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α-Glucosidase inhibitors prevent diet-induced increases in intestinal sugar transport in diabetic mice

Donatella M. Casirola, Ronaldo P. Ferraris*

Department of Pharmacology and Physiology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ 07101-1709, USA

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Abstract

The recommended diet for diabetes mellitus is rich in complex carbohydrates. We have previously shown that high carbohydrate levels in the intestinal lumen induce adaptive increases in sugar absorption which in turn exacerbate postprandial hyperglycemia in diabetic mice. α-Glucosidase inhibitors (AGIs) hinder digestion of complex carbohydrates and therefore alleviate postprandial glycemic excursions. In this study, we tested the hypothesis that AGIs prevent the carbohydrate-induced upregulation of intestinal glucose and fructose transport in diabetes. Streptozotocin-diabetic mice were fed the following isocaloric diets: high carbohydrate (H), H plus acarbose (HA), H plus deoxynojirimycin (HD), and low carbohydrate (L), then nutrient uptakes were determined after 2 and 4 weeks. Body weight, intestinal weight, and length were independent of diet. Fasting and postprandial blood glucose levels were lower in HA and HD than in H mice. Uptakes of D-glucose and D-fructose were 2 to 3 times greater in H than in L mice, but HA and HM diets gradually reduced D-glucose uptakes to rates similar to L mice. Only HA diets reduced D-fructose uptake. Intestinal proline, aspartate, and glutamine uptakes were each greater in L than in H, HA, and HD mice. α-Glucosidase inhibitors did not alter intestinal permeability and amino acid transport rates. α-Glucosidase inhibitor–inhibitable increases in total intestinal absorptive capacity for sugars were due to carbohydrate-induced increases in V_{max} of glucose transport. Clearly, one potential mechanism by which AGIs blunt postprandial glycemic excursions and lower fasting blood glucose concentrations in individuals consuming carbohydrate-containing diets is by preventing carbohydrate-induced increases in intestinal sugar transport.

1. Introduction

Inhibitors of intestinal α-glucosidases (AGIs) are used in the treatment of non–insulin-dependent diabetes mellitus (NIDDM) and represent a huge fraction of the antidiabetic drug market (global turnover of the AGI acarbose is around $300 million [1]). α-Glucosidase inhibitors are remarkably effective in the prevention of cardiovascular complications of hyperglycemia. In human subjects with impaired glucose tolerance, the AGI acarbose is associated with a 49% relative reduction in the risk of cardiovascular disease, particularly myocardial infarction. Acarbose is also associated with a 34% relative risk reduction in the incidence of hypertension [2]. α-Glucosidase inhibitors work by reducing the rate of breakdown of complex carbohydrates in the gut, thus reducing the availability of absorbable monosaccharides, such as glucose. Although their reductive effect on intestinal luminal concentrations of sugars and therefore on subsequent postprandial plasma glucose levels is well known, other potential mechanisms underlying the effects of AGIs are not known.

The recommended diet for NIDDM patients is often rich in complex carbohydrates and fiber, where monounsaturated fatty acids and carbohydrates provide 60% to 70% of daily energy intake [3]. A problem often overlooked is that intestinal transport adapts to dietary substrate concentration. In fact, high sugar and high protein concentrations in the lumen upregulate the specific transport of sugars and amino acids, respectively (see Ref [4]); dietary lipids modify the RNA abundance of the brush-border fatty acid transporter [5].

Because high-carbohydrate diets increase the absorption rate of sugars (see Ref [4]), the carbohydrate-related increase in sugar absorption during diabetes [6] may exacerbate postprandial plasma glucose concentrations

* Corresponding author. Tel.: +1 973 9724519; fax: +1 973 9727950.
E-mail address: ferraris@umdnj.edu (R.P. Ferraris).

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already abnormally high in diabetes. Diabetes is also thought to cause intestinal hypertrophy and hyperplasia [6,7], thus nonspecifically increasing sugar transport. Increases in luminal carbohydrate concentrations such as those arising from a high-carbohydrate diet specifically stimulate sugar transport [8]. We have shown in diabetic mice that diet-induced increases in sugar uptake rate combined with an enlarged absorptive mucosa resulted in much higher rates of total intestinal sugar absorption than those of nondiabetic mice consuming a high-carbohydrate diet [6].

The intestinal α-glucosidases (glucoamylases) sequentially detach terminal glucose residues from complex carbohydrates and increase the luminal concentration of glucose. This increase is prevented by the AGIs, which are thus a first-line treatment of choice for lowering postprandial hyperglycemia. Because AGIs per se do not affect mRNA and protein expression of the brush-border (SGLT1) and basolateral (GLUT2) glucose transporter [9], they likely regulate intestinal sugar transport by their effect on luminal sugar concentrations.

We therefore tested the hypothesis that AGIs prevent the diet-induced upregulation of glucose and fructose transport in diabetic mice, without affecting the transporters of other nutrients. We chose mice as a model because they have been used often in studies on regulation of intestinal transport in both normal and diabetic condition [10] and because we have successfully used them in our previous research on intestinal absorption in experimental diabetes [6,10].

2. Methods

2.1. Animals and experimental diabetes

Adult male Swiss-Webster (Taconic Farms, Germantown, NY) mice (initial body weight, 37.0 ± 0.2 g) were rendered diabetic by streptozotocin (Sigma-Aldrich, St Louis, MO) in citrate buffer, pH 4.5. The streptozotocin was administered intraperitoneally in 2 consecutive days: the initial dose was 100 and the second was 25 mg/kg body weight, as previously described [6]. All the mice became hyperglycemic, but only the mice whose glycemia was at least 200 mg/dL 1 week from the first injection were assigned to this study. The diabetic mice were maintained at 24°C on a 12:12-h dark-light schedule, with free access to water and a standard rodent diet (Purina Lab Rodent Chow, Purina Mills, Richmond, VA) for at least 10 weeks before any treatment. This duration is sufficient to mimic the chronic diabetic condition in humans [6].

