Trypsin secretion and turnover in patients with acute pancreatitis

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O’Keefe, Stephen J. D., Ronzo B. Lee, Jing Li, Stacie Stevens, Souheil Abou-Assi, and Wen Zhou. Trypsin secretion and turnover in patients with acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 289: G181–G187, 2005. First published February 10, 2005; doi:10.1152/ajpgi.00297.2004.—Studies in humans have shown that pancreatic enzyme secretion is reduced during acute pancreatitis. It is not known, however, whether the reduction is due to impaired synthesis or disruption of the secretory pathway. The rate of secretion and turnover of trypsin was measured in 12 patients with acute pancreatitis of variable etiology and severity (median Ranson’s score 2.5, range 0–5, 4 with severe necrotizing disease) and eight healthy volunteers by 4-h primed/continuous intravenous infusions of 1-13C-labeled L-leucine, and collection of pancreatic secretions by duodenal perfusion and sampling. Trypsin secretion was reduced from 476 ± 73 to 153 ± 60 U/h (means ± SE, P = 0.005) in acute pancreatitis, with the greatest reductions being observed in patients with necrotizing disease (32 ± 7 U/h, P = 0.003). The time for newly labeled trypsin to first appear in digestive juice was not, however, delayed in pancreatitis patients (87.2 ± 11.1 vs. 94.7 ± 4.9 min); on the contrary, there was an early appearance of newly labeled trypsin at 30 min in patients with severe necrotizing pancreatitis (P < 0.05). Calculated zymogen pool turnover was unchanged, but pool size was decreased (P = 0.01). Despite low rates of luminal secretion, trypsin continues to be synthesized in patients with acute pancreatitis. Our findings could be explained by post-Golgi leakage of enzymes from acinar cells or by loss of synthetic function in some cells with preservation in others.

Studies in experimental animals and humans have shown that pancreatic enzyme secretion is reduced during acute pancreatitis (4, 13). This might be explained by an inability of the injured acinar cells to either synthesize or to secrete enzymes. Which of the two is responsible in humans remains unknown, but isotope-labeled studies in rats suggested the reason was not a failure of synthesis but defective secretion (22). The fact that serum enzyme levels become massively raised in the early stages of acute pancreatitis suggests that defective luminal secretion is at least partially responsible.

The question is important to investigate, because it will influence the way we manage patients. If the problem is simply one of secretion, measures taken to prevent the stimulation of enzyme synthesis, such as withholding orenteral feeding and “pancreatic rest,” will prevent the production and release of activated trypsin within the inflammatory mass and so help resolve the disease process. On the other hand, if synthesis is inhibited during acute pancreatitis, it will not matter how you feed patients. Consequently, in the following study, we used the method we developed for the simultaneous measurement of trypsin synthesis and secretion (15), based on in vivo labeling of pancreatic enzyme proteins with intravenous-administered stable isotope-labeled amino acids, to investigate this question in patients with acute pancreatitis.

Methods

Study Design

The trypsin secretory response to enteral feeding and the enrichment of the trypsin with stable isotope-labeled amino acids was measured by duodenal perfusion and aspiration and by in vivo labeling of secreted enzyme with intravenous 13C-labeled leucine tracer as previously detailed (15). Results in patients with acute pancreatitis due to a variety of causes were evaluated by direct comparison to values obtained in age-, sex-, and body mass-matched healthy volunteers studied under similar experimental conditions. Before study, all subjects had been fasted 12 h overnight, and during the study, all received the same dietary infusions.

Patient selection. Subjects were selected from those with documented severe or moderately severe acute pancreatitis, who were referred to the Nutrition Service for nutritional support. The diagnosis was made from a combination of clinical (acute upper abdominal pain), biochemical (serum amylase and lipase increased at least 3 times above the upper limit of normal), and radiological findings (ultrasound and CT evidence of pancreatic swelling, peripancreatic inflammation with or without necrosis). Patients were only eligible if they had failed to improve on the initial hospital management protocol consisting of 48 h of bowel rest, intravenous fluids, and analgesics (1). Enrollment followed the Virginia Commonwealth Institutional Review Board and General Clinical Research Center’s guidelines, and informed, signed consent was obtained from each patient and/or next-of-kin in the case of obtunded or ventilated patients with severe disease in the intensive care unit.

