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Original research article (revised version)

Effects of xylitol on carbohydrate digesting enzymes activity, intestinal glucose absorption and muscle glucose uptake: A multi-mode study

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Running title:
Xylitol and glucose absorption

Word count: 5159 (without references, tables and figures)
ABSTRACT

The present study investigated the possible mechanism(s) behind the effects of xylitol on carbohydrate digesting enzymes activity, muscle glucose uptake and intestinal glucose absorption using in vitro, ex vivo and in vivo experimental models. The effect of increasing concentrations of xylitol (2.5% - 40% or 164.31 mM – 2628.99 mM) on alpha amylase and alpha glucosidase activity in vitro and intestinal glucose absorption and muscle glucose uptake were investigated in ex vivo condition. Additionally, the effects of an oral bolus dose of xylitol (1 g/kg BW) on gastric emptying and intestinal glucose absorption and digesta transit in the different segments of the intestinal tract were investigated in normal and type 2 diabetic rats at 1 hour after the dose administration, when phenol red was used as a recovery marker. Xylitol exhibited concentration-dependent inhibition of alpha amylase (IC$_{50}$ = 1364.04 mM) and alpha glucosidase (IC$_{50}$ = 1127.52 mM) activity in vitro and small intestinal glucose absorption in ex vivo condition. Xylitol also increased dose dependent muscle glucose uptake with and without insulin, although the uptake was not significantly affected by the addition of insulin. Oral single bolus dose of xylitol significantly delayed gastric emptying, inhibited intestinal glucose absorption but increased intestinal digesta transit rate both in normal and diabetic rats compared to their respective controls. The data of this study suggest that xylitol reduces intestinal glucose absorption via inhibiting major carbohydrate digesting enzymes, slowing gastric emptying and fastening intestinal transit rate but increases muscle glucose uptake in normal and type 2 diabetic rats.

Keywords:
Carbohydrate digesting enzymes, Intestinal glucose absorption, Muscle glucose uptake, Type 2 diabetes, Xylitol
List of abbreviations: (alphabetical)

1. DBC, diabetic control
2. DXYL, diabetic xylitol
3. GAI, glucose absorption index
4. GIT, gastrointestinal tract
5. NC, normal control
6. NFBG, non-fasting blood glucose
7. NXYL, normal xylitol
8. PR, phenol red
9. T2D, type 2 diabetes
1. Introduction

Starch from carbohydrates is a major dietary source of glucose, which is produced by the gastrointestinal hydrolysis of starch by α-amylase and α-glucosidase enzymes. Then it is absorbed via small intestinal mucosa and influences postprandial blood glucose levels and hyperglycemic condition in diabetics. Thus, limiting the extent of postprandial glucose production as well as absorption can significantly suppress hyperglycemia as well as other complications in diabetics. This is because, persistent hyperglycemia has been reported as a major culprit for diabetes associated complication in all forms of diabetes, with type 2 diabetes (T2D) having the highest prevalence. About 90-95% of the total diabetic patients are suffering from T2D, which has been defined as a heterogeneous metabolic disorder caused by insulin resistance followed by partial pancreatic beta-cell dysfunction as well as hyperglycemia.

Recently, there has been a growing interest of using nutraceuticals for the management of hyperglycemia as well as T2D, which includes but not limited to medicinal foods, functional foods and sugar alcohols such as xylitol.

Xylitol is a 5 carbon sugar alcohol with lower glycemic index (13 vs 65) and calorific value (2.4 vs 4.0 kcal/g) compared to sucrose. A number of previous studies reported that xylitol has many other potential beneficial effects such as control and prevention of obesity, diabetes and related metabolic disorders. In a recent study, Islam reported that 3 weeks supplementation of 10% dietary xylitol significantly decreased non-fasting blood glucose (NFBG) and serum fructosamine levels; increased serum insulin levels; and improved glucose tolerance ability compared to 10% sucrose in non-diabetic rats. In a more recent study, Islam and Indarjit reported that 5 weeks supplementation of 10% dietary xylitol significantly reduced NFBG and also improved most of the diabetes-related metabolic parameters in a T2D rat model. On the
other hand, Amo et al.\textsuperscript{8} reported that 8-week supplementation of 1 or 2 g of xylitol per 100 kcal diet significantly decreased visceral fat mass and plasma lipid concentration in high fat diet-fed rats.\textsuperscript{8} In another study, Kishore et al.\textsuperscript{10} reported that xylitol prevents non-esterified fatty acid-induced insulin resistance in non-diabetic rats.\textsuperscript{10} In a most recent study, Rahman and Islam\textsuperscript{11} confirmed that xylitol has ability to improve the pancreatic islets morphology to improve T2D in rats. All of the above-mentioned studies reported the anti-diabetic potentials of xylitol, however the effects of xylitol on carbohydrate digesting enzymes activity, intestinal glucose absorption and muscle glucose uptake are still not clear.