The mice were then randomly assigned to 4 treatment groups. One group received a high carbohydrate–low protein (H) diet; 2 groups received the H diet added with either the AGI acarbose (HA) or the AGI deoxynojirimycin (HD); a fourth group received a low carbohydrate–high protein (L) diet (for details, see Diets below). Because each experimental uptake procedure required uptake measurements from 48 intestinal sleeves (12 from each mouse representing a treatment group [see Uptake Measurements below]) and lasted 3 days (one half-day for preparations, one full day for uptake experiments, one half-day for tissue and sample processing), we had to stagger the day of the experiment as it was not possible to determine uptakes from more than one representative of each group on a single day. To accomplish this, 2 batches of 40 mice each were purchased (extra mice were ordered to allow for mortality and failure to induce hyperglycemia) about 2 months apart. From each batch, 8 diabetic mice at a time were selected, 2 for each diet of which one was to be sacrificed at 2 weeks after initial consumption of experimental diets, and another at 4 weeks. For each experimental day, one representative from every group was always sacrificed, and the order of sacrifice was randomized.

We then compared the specific uptakes of D-fructose and D-glucose in these 4 groups. D-Sugar transport in the L group was considered baseline because of the extremely low carbohydrate content of the diet. We did not use nondiabetic controls because we were only interested in the adaptation to diet and AGIs in diabetes mellitus, not in the interactions between diet and diabetes (previously studied by us [6]), in effects of diabetes per se (studied by us [6]), and in effects of AGIs on transport in the absence of diabetes (studied by Paiva et al [9] and Gomez-Zubeldia et al [11]).

In the same groups, we also measured the uptakes of the following amino acids to determine the effect of those diets on transport of nutrients that are not products of α-glucosidase–mediated reactions: L-proline (transported by intestinal sodium iminoacid transporter (SIT) [12]), which is the only amino acid transported by a single specific transporter; L-glutamine (important as a fuel for the enterocytes and transported by several systems [13]); and L-aspartate (transported by ASCT2 [13]).

The care of the animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey.

2.2. α-Glucosidase inhibitors

Two different AGIs were used: acarbose (Bayer, West Haven, CT), which is not absorbed by the small intestine, and deoxynojirimycin (1-deoxynojirimycin hydrochloride, Sigma-Aldrich), which, like its closely related analog miglitol, is absorbed [14-16] by active transport [17]. Both compounds have a very high affinity for intestinal glucosidases [18]. Relative to its inhibition of pancreatic amylase, acarbose is a strong inhibitor of brush-border glucosidases [15]. Its affinity for sucrase is extremely high, more than 15 000 times greater than that of sucrose, the natural substrate of sucrase [18]. α-Glucosidase inhibitor dosage was based on levels that caused maximum reduction of postprandial plasma glucose concentrations in healthy humans (acarbose, 300 mg/d; deoxynojirimycin, 50 mg/d), without the intestinal side effects that set in at higher dosages [14]. This dosage was then adjusted by ratio and

proportion for mouse body weight (assuming that the average human male weighed 70 kg and the male mouse 35 g): acarbose, 0.15 mg/d; deoxynojirimycin, 0.025 mg/d. For optimal effect, AGIs need to be administered orally during meals, so they were mixed with other ingredients in the pelletized diet of experimental mice. The amount of each AGI in the diet was calculated based on the average daily food consumption of 5 g/d for similar-sized mice [6]. Addition of AGIs did not affect feeding rate, which averaged 5.1 ± 0.3, 4.7 ± 0.2, and 4.9 ± 0.4 g/d, over the course of the experiment for mice fed the H, HD, and HA diets, respectively. As in previous studies [6,10], feeding rate was slightly less in mice fed L (4.0 ± 0.2).

2.3. Diets

The diets (Dyets, Bethlehem, PA) were isocaloric and identical to each other except in the carbohydrate and protein components and the addition of AGIs. The H diet had (in g/kg diet) starch, 530; sucrose, 100; and casein, 150. The L diet had (in g/kg diet) starch, 50; sucrose, 0; and casein, 700. Both diets had (in g/kg diet) vegetable oil, 70; cellulose, 50; vitamin mix [6], 10; mineral mix [6], 40; and Brewer's yeast, 20. The HA and HD diets were obtained by mixing acarbose (30 mg/kg diet) and deoxynojirimycin (5 mg/kg diet), respectively, in the H diet. Thus, AGIs were taken (0.03 mg/g diet × 5 g/d consumption = 0.15 mg acarbose per mouse per day) with a diet rich in complex carbohydrates, designed to elicit an adaptive upregulation of sugar transport as shown in previous work [19]. The L diet was isocaloric with the others but had a minimum amount of carbohydrates. It was designed to downregulate sugar absorption to baseline levels, similar to sugar transport rates obtained when rodents are fed carbohydrate-free diets. Mice were killed 2 and 4 weeks after initial consumption of the experimental diets.

