

Duodenal juice total protein and pancreatic enzyme synthesis, turnover, and secretion in patients after acute pancreatitis

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Abstract

It is controversial whether acute pancreatitis has longterm effects on pancreatic function. Pancreatic enzyme synthesis, turnover, and secretion were measured in 10 patients in clinical remission who had had one or more (one to six) attacks of acute alcoholic pancreatitis. The studies were done between two and 29 months after the most recent attack. A control group included five patients with no evidence of pancreatic disease. A four hour primed/continuous intravenous infusion of [¹⁴C]L-leucine tracer was given with secretin (2 U/kg/h) and cholecystokinin (0.5 U/kg/h) and secreted duodenal juice aspirated. Amylase and trypsin were extracted from duodenal juice by affinity chromatography, permitting measurement of the rate of isotope incorporation into total protein, amylase, and trypsin. The results showed non-parallel changes in enzyme synthesis and turnover with decreases in total enzyme protein and amylase synthesis and turnover but preservation of trypsin synthesis and turnover. The low turnover rates may be ascribed to continuing pancreatic cell malfunction after recovery from acute alcoholic pancreatitis and suggest that the decreased amylase secretion rates are partly a consequence of impaired amylase synthesis and not simply because of loss of pancreatic tissue.

(Gut 1993; 34: 1261-1266)

The effect of acute pancreatitis on pancreatic function is unclear with some¹ but not all^{2,3} studies showing complete recovery as judged by secretory testing. Secretory tests may not be suitable, however, for assessing pancreatic function as preliminary studies have suggested that enzyme synthesis may be changed before changes in secretion are identified.⁴

In a previous study, we used a primed/4 hour continuous intravenous infusion of ¹⁴C labelled leucine to compare synthesis rates in patients with chronic calcific pancreatitis with those in patients with no pancreatic disease.⁵ The results showed that protein secreted during the first hour of stimulation was derived from pancreatic stores and that the turnover rate of stores was approximately 37%/h in normal human subjects. Calculation of these results assumed, however, that pancreatic juice protein was equivalent to enzyme protein. While this is a reasonable assumption in normal subjects, it can lead to significant error in patients with inflammatory disease, such as pancreatitis, where juice may be contaminated with exudative proteins.⁵

To exclude such possible error a method was developed for the extraction of pure enzyme protein from aspirated duodenal juice, based on affinity chromatography for both amylase⁶ and trypsin.⁷ These developments have now allowed us to perform measurements of total protein, amylase, and trypsin turnover in 10 patients who have experienced acute attacks of alcohol induced pancreatitis. In this study we have used this technique to assess whether there are any longterm changes in pancreatic function after recovery from acute alcoholic pancreatitis.

Patients

Ten male patients in clinical remission after between one and six attacks of clinically acute alcoholic pancreatitis were studied between two and 29 months after the most recent attack. Table I gives the clinical details of the patients. None of the patients, however, were overtly diabetic, had clinical steatorrhoea, or were taking pancreatic enzyme supplements. Five normal healthy men were selected as controls for the study. The controls had no history or symptoms suggestive of pancreatitis. There was no significant difference between the control and patient group with regard to age and body weight. The procedure was reviewed and accepted by the Hospital Medical Ethics and Radiation Safety Committees and informed signed consent was obtained from each patient.

Methods

We have shown that it would be possible to measure pancreatic enzyme secretion and synthesis rates simultaneously if approximately 50 µCi of a ¹⁴C labelled amino acid tracer was infused intravenously for four hours together with secretin and cholecystokinin.⁸

PROCEDURE

The procedure has been previously described⁶ in detail. After an overnight fast, subjects had an endoscopy and insertion of two Rüsck tubes, one into the duodenum and the other into the stomach. Constant suction was then applied to both tubes. The position of the tubes was frequently checked fluoroscopically during infusion. After intravenous administration of a pulse dose of 10 µCi [¹⁴C]L-leucine, a four hour intravenous infusion consisting of 500 ml 0.45% sodium chloride, 50 µCi [¹⁴C]L-leucine tracer, secretin (2.0 Crick-Harper-Raper U/kg/h), and cholecystokinin (0.5 CHR U/kg/h) was started. Gastric juice was discarded.