Controls. Normal, healthy adult volunteers of similar age, sex, and nutritional composition were recruited as control subjects. Subjects with a history of chronic medical disease, abdominal surgery, or alcohol abuse were specifically excluded.

Isotope methodology. Details of the method for the simultaneous measurement of in vivo pancreatic enzyme turnover and secretion have been published previously (15, 18). In the present study, we used a physiological stimulus for enzyme secretion in the form of a constant duodenal infusion of a commercial elemental formula diet (Vivonex TEN, Novartis, St. Louis Park, MN), given at a rate that would cover normal nutritional requirements (amino acids, 1.5 g·kg−1·day−1; energy, 30 kcal·kg−1·day−1). Enzyme secretions were labeled by a primed (1 mg/kg ideal body wt), 4-h continuous (1 mg·kg·ideal body wt−1·h−1) infusion of 13C-labeled L-leucine (lot #MTI-10414-S4, chemical and isotopic purity >99%, MassTrace, Address for reprint requests and other correspondence: S. J. D. O’Keefe, Division of Gastroenterology, Univ. of Pittsburgh Medical School, Pittsburgh, PA 15213 (E-mail: sjokeefe@pitt.edu).

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Woburn, MA). The stability of isotope labeling of the precursor pool was assessed by measuring isotope enrichment of plasma α-ketoisocaproic acid [an oxidative product of intracellular leucine (6)] in the plasma at 60, 120, and 240 min into the isotope infusion.

Pancreatic secretions released into the duodenum were recovered by a double lumen nasoduodenal tube placed manually with fluoroscopic guidance, or by transnasal endoscopy (17), such that the proximal perfusion port was adjacent to the papilla of Vater and the aspiration port was located 20 cm distally. Throughout the 4 h, the duodenal segment was perfused with a mixture of the enteral feed and normal saline at 300 ml/h, while mild suction (80 mmHg) was applied to the distal port. To measure the completeness of enzyme recoveries in aspirates and thus calculate secretion rates, the perfusate contained a marker (polyethylene glycol, average molecular mass 3,350 Da, 5 g/l; Sigma Chemical, St. Louis, MO).

Aspirations were collected on ice before transport to the laboratory for immediate measurement of trypsin concentration. Duplicate samples were stabilized with aprotonin solution for amylase and lipase measurement, and the remainder was stored at −80°C for the later extraction of trypsin and measurement of isotope enrichment (see Trypsin extraction and purification).

Plasma substrates and hormones. Additional blood samples were taken at 0, 120, and 240 min for the measurement of glucose, insulin, and CCK as previously (19).

Analysis of Samples

Pancreatic enzyme concentrations. Trypsin, amylase, and lipase concentrations were measured as previously (15, 18, 19). Trypsin concentrations were measured by the quantity of hydrogen ions liberated after incubation with α-benzoyl-l-arginine ethyl ester (Sigma). Amylase and lipase were measured by automated Vitros dry multilayered slide technique (Johnson and Johnson Clinical Diagnostics, Rochester, NY). Concentrations were converted to secretory outputs by measuring the PEG concentration in the samples by turbidity/spectrophotometry and using marker-dilution calculations to calculate recoveries and therefore enzyme secretion rates.