In some previous studies, it has been reported that xylitol consumption significantly reduced food intake in normal humans\textsuperscript{12} and diabetic rats\textsuperscript{9}. Slower gastric emptying\textsuperscript{12,13} and more accelerated intestinal transit\textsuperscript{14} were observed in normal human subjected when xylitol was supplied as single oral dose (30 g in 200 ml water) compared to the similar dose of glucose. Hence, xylitol might reduce NFBG levels not only by reducing food intake but also by slowing gastric emptying and accelerating nutrient transit time both in normal and diabetic conditions. Furthermore, the insulinotropic effect of xylitol in diabetic condition\textsuperscript{9} may improve circulating glucose uptake, especially in muscle and fat cells to ameliorate hyperglycemia in diabetics. From the above-mentioned studies, it is not clear whether xylitol has any additional effects on the absorption of glucose from the different segments of the intestinal tract and on the muscle glucose uptake at the post absorption period.

Hence, the present study was conducted to examine the effects of xylitol on intestinal glucose absorption as well as muscle glucose uptake using three different set of experiments: (1) \textit{in vitro}: effects of xylitol on $\alpha$-amylase and $\alpha$-glucosidase activity; (2) \textit{ex-vivo}: effects of xylitol on glucose absorption in isolated rat jejunum and on glucose uptake in isolated psoas muscles of
normoglycemic rats; and (3) in vivo: effects of xylitol on glucose absorption and digesta transit in the different segments of gastrointestinal tracts of normal and type 2 diabetic rats.

2. Materials and methods

2.1 Chemicals and reagents

The α-amylase, α-glucosidase, streptozotocin, 3,5-dinitrosalicylic acid, di-basic sodium phosphate, paranitrophenyl-α-D-glucopyranoside and citric acid were purchased from Sigma Aldrich, Germany. Mono-basic sodium phosphate, sodium hydroxide, sodium bicarbonate, sodium chloride, potassium chloride, calcium chloride di-hydrate, mono-basic potassium phosphate, magnesium sulphate, sodium hydrogen carbonate, phenol red, sodium citrate and sodium potassium tartrate were purchased from Merck, South Africa. Xylitol (food grade) was kindly supplied by Sweet Nothings, South Africa. Starch, glucose and fructose were purchased from Associated Chemical Enterprise, South Africa, while metformin and Novo rapid insulin were purchased from a local pharmacy store (Pharmed) in Durban, South Africa.

2.2 In vitro study

2.2.1 Measurement of the alpha amylase inhibitory activity

The effect of xylitol on alpha amylase activity was determined using a method described previously\textsuperscript{15} with slight modifications. Reducing sugars resulting from starch hydrolysis by α-amylase enzyme can reduce yellow 3,5-dinitrosalicylic acid (DNSA) to a reddish-brown 3-aminophenol-5-nitrosalicylic acid, which can be monitored at 540 nm. Briefly, 1 mL of the different concentrations of xylitol (164.31 – 2628.99 mM) or 0.37 mM acarbose or 0.37 mM of quercetin and 1 mL of 4 U/mL α-amylase (in 20 mM sodium phosphate buffer, pH 6.9) was incubated for
30 min at 37 °C in test tubes, after which 1 mL of 1% starch solution was added in each tube and the mixture was incubated for 1 h at 37 °C. A 1 mL of DNSA reagent was added in each tube and boiled for 10 min in a boiling water bath and the absorbance was measured at 540 nm after cooling in a spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan). Percentage inhibition was determined according to the following formula:

\[
\% \text{ Inhibition} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100
\]

