HOKKAIDO UNIVERSITY

Title	The Corn Protein, Zein Hydrolysate, Administered into the Ileum Attenuates Hyperglycemia via Its Dual Action on Glucagon-Like Peptide-1 Secretion and Dipeptidyl Peptidase-IV Activity in Rats
Author(s)	Mochida, Taisuke; Hira, Tohru; Hara, Hiroshi
Citation	Endocrinology, 151(7), 3095-3104 https://doi.org/10.1210/en.2009-1510
Issue Date	2010
Doc URL	https://hdl.handle.net/2115/85405
Type	journal article
File Information	Endocrinology_151_3095.pdf

Title

The corn protein, Zein, hydrolysate administered into the ileum attenuates hyperglycemia via its dual

action on GLP-1 secretion and DPP-IV activity in rats

Precis

Corn Zein hydrolysate both induces GLP-1 secretion and reduces DPP-IV activity in rats

Short Title

GLP-1 releasing peptide on glycemic control

Authors

Taisuke Mochida, Tohru Hira, and Hiroshi Hara

Affiliations

Division of Bio-Systems and Sustainability, Graduate School of Agriculture, and Division of Applied

Biosciences, Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

Corresponding author

Tohru Hira

email: hira@chem.agr.hokudai.ac.jp

TEL: +81-11-7-6-2811, FAX: +81-11-706-2504

Key words

Glucagon-like peptide-1; Dipeptidyl peptidase-IV; Dietary peptide

Abstract

1   **Abstract**

2   We previously showed that a hydrolysate prepared from corn zein (ZeinH) strongly stimulates

3   GLP-1 secretion from the murine GLP-1-producing enteroendocrine cell line and in the rat small

4   intestine, especially in the ileum. Here, we investigated whether ZeinH administered into the ileum

5   affects glucose tolerance via stimulating GLP-1 secretion. To observe the effect of luminal ZeinH

6   itself on GLP-1 secretion and glycemia, intraperitoneal glucose tolerance tests were performed in

7   conscious rats with ileal and jugular catheters, and plasma glucose, insulin, and GLP-1 (total and

8   active) were measured. In addition, plasma dipeptidyl peptidase-IV (DPP-IV) activities in the ileal

9   vein were measured after the administration of ZeinH into the ileal-ligated loop in anesthetized rats.

10   The ileal administration of ZeinH attenuated the glucose-induced hyperglycemia accompanied by the

11   enhancement of insulin secretion, whereas meat hydrolysate (MHY) neither induced insulin secretion

12   nor attenuated hyperglycemia. The anti-hyperglycemic effect was also demonstrated by the oral

13   administration of ZeinH. From these results, it was predicted that the GLP-1-releasing potency of

14   ZeinH was higher than that of MHY. However, both peptides induced similar increase in total GLP-1

15   concentration after the ileal administration. In contrast, active GLP-1 concentration was increased

16   only in ZeinH-treated rats. In anesthetized rats, ileal administration of ZeinH but not MHY decreased

17   plasma DPP-IV activity in the ileal vein. These results indicate that the ileal administration of a

18   dietary peptide, ZeinH, has the dual functions of inducing GLP-1 secretion and inhibiting GLP-1

19   degradation, resulting in the enhancement of insulin secretion and the prevention of hyperglycemia in

20   rats.

21

22

23

24

25

26

1

Introduction

Glucagon-like peptide-1 (GLP-1) is one of the gut hormones that are released in response to nutrient ingestion. GLP-1 stimulates insulin secretion from pancreatic β-cells in the presence of plasma glucose (1). The enhancement of insulin secretion by gut-derived factors, such as GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), was termed the "incretin effect" (2). GLP-1 also stimulates β-cell proliferation, delays gastric emptying and reduces food intake in rats and humans (2, 3). Recently, stable GLP-1 analogs and dipeptidyl peptidase-IV (DPP-IV) inhibitors that protect active GLP-1 from cleavage by DPP-IV are currently used as additional drugs to control postprandial glycemia in type-2 diabetes (4, 5).

GLP-1 is produced by enteroendocrine L cells, which are located predominantly in the lower part of the intestine (ileum, cecum and colon) (6). After its release into the mesenteric and portal circulation, intact GLP-1 is rapidly degraded (inactivated) by DPP-IV in the plasma (7). It is also inactivated by DPP-IV during passage across the hepatic bed and in the peripheral tissues (8). Despite its short half-life, it is estimated that the incretin effect accounts for $\geq 50\%$ of insulin release after glucose ingestion (9). In addition, GLP-1R$^{-/-}$ mice are characterized by mild fasting hyperglycemia and abnormal glucose tolerance (2). Therefore, GLP-1 secretion triggered by luminal nutrients is potentially important for the postprandial regulation of glucose homeostasis.

Recently, attention has been focused on whether it would be possible to utilize the endogenous GLP-1 stored in L cells to improve glucose tolerance (10). Indeed, several pharmacological compounds, including Berberine (an isoquinoline alkaloid originally isolated from *Coptidis rhizome*) (11), TGR5 (a bile acid-sensing GPCR) agonists (12) and GPR119 agonists (13), are effective at improving glucose tolerance through the enhancement of GLP-1 secretion. Therefore, potent luminal stimulants of GLP-1 secretion are of interest and have a great potential for obtaining better glycemic control in subjects with normal and abnormal glucose tolerance.

Glucose and fatty acids are well known as strong stimulants of GLP-1 secretion (14, 15), but

3

peptones also induce GLP-1 secretion in rats (16) and enteroendocrine cell lines (17), and whey

preload enhances GLP-1 secretion in humans (18). Recently, we found that a hydrolysate prepared

from zein, a major corn protein, potently stimulates GLP-1 secretion in the murine GLP-1-producing

enteroendocrine cell line GLUTag and in the rat small intestine (19). Zein hydrolysate (ZeinH) in the

duodenum indirectly stimulates GLP-1 secretion from ileal L cells probably via the afferent vagus, but

in the ileum, ZeinH directly acts on ileal L cells to induce GLP-1 secretion as well as to enhance

fat-induced GLP-1 secretion (20).

The purpose of the present study was to investigate whether the potent GLP-1-releasing peptide

ZeinH affects glycemia via the stimulation of GLP-1 secretion. We examined the effect of ileal ZeinH

on plasma glucose, insulin, and total and active GLP-1 secretion in conscious rats using the

intraperitoneal glucose tolerance test (IPGTT). The effect of luminal ZeinH on plasma DPP-IV

activity in the ileal vein was also examined by using the ileal-ligated loop in anesthetized rats. Since

GLP-1 has the potential to improve pancreatic β-cell function, controlling endogenous GLP-1

secretion by luminal dietary peptides could provide a novel strategy for the prevention and treatment

of obesity and diabetes.