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Accepted for publication
19 January 1993

TABLE I Patients after acute pancreatitis

Patient	Age (y)	Weight (kg)	Duration alcohol abuse (y)	Age first attack (y)	Acute attacks (No)	Time between most recent acute attack and date study performed (months)
1	32	61	14	31	4	2
2	30	70	3	28	5	5
3	48	86	30	46	1	29
4	27	72	3	26	1	15
5	36	74	18	32	5	6
6	54	95	34	51	3	7
7	30	69	14	29	2	7
8	26	51	7	22	6	6
9	35	83	15	35	1	4
10	55	78	27	55	1	5

Duodenal contents were separated into 30 minute collections. Samples were collected on ice, the volume measured, and samples immediately assayed for amylase, trypsin, lipase, and bicarbonate. Five ml samples of duodenal juice were mixed with an equal volume of 10% trichloroacetic acid (TCA) and trasylol (10% vol/vol) added to the remainder. Samples were stored frozen (-20°C). Venous blood samples were taken at 1, 2.5, and 4 hours for measurement of plasma total amino acid concentrations, plasma leucine specific activity, and plasma protein synthesis rates.

THEORY AND CALCULATIONS

Previous studies have shown that during a primed/continuous four hour infusion of ^{14}C labelled leucine hormonally stimulated pancreatic enzyme secretion only becomes labelled after a delay period of approximately 100 minutes.⁹ As the time taken for secreted enzyme to pass into the duodenum is relatively small (three to four minutes),¹⁰ the delay period may be used as a measure of the time taken for the synthesis of new enzymes by the acinar cell. After the delay period there is a linear increase in the labelling of secreted enzymes curving off to a plateau value after four hours at which time pancreatic enzyme stores (zymogen) will be fully labelled at a specific activity equivalent to that of the precursor amino acid pool.⁶ Measurement of the rate of increase in the linear part of the curve

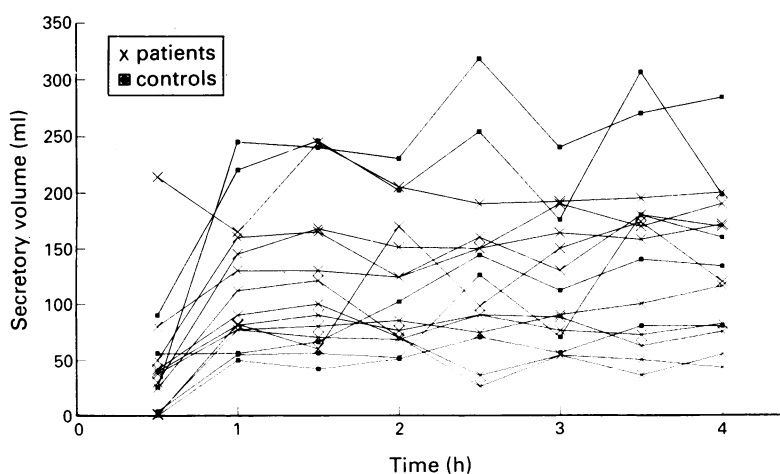


Figure 1: Secretory volumes of aspirated juice in post-acute pancreatitis patients and controls.

will permit calculation of the fractional turnover rate of the zymogen pool from the equation:

$$\text{enzyme pool turnover} = \frac{\Delta \text{SA}_{\text{protein}}}{\text{SA}_{\text{plasma max}}}$$

where, $\Delta \text{SA}_{\text{protein}}$ is the rate of increase of specific activity of bound leucine in protein and $\text{SA}_{\text{plasma max}}$ is the plateau specific activity of plasma leucine during the four hour infusion.¹¹ This assumes that the plasma free amino acid pool is representative of the precursor for pancreatic enzyme synthesis. Support for this assumption was obtained from a similar study in pigs that showed that amino acids derived from the extracellular space were used directly for pancreatic enzyme synthesis.¹² Finally pancreatic enzyme pool size can be calculated from the equation:

$$\text{pancreatic enzyme pool} = \frac{\text{enzyme secretion (U/h)}}{\text{enzyme turnover (\%/h)}}$$

SAMPLE ANALYSIS

Methods for the measurement of duodenal juice total protein specific radioactivities, pancreatic amylase and trypsin specific radioactivities, and plasma leucine specific radioactivities have been described.⁶

STATISTICAL ANALYSIS

Means of the control and post-acute pancreatitis groups were compared using the Student's *t* test for unpaired data. Correlation studies were performed using linear regression and Spearman's correlation coefficients were calculated.

