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1,5-Anhydro-D-fructose increases glucose tolerance by increasing glucagon-like peptide-1 and insulin in mice

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Abstract

Besides being degraded to glucose-6-phosphate and to free glucose, glycogen is degraded by α-1,4-glucan lyase to 1,5-anhydro-D-fructose. We examined the influence of 1,5-anhydro-D-fructose on glucose-stimulated insulin secretion in vivo and in vitro in mice. When administered together with i.v. glucose (1 g/kg), 1,5-anhydro-D-fructose did not affect (at 0.2 g/kg) or inhibited (at 1 g/kg) insulin secretion without affecting glucose elimination. When incubated with isolated islets, 1,5-anhydro-D-fructose at < 16.7 mmol/l, did not affect glucose (11.1 mM)-stimulated insulin secretion but inhibited insulin secretion at 16.7 mmol/l. When given through a gastric gavage (150 mg/mouse) together with glucose (150 mg/mouse), 1,5-anhydro-D-fructose increased glucose tolerance and insulin secretion. Furthermore, 1,5-anhydro-D-fructose potentiated the increase in plasma levels of the gut hormone, glucagon-like peptide-1 (GLP-1). We therefore conclude that when given enterally, but not parenterally, 1,5-anhydro-D-fructose increases glucose tolerance in mice by increasing insulin secretion due to increased plasma levels of GLP-1. The sugar may therefore be explored for increasing endogenous GLP-1 secretion in the treatment of type 2 diabetes.

Keywords: Insulin secretion; Glucose elimination; 1,5-Anhydro-D-fructose; Glucagon-like peptide-1; (Mouse)

1. Introduction

Glycogen is the main polymer of carbohydrate in liver and muscles for carbon and energy storage and as a dynamic pool for maintaining glycemia homeostasis. Both the synthesis and degradation of glycogen are known to be under rigid control at both enzymatic and hormone levels and several diseases are known to be related to metabolic disorders of glycogen (Larner, 1990; Mathews and van Holde, 1990). It is well established that the breakdown of glycogen is catalysed by α-glucosidase to free glucose and by glycogen phosphorylase to glucose-1-phosphate, which can be converted to free glucose by a phosphatase (Larner, 1990). Further understanding of these processes is continually made with discoveries of novel proteins, such as glycogenin (Alonso et al., 1995) and protein targeting to glycogen (Printen et al., 1997). In addition, a third glycogen degradation route, called the Anhydrofructose Pathway, has been unveiled. In this route, glycogen is first converted to 1,5-anhydro-D-fructose by α-1,4-glucan lyase, the formed 1,5-anhydro-D-fructose is reduced by a NADPH-dependent 1,5-anhydro-D-fructose specific reductase to 1,5-anhydro-D-glucitol, which may further be phosphorylated to 1,5-anhydro-D-glucitol 6-phosphate by a kinase (Shiga et al., 1999; Yu et al., 1999). The chemical structure of 1,5-anhydro-D-fructose is given in Fig. 1. This alternative glycogen degrading route has been demonstrated in *Escherichia coli*, fungi and algae (for review see Yu et al., 1999) and also in livers in rats (Kametani et al., 1996). Little is known about the physiological importance of this alternative glycogen degrading route in mammals. The aim of the current study was to examine the effect of 1,5-anhydro-D-fructose on glucose homeostasis and the secretion of insulin in mice. The results obtained indicate that 1,5-anhydro-D-fructose increases glucose tolerance and insulin secretion following gastric but not intravenous glucose administration. This finding furthered us to investigate whether 1,5-anhydro-D-fructose affects plasma levels

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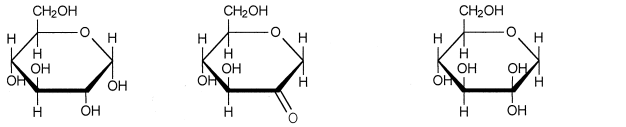

Fig. 1. The structures of 1,5-anhydro-D-fructose compared with the structure of α-D-glucose and 1,5-anhydro-D-fructose hydrate. Note that 1,5-anhydro-D-fructose exists as 1,5-anhydro-D-fructose hydrate in aqueous solution.

of glucagon-like peptide-1 (GLP-1) following gastric glucose, since GLP-1 is a main gut hormone released by enteral glucose and regulating islet hormone secretion (Ahrén, 1998).