Trypsin extraction and purification. Affinity chromatography was used to extract pure trypsin from duodenal juice (14). Briefly, a commercially available trypsin inhibitor-agarose medium (Sigma Chemical) was held in an affinity column perfused by a low pressure chromatography system (Biologic LP System, Bio-Rad, Chicago, IL). Five-milliliter juice samples were loaded onto the column. The non-trypsin proteins were washed off with 0.05 M Tris-HCl, 0.5 M NaCl buffer, pH 8.0. Trypsin was released from the antibody by subsequent perfusion with 0.025 M citrate, 0.025 M CaCl<sub>2</sub> buffer at pH 2.5. The eluted fraction containing the trypsin peak was then desalted by gel filtration (HiPrep 26/10, Amersham Pharmacia Biotech, Piscataway, NJ). The protein was then dried by rotary evaporation and then hydrolyzed by incubation with 6 M HCl at 100°C for 24 h to release free amino acids so that the isotopic enrichment of leucine with 13C could be measured as described in Isotopic enrichment measurements. Isotopic enrichment measurements. Measurement of the 13C isotope enrichment of free leucine derived from duodenal juice trypsin and plasma 1-13C α-ketoisocapric acid (1-13C KIC) was measured by mass spectrometry but by different methods.

Plasma KIC enrichments were measured by electrical impact gas chromatographic (GC)-mass spectrometry (MS) commercially by Metabolic Solutions (Nashua, NH). Samples were deproteinized with acetonitrile prior to derivatization. 1-13C KIC and unlabeled KIC were assessed by conversion to a 1-t-butyl-silylquinoxalinol derivative according to a modification of the method of Schwarz et al. (21). A Hewlett-Packard 5890 GC coupled to a 5989A MS was autotuned in electron impact mode according to the manufacturer’s specifications. A standard curve was prepared comparing unlabeled KIC to varying amounts of 1-13C KIC (99%). Selective ion monitoring was used to analyze unlabeled KIC (m/z = 259) and isotope labeled KIC (m/z = 260, M + 1). The ratio of the peak area counts for m/z 259 and m/z 260 was used to calculate mole fraction.

Free leucine derived from trypsin hydrolysis was processed using the same instrument in our laboratory but by employing positive chemical ionization GC-MS because the leucine masses in patients with pancreatitis were small. The method was based on n-propyl esterification and heptafluorobutyrimide derivitization (5), monitoring ions at m/e 328 to 329.

Calculations

Pancreatic enzyme secretion rates. Measurement of enzyme (en) activity and PEG marker concentrations (PEG<sub>out</sub>) in duodenal juice samples allowed calculation of enzyme secretion rates from the relationship enzyme secretion (u/h) = (enz) × (PEG<sub>in</sub>/PEG<sub>out</sub>) × (PEG infusion rate), where PEG<sub>in</sub> = PEG concentration in perfusate. Trypsin turnover. The isotope enrichment curve over 4 h for trypsin protein was modeled mathematically as before (15) to obtain objective measurements of 1) the time it takes for newly synthesized enzyme to first appear in secreted juice (“synthesis time”), 2) the slope of the isotope enrichment curve, and 3) the estimated plateau enrichment. A simple, nonlinear exponential model with delay parameter (D; where D = synthesis time) provided the best fit for the complete data (Fig. 1). In fitting the isotope data for each subject to this model, the following definitions were made: 1) the isotopic enrichment of leucine in the precursor amino acid pool for trypsin synthesis (SA<sub>precursor</sub>) will be equal to the estimated plateau value (MX) for isotope enrichment of leucine bound in trypsin (ESA) from the precursor-product relationship, 2) the slope of the curve is the slope at the point where the ESA has achieved 50% of MX (i.e., time = half-time, in min) and is given by d(ESA)/dt = A × MX/2, where A is the estimated rate parameter from the exponential model, 3) intracellular enzyme pool turnover rate (i.e., “zymogen turnover”; EPTR, %/h) as is given by 100 × (A/2) × 60, and 4) intracellular enzyme pool size (i.e., “zymogen stores”) equals enzyme secretion divided by EPTR. Curve fitting was accomplished using the SAS statistical package procedure NLIN (SAS, Cary, NC). Goodness of fit was assessed by r², and the model was only used for calculations in subjects whose r² exceeded 0.8.

