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Chronic acarbose-feeding increases GLUT1 protein without changing intestinal glucose absorption function

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Abstract

As α-glucosidase inhibitor, the antidiabetic drug acarbose reduces postprandial glucose levels by retarding the intestinal digestion of polysaccharides. However, it is unknown if acarbose also affects the expression of intestinal glucose transporters, especially the Na^+-glucose cotransporter (SGLT1) and the glucose transporters GLUT1 and GLUT2. To unravel this question, Wistar rats received standard powdered chow either without (control) or with acarbose (40 mg acarbose/100 g chow) for 40 days. While food intake was slightly enhanced by acarbose, the drug had no influence on weight gain or plasma glucose and insulin levels. The acarbose-treatment did not alter the SGLT1 and GLUT2 gene expression in both upper and middle small intestine, whereas GLUT1 protein was increased by 75% in middle small intestine. Despite the territorial change in GLUT1 protein, the intestinal glucose absorption in an acarbose-free perfusion study was unaltered. In conclusion, the chronic use of acarbose did not alter the acarbose-free glucose absorption profile.

Keywords: Acarbose; Intestinal glucose transport; SGLT1; GLUT1; GLUT2; Diabetes mellitus

1. Introduction

According to a report of the World Health Organization (WHO) in the year 1998, a recent estimation predicts that the number of persons with diabetes worldwide will more than double, from 140 million to 300 million in the next 25 years (King et al., 1998). This outlook clearly demands strategies for the monitoring, prevention and control of diabetes, especially the more dominating type 2 diabetes (non-insulin-dependent diabetes mellitus).

Pharmacological approaches to type 2 diabetes are including sulfonylureas (e.g. glibenclamide, glibonuride, glisoxepid, glimepiride) as well as the nonsulfonylurea agents meglintinides (e.g. repaglinide) to enhance insulin secretion from pancreatic β-cells and exogenous insulin supply to increase the plasma insulin level (Wolffenbuttel and Graal, 1996; Wagman and Nuss, 2001). Additional options are the biguanide metformin, which reduces the hepatic glucose release and increases peripheral glucose utilization, as well as the thiazolidinediones or glitazones (e.g. troglitazone, rosiglitazone, pioglitazone), which improve peripheral insulin sensitivity and reducing insulin resistance (Day, 1999; Rendell and Kirchain, 2000). Other drugs are the α-glucosidase inhibitors acarbose, miglitol and voglibose, which are all causing a delay of the absorption of glucose from the intestine (Lebovitz, 1998; Evans and Krentz, 1999).

Acarbose (*O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-α-D-glucopyranosyl-(1,4)-*O*-α-D-glucopyranosyl-(1,4)-*O*-α-D-glucopyranose) is a nitrogen-containing pseudotetrasaccharide and was originally found in culture broth of Actinoplanes strain SE50 (Lebovitz, 1998). Due to its sugar-like structure, acarbose is recognized by various α-glucosidases of the gastrointestinal tract (e.g. sucrase, maltase) and thus interferes with the enzymatic hydrolysis of their original disaccharide substrates (e.g. sucrose, maltose). As a result, acarbose is delaying the degradation of complex carbohydrates to absorbable monosaccharides and decreases the postprandial plasma glucose and plasma insulin rise when taken before a meal (Caspary, 1981; Chiasson, 1996). Acarbose is only poorly absorbed in the small intestine, but is metabolized by bacterial enzymes in the large intestine. The metabolites formed are several intermediates and four methylpyrogallol derivatives, and 35% of an oral dose appears as these metabolites in the urine (Krause and Ahr, 1996; Puls, 1996).

The absorption of glucose in the intestine involves certain integral membrane proteins, which catalyze the transport of glucose across the cellular membranes (Olson and

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Pessin, 1996; Takata, 1996). In the small intestine, a Na^+-glucose cotransporter (SGLT1) is localized at the apical plasma (brush border) membrane of the absorptive mucosal epithelial cells (Takata, 1996; Martin et al., 2000). The SGLT1 transports glucose against a concentration gradient by a secondary active transport mechanism in which glucose is cotransported with sodium ions due to a down-hill Na^+-gradient (Bell et al., 1990). At the basolateral membrane of these cells, the glucose transporter proteins GLUT1 and GLUT2 ensure the transport of glucose across the membrane by facilitative diffusion, a form of passive transport (Takata, 1996; Bell et al., 1990). GLUT1 has a high affinity to glucose (1–2 mM), but a low transportation rate, while GLUT2 has a low affinity for glucose (20 mM), but a high capacity and is slightly more abundant in the membrane than GLUT1 (Thorens, 1992). The expression of these glucose transporter proteins and their abundance in the plasma membrane of epithelial cells depends on physiological and pathological conditions, e.g. diabetes causes an upregulation of SGLT1 and GLUT2, and a downregulation of GLUT1.

