Low dose of acarbose does not delay digestion of starch but reduces its bioavailability

Renate E. Wachters-Hagedoorn
Marion G. Priebe
Janneke A.J. Heimweg
A. Marius Heiner
Henk Elzinga
Frans Stellaard
Roel J. Vonk

Adapted from: *Diabetic Medicine* 2007, 24 (6):600–606
Abstract

Aims
Slowly digestible starch is associated with beneficial health effects. The antihyperglycemic drug acarbose has the potential to retard starch digestion since it inhibits α-amylase and α-glucosidases. We tested the hypothesis that a low dose of acarbose delays the rate of digestion of rapidly digestible starch without reducing its bioavailability and thereby increasing resistant starch flux into the colon.

Methods
In a crossover study 7 healthy males ingested corn pasta (50.3 g dry weight), naturally enriched with $^{13}$C, with and without 12.5 mg acarbose. Plasma glucose and insulin concentrations as well as $^{13}$CO$_2$ and hydrogen excretion in breath were monitored during 6 h after ingestion of the test meals. Using a primed continuous infusion of D-[6,6-$^2$H$_2$] glucose, the rate of appearance of starch derived glucose was estimated, reflecting intestinal glucose absorption.

Results
Areas under the 2 h postprandial curves of plasma glucose and insulin concentrations were significantly decreased by acarbose (-58.1 ± 8.2 % and -72.7 ± 7.4 % respectively). Acarbose reduced the overall 6 h appearance of exogenous glucose (bioavailability) by 22 ± 7% (mean ± SE) and the 6 h cumulative $^{13}$CO$_2$ excretion by 30 ± 6.

Conclusions
These data show that in healthy volunteers even a low dose of 12.5 mg acarbose decreases the appearance of starch derived glucose substantially. Reduced bioavailability seems to contribute to this decrease to a greater extent than delay of digestion. This implies that the treatment effect of acarbose could in part be ascribed to the metabolic effects of colonic starch fermentation.
Introduction

Starchy foods are an important source of carbohydrates, but differ considerably in their physiological and metabolic response and consequently also in their potential health benefits. The glycemic response is widely used to categorize foods and is applied in studies to correlate food intake with the incidence of chronic diseases. Foods that elicit a low glycemic response (low glycemic index foods) are associated with the prevention of diabetes (1;2), coronary disease (3;4) and enhanced weight control (5;6). However, a low glycemic response can be caused by a high fat or fructose content which is not regarded as desirable due to the risk of promoting adiposity (7;8). In starchy foods the rate of starch digestion and the subsequent influx rate of glucose is the major determinant of the glycemic response.

However, the choice of starchy foods with a high content of slowly digestible starch is limited. Alteration of processing methods addition of viscous fiber or whole cereal grains and use of high amylose varieties are strategies applied to increase the availability of starchy foods with beneficial characteristics (9). Retarding starch digestion could also be achieved by compounds that inhibit the activity of intestinal enzymes responsible for hydrolysis of starch. Acarbose, a pseudotetrasaccharide of microbial origin, inhibits intestinal \( \alpha \)-glucosidases and pancreatic \( \alpha \)-amylase by reversibly binding to these enzymes (10;11). It is applied in the treatment of postprandial hyperglycemia of diabetic patients in doses of 50–200 mg per meal and its expected and intended mode of action is to delay carbohydrate digestion and absorption without induction of malabsorption (11). We aimed to investigate whether addition of a low dose of acarbose to rapidly digestible starch can change the digestive profile to that of a slowly digestible starch. To achieve the alteration of the digestion profile and without changing the starch flux into the colon, a dose as low as 12.5 mg of acarbose was used. The hypothesis was tested that this dose delays digestion and absorption of rapidly digestible starch without reducing total bioavailability of starch. The dual isotope technique was applied to measure the rate of appearance of starch derived glucose, reflecting intestinal starch digestion and glucose absorption.

Subjects and methods

Subjects
Seven healthy male subjects [age 23.4 ± 1.0 yr (mean ± SEM), body mass index 21.6 ± 1.1 kg/m\(^2\)] were recruited by advertising. The criteria for exclusion were use of
medications, blood donation in the previous 6 months, use of antibiotics in the last 3 months, gastrointestinal symptoms, diabetes mellitus and gastrointestinal surgery. Approval was obtained from the Medical Ethics Committee of the University Medical Centre in Groningen and each subject gave written informed consent for the study.

