

## $\alpha$ -Amylase (EC 3.2.1.1) susceptibility rather than viscosity or gastric emptying rate controls plasma responses to starch in healthy humans

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The relationship between starch  $\alpha$ -amylase (EC 3.2.1.1) susceptibility, plasma responses and gastric emptying rates has been investigated in humans. Nine randomly chosen healthy subjects were given three carbohydrate test meals (25 g starch or equivalent glucose units): two maize starch pastes with (a) 240 (S24) or (b) 500 (S50) g amylose/kg, and a glucose solution (GS). At 30 min, in vitro starch  $\alpha$ -amylolysis was 48 (SD 4)% for S24 and 35 (SD 4)% for S50. Test meals differed in viscosity (mPa·s: S24, 54000; S50, 190; GS, 4). Carbohydrates were labelled with <sup>99m</sup>Techetium and isotope gastric emptying was measured by external gamma counting. Carbohydrate isotopic gastric emptying patterns were exponential. Half gastric emptying time (min) was significantly ( $P < 0.05$ ) shorter for S50 (19 (SD 2)) than for GS (26 (SD 2)) or S24 (29 (SD 2)). No correlation was found between half gastric emptying time and plasma response values. Values for peak insulin (pmol/l) above fasting were significantly ( $P < 0.05$ ) different: GS, 306 (SD 11); S24, 227 (SD 11); S50, 187 (SD 11). It is concluded that  $\alpha$ -amylase susceptibility of the test carbohydrates is a determining factor in the insulin response of healthy subjects, while viscosity of the test meals and gastric emptying rate have no effect.

$\alpha$ -Amylase hydrolysis: Gastric emptying: Starch: Viscosity

Glucose and insulin responses to starchy foods may vary considerably in diabetics as well as in normal subjects (Crapo *et al.* 1977; Jenkins *et al.* 1981; Bornet *et al.* 1987). Recently we have shown the importance of the botanical origin of the starch and modifications of the starch during domestic or industrial processing on insulinaemic plasma responses to starch in healthy subjects (Fontvielle *et al.* 1988; Bornet *et al.* 1989). Bioavailability of starch in food measured by in vitro  $\alpha$ -amylolysis has often been correlated with plasma responses to starchy food (O'Dea *et al.* 1980; Jenkins *et al.* 1982; Bornet *et al.* 1989), suggesting that in vivo amylolysis plays a major role in plasma responses to starchy foods. Nevertheless, gastric emptying is a limiting factor to small intestinal enzymic digestion and absorption. Thus, the wide differences between intra- and inter-individual incremental plasma glucose responses after an oral glucose tolerance test have been attributed to the wide variations in glucose gastric emptying among subjects (Thompson *et al.* 1982). Glycaemic and insulinaemic indices of carbohydrate foods have recently been correlated with their half gastric emptying time ( $T_{1/2}$ ) (Mourot *et al.* 1988). The efficiency of viscous fibre in reducing carbohydrate-associated plasma glucose and insulin responses has been attributed in part to its ability to reduce carbohydrate gastric emptying in normal subjects (Holt *et al.* 1979; Schwartz *et al.* 1982; Flourié *et al.* 1985; Sandhu *et al.* 1987), as well as in diabetics (Ray *et al.* 1983) or in subjects with dumping syndrome (Holt *et al.* 1979; Leeds *et al.* 1981; Lawaetz *et al.* 1983). Effects of viscosity on gastric function and on carbohydrate metabolism have been extensively studied with soluble fibres such as guar or

pectin, but very seldom with starch pastes (Erhlein & Prove, 1980) which can also exhibit high viscosity (Doublier, 1987). The aim of the present study was to investigate in humans the relationships between in vitro  $\alpha$ -amylase (EC 3.2.1.1) susceptibility of carbohydrates and their plasma responses while controlling in vitro carbohydrate viscosity of test meals and measuring their carbohydrate gastric emptying rate.

#### MATERIALS AND METHODS

##### *Subjects and experimental design*

Nine young healthy volunteers (six men and three women, aged 24.0 (SD 2.4) years, mean body mass index 21.3 (SD 2.7) kg/m<sup>2</sup>) without any history of gastrointestinal disease or diabetes gave their written informed consent to participate in a study approved by the Ethics Committee of the University Hospital of Nantes.

Two starch pastes and a glucose solution were tested. Each meal (300 ml, weight 320 g) contained 25 g starch (on a dry basis, determined by drying a portion at 103° for 12 h) or the equivalent glucose units (i.e. 27.8 g anhydroglucose, 460 kJ).

Both native maize starches (Roquette Frères S.A., Lestrem), which differed in amylose content (240 g/kg S24, 500 g/kg S50), were tested after being made into pastes. These were obtained by cooking 58.5 g (dry basis) starch in salted Volvic® water (5 g/l; 715 ml) for 20 min from 20° to 96° in a double-walled round-bottom vessel. The temperature was maintained at 96° for 5 min. The isotopic marker used for the starches was <sup>99m</sup>Technetium-labelled human serum albumin (<sup>99m</sup>Tc-SHA; TCK8; ORIS Industrie; 1 mCi per test meal) which was added to the starch suspension before cooking. The suspension was stirred by an anchor-shaped blade rotating at 200 rev./min. The starch paste was flavoured with aspartame and lemon extract (2 g/kg). After they were cooked, the starch pastes were tested at 37° after a 10-min cooling period.

