

# Serum immunoreactive trypsin concentration after a Lundh meal

## Its value in the diagnosis of pancreatic disease

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**SUMMARY** The changes in serum trypsin concentration have been measured in 47 subjects for up to 2 hours after a Lundh meal. In 18 healthy controls, mean fasting trypsin concentration was  $285 \pm 125$  ng/ml (mean  $\pm 2$  SD). The maximum increase after the Lundh meal (the trypsin response ratio) was  $6.7 \pm 7.5\%$ .

Six patients with chronic renal failure had elevated fasting serum trypsin concentrations (range 460-1100 ng/ml) but trypsin response ratios fell within the control range.

Of five patients with relapsing pancreatitis, two had raised and three normal or low fasting trypsin. After stimulation two had elevated trypsin response ratios; one of the two had evidence of main duct obstruction.

Eleven out of 12 patients with chronic pancreatitis (with or without insufficiency) had low fasting trypsin concentrations (range 0-120 ng/ml). Seven of the 12 also had raised trypsin response ratios.

In six patients with cancer of the pancreas, fasting trypsin was low in three, normal in two, and raised in one. Both patients with a normal fasting level had a raised trypsin response ratio.

The combination of a single estimation of fasting serum trypsin concentration followed by serial measurements after a Lundh meal provides a useful screening test for chronic pancreatic disease.

The diagnosis of chronic pancreatic disease presents considerable problems to the clinician. No single test has yet been shown to be completely reliable. The more invasive tests such as isotope scanning, endoscopic retrograde cholangiopancreatography (ERCP), and duodenal aspiration tests, although helpful, are not always accurate (Mottaleb *et al.*, 1973; Youngs *et al.*, 1973; Cotton *et al.*, 1975; Reuben *et al.*, 1976). Computerised axial tomography of the pancreas is of limited application (Fawcitt *et al.*, 1978), and ultrasonography remains to be satisfactorily assessed.

Single estimations of serum amylase and lipase, although useful in the diagnosis of acute pancreatitis, are not helpful in chronic pancreatitis or carcinoma of the pancreas (Burton *et al.*, 1960). Radioimmunoassay of the serum trypsin concentration in man appears to offer better discrimination, low fasting levels being associated with chronic pancreatitis, and

high levels with cancer of the pancreas (Elias *et al.*, 1977). Both groups, however, show considerable overlap.

Serial changes in blood levels of pancreatic enzymes after stimulation will distinguish duct obstruction from pancreatic atrophy in dogs (Popper and Necheles, 1943). Serum amylase and lipase estimations after pancreatic stimulation, however, have not proved of value in the diagnosis of either carcinoma of the pancreas or chronic pancreatitis in man (Dreiling and Richman, 1954). We have measured the changes in serum trypsin concentration after a Lundh meal in an attempt to improve the diagnostic sensitivity of this test.

### Material and methods

#### PATIENTS

Forty-seven subjects were studied, 31 males and 16 females. Eighteen were healthy laboratory staff. The diagnosis of chronic pancreatitis was made on the basis of a typical history, characteristic abnormalities

of the pancreatic ducts at endoscopic pancreatography (Rohrman *et al.*, 1974), and the presence of two or more of the following: (a) pancreatic calcification on plain radiographs of the abdomen (Sarles, 1974), (b) abnormal  $^{75}\text{Se}$ -selenomethionine pancreatic scanning (Youngs *et al.*, 1973), (c) diminished trypsin output after a standard Lundh test meal, and (d) nodularity of the pancreas at laparotomy. Six of these patients (group A) had a normal glucose tolerance test and faecal fat excretion of less than 18 mmol per day. The other six patients (group B) had evidence of pancreatic insufficiency with faecal fat excretion greater than 18 mmol per day; two of these were frankly diabetic. In nine of the 12 patients a history of alcoholism was obtained.

The diagnosis of relapsing pancreatitis was made in five patients with recurrent episodes of abdominal pain associated with raised serum amylase activities. Gallstones were present in three, and one had a history of alcoholism.