**Test meals**
Each test meal consisted of 50.3 g (dry weight) corn pasta (90.3 % carbohydrates, Honig, Koog aan de Zaan, The Netherlands) cooked for 10 min in 1 L water. *In vitro* characteristics of corn pasta were measured according to the method of Englyst (12) and showed that 89.0 % of total carbohydrate content of the cooked product consisted of rapidly available glucose, 6.8 % of slowly available glucose and 4.2 % of resistant starch. The $^{13}$C abundance (atom %) of the corn pasta measured with total combustion using an on-line coupled elemental analyzer (TracerMAT, Thermo Finnigan, Bremen, Germany) was 1.09833. During one of the study days the test meal was ingested together with 12.5 mg acarbose (Bayer AG, Leverkusen, Germany).

**Study protocol**
The study was performed in a crossover manner, with each subject studied on two occasions at least one week apart. The subjects were asked to refrain from consuming foods enriched in $^{13}$C, such as cane sugar, corn, corn products and pineapple, for the three days preceding the experiments and from alcohol and strenuous exercise for 24 h before each study day. The subject’s food intake after 5 p.m. the day before each experiment was individually standardized. Subjects fasted and drank only water, coffee or tea without sugar and milk from 10 p.m. the night before the study and arrived at 8 a.m. on both study days in the Medical Centre. Cannulas were inserted into veins in both forearms, one for collection of blood, kept patent with heparin (50 IE/mL) and the other for infusion of D-[6,6-$^{2}$H$_2$] glucose (98 % $^2$H ape) (Isotec Inc, Miamisburg, OH, USA). Throughout the study, subjects were encouraged to relax by reading or watching videos.

A primed-continuous infusion of D-[6,6-$^{2}$H$_2$] glucose [prime: 342 mg, continuous: 3.5 mg/min (9.5 mg/mL) was started at time minus 120 min and blood and breath samples were taken at frequent intervals for 8 h. 120 min after the beginning of the infusion (t = 0), the test meal, corn pasta (CP) or CP with 12.5 mg of acarbose (CPac), was ingested. Acarbose with 100 mL of tap water was taken with the first bite of the test meal.
Sample collection
Blood was collected throughout the study into tubes containing sodium fluoride potassium oxalate. After centrifugation at 4 °C the samples were stored at −20 °C until assayed. Breath samples were collected by breathing through a straw into 10-mL exetainers (Labco limited, Buckinghamshire, United Kingdom). Basal blood and breath samples were collected before the beginning of the infusion. Blood samples were taken every 30 min for 90 min, every 15 min for the following 150 min, and every 30 min in the 240 min thereafter. Breath samples were collected every 30 min for 90 min and every 15 min in the 390 min thereafter.

Analytical procedures
Glucose was measured with an eca-180 glucose analyzer (Medingen, Dresden, Germany). The inter-assay and intra-assay coefficient of variation was 3 % and 1 %, respectively. Insulin concentrations were measured in duplicate using a commercially available radioimmunoassay (Diagnostic Systems Laboratories, Webster, Texas, USA). The inter-assay and intra-assay coefficient of variation was 9.9 % and 4.5 %, respectively. The derivatization of plasma glucose to glucose pentaacetate for the analysis of the isotopic enrichment of plasma glucose is described in detail elsewhere (13), we made only some minor modifications. In short, glucose was extracted with ethanol. The extract was dried under nitrogen gas and thereafter glucose was derivatized to its pentaacetate-ester using acetic acid anhydride-pyridine. After evaporation of the reagent, the derivative was dissolved in 1250 μL acetone. The $^{13}$C/$^{12}$C isotope ratio measurement of the glucose penta-acetate derivative was determined by Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry (GC/C/IRMS) and the $^2$H enrichment was measured by Gas Chromatography/Mass Spectrometry (GC/MS) under conditions previously described (13). All plasma samples of one subject were analyzed together to eliminate the effects of inter-batch variation. Analysis of $^{13}$C abundance in breath CO$_2$ was performed using gas IRMS (Tracermat, Thermo Finnigan, Bremen, Germany) measuring the $^{13}$C/$^{12}$C ratio versus the international standard Pee Dee Belemnite ($\delta^{13}$C$_{PDB}$) in per mill. Breath hydrogen analysis was performed using gas chromatography (HP 6890 Agilent, Hewlett Packard Co, Palo Alto, USA), using a CP-Molsieve 5A column of 25 m × 0.53 mm (50 μm film thickness) (Chrompack International B.V., Bergen op Zoom, The Netherlands).
Calculations
The molar percent enrichment of [6,6-2H₂] glucose and the 13C atom percentage were calculated as previously described (13) and smoothed using the Optimized Optimal Segments (OOPSEG) program developed by Bradley et al (14). The rate at which glucose appeared in plasma (RaT) from exogenous (meal) and endogenous (hepatic) sources was calculated using the non-steady state equation of Steele (15) as modified by De Bodo (16). Identical behavior of labelled and unlabelled glucose molecules was assumed. The effective volume of distribution was assumed to be 200 mL/kg and the pool fraction value 0.75 (17). The rate of appearance of exogenous glucose (RaEx) was estimated as described by Tissot et al (17). The time to peak was defined as time period between the intake of the test meal and the appearance of peak plasma concentration.