The glucose solution was flavoured with lemon extract and tested at 37°. The isotopic marker used for the glucose solution (GS) was <sup>99m</sup>Technetium-labelled, diethylenetriaminepentaacetic acid (<sup>99m</sup>Tc-DTPA; TCK6; ORIS Industrie; 1 mCi per glucose test meal).

The order of the three meals was randomly assigned to each volunteer during a 3-week test period using a Latin square experimental design; meals were taken at 09.00 hours within a 4 min period, after overnight fasting.

##### *Blood samples and gastric emptying procedure*

Blood samples were withdrawn 30 min before the test meal and every 15 min for 1 h following the meal, then every 30 min for a further 2 h. Samples were immediately centrifuged, frozen and stored for analysis. Plasma glucose was assayed using a glucose oxidase (EC 1.1.3.4) method (Beckman Autoanalyzer II; Beckman, Fullerton, CA, USA; intra-assay repeatability 2%). Plasma insulin was tested by radioimmunoassay (anti-insulin antibody; Novo Industri, Copenhagen, Denmark) using a charcoal separation method (intra-assay repeatability 6%).

The positive (or negative) differences between the maximal (or the minimal) glycaemic value and the fasting value were calculated for each plasma curve ( $\Delta$  peaks). The times of plasma glucose peaks (positive and negative) were also noted. The areas below (or above) the fasting glucose value for 0–180 min were calculated using the trapezoidal method. The insulinaemic characteristics were calculated similarly from the respective insulin curves.

At 3 min after test carbohydrate ingestion, the subject was seated upright in front of a gamma-camera head (Rota-camera, Siemens, W. Germany) fitted with a medium-energy parallel-hole collimator. Marker activity was detected using a specific window setting at the 140 (SD 10) keV photopeak of <sup>99m</sup>Tc. Images (60 s) of the stomach were taken at 10-min

intervals for 3 h, from front and rear of the body. Each complete scan took 3 min. Between scans the subject sat in a chair. Three external  $^{99m}\text{Tc}$  point sources were taped to the abdomen and thorax to allow the accurate horizontal and vertical gamma-camera head repositioning over the stomach area of the subject with the aid of an oscilloscope display. The scintillation camera was interfaced to a computer system (Digital Design), and all images were stored on magnetic disk for subsequent data processing. A mouse system was used to select a region of interest (ROI) corresponding to the stomach. Radioactivity within this ROI was computed for each image and stored. Values were corrected for radioactive decay. Geometric means of the anterior and posterior values were calculated to correct for attenuation and the effect of depth within the body (Tothill *et al.* 1978, 1980; Christian *et al.* 1980, 1983). The radioactivity observed at each scan interval was expressed as a percentage of the radioactivity obtained in the first scan (7 min after start of test meal) (Tothill *et al.* 1978, 1980).

Marker gastric emptying values were expressed as the percentage of marker remaining at each image time. Curves were fitted using a non-linear least squares method to a power exponential model (Elashoff *et al.* 1982), which permitted  $T_{1/2}$  to be calculated, and an  $\alpha$ -parameter, which describes the shape of the power exponential curve.

Using the values published by Siegel *et al.* (1983), and considering the negligible absorbance of the marker by the gastrointestinal tract (Chaudhuri, 1974), the estimated whole body radiation dose was 20 mrad, assuming a retention time of 31 h in the digestive tract, and 320 mrad for the large intestine (critical organ), assuming a retention time of 17 h in the large intestine.

#### *In vitro carbohydrate characteristics*

*$\alpha$ -Amylase hydrolysis.* Starch pastes were tested *in vitro* in order to investigate their susceptibility to  $\alpha$ -amylase. A portion of freshly prepared starch paste (18 g starch) was submitted to  $\alpha$ -amylase hydrolysis with 3000 units hog pancreatic  $\alpha$ -amylase (Merck, Darmstadt, W. Germany) with constant stirring (30 rev./min) in 200 ml phosphate buffer (0.005 M, pH 6.9–7.0) for 3 h at 37°. Every 5 min a 0.90 ml sample was mixed with 4.5 ml ethanol (95%)–acetic acid (1.5%) and stored overnight at 4°. Samples were then centrifuged (9000 g) for 10 min. The polysaccharide content of the supernatant fraction was assayed using a sulphuric orcinol automated method (Robin, 1976). The soluble polysaccharide content was plotted in terms of percentage of initial starch content *v.* time. Each  $\alpha$ -amylolysis was performed three times.