Materials and Methods

Materials

Zein hydrolysate (ZeinH) was prepared as described previously (19). Briefly, Zein (Tokyo

Chemical Industry, Tokyo, Japan) (50 g) was suspended in deionized water (500 ml) and adjusted to

pH 7.0. The suspension was shaken for 60 min at 55°C after the addition of papain (250 mg, Papain F;

Asahi Food and Health Care, Tokyo, Japan). It was then treated in boiling water for 20 min to stop the

enzyme reaction. After centrifugation and filtration (0.2-μm pore size), the supernatant was

lyophilized as ZeinH. Meat hydrolysate (MHY) was purchased from Sigma (St. Louis, MO). ZeinH

and MHY had peptide contents of 65.5 and 80.0%, respectively, as determined by the Lowry protein

assay, and they had respective molecular weights of 1,600 and <1200 Da, as described previously (19,

1 21). Total and free amino acid composition of ZeinH and MHY were measured by the method

2 previously described (22) and are shown in Table 1.

3 *Animals*

4 Male Sprague-Dawley rats (7 weeks old) weighing 210–230 g were purchased from Japan SLC

5 (Hamamatsu, Japan). Animals had free access to a semi-purified diet containing 25% casein based on

6 AIN-93G (23) and water in individual cages. All animal experiments were performed after an

7 acclimation period (3–7 d) in a temperature-controlled room maintained at $23 \pm 2°C$ with a 12-h

8 light-dark cycle (0800–2000, light period). The study was approved by the Hokkaido University

9 Animal Committee, and the animals were maintained in accordance with the guidelines for the care

10 and use of laboratory animals of Hokkaido University.

11 *Surgical preparation for in vivo experiments (Experiments 1 and 2)*

12 After a 24-h fast, rats were anesthetized with sodium pentobarbital (40 mg/kg body weight,

13 Nembutal Injection, Dainippon Sumitomo Pharma, Osaka, Japan). The right jugular vein was exposed

14 and a silicone catheter (Silascon No. 00, I.D. 0.5 mm, O.D. 1.0 mm; Dow Corning Co., Kanagawa,

15 Japan) was inserted into the vessel and fixed with a thread. The catheter was prefilled with saline

16 containing heparin (50 IU/ml, Ajinomoto, Tokyo, Japan). Another silicone catheter (Silascon No.00,

17 I.D. 0.5 mm, O.D. 1.0 mm; Dow Corning Co.), whose tip housed the small segment (2–3 mm) of a

18 polyethylene tube (Hibiki Fr No. 4; Kunii Co., Tokyo, Japan), was inserted into the proximal ileum

19 (45 cm distal to the ligament of Treitz) and fixed with a thread. The free ends of both catheters were

20 exteriorized dorsally, which allowed the experiment to be carried out under unanesthetized and

21 unrestrained conditions. Rats were used for *in vivo* experiments (Experiments 1 and 2) after a

22 recovery period (3–4 d). The ileal and jugular catheters were flushed daily with saline and heparinized

23 saline, respectively, to maintain their patency. Because the ileal catheter is thinner than ileal tract, the

24 intestinal flow would not be blocked by the ileal catheter. This was confirmed by observing normal

25 feeding and evacuation behaviors before and after the surgical operation, and also by observing the

26 intestinal flow under the anesthesia before killing rats at the end of experiments.

5

1   *Experiment 1: Effects of ileal peptide administration on plasma glucose and insulin in conscious*

2   *rats during IPGTT*

3   The glucose solution was administered interperitoneally to examine the effect of luminal peptides

4   themselves on GLP-1-mediated glycemia control. Peptides (MHY and ZeinH) and water (as negative

5   control) were administered into the ileal lumen through the catheter to observe direct effects of

6   peptides on GLP-1 secretion in the ileum and subsequent glucose handling. MHY was chosen as

7   another dietary peptide that has GLP-1-releasing activity *in vitro* (17, 24) and *in situ* (16). After a 24-h

8   fast, a basal (-30 min) blood sample (50 μl) was drawn from the jugular catheter. The catheter was

9   refilled with saline containing heparin (50 IU/ml) between each blood sampling. Just after the basal

10   blood collection, deionized water (2 ml) or test liquids (500 mg MHY, 500 mg ZeinH in 2 ml

11   deionized water) were administered into the ileal lumen through the ileal catheter. A blood sample

12   was drawn (0 min), and then glucose was injected intraperitoneally (1 g/kg) 30 min after the ileal

13   administration. Blood samples were drawn into a syringe containing aprotinin (final concentration 200

14   kIU/ml, Wako, Osaka, Japan) with heparin (final concentration 50 IU/ml) at 15, 30 and 60 min after

15   i.p. glucose injection. Plasma was separated by centrifugation at 2,500 x *g* for 15 min at 4°C and

16   frozen at –80°C until glucose and hormone measurements. Plasma glucose and insulin were measured

17   by using the Glucose CII-test kit (Wako) and insulin-ELISA kit (AKRIN-010T; Shibayagi, Gunma,

18   Japan), respectively.

19   *Experiment 2: Effects of ileal peptide administration on plasma GLP-1 in conscious rats (IPGTT)*

20   The effect of ileal ZeinH on GLP-1 secretion was examined in conscious rats in a separated

21   experiment because a large volume of plasma was required to measure glucose and total and active

22   GLP-1. Because changes in total GLP-1, which includes both active (7-37) and inactive (9-37) forms,

23   reflect the release of GLP-1 but not its activity as incretin, we also measured active GLP-1 in identical

24   blood samples. IPGTT was performed in conscious rats as in Experiment 1. Blood samples for

25   glucose and total GLP-1 (80 μl) were drawn into a syringe containing aprotinin (final concentration

26   200 kIU/ml) and heparin (final concentration 50 IU/ml). Blood samples for active GLP-1 (240 μl)

1   were drawn into a syringe containing EDTA (final concentration 1 mg/ml, Dojindo, Kumamoto,

2   Japan), aprotinin (final concentration 500 kIU/ml) and DPP-IV inhibitor (final concentration 50 μM,

3   catalog no. DPP4-010; Linco Research, St. Charles, MO). Total GLP-1 in the plasma (30 μl) was

4   measured by ELISA kit (Yanaihara Institute, Shizuoka, Japan), and active GLP-1 in the plasma (100

5   μl) was measured by another ELISA kit (Linco Research).

6

7   ***Experiment 3: Effects of ileal administration of ZeinH and MHY on plasma DPP-IV activity in the***

8   ***ileal vein of anesthetized rats (in situ experiment)***

9   To examine the effect of ileal ZeinH and MHY on plasma DPP-IV activity, blood samples were

10   collected from the ileal vein through the catheter after the administration of water, MHY and ZeinH

11   into the ligated ileal loop in anesthetized rats. After a 24-h fast, rats were anesthetized with ketamine

12   (80 mg/kg body wt i.p., Ketaral, Daiichi Sankyo, Tokyo, Japan) containing xylazine (12 mg/kg i.p.,

13   MP Biomedicals, Irvine, CA), and a middle abdominal incision was made.

14   The small tip (6–7 mm) of a polyethylene catheter (SP 10; ID 0.28 mm, OD 0.61 mm; Natsume

15   Seisakusyo, Tokyo, Japan) connected to a silicone catheter (Silascon no. 00, ID 0.5 mm, OD 1.0 mm;

16   Dow Corning Co.) was inserted into the ileal vein in the ileal mesenteric tissue. The ileal lumen was

17   washed by flushing with saline. The ligated ileal loop (30 cm in length) was prepared between 5 and

18   35 cm proximal to the cecum, and the proximal and distal ends of the loop were ligated with a silk

19   thread. A basal (–30 min) blood sample (50 μl) was drawn from the ileal vein catheter, and the

20   catheter was refilled with saline containing heparin (50 IU/ml) between each blood sampling.