2.4. Uptake measurements

We measured uptake of D-[^{14}C]glucose, D-[^{14}C]fructose, L-[^{3}H]proline, L-[^{3}H]glutamine, and L-[^{3}H]aspartate in the intestinal mucosa by using the preparation of everted sleeves [20]. Glucose, fructose, and proline uptakes were measured in the proximal, middle, and distal intestinal regions. Aspartate, glutamine, and L-glucose (see below) uptakes were measured in the middle small intestine. Experiments were always carried out between 11:00 AM and 3:00 PM to minimize the effects of diurnal rhythms. The mice (not fasted overnight) were anesthetized and subsequently killed by an overdose of pentobarbital sodium (3.5 mg/kg body weight). The intestine was gently flushed in ice-cold Ringer solution, excised, and everted over a glass rod. One-centimeter-long sleeves were obtained from the proximal (12 cm distal to the pylorus), distal (12 cm proximal to the cecum), and middle (about 50% of total intestinal length) small intestine; tied on a steel rod; and incubated as described in Ref [21]. Within a region, determinations of solute uptake were assigned to sleeves in a random manner. Incubation times were 1 minute for D-glucose and 2 minutes for D-fructose and the amino acids, following the criteria of Ref [20]. Test nutrient concentrations were chosen to yield the V_{max}, a condition where

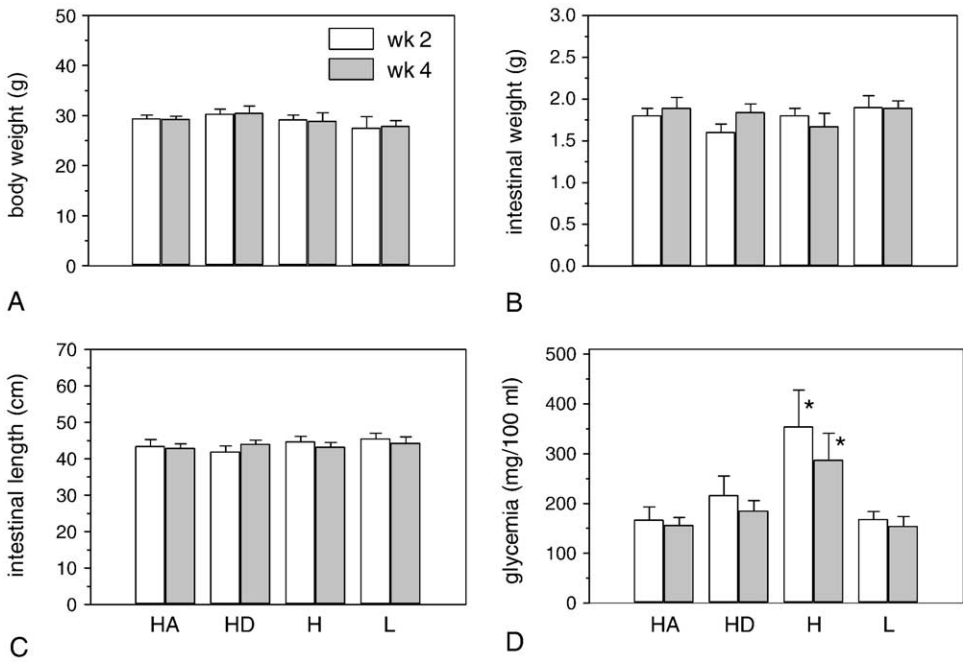

Fig. 1. Body weight (A), small intestinal weight (B), small intestinal length (C), and glycemia (D) at the time of death in streptozotocin diabetic mice receiving 4 different diets. The diets were as follows: H, high carbohydrate–low protein; HA, H diet added with acarbose (30 mg/kg diet); HD, H diet added with deoxynojirimycin (5 mg/kg body weight); and L, low carbohydrate–high protein diet. Bars represent means ± SE (n = 6). Asterisks denote differences ($P < .05$ by 1-way ANOVA followed by Fisher's protected least significant difference test) from other diet groups with the same treatment duration.

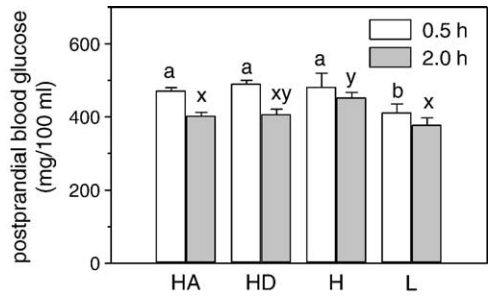

Fig. 2. Postprandial blood glucose in streptozotocin diabetic mice receiving 4 different diets (H, HA, HD, L; see legend to Fig. 1). Within the same duration, bars with different superscript letters are significantly different from each other ($P < .05$).

unstirred layer effects are minimal: 50 mmol/L for glucose, fructose, proline, and glutamine; 25 mmol/L for aspartate [20,22,23]. L-[^{3}H]Glucose uptake was used to correct for adherent fluid and for the diffusive component of total D-glucose and D-fructose uptake [20]. [^{14}C]Polyethylene glycol (molecular weight, 4000) was used to correct amino acid uptake for the radioactivity in the adherent fluid. Radioisotopes were from Perkin Elmer (formerly New England Nuclear, Boston, MA).

Transport results were expressed as per milligram of small intestinal weight to detect specific changes in the transport rate, as per centimeter of small intestine to detect changes in mucosal mass affecting uptake, and as total absorptive capacity of the small intestine. Total intestinal capacity was obtained by integrating transport per centimeter along the length of the small intestine, according to Ref [24].

2.5. Statistical analysis

Results are expressed as means ± SE (n = number of mice) and were analyzed by 1-way analysis of variance (ANOVA). Analysis of variance results were considered significant at P values < .05. Post-ANOVA analysis was done by Fisher's protected least significant difference test.

3. Results

3.1. Clinical parameters

3.1.1. Body weight, intestinal weight, intestinal length

There was no significant ($P > .40$ in all cases) influence by diet on body weight, intestinal weight, and intestinal length at 2 and 4 weeks after initial consumption of experimental diets, indicating that these parameters were independent of both the assigned diet and AGI treatment (Fig. 1A-C). The overall average body weight, intestinal weight, and intestinal length in mice fed the various diets were 28.0 ± 0.2 g, 1.7 ± 0.2 g, and 44 ± 6 cm, respectively.