*Viscosity measurements.* The viscosity of the carbohydrate test meal was measured using a coaxial cylinder viscosimeter (Rheomat 30 Contraves, Zürich, Switzerland) with the following specifications for the cylinders: internal radius 22.9 mm, external radius 24.2 mm, height 56.5 mm. Measurements were made at 37° on glucose solution or on freshly prepared starch paste. Each sample was submitted to a shear rate scan from 0 to 662/s and back to 0/s at the same rate of 331/s per min. The same sample was then submitted to shear rate decreasing step by step from 662 to about 1/s; each step was maintained for the time needed to reach an equilibrium of the shear stress, always less than 20 s (Doublier, 1987).

*Homogeneity and stability of starch paste labelling.* Homogeneity of labelling was checked for both starch pastes. Gastric stability of starch labelling was studied *in vivo* in rats, twelve rats (180–220 g) being used for each experiment. They were deprived of food, but not water, for 48 h before the experiment. At zero time, rats began to eat *ad lib.* a freshly prepared labelled starch paste at 37°. After 15 min, the test meal was removed and the exact amount ingested was weighed. Six randomly selected rats were killed by pentobarbital intracardiac injection after 30 min and the remainder at 60 min. The stomach was isolated immediately by ligatures at the pylorus and cardia. Stomach contents were weighed.

Specific radioactivity was measured simultaneously on twelve random samples (1 ml) of

prepared labelled starch paste and on samples of total stomach contents. Radioactivity of all samples was counted simultaneously on the  $^{99m}\text{Tc}$  channel of a well gamma-counter and corrected for radioactive decay. Sample dry matter was determined by drying at  $103^\circ$  for 12 h. The coefficient of variation (SD/mean) of dry mass activity of twelve starch paste samples represented the homogeneity of starch paste labelling. The starch paste labelling stability in the stomach at 30 or 60 min was calculated as the ratio of the dry matter activity of stomach contents at 30 or 60 min to the mean of the dry matter activity of starch paste, expressed as a percentage.

#### *Statistical methods*

Analysis of variance (ANOVA) was used for human values (plasma and gastric emptying variables) to test the effect of subjects, test meal, and order of carbohydrate meals. Multiple comparisons between test meal values were made using the method of Newman-Keuls (Winer, 1971).

Analysis for the significance of differences between starch paste labelling results was performed using the Mann Whitney U test (Winer, 1971).

Linear correlations between individual gastric emptying and plasma results were calculated using the least square method.

Results are expressed as means and standard deviations.

## RESULTS

### *Starch pastes $\alpha$ -amylolysis*

The kinetics of in vitro susceptibility of the two starch pastes submitted to  $\alpha$ -amylolysis are shown in Fig. 1. The amount of soluble oligosaccharides formed increased rapidly in the first 30 min. A maximal plateau level was progressively reached after 60 min. S50 was the least susceptible starch paste. At time 30 min, 35 (SD 4)% of S50 starch paste was solubilized v. 48 (SD 4)% for S24 starch paste.

### *Viscosity of carbohydrate test meals*

The flow curves obtained at  $37^\circ$  with 86 g glucose/l solution and 78 g starch/kg pastes are shown in Fig. 2.

Whatever the shear rate, the apparent viscosity of glucose solution was constant at 4 mPa·s. Starch paste curves were characterized by a pronounced shear thinning behaviour. At a low shear rate (about 1/s) apparent viscosity was 190 mPa·s and 54000 mPa·s for S50 and S24 respectively. At higher shear rates both apparent viscosities decreased. At a shear rate of 100/s the least viscous starch paste was S50 with an apparent viscosity of 16 mPa·s, v. 2500 mPa·s for S24. Largely different viscosities persisted between starch pastes whatever the shear rate.

The physicochemical characteristics of carbohydrate test meals are summarized in Table 1.

### *Starch paste labelling*

In vitro measurements of labelling homogeneity, expressed as the coefficient of variation of dry matter activity of starch pastes (twelve observations), was 3.9% for S24 and 3.8% for S50. The starch paste labelling stability in the stomach at 60 min, assayed using rats, approached 100% (Table 2).

### *Plasma responses*

The mean plasma glucose responses observed within 180 min after the starch paste and glucose tolerance tests are shown in Fig. 3, with the mean postprandial plasma glucose variations summarized in Table 3. The mean glycaemic  $\Delta$  peak value occurred within

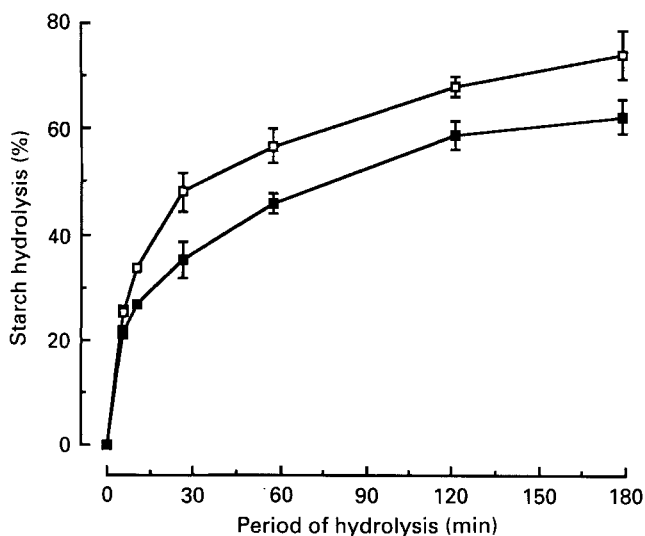


Fig. 1. In vitro starch hydrolysis kinetics (means and standard deviations represented by vertical bars for three determinations), using 3000 U hog pancreatic  $\alpha$ -amylase (*EC* 3.2.1.1) at 37°. (■), S50 starch paste (500 g amylose/kg maize starch); (□), S24 starch paste (240 g amylose/kg maize starch). (For details of test meals, see Table 1 and p. 208.)