The six patients with carcinoma of the pancreas underwent laparotomy, and two had pancreatic biopsies showing adenocarcinoma. The duration of symptoms ranged from one month to three years. All had gross steatorrhoea and two were diabetic.

All six patients with chronic renal failure had creatinine clearance levels less than 15 ml per minute. None had any evidence of pancreatic disease.

#### METHODS

After an overnight fast, a sample of venous blood was taken. A Lundh meal comprising 40 g glucose, 18 g corn oil, and 15 g Casilan made up to 200 ml with water was then drunk over 2-3 minutes. Blood samples were collected at 30-minute intervals for 2 hours after the test meal. Serum was stored at  $-20^{\circ}\text{C}$  before estimation. Trypsin concentration was determined by a radioimmunoassay (RIA-gnost trypsin, Hoechst Pharmaceuticals, UK). Serum trypsin was stable for up to 3 months at  $-20^{\circ}\text{C}$ . The within-assay variance was 6.8% for the low concentration ( $\approx 200$  ng/ml) and 2.7% for the high concentration ( $\approx 600$  ng/ml) test sera. Over a three-month period the between-assay variance was 5.2% and 7.3% for the low and high concentration test sera respectively. The sensitivity of the assay was 34 ng/ml (95% confidence limits). Serum  $\alpha$  amylase activity was measured by the Phadebas method or the Amylochrome method. Results from both were similar. Serum trypsin concentration and  $\alpha$  amylase activity after the test meal were compared with the fasting levels using a paired *t* test.

#### Results

##### NORMAL SUBJECTS

In the 18 healthy control subjects, the mean fasting

serum trypsin concentration was  $285 \pm 125$  ng/ml (mean  $\pm 2$  SD). After the test meal, the mean serum trypsin concentrations at 30, 60, and 120 minutes were 270, 280 and 290 ng/ml respectively. Pancreatic stimulation with the test meal did not cause a significant change in the serum trypsin concentration. The mean fasting serum amylase activity was  $193 \pm 37$  IU/l (mean  $\pm 2$  SD). After stimulation, mean serum amylase at 30, 60, and 120 minutes were 187, 195, and 200 respectively. Pancreatic stimulation did not cause a significant change in the serum amylase activity.

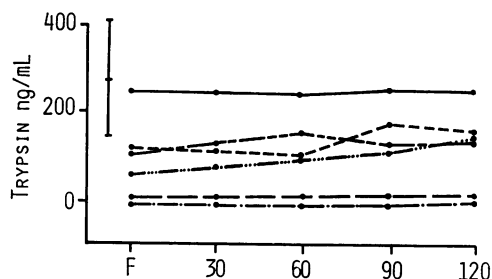


Fig. 1 Serum trypsin concentrations in six patients with chronic pancreatitis without insufficiency (group A) after a Lundh meal. Bar represents mean  $\pm 2$  SD for the control group. Five of the patients have a subnormal fasting level. The other patient with a normal response had a cyst in the tail of the pancreas at ERCP.

#### CHRONIC PANCREATITIS

##### Group A: without pancreatic insufficiency (Fig. 1)

The fasting serum trypsin concentrations were subnormal in five of the six patients. In two, trypsin concentration was undetectable throughout. Three patients showed a significant rise in trypsin concentration after stimulation; of these, one had a cyst in the head of the pancreas shown at ERCP. Only one of the six patients had a normal fasting serum trypsin concentration, and this patient had no significant change after stimulation. The pancreatic duct at ERCP was normal except for a small parenchymal blush in the tail of the gland.

Fasting serum amylase activities were normal in three of the patients and raised in the remaining three. There were no significant increases in the serum amylase levels after pancreatic stimulation.

##### Group B: with pancreatic insufficiency (Fig. 2)

All six patients in this group had fasting serum trypsin concentrations below normal. After pancreatic stimulation a significant rise was seen in four; in the remaining two, serum trypsin was undetectable throughout.