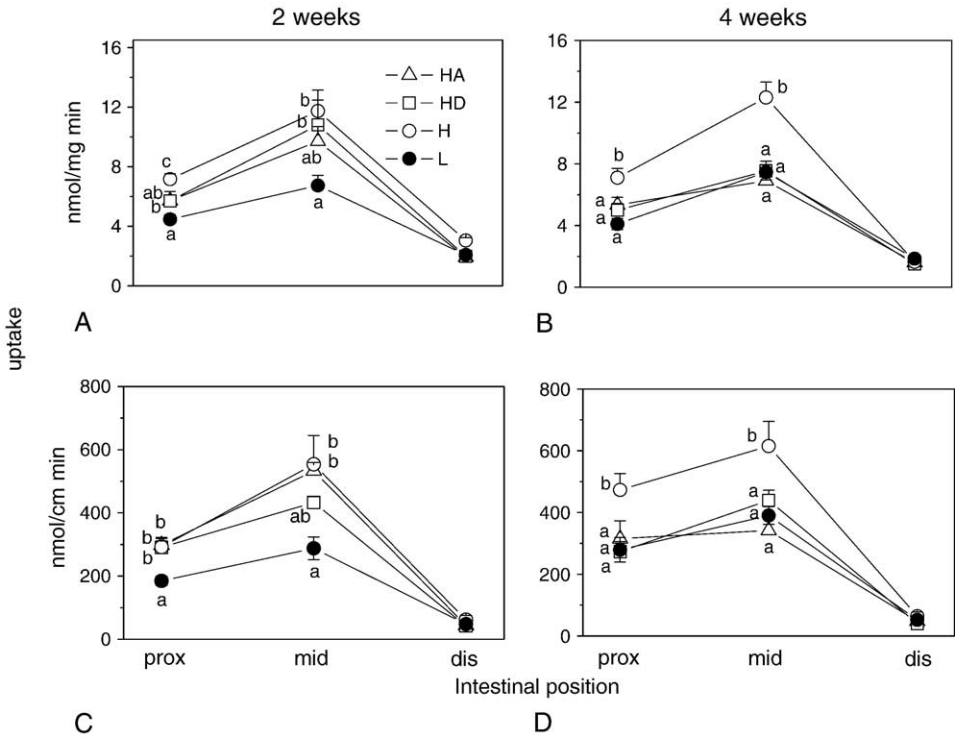

Fig. 3. D-Glucose transport per milligram and per centimeter of small intestine in streptozotocin diabetic mice receiving 4 different diets. Points represent mean D-glucose uptake ± SE (n = 6). H, HA, HD, L as in Fig. 1. Pro, mid, dis indicate proximal, middle, and distal small intestine, respectively. Different superscripts denote significant differences within each intestinal region. After 2 weeks, uptake per milligram in the proximal small intestine was lower in HA and HD than in H, but higher than in L. Uptake per centimeter had a similar trend (C). After 4 weeks, uptake in HA and HD was lower than in H and similar to L, both expressed as per milligram (B) and per centimeter (D). Acarbose and deoxynojirimycin block the dietary carbohydrate-induced increases in glucose transport.

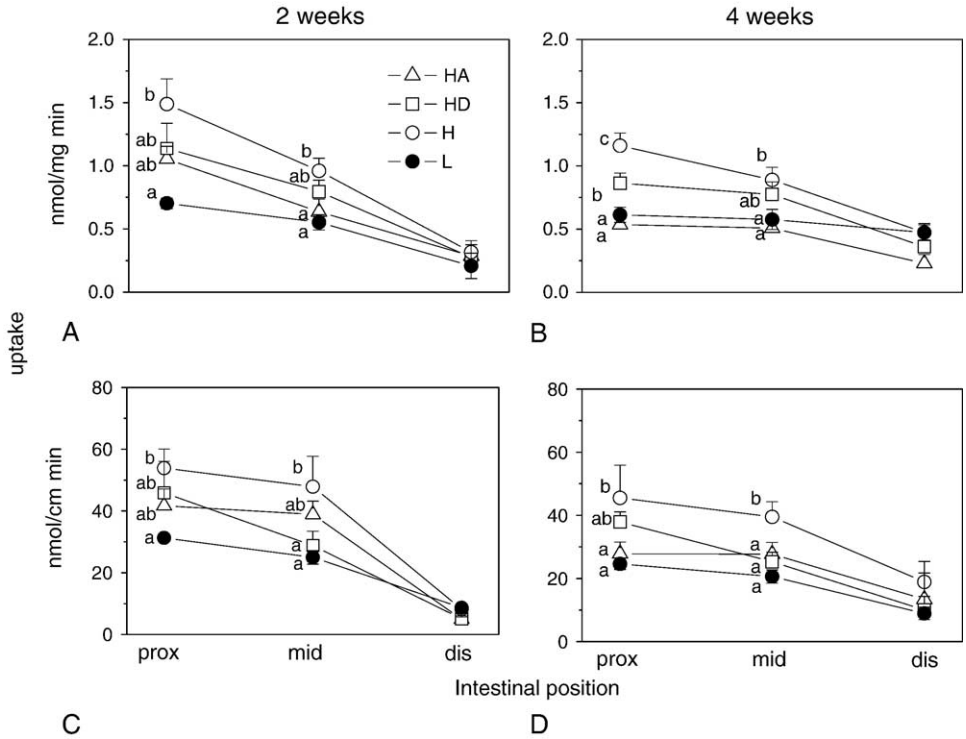

Fig. 4. D-Fructose transport per milligram and per centimeter of small intestine in streptozotocin diabetic mice receiving 4 different diets. Points represent mean D-fructose uptake ± SE (n = 6). H, HA, HD, L as in Fig. 1. Different superscripts denote significant differences in each intestinal region. After 2 weeks, HA and HD uptakes in the proximal small intestine tended to be lower than that of H (A and C). After 4 weeks, uptake in HA and HD was lower than that in H and similar to L, both expressed as per milligram (B) and per centimeter (D). Acarbose prevents diet-induced increases in fructose transport. Deoxynojirimycin is a less effective inhibitor of fructose than of glucose transport.