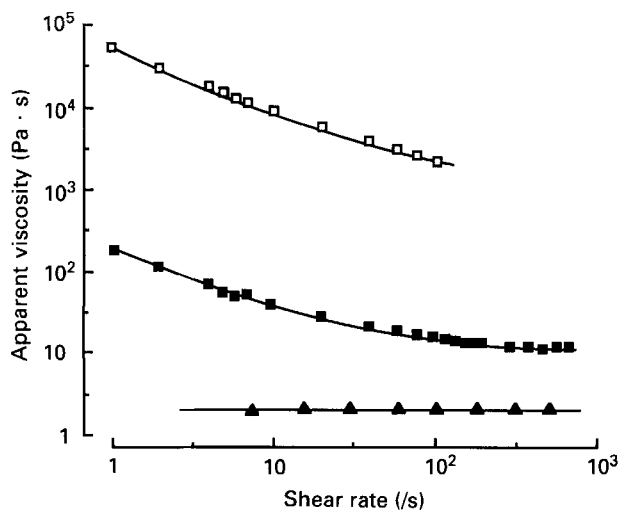


Fig. 2. Flow curves (log basis) obtained at 37° using a coaxial cylinder viscosimeter (Rheomat 30). (▲), Glucose solution; (■), S50 starch paste (500 g amylose/kg maize starch); (□), S24 starch paste (240 g amylose/kg maize starch). (For details of test meals, see Table 1 and p. 208.)

40 min after the start of the tests. The mean  $\Delta$  plasma glucose responses above baseline fasting levels and the negative areas above the glycaemic curve within the 0–180 min time-interval were significantly ( $P < 0.05$ ) higher after the glucose tolerance test than after ingesting starch pastes.

The mean plasma insulin profiles observed within 180 min after the starch paste and glucose tolerance tests are shown in Fig. 3 and summarized in Table 4. From 15 min to 45 min after the start of the tests, insulin levels differed significantly between the glucose tolerance test and the starch paste tests. Insulin areas under the curves from the 0–15 min

Table 1. *Physicochemical characteristics of carbohydrate test meals and comparison with glucose solution (GS)*

	Viscosity	Apparent viscosity ratio at 1/s shear rate (carbohydrate test meals: GS)	$\alpha$ -Amylase rate (EC 3.2.1.1) hydrolysis	In vitro percentage starch hydrolysis ratio at 30 min (carbohydrate test meals: GS*)
Glucose solution	Low	1	High	1
Starch paste				
240 g Amylose/kg maize starch (S24)	High	12500	Medium	0.48
500 g Amylose/kg maize starch (S50)	Medium	50	Low	0.35

\* Assuming 100% hydrolysis.

Table 2. *Characteristics of starch paste labelling in vivo in the rat*  
(Mean values and standard deviations for six observations)

Starch paste (g amylose/kg maize starch)...	240 (S24)		500 (S50)	
	Mean	SD	Mean	SD
Amount of starch paste ingested (g dry matter)	0.62	0.29	0.94	0.17
Stomach content dry matter remaining after ingestion time (% dry matter ingested)				
30 min	51.5	7.9	48.5	8.0
60 min	13.1	9.6	19.6	8.4
Stomach content dry matter activity (% of initial starch paste dry matter mass activity)				
30 min	98	7	105	4
60 min	102	5	103	3

There were no significant differences between starch pastes.

time-interval to the 0–180 min time-interval were significantly ( $P < 0.05$ ) higher for the glucose solution than for either starch paste. After the S50 starch paste, the mean insulinaemic  $\Delta$  peak value above baseline fasting levels was significantly ( $P < 0.05$ ) lower than that of S24 starch paste. From the 0–60 min time-interval to the 0–120 min time-interval, insulin areas under the starch curves differed significantly.

#### *Isotopic gastric emptying*

The marker gastric emptying kinetics for the three carbohydrate meals are shown in Fig. 4. The percentages of tracer remaining isotopic after the glucose tolerance and S24 starch paste tests were significantly higher than that after the S50 starch paste test at 30 min and 40 min after the first scan. The difference between the percentages of the isotopic tracer remaining after the two starch pastes remained significant until 80 min after the first scan. The mean characteristics of fitted gastric emptying curves are summarized in Table 5.

