JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
SHORT REPORT

Fructose induces glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1 and insulin secretion: Role of adenosine triphosphate-sensitive K^+ channels

Yusuke Seino[1]*, Hidetada Ogata[2], Ryuya Maekawa[2], Takako Izumoto[2], Atsushi Iida[2], Norio Harada[3], Takashi Miki[4], Susumu Seino[5], Nobuya Inagaki[3], Shin Tsunekawa[2], Yutaka Oiso[2], Yoji Hamada[1]

Departments of [1]Metabolic Medicine, and [2]Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, Nagoya, [3]Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, [4]Department of Medical Physiology, Graduate School of Medicine, Chiba University, Chiba, and [5]Division of Molecular and Metabolic Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Keywords
Adenosine triphosphate-sensitive K^+ channel, Fructose, Hormone secretion

***Corresponence**
Yusuke Seino
Tel.: +81-52-744-2191
Fax: +81-52-744-2191
E-mail address: yusuke@med.nagoya-u.ac.jp

J Diabetes Invest 2015; 6: 522–526

doi: 10.1111/jdi.12356

ABSTRACT
Adenosine triphosphate-sensitive K^+ (K_{ATP}) channels play an essential role in glucose-induced insulin secretion from pancreatic β-cells. It was recently reported that the K_{ATP} channel is also found in the enteroendocrine K-cells and L-cells that secrete glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), respectively. In the present study, we investigated the involvement of the K_{ATP} channel in fructose-induced GIP, GLP-1 and insulin secretion in mice. Fructose stimulated GIP secretion, but pretreatment with diazoxide, a K_{ATP} channel activator, did not affect fructose-induced GIP secretion under streptozotocin-induced hyperglycemic conditions. Fructose significantly stimulated insulin secretion in *Kir6.2*$^{+/+}$ mice, but not in mice lacking K_{ATP} channels (*Kir6.2*$^{-/-}$), and fructose stimulated GLP-1 secretion in both *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice under the normoglycemic condition. In addition, diazoxide completely blocked fructose-induced insulin secretion in *Kir6.2*$^{+/+}$ mice and in MIN6-K8 β-cells. These results show that fructose-induced GIP and GLP-1 secretion is K_{ATP} channel-independent and that fructose-induced insulin secretion is K_{ATP} channel-dependent.

INTRODUCTION
Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretin hormones secreted from enteroendocrine K-cells and L-cells by nutrients such as carbohydrate[1,2].

Adenosine triphosphate-sensitive K^+ (K_{ATP}) channels play an important role in glucose-induced insulin secretion from pancreatic β-cells[3]. It has been reported that K-cells and L-cells express glucokinase and K_{ATP} channels identical to those expressed in pancreatic β-cells[4,5]. In addition, facilitative glucose transporter 5 (GLUT5), which absorbs fructose from intestinal lumen to cytosol[6], is abundantly expressed in K-cells, L-cells and β-cells. However, the role of fructose and the involvement of the K_{ATP} channel in the secretion of GIP, GLP-1 and insulin *in vivo* are poorly understood.

In the present study, we investigated the contributions of fructose and the K_{ATP} channel in the secretion of these hormones utilizing K_{ATP} channel-deficient mice.

MATERIALS AND METHODS
Mice
C57BL/6J mice (*Kir6.2*$^{+/+}$ mice) and mice lacking the K_{ATP} channel (*Kir6.2*$^{-/-}$ mice)[3] were used. We carried out all animal experiments according to the protocol approved by the Nagoya University Institutional Animal Care and Use Committee.

Plasma Biochemical Analyses
Blood glucose levels were measured with ANTSENSE II (Bayer Medical, Leverkusen, Germany). Plasma total GIP and GLP-1 levels were measured using the GIP (TOTAL) ELISA kit (Merck

Received 5 November 2014; revised 24 February 2015; accepted 16 March 2015

Millipore, Billerica, MA, USA) and an electrochemiluminescent sandwich immunoassay (Meso Scale Discovery, Gaithersburg, MD, USA) as previously described[7,8]. Plasma insulin levels were determined by an ELISA kit (Morinaga, Tokyo, Japan).

