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$^{13}CO_2$ breath test to measure the hydrolysis of various starch formulations in healthy subjects

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Abstract

$^{13}CO_2$ starch breath test was used to study the effect of physicochemical characteristics of starch digestion. As starch is hydrolysed to glucose, which is subsequently oxidised to CO_2, differences in $^{13}CO_2$ excretion after ingestion of different starch products must be caused by differences in hydrolysis rate. To study the effect of the degree of chain branching, waxy starch, containing 98% amylopectin, was compared with high amylose starch, containing 30% amylopectin, and normal crystalline starch, containing 74% amylopectin. The effect of the extent of gelatinisation was studied by comparing extruded starch and crystalline starch. Finally, the possible inhibitory effect of adding wheat fibre to extruded starch on the hydrolysis rate was studied. The $^{13}CO_2$ excretion from two to four hours after intake of crystalline starch was significantly lower than that of extruded starch. Waxy starch was hydrolysed much faster than high amylose starch, but there was no significant difference between waxy starch and normal crystalline starch. Addition of wheat fibre did not influence the hydrolysis rate. The $^{13}CO_2$ starch breath test is an attractive test for the study of factors affecting carbohydrate assimilation.

In vitro studies and observations on the glycaemic responses after ingestion of different starchy foods have shown that the rate of starch assimilation in normal and in diabetic subjects is influenced by the chemical and physical properties of the starch preparations. The amylose/amylopectin ratio,[1 2] the physical form,[3 4] the particle size,[5 6] and the method of food processing[6-10] are important factors influencing starch hydrolysis and postprandial glycaemic response in diabetic subjects. It has also been suggested that complete starch absorption may be a risk factor for the development of diverticular disease.[11] Besides possible preventive effects against diseases such as diabetes and diverticular disease, slowly digested carbohydrates can also be used therapeutically in the dumping syndrome.

The $^{13}CO_2$ starch breath test has been shown to be a reliable test for the study of carbohydrate absorption. Using naturally ^{13}C-enriched corn starch and glucose, the $^{13}CO_2$ excretion after intake of crystalline starch was slower than after intake of glucose,[12] indicating that the hydrolysis rate of starch, and not the absorption of the monosaccharides, is the rate limiting step in starch assimilation. It was also shown that there is a difference in starch assimilation between normal subjects and patients with pancreatic disease.[13]

The aim of this study was to determine the effect of the amylose/amylopectin ratio and the degree of gelatinisation of starch on its hydrolysis rate, by measuring the rate of $^{13}CO_2$ appearance in breath after the intake of different corn starch preparations by healthy subjects. We also studied the influence of the addition of wheat bran on the starch absorption rate.

Methods

SUBJECTS

Eleven healthy volunteers, seven women and four men, aged 22 to 27 years were studied. All subjects had a body mass index between percentile 5 and 75, according to the values of Cronk and Roche.[14] Informed consent was obtained from all subjects, and the study protocol was approved by the ethical committee of the Leuven University.

SUBSTRATES

All starch preparations were corn derived. The following starch preparations were supplied by Cerestar, Vilvoorde, Belgium: normal crystalline starch, 'waxy starch', which originates from a hybrid of Zea Mays, and starch treated by extrusion cooking, which is called extruded starch in the text. High amylose corn was obtained from Sigma, St Louis, USA, and wheatbran (Fiberform®) was obtained from Duphar, Brussels, Belgium.

The method of Wootton *et al*[15] was used to study the gelatinisation of the starch substrates. The percentage of gelatinisation and the amylopectin content of the various starch preparations are shown in the Table.

Corn carbohydrates are known to be naturally enriched in ^{13}C. The ^{13}C-enrichment was $-10{\cdot}804$ δ for crystalline starch, $-10{\cdot}194$ δ for waxy starch, $-10{\cdot}827$ δ for extruded starch, and $-9{\cdot}720$ for high amylose starch. All values are expressed as δ^{13}_{PDB},[16] and have been obtained by combustion[17] and subsequent analysis of the $^{13}CO_2/^{12}CO_2$ ratio.