2. Methods

2.1. Animals

Non-fasted NMRI mice (Bomholdtgaard Breeding and Research Center, Ry, Denmark), weighing 20–25 g were used throughout the study. The animals were fed a standard pellet diet and tap water ad libitum.

2.2. Intravenous glucose tolerance test

The mice were anesthetized with an intraperitoneal injection of midazolam (Dormicum®, Hoffman-La-Roche, Basel, Switzerland, 0.4 mg/mouse) and a combination of fluanison (0.9 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm®, Janssen, Beerse, Belgium). Thereafter, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes, whereafter D-glucose (British Drug Houses, Poole, UK; 1 g/kg) was injected rapidly intravenously either alone or together with 1,5-anhydro-D-fructose (Danisco, Copenhagen, Denmark; 0.2 or 1 g/kg); in one series of experiments, 1,5-anhydro-D-fructose was given alone (1 g/kg). The volume load was 10 μl/g body weight. New blood samples were taken after 1, 5, 10, 20, 30 and 50 min. Blood samples were taken as above. Following immediate centrifugation at 4°C, plasma was separated and stored at −20°C until analysis.

2.3. Gastric glucose tolerance test

The mice were fasted overnight and anesthetized as above. After induction of anesthesia, D-glucose (150 mg/mouse in 0.5 ml) was administered alone or together with 1,5-anhydro-D-fructose (75 or 150 mg/mouse) through a gavage tube (outer diameter 1.2 mm) placed in the stomach. In one series of experiments, 1,5-anhydro-D-fructose was given along through the gastric gavage (150 mg/mouse). Blood samples were taken before administration and after 15, 30, 60, 90 and 120 min, or in the experimental series measuring plasma GLP-1, before and after 15, 30 and 60 min, and treated as above.

2.4. Insulin secretion in vitro

Pancreatic islets were isolated from eight mice with the collagenase isolation technique. In brief, the pancreas was filled retrogradely through the pancreatic duct with 3 ml of Hank's Balanced Salt Solution (Sigma), supplemented with 0.3 mg/ml of Collagenase P (activity 1.86 U/mg; Boehringer Mannheim, Mannheim, Germany). The pancreas was subsequently removed and incubated in the same solution for 20 min at 37°C. After rinsing, the islets were handpicked under a stereomicroscope and incubated overnight in RPMI 1640 medium supplemented with 10% fetal calf serum, 2.05 mmol/l L-glutamine, 2.5 μg/ml amphotericin B (GIBCO BRL, Paisley, Scotland), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Biol Ind, Beit Haemek, Israel) at 37°C in humidified air equilibrated with 5% CO_2. Following the overnight incubation, the islets were washed three times and then pre-incubated for 60 min at 37°C in a HEPES medium (pH 7.36) supplemented with 0.1% human serum albumin (Sigma) and 3.3 mmol/l glucose. The medium consisted of (in mmol/l): 125 NaCl, 5.9 KCl, 1.2 $MgCl_2$, 1.28 $CaCl_2$ (all Sigma), and 25 HEPES (Boehringer Mannheim). After the pre-incubation, groups of three islets were transferred into separate chambers containing 200 μl of the medium supplemented with glucose and 1,5-anhydro-D-fructose at various concentrations. Following incubation at 37°C for 60 min, 25 μl of the medium were collected from each chamber and stored at −20°C until analysis.

2.5. Analysis

Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ^{125}I-labeled porcine insulin as tracer and rat insulin as standard (Linco Research, St. Charles, MO, USA). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l and the coefficiency of variation is less than 3% at both low and high levels. Plasma glucose was determined with the glucose oxidase method. Plasma GLP-1 was measured by a radioimmunoassay after extraction of plasma samples with ethanol; 400 μl 0.05 mol/l sodium phosphate buffer, pH 7.5, containing 6% albumin and 0.1 mol/l NaCl was added to 100 μl mouse plasma on ice and mixed well. The mixture was then extracted with 70% ethanol (vol/vol, final dilution), and after vacuum centrifugation the residue was reconstituted in assay buffer and assayed as previously described (Ørskov et al.,

1994). The antiserum (code no. 89390) raised in rabbits against synthetic GLP-1 is highly specific for C-terminal intestinal GLP-1, and recognizes mouse GLP-1. The sensitivity using this procedure is 5 pmol/l, and the intra-assay coefficient of variation is 10%. The recovery of GLP-1 added to mouse plasma is within ±20% of expected values.

