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Author(s)	HIRA, Tohru; MURAMATSU, Maya; OKUNO, Masahiro; HARA, Hiroshi
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1 **Title**

2 GLP-1 secretion in response to oral and luminal palatinose (isomaltulose) in rats

3 **Authors**

4 Tohru Hira¹, Maya Muramatsu², Masahiro Okuno³, Hiroshi Hara¹

5

6 **Affiliations**

7 ¹ Research Faculty of Agriculture, Hokkaido University

8 Kita-9, Nishi-9, Kita-ku, Sapporo, 060-8589, Japan

9 ² Graduate School of Agriculture, Hokkaido University

10 Kita-9, Nishi-9, Kita-ku, Sapporo, 060-8589, Japan

11 ³ Product Development Section, Mitsui Sugar Co., Ltd.

12 1-2-14, Honson, Chigasaki, Kanagawa, 253-0042, Japan

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14 **Number of Figures~ 3**

15

16 **Running head**

17 Palatinose induces GLP-1 secretion in rats

18

19 **Corresponding author**

20 Tohru Hira

21 Laboratory of Nutritional Biochemistry, Research Faculty of Agriculture, Hokkaido

22 University, Kita-9, Nishi-9, Kita-ku, Sapporo, 060-8589, Japan

23 Email: hira@chem.agr.hokudai.ac.jp

24 Tel & Fax: +81-11-706-2811

25

25 「ラットにおけるパラチノースの経口投与、腸管内投与に対する GLP-1 分泌応答」

26 比良 徹¹、村松 茉耶²、奥野 雅浩³、原 博¹

27 ¹北海道大学 大学院農学研究院 応用生命科学部門 食品科学分野 食品栄養学研究室

28 ²北海道大学 大学院農学院 応用生物科学専攻 食資源科学講座

29 ³三井製糖株式会社 開発本部 商品開発部

30 連絡先: 比良 徹、email: hira@chem.agr.hokudai.ac.jp

31 〒060-8589 札幌市北区北9条西9丁目

32 電話、ファックス: 011-706-2811

33 <要約>

34 消化管ホルモン Glucagon-like peptide-1 (GLP-1)は、糖質や脂質、ペプチドの摂取により、下
35 部消化管に多く分布する GLP-1 産生細胞より分泌される。消化速度の遅い二糖類パラチノース
36 (isomaltulose)が、GLP-1 の分泌を促進するどうか、またその機構について、ラットおよび GLP-1
37 産生細胞株を用いて検討した。

38 SD系雄性ラットにおいて、パラチノースまたはスクロース溶液を、無麻酔下で経口投与、または
39 麻酔下で空腸、回腸に直接投与し、門脈血中のグルコース、GLP-1 濃度を経時的に測定した。
40 また、マウス由来 GLP-1 産生細胞株をパラチノース溶液に暴露し、上清中の GLP-1 濃度を測定
41 した。

42 覚醒ラットにおいて、パラチノース経口投与群の血中 GLP-1 濃度は、スクロース投与群よりも
43 高値を示した。麻酔下ラットにおいては、パラチノース、スクロースともに回腸投与の方が空腸投
44 与よりも強く GLP-1 分泌を誘導した。血中グルコース濃度は、いずれの試験においてもパラチノ
45 ース投与群が低値を示した。グルコースは GLP-1 産生細胞株からの GLP-1 分泌を促進したが、
46 パラチノース、スクロースは促進しなかった。以上より、パラチノースがラット腸管において GLP-1
47 分泌を刺激することが見出され、その作用はパラチノースより遊離したグルコースへの寄与が大
48 きいことが示唆された。

