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Carbohydrate digestion and release of pancreatic polypeptide in health and diabetes mellitus

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SUMMARY **The effects of meal volume and luminal digestion of carbohydrates on the release of pancreatic polypeptide (HPP) were investigated in eight healthy subjects and in six patients who had non-insulin dependent diabetes mellitus. On one occasion each subject ingested a placebo with 200 ml water and a starch (50 g) pudding meal (400 ml) 30 minutes later. On another occasion an amylase inhibitor that retards intraluminal starch digestion was given with the water and starch. In normal subjects, water caused a moderate rise in HPP plasma levels (16·9 (10·9) pg/ml; $p<0·02$) and ingestion of starch increased HPP in a double peaked pattern. The mean increments of the peaks were 45·0 (15·2) pg/ml ($p<0·02$) and 41·1 (17·3) pg/ml ($p<0·05$), respectively. In the diabetic subjects, the HPP concentrations did not increase in response to water. After ingestion of starch the diabetics had two peaks of HPP that were similar in magnitude, but the early postprandial peak was delayed significantly compared to normal subjects (37·5 (5·1) min *v* 23·4 (3·9) min; $p<0·05$). The amylase inhibitor (5 or 10 g) reduced the early postprandial HPP peak by 79% ($p<0·05$) in normal subjects and 4 g of the inhibitor reduced the early HPP peak by 58% ($p<0·05$) in the diabetics. In both groups ingestion of the amylase inhibitor abolished the late HPP peak ($p<0·05$). In conclusion, carbohydrate induced HPP release is dependent on undisturbed intraluminal starch digestion.**

Human pancreatic polypeptide (HPP) is a 36-amino acid polypeptide formed mainly in the pancreas.[1,2] The physiological role of HPP is uncertain, but it is probably involved in the regulation of pancreatic function. Postprandially, HPP is released by stimuli including cephalic stimulation, gut distension and presence of nutrients in the gastrointestinal lumen.

While it is assumed that cephalic or distension induced HPP release is exclusively or mainly mediated by the vagus, regulation of nutrient induced HPP release is less well understood.[3] For example, it is unknown whether digestion of nutrients within the gut lumen is necessary to release HPP from the pancreas postprandially.

Our aims were to determine if HPP is released by a non-caloric volume stimulus (water) or intraluminal digestion of carbohydrate in healthy volunteers and in subjects with diabetes mellitus. The response to reduced and normal rates of intraluminal carbohydrate digestion were measured by giving a starch meal with and without an amylase inhibitor that decreases starch digestion.[4]

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Methods

SUBJECTS

The protocol was approved by the Mayo Institutional Review Board and informed written consent was obtained from all participants. Eight normal subjects (five men, age range 19 to 47 years) and six diabetic patients (four men, age range 53 to 65 years) participated in this study. Each patient had the onset of non-insulin-dependent diabetes mellitus (type II) after the age of 40 years which was present for eight to 29 (mean 17·8) years. No patient had a history of ketoacidosis nor clinical evidence of autonomic or somatic neuropathy or other diabetic complications (history, physical examination). All of the patients met the National Diabetes Data Group criteria for non-insulin-dependent (type II) diabetes mellitus.[5] Each normal and diabetic subject was an H_2 producer

in response to malabsorbed carbohydrate. One week before the study each subject had a breath H_2 response of >10 ppm over baseline in response to ingesting 20 g of lactulose.

EXPERIMENTAL DESIGN

In each subject the HPP response to a test meal was determined on two separate days. The test meal consisted of two courses. The first course was 200 ml water. The second course, given 30 min later, was 400 ml pure starch pudding that contained 50 g rice starch.

On one occasion, the test meal was administered without, and on the other occasion with a specific, inhibitor of human salivary and pancreatic α amylases[6] that we purified from Great Northern white beans.[7] Ingestion of 4 to 10 g of this inhibitor with 50 g starch[7 8] 2·9[9] to 6·0[10] g with mixed carbohydrate, fat and protein meals or perfusion of 2 to 5 mg per ml into the duodenum[7] causes >95% inhibition of pancreatic amylase secretion, decreases intraluminal digestion of starch in the human small intestine and reduces postprandial glycaemia. Five or 10 g of the inhibitor was given to normal subjects (n=4 each) and 4 g was given to diabetic subjects (n=6); 20% of the total inhibitor dose was added to the water and 80% to the starch pudding. The lower dose in diabetics was chosen because in normal subjects both inhibitor doses had similar effects of intaluminal amylase activity and starch digestion as well as postprandial glycaemia, but caused dose dependent diarrhoea.[4 8] Further, in diabetics the 4 g dose significantly reduced postprandial glucose, but caused less gastrointestinal symptoms than the high doses.[10]

Blood samples were taken before water was ingested, before the starch was ingested and postprandially every quarter hour for the first 90 minutes, every half hour from 90 to 300 minutes and hourly from 300 to 420 minutes. To avoid cephalic stimulation of HPP release, water drinks and starch meals were kept in a separate room and only taken into the study area immediately before their ingestion. End expiratory breath samples were taken every 15 minutes throughout the experiment.