Acarbose is widely used as a drug to treat diabetes type 2 subjects. Although there are studies concerning the acute effects of acarbose on glucose intestinal transport (Hirsh et al., 1997), there is no information available about its chronic effect on the intestinal glucose absorption system and its impact on the expression of SGLT1, GLUT1 or GLUT2. Accordingly, the purpose of this study was to investigate the action of chronic acarbose treatment in this context. Over a period of 40 days, female Wistar rats were given either a normal diet or an acarbose-enriched diet (40 mg acarbose/100 g chow) and then analyzed. In comparison with the standard-fed control, acarbose-treated rats showed a slightly higher food intake, but no changes in the final body weight as well as plasma glucose and insulin levels. In addition, in a perfusion study without acarbose being present, the acarbose-fed animals showed the same glucose absorption rate as the control animals. In line with this finding, Northern blot analysis for SGLT1 mRNA and Western blot analysis for GLUT2 protein in selected intestinal areas—upper and middle small intestine—showed no differences between the two animal groups. However, although unaffected in the upper small intestine, Western blot analysis for GLUT1 protein demonstrated a clear increase by 75% in the middle small intestine of acarbose-fed rats. Apparently, chronic acarbose-feeding has a selective impact on glucose transporter proteins, and the upregulation of GLUT1 protein observed is likely important to preserve the total glucose absorption capacity of the small intestine.

2. Materials and methods

2.1. Chemicals, antibodies and probes

Acarbose was a gift from Bayer (Wuppertal, Germany). Polyclonal rabbit antisera against GLUT1 and GLUT2 were raised in our lab by coupling a peptide, with an amino acid sequence identical either to the C-terminal 15 amino acids of rat GLUT1 or to the C-terminal 10 amino acids of rat GLUT2, to keyhole limpet hemocyanin and injecting the antigen-conjugates into male New Zealand white rabbits. A 2.4 kb cDNA probe specific for rat SGLT1 was generously provided by Dr. M. Kasahara (Teikyo University, Japan) and ^{32}P-labeled with the "Random Primers DNA Labeling System Kit" from Gibco-BRL (NY, USA). ^{32}P-labeled 18 S c-rRNA was generated using the "Maxi Script In vitro transcription kit" from Ambion (Austin, TX, USA).

2.2. Animal care

Female Wistar rats (172–184 g; 2 months old) had an adaptation period of 7 days and were all fed with standard powdered chow (from NUVILAB, Colombo, Brazil) during that time. Then, for the following 40 days one group of animals was held on the standard chow diet (control group), while another group received standard chow supplemented with acarbose (0.04% [w/w]) in parallel. Body weight and food intake were monitored during the initial adaptation period and during the experimental treatment period. After 40 days, rats were anesthetized with sodium pentobarbital (50 mg/kg body weight; injected intraperitonial) and used for blood collection or intestinal glucose absorption assay (see below), or sacrificed for the intestinal sampling and detection of SGLT1, GLUT1 and GLUT2 (see below). All experimental procedures were performed at 8:00 p.m. that means 1 h of dark period. The experimental protocol (protocol #85/99) was approved by the Ethical Committee for Animal Research (CEEA) of the Institute of Biomedical Sciences, University of São Paulo.

2.3. Determination of plasma glucose and insulin levels

Blood (1 ml) was collected from the inferior vena cava of the anesthetized animals by using heparin-treated syringes, and samples centrifuged at $2000 \times g$ for 10 min at 4 °C. The obtained plasma samples were assayed for glucose by the glucose oxidation method with a kit from Analisa Diagnostica (Belo Horizonte, Brazil), and insulin levels were measured with the radioimmunoassay "Coat-A-Count" from Diagnostic Products (Los Angeles, USA); both kits were handled according to the given instructions from the suppliers.