BREATH TESTS

After an overnight fast of approximately 13 hours, the subjects received an oral load of 50 g carbohydrates in 250 ml water. Six subjects underwent four tests with crystalline starch, waxy starch, extruded starch, and extruded starch plus wheatbran as substrate. Five other subjects underwent two tests – that is, with waxy starch and high amylose starch. There was an

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TABLE *Characteristics of studied starch preparations*

Starch preparation	*Amylopectin content* %	*Degree of gelatinisation* %
Normal crystalline	74	1
Waxy	98	4·5
Extruded	74	79
High amylose	30	3

interval of at least three days between two tests. The subjects were not allowed to smoke on the day of the test. The substrates were administered in random order. A breath sample was taken in an aluminium coated low density polyethylene bag 10 and five minutes before ingestion of the test carbohydrate, and subsequently every 30 minutes for eight hours. The subjects were kept completely rested during the test. The H_2 concentration of each sample was measured (in ppm), by injecting 20 ml in a H_2 monitor (GMI, Renfrew, Scotland). Pure CO_2 from the samples was obtained by cryogenic trapping over liquid N_2 after removal of water by a methanol-CO_2-ice trap. The ratio $^{13}C/^{12}C$ was measured by isotope ratio mass spectrometry (Finnigan MAT 250) and expressed as δ^{13}PDB value. Assuming a total CO_2 production of 300 mmol/m^2 BSA/h, the amount of ^{13}C recovered in breath was calculated as percentage of the administered dose. Body surface area was estimated by the formula of Haycock.[18]

STATISTICAL ANALYSIS

Values are given as means (SEM). Student's *t* test for paired samples and signed-rank test statistics were used for comparing differences between two groups for normally distributed and not normally distributed data respectively. The Wilks-Shapiro test was used to evaluate normality. Tukey's test for multiple comparisons was used to compare means of more than two groups.

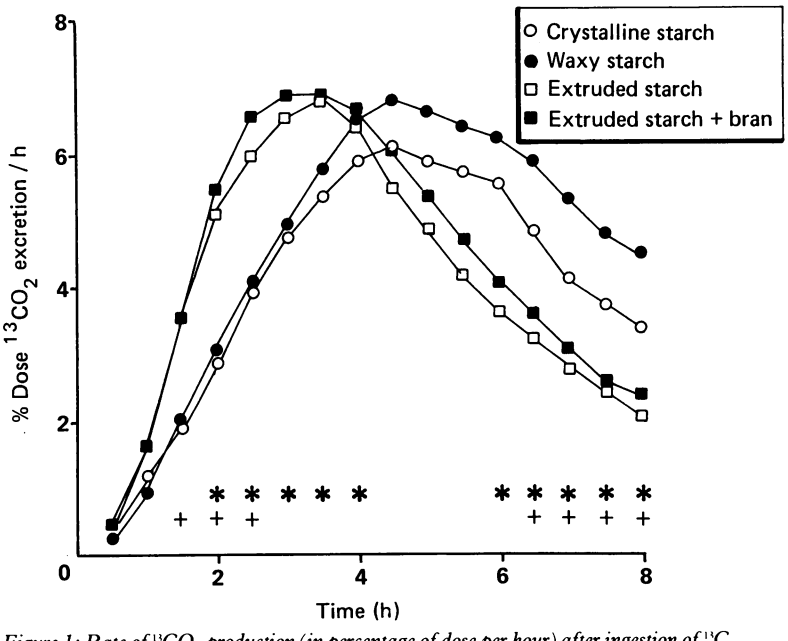

Figure 1: Rate of $^{13}CO_2$ production (in percentage of dose per hour) after ingestion of ^{13}C labelled starch products. (: difference between crystalline and extruded starch significant at p=0·05 level; †: difference between waxy and extruded starch significant at p=0·05 level.)*

Results

$^{13}CO_2$ BREATH TESTS

The mean rate of $^{13}CO_2$ appearance in breath after ingestion of crystalline starch, waxy starch, extruded starch and extruded starch plus wheatbran, is shown in Figure 1. At the beginning of the test (from two to four hours) the $^{13}CO_2$ excretion after ingestion of crystalline starch was significantly lower ($p \leq 0{\cdot}05$) than after intake of extruded starch. This proportion reversed from six to eight hours after intake of the substrates. This means that the hydrolysis rate of crystalline starch is slower than the hydrolysis rate of extruded starch. The oxidation rate, and hence the hydrolysis rate of extruded starch was significantly faster than that of waxy starch between one and a half hours and two and a half hours and significantly slower from six and a half hours to eight hours after substrate ingestion.

Although the mean curve of $^{13}CO_2$ excretion after ingestion of waxy starch was somewhat higher than that after ingestion of crystalline starch, the difference was never statistically significant. The curve of the $^{13}CO_2$ excretion after intake of extruded starch was not different from that after intake of extruded starch plus wheatbran.

If the individual results are compared, the difference between the $^{13}CO_2$ excretion curves after intake of extruded starch with and without addition of wheatbran and the other starch preparations was present in five of the six subjects. Figure 2 shows the cumulative $^{13}CO_2$ excretion after intake of the different substrates. From two hours to five and a half hours after the intake of crystalline starch, the cumulative $^{13}CO_2$ excretion was significantly lower than after the intake of extruded starch. Comparable differences were found between waxy starch and extruded starch between one hour and three and a half hours.