3.1.2. Glycemia

Fasting blood glucose concentrations, determined during daytime hours when mice were not actively feeding [25], were affected by diet. At 2 weeks (P = .003) and again at 4 weeks (P = .015), blood glucose in HA, HD, and L mice were each significantly lower than that of H (Fig. 1D).

Postprandial blood glucose concentrations of diabetic mice fed H, HD, and HA were elevated within 30 minutes after feeding (Fig. 2). Mice fed the L diet, however, had lower (P < .01) blood glucose concentrations than those fed the other diets. At 2 hours postprandial, blood glucose concentrations in H mice stayed high, but those in HA and HD decreased significantly (P < .05) to levels exhibited by L mice. Hence, consumption of AGIs with H diets lowered blood glucose concentrations at 2 hours compared to those at 0.5 hour. We did not analyze blood glucose concentrations at other time points to minimize stress on the mice.

3.2. Nutrient uptake

3.2.1. D-Glucose

Rates of intestinal glucose uptake were almost 2 times greater in H than in L mice in the proximal (P < .005 for per milligram and per centimeter) and middle (P < .001) intestines at 2 weeks (Fig. 3A,C). At 4 weeks, intestinal glucose uptakes were also about 2 times greater in H mice (Fig. 3B,D). Addition of AGIs to the H diet resulted in a significant reduction in glucose uptake rates in the proximal (P < .05) small intestine after 2 weeks, and in both proximal (P < .002) and middle (P < .0001) intestines after 4 weeks. After 4 weeks, uptake rates in HA and HD mice clearly migrated from being somewhat similar to rates exhibited by H mice to rates exhibited by L mice. Hence, AGI consumption clearly reduced glucose uptake to rates similar to those in mice consuming the L diet (Fig. 3B). As expected, uptakes were greatest in the middle intestine.

Because similar results were obtained when glucose uptake was expressed per centimeter (Fig. 3C,D), diet probably did not alter the weight of intestinal sleeves. In fact, by dividing the small intestinal weight by its length in each mouse, the ratio in milligrams per centimeter was independent of diet. At 2 weeks, HA, 43 ± 2; HD, 39 ± 2; H, 39 ± 1; L, 41 ± 3 (P = .53). At 4 weeks, HA, 44 ± 2; HD, 42 ± 2; H, 38 ± 3; L, 43 ± 2 (P = .37). There was no difference either in weight per centimeter between mice sacrificed at 2 and 4 weeks (P = .43).

3.2.2. D-Fructose

Unlike glucose, fructose uptakes were greatest in the proximal intestine. Except in the distal intestine, fructose uptake in L mice was always less than half of that in H mice 2 (Fig. 4A,C) and 4 (Fig. 4B,D) weeks after consumption of experimental diets. After 2 weeks, acarbose in particular reduced fructose uptake per milligram in the middle (P < .05) but not in the proximal and distal regions.

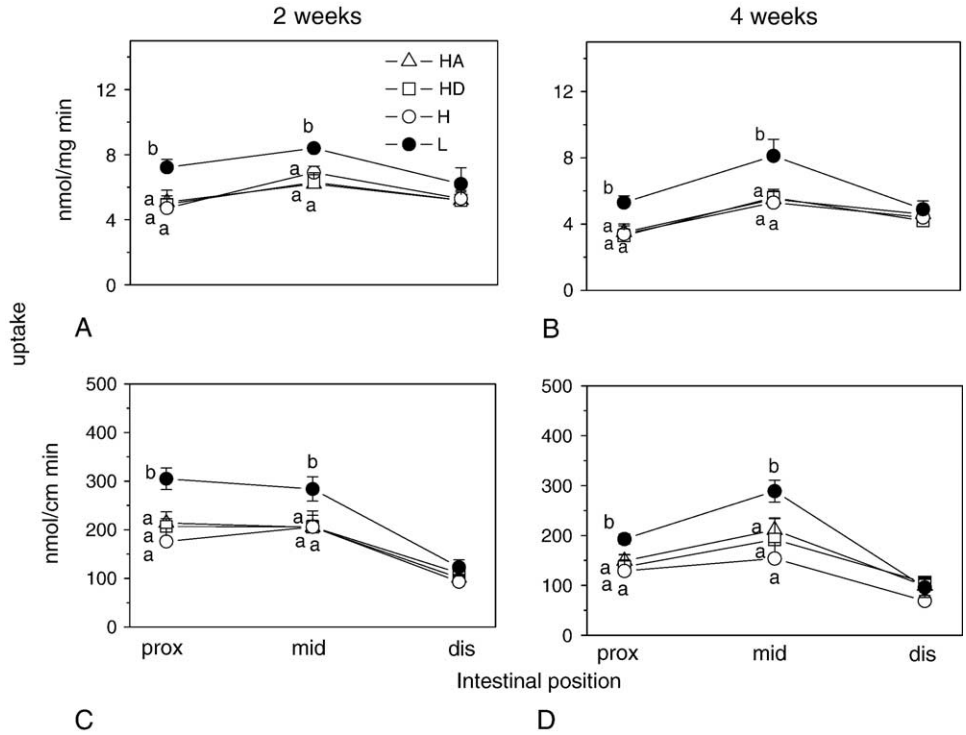

Fig. 5. L-Proline transport per milligram and per centimeter of small intestine in streptozotocin diabetic mice receiving 4 different diets. Points represent mean L-proline uptake ± SE (n = 6). H, HA, HD, L as in Fig. 1. Different superscripts denote significant differences in each intestinal region. After 2 weeks, in the proximal and middle small intestine HA and HD uptake were similar to that of H and significantly lower than that of L, expressed as either per milligram (A) or per centimeter (C). These differences were unchanged after 4 weeks (B and D). Proline uptake is regulated only by dietary effects.