2.4. Glucose absorption assay

Taking 2.0 cm after the piloro as starting point, 21 cm of the small intestine of anesthetized rats were cannulated at both ends. The inlet catheter was connected to a reservoir containing gassed (95% O_2, 5% CO_2) Krebs–Ringer buffer (pH 7.4, 37 °C), and after setting a flow rate of 7 ml/min for the system, the intestine was washed with this buffer for 15 min. Body temperature was maintained at 37 °C throughout

the experiment using a heated blanket. After the wash, the buffer solution was exchanged, using now gassed Krebs–Ringer buffer with 5 mM D-glucose, and the perfusion was continued for 1 min to get rid off the remaining (glucose-free) washing buffer in the system. Then, the system was closed, by connecting the outlet catheter to the reservoir, and the perfusion continued for 30 min in a recirculating way. During that period, 50 μl samples were taken from the reservoir every minute and the glucose concentration measured as described above (Section 2.3). After 30 min, the perfused segment was removed, and proximal and terminal 2.0 cm, which were used to hold the catheter, were discarded. The remained loop was opened longitudinally to form a flat sheet, dried overnight at 100 °C and weighed to determine the dry weight. The glucose absorption was calculated as luminal loss of the sugar and expressed as μmol glucose absorbed per min per g dry weight per ml (μmol min^{-1} g^{-1} ml^{-1}).

2.5. Intestinal sampling

Using the same parameters of the perfusion study, the intestinal segment was removed from anesthetized rats and cleared of food residue by washing with diethyl pyrocarbonate-containing saline. Proximal and terminal 2 cm pieces of the isolated loop were discarded, and 3 cm pieces were cut at the beginning (upper small intestine) and at the end (middle small intestine), yielding for each part a longitudinal tissue strip weighing 0.1 g, which were immediately processed for the mRNA analysis of SGLT1 (see below). The remaining tissue was snap-frozen in liquid nitrogen for the protein analysis of GLUT1 and GLUT2 (see below).

2.6. Detection of SGLT1 by Northern blotting

Total RNA was extracted from the 0.1 g tissue samples (isolated as described above), using Trizol® reagent (Gibco BRL, NY, USA) according to manufacturer's instructions. RNA samples (20 μg) were first separated by electrophoresis in a 1.2% agarose gel containing 2.2 M formaldehyde, and then blotted onto a nylon membrane by neutral capillary transfer for 18 h, using 20 × 0.15 M NaCl; 0.015 M sodium citrate; pH 7.0 (SSC)-buffer as carrier. After fixing the membrane for 2 h under vacuum at 80 °C and blocking with pre-hybridization buffer (0.5 M $NaPO_4$, pH 6.8, 1% SDS, 1% bovine serum albumin [BSA], 1 mM EDTA, 100 μg/ml denaturated salmon sperm DNA) for 4 h at 42 °C, hybridization was carried out by adding a ^{32}P-labeled rat SGLT1 cDNA probe ($1-2 \times 10^6$ cpm/ml) and a further incubation for 16 h at 42 °C. Then, the membrane was washed (two washes with washing buffer 1 [0.1% sodium dodecyl sulfate (SDS), 2 × SSC] for 5 min at 25 °C, one wash with washing buffer 2 [1% SDS, 0.1 × SSC] for 15 min at 50 °C) and exposed to autoradiography. After that, the membrane was stripped by washing two times at high stringency conditions and rehybridized with ^{32}P-labeled cRNA probes of 18 S rRNA, followed by another autoradiography. The autoradiograms were analyzed by densitometric scanning (Image Master 1D®, Pharmacia Biotech, Upsalla, SW), and the obtained arbitrary units (AU) for SGLT1 normalized with the corresponding 18 S rRNA value.

2.7. Detection of GLUT1 and GLUT2 by Western blotting

The remaining longitudinal tissue samples of either upper or middle small intestine material were each homogenized in 1:10 weight/volume buffer (10 mM Tris–HCl, 1 mM