49

49 **Summary**

50 Palatinose (isomaltulose), a slowly digested disaccharide, is used as a non-cariogenic
51 sugar and as a sucrose substitute in several foods. Because of its ability to lower
52 postprandial glycemia, palatinose may be beneficial as a treatment for impaired glucose
53 metabolism. Glucagon-like peptide-1 (GLP-1) improves glycemia via enhancing pancreatic
54 beta-cell functions. The secretion of GLP-1 is stimulated by sugars, including glucose and
55 artificial sweeteners. In this study, we examined whether palatinose induced GLP-1
56 secretion *in vivo* and *in vitro*. Firstly, portal GLP-1 and glucose were measured after oral
57 administration of palatinose or sucrose in conscious rats. Secondly, portal GLP-1 and
58 glucose were measured after jejunal or ileal administration of each sugar in anesthetized
59 rats. Finally, GLUTag, a murine GLP-1-producing cell line, was exposed to several sugars,
60 including palatinose and sucrose, to observe the direct effect of these sugars on GLP-1
61 secretion. Compared with sucrose, palatinose enhanced portal GLP-1 level when
62 administered orally in conscious rats. Both palatinose and sucrose induced a significant
63 increase in portal GLP-1 after jejunal or ileal administration of each sugar in anesthetized
64 rats. Ileal administration triggered a greater response than did jejunal administration.
65 Glycemic responses were higher in sucrose-treated rats than in palatinose-treated rats in
66 every experiment. In GLUTag cells, glucose induced a significant increase in GLP-1
67 secretion, but neither sucrose nor palatinose had an effect. These data demonstrate that
68 luminal palatinose induces GLP-1 secretion in rat. However, it is likely that GLP-1 secretion
69 is triggered mainly by glucose released in the lumen rather than by palatinose itself.

70

71 **Key words**

72 Palatinose, Isomaltulose, GLP-1, enteroendocrine cells

73

74

74 Introduction

75 Achieving glycemic control is critical for the prevention and treatment of diabetes and
76 other disorders of glucose metabolism. Inhibitors of α -amylase or α -glucosidase are used
77 to prevent postprandial hyperglycemia by delaying carbohydrate metabolism and thereby
78 reducing glucose absorption from the gut. Delaying carbohydrate metabolism also
79 enhances the secretion of a gut hormone, glucagon-like peptide-1 (GLP-1) (1). When intact
80 carbohydrates reach the distal small intestine, they are slowly digested there, and glucose
81 is released into the lumen. The middle and distal regions of the small intestine contain
82 'L-type' enteroendocrine cells that produce GLP-1, glucagon-like peptide-2, and/or
83 peptide-YY (2). Luminal glucose is a potent stimulator of GLP-1 secretion from L cells (3,4).

84 GLP-1 has several biological functions: it protects pancreatic β -cells, and it enhances
85 β -cell proliferation and the release of insulin. GLP-1 attenuates hyperglycemia acutely via
86 insulin release; in addition, long-term treatment with GLP-1 or its analogue improves
87 insulin-sensitivity in animal models and in human subjects (5-8). Given the functions of
88 GLP-1 and the location of the cells that secrete it, it is important to note that luminal
89 glucose in the distal intestine, but not in the proximal small intestine, can be beneficial for
90 glycemic control.

91 Palatinose (6-O-D-glucopyranosyl-D-fructose, or isomaltulose) is a disaccharide that
92 consists of glucose and fructose connected through an α -1,6-glucosidic bond. Sucrose
93 consists of glucose and fructose connected through an α -1,2-glucosidic bond. Unlike
94 sucrose, palatinose is slowly hydrolyzed by brush-border isomaltase (9,10), however, it is
95 completely digested and absorbed as monosaccharides (glucose and fructose) in the small
96 intestine (11). Several papers have demonstrated that palatinose treatment is effective in
97 preventing postprandial hyperglycemia and in improving insulin sensitivity (12-16). These
98 effects are primarily explained by the slower release and absorption of glucose. It has also
99 been demonstrated that palatinose itself inhibits glucose absorption in an everted intestinal

100 sac model (17). Although both palatinose and GLP-1 have beneficial effects on glycemia,
101 the effect of palatinose on GLP-1 secretion is unknown.

102 In the present study we examined whether oral and luminal palatinose induce GLP-1
103 secretion in rats, and we determined whether palatinose itself directly stimulates GLP-1
104 secretion from GLP-1-producing enteroendocrine cells.

105

106 **Materials and Methods**

107 **Animals**

108 Male Sprague-Dawley rats (7 weeks old) weighing 210–230 g were purchased from
109 Japan SLC (Hamamatsu, Japan). Rats had free access to water and to a semi-purified diet
110 containing 25% casein based on AIN-93G (18). They were housed in individual cages in a
111 temperature-controlled room maintained at $23 \pm 2^\circ\text{C}$ with a 12-h light–dark cycle
112 (0800–2000, light period). The study was approved by the Hokkaido University Animal
113 Committee, and the rats were maintained in accordance with the Hokkaido University
114 guidelines for the care and use of laboratory animals.

115

116 Experiment 1~ Effects of oral administration of palatinose or sucrose on GLP-1 secretion
117 and glycemia in conscious rats.