21   Deionized water (2 ml) or test liquids (500 mg MHY, 500 mg ZeinH in 2 ml) were then directly

22   administered into the loop. Blood was collected (0 min) 30 min after the sample administration, and

23   glucose was administered intraperitoneally (1 g/kg) to reproduce the experimental conditions of

24   Experiments 1 and 2. Blood samples were collected through the ileal vein catheter at 15, 30 and 60

25   min after the glucose injection.

26   During the experiment, additional ketamine/xylazine was injected to keep the rats anesthetized,

1 and body temperature was maintained with heating pads. Blood samples for plasma DPP-IV activity

2 measurement were drawn into a syringe containing heparin (final concentration 50 IU/ml,

3 Ajinomoto).

4 ***Plasma DPP-IV activity***

5   DPP-IV activity was determined based on the rate of hydrolysis of a surrogate substrate,

6 Gly-Pro-*p*-nitroaniline (Gly-Pro-*p*NA, Sigma) (25, 26). A 5-µl aliquot of plasma was added to each

7 well of a flat-bottom 96-well plate, followed by the addition of 80 µl of assay buffer (0.25 M Tris-HCl

8 buffer, pH 8.0). The reaction was initiated by the addition of 80 µl of 1.6 mM Gly-Pro-*p*NA in

9 deionized water. After an incubation at 37°C for 60 min, 40 µl of 1 M acetate (pH 5.2) was added to

10 stop the reaction, and the absorbance at 405 nm was measured (A) using a microplate reader (model

11 680; Bio-Rad Laboratories, Mississauga, ON, Canada). To correct for the influence of hemolysis, a

12 negative control was also prepared for each plasma sample, in which plasma was finally added to the

13 mixture of assay buffer containing substrate and acetate after a 60-min incubation, and the absorbance

14 at 405 nm was measured (B). Plasma DPP-IV activity was determined as the liberation of *p*NA from

15 Gly-Pro-*p*NA by plasma DPP-IV, by subtracting the absorbance (B) from (A). One unit is defined as

16 the liberation of 1 µmol Gly-Pro-*p*NA in one minute.

17

18 ***Experiment 4: Effects of oral ZeinH administration on plasma glucose in conscious rats (IPGTT)***

19   Rats were trained daily by an orogastric administration with distilled water using a feeding tube

20 (Safeed Feeding Tube Fr. 5, 40 cm; Terumo, Tokyo, Japan) during acclimation period. After a 24-h

21 fast, a basal (-15 min) blood was collected (30 µl) from the tail vein, and then deionized water (8

22 ml/kg) or 250, 500 mg/ml of ZeinH (2 g/kg, 4 g/kg) were orally administered into the stomach by

23 using a feeding tube. Glucose was injected (1 g/kg i.p.) 15 min after the oral administration, and tail

24 vein blood was collected just before (0 min), and 15, 30, 60, 90 and 120 min after the glucose

25 injection. Blood samples were heparinized and plasma glucose concentrations were measured as

26 above.

Statistical analysis

Results are expressed as means ± SE. Statistical significance was assessed by one-way or two-way

ANOVA, and significant differences among mean values were determined by the Fisher's post hoc

test ($P < 0.05$) or Dunnett's post hoc test ($P < 0.05$).

Results

Experiment 1: Effect of ileal administration of ZeinH and MHY on plasma glucose and insulin

concentrations during IPGTT

We first examined the effect of ileal ZeinH administration on plasma glucose and insulin

concentrations under IPGTT in conscious rats. Because oral glucose loading can enhance endogenous

GLP-1 secretion, and in order to observe the effect of luminal ZeinH itself on GLP-1 secretion and

glycemia, we performed IPGTT in the present study. Since plasma glucose returned to nearly baseline

level at 60 min after i.p. glucose injection (1 g/kg) in preliminary study, blood samples were collected

up to 60 min.

Ileal administration of test liquids slightly increased plasma glucose concentrations (from –30 to 0

min), as shown in Figure 1A. Glucose concentrations at 15 and 30 min in MHY-treated rats were

slightly but not significantly lower than those in control rats. In contrast, glucose concentrations at 15

and 30 min in ZeinH-treated rats were significantly lower than the values in control rats. The glucose

concentration returned to nearly baseline level at 30 min in ZeinH-treated rats.

The plasma insulin concentration was not affected by ileal administration of water, and it increased

from 0.09 nM (0 min) to 0.47 nM at 15 min after i.p. glucose injection in control rats (Fig. 1B). The

treatment with MHY slightly but not significantly enhanced insulin concentrations at 0 and 15 min

compared with control rats. In contrast, ileal administration of ZeinH significantly increased insulin

concentrations at 0 min and 15 min by 6.3- and 2.4-fold, respectively, compared with control rats.

Insulin concentrations also tended to be higher in ZeinH-treated rats compared with control and

1 MHY-treated rats at 30 and 60 min.

2 **Experiment 2: Effects of ileal ZeinH and MHY on plasma total and active GLP-1, and glucose**

3 **concentrations during IPGTT**

4 We next examined whether ileal ZeinH or MHY stimulated GLP-1 secretion under the same

5 conditions as in Experiment 1. Plasma glucose concentrations at 15 and 30 min in ZeinH-treated rats,

6 but not in MHY-treated rats, were significantly lower than those in control rats. These results are

7 consistent with the results shown in Figure 1A.

8 Basal values of total GLP-1 were 3.20–3.78 nM, and changes in total GLP-1 levels (ΔGLP-1) in

9 the jugular vein plasma are presented in Figure 2B, because changes after the administration of test

10 liquids were relatively small compared to basal total GLP-1 levels as observed in our previous study

11 (19). The ileal administration of water did not cause any significant changes in the total GLP-1

12 concentration throughout the blood collection period. In contrast, the ileal administration of ZeinH

13 and MHY induced a significant and sustained increase in total GLP-1 concentration during the period

14 from 0 min to 60 min, except for the time point at 15 min in MHY-treated rats. The increment of total

15 GLP-1 was slightly higher in ZeinH-treated than in MHY-treated rats. Total GLP-1 levels were

16 increased by both MHY- and ZeinH-treatments in a similar manner. These results were not correlated

17 with the insulin or glucose responses shown in Figure 1.

18 To estimate the activity of GLP-1 as an incretin, plasma active GLP-1 concentrations in the jugular

19 plasma were also measured (Fig. 2C). Basal active GLP-1 concentrations were not significantly

20 different among the three groups (5.78–6.25 pM). Ileal administration of water (at –30 min) and i.p.