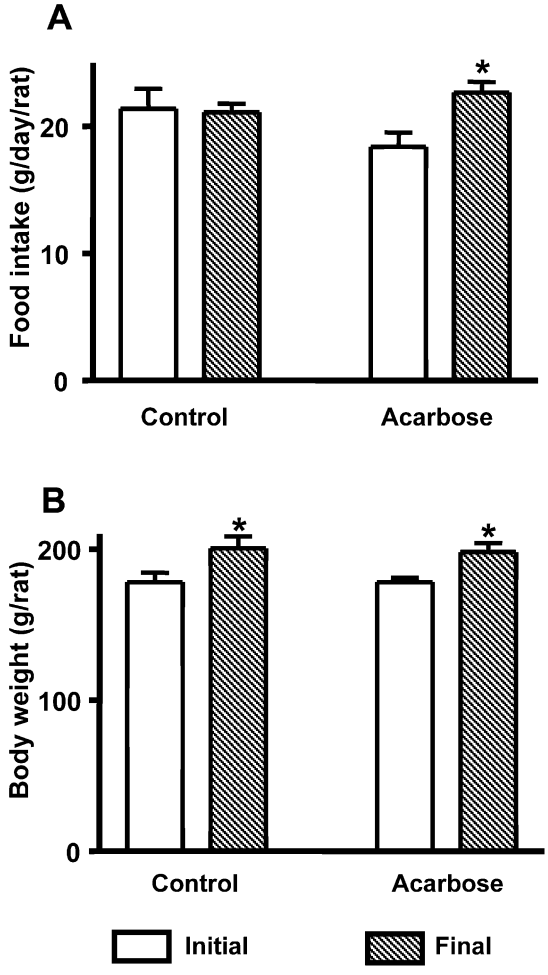

Fig. 1. Effect of acarbose on food intake and weight gain in Wistar rats. After 7 days of adaptation period, female Wistar rats received either a standard powdered chow (control) or acarbose-supplemented chow (0.04% [w/w], acarbose) for the experimental period of 40 days. The food intake and body weight were recorded during those periods. (A) The food intake for both the control (left side bars) and the acarbose group (right side bars) immediately before (initially, open bars) and after (finally, filled bars) the experimental period. Data are means ± S.E.M.; $n = 15$. * $P < 0.05$ versus initial. (B) The body weight of the animals of the control group (left side bars) and acarbose group (right side bars) immediately before (initially, open bars) and after (finally, shaded bars) the experimental period. Data are means ± S.E.M.; $n = 15$. * $P < 0.01$ versus initial.