118 Rats were anesthetized with sodium pentobarbital (40 mg/kg body weight, Nembutal
119 Injection, Dainippon Sumitomo Pharma, Osaka, Japan) and subjected to a laparotomy. A
120 small tip (7-8 mm) of a polyethylene catheter (SP 28; I.D. 0.4 mm, O.D. 0.8 mm; Natsume
121 Seisakusyo, Tokyo, Japan) connected to a silicone catheter (Silascon No.00, I.D. 0.5 mm,
122 O.D. 1.0 mm; Dow Corning Co., Kanagawa, Japan) was inserted into the portal vein. The
123 catheter was prefilled with saline containing heparin (50 IU/ml; Ajinomoto, Tokyo, Japan).
124 The free end of the portal catheter was exteriorized dorsally, which allowed the collection of
125 portal blood under unanesthetized and unrestrained conditions. Rats had a 2-day recovery

126 period before the onset of the experiment. The portal catheter was flushed daily with
127 heparinized saline to maintain patency.

128 After an overnight fast, rats received an oral dose of palatinose solution (4 g/kg body
129 weight, Mitsui Sugar Co., Ltd., Tokyo, Japan) or sucrose solution (4 g/kg body weight) via
130 a feeding tube (Safeed feeding tube Fr. 5, TERUMO. Co., Tokyo, Japan) inserted directly
131 into the stomach. The dose of test sugars at 4 g/kg was comparable to the dose of glucose
132 that is widely used in the oral glucose tolerance test (2 g/kg). Blood samples (100 μ l) were
133 drawn into a syringe containing EDTA (final concentration 1 mg/ml), aprotinin (final
134 concentration 500 kIU/ml, Wako Pure Chemical Industries, Ltd, Osaka, Japan) and
135 Diprotin A, a dipeptidyl peptidase IV inhibitor (final concentration 100 μ M, PEPTIDE
136 Institute Inc., Osaka, Japan) through the portal catheter before (0 min) and after (15, 30, 60,
137 90, 120, 150, 180, 210 and 240 min) the sample administration. Plasma was separated
138 from blood samples by centrifugation at 2,500 x g for 15 min at 4°C and was then frozen at
139 -80°C until GLP-1 and glucose were measured. The plasma concentration of GLP-1 and
140 insulin were measured by the GLP-1(Active) ELISA kit (Shibayagi Co. Ltd., Gunma,
141 Japan) and by rat insulin ELISA kit (U-type, Shibayagi Co. Ltd.), respectively, and plasma
142 glucose was measured by the Glucose CII-test kit (Wako).

143

144 Experiment 2~ Effects of luminal administration of palatinose or sucrose on GLP-1
145 secretion and glycemia in anesthetized rats.

146 After an overnight fast, rats were anesthetized with ketamine (80 mg/kg body weight,
147 Ketalar, Daiichi Sankyo, Tokyo, Japan) mixed with xylazine (12 mg/kg, Sigma). A catheter
148 was inserted into the portal vein, as described above. The jejunal ligated loop, including the
149 duodenum and jejunum, was prepared between the end of the pylorus and 45 cm distal to
150 the ligament of Treitz. The ileal ligated loop (~45 cm) was prepared between a section 45
151 cm distal to the ligament of Treitz and the terminal ileum. The proximal and distal ends of

152 the loop were ligated with a silk thread. After basal (0 min) blood collection, a palatinose or
153 sucrose solution (2 g/ 4 ml/ kg) was directly administered into the loop. The dose was set at
154 half of the oral administration described above. Portal blood was collected through the
155 portal catheter at 15, 30, 60, 90 and 120 min after administering the solutions. During the
156 experiment, additional ketamine (40 mg/kg) mixed with xylazine (6 mg/kg) was injected to
157 keep the rats anesthetized, and body temperature was maintained using a heating pad.
158 Plasma GLP-1 and glucose were measured as described above.

159

160 Experiment 3~ Effects of palatinose, sucrose and various sugars on GLP-1 secretion in a
161 GLP-1-producing enteroendocrine cell line.