Deoxynojirimycin was less effective and only tended to reduce fructose uptakes per milligram in the proximal ($P < .10$) and middle ($P < .20$) intestines. At 4 weeks, both AGIs clearly decreased rates of fructose uptake (Fig. 4B), but the effect of acarbose ($P < .001$ for proximal, $P < .01$ for middle) was much more dramatic than that of deoxynojirimycin ($P < .01$ for proximal). In fact, fructose uptakes in HA mice were less than 50% those of H mice and even tended to be lower than those of L mice. Similar patterns were observed for uptakes per centimeter of intestine.

3.2.3. L-Glucose

Diet had no effect on L-glucose uptake at 2 ($P > .90$) and 4 weeks ($P > .50$). Average (of 2 and 4 weeks) L-glucose uptake in the middle intestine was as follows: HA, 0.021 ± 0.003; HD, 0.017 ± 0.003; H, 0.024 ± 0.002; L, 0.021 ± 0.003 μL/[min · cm]). Because L-glucose is not recognized by any transporter, L-glucose uptake is an index of membrane permeability to sugars. Hence, AGIs did not alter membrane permeability characteristics.

3.2.4. L-Proline

The pattern was opposite that found for sugars, so that at 2 weeks L-proline uptake in the proximal (Fig. 5A,C; $P < .005$) and middle ($P < .05$) intestine was about 50% greater in mice fed L than in mice fed H diets. Very similar patterns were observed at 4 weeks (Fig. 5B,D). Intestinal proline uptake was similar among H, HA, and HD mice in all regions and at both time intervals. Hence, addition of AGIs to the H diet clearly had no effect on proline uptake, indicating that the inhibitory effect of AGIs on uptake is specific for the monosaccharides glucose and fructose.

3.2.5. L-Aspartate

Intestinal aspartate uptake in mice fed L diet was about 70% greater than that in mice fed H at 2 ($P < .05$) and 4 ($P < .05$) weeks of treatment (Table 1). Uptakes of aspartate in mice treated with AGIs were similar to those fed the H diet in both time intervals. Hence, addition of AGIs to the H diet did not affect aspartate uptake.

Table 1
Effect of AGIs on intestinal aspartate and glutamine transport

Treatment	Aspartate		Glutamine	
	2 wk	4 wk	2 wk	4 wk
nmol/mg · min				
HA	2.90 ± 0.35	2.03 ± 0.25	7.79 ± 1.35	8.19 ± 0.50
HD	2.98 ± 0.19	1.61 ± 0.28	7.20 ± 1.88	7.77 ± 0.19
H	2.57 ± 0.34	1.79 ± 0.34	7.63 ± 0.40	8.22 ± 1.02
L	4.63* ± 0.92	3.00* ± 0.32	11.50* ± 0.92	10.5* ± 0.77
nmol/cm · min				
HA	93.0 ± 10.6	84.3 ± 5.4	353.5 ± 29.1	230.3 ± 22
HD	80.1 ± 4.6	63.0 ± 8.4	237.6 ± 38.8	218.8 ± 21
H	65.7 ± 9.5	55.4 ± 8.8	231.2 ± 12.0	192.8 ± 30
L	156.0* ± 34.0	99.6* ± 17.8	353.5* ± 29.1	321.4* ± 36

* $P < .05$ by 1-way ANOVA as compared to the other dietary treatments.

3.2.6. L-Glutamine

The effect of diet was also significant for glutamine uptake measured in the middle intestine at 2 ($P < .05$) and 4 ($P < .05$) weeks. Uptake was 40% greater in L mice compared to those in the H, HA, and HD groups (Table 1). Addition of AGIs to the H diet did not affect glutamine uptake.

3.2.7. Total intestinal transport capacity

By integrating uptake per centimeters along the length of the small intestine, we calculated total intestinal uptake capacities for glucose, fructose, and proline 4 weeks after initial consumption of the diets (Fig. 6). Total transport capacity for glucose in both the HA and HD mice was similar to that of the L mice and about 30% less than that of the H mice ($P = .006$), indicating that chronic treatment with either of the AGIs lowered intestinal glucose uptake capacity to levels typical of intestines from mice fed a low-carbohydrate diet. Total intestinal uptake capacity for fructose ($P = .024$) decreased markedly in mice fed HA but not in mice fed HD.