CO₂ production was assumed to be 300 mmol/m² body surface area (BSA) per hour. BSA was calculated according to the classic weight-height formula of Haycock et al (18). The 13CO₂ excretion in breath was expressed as percentage of the administered dose per hour (%dose/h) and as a cumulative percentage of the administered dose (cum %dose) over time. Hydrogen results were expressed as parts per million (ppm). A sustained increase in hydrogen of more than 10 ppm was regarded to indicate arrival of carbohydrates in the colon (19).

Using the trapezoidal rule (20) the incremental areas under the postprandial curves (AUCs) for glucose, insulin, RaEx, RaT and 13CO₂ were calculated. Areas below baseline were not included. For the AUC calculations RaEx values were multiplied by bodyweight and expressed as percentage of the administered dose of glucose equivalents (cum %dose). To be able to judge whether digestion was delayed the 0–120 min, 120–240 min and 240–360 min AUC of RaEx were calculated and compared.

Statistics
All values were presented as mean ± SEM. All samples were tested for normal distribution by the Kolmogoroff-Smirnoff test. Rates are expressed as milligrams per kilogram total body weight per minute (mg/kg/min). Differences between the results of the test meals were assessed with the two-tailed paired t-test. The analyses were performed with the statistical program SPSS 11.0 for Windows software (SPSS inc., Chicago, IL, USA). P < 0.05 was considered to be significant.
Results

Postprandial plasma glucose and insulin responses

Fasting glucose and insulin concentrations were not significantly different between the corn pasta (CP) and corn pasta plus acarbose (CPac) study days. Postprandial plasma glucose concentration are shown in Figure 1. The AUC from 0–120 min was significantly lower for CPac (25.7 ± 7.1 mmol/L/2h) than for CP (65.3 ± 17.3; \( P = 0.034 \)). Also, the peak glucose concentration was significantly lower for CPac (6.0 ± 0.2 mmol/L) than for CP (6.7 ± 0.3; \( P = 0.007 \)).

Figure 1 Mean (± sem) postprandial plasma glucose concentrations in seven healthy subjects after ingestion of 50.3 g (dry weight) corn pasta with (●) and without 12.5 mg of acarbose (O).

Figure 2 shows the postprandial insulin concentrations. The AUC from 0–120 min was significantly lower for CPac (153 ± 47 mU/L/2h) than for CP (598 ± 127; \( P = 0.003 \)). Also, the peak insulin concentration was significantly lower for CPac (9.6 ± 1.2 mU/L) than for CP (18.2 ± 4.3 mU/L; \( P = 0.036 \)).
Figure 2 Mean (± SEM) postprandial plasma insulin concentrations in seven healthy subjects after ingestion of 50.3 g (dry weight) corn pasta with (●) and without 12.5 mg of acarbose (○).

Rate of total glucose appearance (RaT)
The RaT before ingestion of the test meals was not significantly different between the cp and cpac study days. The peak RaT after ingestion of CP (3.8 ± 0.3 mg/kg/min) did not differ from that after cp (3.1 ± 0.3; P = 0.78) nor did the times to reach peak RaT (38.6 ± 3.0 and 32.1 ± 11.4 min respectively; P = 0.569). The AUC in the first 2 h was significantly higher for cp (27.1 ± 1.9 mg/kg/2h) than for cpac (23.1 ± 1.1; P < 0.001).