Statistical Evaluation

Results are presented throughout as group means ± SE. Results in patients were evaluated by comparison with those of the healthy volunteers, who, hereafter, are termed “controls”. The significance of the observed differences in group mean values was determined by Student’s t-testing if the data were normally distributed or by non-parametric testing if it was not, employing the Mann-Whitney rank sum test for unpaired data (e.g., patients vs. controls). Post hoc subgroup analysis was performed by analysis of variance with Bonferroni/Dunn correction. Probabilities of <0.05 were accepted as significant; unless post hoc analyses were used, in which case higher probabilities were demanded.

RESULTS

Patients

All 12 enrolled patients completed the study; 63% were male, 54% Caucasian, and 38% African American, with a median age of 47 (range 18–77) yr (Table 1). The cause of acute pancreatitis was acute alcohol toxicity in four; three gave a history of at least one earlier attack. Three others gave a history of biliary dysfunction and gallstones, but none had gallstone impaction demonstrated at the time of admission or study. Three had complicated critical illness, where the cause of acute pancreatitis was unclear but probably related ischemia or drugs. Severity scores varied from Ranson’s 0 to 5, with four
being studied after the development of pancreatic necrosis. Forty-six percent of patients were obese with a body mass index (BMI) of >30 kg/m².

The study was performed at a median of 5 days following initial diagnosis, with a range of 2–22 days. Seven of the patients were studied following failure to respond to our usual protocol for acute pancreatitis (1) of 48 h bowel rest, intravenous fluids, and analgesics, four following total parenteral nutrition and bowel rest for 3–7 days and 1 following tube feeding for 5 days. All patients had massive elevations of serum lipase levels (median 6,147, range 1,345–69,260 U/l; normal <300) and milder increases in amylase concentrations (median 449 U/l, range 289–5,268 U/l; normal <150).

The four patients with necrotizing pancreatitis were studied in the intensive care unit at variable times (4, 5, 8, and 13 days) during the course of their disease. Table 2 shows that lipase concentrations were significantly higher (P = 0.006, Mann Whitney U-test) in this group compared with patients with less severe pancreatitis. All patients tolerated the study without change in symptoms or signs, although one with necrotizing disease (patient 10) developed extension of his disease after advancement of enteral feeding the following day (patient 10). Three of the four patients with necrotizing pancreatitis needed late surgery for evacuation and drainage of necrotic fluid collections and, after extended lengths of stay, made full recoveries. One patient with necrotizing disease (patient 1) died of uncontrolled sepsis and respiratory failure a week after study following surgical intervention at day 14.

Controls

Eight healthy volunteers (6 male, 2 female) were studied for control purposes. There was no significant difference in the mean age (30.0 ± 3.4 vs. 45.1 ± 5.4 yr; P = 0.07) and body mass (27.4 ± 2.1 vs. 28.8 ± 2.0 kg/m²; P = 0.64) and sex ratio (male/female 6:2 vs. 8:4) between controls and patients.

Plasma Concentrations

Figure 2 illustrates the relative changes in plasma CCK during 4 h of dietary infusions. CCK levels were significantly higher in patients than in healthy volunteers at baseline (P = 0.02). Although levels remained numerically higher during the nutrient infusions, the significance of the difference was lost (P = 0.08 at 240 min).