2.6. Statistics

Means ± S.E.M. are shown. The suprabasal area under the curve for plasma insulin levels ($AUC_{insulin}$) was calculated by the trapezoid rule. The glucose elimination rate after the intravenous glucose injection (K_G) was calculated using the $t_{1/2}$ for the min 1–20 after glucose injection after logarithmic transformation of the individual plasma glucose values. Statistical analyses were performed with the SPSS for Windows system. Statistical comparisons between groups were performed with Student's *t*-test.

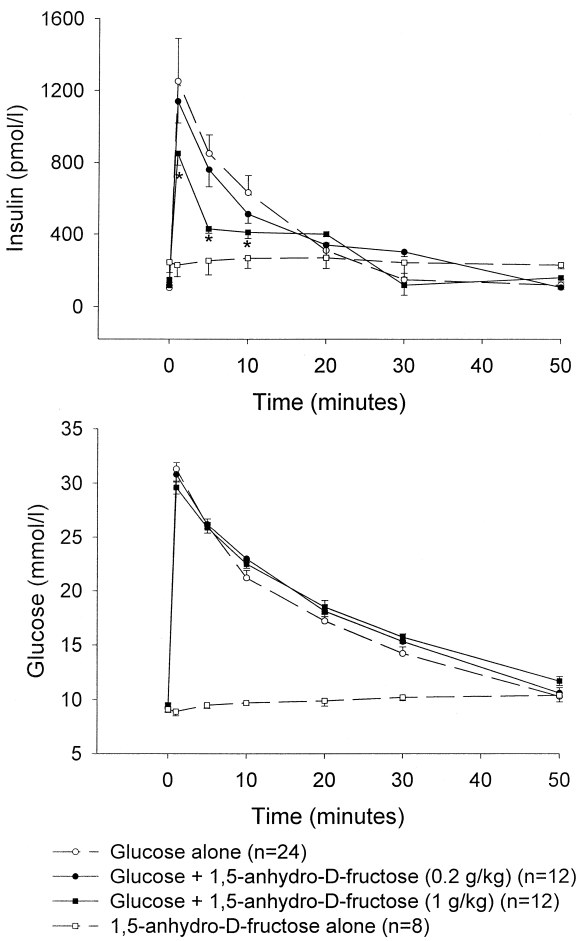

Fig. 2. Plasma levels of insulin (upper panel) and glucose (lower panel) immediately before and at 1, 5, 10, 20, 30 and 50 min after an i.v. injection of glucose (1 g/kg) with or without addition of 1,5-anhydro-D-fructose at 0.2 or 1 g/kg in anesthetized mice or after i.v. injection of 1,5-anhydro-D-fructose alone (1 g/kg). Means ± S.E.M. are shown; *n* indicates number of mice; asterisks indicate the probability level of random difference between animals injected with glucose alone versus animals injected with glucose plus 1,5-anhydro-D-fructose at 1 g/kg; * $P < 0.05$.

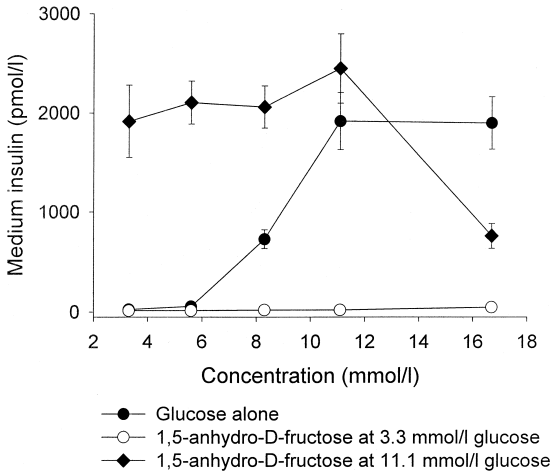

Fig. 3. Medium concentration of insulin after 60 min incubation in presence of different concentrations of glucose or 1,5-anhydro-D-fructose (at 3.3 or 11.1 mmol/l glucose) in overnight cultured isolated mouse islets. Values are mean ± S.E.M. There were 24 observations in each point; each observation being an incubation of three islets; eight mice were used.