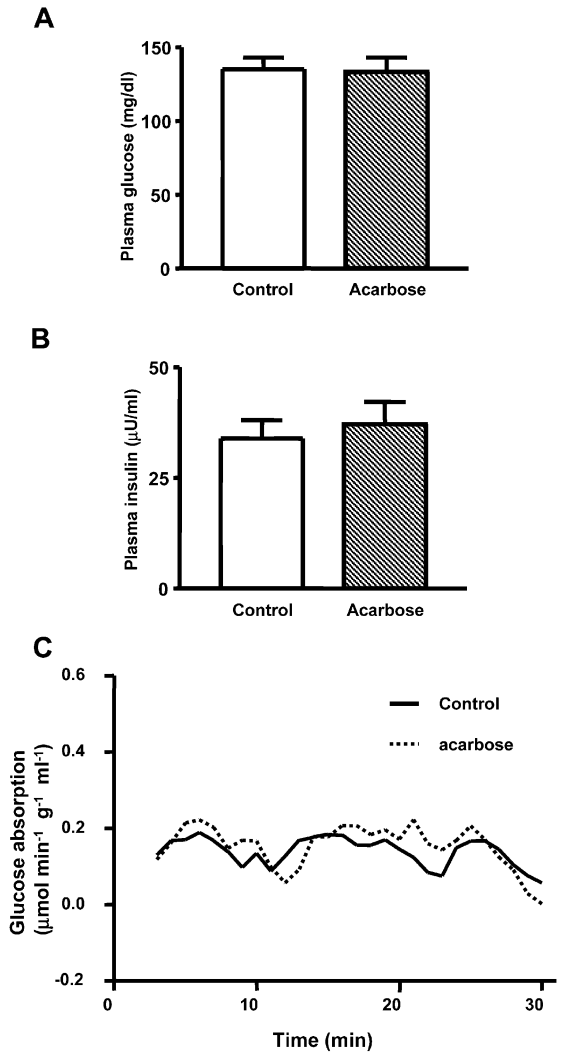

Fig. 2. Comparison of glucose homeostasis in distinctly treated Wistar rats. Female Wistar rats received either a standard powdered chow (control) or acarbose-supplemented chow (0.04% [w/w], acarbose) for 40 days. Then, blood samples were taken and the plasma levels of glucose (A) and insulin (B) were determined in both animal groups, using corresponding standards in parallel for a quantification; values are means ± S.E.M.; $n = 10$. In parallel, animals from both groups were also used for a measurement of the intestinal glucose absorption (C). In each animal, a 21-cm segment of the small intestine was washed and perfused in a closed system with gassed (95% O_2, 5% CO_2) Krebs–Ringer buffer containing 5 mM D-glucose (pH 7.4, 37 °C) at flow rate of about 7 ml/min. Over a period of 30 min, 50 μl samples were taken every minute to follow the reduction of the glucose concentration in the buffer due to glucose absorption. Note that 'acarbose' indicates the acarbose-fed animal group analyzed, and that no acarbose is present during the perfusion study. Values are means ± S.E.M.; $n = 5$.

EDTA, 250 mM sucrose, 5 μg/ml leupeptin, 15 μg/ml aprotinin, pH 7.4), using a Polytron® homogenizer (Brinkman Instruments, Westbury, NJ, USA) set at 24,000 rpm for 30 s. After centrifugation (3000 × g for 15 min), the supernatant was centrifuged (12,000 × g for 20 min), and the obtained pellet suspended in 450 μl buffer, resulting in a plasma membrane rich fraction. The protein content of each fraction was quantified with the method of Lowry, and the Western blotting analysis was performed as previously described. Briefly, equal amounts of membrane protein (100 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 10% polyacrylamide) and then electro-transferred onto nitrocellulose membrane. After blocking with non-fat milk, the membranes were first incubated with the anti-GLUT1 or anti-GLUT2 antibodies, and then with ^{125}I-labeled protein-A (Amersham International, Amersham, UK). After a final washing step, the membranes were exposed to autoradiography for 10 days at −70 °C. The obtained signals were quantified by densitometry (Image Master 1D®, Pharmacia Biotech, Upsalla, SW), and the results expressed as arbitrary units (AU) per μg membrane protein sample.

2.8. Statistical analysis

All values are means ± S.E.M., with a n-value of 4–15. Significant differences in the experimental results were determined using Student's t-test.

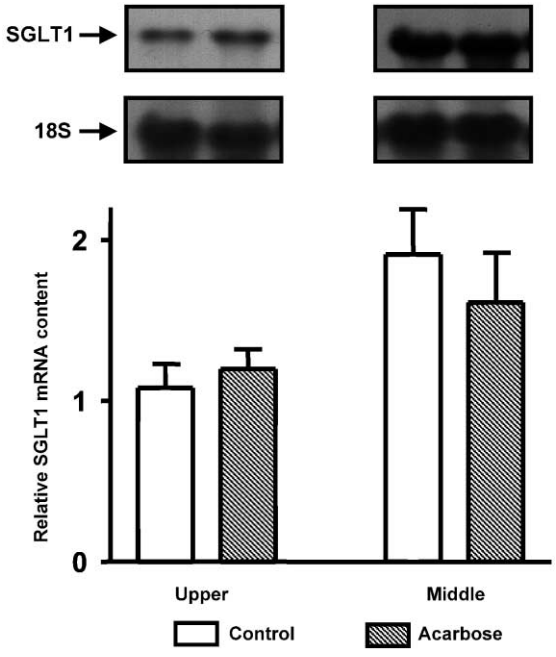

Fig. 3. SGLT1 mRNA expression in the intestine of distinctly treated Wistar rats. Female Wistar rats received either a standard powdered chow (control) or acarbose-supplemented chow (0.04% [w/w], acarbose). After 40 days, the animals were sacrificed and each isolated intestine was used to obtain a tissue sample (0.1 g) of the upper and middle small intestine. Total RNA was isolated from each sample and subjected to Northern blotting, using specific [^{32}P]-labeled probes for the detection of SGLT1 mRNA (SGLT1) and the 18 S rRNA (18 S). The top shows representative signals obtained by autoradiography for SGLT1 mRNA and 18 S rRNA; after a densitometric scanning, the obtained arbitrary units (AU) for SGLT1 were normalized with the corresponding 18 S rRNA value to give the relative SGLT1 mRNA content in each sample (bottom). Data are means ± S.E.M.; $n = 5$.