21 glucose injection (at 0 min) did not cause any significant changes in the active GLP-1 concentration

22 (Fig. 2C). Ileal administration of MHY showed only a tendency to increase the active GLP-1

23 concentration at 0 min. ZeinH administered into the ileum sharply increased the active GLP-1

24 concentration from 5.9 pM at –30 min to 19.3 pM at 0 min, and the values at 0 min and 15 min in

25 ZeinH-treated rats were significantly higher than those in control rats. In contrast to the total GLP-1

26 responses (Fig. 2B), active GLP-1 responses were correlated with insulin and glucose responses. The

10

discrepancy between total and active GLP-1 responses induced by ileal MHY and ZeinH might be
explained by differences in plasma DPP-IV activity. Therefore, we next examined the effect of ileal
administration of ZeinH and MHY on plasma DPP-IV activity in the ileal vein.

Experiment 3: Effects of ileal ZeinH and MHY on plasma DPP-IV activity in the ileal vein of anesthetized rats

By using a catheter inserted into the ileal vein as described previously (19), we collected ileal vein blood before and after ileal administration of test agents. Statistically significant differences were not observed in basal plasma DPP-IV activity (24.0–28.3 mU/ml) among the three groups. The administration of water (control) or MHY into the ligated ileal loop (–30 min) and i.p. injection of glucose (0 min) slightly but not significantly reduced plasma DPP-IV activity (Fig. 3). In contrast, DPP-IV activity at 0 and 15 min in ZeinH-treated rats significantly decreased after ileal administration, and then recovered gradually but did not reached the basal level by 60 min. ZeinH-induced decrements of DPP-IV activity were 26.8% (at 0 min) and 22.1% (at 15 min) compared to the basal level. Values in ZeinH-treated rats were slightly or significantly lower than those in other two groups throughout the experimental period. This finding demonstrates that ZeinH administration into the ileal loop decreased plasma DPP-IV activity in the ileal vein in anesthetized rats.

Experiment 4: Effects of oral ZeinH on glycemia during IPGTT in conscious rats

It was examined the effect of oral administration of ZeinH on the glycemia under IPGTT in conscious rats. ZeinH was administered at the dose of 2, 4 g/kg body weight. The dose of 2 g/kg is comparable to that of 500 mg/rat with the body weight of 250 g in the experiments above. In all three groups, elevated glucose levels at 15 min were gradually lowered from 30 min to 120 min after the glucose injection. Rats treated with oral ZeinH showed significantly lower glucose levels in a dose-dependent manner at 15 and 30 min after the glucose injection. The elevation of glucose concentration at 15 min in 4 g/kg ZeinH-treated rats was around half of that in control rats.

Discussion

Enteroendocrine L cells secrete GLP-1 in response to luminal nutrients, which serves important physiological roles in the maintenance of normal glucose homeostasis, including the potentiation of glucose-stimulated insulin release (1, 2). Exogenously administered GLP-1 exerts powerful anti-diabetic effects, even in type 2 diabetic patients with secondary failure to sulfonylureas (27). Indeed, stable GLP-1 analogs and DPP-IV inhibitors are already licensed for the treatment of type 2 diabetes (28, 29). Therefore, targeting GLP-1 secretion from L cells can provide new opportunities for the improvement of glucose tolerance.

In our previous *in situ* study, the GLP-1-releasing potency of ZeinH was highest in the ileum, where GLP-1-producing L cells are found at a higher density than in the duodenum or the jejunum (19). The interposition of the ileal segment within the jejunum (ileal interposition) has been reported to enhance GLP-1 release and to prevent hyperglycemia via the enhancement of insulin secretion after oral glucose load in non-diabetic rats (30). In that study, orally administered glucose might stimulate GLP-1 secretion from L cells in the interpositioned ileum before glucose absorption in the jejunum. This raises the possibility that pre-stimulation of GLP-1 secretion by ileally administered ZeinH can prevent hyperglycemia *in vivo*.

As expected, ZeinH administered into the ileum effectively attenuated the glycemic response induced by i.p. glucose injection in conscious rats (Fig. 1A). This was confirmed in a repeated experiment (Fig. 2A). Enhanced insulin secretion (Fig. 1B) before and after the glucose injection in ZeinH-treated rats could be responsible for the attenuation of hyperglycemia. Because glucose was injected intraperitoneally in the present study, inhibition of glucose absorption could not be involved in such effect. Therefore, these results demonstrate that a dietary peptide in the ileum can contribute to the prevention of hyperglycemia by enhancing insulin secretion. In contrast, ileal MHY had only a small effect on reducing plasma glucose (Fig. 1A). This reflected insufficient insulin secretion in MHY-treated rats, which was similar to that in control rats (Fig. 1B). MHY is a dietary peptide that has GLP-1-releasing activity *in situ* (16) and *in vitro* (17, 24). In our previous study, MHY was less

effective at inducing GLP-1 secretion than was ZeinH in GLUTag cells (19). Therefore, we expected

MHY to induce less GLP-1 secretion than ZeinH in the present study, and this would result in

different insulin and glucose responses.

Ileal administration of both ZeinH and MHY induced significant and sustaining increases in total

GLP-1 in conscious rats (Fig. 2B). The secretory response of total GLP-1 in MHY-treated rats was

unexpectedly similar to that in ZeinH-treated rats. Although MHY induced less GLP-1 secretion than

ZeinH *in vitro* at lower concentration (5 mg/ml) (19), the secretion of GLP-1 from ileal L cells might

be maximally stimulated by those peptides at the 500 mg/rat in the present study.

In contrast to total GLP-1, the active GLP-1 level was significantly enhanced only by the ileal

administration of ZeinH but not by that of MHY (Fig. 2C). This seems to be reflected in the insulin

and glucose responses (Fig. 1 and Fig. 2A), but it is not consistent with the total GLP-1 response (Fig.

2B). Since total GLP-1 includes both the active form (7-37) and the partially degraded form (9-37) of

GLP-1, the sustained increases of total GLP-1 observed in Fig. 2B might reflect secreted active

GLP-1 and accumulated inactive GLP-1. It is possible that large differences between active GLP-1

secretion in ZeinH and MHY treatments result in the small difference observed in total GLP-1.

Changes in active GLP-1 reflect both secreted GLP-1 and biologically active GLP-1 that has not

yet been degraded by DPP-IV. The difference between the active GLP-1 (at 0–15 min) levels in

MHY- and ZeinH-treated rats could come from the degree of degradation of active GLP-1. That is,

the degradation of secreted GLP-1 might be either reduced in ZeinH-treated rats or enhanced in

MHY-treated rats. Thus, the present results suggest that the efficacy of enhancing GLP-1 secretion is

not necessarily linked to the increase in active GLP-1. Ileal administration of ZeinH significantly

increased insulin level at 0 min before the glucose injection without hypoglycemia (Fig. 1B). Such

increase in insulin induced by enhanced GLP-1 would be insufficient to cause hypoglycemia in the

basal state.

