fasting glucose, and glucose intolerance were significantly improved by both agents at 10 mg/kg/day ($P < 0.001$, Fig. 2B and C). Insulin resistance induced by HFD + STZ decreased by 10 mg/kg/day of GSK2656157 (Figs. 2D and S3). Suppression of stimulated insulin levels by HFD + STZ was not significant, but enhancement of them by either agent was significant at 10 mg/kg/day ($P < 0.01$, Fig. 2E). When insulin secretion was adjusted with glucose levels, HFD + STZ significantly inhibited fasting insulin secretion (Fig. 2F) and both agents markedly increased GSIS (Fig. 2G). All these effects were not significant at lower or higher doses than 10 mg/kg/day.

When we examined pancreatic tissues, HFD + STZ increased pancreatic weights, and only 16 mg/kg/day of GSK2656157 significantly reduced it similar to the control levels ($P < 0.05$, Fig. 2H). However, GSK2656157 did not affect either beta cell mass or apoptosis (Fig. 2I and J). GSK2656157 and glimepiride at 10 mg/kg/day did not change pancreatic insulin contents (Fig. 2K). Analyses after excluding some outliers are presented in Figs. S4 and S5, demonstrating the same results.

4. Discussion

In this study, we demonstrated that PI could recover GSIS and calcium transit in the islets under stress by high-glucose and fatty acid. We speculated that action of PI at low dose was not dependent on EIF2A as we had suggested [6], because its activity was not affected by PI significantly. On the other hand, BIP expression was not increased significantly by PI, which seemed to mediate the effects of PI in our previous study [6]. BIP might not be an effector of PI under the stress of HG + PA, or might have been induced in early phase but we missed it.

Low-dose PI chronically improved GSIS and hyperglycemia in the diabetic mice induced by diet and beta cell destruction, which was comparable to effects of glimepiride, a sulfonylurea which is currently used in type 2 DM. Interestingly, PI also improved insulin resistance significantly, which warrants further evaluation of PI effects on insulin target tissues [9]. Because pancreatic insulin contents were not changed by PI, the enhanced GSIS compensating the hyperglycemia in part did not seem to induce beta cell exhaustion, even though PI-induced insulin synthesis cannot be estimated with current study.

Considering a possibility of PI for clinical application, it would be essential to evaluate whether low-dose PI is detrimental to pancreas and beta cell mass as mentioned in the introduction. We observed that 16 mg/kg/day of GSK2656157 reduced pancreas weight without change in beta cell mass, which suggested that it mainly affected exocrine tissue. However, according to the no influences on body weights, the metabolic outcome seemed minimal. In vitro PI at effective dose for GSIS did not significantly increase apoptotic signal induced by HG + PA, either.

The narrow range of effective PI dose in mice (only 10 mg/kg/day but not 6 or 16 mg/kg/day) might be different in human as in vitro assay [6], and therapeutic ranges should be assessed in pharmacokinetic/pharmacodynamic studies with larger animals.

Several agents are used in patients with type 2 DM, however, there is still unmet need for optimal management [10]. We provided some evidences that low-dose PI has a potential as a novel therapeutic approach for type 2 DM by enhancing GSIS.

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Declaration of Interest

None.

Author Contributions

HSJ is the guarantor of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses. MNK and SHM planned experiments and analyzed data. MJK interpreted data and wrote the manuscript. D-SH, J-WK, and K-HY measured the pancreatic insulin contents. KSP contributed to discussions. HSJ planned the study, interpreted data and edited the manuscript. All authors approved the final version of the manuscript.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2018.12.007.

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