3. Results

3.1. Chronic acarbose-feeding affects food intake, but not body weight

In comparison with the control group, the adding of acarbose to the standard powdered chow did cause a significant change concerning food intake during the 40 days of treatment. As shown in Fig. 1A (left side bars), the control group had immediately before and after the experimental period a constant food intake of about 21 g per day. In contrast, the rats subjected to acarbose-treatment showed with a daily food consumption of 18.4 ± 1.1 g, a slightly lower food intake at the end of the adaptation period than the control group, but had a significant higher food intake after adding acarbose to the standard chow for 40 days (Fig. 1A, right side bars). Here, the final food intake reached 22.7 ± 0.8 g per day.

However, in comparison with the control group, the increased food consumption within the acarbose group did not cause differences in the final body weight. As shown in Fig. 1B (open bars), the control and the acarbose group started with a similar body weight of about 178 g per animal. And after the experimental treatment period of 40 days, animals of both groups had gained similar weight as it is indicated by the final body weight per rat—200 ± 8 g per control rat and 198 ± 7 g per acarbose-fed animal (Fig. 1B, shaded bars).

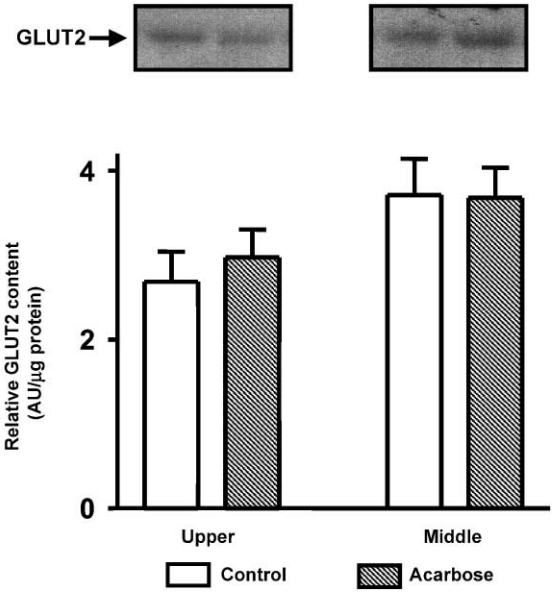

Fig. 4. GLUT2 protein expression in the intestine of distinctly treated Wistar rats. Female Wistar rats received either a standard powdered chow (control) or acarbose-supplemented chow (0.04% [w/w], acarbose). After 40 days, the animals were sacrificed and each isolated intestine was used to obtain a tissue sample of the upper and middle small intestine. After homogenization and preparation of a plasma membrane fraction, protein samples (100 μg) were separated by SDS-PAGE and further analyzed by Western blotting, using a polyclonal rabbit antiserum specific against GLUT2; the immunocomplexes formed were detected by using [^{125}I]-labeled protein A and visualized by autoradiography. The top shows a representative autoradiography; after densitometric scanning, the arbitrary units (AU) for each GLUT2 signal were related to the amount of sample protein (bottom). Data are means ± S.E.M.; $n = 4$.

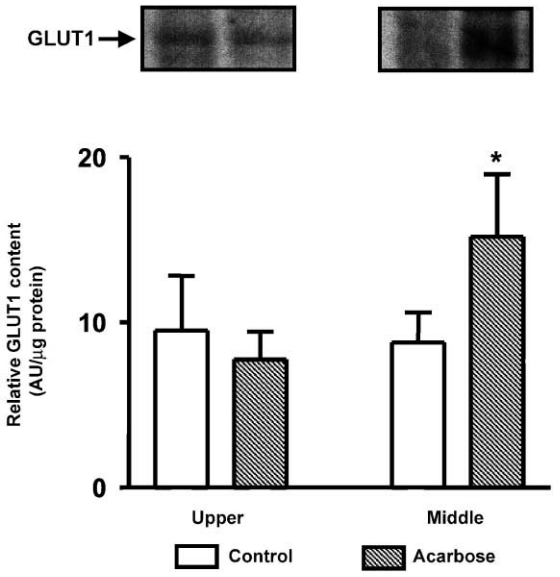

Fig. 5. GLUT1 protein expression in the intestine of distinctly treated Wistar rats. Female Wistar rats received either a standard powdered chow (control) or acarbose-supplemented chow (0.04% [w/w], acarbose). After 40 days, the animals were sacrificed and each isolated intestine was used to obtain a tissue sample of the upper and middle small intestine. After homogenization and preparation of a plasma membrane fraction, protein samples (100 μg) were separated by SDS-PAGE and further analyzed by Western blotting, using a polyclonal rabbit antiserum specific against GLUT1; the immunocomplexes formed were detected by using [^{125}I]-labeled protein A and visualized by autoradiography. The top shows a representative autoradiography; after densitometric scanning, the arbitrary units (AU) for each GLUT1 signal were related to the amount of sample protein (bottom). Data are means ± S.E.M.; $n = 4$. * $P < 0.05$ versus control.