162 GLUTag cells (a gift from Dr. D. J. Drucker, University of Toronto, Toronto, Canada), a
163 murine GLP-1-producing enteroendocrine cell line, were grown in Dulbecco's modified
164 Eagle's medium (Invitrogen, Cat. No. 12100-038) supplemented with 10% fetal bovine
165 serum, 50 IU/ml penicillin, and 500 µg/ml streptomycin in a humidified 5% CO₂ atmosphere
166 at 37°C. Cells were routinely subcultured by trypsinization after reaching 80–90%
167 confluency. GLUTag cells were grown in 48-well culture plates at a density of 1.25×10^5
168 cells/well for 2-3 days until they reached 80-90% confluency. Cells were washed twice with
169 HEPES buffer (140 mM NaCl, 4.5 mM KCl, 20 mM HEPES, 1.2 mM CaCl₂, 1.2 mM MgCl₂,
170 and 0.1% bovine serum albumin, pH 7.4) to remove the culture media and were then
171 exposed to test agents dissolved in the same buffer for 60 min at 37°C. Based on the
172 results in previous papers (19, 20), all sugars were used at 20 mM (in HEPES buffer). A 70
173 mM KCl solution (74.5 mM NaCl, 70 mM KCl, 20 mM HEPES, 1.2 mM CaCl₂, 1.2 mM MgCl₂,
174 and 0.1 % bovine serum albumin, pH 7.4) was used as a depolarization stimulus.
175 Supernatants were collected from the wells, centrifuged at 800 x g for 5 min at 4°C to
176 remove remaining cells, and then stored at –50°C until the GLP-1 concentration was
177 measured with a commercial enzyme-immunoassay (EIA) kit (Yanaihara Institute Inc.,

178 Shizuoka, Japan).

179

180 Statistical analysis

181 Results are expressed as means \pm SEM. Statistical significance was assessed using
182 one-way or two-way ANOVA, and significant differences among mean values were
183 determined by Student's t-test or Fisher's LSD test ($P < 0.05$).

184

185 **Results**

186 Portal blood samples were collected through the catheter before and after the intragastric
187 administration of a palatinose or sucrose solution in conscious rats. After the sucrose
188 administration, the plasma GLP-1 concentration (Fig. 1A) was not significantly changed
189 throughout the experimental period (0-240 min). GLP-1 levels in palatinose-treated rats
190 were elevated at all times except for 150 min after treatment. Although statistically
191 significant differences between treatments at each time point were not detected, two-way
192 ANOVA indicated a significant difference in plasma GLP-1 between palatinose-treated rats
193 and sucrose-treated rats. The area under the curve (AUC) of plasma GLP-1 in
194 palatinose-treated rats (34.5 ± 10.3 nM*4hr) was around 1.5-times higher than that of
195 sucrose-treated rats (20.1 ± 1.7 nM*4hr), but statistically significant difference was not
196 detected.

197 Plasma glucose (Fig. 1B) was immediately increased 15 min after intragastric
198 administration of sucrose. The glucose level peaked at approximately 200 mg/dl 15 to 30
199 min after the administration of sucrose and then gradually decreased. In contrast, glucose
200 levels remained below 160 mg/dl throughout the experimental period in the
201 palatinose-treated rats. Plasma glucose levels in palatinose-treated rats were considerably
202 lower than those in sucrose-treated rats from 15 to 60 min; conversely, plasma glucose
203 levels in palatinose-treated rats were slightly higher than those in sucrose-treated rats at

204 180 and 210 min. Changes in plasma insulin in sucrose-treated rats (Fig. 1C) were almost
205 similar to those in plasma glucose (Fig. 1B). Insulin levels were gradually increased until
206 210 min in palatinose-treated rats. Peak insulin levels were similar between two
207 treatments. Fig. 1

208 Plasma GLP-1 levels (Fig. 2A) gradually increased until 90 min after either the jejunal or
209 ileal administration of test sugars in anesthetized rats. In the jejunum, the administration of
210 sucrose and palatinose induced similar increases in portal GLP-1 for 90 min, but whereas
211 the palatinose infusion continued to enhance portal GLP-1, the sucrose infusion did not.
212 GLP-1 levels at 90 min after the administration of either sugar and at 120 min after the
213 administration of palatinose were significantly higher than basal levels. In the ileum, both
214 sugars caused a higher increase in portal GLP-1 than they did in the jejunum. GLP-1 levels
215 at 60, 90, 120 min in sucrose-treated rats were significantly higher than the basal level, and
216 the levels at 90 and 120 min in palatinose-treated rats were also significantly higher than
217 the basal level. Increments of GLP-1 in palatinose-treated rats were relatively small
218 compared with those in sucrose-treated rats. Fig. 2