#### *Correlations*

No correlation was found between individual  $T_{1/2}$  values and individual plasma responses (Fig. 5). However, there was a positive relationship between carbohydrate bioavailability

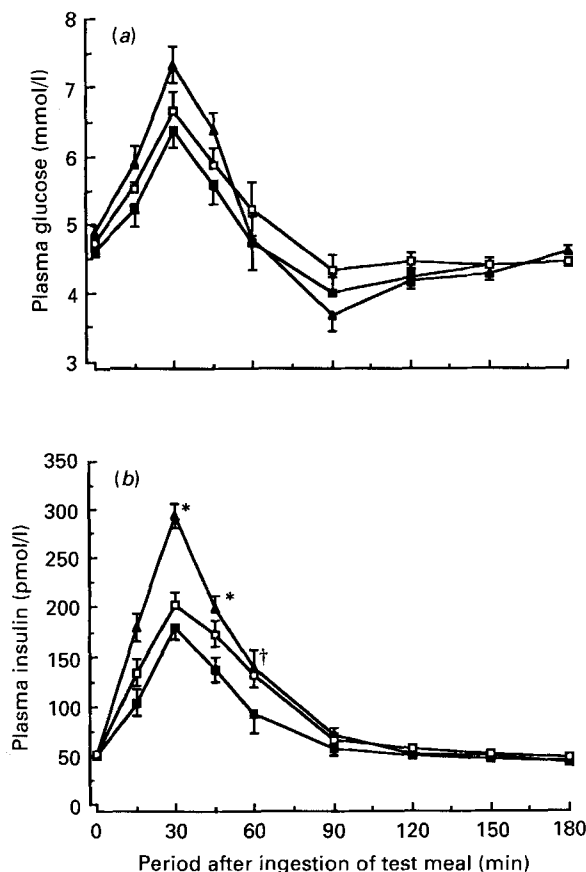


Fig. 3. Plasma glucose (a) and insulin (b) changes (means and standard deviations represented by vertical bars) in nine healthy human volunteers for 180 min after receiving carbohydrate test meals. (▲), Glucose solution (GS); (■), S50 starch paste (500 g amylose/kg maize starch); (□), S24 starch paste (240 g amylose/kg maize starch). (For details of test meals, see Table 1 and p. 208.) Each carbohydrate test contained 25 g starch or equivalent glucose units. \*Mean values for GS and starch pastes were significantly different ( $P < 0.05$ ). †Mean values for GS and S50 were significantly different ( $P < 0.05$ ).

(glucose or oligosaccharides solubilized by *in vitro*  $\alpha$ -amylase at 30 min) and the glucose  $\Delta$  peak, insulin  $\Delta$  peak and insulin area below the 0–180 min curve values (Fig. 6).

#### DISCUSSION

Our results confirm that glucose and insulin increments after carbohydrate test meals are closely related to their  $\alpha$ -amylase susceptibility. With regard to the substantial differences in plasma responses observed after the three meals,  $\alpha$ -amylase *in vitro* susceptibility rather than viscosity seems to play the major role.

Gastric emptying was characteristic of a liquid emptying behaviour. Whatever their viscosity, the same pattern of emptying was observed for the three meals and the differences were small, although statistically significant, between S50 and the two other carbohydrates. Therefore, the relevance of these differences in gastric emptying rates should be questioned; it seems likely that in more physiological conditions with a solid–liquid meal containing lipids, proteins, and acidic foods, such differences in gastric emptying rates would probably

Table 3. *Plasma glucose responses in healthy human volunteers after ingestion of carbohydrate test meals\**  
(Mean values and standard deviations)

	Glucose solution		Maize starch pastes (g amylose/kg)			
			240 (S24)		500 (S50)	
	Mean	SD	Mean	SD	Mean	SD
Plasma glucose (mmol/l)						
Fasting	4.9 <sup>a</sup>	0.1	4.7 <sup>a</sup>	0.1	4.6 <sup>a</sup>	0.1
Δ Peak above fasting value†	2.6 <sup>a</sup>	0.2	2.2 <sup>a</sup>	0.2	1.9 <sup>a</sup>	0.2
Δ Peak below fasting value‡	-1.4 <sup>a</sup>	0.2	-0.8 <sup>b</sup>	0.2	-0.7 <sup>b</sup>	0.2
Peaking time (min)						
Peak above fasting value	30 <sup>a</sup>	4	38 <sup>a</sup>	4	32 <sup>a</sup>	4
Peak below fasting value	87 <sup>a</sup>	6	107 <sup>a</sup>	6	98 <sup>a</sup>	6
Area below 0-180 min curve above fasting value ((mmol/l)·min)	77.7 <sup>a</sup>	10.5	81.6 <sup>a</sup>	10.5	58.8 <sup>a</sup>	10.5
Area above 0-180 min curve below fasting value ((mmol/l)·min)	-94.4 <sup>a</sup>	9.4	-46.6 <sup>b</sup>	9.4	-46.1 <sup>b</sup>	9.4

<sup>a, b</sup> Within columns, mean values with different superscript letters were significantly different (ANOVA):  $P < 0.05$ .

\* For details, see Table 1 and p. 208.