We examined whether ileal ZeinH and MHY affect plasma DPP-IV activity in the ileal vein of

anesthetized rats (Experiment 3). It has been reported that approximately half of the secreted active

1   GLP-1 is already degraded before it enters the systemic circulation (7). DPP-IV exists not only in

2   multiple tissues, such as the liver, kidney and intestine, as a cell-surface membrane-bound protein but

3   also in plasma as a soluble form (31). In the present study, blood collections from the ileal vein

4   enabled us to observe DPP-IV activity in the blood where GLP-1 is released and initial

5   DPP-IV-mediated degradation occurs. Interestingly, ileal administration of ZeinH decreased plasma

6   DPP-IV activity (at 0–15 min), whereas MHY did not cause significant changes in DPP-IV activity

7   (Fig. 3). The slight reductions in the control and MHY groups might be due to the effect of luminal

8   administration. This is the first demonstration that a luminal peptide acutely affects plasma DPP-IV

9   activity. Although the experimental design was not identical to that of Experiment 1, the decrease in

10   plasma DPP-IV activity was coincident with the increase in active GLP-1 (at 0 min) (Fig. 2C) in

11   ZeinH-treated rats. This suggests that the ZeinH-induced increase in active GLP-1 was a consequence

12   of both the stimulation of GLP-1 secretion from L cells and the acute reduction of DPP-IV-mediated

13   degradation of GLP-1. In contrast, MHY only increased total GLP-1 but not active GLP-1, due to the

14   lack of a reduction of DPP-IV activity, which results in a failure of stimulating insulin secretion. A

15   previous paper reported that partial inhibition (20–30%) of plasma DPP-IV activity is sufficient to

16   increase active GLP-1 and to attenuate hyperglycemia by enhancing the incretin effect during the oral

17   glucose tolerance test in lean Zucker rats (32). Therefore, it is likely that the decrement of plasma

18   DPP-IV activity (26.8% at 0 min, 22.1% at 15 min) by luminal ZeinH was sufficient to maintain

19   secreted GLP-1 in the active form.

20   The mechanism by which ileal ZeinH decreases plasma DPP-IV activity remains to be determined.

21   One possible explanation is the competitive inhibition of DPP-IV by absorbed peptides derived from

22   luminal ZeinH. It has been reported that ingestion of whey protein (WP) inhibits DPP-IV activity in

23   the small intestine in mice (33). In that report, small fragments (di- and tri-peptides) generated by the

24   luminal digestion of whey protein might have become substrates of DPP-IV within the intestinal wall

25   and decreased its activity as competitive inhibitors. In addition, tri-peptides such as diprotin A

26   (Ile-Pro-Ile) and diprotin B (Val-Pro-Leu) have been shown to block DPP-IV-mediated GLP-1

1 degradation *in vitro* (34, 35) as competitive substrates (36). These reports support our hypothesis that

2 absorbed fragments of ZeinH inhibit DPP-IV activity in the ileal vein. Although ZeinH might be

3 insusceptible to luminal digestion in the ligated ileal loop, brush-border membrane peptidases could

4 hydrolyze it to some degree, resulting in the generation of small peptides that are absorbed and inhibit

5 plasma DPP-IV. The WP-induced decrease of DPP-IV activity was observed only in the small

6 intestinal tissue but not in plasma of the orbital vein (33). In contrast, we could observe a decrease of

7 plasma DPP-IV activity in ZeinH-treated rats by using ileal vein cannulation (Fig. 3).

8 　　Zein is a well-known indigestible protein due to its strong hydrophobicity. Although it is slightly

9 less than MHY, ZeinH contained Pro and Ala (Table 1) as well as another Zein hydrolysate prepared

10 with alcalase (37). Because DPP-IV has preferential specificity for X-proline and X-alanine sequences

11 in the N-terminal of small peptides (38), such peptides contained in ZeinH or generated after the

12 digestion of ZeinH might be responsible for DPP-IV inhibition. Free amino acids content was less in

13 ZeinH than MHY, suggesting free amino acids are not responsible for GLP-1 secretion. ZeinH was

14 rich in Leu and Glu (including Gln) compared to MHY. As Leu and Gln are reported to stimulate

15 GLP-1 secretion (39, 40), these amino acids themselves liberated during luminal digestion or peptides

16 containing these amino acids might be involved in ZeinH-induced GLP-1 secretion. Further

17 investigations will be required to clarify active peptides that induce GLP-1 secretion and inhibit

18 DPP-IV, respectively. As a GLP-1-independent mechanism by which luminal ZeinH attenuates

19 hyperglycemina, it is interesting to note that small peptides and free amino acids absorbed from

20 luminal ZeinH might directly stimulate insulin release, since free leucine and arginine are known to

21 have such functions (41).

22 　　Oral ZeinH dose-dependently prevented hyperglycemia under IPGTT in conscious rats (Fig. 4).

23 This result further evidences the anti-hyperglycemic effect of ZeinH under physiological condition,

24 and also provides the possibility of application in humans. Although it was predicted that higher dose

25 of ZeinH was required in case of oral administration than that of ileal administration (500 mg/rat), the

26 result in Fig. 4 demonstrated that oral ZeinH at 2 g/kg (~500 mg/rat) is enough to exert its

15

1   anti-hyperglycemic activity. It is possible that orally administered ZeinH functioned not only in the

2   ileum to stimulate GLP-1 secretion but also in the upper small intestine to stimulate both GLP-1 and

3   GIP secretion. ZeinH in the duodenum and jejunum induced significant increase in GLP-1 secretion,

4   but smaller than in the ileum in our previous study (19). GIP is another incretin hormone, which is

5   released from enteroendocrine K cells in the duodenum and jejunum by direct contact with luminal

6   nutrients (42, 43). A previous paper has demonstrated peptone-induced GIP secretion in rats (44). If

7   DPP-IV inhibition by ZeinH was occurred in the upper small intestine, degradation of GIP could be

8   prevented as well as that of GLP-1. However, further investigations will be necessary in future to

9   assess these speculations and applications.

10   Single oral administration of wheat albumin prevents postprandial hyperglycemia in healthy

11   subjects through its α-amylase inhibitory activity (45). In pharmacological therapy, a single oral

12   administration of an α-glucosidase inhibitor, such as acarbose and voglibose, attenuates postprandial

13   glucose and insulin levels (46, 47). The anti-hyperglycemic effects of these compounds depend

14   primarily on the inhibition of carbohydrate digestion and absorption in the small intestine. On the

15   other hand, recent studies have demonstrated that the stimulation of GLP-1 secretion by luminal

16   compounds such as berberin (11), TGR5 agonists (12) and GPR119 agonists (13) is effective for

17   glycemic control. With regard to nutrients, oral glutamine increases GLP-1 and insulin in humans (40).

18   Therefore, such strategies based on an enhancement of endogenous GLP-1 secretion are promising for

19   the prevention of hyperglycemia.