219 Basal glucose levels were 200-250 mg/dl in all treatments (Fig. 2B) and exceeded the
220 basal values in conscious rats (Fig. 1B). Previous reports have attributed this effect to
221 anesthesia (21,22). Indeed, glucose levels in the mesenteric vein were also ~200 mg/dl in
222 our preliminary study, which used the same anesthetic conditions as the present study.
223 However, changes in the glucose level in the portal vein seem to reflect glucose absorption
224 appropriately in the present study because ketamine-induced hyperglycemia is explained
225 by the inhibitory effect of the anesthetic on glucose-induced insulin secretion (22). This
226 inhibition might be also responsible for lower basal GLP-1 in anesthetized rats than
227 conscious rats since insulin reportedly induces GLP-1 secretion (23). Plasma glucose
228 increased greatly after the jejunal administration of sucrose, and values from 30 to 120 min
229 were significantly higher than the basal value. Jejunal palatinose induced a gradual

230 increase in plasma glucose, which reached statistical significance at 90 and 120 min.
231 Increments were smaller than those in sucrose-treated rats. Ileal infusion of sucrose or
232 palatinose induced gradual and similar increases in portal glucose. Increments induced by
233 each sugar in the ileum were smaller than those induced in the jejunum.

Fig. 3

234 To see the direct effect of different sugars on GLP-1-producing cells, GLUTag cells were
235 exposed to palatinose, sucrose, and other saccharides. Glucose, sucralose, and a
236 depolarization stimulus (70 mM KCl) induced a 2- to 2.5-fold increase in GLP-1 secretion;
237 this increase confirms that GLUTag cells are susceptible to physiological stimuli, including
238 sugars (19,20). Sucrose, palatinose, and fructose, all at the same concentration as glucose
239 (20 mM), did not trigger an increase in GLP-1 secretion in GLUTag cells.

240

241 Discussion

242 Palatinose is known as one of the slowly digested sugars (9,11). Several studies have
243 demonstrated its usefulness for glycemic control in animals and humans (12-16). GLP-1
244 has also gained popularity as a means for preventing and treating glycemic disorders,
245 including type-2 diabetes. Its therapeutic value results from its protective and proliferative
246 effects on pancreatic β -cells and its ability to stimulate insulin secretion. GLP-1 secretion is
247 triggered by luminal glucose (4,19) and sweeteners (20) via sugar sensors on
248 GLP-1-producing enteroendocrine cells.

249 Palatinose is slowly digested so that glucose is liberated in the ileal lumen. Palatinose is
250 as sweet as glucose and about half as sweet as sucrose (11). Its sweetness level and its
251 ability to generate glucose suggest that palatinose may stimulate GLP-1 secretion. No
252 published reports have investigated GLP-1 secretion in response to palatinose. In the
253 present study, we examined whether palatinose induces GLP-1 secretion *in vivo* (rats) and
254 *in vitro* (GLP-1-producing enteroendocrine cells).

255 We found that portal GLP-1 levels were higher in rats treated with palatinose than in those

256 received sucrose (Fig. 1A). Although increases in GLP-1 after oral administration of
257 palatinose in conscious rats were statistically insignificant, *in situ* experiment (Fig. 2A)
258 revealed that luminal palatinose induced GLP-1 secretion in rats. In contrast, oral sucrose
259 did not enhance portal GLP-1 levels (Fig. 1A). Because direct administration of sucrose
260 into the jejunum or ileum induced a significant increase in GLP-1 (Fig. 2A), the failure of
261 oral sucrose to increase GLP-1 might be due to an insufficient delivery of sucrose into the
262 middle and distal intestinal lumen to trigger GLP-1 secretion from L cells. Sucrose passed
263 through the stomach can be easily hydrolyzed by brush-border sucrase and immediately
264 absorbed in the upper small intestine. In contrast, palatinose is slowly digested by
265 brush-border isomaltase so that it can reach the middle and distal small intestine.
266 Therefore, it is likely that palatinose itself or glucose liberated from palatinose directly
267 stimulated GLP-1 secretion from L cells. Previous studies using α -glucosidase inhibitors
268 have also suggested that a delayed release of glucose in the distal small intestine
269 enhances GLP-1 secretion (1,3,24). Our *in vitro* study using murine GLP-1-producing
270 GLUTag cells supports the hypothesis that liberated glucose is responsible for stimulating
271 GLP-1 secretion because neither palatinose nor sucrose had an effect (Fig. 3).

272 Portal glucose levels were lower in palatinose-treated rats than in sucrose-treated rats in
273 the first hour of the experiment (Fig. 1B). This reflects the lower digestibility of palatinose
274 than sucrose. Sustained glucose levels in palatinose-treated rats also indicate that the
275 sugar is slowly digested and absorbed not only in the proximal small intestine but also in
276 the distal region. Insulin responses showed almost similar pattern to glucose responses in
277 each treatment (Fig. 1C). However, differences in insulin level between two treatments are
278 smaller than those in glucose level. Higher GLP-1 levels after palatinose administration
279 might be responsible for enhanced insulin responses.