Results

Results were analysed with and without values obtained for the two patients studied 15 and 29 months after their most recent acute attack of pancreatitis. Excluding these two patients made no difference, however, as they do not represent the outlying points. Mean and standard deviation figures therefore include these two patients.

TOTAL PROTEIN, ENZYME, AND BICARBONATE SECRETION

Figure 1 shows that individual secretory volumes were reasonably constant in patients and controls during the four hour infusion study, showing effective continual hormonal stimulation and satisfactory recovery. Data for enzyme secretion paralleled changes in secretory volume. There was no significant difference in mean total protein and trypsin secretion between the control and post-acute pancreatitis groups (Fig 2). Amylase secretion was significantly ($p < 0.05$) lower, however, in the post-acute pancreatitis patients (Fig 2). Mean lipase secretion was not significantly different in the two groups – that is, mean (SD) $94.1 (46.0) \text{ IU} \times 10^3/\text{h}$ in the control group and $56.4 (33.0) \text{ IU} \times 10^3/\text{h}$ in the post-acute pancreatitis group. Similarly, there was no significant difference in bicarbonate secretion between the two groups – that is, mean (SD) 27.0

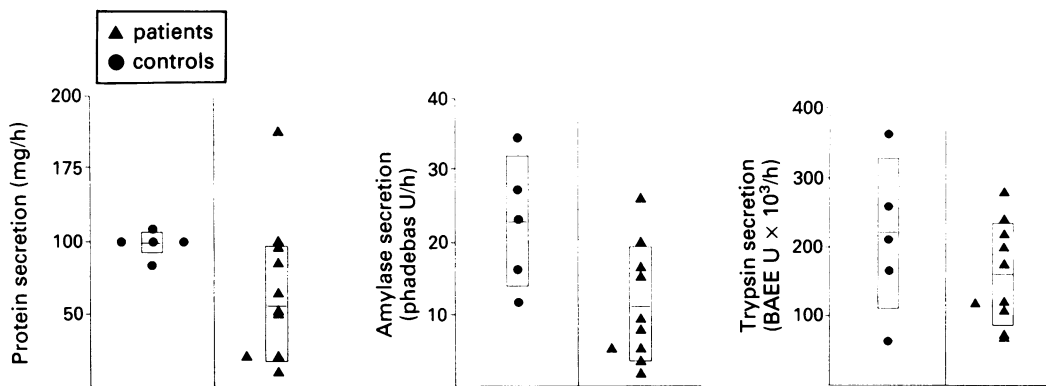


Figure 2: Pancreatic total protein amylase and trypsin secretion measured in the aspirated duodenal juice of five controls and 10 post-acute pancreatitis patients. Boxes show means and standard deviations. There was no significant difference in mean total protein and trypsin secretion between the post-acute pancreatitis patients and the controls. Mean amylase secretion, however, was significantly ($p < 0.05$) lower in the post-acute pancreatitis patients than it was in the controls.

(15.5) mmol/h in the control group and 15.4 (7.7) mmol/h in the post-acute pancreatitis group.

There was a positive correlation between the number of months elapsed since a patient's most recent attack and amylase secretion. Spearman's correlation coefficient was found to be 0.75 ($p < 0.05$). There was only one patient, however, who had a mean amylase secretion rate above the control mean value. This was the patient who was studied 29 months after his most recent acute attack. Only three other patients had amylase secretion rates that fell within the control range. These patients were studied 15, 6, and 4 months after their most recent acute attack. Other patients studied at 5, 6, and 7 months after their most recent acute attack had mean amylase secretion rates below the control range. If the patients studied at 15 and 29 months were excluded, there was no correlation between the number of months elapsed since a patient's most recent attack and amylase secretion.