Induction of Diabetes

As described previously[7], streptozotocin (STZ; 150 mg/kg bodyweight) was given intraperitoneally to *Kir6.2*$^{+/+}$ mice after a 16-h fast.

Diazoxide and Fructose Administration

After 16 h of food deprivation, 240 mg/kg bodyweight of diazoxide (Wako, Osaka, Japan) was given orally[7]. 90 min after diazoxide administration, 6 g/kg bodyweight of fructose was given orally.

MIN6 Experiment

MIN6-K8 β-cells were cultured and stimulated for 30 min by various materials after pre-incubation for 30 min in HEPES-Krebs buffer with 2.8 mmol/L glucose, and released insulin was evaluated by insulin assay kit as previously reported[9].

Statistical Analysis

Statistical analysis was carried out by unpaired, two-tailed Student's *t*-test or two-way ANOVA.

RESULTS

Fructose Induces GIP Secretion in the Diabetic State

We first examined whether fructose stimulates GIP secretion. In *Kir6.2*$^{+/+}$ mice, fructose tended to, but not significantly, stimulate GIP secretion in a normal state, but significantly enhanced the GIP secretion in the STZ-induced diabetic state (Figure 1a). To investigate the involvement of the K_{ATP} channel in fructose-induced GIP secretion in the diabetic state, we examined the effect of the K_{ATP} channel activator, diazoxide, on fructose-induced GIP secretion. Pretreatment of diazoxide did not affect fructose-induced GIP secretion in the diabetic state (Figure 1b). Fructose-induced GLP-1 levels at 15 min were not different under the normoglycemic condition and hyperglycemic condition (Figure 1c).

K_{ATP} Channels Are Not Involved in Fructose-Induced GLP-1 Secretion *In Vivo*

We next investigated whether the K_{ATP} channel participates in fructose-induced GLP-1 secretion *in vivo*, by utilizing *Kir6.2*$^{-/-}$ mice. Both in *Kir6.2*$^{+/+}$ and *Kir6.2*$^{-/-}$ mice, fructose significantly stimulated GLP-1 secretion more than twofold at 15 min of fructose administration (Figure 2b). In contrast, fructose did not stimulate GIP secretion in *Kir6.2*$^{-/-}$ mice at all (Figure 2a).

K_{ATP} Channels Are Involved in Fructose-Induced Insulin Secretion *In Vivo* and *In Vitro*

To assess whether fructose-induced insulin secretion requires the K_{ATP} channel pathway, we investigated blood glucose levels