280 In the ligated loop experiment, both of the test sugars (at the half dose of the oral
281 administration) induced a higher GLP-1 secretion in the ileum than in the jejunum (Fig. 2A).

282 This result is due to the distribution of GLP-1 producing cells in the intestine; the number of
283 GLP-1-producing cells is higher in distal intestine than in the proximal intestine (2,25).
284 Higher GLP-1 secretion by ileal sucrose than ileal palatinose might result from the
285 difference in the digestibility of these sugars and from the lower degree of glucose
286 transport in the ileum than in the jejunum. Sucrose is digested more rapidly than palatinose,
287 but the rate of glucose absorption was similar in both treatments (Fig. 2B). Therefore, it is
288 likely that sucrose treatment caused more glucose to accumulate in the ileal lumen,
289 thereby inducing a higher level of GLP-1 secretion than palatinose. The difference in
290 GLP-1 secretion between conscious rats and anesthetized rats suggests that the delivery
291 of sugars into the intestinal lumen (mediated by gastric emptying) is an important factor for
292 stimulating GLP-1 secretion *in vivo*.

293 Recent papers report that the sweet-taste receptor (T1R2/3) is involved in sugar-induced
294 GLP-1 secretion (20,26,27). In the present study, glucose and sucralose (both at 20 mM)
295 induced significant GLP-1 secretion in GLUTag cells; however, sucrose, palatinose, and
296 fructose (all at 20 mM) did not (Fig. 3). Based on these results, glucose, as a product of the
297 digestion of sucrose or palatinose, may mainly be responsible for stimulating GLP-1
298 secretion *in vivo*. A previous paper (28) demonstrated fructose-induced GLP-1 secretion in
299 GLUTag cells with less potency than glucose, suggesting that higher concentration than 20
300 mM is necessary for fructose to induce significant increase in GLP-1 secretion in our
301 experimental conditions. In case of disaccharides such as sucrose and palatinose,
302 degradation to monosaccharide might be important to stimulate GLP-1 secretion. However,
303 slight increases in GLP-1 by fructose and palatinose (Fig. 3) also suggest possible
304 involvement of these components in palatinose-induced GLP-1 secretion in the intestine.
305 Further examinations are necessary to elucidate the hypothesis.

306 In summary, portal GLP-1 concentrations after oral administration of palatinose were
307 higher compared to those after sucrose administration in conscious rats. Both palatinose

308 and sucrose directly administered into the ileum induced a higher GLP-1 secretion than did
309 their administration into the jejunum. Portal glucose concentrations were lower in
310 palatinose-treated rats than in sucrose-treated rats. In GLP-1 producing cells, glucose, but
311 not palatinose or sucrose, induced significant GLP-1 secretion. These results suggest that
312 glucose released from palatinose in the ileum is mainly responsible for stimulating
313 GLP-1-producing cells to secrete GLP-1.

314

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403

403 **Figure legends**

404 Fig. 1. GLP-1, glucose and insulin concentrations in the portal vein after intragastric
405 administration of palatinose or sucrose in conscious rats.

406 Palatinose (4 g/kg, closed circle) or sucrose (4 g/kg, open circle) was orally administered
407 into the stomach of conscious rats. Blood samples were collected through the portal vein
408 catheter before (0 min) and after the oral sugar administration. Values are means \pm SEM of
409 7-8 rats in each group. Two-way ANOVA P values for GLP-1 (A) were 0.99 for time, 0.043
410 for treatment, and 0.93 for treatment x time. The values for glucose (B) were all <0.01 . The
411 values for insulin (C) were 0.02 for time, 0.69 for treatment, and 0.38 for treatment x time.
412 Asterisk (*) signs indicate significant differences between treatments (Student's t-test, $P <$
413 0.05).

414

415 Fig. 2. Plasma GLP-1 and glucose after the instillation of a palatinose or sucrose solution in
416 the ligated jejunal or ileal loop in anesthetized rats.