APPEARANCE OF LABELLED PROTEIN, AMYLASE, AND TRYPSIN IN DUODENAL JUICE

Comparing the post-acute pancreatitis patients with the controls, the time taken for labelled total protein to appear in the duodenal juice was significantly ($p < 0.05$) longer in the post-acute

TABLE II Time elapsed (min) between the start of the intravenous infusion and the first appearance of labelled protein in duodenal juice

	Total protein		Amylase		Trypsin	
	Controls	Patients	Controls	Patients	Controls	Patients
Mean	27.8	57.8*	28.8	54.1**	41.6	43.4
SEM	6.0	8.7	4.5	4.8	12.2	4.0

* $p < 0.05$; ** $p < 0.01$.

TABLE III Comparison of amylase secretion and synthesis (mean (SEM)) in patients and controls

	Control (n=5)	One attack (n=4)	Multiple attacks (n=6)
Amylase secretion (phadebas U/h)	22.7 (4.1)	17.8 (3.6)	6.7 (2.2)**‡
Amylase synthesis (min)†	28.8 (4.5)	51.8 (9.1)*	55.7 (6.2)**

†Measured as the time taken for labelled amylase to appear in the duodenal juice; significantly different with control * $p < 0.05$, ** $p < 0.01$; ‡significantly different to one attack $p < 0.05$.

pancreatitis patients (Table II, Fig 3). Similarly, the time taken for labelled amylase to appear in duodenal juice was significantly ($p < 0.01$) longer in the post-acute pancreatitis patients. There was no significant difference, however, in the time taken for labelled trypsin to appear in the duodenal juice of the post-acute pancreatitis patients.

DUODENAL JUICE TOTAL PROTEIN, AMYLASE, AND TRYPSIN TURNOVER RATE

The turnover rate of duodenal juice protein was 33% lower in the post-acute pancreatitis patients compared with controls ($p < 0.05$) (Fig 4). Similarly the amylase turnover rate was 37% lower ($p < 0.01$). The trypsin turnover rate, however, was not significantly changed.

TOTAL PROTEIN, AMYLASE, AND TRYPSIN POOL SIZES

There was no significant difference in total protein, amylase, and trypsin pool sizes between the post-acute pancreatitis patients and the controls. Mean (SD) values were as follows: total protein (mg): 301 (61) in the control group and 380 (394) in the post-acute pancreatitis group; amylase (phadebas U): 92 (44) in the control group and 82 (72) in the post-acute pancreatitis group; and trypsin 999 (484) and 803 (553) in the post-acute pancreatitis group.

THE EFFECTS OF ONE v MULTIPLE ACUTE ATTACKS

The pancreatitis patients were divided into two groups - that is, those who had had one acute attack and those who had had multiple attacks. When comparing the group of patients who had had a single attack with the control group, there was no significant difference in amylase secretion (Table III). By contrast, the time taken for labelled amylase to appear in the duodenal juice was longer in the patients ($p < 0.05$). There was a significant difference in both secretion ($p < 0.05$) and synthesis ($p < 0.01$) between the multiple attack group and controls.