Although blood glucose concentrations were significantly higher in patients before and during the 4-h infusions (baseline

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ranson’s Score</th>
<th>Etiology</th>
<th>BMI, kg/m²</th>
<th>Age, yr</th>
<th>Sex</th>
<th>CT Scan</th>
<th>Amylase, IU/l</th>
<th>Lipase, IU/l</th>
<th>Glucose, mg/dl</th>
<th>Study, day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Alcohol</td>
<td>30.5</td>
<td>47</td>
<td>M</td>
<td>Severe, phlegmon – necrosis +</td>
<td>922</td>
<td>7473</td>
<td>280</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Pancreas divisum, 5-ASA</td>
<td>28.8</td>
<td>40</td>
<td>F</td>
<td>Bulky, stranding mild</td>
<td>302</td>
<td>5032</td>
<td>118</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Alcohol</td>
<td>27.3</td>
<td>71</td>
<td>M</td>
<td>Swollen, peripancreatic collection fat stranding</td>
<td>589</td>
<td>7206</td>
<td>112</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>Alcohol</td>
<td>30.9</td>
<td>34</td>
<td>M</td>
<td>Not done</td>
<td>393</td>
<td>6172</td>
<td>148</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Azothiaprine, hypoxia</td>
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<td>66</td>
<td>F</td>
<td>Edema</td>
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<td>2696</td>
<td>268</td>
<td>2</td>
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<tr>
<td>6</td>
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<td>Idiopathic, ?stones</td>
<td>23.6</td>
<td>28</td>
<td>M</td>
<td>Edema</td>
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<td>3182</td>
<td>145</td>
<td>3</td>
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<td>7</td>
<td>4</td>
<td>Idiopathic, ?stones</td>
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<td>77</td>
<td>F</td>
<td>Edema</td>
<td>456</td>
<td>4027</td>
<td>90</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Trauma/drugs/hypoxia</td>
<td>23.0</td>
<td>18</td>
<td>M</td>
<td>Sludge</td>
<td>289</td>
<td>1345</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>Alcohol/drugs/ischemia</td>
<td>18.4</td>
<td>51</td>
<td>M</td>
<td>Enlarged pancreas</td>
<td>805</td>
<td>5838</td>
<td>149</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>Idiopathic</td>
<td>26.0</td>
<td>64</td>
<td>M</td>
<td>Necrosis</td>
<td>1715</td>
<td>40000</td>
<td>164</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>Hypertriglyceridemia</td>
<td>47.9</td>
<td>36</td>
<td>M</td>
<td>Severe, phlegmon, fat stranding necrosis</td>
<td>427</td>
<td>14925</td>
<td>293</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>Ideopathic/Accutane</td>
<td>22.7</td>
<td>19</td>
<td>F</td>
<td>Necrosis</td>
<td>5268</td>
<td>69260</td>
<td>114</td>
<td>5</td>
</tr>
</tbody>
</table>

BMI, body mass index; M, male; F, female.
glucose $215 \pm 79$ mg/dl in patients, $92.4 \pm 5.8$ in controls, 240 min glucose $166 \pm 12$ vs. $105 \pm 5$, $P = 0.02$), insulin concentrations were not (baseline $7.7 \pm 2.0$ vs. $8.2 \pm 2.8$ U/l, 240 min $18.6 \pm 7.8$ vs. $17.8 \pm 4.2$ U/l), respectively.

Pancreatic Enzyme Secretion

Trypsin ($P = 0.005$), amylase ($P = 0.03$), and lipase ($P = 0.005$) secretion rates were all significantly suppressed in patients (Mann-Whitney test), the greatest difference being noted in lipase, which was five times lower. However, when patients were subdivided into those with mild/moderately severe disease and those with severe necrotizing pancreatitis, a post hoc analysis (ANOVA with Bonferroni/Dunn correction) indicated that only trypsin secretion was significantly lower than controls in the mild/moderate group, whereas both trypsin and lipase were significantly lower in the necrotizing group (Table 2). Post hoc subgroup analysis also showed that the greater the severity of disease, the greater the reduction in enzyme secretion [e.g., for trypsin, secretion was 59% reduced in patients with mild and moderate acute pancreatitis ($P = 0.01$), and 93% reduced in patients with necrotizing disease ($P = 0.002$)]. The etiology of disease had no profound influence, but on average, suppression of secretion was less in the patients with trauma- and drug-associated pancreatitis.