20   The results of the present study provide the basis for a novel strategy for glycemic control via both

21   stimulating endogenous GLP-1secretion and reducing the degradation of secreted GLP-1. At this time,

22   the anti-hyperglycemic effect is specific for ZeinH. The identification of other peptides with similar

23   functions as ZeinH will be also beneficial for future applications. Although the reduction of DPP-IV

24   activity was observed in the ileal vein in the present study, some reports suggest that GLP-1 acts

25   locally at the site of secretion. The local action of GLP-1 involves the activation of nerve fibers in

26   close proximity to the L cells (7), and postprandial β-cell stimulation by GLP-1 is evoked via a neural

reflex triggered in the hepatoportal system (48, 49).

   In summary, we found that ileal administration of ZeinH but not MHY attenuated hyperglycemia by enhancing insulin secretion during IPGTT in conscious rats. Although ZeinH and MHY induced similar increases in total GLP-1, active GLP-1 level was increased only in ZeinH-treated rats. The ileal administration of ZeinH, but not MHY decreased plasma DPP-IV activity in the ileal vein in anesthetized rats. These results indicate that ileal administration of ZeinH both induced GLP-1 secretion and reduced plasma DPP-IV activity, resulting in enhanced insulin secretion. The anti-hyperglycemic activity of ZeinH was also demonstrated in IPGTT with oral administration of ZeinH. Our findings highlight a novel nutritional strategy to improve glycemic control by utilizing the endogenous GLP-1.

References

1. **Drucker DJ** 2007 The role of gut hormones in glucose homeostasis. J Clin Invest 117:24-32

2. **Baggio LL, Drucker DJ** 2007 Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131-2157

3. **Holst JJ** 2007 The physiology of glucagon-like peptide 1. Physiol Rev 87:1409-1439

4. **Drucker DJ, Nauck MA** 2006 The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368:1696-1705

5. **Gentilella R, Bianchi C, Rossi A, Rotella CM** 2009 Exenatide: a review from pharmacology to clinical practice. Diabetes Obes Metab 11:544-556

6. **Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B** 1992 Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22:283-291

7. **Hansen L, Deacon CF, Orskov C, Holst JJ** 1999 Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 140:5356-5363

8. **Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ** 1996 Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. Am J Physiol 271:E458-E464

9. **Tseng CC, Zhang XY, Wolfe MM** 1999 Effect of GIP and GLP-1 antagonists on insulin release in the rat. Am J Physiol 276: E1049-E1054

10. **Tolhurst G, Reimann F, Gribble FM** 2009 Nutritional regulation of glucagon-like peptide-1 secretion. J Physiol 587:27-32

11. **Lu SS, Yu YL, Zhu HJ, Liu XD, Liu L, Liu YW, Wang P, Xie L, Wang GJ** 2009 Berberine promotes glucagon-like peptide-1 (7-36) amide secretion in streptozotocin-induced diabetic rats. J Endocrinol 200:159-165

12. **Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K** 2009 TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 10: 167-177

13. **Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D, Chen R, Jones RM, Behan DP, Leonard J** 2008 A Role for Intestinal Endocrine Cell-Expressed G Protein-Coupled Receptor 119 in Glycemic Control by Enhancing Glucagon-Like Peptide-1 and Glucose-Dependent Insulinotropic Peptide Release. Endocrinology 149:2038-2047

14. **Anini Y, Fu-Cheng X, Cuber JC, Kervran A, Chariot J, Roz C** 1999 Comparison of the postprandial release of peptide YY and proglucagonderived peptides in the rat. Pflu¨gers Arch 438:299-306

15. **Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V** 1993 Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. J Endocrinol 138:159-166

16. **Dumoulin V, Moro F, Barcelo A, Dakka T, Cuber JC** 1998 Peptide YY, glucagon-like

peptide-1, and neurotensin responses to luminal factors in the isolated vascularly perfused rat ileum. Endocrinology 139:3780-3786

17. **Cordier-Bussat M, Bernard C, Levenez F, Klages N, Laser-Ritz B, Philippe J, Chayvialle JA, Cuber JC** 1998 Peptones stimulate both the secretion of the incretin hormone glucagon-like peptide 1 and the transcription of the proglucagon gene. Diabetes 47:1038-1045

18. **Hall WL, Millward DJ, Long SJ, Morgan LM** 2003 Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. Br J Nutr 89:239-248

19. **Hira T, Mochida T, Miyashita K, Hara H** 2009 GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats. Am J Physiol Gastrointest Liver Physiol 297: G663-G671

20. **Brubaker PL, Anini Y** 2003 Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. Can J Physiol Pharmacol 81:1005-1012

21. **Cuber JC, Bernard G, Fushiki T, Bernard C, Yamanishi R, Sugimoto E, Chayvialle JA** 1990 Luminal CCK-releasing factors in the isolated vascularly perfused rat duodenojejunum. Am J Physiol Gastrointest Liver Physiol 259:G191-G197

22. **Cohen SA, Bidlingmeyer BA, Tarvin TL** 1986 PITC derivatives in amino acid analysis. Nature 320:769-70

23. **Reeves PG, Nielsen FH, Fahey GC Jr** 1993 AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123:1939-1951

24. **Reimer RA** 2006 Meat hydrolysate and essential amino acid-induced glucagon-like peptide-1 secretion, in the human NCI-H716 enteroendocrine cell line, is regulated by extracellular signal-regulated kinase1/2 and p38 mitogen-activated protein kinases. J Endocrinol 191:159-170

25. **Flock G, Baggio LL, Longuet C, Drucker DJ** 2007 Incretin receptors for glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide are essential for the sustained metabolic actions of vildagliptin in mice. Diabetes 56:3006-3013

26. **Karl T, Chwalisz WT, Wedekind D, Hedrich HJ, Hoffmann T, Jacobs R, Pabst R, von Hörsten S** 2003 Localization, transmission, spontaneous mutations, and variation of function of the Dpp4 (Dipeptidyl-peptidase IV; CD26) gene in rats. Regul Pept 115:81-90

27. **Nauck MA, Sauerwald A, Ritzel R, Holst JJ, Schmiegel W** 1998 Influence of glucagon-like peptide 1 on fasting glycemia in type 2 diabetic patients treated with insulin after sulfonylurea secondary failure. Diabetes Care 21:1925-1931

28. **Holst JJ, Vilsbøll T, Deacon CF** 2009 The incretin system and its role in type 2 diabetes mellitus. Mol Cell Endocrinol 297:127-36

29. **Lovshin JA, Drucker DJ** 2009 Incretin-based therapies for type 2 diabetes mellitus. Nat Rev Endocrinol 5:262-269

30. **Strader AD, Clausen TR, Goodin SZ, Wendt D** 2009 Ileal Interposition Improves Glucose Tolerance in Low Dose Streptozotocin-treated Diabetic and Euglycemic Rats. Obes Surg 19:96-104

31. **Deacon CF** 2004 What do we know about the secretion and degradation of incretin hormones? Regul Pept 128:117-124

32. **Tanaka-Amino K, Matsumoto K, Hatakeyama Y, Shima I, Takakura S, Muto S** 2008 ASP4000, a novel, selective, dipeptidyl peptidase 4 inhibitor with antihyperglycemic activity. Eur J Pharmacol 590:444-449

33. **Gunnarsson PT, Winzell MS, Deacon CF, Larsen MO, Jelic K, Carr RD, Ahrén B** 2006 Glucose-induced incretin hormone release and inactivation are differently modulated by oral fat and protein in mice. Endocrinology 147:3173-3180