ANALYSES AND STATISTICS

Plasma concentrations of HPP were measured by radioimmunoassay as previously described.[11] Breath H_2 concentrations were assessed by gas chromotography as previously reported.[8]

Within each group the effect of water (volume) was assessed by subtracting preprandial values from the values obtained 30 minutes after water ingestion (immediately before ingestion of the starch meal) and evaluating the difference by paired *t* tests. The effects of the amylase inhibitor on the response to water was assessed by comparing the increments obtained for the placebo studies with the increments obtained with the inhibitor by using paired *t* tests.[12] Ingestion of the starch pudding regularly induced a double peaked increase in HPP levels with an early peak within the first postprandial hour, and a later peak between three and five hours postprandially. Thus, to assess the early increase in HPP levels, prestarch values were subtracted from poststarch values, and increments were integrated over the first hour after starch ingestion. Similarly, to assess the late increase, integrated increments were calculated for the period between 180 and 300 min after starch ingestion. These integrated responses were compared for placebo and inhibitor studies by using paired *t* tests.[12] Because the two inhibitor doses used in normal subjects had virtually identical effects, overall effects of both doses are presented unless indicated otherwise. Time intervals to HPP peaks were compared for normal subjects and diabetics by using unpaired *t* tests.[12] Data are presented as mean (SE).

Results

NORMAL SUBJECTS

Fasting levels of HPP did not differ significantly before ingestion of the test meals with placebo (77·6 (10·8) pg/ml) or amylase inhibitor (51·2 (8·3) pg/ml). Water without amylase inhibitor induced a moderate rise in mean HPP of 16·9 (10·9) pg/ml (22%) over the preprandial level ($p<0·02$; Fig. 1, left panel).

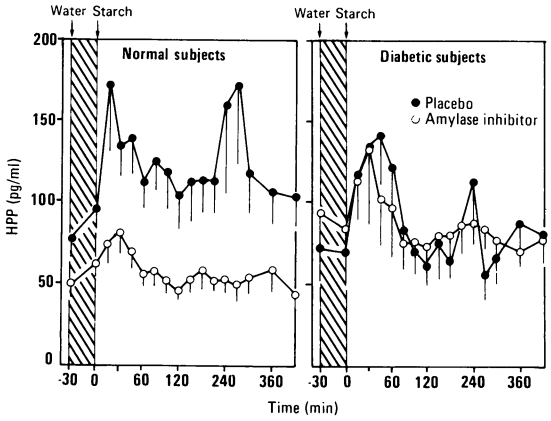

Fig. 1 Left panel: *Plasma HPP concentrations before ingestion of water (first arrow) and starch (second arrow) without (closed symbols) or with (open symbols) amylase inhibitor in normal subjects. Mean of n=8 experiments (SE). Right panel: Plasma HPP concentrations before ingestion of water (first arrow) and starch (second arrow) without (closed symbols) or with (open symbols) amylase inhibitor in diabetic subjects. Mean of n=8 experiments (SE).*

Ingestion of the starch pudding subsequently caused a double peaked increase in HPP concentrations (Fig. 1, left panel). The mean of maximal HPP levels during the first hour (early peak) was 190·6 (43·2) pg/ml and this peak occurred 15 to 45 min (mean 23·4 (3·9) min) postprandially. The mean increment during the first hour following starch ingestion was 45·0 (15·2) pg/ml ($p<0·02$; Fig. 2, left panel). Mean HPP values of the second peak which occurred during the fourth and fifth hours (mean 227 (17) min) were similar to the values of the first peak (peak concentration=221·5 (60·6) pg/ml; mean increment=41·1 (17·3) pg/ml; $p<0·05$ *v* preprandial and the second and third postprandial hours; (Fig. 2, left panel). Ingestion of the placebo meal did not induce a significant increase in breath H_2 above preprandial concentrations (7·8 (1·1) ppm).

Ingestion of water and the ingestion of the combination of water and the amylase inhibitor was associated with a similar rise in HPP over preprandial levels (Fig. 2, left panel). By contrast, addition of the amylase inhibitor to the starch pudding decreased ($p<0·05$) the integrated HPP response during the first hour by 79% and completely abolished the late peak. Breath H_2 levels increase significantly in response to the starch meal with amylase inhibitor (from 5·8 (0·9) ppm to 21·3 (3·6) ppm; $p<0·001$).