The fasting serum amylase activities were normal in three and showed no significant increase after

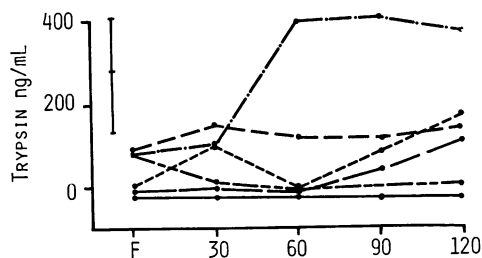


Fig. 2 Changes in serum trypsin concentration in six patients with chronic pancreatitis and insufficiency. All six have subnormal fasting trypsin concentrations. Bar represents mean  $\pm$  2 SD for the control group.

stimulation. In the other three patients, fasting serum amylase was raised; after stimulation all three levels fell.

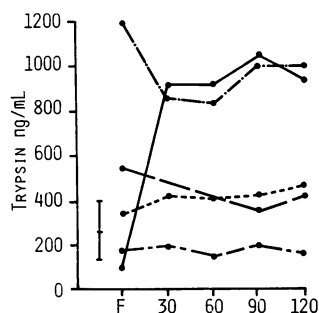


Fig. 3 Changes in serum trypsin concentration in relapsing pancreatitis. The two patients with raised fasting levels were studied within three days of the acute attack. One patient with a low fasting trypsin showed a marked rise at 30 minutes associated with severe pain. A large pseudocyst was later found with obstruction of pancreatic duct drainage. Bar represents mean  $\pm$  2 SD for the control group.

#### RELAPSING PANCREATITIS (Fig. 3)

Fasting serum trypsin concentrations were raised in the two patients who were studied within three days of an acute attack of pancreatitis. In two patients studied after three days but within one week of the attack, fasting serum trypsin concentrations were normal. Both patients with raised fasting trypsin concentrations showed a fall after stimulation. One of the two patients with normal fasting trypsin concentrations, however, showed a significant post-stimulation rise.

The fifth patient was studied within one week of the

acute attack. Fasting serum trypsin concentration was subnormal, but after stimulation a marked rise was seen and an attack of pancreatitis was precipitated. At laparotomy a large pseudocyst was found obstructing the main pancreatic duct.

Fasting serum amylase activities were raised in all five patients and showed no increase after stimulation.

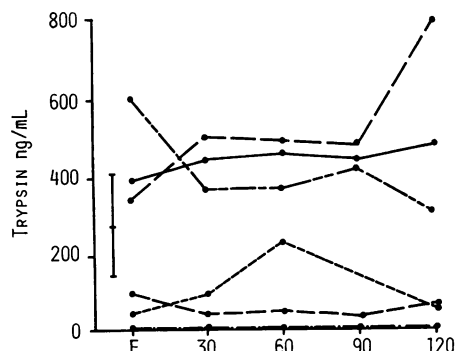


Fig. 4 Changes in serum trypsin in six patients with carcinoma of the pancreas after a Lundh meal. Bar represents mean  $\pm$  2 SD for the control group.

#### CARCINOMA OF THE PANCREAS (Fig. 4)

The fasting serum trypsin concentrations were low in three out of six patients, normal in two, and raised in one. This was not related to the length of history. All six patients had cancer involving the head or both head and body of the pancreas.

After stimulation, both patients with a normal fasting trypsin concentration showed a significant rise.

Serum amylase was measured in three and was low in one patient (56 IU/l) and normal in two (175 and 335 IU/l). Pancreatic stimulation caused no increase in serum amylase activities.

#### CHRONIC RENAL FAILURE

Fasting serum trypsin concentration was raised in all six patients (range 460-1100 ng/ml), but none of these showed a significant rise after pancreatic stimulation.

Fasting serum amylase was also elevated in all six patients (range 390-940 IU/l), and no post-stimulation increase was observed. The serum trypsin concentrations correlated well with the amylase levels.