To ensure that the low trypsin secretion rates in acute pancreatitis patients were not due to incomplete conversion of trypsinogen to trypsin in duodenal juice, we repeated measurements in one patient (5 time points) and one volunteer (3 time points) after the addition of 0.28 U enterokinase (Sigma: cat #E5510). Results demonstrated that there was no increase in trypsin activity in the acute pancreatitis patient [$12.8 \pm 3.8$ U/ml benzyl-L-arginine ethyl ester (BAEE) before addition of enterokinase and $11.5 \pm 4.0$ U after]. Interestingly, the trypsin activity from the healthy volunteers actually decreased from $1,124 \pm 157$ to $753 \pm 161$ U after enterokinase addition. This phenomenon has been previously reported by Kunitz (9) and is thought to be due to secondary trypsin inactivation.

Isotope Studies

Plasma enrichment. Dietary leucine intake during the studies was similar for patients and controls ($1.232 \pm 0.049$ vs. $1.233 \pm 0.438 \mu$mol·kg$^{-1}$·min$^{-1}$, respectively), as was the rate of $^{13}$C isotope-labeled leucine infusion ($0.987 \pm 0.024$ vs. $0.953 \pm 0.029 \mu$mol·kg$^{-1}$·min$^{-1}$). Figure 3 illustrates the isotopic stability in the plasma during the 4-h studies. The mean values for $^{13}$C were not significantly different among the intervals 60, 120, and 240 min, namely during the period that the uptake of isotope into secreted enzymes was measured, indicating the achievement of an isotopic steady state in the intracellular amino acid pool (6). Subgroup analysis showed that the stability was similar in patients with acute pancreatitis and controls (Fig. 3).

Trypsin turnover. The time course for $^{13}$C-labeled leucine incorporation into trypsin extracted from duodenal juice for controls and all patients subdivided into those with mild and moderate disease ($n = 8$) and those with severe necrotizing disease was similar for patients and controls (Fig. 3).

### Table 2. Serum pancreatic enzyme concentrations and duodenal secretion rates

<table>
<thead>
<tr>
<th>Serum Concentrations, U/l</th>
<th>Duodenal Secretion Rates, U/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase</td>
<td>Amylase</td>
</tr>
<tr>
<td>Controls ($n = 8$)</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
</tr>
<tr>
<td>Mild/moderate ($n = 8$)</td>
<td></td>
</tr>
<tr>
<td>Necrotizing ($n = 4$)</td>
<td></td>
</tr>
</tbody>
</table>

$n$, Number of subjects. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.005$ vs. controls; §$P < 0.01$ vs. mild/moderate: post hoc analysis, ANOVA with Bonferroni/Dunn correction.

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![Fig. 2](http://ajpgi.physiology.org/) Group means ± SE results of plasma CCK concentrations before, during, and at the end of the 4-h studies showing significantly higher CCK concentrations in patients at baseline ($*P = 0.02$) and nonsignificant elevation during nutrient infusion ($P = 0.08$) compared with healthy volunteers (controls).

![Fig. 3](http://ajpgi.physiology.org/) Plasma isotopic stability data showing no significant differences in the mean enrichments of plasma $\alpha$-ketoisocaproic acid (KIC) with $^{13}$C at 60, 120, and 240 min for both patients and controls during the primed, continuous intravenous infusion of $^{13}$C-labeled leucine. Values are given as percent excess.
patients (\(n = 4\)) is illustrated on Fig. 4. Visual inspection suggests there were no major differences in the isotopic incorporation rates among healthy subjects, patients with mild/moderate pancreatitis, and patients with severe necrotizing pancreatitis, with the exception of the early appearance of newly labeled trypsin in the necrotizing group at 30 and 60 min. Statistical analysis confirmed that the enrichment for the necrotizing group was significantly higher than both controls and patients with mild/moderate disease at 60 min (\(P = 0.001\) and 0.003, respectively, by ANOVA with Bonferroni/Dunn correction for post hoc analysis).