† Difference between maximal and fasting values.

‡ Difference between minimal and fasting values.

Table 4. *Plasma insulin responses in healthy human volunteers after ingestion of carbohydrate test meals\**  
(Mean values and standard deviations)

	Glucose solution		Maize starch pastes (g amylose/kg)			
			240 (S24)		500 (S50)	
	Mean	SD	Mean	SD	Mean	SD
Plasma insulin (pmol/l)						
Fasting	51.7 <sup>a</sup>	2.2	51.7 <sup>a</sup>	2.2	50.2 <sup>a</sup>	2.2
Δ Peak above fasting value†	306.4 <sup>a</sup>	10.8	226.7 <sup>b</sup>	10.8	186.6 <sup>c</sup>	10.8
Peaking time (min)						
Peak above fasting value	30 <sup>a</sup>	4	32 <sup>a</sup>	4	33 <sup>a</sup>	4
Area below 0-180 min curve above fasting value ((nmol/l)·min)						
0-15 min	1.0 <sup>a</sup>	0.1	0.6 <sup>b</sup>	0.1	0.4 <sup>b</sup>	0.1
0-30 min	3.7 <sup>a</sup>	0.2	2.4 <sup>b</sup>	0.2	1.8 <sup>b</sup>	0.2
0-45 min	6.6 <sup>a</sup>	0.3	4.4 <sup>b</sup>	0.3	3.4 <sup>c</sup>	0.3
0-60 min	8.4 <sup>a</sup>	0.4	5.9 <sup>b</sup>	0.4	4.3 <sup>c</sup>	0.4
0-90 min	10.0 <sup>a</sup>	0.7	7.3 <sup>b</sup>	0.7	5.1 <sup>c</sup>	0.7
0-120 min	10.3 <sup>a</sup>	0.8	7.6 <sup>b</sup>	0.8	5.2 <sup>b</sup>	0.8
0-150 min	10.4 <sup>a</sup>	0.8	7.7 <sup>b</sup>	0.8	5.3 <sup>b</sup>	0.8
0-180 min	10.5 <sup>a</sup>	0.9	7.8 <sup>b</sup>	0.9	5.3 <sup>b</sup>	0.9

<sup>a, b, c</sup> Within columns, means values with different superscript letters were significantly different (ANOVA):  $P < 0.05$ .

\* For details, see Table 1 and p. 208.

† Difference between maximal and fasting values.



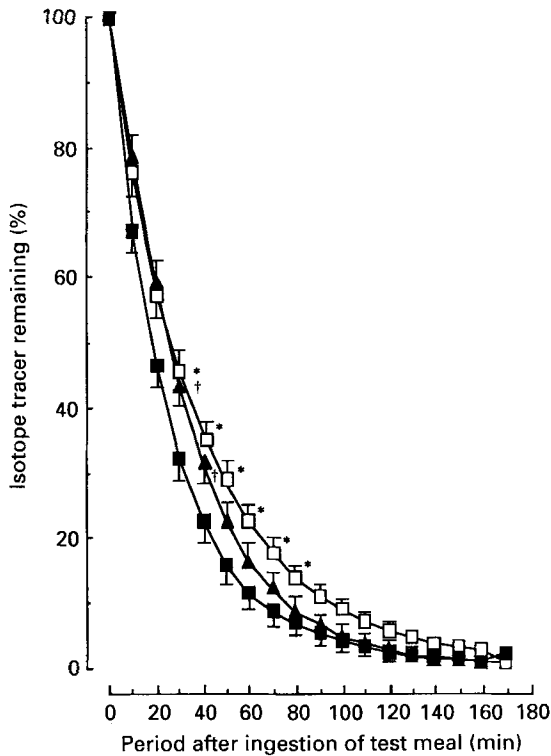


Fig. 4. Percentages (means and standard deviations represented by vertical bars) of gastric  $^{99m}\text{Tc}$  activity remaining in nine healthy human volunteers, within 170 min after ingestion of labelled test meals. ( $\blacktriangle$ ), Glucose solution (GS); ( $\blacksquare$ ), S50 starch paste (500 g amylose/kg maize starch); ( $\square$ ), S24 starch paste (240 g amylose/kg maize starch). (For details of test meals, see Table 1 and p. 208.) \*Mean values for S24 and S50 were significantly different ( $P < 0.05$ ). †Mean values for GS and S50 were significantly different ( $P < 0.05$ ).

disappear. However, in order to identify the different factors governing plasma responses to starchy foods we decided to use pure carbohydrate meals (starch and glucose). This choice ensured a specific and correct isotopic labelling of glucose solution and starch paste, and enabled a valid measurement of *in vitro* viscosity.