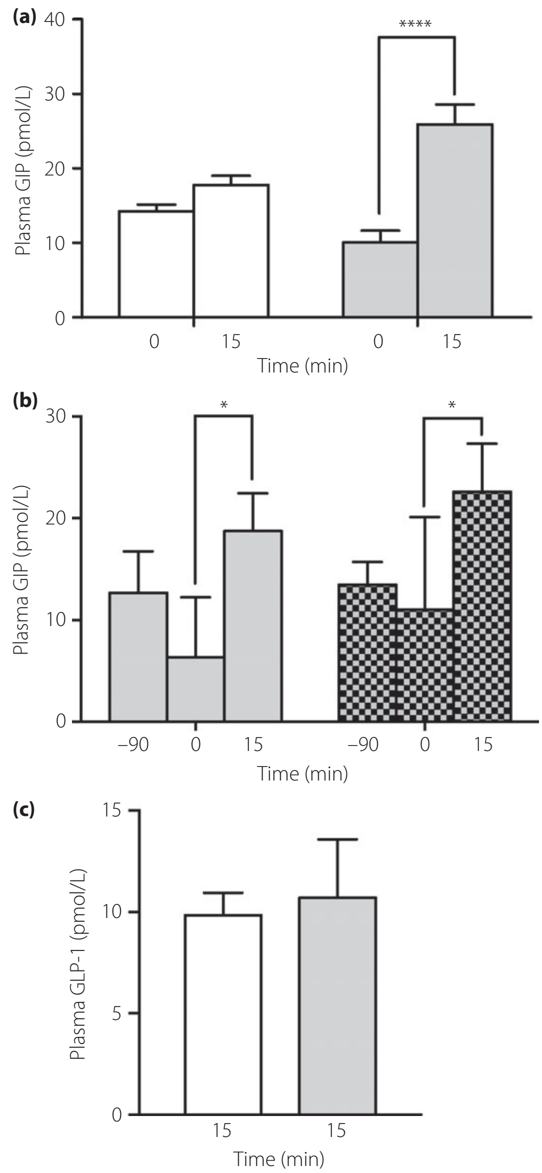

Figure 1 | Fructose-induced glucose-dependent insulinotropic polypeptide (GIP) secretion. (a) Plasma GIP levels on the oral administration of 6 g/kg fructose in the control mice (white bar; n = 17) or the diabetic mice (gray bar; n = 15). (b) Plasma GIP levels on the oral administration of 6 g/kg fructose in the streptozotocin-induced diabetic mice pretreated with vehicle (gray bar; n = 6) or pretreated with diazoxide (gray checked bar; n = 7). (c) Plasma glucagon-like peptide-1 (GLP-1) levels on the oral administration of 6 g/kg fructose in the control mice (white bar; n = 6) or the diabetic mice (gray bar; n = 6; *P < 0.05, ****P < 0.0001). Data are expressed as means ± standard error of the mean.

and serum insulin levels during oral fructose tolerance test in both *Kir6.2*$^{+/+}$ and *Kir6.2*$^{-/-}$ mice. The blood glucose levels were significantly higher in *Kir6.2*$^{-/-}$ mice than in *Kir6.2*$^{+/+}$ mice (Figure 2c). Fructose significantly stimulated insulin secretion in *Kir6.2*$^{+/+}$ mice at 15 min, but not in *Kir6.2*$^{-/-}$ mice at