2.2.2 Measurement of the alpha glucosidase inhibitory activity

The effect of xylitol on alpha glucosidase activity was measured according to a previously described method with slight modifications. Briefly, 0.5 mL of the different concentrations of xylitol (164.31 – 2628.99 mM) or 0.37 mM acarbose or 0.37 mM of quercetin and 1 mL of 1 U/mL α-glucosidase in assay buffer (0.1 M sodium phosphate buffer, pH 6.9) was incubated for 10 min at 25 °C in test tubes. Reaction was started with a 0.5 mL of substrate (paranitrophenyl-α-D-glucopyranoside) at 25 °C and thereafter stopped with 2 mL of 0.2 M sodium bicarbonate after a 5 min incubation period. Absorbance was measured at 405 nm using above-mentioned spectrophotometer and percentage inhibition was determined as the inhibition of release of yellow p-Nitro phenol from substrate by enzymatic action. Percentage inhibition of α-glucosidase was calculated using the following formula:

\[
\% \text{ Inhibition} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100
\]

2.3 Ex-vivo study
2.3.1 Animals

Five adult male Spagrue-Dawley rats with mean body weight 201.12 ± 12.48 g were procured from the Biomedical Resource Center located at the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa. The animals were fasted over-night (12 hours) and euthanized by halothane anesthesia. The abdominal wall was dissected and the whole gastrointestinal tract (GIT) and parts of the psoas muscle were collected and immediately used for glucose absorption and glucose uptake study, respectively. All animal procedures were carried out according to the rules and regulations of the Experimental Animal Ethics Committee of the University of KwaZulu-Natal, Durban, South Africa (Ethical approval number: 097/13/Animal).

2.3.2 Measurement of glucose absorption in isolated rat jejunum

The effect of xylitol on glucose absorption by isolated rat intestine was measured by monitoring the reduction of glucose concentration in incubation solution containing isolated rat jejunum and test samples according to a previously published method with slight modifications. Jejunal segments of the collected GIT were cut into small portions of 5 cm and rinsed with 2 mL of Kreb’s buffer (118 mM NaCl, 5 mM KCl, 1.328 mM CaCl₂·2H₂O, 1.2 mM KH₂PO₄, 1.2mM MgSO₄ and 25 mM NaHCO₃) through the jejunal lumen using a sterile syringe. Each segment was first inverted to expose the villi, and then incubated in carbon dioxide (CO₂) incubation tube containing 8 mL of Kreb’s buffer and 11.1 mM glucose and different concentrations of xylitol (657.25 mM, 1314.50 mM and 2628.99 mM) when glucose with Kreb’s buffer was used as a control. A 1 mL sample was collected from each incubation tube before and after a 2 h incubation in a Steri-Cult CO₂ incubator (Labotec, South Africa) at 5% CO₂, 95% Oxygen and
37 °C condition and glucose concentration was measured using an automated Chemistry Analyzer (Labmax Plenno, Labtest Inc., Costa Brava, Brazil). The intestinal glucose absorption was calculated as the amount of glucose (mg) absorbed per centimeter of rat jejunum using the following formula:

\[
\text{Intestinal glucose absorption} = \frac{(GC_1 - GC_2)}{5 \text{ cm of jejunum}}
\]

Where, “GC}_1” and “GC}_2” are glucose concentrations (mg/dL) before and after incubation, respectively.

2.3.3 Measurement of glucose uptake in isolated rat psoas muscles

The effect of xylitol on glucose uptake in isolated rat psoas muscles was determined according to the method described in a previous study\textsuperscript{18} with slight modifications. Briefly, the collected psoas muscle was immediately rinsed with Kreb's buffer and cut into small pieces of equal weight (500 mg). Each piece was then incubated in 8 ml of Krebs’ buffer, containing 11.1 mM glucose (control) and increasing concentrations of xylitol (657.25 mM, 1314.50 mM and 2628.99 mM) with and without insulin (50 mU/ml). Incubation period was for 1 h in a CO\textsubscript{2} incubator at 5% CO\textsubscript{2}, 95% Oxygen and 37 °C condition. A 1 mg/mL metformin was used as positive control. A 1 mL aliquot was collected from each incubation tube before and after the incubation period and the glucose concentration was measured. Muscle glucose uptake was calculated as the amount of glucose (mg) taken up per gram of muscle tissue using the following formula:

\[
\text{Muscle glucose uptake} = \frac{(GC_1 - GC_2)}{0.5 \text{ g of muscle tissue}}
\]
Where, “GC₁” and “GC₂” are glucose concentrations before and after incubation, respectively.