At 30 min after an oral administration of  $^{99m}\text{Tc}$ -DTPA-labelled saline solution, less than 1% of the radioactivity adheres to the mucosa (Chaudhuri, 1974); thus for the glucose solution  $^{99m}\text{Tc}$ -DTPA labelling was satisfactory. In a preliminary study we tried to evaluate starch gastric marker stability in humans. However, because of great difficulties in isolating labelled starch from mucus in gastric contents, even after a high-speed prolonged centrifugation, we were compelled to perform these stability studies in rats. Although times of ingestion in rats differed from those in humans, starch paste gastric emptying rates were similar. More than 80% of ingested starches were emptied at 60 min in both studies. Starch labelling was homogeneous and, thus, we assumed from the *in vivo* animal experiments that it was stable in the human stomach during the first hour after the beginning of ingestion.

The cooking procedures were identical for both starches. Differences in viscosity and  $\alpha$ -amylase susceptibility between starches result from their botanical origin and amylose content. When amylose content increases (S50), starch molecules are dispersed with greater difficulty (Guilbot & Mercier, 1985), thus lowering their viscosity and bioavailability. S24, which was the most gelatinized starch, gave the highest viscosity and the highest  $\alpha$ -amylase

Table 5. *Isotope gastric emptying in healthy human volunteers after ingestion of labelled carbohydrate test meals\**  
(Mean values and standard deviations)

	Maize starch pastes (g amylose/kg)							
	Glucose solution		240 (S24)				500 (S50)	
	Mean	SD	Mean	SD	Mean	sd		
$\alpha$ -Parameter†	1.18 <sup>a</sup>	0.09	1.04 <sup>a</sup>	0.09	1.00 <sup>a</sup>	0.09		
$r^2$	0.997 <sup>a</sup>	0.003	0.994 <sup>a</sup>	0.003	0.989 <sup>a</sup>	0.003		
$T_{1/2}‡$	26 <sup>a</sup>	2	29 <sup>a</sup>	2	19 <sup>b</sup>			

<sup>a, b</sup> Within columns, mean values with different superscript letters were significantly different (ANOVA):  $P < 0.05$ .

\* For details see Tables 1 and 2 and p. 208.

† Gastric emptying values were fitted using power exponential model and non-linear least squares of geometric means of anterior and posterior values.

‡ Half gastric emptying time.

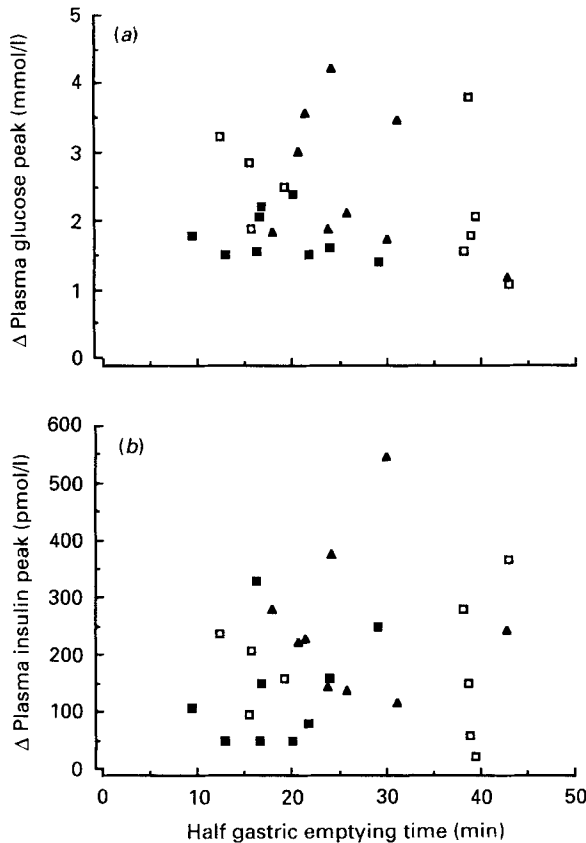


Fig. 5. Individual  $\Delta$  plasma glucose peak (a) and  $\Delta$  plasma insulin peak (b) values v. individual half gastric emptying time of a glucose solution (▲); S24 starch paste (240 g amylose/kg maize starch) (□); and S50 starch paste (500 g amylose/kg maize starch) (■), observed for nine healthy human volunteers. (For details of test meals, see Table 1 and p. 208.) No correlation was found between  $\Delta$  plasma peak values and half gastric emptying time for any of the carbohydrate test meals.