Discussion

We have shown that after acute pancreatitis,

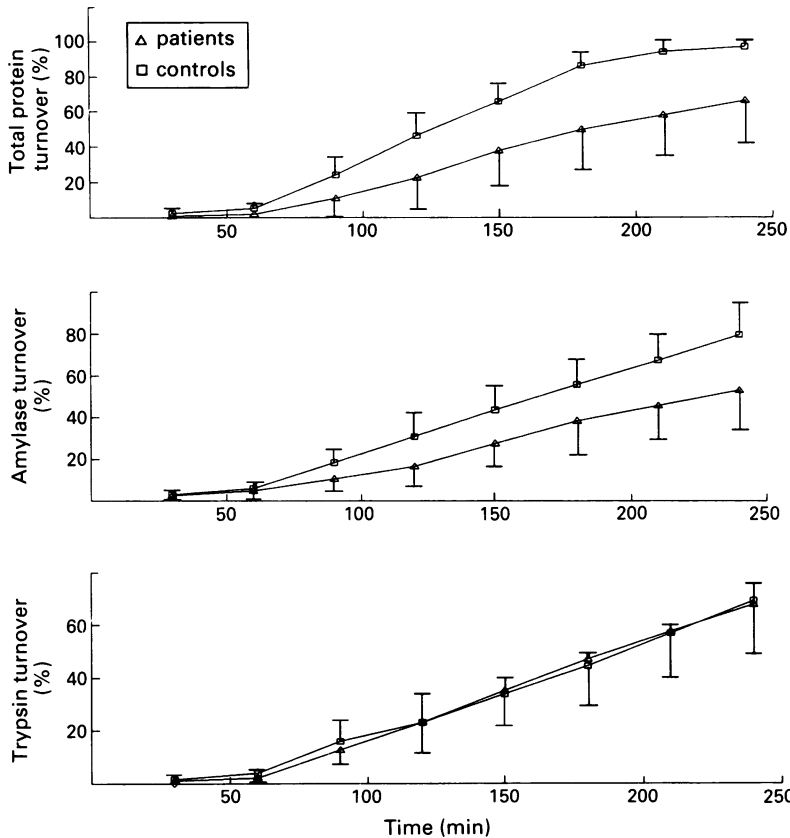


Figure 3: Pancreatic total protein, amylase, and trypsin turnover measured in the aspirated duodenal juice of five controls and 10 post-acute pancreatitis patients. Comparing the post-acute pancreatitis patients and the controls, there was a significant difference in the time taken for labelled total protein ($p < 0.05$) and amylase ($p < 0.01$) to appear in the duodenal juice. There was no significant difference, however, in the time taken for labelled trypsin to appear in the duodenal juice of post-acute pancreatitis patients.

amylase secretion was decreased whereas trypsin, lipase, and bicarbonate secretion were not. Patients after acute pancreatitis had slower duodenal juice protein and amylase turnover rates but trypsin turnover was not different with controls. The decreased total protein and amylase turnover rates are probably a result of a reduction in synthetic rates as the time taken for labelled total protein and amylase to appear in the duodenal juice was significantly longer in the the post-acute pancreatitis patients. There was no correlation between duodenal juice protein,

enzyme turnover rates, and enzyme secretion. There was a positive correlation, however, between the number of months elapsed from the most recent acute attack and amylase secretion.

It is possible that if a large group of post-acute pancreatitis patients was studied, trypsin, lipase, and bicarbonate secretion may also be found to be significantly decreased. When mean enzyme and bicarbonate secretion in the post-acute pancreatitis patients, however, were expressed as a percentage of those in the control group, amylase secretion was the lowest followed by bicarbonate, lipase, and then trypsin. Our results are in agreement with those of Marks and Thompssett who found that the earliest and most frequent finding in patients with pancreatic disease was a decrease in amylase secretion.¹³ This finding was confirmed by Burton *et al*¹⁴ and Amman *et al*.¹⁵ The secretion of amylase, however, was normal or less severely impaired than the secretion of lipase or of proteolytic enzymes in pancreatic disease in a number of other studies.¹⁶⁻²⁰ The discrepancies among published reports are partly a result of the very considerable scatter of ratios between the different enzymes in normal subjects.²¹⁻²³

Non-parallel enzyme secretion has been shown in patients with normal pancreatic function under certain stimulatory conditions.²⁴ Non-parallel enzyme synthesis has been reported in rats fed protein rich or carbohydrate rich diets.²⁵ A protein rich diet has been shown to augment proteolytic proenzyme synthesis and decrease amylase and lipase synthesis, whereas a carbohydrate rich diet only decreased the synthesis rate of anodic chymotrypsinogen. Infusion of caerulein has also been reported to cause divergent changes in pancreatic enzyme synthesis under certain conditions,²⁶ as has intraduodenal administration of oleic acid.²⁷ This study is the first to suggest non-parallel enzyme synthesis in man.