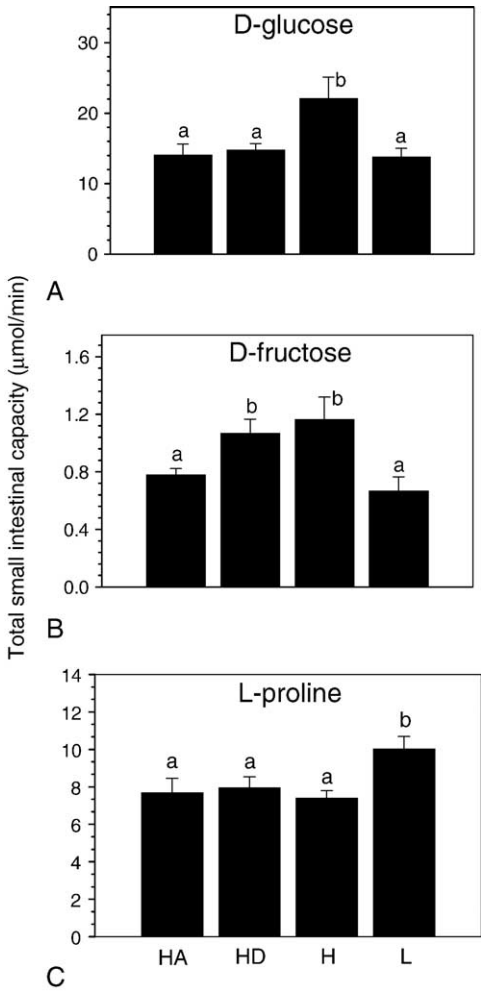

Fig. 6. Total small intestinal transport capacity for D-glucose, D-fructose, and L-proline of streptozotocin diabetic mice receiving 4 different diets for 4 weeks. Bars represent mean uptake ± SE ($n = 6$). H, HA, HD, L as in Fig. 1. Different superscripts denote significant differences. D-Glucose (A), D-fructose (B), and L-proline (C). Total transport capacity for glucose was upregulated by the H diet and lowered to L values by HA and HD. Total transport capacity for D-fructose was upregulated by the H diet and lowered by HA but not by HD to L values. L-Proline was upregulated by the L diet and unaffected by HA and HD.

Proline uptake capacity by HA and HD mice was similar to that of H and was lower than that of L ($P = .021$), indicating that AGI consumption had no effect on the uptake of proline. The significant effect of treatment could only be ascribed to the imino transporter increasing in abundance in response to the high protein in the L diet.

4. Discussion

Although AGIs are known to reduce postprandial blood glucose concentrations by decreasing the rate of starch hydrolysis and the absorption of glucose, their effects on the *regulatory mechanisms* of intestinal glucose transport have never been studied, and no study has described their potential in the regulation of other transport systems, such as that of fructose or amino acids.

In this study, we found that carbohydrate-induced increases in sugar transport by the small intestine of diabetic mice are prevented by AGIs. Because sugar transport rates in carbohydrate-fed but AGI-treated mice are similar to those elicited by a low-carbohydrate diet, the mechanism underlying the AGI effect may be reductions in luminal carbohydrate concentrations. It is also possible that AGIs inhibit sugar transport directly, but it has been shown that acarbose itself had no effect on glucose transport in isolated intestine from nondiabetic rats [9]. There are 2 corollary and equally novel findings: first, the adaptive response to AGIs is based on specific changes in transport V_{max} because AGIs do not interfere with adaptations of other Na^+-dependent transporters such as those of amino acids, and second, acarbose may be a more effective AGI than deoxynojirimycin and its analogs like miglitol because it markedly inhibits not only glucose but also fructose transport. This study also confirmed, in the mouse model, that treatment with AGIs decreases plasma glucose concentrations. Clearly, one potential mechanism by which AGIs blunt postprandial glycemic excursions and lower fasting blood glucose concentrations in individuals consuming carbohydrate-containing diets is inhibition of carbohydrate-induced increases in intestinal sugar transport.

4.1. Diabetes, AGIs, and intestinal absorption

Most of the large number of studies on effects of diabetes on intestinal transport reported increases in sugar transport because of increases in absorptive mucosal mass and/or in site density of transporters [4]. There has only been one research on human NIDDM subjects. In brush-border membrane vesicles from duodenal biopsies of patients with NIDDM, α-glucosidase activities, Na^+-dependent glucose transport, and SGLT1 and GLUT5 protein levels were

3-fold higher than those from healthy controls [26]. There was also a 3-fold increase in SGLT1, GLUT5, and GLUT2 mRNA in the diabetic intestine. These increases in SGLT1, GLUT5, and GLUT2 expression resulted in increased absorptive capacity of the diabetic intestine to sugars. As GLUT5 protein increased, intestinal fructose transport also probably increased in NIDDM. In diabetic mice [6] and rats [27], intestinal fructose transport increased.

α-Glucosidase inhibitors can be used for the treatment of diabetes, obesity, and hyperlipoproteinemia [15]. Competitive, high-affinity inhibitors of intestinal α-glucosidases delay the degradation of complex carbohydrates to absorbable monosaccharides, and thus decrease the rate of D-sugar absorption [18]. Acarbose inhibits pancreatic amylase rather weakly, but is a strong inhibitor of brush-border glucosidases [15] which increase with diabetes [26] and diet [4]. Its affinity for sucrase is about 10^5 times greater than that of sucrose [22]. 1-Deoxynojirimycin has a similar affinity for sucrase, but, in contrast to acarbose, is almost completely absorbed from the intestine of rat [15] and has no effect on pancreatic amylase in vitro. In human diabetic patients, both fasting and postprandial blood glucose decreased significantly in the first 3 months of AGI treatment and remained constant thereafter; both values rose again when acarbose was discontinued [28]. In healthy volunteers, single morning doses of deoxynojirimycin caused a dose-dependent reduction in blood glucose and serum insulin. The 40-mg dosage prevented the postprandial increase in blood glucose and serum insulin altogether; higher dosages perturbed intestinal function. The effect persisted 6 to 12 hours after administration of the drug [14]. Acarbose (at 100-200 mg with each meal) inhibited postprandial increases in glucose, insulin, and triglycerides; C-peptide and pancreozymin plasma concentrations were reduced, whereas secretion of somatostatin increased and that of gastrin decreased [29].

There have been 2 studies on the effect of AGIs and diet on blood glucose concentrations. Addition of AGI to a high-carbohydrate meal significantly reduced the magnitude of changes in postprandial plasma glucose concentrations in diabetic, but not in nondiabetic, rats [30]. This study, however, did not investigate the mechanisms underlying the acarbose effect. As would be expected from an AGI, acarbose reduced hyperglycemia after an oral sucrose or starch load, but not after a glucose load [31], indicating that its hypoglycemic effect is linked to its inhibition of sucrose digestion.