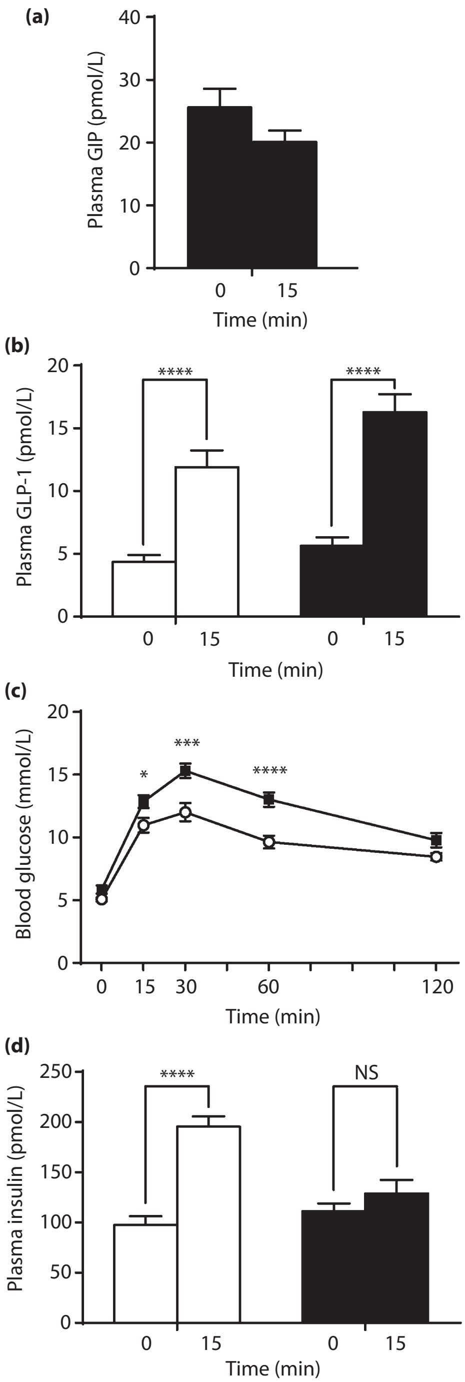

Figure 2 | Effects of adenosine triphosphate-sensitive K^+ (K_{ATP}) channel on fructose-induced glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and insulin secretion. (a) Plasma GIP levels on the oral administration of 6 g/kg fructose in *Kir6.2*$^{-/-}$ mice (black bar; n = 13). (b) Plasma GLP-1 levels on the oral administration of 6 g/kg fructose in *Kir6.2*$^{+/+}$ mice (white bar; n = 12) and *Kir6.2*$^{-/-}$ mice (black bar; n = 13; ****P < 0.0001 relative to 0 min). (c) Blood glucose levels during oral fructose tolerance test in *Kir6.2*$^{+/+}$ mice (open circle; n = 5) in *Kir6.2*$^{-/-}$ mice (solid square; n = 6; *P < 0.05, ***P < 0.001, ****P < 0.0001 compared with *Kir6.2*$^{+/+}$ mice at the indicated time-points). (d) Plasma insulin levels on the oral administration of 6 g/kg fructose in *Kir6.2*$^{+/+}$ mice (white bar; n = 12) and *Kir6.2*$^{-/-}$ mice (black bar; n = 13; ****P < 0.0001 relative to 0 min). Data are expressed as means ± standard error of the mean. NS, not significant.

all (Figure 2d). Basal levels of insulin were not decreased by pretreatment of diazoxide in *Kir6.2*$^{-/-}$ mice, but were decreased in *Kir6.2*$^{+/+}$ mice (Figure 3a,b). Fructose significantly stimulated insulin secretion in *Kir6.2*$^{+/+}$ mice pretreated with vehicle at 15 min, but did not stimulate insulin secretion in *Kir6.2*$^{+/+}$ mice pretreated with diazoxide or in *Kir6.2*$^{-/-}$ mice pretreated with vehicle and diazoxide at 15 min (Figure 3a,b). To assess whether fructose directly stimulates insulin secretion, we investigated insulin secretion using MIN6-K8 β-cells[9]. Diazoxide tended to decrease insulin secretion at 8.3 mmol/L glucose (P = 0.05). The addition of 20 mmol/L fructose significantly potentiated insulin secretion at 8.3 mmol/L glucose, and diazoxide completely blocked the insulin response (Figure 3c).

Pretreatment of diazoxide did not affect fructose-induced GLP-1 secretion at 15 min in either *Kir6.2*$^{+/+}$ mice or *Kir6.2*$^{-/-}$ mice (Figure 3d).

DISCUSSION

The mechanism by which fructose stimulates gut hormone secretion is not well known. In the present study, we investigated the role of the K_{ATP} channels in fructose-induced GIP, GLP-1 and insulin secretion *in vivo*.

We previously reported that the K_{ATP} channels in K-cells are in a closed state under the normoglycemic condition *in vivo*, and are in an open state under the hyperglycemic condition[7]. The increase of ATP produced by metabolism of glucose closes the K_{ATP} channels in the K-cells under the hyperglycemic condition and enhances glucose-induced GIP secretion, suggesting that K_{ATP} channels in K-cells contribute to glucose-induced GIP secretion under the hyperglycemic condition. However, the present results show that this mechanism is not involved in fructose-induced GIP secretion in the diabetic state and that the K_{ATP} channels in K-cells do not contribute to fructose-induced GIP secretion under the hyperglycemic condition. In previous reports, 3 g/kg fructose did not stimulate GIP secretion in C57BL/6J mice, but did stimulate GIP secretion in obese type 2 diabetic model *ob*/*ob* mice[10,11]. The mechanism of such fructose-induced GIP secretion in various diabetic models remains to be elucidated.

In the present study, fructose was found to significantly induce GLP-1 secretion in *Kir6.2*$^{-/-}$ mice, and pretreatment of diazoxide did not block fructose-induced GLP-1 secretion at 15 min and fructose-induced GLP-1 secretion was not enhanced under the hyperglycemic condition. These results show that the K_{ATP} channel is not required for fructose-induced GLP-1 secretion *in vivo*. However, a previous *in vitro* study using GLUTag cells found that fructose-induced GLP-1 secretion was entirely K_{ATP} channel-dependent[12]. This discrepancy could be due to the nature of the GLUTag cell line and/