Various animal studies have shown inhibition of pancreatic enzyme secretion by pancreatic proteases in the duodenum.^{28,29} In the rat, this negative feedback control is mediated by cholecystokinin,³⁰⁻³³ which is probably released by protease sensitive proteins from either duodenal mucosa or pancreatic juice.^{30,33,34} There are

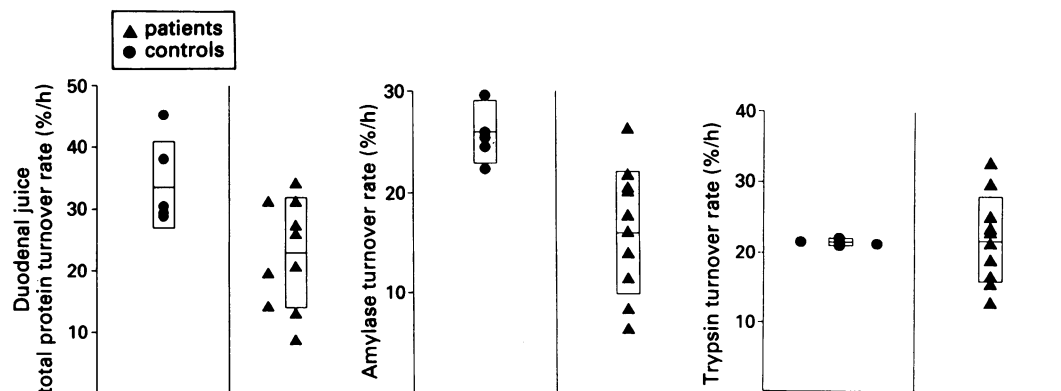


Figure 4: The pancreatic total protein, amylase, and trypsin turnover rates measured as a percentage of the intracellular total protein pool renewed per hour in the aspirated duodenal juice of five controls and 10 post-acute pancreatitis patients. Boxes show means and standard deviations. The mean total protein ($p < 0.05$) and amylase ($p < 0.01$) turnover rates were significantly lower in the post-acute pancreatitis patients than it was in the controls, however, there was no significant difference in mean trypsin turnover between the post-acute pancreatitis patients and the controls.

conflicting reports as to whether negative feedback inhibition exists in humans^{35,36} and whether it is mediated by cholecystokinin.^{37,38} Should negative feedback inhibition exist in man, however, amylase and lipase would not be involved and would therefore not be affected by compensatory mechanisms in the way that proteases are. Negative feedback inhibition would therefore explain the greater sensitivity of amylase secretion for the detection of pancreatic disease. One would then expect, however, lipase secretion to be an equally sensitive measurement. Although lipase secretion was not significantly lower in the post-acute pancreatitis patients, when mean enzyme secretion was expressed as a percentage of the control mean lipase secretion was lower than trypsin.

The lack of correlation between turnover of enzyme pools and secretion is perhaps due to interindividual variation in enzyme pool sizes. A large enzyme pool turning over at the same rate as a small one would produce more new enzyme, which would result in a greater amount of enzyme being available for secretion. Enzyme turnover would correlate with enzyme secretion only if pool sizes were similar.

The correlation between amylase secretion and the time elapsed since a patient's most recent attack, shows that amylase secretion is low after an acute attack, but improves with time. Individual results, however, show that the rate of recovery varies. Comparison of amylase secretion and synthesis results of patients who had had only one acute attack and those who had had two or more, shows that synthetic studies may be more sensitive than the standard pancreatic function test, which only measures secretion in detecting pancreatitis. Further study, with larger numbers is required to compare the relative sensitivity of secretion and synthesis measurements. Control amylase synthesis times obtained in this study are shorter than those obtained previously.³⁸ This may be because of the different types and doses of cholecystokinin used.^{39,40}

The most important finding of this study is the reduction in amylase synthesis and turnover in patients studied between 2 and 29 months after an attack of acute pancreatitis. This suggests that the decreased amylase secretion seen in the post-acute pancreatitis patients is as a result of not only possible loss of pancreatic tissue with chronic change but also because of prolonged pancreatic cell malfunction after an acute attack.

The authors are greatly indebted to Professor R E Kirsch for his helpful discussions and the use of his laboratory. The authors also wish to thank Dr G O Young for critically reviewing this manuscript, the Institute for Biostatistics of the South African Medical Research Council for performing the statistical analysis and Mrs Nel Tigler-Wybrandt, Mrs Sharon Bridger, and Mrs Elspeth Burke for performing the enzyme activity and bicarbonate assays.

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