3. Results

3.1. Intravenous glucose tolerance test

Fig. 2 shows that basal plasma levels of insulin or glucose were not affected by 1,5-anhydro-D-fructose when the sugar was given alone (1 g/kg). However, when given together with glucose (1 g/kg), 1,5-anhydro-D-fructose inhibited glucose-stimulated insulin secretion when given at 1 g/kg, but not when given at 0.2 g/kg. Thus, the area under the suprabasal insulin curve during the 50-min study period, $AUC_{insulin}$, which was 14.4 ± 2.1 nmol/l × 50 min in the controls given glucose alone and 14.6 ± 1.9 nmol/l × 50 min in mice given glucose and 1,5-anhydro-D-fructose at 0.2 g/kg, was reduced to 8.6 ± 1.9 nmol/l × 50 min, i.e. by 40%, in mice given glucose and 1,5-anhydro-D-fructose at 1 g/kg ($P = 0.021$). In contrast, glucose elimination after the intravenous glucose administration was not affected by 1,5-anhydro-D-fructose at either of the two dose levels. Thus, the glucose elimination rate, determined as K_G, was 2.3 ± 0.2%/min in the animals injected with glucose alone versus 2.2 ± 0.2%/min after injection of glucose together with 1,5-anhydro-D-fructose at 0.2 g/kg and 2.1 ± 0.3%/min after injection of glucose together with 1,5-anhydro-D-fructose at 1 g/kg (NS). We have previously shown that intravenous administration of vehicle to anesthetized mice does not change plasma levels of insulin or glucose during a 50-min study period (Filipsson et al., 1998).

3.2. Insulin secretion in vitro

Fig. 3 shows that 1,5-anhydro-D-fructose did not affect glucose-stimulated insulin secretion when added to isolated mouse islets at dose levels of 11.1 mmol/l or below. However, added at the high dose level of 16.7 mmol/l, 1,5-anhydro-D-fructose inhibited insulin secretion ($P < 0.001$).

3.3. Gastric glucose tolerance test

When 1,5-anhydro-D-fructose was given alone through a gastric gavage, plasma levels of insulin or glucose were not significantly affected (Fig. 4). However, when 1,5-anhydro-D-fructose was given together with glucose, the plasma insulin levels were increased in comparison when glucose was given alone (Fig. 4, upper panel). Thus, the