DIABETIC SUBJECTS

In diabetics, consumption of 200 ml water did not alter fasting HPP levels (70·6 (20·4) *v* 72·6 (21·4) pg/ml; Fig. 1, right panel). Ingestion of the starch pudding induced a double peaked response, but the first peak occurred after 37·5 (5·1) min, which was significantly later than in normal subjects ($p<0·05$). The mean of the maximal HPP concentration of each diabetic patient during the first hour (the first peak) was 150·0 (34·5) pg/ml ($p<0·005$ *v* preprandial) and the mean increment over prestarch concentration during the first hour after starch ingestion was 60·2 (10·4) pg/ml (Fig. 2, right panel). Similar to normal subjects, diabetics had a second peak that occurred after 223 (10) min (Fig. 1, right panel) and had a mean peak concentration of 116·8 (27·2) pg/ml ($p<0·01$ *v* preprandial) and a mean increment of 24·0 (4·7) pg/ml (Fig. 2, right panel) during the fourth and fifth hours. The placebo meal did not alter postprandial breath H_2 concentrations compared to preprandial levels (2·3 (0·3) ppm).

The combination of water and amylase inhibitor did not raise HPP levels above fasting levels (94·4 (20·8) *v* 83·8 (22·4) pg/ml; Fig. 2, right panel). As in the normal subjects, HPP concentration in response to starch was significantly decreased by the inhibitor (Figs 1 and 2, right panels). The mean increment during the first hour was reduced by 58% ($p<0·05$; Fig. 2, right panel) and the late peak was abolished. With amylase inhibitor, there was a slight, but significant, increase in postprandial breath H_2 concentrations (from 2·0 (0·2) ppm to 6·8 (2·0) ppm; $p<0·05$).

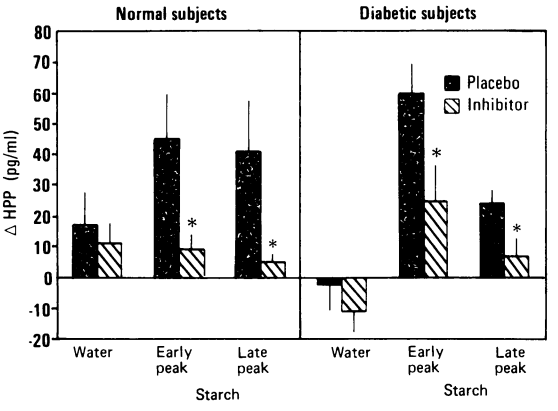

Fig. 2 Left panel: *Increment response of plasma HPP to ingestion of water (left) and starch (middle: mean integrated increment during the first 60 min after starch; right: mean integrated increment during the fourth and fifth hours following starch), without (hatched bars) or with (gray bars) amylase inhibitors in normal subjects. *$p<0·05$ v placebo.* Right panel: *Increment response of plasma HPP to ingestion of water (left) and starch (middle: mean integrated increment during the first 60 min after starch; right: mean integrated increment during the fourth and fifth hours following starch), without (hatched bars) or with (gray bars) amylase inhibitor in diabetic subjects. *$p<0·05$ v placebo.*

Discussion

In healthy subjects HPP plasma concentrations increased slightly in response to the volume load and increased markedly in a double peaked pattern in response to the starch meal. In comparison with healthy subjects, patients with longstanding type II diabetes mellitus had a diminished or absent HPP response to the non-caloric volume stimulus and a quantitatively normal, but delayed carbohydrate induced first peak of HPP concentrations. When intraluminal carbohydrate digestion was inhibited, the postprandial response to starch was markedly reduced in both normals and diabetics. These findings suggest that different mechanisms mediate postprandial HPP release in response to the volume stimulus and the carbohydrate stimulus after ingestion of a carbohydrate meal.

Postprandial release of HPP has been assumed to be mediated mainly by vagal cholinergic[13,14] and partly by non-vagal cholinergic[15,16] pathways and elicited by mechanisms such as cephalic stimula-

tion[16 17], gastroduodenal distension,[6 18–22] and presence of nutrients in the gastrointestinal lumen.[22–26] The initial rise in postprandial HPP concentration is believed to be secondary to gastroduodenal distension induced by the meal volume, mediated by a vagal reflex and to comprise approximately 10-20% of the overall postprandial HPP increase.[3 27 28]

Our observations that in normal subjects water alone and the starch meal with the amylase inhibitor induced an increase in HPP during the early postprandial phase are consistent with this hypothesis. However, as amylase inhibition markedly reduced the early peak and completely abolished the late peak of HPP we believe that intraluminal digestion of complex carbohydrate is a major factor for the intestinal phase of the HPP response to ingesting starch. We have previously shown[7] that ingestion of the inhibitor delays the digestion of starch intraluminally. Consequently the absorption of carbohydrate is reduced and the postprandial rise in plasma glucose is decreased. Therefore, luminal generation of the products of carbohydrate digestion or the absorption or both are probably necessary for carbohydrate induced HPP release.