4.2. Adaptation of D-sugar transport to AGIs is similar to that elicited by a low-carbohydrate diet

The H diet upregulated glucose uptake, confirming that the small intestine of the diabetic mice can adapt to dietary changes, as previously demonstrated [6]. Unfortunately, chronic diabetes increases small intestinal mucosal hyperplasia, with an increased number of transporting enterocytes [6,32,33], thus amplifying the response.

Because the proximal to mid small intestine is the main site for glucose transport, the AGIs are particularly effective in these regions. By inhibiting the breakdown of complex carbohydrates of the H diet, acarbose and deoxynojirimycin induce adaptive decreases in the V_{max} of glucose transport. Because membrane permeability to glucose and intestinal weight were not affected, the decrease in V_{max} of glucose transport likely reflected a decrease in SGLT1 number per enterocyte [8]. This finding is strikingly similar to parallel decreases in V_{max} of glucose transport and site density of SGLT1 in response to a diet low in carbohydrate and, presumably, to low luminal glucose concentrations. Because it inhibits carbohydrate breakdown, acarbose has been shown to reduce disaccharide concentrations in the intestinal lumen [11].

The effect of AGIs on fructose uptake is most effective in the proximal SI, which is the main site for both specific fructose transport and for α-glucosidase activity. Their effect on fructose uptake seems to be slower and less marked than that on glucose perhaps because of the smaller contribution of α-glucosidases to changes in fructose concentration in the lumen.

4.3. Effect of experimental duration

It was not clear to us why fructose uptake would decrease slightly at 4 weeks relative to that at 2 weeks of treatment, especially that in the H diet in the proximal intestine (compare panels A and B of Fig. 4). Uptake rates of certain nutrients (proline and aspartate) but not of others (glucose) also tended to be lower at 4 weeks, typically in the proximal intestine. We ascribe this variation mainly to experimental variability. We note, however, that the effects of diet and of AGI treatments were highly consistent between the 2-week and 4-week durations: sugar uptakes always greater in H mice, amino acid uptakes always greater in L mice, and AGI treatments typically reducing sugar uptakes in H mice. It also seems clear, particularly with glucose uptakes, that long-term (4-week) consumption of AGIs improves their ability to inhibit sugar absorption by the small intestine.

4.4. α-Glucosidase inhibitors reduce total intestinal transport capacity for sugars

Total transport capacity for each nutrient varies as a function of (1) specific changes in the number of its transporters and (2) total intestinal mass [34]:

$$\sum J/\mathrm{BW}^{0.75} = \left(\mathrm{IW}/\mathrm{BW}^{0.75}\right)\left(\sum J/\mathrm{IW}\right)$$

where $\sum J$ is the total intestinal transport capacity for a given nutrient, BW is body weight, and IW is intestinal weight.

The first term of the equation ($\sum J/\mathrm{BW}^{0.75}$) is the total intestinal capacity as normalized to metabolic mass, which is (body weight)$^{0.75}$ [4,31]. This normalization accounts for the fact that metabolism changes as a function of metabolic

Table 2
Physiological and anatomical contributions to total intestinal absorptive capacity for D-glucose, D-fructose, and L-proline

	Treatment			
	HA	HD	H	L
$BW^{0.75}$	12.58	12.97	12.43	12.13
IW	1.89	1.84	1.67	1.89
$IW/BW^{0.75}$	0.15	0.14	0.13	0.16
ΣJ glucose	5.189	2.952	10.345	4.252
$\Sigma J/BW^{0.75}$	0.412	0.228	0.832	0.350
$\Sigma J/IW$	2.746	1.604	6.195	2.250
ΣJ fructose	0.782	1.068	1.167	0.670
$\Sigma J/BW^{0.75}$	0.062	0.082	0.094	0.055
$\Sigma J/IW$	0.414	0.58	0.699	0.354
ΣJ proline	7.700	7.962	7.408	10.039
$\Sigma J/BW^{0.75}$	0.612	0.614	0.596	0.828
$\Sigma J/IW$	4.074	4.327	4.436	5.312

mass, so the whole term represents how much nutrient flows per unit metabolic weight [4,31]. The second term, $IW/BW^{0.75}$, is the "anatomical" factor, which represents changes in intestinal mass relative to body weight; $\sum J/IW$ is the "physiological" factor, which represents changes in the ability of the small intestine to transport nutrients (see Ref [4] for details on the equation and a discussion of its components).

The anatomic factor does not vary with treatment, indicating that the adaptation to diet composition and to AGIs does not influence intestinal mass. The physiological factor changes with treatment for each of the specific nutrients. For D-glucose, the physiological factor (~6.2) is highest in the H diet and is markedly decreased by the HA, HD, and L diets (physiological factors, ~1.6-2.7). There is a similar, although less, marked effect in the case of D-fructose. As compared to the H group (physiological factor, ~0.7), the physiological factor decreased in the L and HA groups (~0.35-0.41), but is only slightly lower in the HD (physiological factor, ~0.58). For proline, the physiological factor is highest in the L diet, which has a greater protein content, and lower in the other 3 diet groups (Table 2). Hence, carbohydrate-induced increases in total intestinal absorptive capacity for glucose are prevented by AGIs; those for fructose are prevented by acarbose.

We can conclude that the small intestine adapts to chronic AGI treatment by decreasing the V_{max} of sugar transport to rates elicited by a low-carbohydrate diet. The immediate future plans for this study will be to assess by c-DNA micro-array techniques what genes are up- or downregulated by the different dietary treatments in the diabetic mice and thus relate gene expression to function.

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