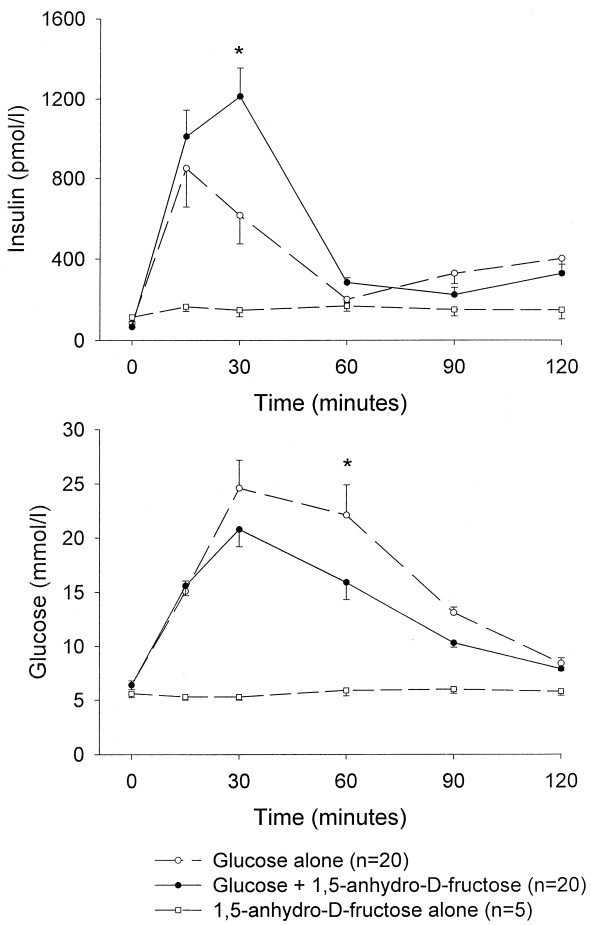

Fig. 4. Plasma insulin (upper panel) and glucose (lower panel) immediately before and at 15, 30, 60, 90 and 120 min after administration of glucose (150 mg/mouse) or 1,5-anhydro-D-fructose (150 mg/mouse) alone or the two sugars together through a gastric gavage in anaesthetised mice. Means ± S.E.M. are shown; *n* indicates number of mice in each group; asterisks indicate the probability level of random difference in the groups injected with glucose with or without 1,5-anhydro-D-fructose; * $P < 0.05$.

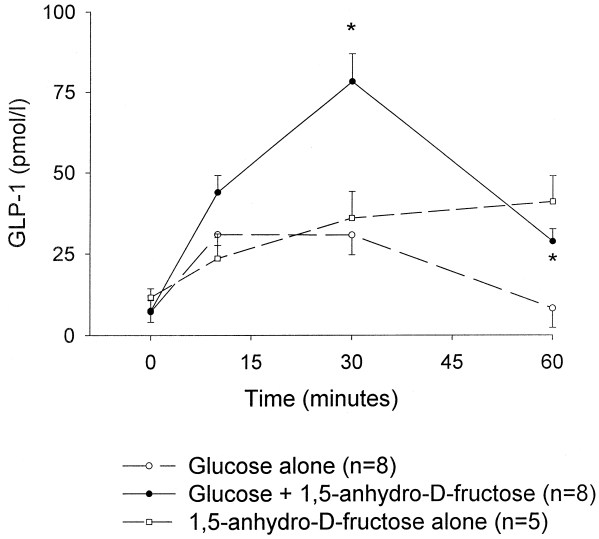

Fig. 5. Plasma GLP-1 immediately before and at 15, 30 and 60 min after administration of glucose (150 mg/mouse) or 1,5-anhydro-D-fructose (150 mg/mouse) alone or the two sugars together through a gastric gavage in anaesthetised mice. Means ± S.E.M. are shown; *n* indicates number of observations, in each observation plasma from two mice were pooled; asterisks indicate the probability level of random difference in the groups injected with glucose with or without 1,5-anhydro-D-fructose; * $P < 0.05$.

suprabasal $AUC_{insulin}$ during the first 60 min after administration was increased by 1,5-anhydro-D-fructose from 20.3 ± 2.3 nmol/l × 60 min in controls to 32.9 ± 2.6 nmol/l × 60 min in mice given glucose and 1,5-anhydro-D-fructose ($P = 0.018$). This was accompanied by increased glucose elimination (Fig. 4, lower panel), as evidenced by higher 60 min glucose value in the control group (22.1 ± 2.8 mmol/l) than in mice given glucose with 1,5-anhydro-D-fructose (15.5 ± 1.6 mmol/l; $P = 0.021$).

3.4. Plasma GLP-1 after gastric gavage

Since 1,5-anhydro-D-fructose increased insulin secretion when administered through gastric gavage but not when administered intravenously, we examined in a separate experimental series whether the enteral administration of the sugar increases plasma levels of GLP-1, which is a gut hormone released by enteral glucose and stimulating insulin secretion (Ahrén, 1998). In this experimental series, plasma from two mice was pooled since 100 μl plasma were required for the assay in each sample. The results show that adminstration of glucose and 1,5-anhydro-D-fructose alone through gastric gavage both increased plasma levels of GLP-1 (Fig. 5). Furthermore, the increase in GLP-1 levels was potentiated by the combined aministration of 1,5-anhydro-D-fructose and glucose (Fig. 5). Thus, both the 30 min (30.8 ± 6.1 versus 78.2 ± 8.6 pmol/l) and the 60 min (8.2 ± 5.9 versus 28.8 ± 3.8 pmol/l) values were higher after administration of glucose with 1,5-