417 Palatinose (Pal: 2 g/kg, closed circle) or sucrose (Suc: 2 g/kg, open circle) was directly
418 administered into the jejunum (Jej: thinner line) or ileum (Ile: thicker line) of anesthetized
419 rats. Blood samples were collected through the portal vein catheter before (0 min) and after
420 the sugar administration. Values are means \pm SEM of 7-8 rats in each group. Two-way
421 ANOVA P values for GLP-1 (A) were < 0.01 for time and for treatment, and 0.898 for
422 treatment x time. Two-way ANOVA P values for glucose (B) were < 0.01 for time and for
423 treatment, and 0.549 for treatment x time. Plots with a plus (+) sign differ significantly from
424 the value at 0 min in each group (Fisher's test, $P < 0.05$). Plots at the same time point not
425 sharing the same letter (a, ab, b) differ significantly between treatments (Fisher's test, $P <$
426 0.05).

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428

428 Fig. 3 GLP-1 secretion in response to various sugars in GLUTag cells

429 GLUTag cells were exposed to various sugars (20 mM), a sweetener (sucralose at 20
430 mM) or 70 mM KCl for 60 min. The supernatant was collected and the GLP-1 concentration
431 was measured with GLP-1 EIA. Values are relative (%) to control GLP-1 secretion and
432 expressed as means \pm SEM (n = 6-8). Values not sharing the same letter differ significantly
433 (P < 0.05 by Fisher's test).

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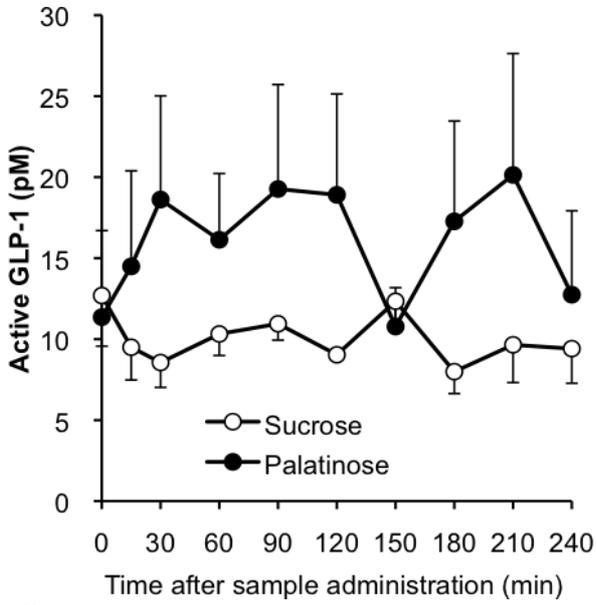
436

436 **FIGURE 1**

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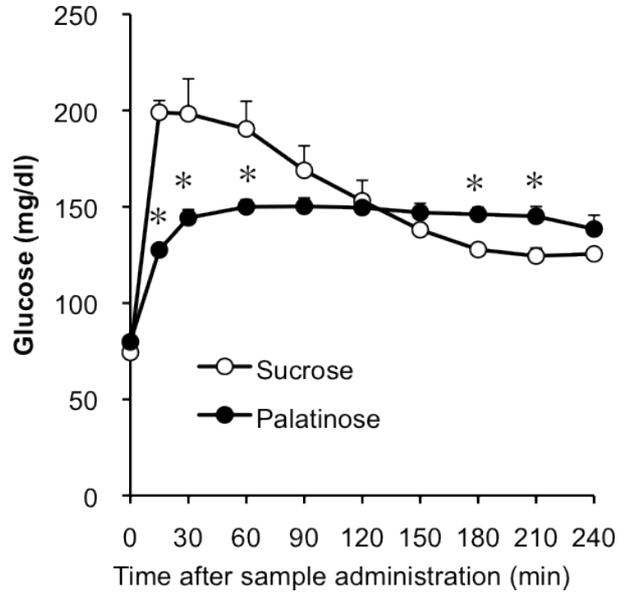
438