#### TRYPSIN RESPONSE RATIO

Marked rises in serum pancreatic enzymes occur after pancreatic stimulation in animals with pancreatic duct ligation (Popper and Necheles, 1943). To

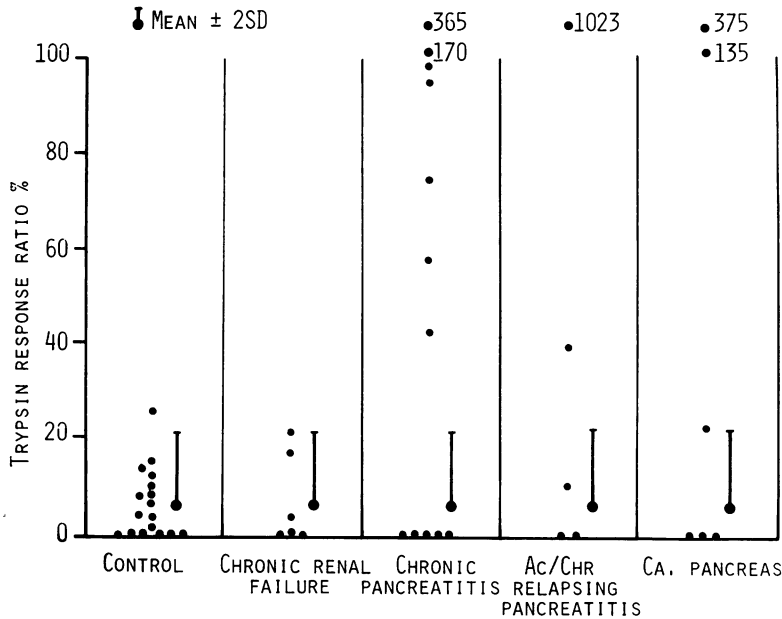


Fig. 5 Trypsin response ratio (ie maximum rise above fasting serum trypsin concentration). Bar represents mean  $\pm$  2 SD for the control group.

quantify these changes, the highest serum trypsin concentration reached during the test has been calculated as a percentage rise above the fasting level. This value was defined as the trypsin response ratio.

Figure 5 shows the trypsin response ratio within the patient groups.

In the 18 normal controls, mean trypsin response ratio was  $6.7 \pm 7.5\%$  (mean  $\pm$  2 SD).

In six patients with chronic renal failure in whom the serum trypsin concentration was presumably due to impaired renal excretion, the trypsin response ratios all fall within the normal range.

Seven out of 12 patients with chronic pancreatitis had raised trypsin response ratios. Three of these had evidence of main pancreatic duct obstruction: one had pancreatic duct stones, one a cyst obstructing drainage at the head of the pancreas, and the third marked nodularity confined to the head of the pancreas with distal obstruction at laparotomy.

Two out of five patients with relapsing pancreatitis had raised trypsin responses. One was shown to have a large pseudocyst obstructing the pancreatic duct; the other had multiple gallstones at the lower end of the common bile duct.

Three out of six patients with carcinoma of the head of the pancreas had increased ratios. It is noteworthy that in two of these the serum fasting trypsin concentration was normal.

## Discussion

A single estimation of fasting serum trypsin concentration has previously been shown to be of value in the diagnosis of chronic pancreatic disease (Elias *et al.*, 1977). This is unlike the other commonly measured pancreatic enzyme  $\alpha$  amylase, which although useful in the diagnosis of acute pancreatitis is often normal in chronic pancreatic disease. The greater diagnostic value of trypsin in chronic pancreatic disease presumably reflects the fact that the enzyme is present only in the pancreas. The mean fasting serum trypsin concentration in our controls,  $285 \pm 134$  ng/ml, was similar to that previously reported by Elias *et al.* (1977),  $273 \pm 134$  ng/ml, and Adrian *et al.* (1978),  $286 \pm 26$  ng/ml (mean  $\pm$  1 SEM). All six patients with chronic pancreatitis and insufficiency and five out of six without insufficiency had subnormal levels of fasting trypsin; serum trypsin was normal in one. Adrian *et al.*, however, reported high levels in patients without, and low levels in cases with pancreatic insufficiency. Serum trypsin concentration reflects not only the total mass of functioning pancreatic tissue but presumably also leakage from damaged acini. The high levels of serum trypsin measured in some cases of chronic pancreatitis may reflect leakage from inflamed foci within the gland. Our findings in chronic renal failure and in cancer of the pancreas in general agree with those previously reported (Elias *et al.*, 1977). In