The results of the comparison between high amylose starch and waxy starch (high-amylopectin) are shown in Figure 3. The $^{13}CO_2$ excretion rate after ingestion of high-amylose starch was markedly lower than after ingestion of waxy starch. This difference was statistically significant ($p \leq 0{\cdot}05$) between one and a half hours and seven hours.

H_2 BREATH TEST

In three of the five subjects who took high amylose starch, there was a rise in breath H_2 of 22, 11, and 7 ppm above basal level respectively. In one of these subjects, however, there was also a rise in breath H_2 of 12 ppm above basal level after intake of glucose. This difference did not reach statistical significance.

No significant increase of breath H_2 was found after intake of any of the other starch preparations during the study period.

Discussion

In this study a $^{13}CO_2$ breath test was used to study the digestibility of different starch preparations.

Since the work of Lacroix *et al*[19] who measured $^{13}CO_2$ excretion in breath after ingestion of

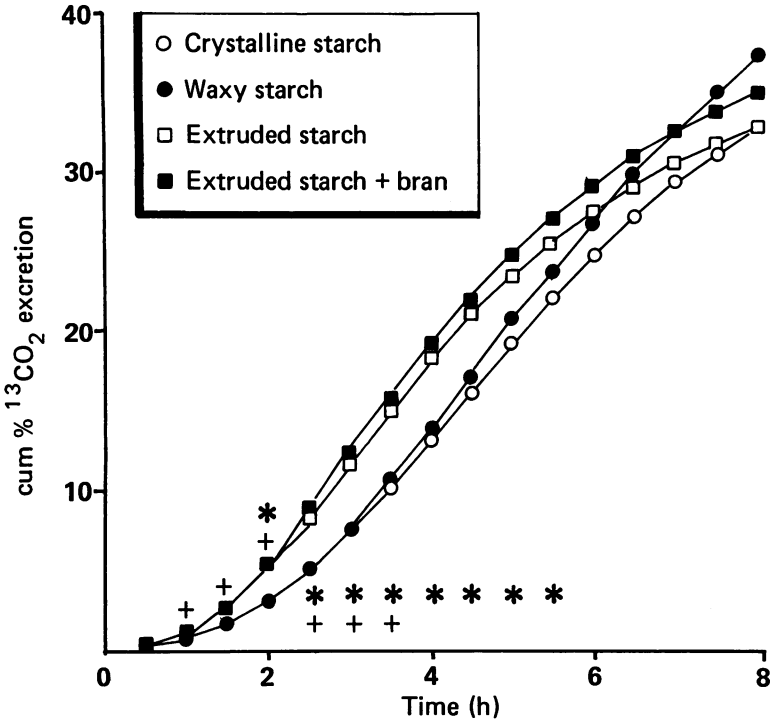

Figure 2: Cumulative $^{13}CO_2$ excretion in percentage of dose after ingestion of ^{13}C labelled starch products. (: difference between crystalline and extruded starch significant at p=0·05 level; †: difference between waxy and extruded starch significant at p=0·05 level.)*

naturally ^{13}C-labelled glucose, $^{13}CO_2$ breath tests have been used in gastrointestinal and nutritional research.[20-22] We recently evaluated a $^{13}CO_2$ lactose breath test as a diagnostic test of lactase deficiency,[23] and used naturally ^{13}C-labelled starch for a study of starch digestion in patients with pancreatic disease.[13] This $^{13}CO_2$ breath test is based on the fact that digestion of all starch preparations consists of hydrolysis into glucose that is absorbed and further metabolised to CO_2. As each subject is used as his own control, and as it may be assumed that the rate of glucose oxidation is comparable in each test because the same fasting period and the same resting conditions were used, differences in $^{13}CO_2$ excretion between different starch preparations must be due to differences in hydrolysis rate.