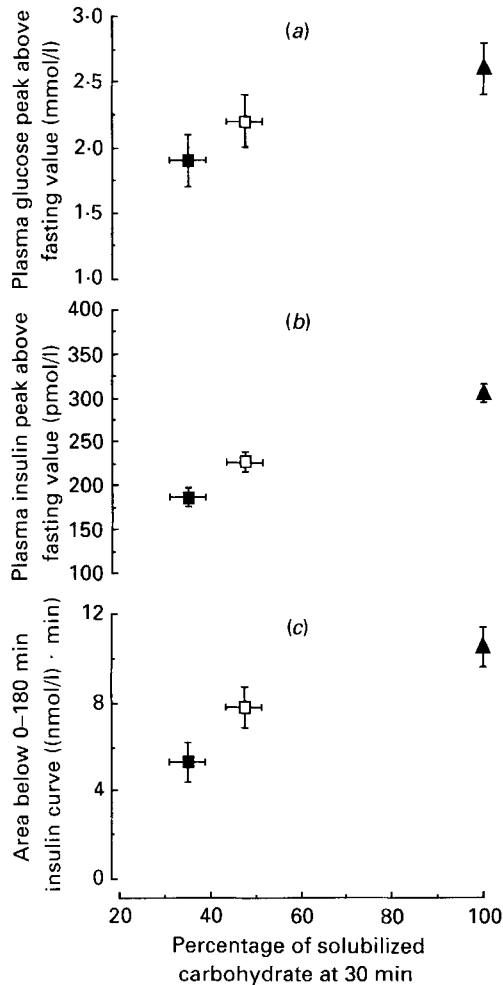


Fig. 6. Relationships between *in vitro* percentage starch hydrolysis at 30 min (assuming 100% for glucose solution (GS)) (means and standard deviations represented by horizontal bars) and (a),  $\Delta$  plasma glucose peak values (means and standard deviations represented by vertical bars, mmol/l); (b),  $\Delta$  plasma insulin peak values (means and standard deviations represented by vertical bars, pmol/l); (c) area below 0-180 min insulin curve (means and standard deviations represented by vertical bars ((nmol/l)·min)) for GS ( $\blacktriangle$ ); S24 starch paste (240 g amylose/kg maize starch) ( $\square$ ); and S50 starch paste (500 g amylose/kg maize starch) ( $\blacksquare$ ) in nine healthy human volunteers. (For details of test meals, see Table 1 and p. 208.)

susceptibility. Glucose solution was tested as a digestible carbohydrate with a low viscosity and a high bioavailability (100%). We could not test a high viscosity, low  $\alpha$ -amylase susceptibility starch product because it is impossible to obtain such a product using only starch molecules.

Starch pastes had a non-Newtonian rheological behaviour (i.e. apparent viscosity depends on shear rate; Doublier, 1987). Starch apparent viscosity ratio was adjusted so as not to depend on shear rate. Although the digestive shear rates and hence the *in vivo* starch paste viscosities are unknown, the apparent viscosity ratio can be assumed to be the same as that in the stomach.

Whatever the viscosity, carbohydrate gastric emptying patterns were similar and

exponential ( $\alpha$ -parameter about 1), which is characteristic of a liquid emptying behaviour (Hunt & Stubbs 1975; Grimes & Goddard, 1977). These results are in disagreement with those of Erhlein & Prove (1980), who studied the effect of the viscosity of potato-granule meals on gastric emptying in dogs via a duodenal cannula. The emptying curves of medium ( $10^5$  mPa·s)- and high ( $10^6$  mPa·s)-viscosity meals followed a sigmoidal pattern. It is difficult to compare these two experiments which were conducted using different techniques for gastric emptying measurements. In the canine experiments, the effect of the intestine on gastric motility and emptying which might be produced by starch hydrolysis were eliminated.

The  $T_{1/2}$  for the isotopic marker used for the glucose solution tested was in agreement with previous studies in humans (Hunt & Stubbs, 1975; Brener *et al.* 1983). The  $T_{1/2}$  for the isotopic marker used for S50 starch paste was significantly shorter than that of the glucose solution. The apparent viscosity ratio, S50:GS, was nevertheless high (50/1). The difference in gastric emptying has been related to the difference in osmotic pressure which influences gastric emptying via duodeno-gastric feedback. Using the intubation technique, Hunt (1960) had shown in adults that 20 min after ingestion the intragastric volume of a meal containing starch was less than that for a meal containing glucose. Both carbohydrates were emptied more slowly than water, with the implication that both starch and glucose excited receptors and slowed gastric emptying. Husband *et al.* (1970) showed that in newborn infants, a boiled starch test meal (waxy sorghum) left the stomach more rapidly than glucose solution. They suggested that the rapid emptying of the starch test meal is related to the relatively low secretion of pancreatic amylase, which in turn results in slow hydrolysis of starch. The significant difference in  $T_{1/2}$  between S24 and S50 starch pastes might be explained by two mechanisms: (a) a greater inhibitory influence of the intestine on gastric motility for S24 starch paste due to the higher  $\alpha$ -amylase susceptibility of S24, (b) a modulating effect of viscosity on gastric motility (apparent viscosity ratio S24:S50 is 250). With such a difference viscosity can become a factor in slowing down carbohydrate gastric emptying. For pectin-induced viscosity, several authors (Flourié *et al.* 1985; Rainbird & Low, 1986) have suggested that there is a threshold viscosity below which gastric emptying modifications are not seen.

The non-significant relationship between individual  $T_{1/2}$  and plasma glucose or insulin responses for the ingested carbohydrates suggests that either the hydrolysis step is the major physiological determinant of plasma carbohydrate effects or the amount of carbohydrate emptied within the first 10 min determines the carbohydrate fraction of the meal which produces the incremental plasma effect.

Further investigations are now required to elucidate the physiological mechanisms governing solid starchy food plasma responses. Physical characteristics such as particle size or particle stiffness might influence gastric emptying so that these physical characteristics would become determinants of food plasma effects.

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