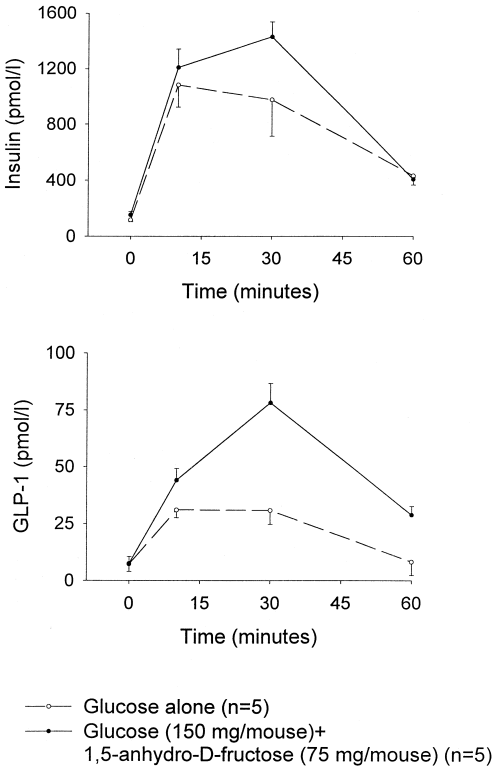

Fig. 6. Plasma insulin (upper panel) and GLP-1 (lower panel) immediately before and at 15, 30 and 60 min after administration of glucose (150 mg/mouse) alone or together with 1,5-anhydro-D-fructose (75 mg/mouse) through a gastric gavage in anaesthetised mice. Means ± S.E.M. are shown; *n* indicates number of observations in each group, in each observation plasma from two mice were pooled.

anhydro-D-fructose than after administration of glucose alone ($P < 0.05$ for both).

3.5. Effects of a low dose of 1,5-anhydro-D-fructose

Fig. 6 shows that also when the dose of 1,5-anhydro-D-fructose was reduced to 75 mg/mouse, the sugar potentiated the glucose-induced increase in plasma levels of both insulin and GLP-1 ($P < 0.05$ for both 30 min values).

4. Discussion

1,5-Anhydro-D-fructose is a monosugar formed by action of α-1,4-glucan lyase on glycogen and related substrates, such as maltose, maltosaccharides, and industrially, 1,5-anhydro-D-fructose can be produced by the glucan lyase using starch as substrate (Yu et al., 1999). In fungi and algae, 1,5-anhydro-D-fructose is further converted to antibiotics under stress conditions (Baute et al., 1988; Broberg et al., 1999), while in mammals and *E. coli* 1,5-anhydro-D-fructose is reduced by NADPH-dependent reductase to 1,5-anhydro-D-glucitol (Sakuma et al., 1998; Yu et al., 1999). This polyol may be phosphorylated or filtered directly into the preurine, where it competes with glucose for tubular reabsorption (Yamanouchi et al., 1992).

The function of 1,5-anhydro-D-fructose is not known. Since it represents an alternative degradation pathway of glycogen, we examined its influence on insulin secretion and glucose disposal in mice. We found that at high dose levels, 1,5-anhydro-D-fructose inhibited glucose-stimulated insulin secretion during an intravenous glucose tolerance test in vivo and after raising the glucose level in the incubation medium when isolated islets were incubated in vitro. Although not examined further in this study, inhibition of insulin secretion from islets may be due to inhibition of glucokinase and hexokinase by 1,5-anhydro-D-fructose, thereby impairing islet glucose phosphorylation, as has been demonstrated in vitro in enzyme solutions (Taguchi et al., 1993). It should be emphasized, however, that inhibition by 1,5-anhydro-D-fructose of glucose-stimulated insulin secretion from isolated islets was observed only when 1,5-anhydro-D-fructose was given at a high dose level. It may be surprising that in spite of 40% inhibition of glucose-stimulated insulin secretion after administration of 1,5-anhydro-D-fructose during the intravenous glucose tolerance test, glucose elimination was not altered. This is, however, an expected finding in rodents, where, for example, marked (> 70%) inhibition of glucose-stimulated insulin secretion is not accompanied by impaired glucose elimination, as shown in rats (Laury et al., 1991; Balkan and Dunning, 1995), and circulating insulin contributes only by approximately 30% to glucose elimination after an intravenous glucose load, as shown in mice (Pacini and Ahrén, 1999).