3.2. Chronic acarbose-feeding does not alter plasma glucose and insulin levels as well as intestinal glucose absorption in an acarbose-free perfusion study

Although a higher food intake was seen for the acarbose-treated animals, the plasma levels of glucose and insulin in those rats remained similar with the corresponding levels of the control group. At the end of the experimental period, a blood glucose level of about 134 mg/dl was found for both the control and the acarbose-treated rats (Fig. 2A). Insulin concentrations were 33.8 ± 4 μU/ml and 36.1 ± 5 μU/ml for control and acarbose group, respectively (Fig. 2B). Interestingly, the adding of acarbose to the standard powdered chow for 40 days had also no impact on the capability of glucose absorption as measured by an in vivo perfusion. As shown in Fig. 2C, during the perfusion of the washed and thus acarbose-free intestine with a standard glucose solution (5 mM D-glucose), the control and the acarbose-fed animals

had similar glucose absorption over the measured period of 30 min. More precisely, the rats of the control group had an average of glucose absorption of 0.147 ± 0.032 μmol min^{-1} g^{-1} ml^{-1}, while the acarbose-fed animals showed a rate of 0.143 ± 0.027 μmol min^{-1} g^{-1} ml^{-1}.

3.3. Chronic acarbose-feeding upregulates GLUT1, but not SGLT1 or GLUT2

A Northern blot analysis of the upper and middle small intestine from the two different animal groups showed no difference in the relative amount of SGLT1 mRNA between the control and the acarbose-treated group (Fig. 3). Also, a Western blot analysis of those intestinal segments did not reveal any differences concerning the expression of GLUT2 protein (Fig. 4). In another Western blot analysis for GLUT1, no difference in the relative amount of that glucose transporter protein was found in the upper small intestine samples from the control group and the acarbose-fed group (Fig. 5, left side). However, in comparison with the control group, acarbose-feeding caused an upregulation of GLUT1 protein by 75% in the middle small intestine (Fig. 5, right side). In this segment, the control animals had only a relative GLUT1 content of 8.79 ± 1.81 AU/μg, while acarbose-fed animals showed a relative GLUT1 content of 15.1 ± 3.79 AU/μg; this was also almost twice as much as the corresponding GLUT1 content of 7.76 ± 1.67 AU/μg in the upper small intestine (Fig. 5, filled bar right versus left). Thus, chronic acarbose-feding caused a selective upregulation of GLUT1 protein in the middle small intestine of Wistar rats.

4. Discussion

Acarbose has become a common drug for the treatment of type 2 diabetes due to its inhibitory impact on α-glucosidases in the intestine (Clissold and Edwards, 1988; Rachman and Turner, 1995). Taken orally before a meal, acarbose delays the digestion of complex carbohydrates and decreases the postprandial plasma glucose rise (Puls et al., 1984). As changes in glucose concentrations are known to modulate the expression of cellular glucose transporter proteins, it is tempting to assume that a chronic acarbose-treatment will alter the expression of the glucose transporter proteins SGLT1, GLUT1 or GLUT2 in the intestine. However, this point has never been investigated and was targeted for the first time by this study, with the main finding that acarbose caused indeed an upregulation of GLUT1 in the middle small intestine of Wistar rats after an acarbose-feeding for 40 days.