2.4 In vivo study

2.4.1 Animals

Twenty-four seven-week-old male Sprague-Dawley (SD) rats with mean body weight 222.08 ±14.49 g were procured from the Biomedical Resource Center located at the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa. Animals were randomly divided into four groups, namely normal control (NC), normal xylitol (NXYL), diabetic control (DBC) and diabetic xylitol (DXYL). Each normal animal group had five animals and each diabetic animal group had seven animals. All animals were fed with a commercial rat pellet diet and were maintained according to the rules and regulations of the Experimental Animal Ethics Committee of the University of KwaZulu-Natal, South Africa during the entire experimental period (Ethical approval number: 097/13/Animal).

2.4.2 Induction of diabetes

In order to induced T2D,¹⁹ during the first 2 weeks of the experiment, the animals in the DBC and DXYL groups were supplied with a 10% fructose solution to induce insulin resistance while the animals in the NC and NXYL groups were supplied with normal drinking water. Thereafter, animals in the DBC and DXYL groups were injected (i.p.) with a low dose of streptozotocin (40 mg/kg body weight) dissolved in citrate buffer (pH 4.5) to induce partial pancreatic β-cell dysfunction.¹⁹ Animals in the NC and NXYL groups were injected with similar volume of citrate buffer only. One week after the streptozotocin injection, NFBG levels of all animals were measured using a portable Glucometer (Glucoplus Inc., Saint-Laurent, Quebec, Canada).
Animals with NFBG levels $\geq 300$ mg/dl were considered as diabetic and included in the study.\textsuperscript{19} Animals with NFBG levels $< 300$ mg/dl were excluded from the study.

2.4.3 Feeding and sampling

One week after the streptozotocin injection and confirmation of diabetes, all animals were fasted overnight (16 hours) with free access to drinking water only. After fasting, the animals in the NXYL and DXYL groups were administered with an oral bolus dose of xylitol (1 g per kg body weight) with glucose (2 g per kg body weight) containing 0.05\% (w/v) phenol red (recovery marker). Only glucose with phenol red (PR) was administered to the animals in NC and DBC groups. Animals were then sacrificed using halothane anesthesia exactly 1 h after the dose administration, without any access to food or drinking water. Gastro-intestinal tract (GIT) from each animal was quickly removed, frozen immediately in liquid nitrogen to prevent the movement of the contents, and preserved immediately at $-30^\circ$C for further analysis.

2.4.4 Sample preparation

Each GIT was thawed and divided into eight segments: stomach; 1st, 2nd, 3rd, and 4th quarter of small intestine; cecum; proximal and distal half of the colon. Content weight of each segment was determined by subtracting the weight of the segment without content from the respective weight with content. Contents and tissues were collected and individually homogenized in ice cold normal saline (Ultra Turrax Tube Drive Work Station homogenizer, IKA Laboratory equipment, Staufen, Germany) and centrifuged twice at 15,000 rpm for 30 min (Hettich Mikro 200 microcentrifuge, Hettich Lab Technology, Tuttlingen, Germany) according to the method reported previously with slight modifications.\textsuperscript{20} Phenol red concentration was determined
spectrophotometrically (Spectrostar Nano, Bmg Labtech, Offenburg, Germany) with bile acid
correction in the supernatants of contents and segments according to a previously published
method. Briefly, a 30 µL of supernatant or phenol red standard (concentrations 0.0038% -
0.00025%) was mixed with 210 µL of 0.1 M dibasic sodium phosphate solution (pH 10.5). The
optical density at 420 nm was subtracted from the optical density at 620 nm (for bile acid
correction) to get the final optical density. The concentration as well as the amount of recovered
phenol red was calculated from the standard curve. Glucose concentration in the intestinal
contents was measured using an Automated Chemistry Analyzer (Labmax Plenno, Labtest Inc.,
Costa Brava, Brazil) using commercial assay kits.