34. **Kieffer TJ, McIntosh CHS, Pederson RA** 1995 Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 136:3585-3596

35. **Deacon CF, Johnsen AH, Holst JJ** 1995 Degradation of glucagon-like peptide-1 by human

plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J Clin Endocrinol Metab 80:952-957

36. **Rahfeld J, Schierhorn M, Hartrodt B, Neubert K, Heins J** 1991 Are diprotin A (Ile-Pro-Ile) and diprotin B (Val-Pro-Leu) inhibitors or substrates for dipeptidyl peptidase IV? Biochim Biophys Acta 1076:314-316

37. **Zhu L, Chen J, Tang X, Xiong YL** 2008 Reducing, radical scavenging, and chelation properties of in vitro digests of alcalase-treated zein hydrolysate. J Agric Food Chem 56:2714-2721

38. **Demuth HU, McIntosh CH, Pederson RA** 2005 Type 2 diabetes--therapy with dipeptidyl peptidase IV inhibitors. Biochim Biophys Acta 1751:33-44

39. **Chen Q, Reimer RA.** 2009 Dairy protein and leucine alter GLP-1 release and mRNA of genes involved in intestinal lipid metabolism in vitro. Nutrition 25(3):340-349

40. **Greenfield JR, Farooqi IS, Keogh JM, Henning E, Habib AM, Blackwood A, Reimann F, Holst JJ, Gribble FM** 2009 Oral glutamine increases circulating glucagon-like peptide 1, glucagon, and insulin concentrations in lean, obese, and type 2 diabetic subjects. Am J Clin Nutr 89:106-113

41. **Ball AJ, Flatt PR, McClenaghan NH** 2004 Acute and long-term effects of nateglinide on insulin secretory pathways. Br J Pharmacol 142:367-373

42. **Parker HE, Habib AM, Rogers GJ, Gribble FM, Reimann F** 2008 Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. Diabetologia 52:289-298

43. **Mortensen K, Christensen LL, Holst JJ, Orskov C** 2003 GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine Regul Pept 114:189-196

44. **Wolfe MM, Zhao KB, Glazier KD, Jarboe LA, Tseng CC** 2000 Regulation of glucose-dependent insulinotropic polypeptide release by protein in the rat. Am J Physiol Gastrointest Liver Physiol 279:G561-G566

1 45. **Kodama T, Miyazaki T, Kitamura I, Suzuki Y, Namba Y, Sakurai J, Torikai Y, Inoue S**

2   2005 Effects of single and long-term administration of wheat albumin on blood glucose control:

3   randomized controlled clinical trials. Eur J Clin Nutr 59:384-392

4 46. **Shimabukuro M, Higa N, Chinen I, Yamakawa K, Takasu N** 2006 Effects of a Single

5   Administration of Acarbose on Postprandial Glucose Excursion and Endothelial Dysfunction in

6   Type 2 Diabetic Patients: A Randomized Crossover Study. J Clin Endocrinol Metab 91:837-842

7 47. **Mori Y, Kitahara Y, Miura K, Tajima N** 2004 Comparison of voglibose and nateglinide for

8   their acute effects on insulin secretion and free fatty acid levels in OLETF rat portal blood after

9   sucrose loading. Endocrine 23:39-43

10 48. **Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Niijima A** 1996 Vagal

11   hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. Am J Physiol

12   271:E808-E813

13 49. **Balkan B, Li X** 2000 Portal GLP-1 administration in rats augments the insulin response to

14   glucose via neuronal mechanisms. Am J Physiol Regul Integr Comp Physiol 279:R1449-R1454

15

16

1   **Figure Legends**

2   **Figure 1. Plasma glucose and insulin levels during the intraperitoneal glucose tolerance test**

3   **(IPGTT) in conscious rats after the ileal administration of water, MHY or ZeinH.**

4   Water (2 ml, open square), MHY (500 mg/2 ml, closed triangle), or ZeinH (500 mg/2 ml, closed

5   circle) was administered into the ileum 30 min prior to intraperitoneal glucose injection (1 g/kg).

6   Blood samples were collected from the jugular vein before (–30, 0 min) and after (15, 30, 60 min) the

7   glucose injection. Values are displayed as the means ± SEM of 6 rats in each group. Two-way

8   ANOVA P values for glucose (A) and insulin (B) were 0.14 and < 0.01 for the treatments; both < 0.01

9   for time; and < 0.05 and 0.26 for treatment x time, respectively. Plots at the same time point not

10   sharing the same letter differed significantly between treatments (Fisher's PLSD test, P < 0.05).

11

12   **Figure 2. Plasma glucose, total GLP-1 and active GLP-1 levels during IPGTT in conscious rats**

13   **after ileal administration of water, MHY or ZeinH.**

14   Water (2 ml, open square), MHY (500 mg/2 ml, closed triangle), or ZeinH (500 mg/2 ml, closed

15   circle) was administered into the ileum 30 min prior to intraperitoneal glucose injection (1 g/kg).

16   Blood samples were collected from the jugular vein before (–30, 0 min) and after (15, 30, 60 min) the

17   glucose injection. Values are displayed as the means ± SEM of 6–8 rats in each group. Two-way

18   ANOVA P values for glucose (A), total GLP-1 (B) and active GLP-1 (C) were all <0.01 for the

19   treatment; < 0.01, < 0.01, and < 0.05 for time; and 0.07, 0.23, and 0.11 for treatment x time,

20   respectively. Plus (+) signs indicate significant differences from the basal value (–30 min) in each

21   group (Dunnett's test, P < 0.05). Plots at the same time point not sharing the same letter differ

22   significantly between treatments (Fisher's PLSD test, P < 0.05).

23

24

1   **Figure 3. Changes in plasma DPP-IV activity in the ileal vein in anesthetized rats after ileal**

2   **administration of water, MHY or ZeinH.**

3   Water (2 ml, open square), MHY (500 mg/2 ml, closed triangle), or ZeinH (500 mg/2 ml, closed

4   circle) was administered into the ligated ileal loop at –30 min, and then glucose (1 g/kg) was injected

5   intraperitoneally at 0 min. Blood samples were collected through the ileal vein catheter before (–30, 0

6   min) and after (15, 30, 60 min) the glucose injection. Two-way ANOVA P values were < 0.05, < 0.05,

7   and 0.76 for treatment, time and treatment x time, respectively. Values are displayed as the means ±

8   SEM of 5–7 rats in each group and are expressed as the percentage of basal (–30 min) activities. *, P

9   < 0.05 compared with basal levels (–30 min) (Dunnett's test, P < 0.05). Plots at the same time point

10   not sharing the same letter differ significantly between treatments (Fisher's PLSD test, P < 0.05).