Rate of appearance of exogenous glucose (RaEx)
The kinetics of the RaEx are shown in Figure 3A. The peak RaEx after ingestion of CP (2.4 ± 0.4 mg/kg/min) was significantly higher than after ingestion of cpac (1.6 ± 0.2; P = 0.015). Times to reach the peak did not differ significantly between CP and cpac. The AUC in the first 2 h was significantly higher for cp (44.2 ± 5.7 cum %dose/2h) than for cpac (30.7 ± 3.2; P = 0.01) as was the AUC in the time period between 2 and 4 h (30.6 ± 2.3 and 22.5 ± 2.4 cum %dose/2h respectively, P = 0.02) (Figure 3B).
Figure 3A Mean (± SEM) postprandial rate of appearance of exogenous glucose in seven healthy subjects after ingestion of 50.3 g (dry weight) corn pasta with (●) and without 12.5 mg of acarbose (O).

Figure 3B Areas under the curves, calculated from the percentage of the dose glucose equivalents administered, for the time periods of 0–120 and 120–240 min and 240–360 min (white: corn pasta, black: corn pasta with 12.5 mg acarbose). Two-tailed paired t-test showed significant differences between both test meals. *P < 0.05
$^{13}\text{CO}_2$ and hydrogen

Figure 4 shows the $^{13}\text{CO}_2$ excretion in breath. Acarbose significantly reduced the percentage administered dose excreted as $^{13}\text{CO}_2$ in breath (CP: 37.5 ± 2.8 cum %dose/6h; CPac: 27.2 ± 2.2; $P = 0.004$) over the 360 min after ingestion of the test meal (Figure 5). Fasting hydrogen excretion was not different between the CP (21.4 ± 4.1 ppm) and CPac (18.7 ± 3.1 ppm) study days. During the 6-hour period of the experiment the hydrogen excretion did not increase on both study days but declined to a nadir of 11.2 ± 1.1 ppm after CP and 8.9 ± 1.3 ppm after CPac.

The AUC in the last 2 h was not significantly different between both meals (CP 14.0 ± 1.8, CPac 14.8 ± 1.5 cum %dose/2h; $P = 0.7$). The percentage administered dose that appeared up to 360 min after ingestion was significantly higher for CP (88.8 ± 7.8 cum %dose/6h) than for CPac (64.8 ± 5.2; $P = 0.009$) (Figure 5).

*Figure 4 Mean (± SEM) postprandial $^{13}\text{CO}_2$ excretion in breath in seven healthy subjects after ingestion of 50.3 g (dry weight) corn pasta with (●) and without 12.5 mg of acarbose (O).*
**Figure 5** Mean (± SEM) percentage administered dose glucose equivalents that appeared up to 360 min in plasma (left panel) and that was excreted over 360 min as $^{13}$CO$_2$ in breath (right panel) after ingestion of 50.3 g (dry weight) corn pasta with (black column) and without 12.5 mg of acarbose (white column). Two-tailed paired t-test showed significant differences between both test meals. *$P < 0.05$*

**Discussion**

In view of the possible beneficial effects of slowly digestible starch it is of interest to know whether addition of a small dose of acarbose to rapidly digestible starch can change the digestive profile to that of a slowly digestible starch. If this is achieved one would expect with acarbose a lower influx rate of starch derived glucose in the early postprandial period and a higher influx rate in the late postprandial period, resulting in approximately the same bioavailability of glucose as without acarbose. However, in our study we found that a low dose of 12.5 mg of acarbose added to rapidly digestible corn pasta significantly reduced the rate of appearance of exogenous glucose in the first four hours after ingestion whereas in the postprandial period between 4 and 6 h the RaEx was the same. Consequently the bioavailability of starch during the 6 hour test period was reduced by 22 %. Also, the percentage of the ingested dose that was excreted as $^{13}$CO$_2$ during the study period was significantly reduced by acarbose (cp: 37.5 ± 2.8 %dose; cpac: 27.2 ± 2.2 %dose; $P = 0.004$). Similar data were found by Achour et al (21) comparing digestible and partially indigestible cornstarch: the percentage of the
ingested dose excreted after ingestion of digestible starch was 35.3 ± 3.0 and that after partially indigestible starch 28.2 ± 1.8 over a period of 8 h.