2.4.5 Calculations
Gastric emptying, glucose absorption index (GAI) and digesta transit were calculated using
formula described by Islam and Sakaguchi, and expressed in percentage.
Gastric emptying, denoting the emptying time of stomach content was calculated using the
following formula:

\[
\text{Gastric emptying} \% = \left(\frac{A - B}{A}\right) \times 100.
\]

Where, “A” is the total amount of PR (g) recovered from GIT; and “B” is the total amount of PR
(g) recovered from the stomach.
GAI denotes the degree of glucose absorption in each GIT segment. It is the percentage amount of the glucose absorbed passing through a given segment of GIT, and was calculated using the following formula:

\[
\text{GAI} \text{ (%) in a given segment of the GIT} = (1 - (\frac{a}{b}) \div (\frac{c}{d})) \times 100.
\]

Where, “a” is the amount of glucose (g) recovered from that segment; “b” is the amount of PR (g) recovered from the same segment; “c” is the amount of glucose (g) given to corresponding animal; and “d” is the amount of PR (g) given to the corresponding animal.

Digesta transit in a particular segment of the intestine is the percentage ratio of the amount of content leaving that segment to the amount reaching the same segment. It was calculated using the following formula:

\[
\text{Digesta transit in a given segment} \text{ (%)} = (\frac{a}{b}) \times 100.
\]

Where “a” is the amount of PR (g) recovered from that particular segment of the GIT to the distal colon excluding the amount of PR (g) recovered from that particular segment and “b” is the amount of PR (g) recovered from that particular segment of the GIT to the distal colon.

3. Results

3.1 In vitro study

3.1.1 Effects of xylitol on alpha amylase and alpha glucosidase activity
The data for *in vitro* α-amylase and α-glucosidase activity are presented both in Fig. 1 and Table 1. The data showed increasing dose-dependent effect of xylitol on α-amylase (a) and α-glucosidase (b) activity from 164.31 mM to 2628.99 mM with corresponding IC$_{50}$ values of 1364.04 mM and 1127.52 mM respectively (Fig. 1 and Table 1). Although no difference of α-amylase activity was observed for 1314.49, 1971.74 and 2628.99 mM xylitol, their inhibitory activities were significantly higher than the other lower dosages (164.31, 328.62, 657.25 mM) used in the study (Fig. 1b). On the other hand, significant and dose-dependent α-glucosidase activity was observed for different dosage with no difference was observed between 1971.74 and 2628.99 mM of xylitol (Fig. 1a). According to the alpha amylase and alpha glucosidase inhibitory effects of acarbose and quercetin (Fig. 1), their IC$_{50}$ values were significantly lower than the xylitol (Table 1).

**3.2 Ex vivo study**

3.2.1 *Effects of xylitol on glucose absorption in isolated rat jejunum*

Data showing the effects of xylitol on glucose absorption in isolated rat jejunum are presented in Fig. 2. The results showed that the amount of glucose absorbed by isolated rat jejunum in the presence of xylitol was concentration-dependent. This was lowest at 2628.99 mM (1.75 ± 0.46 mg/cm jejunum), which was significantly different ($p < 0.05$) from the control and 657.25 mM xylitol (3.27 ± 0.46 and 3.27 ± 0.67 mg/cm jejunum), respectively but they are significantly different from the result for 1314.49 mM xylitol (2.90 ± 0.78 mg/cm jejunum) (Fig. 2).

3.2.2 *Effects of xylitol on glucose uptake by isolated rat psoas muscle*
The data for the effects of xylitol on glucose uptake with or without insulin in isolated rat psoas muscle are presented in Fig. 3. Induction of glucose uptake was observed with the increasing concentrations of xylitol with or without insulin when only significantly higher \( (p < 0.05) \) absorption was observed with 2628.49 mM xylitol and metformin compared to the control.

3.3 In vivo study

3.3.1 Effects of xylitol on gastric emptying

The data of gastric emptying are presented in Fig. 4. Induction of diabetes increased gastric emptying, but was significantly reduced \( (p < 0.05) \) after feeding xylitol. Feeding of xylitol was also reduced gastric emptying in non-diabetic rats.

3.3.2 Effects of xylitol on intestinal glucose absorption

The data of intestinal glucose absorption index (GAI) are presented in Fig. 5. The GAI was greatly affected by the administration of xylitol in both normal and diabetic animals. Feeding of xylitol significantly reduced \( (p < 0.05) \) glucose absorption in the 1\textsuperscript{st} quarter of the small intestine of normal and diabetic rats when significantly and relatively lower glucose absorption were observed in the 2\textsuperscript{nd} and 3\textsuperscript{rd} quarters of small intestine and distal colon of the xylitol fed normal and diabetic rats, respectively compared to their respective controls (Fig. 5). Feeding xylitol did not significantly affect the absorption of glucose from 4\textsuperscript{th} quarter of the small intestine to the proximal colon of the last intestine.