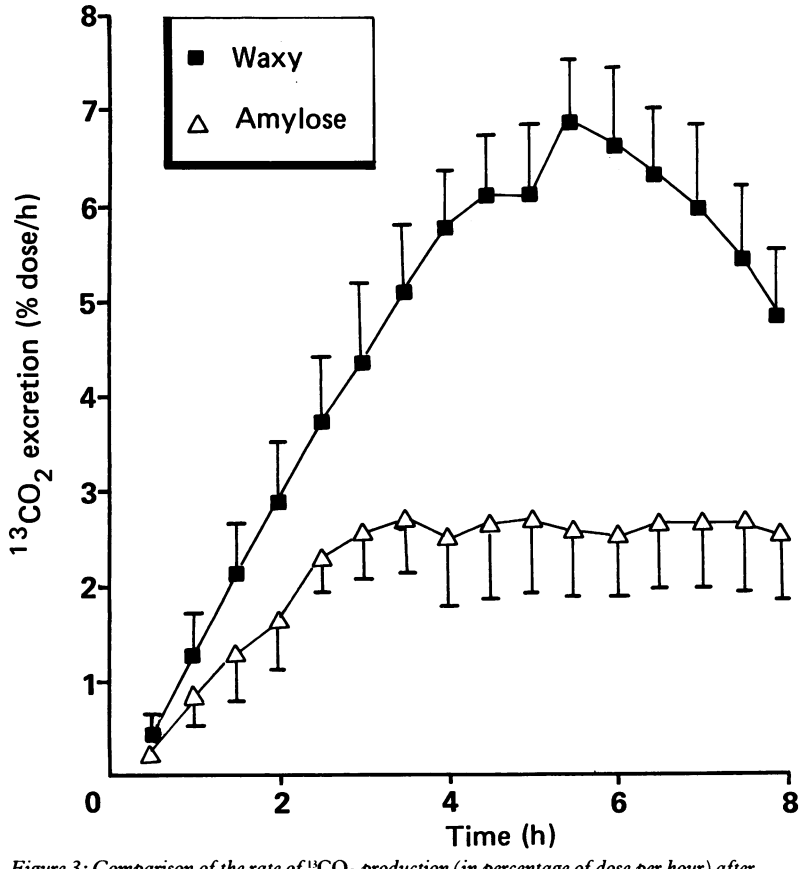

Figure 3: Comparison of the rate of $^{13}CO_2$ production (in percentage of dose per hour) after ingestion of waxy starch and high amylose starch (mean (SEM)).

In this study we used the $^{13}CO_2$ breath test to evaluate the influence of the degree of gelatinisation and the degree of side branching on the hydrolysis rate of starch. Starch with a high degree of gelatinisation, obtained by extrusion processing, is more rapidly hydrolysed than native starch, which is less susceptible to pancreatic amylase because of its crystalline structure. With regard to the effect of the amylose/amylopectin ratio, no difference was observed between starch containing 98% and starch containing 74% amylopectin. Hydrolysis of a high amylose starch preparation (30% amylopectin), however, was strikingly different. These findings are in agreement with the observations of Behall *et al*[2] and the study of Goddard *et al.*[1] The latter study even found a difference in glycaemic response between starch containing 23–25% amylose and pure amylopectin starch. The starch preparations used in their study were cooked, while we compared the raw products. Processing by heat possibly enhances the difference in digestibility between no amylose and low amylose starch.

In this study we also found that addition of wheat bran to a starch load does not influence the hydrolysis rate. These results are in agreement with previous observations.[6] Shaheen and Fleming[24] only found an influence of fibre on the *in vitro* hydrolysis rate if it presented a physical barrier that limited the contact between hydrolytic enzymes and starch.

Another factor that is known to influence the postprandial glycaemic response after a starch meal is the gastric emptying rate, which can be influenced by differences in osmolarity,[25] the presence of viscous fibres[26-28] and the particle size.[29] We did not measure the rate of gastric emptying in this study. The forms in which the starch loads were administered, however, were very comparable with each other in terms of osmotic load, particle size and presence of viscous fibres. Therefore the gastric emptying rate would not be expected to differ between the various compounds. During the past few years the effect of starch structure on starch digestion rate has been studied by several groups. In most of these studies the rise in blood glucose and insulin concentrations were used as parameters. The complexity of the carbohydrates,[30] the way of processing,[7 8 31] the degree of gelatinisation,[32] the particle size,[5] and the amylose content[1 2] are the most important factors that were shown to influence postprandial blood glucose and insulin concentrations. Using the postprandial glycaemic response as a parameter, a variety of starch containing foods were compared for their rate of absorption, and a classification was developed in terms of their glycaemic index.[33 34]

Although this method is simple and useful, different authors use different definitions of 'glycaemic index'. The reference carbohydrate sometimes is glucose, sometimes bread.[34] Even the same authors use the total area under the blood glucose curve in one study,[8] and the incremental area under the blood glucose curve in another study.[35]

The $^{13}CO_2$ starch breath test we used in this study is a dynamic test which gives information on the metabolism of a substrate over a long period, while the glycaemic response is only valuable in the first one or two hours of the test. The H_2 breath test has been advocated to quantify the amount of colonic degradation of non-absorbed carbohydrates,[36,37] but cannot be used to measure the rate of digestion of carbohydrates in the small intestine.

In contrast with the H_2 breath test, the $^{13}CO_2$ breath test gives no information on the presence of malabsorption – that is, the appearance of substrate in the caecum. For this purpose, it has to be combined with the H_2 breath test.

It may be concluded that the $^{13}CO_2$ breath test is an attractive technique for the study of factors affecting carbohydrate absorption. This study confirms the importance of the degree of gelatinisation and the degree of side branching on the rate of starch hydrolysis. It also shows that addition of wheat bran has no influence on the digestion rate.

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