Beside this novel finding, most other investigated parameters are reflecting the expected effects of acarbose- as a α-glucosidase inhibitor, acarbose should mainly affect rises in postprandial plasma glucose, but not change fasting plasma glucose and insulin levels as well as body weight (Lebovitz, 1998). And indeed, most studies pointed out already a relative neutral effect of acarbose on body weight, describing no (Coniff et al., 1994) or only minimal weight loss (Wolever et al., 1997), which was confirmed by our investigation. A change in plasma glucose and insulin levels after acarbose treatment is only documented in studies with type 2 diabetic patients (Mughal et al., 2000) or subjects with impaired glucose tolerance (Chiasson et al., 1996), while in line with our study, healthy dogs showed similar serum glucose and insulin concentrations before and after an acarbose-treatment for several weeks (Robertson et al., 1999). The higher food intake by the acarbose-fed rats in our study seems reasonable as a relative high dose of acarbose was used; accordingly, to overcome the enhanced inhibition of α-glycosidases and reduced digestion, an increase in food consumption was necessary by the animals to compensate the deficit of bioavailable calories in the diet.

The finding of this study that acarbose is only upregulating GLUT1 protein and not affecting the levels of SGLT1 mRNA and GLUT2 protein in parallel indicates that the regulation of those genes in the intestine are triggered by different pathways, allowing at least a selective upregulation for GLUT1. This observation is in line with several other investigations showing that the expression of each glucose transporters is indeed regulated separately. For example, SGLT1 expression can be upregulated by the peptide hormone glucagons-like peptide 2 (GLP-2) (Cheeseman, 1997), and the promoter region of the SGLT1 gene contains binding sites for the transcription factors hepatocyte nuclear factor 1 (HNF1) and for members of the specific protein 1 (Sp1) family of transcription factors (Martin et al., 2000). In contrast, analysis of the GLUT1 promoter region showed an positive impact of cyclic adenosine 3′,5′-monophosphate, irons, hypoxia inducible factor 1 (HIF-1) and phenylephrine (Behrooz and Ismail-Beigi, 1997; Vinals et al., 1997; Montessuit and Thorburn, 1999; Chen et al., 2000), and a repression of gene transcription by Sp3 (Fandos et al., 1999). Glucagon-like peptide-1 (GLP-1) enhances the transcription of the GLUT2 gene (Wang et al., 1999), which has a peroxisomal proliferator response element (PPRE) as well as binding sites for the hepatocyte nuclear factors 1 and 3 (HNF1, HNF3) and the CCAAT/enhancer binding protein alpha (C/EBPα) in its promoter region (Kim and Ahn, 1998; Cha et al., 2000; Kim et al., 2000). But although the specific impact of acarbose on GLUT1 expression was demonstrated by this study, the relevant pathways and mechanism(s) still remain unclear. However, as acarbose causes a delay in carbohydrate digestion and thus distributes the glucose absorption all over the jejunum equally, the found increase of GLUT1 protein in the middle small intestine of acarbose-fed rats is most probably a direct response to altered regional availability of glucose in the intestine. Accordingly, one reason for the upregulation of GLUT1 in the middle small intestine by acarbose might be the locally increased glucose concentration, reaching a critical level which is high enough to stimulate GLUT1 expression, but still too low to have a stimulatory effect on GLUT2 or SGLT1.

It is interesting to note that in our study both animal groups, the control and the acarbose-treated group, showed the same intestinal glucose absorption after the experimental period. Thus, although GLUT1 protein was higher expressed in the middle small intestine of the acarbose group, the enhanced content of GLUT1 had no influence on the glucose absorption rate. This finding underlines not only that glucose absorption in the intestine is mainly depending on SGLT1 and GLUT2 levels, but also indicates that a sudden stop of acarbose-treatment will not cause a dramatic alteration in glucose absorption, e.g. hyperglycemia. Although a direct impact on the intestinal glucose absorption was ruled out, GLUT1 might have a more indirect impact. Considering that under acarbose administration the glucose absorption is reduced in the upper small intestine, but carries on into the lower parts, the GLUT1 upregulation observed may be related to that effect. One explanation could be the necessity to ensure the availability of glucose within the epithelial cells during the interprandial periods. As glucose absorption in the intestine is an energy consuming process, mainly due to the Na^+/K^+-ATPase activity to maintain the Na^+-gradient, the enhanced glucose absorption in this territory should be accompanied by increased import of glucose throughout GLUT1, ensuring the necessary energy production in the interprandial periods. All in all, it seems that the selective upregulation of GLUT1 in the middle small intestine is important for preserving the total intestinal glucose absorption rate.

In summary, the results showed that acarbose treatment increased GLUT1 gene expression in the middle small intestine; however, this did not impair the glucose absorption in the small intestine in the absence of acarbose.

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