4. Discussion
Xylitol is widely used as a sugar substitute because of its several beneficial effects on health compared to other commonly used sweeteners. The lower caloric value (2.4 kcal vs 4.0 kcal/g), insulinenic response, glycemic index (13 vs 65) but similar sweetness compared to sucrose has made it more popular to people, especially diabetics. In a recent study, it has been reported that xylitol exhibits significant hypoglycemic effects in normal rats\textsuperscript{6} and anti-diabetic effects in a T2D rat model\textsuperscript{9}. The present study was conducted to investigate possible mechanisms behind the anti-diabetic effects of xylitol using several \textit{in vitro, ex vivo} and \textit{in vivo} models.

The action of carbohydrate hydrolyzing enzymes has a significant effect on postprandial blood glucose level, and delaying the digestion of carbohydrate like starch and sucrose will translate into lower postprandial blood glucose\textsuperscript{1,23}. The $\alpha$-amylase and $\alpha$-glucosidase inhibitors have gained much popularity as a class of hypoglycemic agents that reduces postprandial blood glucose levels via the above-mentioned mechanism\textsuperscript{24-26}. Results from the present study showed a significant \textit{in vitro} inhibition of $\alpha$-amylase and $\alpha$-glucosidase enzyme activities by xylitol (Fig.1), which corresponds to the results from a study conducted recently\textsuperscript{27} and also indicate that xylitol may possess significant inhibitory effects on carbohydrate digestion. This can translate into lower level of postprandial blood glucose, and may be partly involved in the mechanism behind the reported anti-diabetic effects of xylitol.

Most of the glucose we consumed is absorbed from the small intestinal mucosa, however the absorption capacity of the different small intestinal segments is not the same. Although a previous \textit{in vitro} study suggested the high capacity of glucose absorption across all segments of the small intestine\textsuperscript{28}, another study confirmed that the mid-small intestine (part of the duodenum and jejunum) having the highest glucose absorption capacity\textsuperscript{29}. In the present study, a 2 hour incubation of isolated rat jejunum in a glucose solution showed glucose absorption as high as
3.27 ± 0.46 mg/cm of jejunum without xylitol (Fig. 2). However, in the presence of 2628.99 mM xylitol, there was a significant reduction in the glucose absorption capacity (1.75 ± 0.46 mg/cm of jejunum), which revealed the possible inhibitory potentials of xylitol on intestinal glucose absorption.

Additionally, several in vivo studies have reported the different absorption patterns of glucose from the small intestine. Bogner et al.\textsuperscript{30} reported that glucose absorption was highest in the mid-intestine of female chicks, when Lavin\textsuperscript{28} suggested the ileum as a highest glucose absorption site in the small intestine of the same species. In another study, Riesenfeld et al.\textsuperscript{31} confirmed the reduction of glucose absorption with the increasing distance from the pylorus of chicken. They also explained that the difference in glucose absorption capacity in the different segments of the small intestine may be due to the varied concentrations of glucose. The pattern of glucose absorption in our study (Fig. 5) is also consistent with the findings reported by Pearson et al.\textsuperscript{28}. However, the significantly reduced ($p < 0.05$) GAI in the xylitol fed groups compared to the controls across the small intestinal segments (more pronounced in the 1\textsuperscript{st} and 2\textsuperscript{nd} quarters) correspond to the results of our ex vivo glucose absorption study (Fig. 2). It also supports the inhibitory potentials of xylitol on intestinal glucose absorption in our study.

The rate of nutrient gastric emptying and digesta transit are important factors in gastrointestinal nutrient digestion and absorption.\textsuperscript{9} It has also been reported that faster intestinal transit and delayed gastric emptying might be the cause of slower intestinal nutrient absorption and reduced food intake.\textsuperscript{12,13} In the present study, induction of diabetes appreciably increased the rate of nutrient gastric emptying (Fig. 4). Although negative energy balance resulting from constant hyperglycemia is known as a major reason for the frequent hunger (polyphagia) and increased food intake often observed as a classical symptom of diabetes, when faster gastric emptying may
also contribute to this effect. Previous studies have demonstrated a correlation between gastric emptying rate and eating behavior.\textsuperscript{32,33} In our study, an oral bolus dose of xylitol delayed gastric emptying (Fig. 4) and increased digesta transit rate (Table 1) after 1 h of administration in both normal and diabetic rats, which corresponds to studies previously reported in normal human subjects and experimental animals\textsuperscript{12-14} and may also contribute to lower GAI in the proximal half of the small intestine.