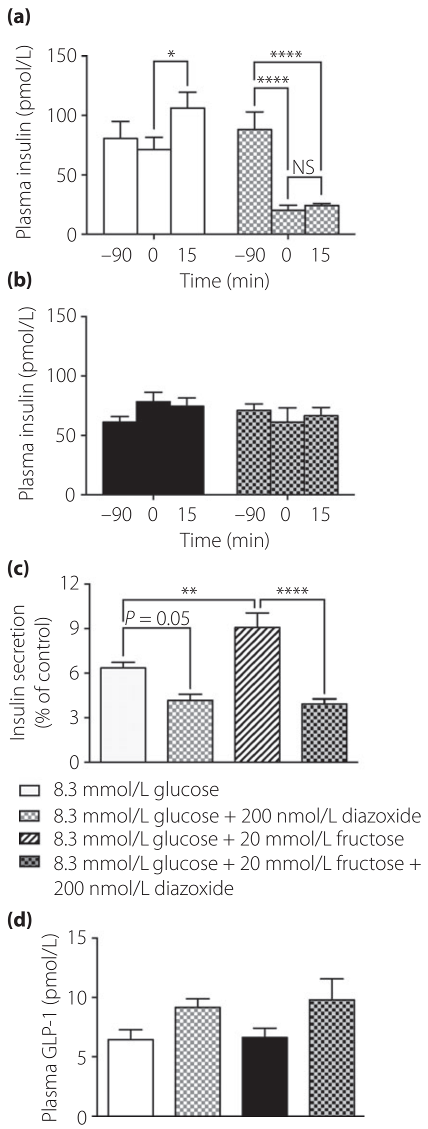

Figure 3 | Effects of diazoxide on fructose-induced insulin or glucagon-like peptide-1 (GLP-1) secretion. (a) Plasma insulin levels on the oral administration of 6 g/kg fructose in *Kir6.2*$^{+/+}$ mice pretreated with vehicle (white bar; $n = 11$) or pretreated with diazoxide (gray checked bar; $n = 9$; $^{*}P < 0.05$, $^{****}P < 0.0001$). (b) Plasma insulin levels on the oral administration of 6 g/kg fructose in *Kir6.2*$^{-/-}$ mice pretreated with vehicle (black bar; $n = 8$) or pretreated with diazoxide (black checked bar; $n = 7$). (c) Effects of fructose and diazoxide on insulin secretion in MIN6-K8 β-cells. Insulin secretion from MIN6-K8 β-cells was normalized by cellular insulin content ($n = 20$ for each experiment; $^{**}P < 0.01$, $^{****}P < 0.0001$). (d) Plasma GLP-1 levels at 15 min on the oral administration of 6 g/kg fructose in *Kir6.2*$^{+/+}$ mice pretreated with vehicle (white bar; $n = 9$) or diazoxide (gray checked bar; $n = 9$) and *Kir6.2*$^{-/-}$ mice pretreated with vehicle (black bar; $n = 8$) or diazoxide (black checked bar; $n = 8$).

or the fact that GLP-1 secretion is regulated by various factors, such as nutrients, intestinal hormones, neuropeptides and neuronal signal *in vivo*[13–16].

It is reported that activation of sweet taste receptors in pancreatic β-cells stimulates insulin secretion through the phospholipase C pathway[17,18]. Kyriazis *et al.* also reported that insulin secretion was not induced by glucose catabolized from fructose, but by activation of the sweet taste receptor in a glucose-dependent manner through transient receptor potential cation channel, subfamily M, member 5[17]. In the present study, the fructose-induced insulin secretion seen in *Kir6.2*$^{+/+}$ mice was not observed at all in *Kir6.2*$^{-/-}$ mice, and dizaoxide completely blocked fructose-induced insulin secretion *in vivo* and *in vitro*. These results show that the K_{ATP} channel in β-cells plays an essential role in the fructose-induced insulin secretion. In contrast, we previously showed that insulin secretion mediated by the vagal nerve *in vivo* was K_{ATP} channel-independent[19], and it was reported previously that insulin secretion through activation of the phospholipase C pathway differed from that induced by carbachol, the activator of the muscarinic receptor[18]. These findings suggest that the K_{ATP} channel-dependent phospholipase C–transient receptor potential cation channel, subfamily M, member 5 pathway is involved in fructose-induced insulin secretion *in vivo*.