11

12   **Figure 4. Plasma glucose level after the oral administration of ZeinH during IPGTT in**

13   **conscious rats.**

14   Water (8 ml/kg; open square) or 250, 500 mg/ml of ZeinH (2 g/kg; open circle, 4 g/kg; closed circle)

15   was administered orogastrically 15 min prior to intraperitoneal glucose injection (1 g/kg). Blood

16   samples were collected from the tail vein before (–15, 0 min) and after (15, 30, 60, 90, 120 min) the

17   glucose injection. Values are displayed as the means ± SEM of 6-7 rats in each group. Two-way

18   ANOVA P values were all < 0.01 for treatment, time and treatment x time, respectively. Plots at the

19   same time point not sharing the same letter differ significantly between treatments (Fisher's PLSD

20   test, P < 0.05).

21

**Table 1.** *Free and total amino acid composition of ZeinH and MHY*

	Free (mg/g)		Total (mg/g)	
	ZeinH	MHY	ZeinH	MHY
Ala	2.9	6.8	55.5	92.4
Arg	-	28.9	11.8	103.4
Asp*	-	3.1	62.9	86.7
Glu**	-	9.6	217.1	143.0
Gly	-	5.9	16.2	258.2
His	-	-	5.0	6.9
Ile	10.7	12.1	30.8	26.3
Leu	8.3	14.1	141.1	46.4
Lys	-	7.5	-	47.4
Met	-	-	8.6	-
Phe	-	9.1	47.5	25.9
Pro	3.6	-	70.3	139.6
Ser	-	5.1	42.9	40.8
Thr	-	4.9	20.9	24.1
Tyr	-	-	37.3	6.1
Val	-	6.3	26.8	33.9

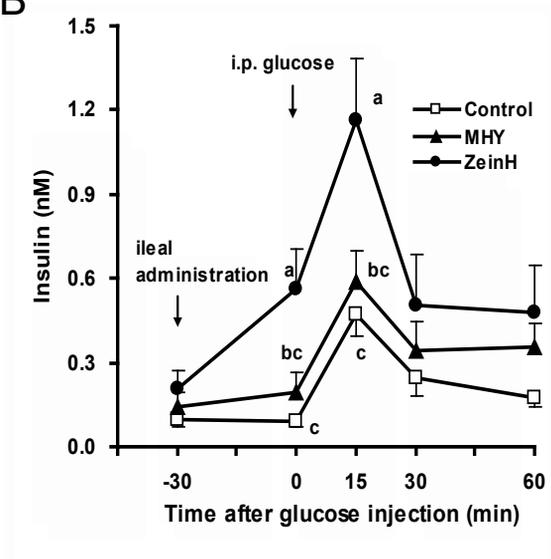Cys and Trp were not detected.
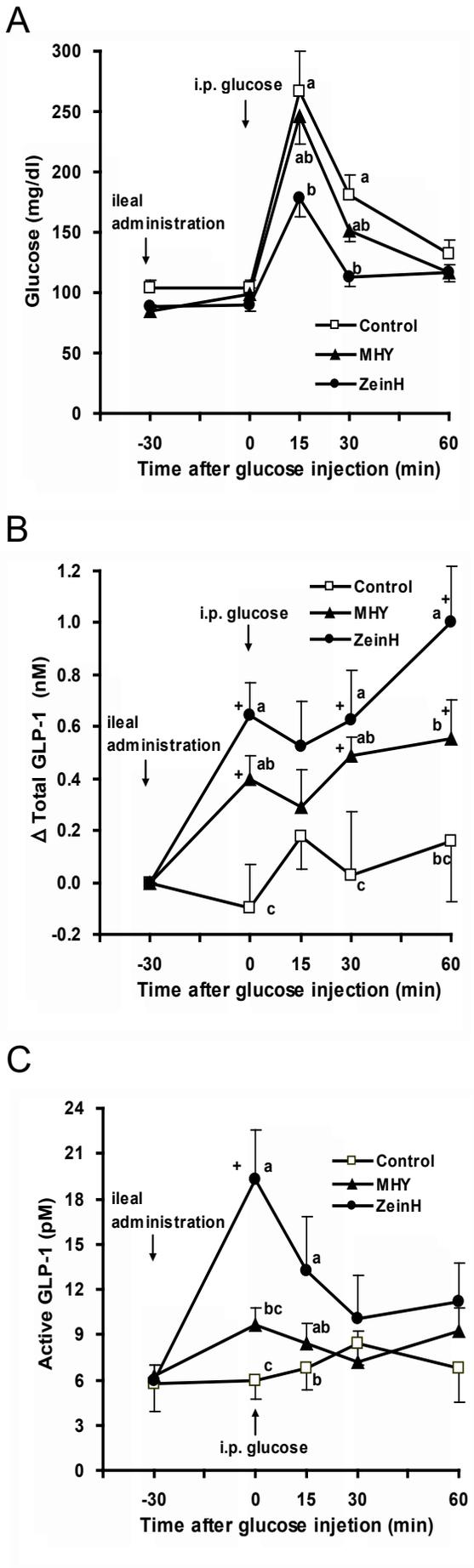-: not detected, *: includes Asn, **: includes Gln
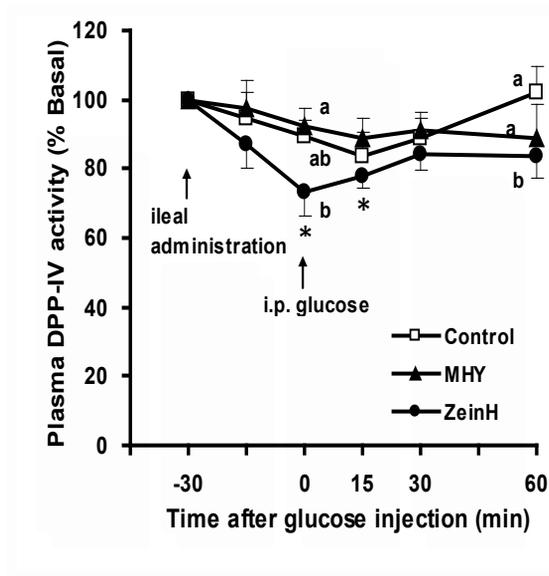
Figure 1

A
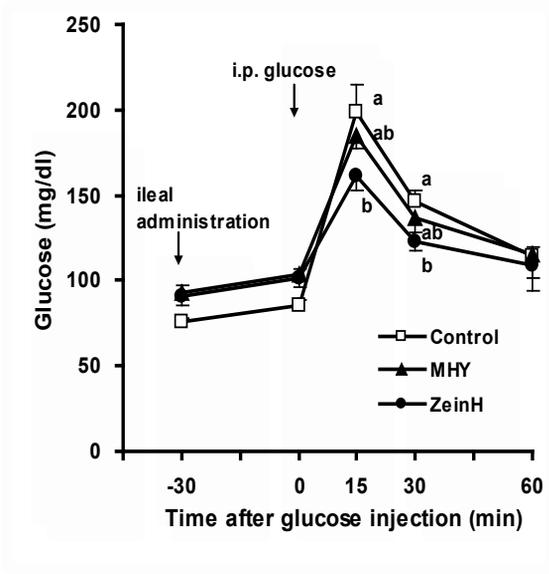
B

Figure 2

Figure 3

Figure 4

Figure 4