Among other functions, insulin produced in the body helps to stimulate the uptake of circulating glucose by active respiratory cells for energy production, thus maintaining blood glucose homeostasis.\textsuperscript{34} Muller-Hess \textit{et al.}\textsuperscript{35} reported that blood glucose and serum insulin was significantly increased by oral administration of 30 or 50 g of xylitol in normal subjects.\textsuperscript{35} Other studies reported that 3 to 5 weeks oral administration of 10\% (657.25 mM) xylitol increased serum insulin in both normal\textsuperscript{6} and diabetic rats\textsuperscript{9,11}. Since inadequate circulating glucose uptake could partly contribute to the observed hyperglycemia in diabetic rats,\textsuperscript{36} it was therefore rationale to investigate the effects of xylitol on glucose uptake in isolated rat psoas muscle. Results from \textit{ex vivo} investigation in the present study suggest significant potentials of xylitol to improve muscle glucose uptake with or without insulin (Fig. 3), which might partly contribute to the anti-diabetic as well as hypoglycemic potential of xylitol.

In summary, results from the \textit{in vitro}, \textit{ex vivo} and \textit{in vivo} sections of the present study suggest that xylitol possesses potential inhibitory effects on the activities of α-glucosidase and α-glucosidase \textit{in vitro}, and intestinal glucose absorption, especially in the duodenal and jejunal segments \textit{ex vivo} and \textit{in vivo} conditions. Our results also suggest that xylitol prolongs gastric emptying and increases intestinal nutrient transit rate \textit{in vivo} in both normal and diabetic conditions, which may partly contribute to its inhibitory potential on intestinal glucose absorption.
absorption. Furthermore, *in vitro* muscle glucose uptake assay of this study suggest that xylitol may also promote the uptake of circulating glucose by muscle tissue.

In conclusion, data of this study suggest that xylitol exhibits its potential hypoglycemic as well as anti-diabetic effects not only by decreasing the activities of carbohydrate digesting enzymes and intestinal glucose absorption but also by delaying gastric emptying, increasing intestinal digesta transit and muscle glucose uptake. Further clinical study can only confirm the similar effects of xylitol in humans.

**Acknowledgements**

The authors would like to thank Research Office, University of KwaZulu-Natal (UKZN), Durban; National Research Foundation (NRF), Pretoria, South Africa for funding this study as well as Dr Mogie Singh, Dr Linda Bester, David Mompe and Shoohana Singh for their technical assistances during this study.

**Conflict of interest**

There is no conflict of interest within this article.
REFERENCES


TABLE CAPTIONS

Table 1
IC₅₀ values for the inhibition of alpha glucosidase and alpha amylase enzymes by xylitol, acarbose and quercetin.

Table 2
Percent digesta transit in the different segments of the GIT during 1 hour experimental period.
1 FIGURE CAPTIONS

2

Figure 1  Fig. 1 Effect of xylitol and standards (acarbose and quercetin) on alpha glucosidase (a) and alpha amylase (b) activities in vitro. Data are presented as mean ± SD of triplicates of analysis. abc Different letters presented beside the bars for a given enzyme are significantly different from each other (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21).

Figure 2  Effect of xylitol on glucose absorption in isolated rat jejunum. Data are presented as mean ± SD of five replicates of analysis. ab Different letters presented above the bars are significantly different from each other (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21).

Figure 3  Effect of xylitol on glucose uptake with or without insulin in isolated rat psoas muscle. Data are presented as mean ± SD of triplicates of analysis. ab or xy Different letters presented above the bars for with or without insulin are significantly different from each other (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21).
Figure 4  Effects of xylitol on gastric emptying in different animal groups at the end of 1 hour experimental period. Data are presented as mean ± SD of five to six animals. Different letters presented above the bars are significantly different from each other group of animals (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.