In conclusion, fructose stimulates GLP-1 secretion under normolglycemia, but enhances GIP secretion under the hyperglycemic condition, both of which modifications are in a K_{ATP} channel-independent manner. K_{ATP} channels play an essential role in the insulin secretion induced by fructose *in vivo*.

ACKNOWLEDGMENTS

We thank Michiko Yamada and Mayumi Katagiri (Nagoya University Graduate School of Medicine) for their technical assistance, and Junichi Miyazaki (Osaka University) for providing MIN6-K8 β-cells. This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sport, Science and Technology, Japan.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; 132: 2131–2157.
2. Seino Y, Yabe D. Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1: incretin actions beyond the pancreas. *J Diabetes Invest* 2013; 4: 108–130.
3. Miki T, Nagashima K, Tashiro F, *et al.* Defective insulin secretion and enhanced insulin action in KATP channel-deficient mice. *Proc Natl Acad Sci USA* 1998; 95: 10402–10406.
4. Parker HE, Habib AM, Rogers GJ, *et al.* Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* 2009; 52: 289–298.
5. Reimann F, Habib AM, Tolhurst G, *et al.* Glucose sensing in L cells: a primary cell study. *Cell Metab* 2008; 8: 532–539.
6. Ferraris RP. Dietary and developmental regulation of intestinal sugar transport. *Biochem J* 2001; 2: 265–276.

7. Ogata H, Seino Y, Harada N, *et al*. KATP channel as well as SGLT1 participates in GIP secretion in the diabetic state. *J Endocrinol* 2014; 222: 191–200.
8. Sakamoto E, Seino Y, Fukami A, *et al*. Ingestion of a moderate high-sucrose diet results in glucose intolerance with reduced liver glucokinase activity and impaired glucagon-like peptide-1 secretion. *J Diabetes Invest* 2012; 3: 432–440.
9. Iwasaki M, Minami K, Shibasaki T, *et al*. Establishment of new clonal pancreatic β-cell lines (MIN6-K) useful for study of incretin/cyclic adenosine monophosphate signaling. *J Diabetes Invest* 2010; 1: 137–142.
10. Kuhre RE, Gribble FM, Hartmann B, *et al*. Fructose stimulates GLP-1 but not GIP secretion in mice, rats, and humans. *Am J Physiol Gastrointest Liver Physiol* 2014; 306: G622–G630.
11. Flatt PR, Kwasowski P, Bailey CJ. Stimulation of gastric inhibitory polypeptide release in ob/ob mice by oral administration of sugars and their analogues. *J Nutr* 1989; 119: 1300–1303.
12. Gribble FM, Williams L, Simpson AK, *et al*. A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLUTag cell line. *Diabetes* 2003; 52: 1147–1154.
13. Brubaker PL. Regulation of intestinal proglucagon-derived peptide secretion by intestinal regulatory peptides. *Endocrinology* 1991; 128: 3175–3182.
14. Roberge JN, Brubaker PL. Regulation of intestinal proglucagon-derived peptide secretion by glucose-dependent insulinotropic peptide in a novel enteroendocrine loop. *Endocrinology* 1993; 133: 233–240.
15. Persson K, Gingerich RL, Nayak S . Reduced GLP-1 and insulin responses and glucose intolerance after gastric glucose in GRP receptor-deleted mice. *Am J Physiol Endocrinol Metab* 2000; 279: E956–E962.
16. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* 1999; 140: 1687–1694.
17. Kyriazis Ga, Soundarapandian MM, Tyrberg B. Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. *Proc Natl Acad Sci USA* 2012; 109: E524–E532.
18. Nakagawa Y, Nagasawa M, Yamada S, *et al*. Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS One* 2009; 4: e5106.
19. Seino Y, Miki T, Fujimoto W, *et al*. Cephalic phase insulin secretion is KATP channel independent. *J Endocrinol* 2013; 218: 25–33.