Previously it was observed that inhibition of intestinal disacchridase activity did not reduce the HPP response to a mixed meal. In that study, however, it is likely that non-carbohydrate nutrients within the duodenum masked any effects that carbohydrates may have on HPP release.[29] As we detected no difference in HPP release between water induced HPP release and the amylase inhibitor in addition to water, we believe it is unlikely that the amylase inhibitor excited non-specific inhibitory effects on endogenous HPP release. Thus, our studies suggest that inhibition of carbohydrate digestion before the luminal generation of glucose reduces carbohydrate induced HPP release.

The results in the diabetic patients indicate that their quantitative HPP response to carbohydrate ingestion was similar to that observed in normal subjects, suggesting that their pancreatic capacity for HPP secretion is not grossly altered. Occurrence of the first HPP peak after carbohydrate ingestion, however, was significantly delayed compared with normal subjects. Because carbohydrate-induced HPP release is dependent on luminal carbohydrate digestion, this delay is probably a consequence of slowed luminal generation and absorption of glucose, most likely caused by retarded gastric emptying of ingested starch. This retardation could not have been caused by a decreased rate of luminal digestion due to impaired pancreatic enzyme secretion because end expiratory breath H_2 concentration did not increase in response to the starch meal. Moreover, pancreatic secretory capacity must be reduced to less than 10% of normal to give rise to a discernable disturbance of digestion[30 31] and our patients had no evidence of such severe exocrine pancreatic insufficiency.

A more likely explanation for the delay in postprandial HPP release in the diabetics is slower than normal gastric emptying of ingested starch. It is conceivable that a retardation of gastric emptying rate in patients with long standing diabetes may reflect an alteration in vagal function.[32] This hypothesis is supported by the observation that ingestion of water, a non-caloric volume stimulus that in healthy individuals elicits a transient, brief 40-50 minute HPP release exclusively *via* vagal cholinergic pathways,[33 34] did not produce an increase in plasma HPP levels in our diabetic subjects. As we did not test these patients for asymptomatic autonomic neuropathy, the question of whether these changes were, indeed, caused by subclinical alteration of the autonomic nervous system remains unanswered. There is evidence, however, that early, largely subclinical disturbances of certain vagal functions may occur in the course of diabetes mellitus. For example, it has been shown that the vagally mediated HPP release in response to hypoglycaemia is disturbed in diabetic patients with or without clinical signs of diabetic autonomic neuropathy[35–40] and in patients with non-diabetic autonomic neuropathy.[41 42] As we have shown previously that in normal subjects the starch meal used in this study evokes only mild reactive hypoglycaemia in the late postprandial period, and no hypoglycaemia in the late postprandial period in diabetic subjects,[8] it is unlikely that hypoglycaemia plays a major role in the generation of the late HPP peak.

In the presence of amylase inhibition, carbohydrate induced HPP release in diabetic patients was also significantly reduced compared with placebo. This reduction was less pronounced than in the experiments with normal subjects receiving larger inhibitor doses. Because carbohydrate malabsorption as assessed by postprandial H_2 production was also smaller in the diabetic group, it is likely that these effects were a result of incomplete amylase inhibition in the patients receiving the 4 g dose.

Plasma concentrations of HPP are age related. Mean basal concentrations of HPP increase approximately 30 pg/ml per decade[43] and after oral glucose ingestion, elderly men and women have a higher concentration of HPP and a larger increase over basal levels, but the time to peak concentration is the same for both age groups and sexes.[44] The trend towards higher basal concentrations and the increased concentration of HPP in the diabetic subjects compared with the normal subjects in response to the amylase inhibitor may have occurred because our diabetic patients were older. Nevertheless, despite the differ-

ence between normal subjects and diabetics in response to the inhibitor, in both groups the amylase inhibitor reduced the HPP response to the starch meal indicating that carbohydrate induced HPP release is in part dependent upon the degree of carbohydrate digestion.

Humoral mediators such as cholecystokinin and gastric inhibitory polypeptide (GIP) also may be involved in regulating the intestinal phase of postprandial liberation of HPP.[24 45–49] For example, physiological levels of GIP have been reported to release HPP in man *via* cholinergically controlled pathways.[47 50] Our observation that postprandial release of GIP after a carbohydrate meals is abolished when intraluminal starch digestion is slowed[4] is consistent with the hypothesis that GIP participates in the control of HPP release.[47 50]

In conclusion, the regulation of the intestinal phase of carbohydrate induced HPP release appears to be dependent upon the rate and completeness of carbohydrate digestion.

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