Figure 5  Glucose absorption index (GAI) in the different GIT segments of different animal groups at the end of 1 hour experimental period. Data are presented as mean ± SD of five to six animals. Different letters presented above the bars for a given segment are significantly different from each other group of animals (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.
### Tables

**Table 1** IC$_{50}$ values for the inhibition of alpha glucosidase and alpha amylase enzymes by xylitol, acarbose and quercetin.

<table>
<thead>
<tr>
<th>Sample/standard</th>
<th>Alpha glucosidase inhibition</th>
<th>Alpha amylase inhibition</th>
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<tr>
<td></td>
<td>IC$_{50}$ values (mM)</td>
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<tr>
<td>Xylitol</td>
<td>1127.52 ± 85.93$^a$</td>
<td>1364.04 ± 171.12$^a$</td>
</tr>
<tr>
<td>Acarbose</td>
<td>0.15 ± 0.01$^b$</td>
<td>0.32 ± 0.03$^b$</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.04 ± 0.01$^b$</td>
<td>0.23 ± 0.02$^b$</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD of triplicates of analysis. $^a,b$ Different superscript letters within a column for a given enzyme are significantly different from each other ($p < 0.05$. Tukey’s HSD post-hoc test, IBM, SPSS, version 21).
Table 2 Percent digesta transit in the different segments of the intestinal tract during 1 hour experimental period.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>1st qtr</th>
<th>2nd qtr</th>
<th>3rd qtr</th>
<th>4th qtr</th>
<th>Cecum</th>
<th>Prox. Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small intestine</td>
<td>Large intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>93.38 ± 1.77</td>
<td>83.23 ± 3.32</td>
<td>71.25 ± 5.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.56 ±5.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.71 ± 3.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.53 ± 8.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NXYL</td>
<td>94.53 ± 2.54</td>
<td>86.94 ± 2.20</td>
<td>81.06 ± 2.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.20 ± 4.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.98 ± 8.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.40 ± 6.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DBC</td>
<td>91.11 ± 1.63</td>
<td>82.65 ± 5.49</td>
<td>70.98 ± 4.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.68±14.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.61 ±6.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>58.61 ± 8.10&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>DXYL</td>
<td>91.54 ± 0.17</td>
<td>84.24 ± 4.14</td>
<td>81.16 ± 4.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.02 ±4.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.31 ±3.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.40 ±4.55&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD of five to six animals. <sup>ab</sup>Different letters presented in each column for a given segment are significantly different from each other group of animals (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.
**FIGURES**

![Graph](image)

**Fig. 1** Effect of xylitol and standards (acarbose and quercetin) on alpha glucosidase (a) and alpha amylase (b) activities in vitro. Data are presented as mean ± SD of triplicates of analysis. *Different letters presented beside the bars for a given enzyme are significantly different from each other (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21)."
**Fig. 2** Effects of xylitol on glucose absorption in isolated rat jejunum. Data are presented as mean ± SD of five replicates of analysis. Different letters presented above the bars are significantly different from each other (p < 0.05. Tukey’s HSD *post-hoc* test, IBM, SPSS, version 21).
**Fig. 3** Effects of xylitol on glucose uptake with or without insulin in isolated rat psoas muscle. Data are presented as mean ± SD of triplicates of analysis. Different letters presented above the bars for with or without insulin are significantly different from each other (p < 0.05. Tukey’s HSD *post-hoc* test, IBM, SPSS, version 21).
Fig. 4 Effects of xylitol on gastric emptying in different animal groups at the end of 1 hour experimental period. Data are presented as mean ± SD of five to six animals. \(^{ab}\) Different letters presented above the bars are significantly different from each other group of animals (p < 0.05. Tukey’s HSD post-hoc test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.
**Fig. 5** Glucose absorption index (GAI) in the different GIT segments of different animal groups at the end of 1 hour experimental period. Data are presented as mean ± SD of five to six animals. Different letters presented above the bars for a given segment are significantly different from each other group of animals (p < 0.05. Tukey’s HSD *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.
Graphical abstract

Carbohydrates

Digestion

Carbohydrate digesting enzymes activities: α-amylase and α-glucosidase

Gastric emptying rate

Digesta transit

Intestinal glucose

Absorption

Xylitol

Possible anti-hyperglycemic mode of action of xylitol

Blood glucose

Pancreas

Insulin

Glucose uptake

Skeletal muscle