The main finding of this work is that gastric administration of 1,5-anhydro-D-fructose potentiated insulin secretion and glucose tolerance during a gastric glucose tolerance test, when given both at the same dose as glucose as when the dose of 1,5-anhydro-D-fructose was reduced by 50%. Since 1,5-anhydro-D-fructose did not potentiate glucose-stimulated insulin secretion directly, we reasoned that an indirect action of 1,5-anhydro-D-fructose had evolved. We therefore examined also the plasma levels of GLP-1 in the mice after gastric glucose, GLP-1 being a gut hormone released by enteral glucose acting as an incretin hormone potentiating insulin secretion (Ahrén, 1998). We found that 1,5-anhydro-D-fructose markedly enhanced the GLP-1 response to gastric glucose, and, therefore, we suggest that the augmented insulin response by gastric 1,5-anhydro-D-fructose is mediated by the increased levels of GLP-1. This would suggest that 1,5-anhydro-D-fructose stimulated GLP-1 secretion from the intestinal L-cells, which is also supported by the finding that 1,5-anhydro-D-fructose increased circulating GLP-1 also when administered by a gastric gavage alone, without glucose. A main mechanism for stimulated GLP-1 secretion is glucose activating the intestinal L-cells through absorption from the luminal side

(Sugiyama et al., 1994). It could be hypothesised that 1,5-anhydro-D-fructose might delay the absorption of glucose in the gut, enabling more glucose to reach the L-cells, in analogy with the pseudotetrasaccharide, acarbose, which increases GLP-1 secretion after oral sucrose by inhibiting enteral α-glucosidase, thereby postponing gut absorption of glucose to more distal parts of the gut with a higher L-cell density (Seifarth et al., 1998). Another possibility is that 1,5-anhydro-D-fructose directly stimulates GLP-1 secretion from the intestinal L-cells. This mechanism is supported by the finding that 1,5-anhydro-D-fructose increased plasma levels of GLP-1 also when given alone. GLP-1 secretion from the L-cells is governed by a sodium-glucose co-transporter mechanism, and carbohydrates that activate this mechanism, like glucose, galactose, methyl-α-glucoside, and 3-*O*-methyl glucose, stimulate GLP-1 secretion, whereas carbohydrates which are not substrates for this luminal sodium/glucose transport, like 2-deoxy-glucose and *N*-acetyl-glucosamine, do not stimulate GLP-1 secretion (Ritzel et al., 1997). In addition, however, a sodium-independent GLP-1 secretion has also been found since fructose stimulates GLP-1 secretion independently from the presence of sodium ions (Ritzel et al., 1997). Whether 1,5-anhydro-D-fructose is a substrate for the sodium/glucose transport mechanism and therefore activates GLP-1 secretion through this pathway or, like fructose, does it through a sodium-independent mechanism, remains to be studied.

Besides being involved in the gut control of postprandial insulin secretion as an incretin hormone, GLP-1 has during recent years been explored for use in the treatment of diabetes, because it exerts antidiabetogenic actions caused by increased insulin secretion, reduced glucagon secretion and inhibited gastric emptying (Nauck, 1998; Nauck et al., 1997; Holst et al., 1998; Ahrén, 1998). For the development of GLP-1 in the treatment of diabetes, however, exogenous GLP-1 administration has major limitations due to both its short half life, being less than 1.5 min in humans (Deacon et al., 1995) and to the requirement of administering the peptide parenterally due to fast gastrointestinal degradation. Attempts to circumvent these limitations include alternative routes of administration, such as buccal administration (Gutniak et al., 1997), combination of GLP-1 administration with inhibition of the GLP-1 degrading enzyme, dipeptidyl peptidase IV (Holst and Deacon, 1998), or the use of dipeptidyl peptidase IV-resistant analogues (Deacon et al., 1998). However, another approach would be to augment endogenous GLP-1 secretion, which would augment insulin secretion after meal intake (Nauck et al., 1997). This would be of particular interest in view of the reduction of GLP-1 secretion after meal, which is seen in diabetics (Toft-Nielsen et al., 1999). The present work suggests that 1,5-anhydro-D-fructose augments endogenous GLP-1 secretion and at the same time increases glucose tolerance and insulin secretion after gastric administration of glucose.

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