a similar number of patients with cancer of the pancreas, three had a low, two normal, and one raised serum trypsin levels.

Popper and others (Popper and Necheles, 1943; Popper *et al.*, 1943) showed in animal experiments that it was possible to detect pancreatic duct obstruction and pancreatic gland atrophy by serial measurements of amylase activity in serum after stimulation of the pancreas. In the presence of duct obstruction, amylase rises after stimulation, whereas the enzyme level remains low throughout the test in pancreatic gland atrophy. Similar estimations in man, however, have not proved useful in cases where either amylase or lipase have been measured (Dreiling and Richman, 1954). It was interesting, therefore, that in both the control group and the group of patients with chronic renal failure, stimulation of the pancreas with a Lundh meal did not cause any significant change in serum trypsin levels since in both these groups no duct obstruction had been anticipated.

Contrary to expectations, however, pancreatic stimulation caused an abnormal increase in trypsin concentration as measured by the trypsin response ratio in seven out of 12 patients (58%) with chronic pancreatitis. Main duct obstruction could be demonstrated in only three patients; the rise in serum trypsin concentration in the remaining four patients is therefore unexplained but may have been due to obstruction at small duct level since these are known to be frequently blocked and fibrosed in chronic pancreatitis (Sarles, 1974). The diagnostic yield of serum trypsin in chronic pancreatitis is, however, not improved by serial measurements, 11 out of 12 patients having had low fasting trypsins measured compared with only seven out of the 12 with raised trypsin response ratios. The single patient with normal fasting serum trypsin concentration also had a normal trypsin response.

Two out of six patients with cancer of the pancreas had normal fasting serum trypsin concentrations. It was of interest, therefore, that the trypsin response ratio was abnormal in both these. The combination of both the serum fasting trypsin concentration and the trypsin response ratio detected all patients with cancer of the pancreas.

In two patients with relapsing pancreatitis and in two with cancer of the pancreas, stimulation of the pancreas resulted in a fall in serum trypsin concentration. Although the mechanism of this fall is unknown, we can speculate that the fasting levels of the enzyme in serum may have been maintained by leakage of the enzyme from damaged acini distal to a partially blocked duct. Subsequent stimulation of the pancreas may then have produced transient relief of this obstruction, thereby causing less enzyme to

escape into the bloodstream.

Fasting serum amylase activity was raised above the control range in both patients with chronic renal failure and in relapsing pancreatitis. In patients with either chronic pancreatitis or cancer of the pancreas, fasting serum amylase activity was either normal or raised; only one patient who had cancer of the pancreas had subnormal levels. No significant changes were observed in any of these groups after pancreatic stimulation, confirming previous findings (Dreiling and Richman, 1954).

In the presence of normal renal function, a single estimation of fasting trypsin concentration in serum has been confirmed as a useful screening test for chronic pancreatic disease. If this is normal and if duct obstruction is suspected, as in cancer of the pancreas, serial measurement of serum trypsin concentration after a Lundh meal to determine the trypsin response ratio is recommended. The test is simple and non-invasive and can be performed in any hospital laboratory equipped for radioimmunoassay techniques.

We thank Professor Dame Sheila Sherlock for permission to study patients under her care, and Hoechst Pharmaceutical UK for their support. G. L-B. is a Medical Research Council Fellow and J. A. S. is in receipt of an MRC grant.

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