A

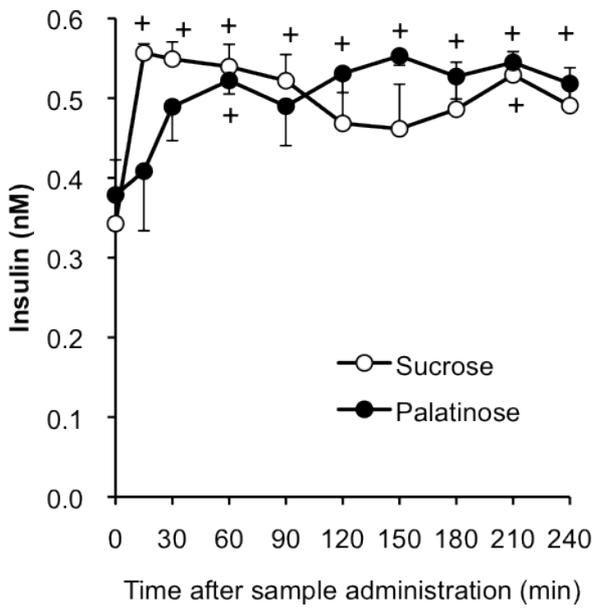


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B



C



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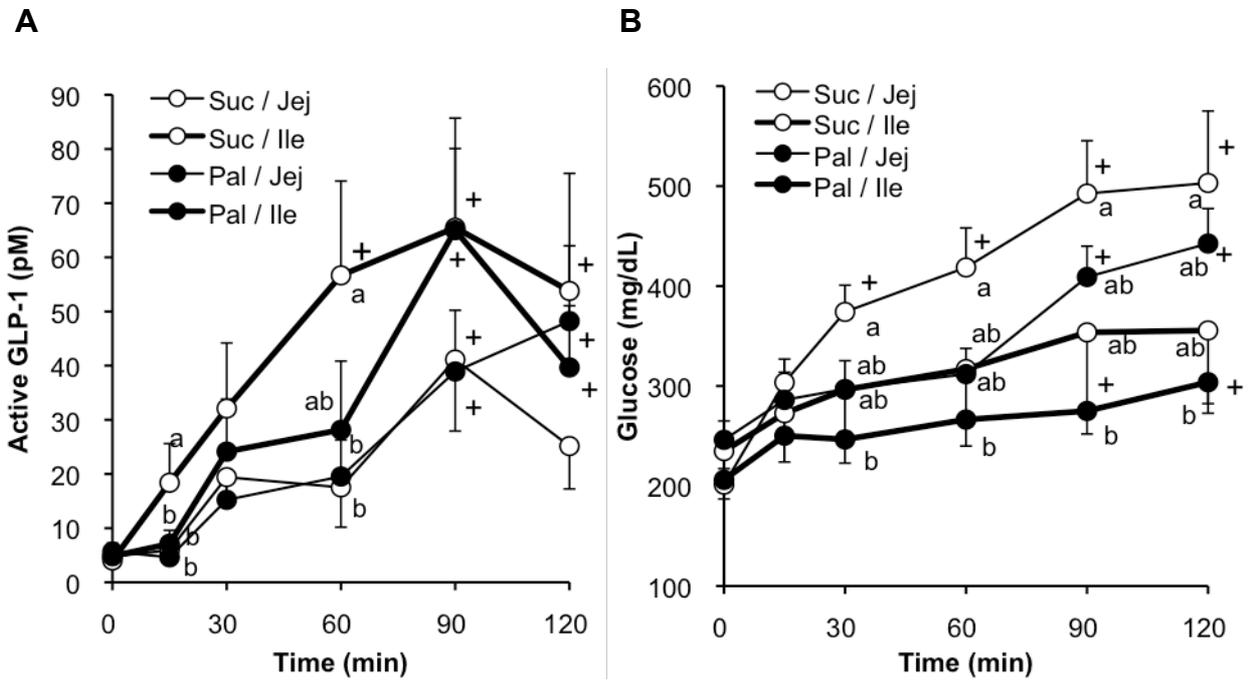
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Hira, Figure 1

442 **FIGURE 2**

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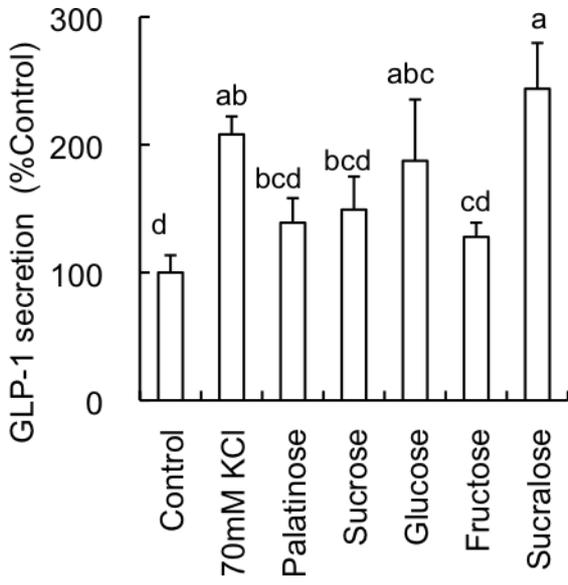
449

Hira, Figure 2

449 **FIGURE 3**

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Hira, Figure 3