Our finding is surprising in the light of the low dose of acarbose used and its expected mode of action. The initial dose of acarbose in the treatment of diabetic patients is recommended to be as low as 50 mg per meal to avoid symptoms of carbohydrate maldigestion (15). Even taking into account that normal meals contain more carbohydrates (e.g. 2 bread rolls contain 100 g carbohydrates) than our test meal, the acarbose to carbohydrate ratio in our experiment (1 : 3.7) was considerably lower than that normally achieved at the start of the acarbose treatment (1 : 2). Higher doses of acarbose than that used in our study can thus be expected to induce starch maldigestion to a much greater extent than observed in this study. Symptoms of carbohydrate maldigestion are reported to decrease during continued treatment which is ascribed to new synthesis of α-glucosidases in the distal small intestine due to delivery of carbohydrates to this part of the intestine (22). It is not likely that this adaptive mechanism is really sufficient to prevent spillover of starch to the colon. Several studies monitoring fermentation parameters indicate that even chronic intake of acarbose from 50 mg onwards results in carbohydrate maldigestion (23–26). Furthermore, a decrease of symptoms can also be explained by an adaptation process in the colon. It has been shown that symptoms occurring after ingestion of indigestible disaccharides are declining due to chronic ingestion (27–30). Factors involved in this decrease of symptoms are hypothesized to be for example changes in the composition of the microbiota or in the metabolic activity of the microbiota.

Hydrogen excretion in breath, which is considered to be indicative for colonic starch fermentation, did not increase in the 6 hour period in our study. Reports about hydrogen excretion due to effects of acarbose are conflicting and depend on the type of carbohydrate in the test meal and the dose of acarbose. Sucrose and dextrimaltose containing meals (33–100 g carbohydrates) resulted in hydrogen excretion with 50–200 mg of acarbose (31;32). After ingestion of starchy meals (50 g carbohydrate) administered with a dose of 200 mg acarbose hydrogen excretion was observed in one (33) but not in another study (34). Anchour et al (21) did not observe any hydrogen production after the ingestion of partly digestible starch in the 8 hour study period. Also fermentation of guar gum, a dietary fiber, did not result in an increase of breath hydrogen despite of increased serum acetate concentrations (35). These observations fit in the general assumption that colonic fermentation of carbohydrates is not always reflected in increased levels of hydrogen in the breath and that this might depend on the type of carbohydrate
as well as on the length of the study period. Thus, the absence of a rise in breath hydrogen in our study does not necessarily contradict our finding of decreased starch bioavailability.

Acarbose is successfully used in the treatment of patients with type 2 diabetes. The STOP-NIDDM trial in persons with impaired glucose tolerance suggested that, besides or in addition to changes in lifestyle, acarbose may reduce the risk of developing type 2 diabetes and the incidence of cardiovascular disease and hypertension (36;37). Discussions concerning the mechanisms involved in the probable effect of acarbose on the development of these chronic diseases are ongoing. Our observation, that even a low dose of acarbose decreases bioavailability of starch adds evidence to the suggestions that metabolic effects of colonic carbohydrate fermentation need to be taken into consideration. There is emerging evidence that colonic fermentation not only affects gut metabolism but also can influence metabolic processes in other tissues and organs. Short- and long-term consumption of resistant starch for example has been shown to increase insulin sensitivity in healthy subjects (38;39). The underlying mechanism of this phenomenon is not known but short-chain fatty acids (SCFAs), like acetate and propionate, which are products of starch fermentation could be involved. These SCFAs are metabolised by the colonic epithelial cells but also enter the portal circulation. The finding that SCFAs are ligands for the G protein-coupled receptor GPR 41 on adipose tissue (40) which secretes signaling peptides influencing among others insulin sensitivity (41), suggests a link between colonic fermentation and peripheral metabolic effects. Further research in this area is needed.

In conclusion, even a low dose of acarbose (12.5 mg) decreases the systemic appearance of starch derived glucose in a substantial way. Reduction of bioavailability seems to contribute to this decrease to a greater extent than a delay of digestion. The metabolic consequences of an increased colonic fermentation of carbohydrates due to the acarbose treatment deserves further attention.

Acknowledgements

This study has been supported by grant GGN.4487 of the Technology Foundation STW, the applied science division of NWO (Utrecht, The Netherlands). This work was also financially supported by the Commission of the European Communities, and specifically the RTD programme ‘Quality of Life and Management of Living Resources’, QLK 1-2001-00431 ‘Stable isotope applications to monitor starch digestion and fermentation for the development of functional foods’
(EUROSTARCH). This work does not necessarily reflect its views and in no way anticipates the Commission’s future policy in this area.

References

Acarbose and starch digestion