For objective analysis of the isotope incorporation curves, the raw data were fitted to the model explained in the Calculations. The sequential data of all but one patient with necrotizing disease (patient 1) were shown to fit well with the model with an \(r^2\) of >0.8. Consequently, the results of our calculations illustrated on Fig. 5 are based on all subjects studied with the exception of this one patient. The analysis shows that there were no major differences in trypsin synthesis time (95 min in controls and 87 min in patients). Second, the slope of the enrichment curve after 90 min was not significantly different between healthy subjects (0.13 ± 0.003 atoms % excess/min) and patients (0.012 ± 0.005). Consequently, the calculations of zymogen turnover were also not significantly different. However, because trypsin secretion rates were significantly lower, calculations of zymogen stores were also significantly lower in patients (\(P < 0.013\); Fig. 5). Post hoc subgroup analysis also could detect no significant differences in the slopes of patients with mild and moderate disease (0.014 ± 0.006 atoms % excess/min) and patients with necrosis (0.006 ± 0.001) compared with controls. The calculated zymogen turnover rates in the subgroups (24.4 ± 4.4 %/h in controls, 28.3 ± 11.3 %/h in mild/moderate patients, and 18.7 ± 7.9 %/h in necrosis patients) were not significantly different. Finally, the zymogen stores were significantly lower in patients with severe necrotizing disease compared with healthy controls. Because secretion was reduced in patients (***\(P = 0.003\)) while turnover was not, calculated zymogen stores were also diminished in patients (**\(P = 0.01\)).

Fig. 5. Illustration of the overall differences in measured trypsin secretion and synthesis and calculated zymogen turnover in patients with acute pancreatitis compared with healthy controls. Because secretion was reduced in patients (***\(P = 0.003\)) while turnover was not, calculated zymogen stores were also diminished in patients (**\(P = 0.01\)).

Our investigations revealed that patients with acute pancreatitis have lower rates of secretion of digestive enzymes compared with healthy volunteers and that the degree of reduction is related to the severity of disease. Our isotope-labeling techniques provided insight into the pathophysiological alterations responsible for the reduced luminal secretion. Unexpectedly, we found that although trypsin secretion into the duodenum was greatly diminished, the time of appearance of newly synthesized trypsin was not delayed; in fact, in patients with the most severe form of the disease, namely necrotizing pancreatitis, newly synthesized enzyme appeared more rapidly.

The differences in trypsin secretion and kinetics detected between patients and healthy subjects could be explained by acute pancreatic damage but also by other factors associated with acute illness, such as inflammation of the small intestine, duration of fasting, and medications that are, in clinical practice, difficult to control for. Focusing on potential pancreatitis-associated explanations, our findings of low secretion (reduced 70–95%) with a normal synthesis time (mean of 89 min) could be due to the maintenance of enzyme synthesis and loss of

**DISCUSSION**

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The differences in trypsin secretion and kinetics detected between patients and healthy subjects could be explained by acute pancreatic damage but also by other factors associated with acute illness, such as inflammation of the small intestine, duration of fasting, and medications that are, in clinical practice, difficult to control for. Focusing on potential pancreatitis-associated explanations, our findings of low secretion (reduced 70–95%) with a normal synthesis time (mean of 89 min) could be due to the maintenance of enzyme synthesis and loss of
70–95% of synthesized enzymes from the secretory pathway or by loss of synthetic function in 70–95% of acinar cells with preservation of function in the surviving cells. The second mechanism would also include decreased overall synthesis with normal trafficking. The additional finding of an early appearance of newly synthesized trypsin at 30 min in patients with severe necrotizing disease could be explained by disturbance of enzyme trafficking within the cell, with leakage into the duct or amplification of the constitutive pathway, both of which would bypass the zymogen pool. Both suggestions are supported by our calculations of zymogen pool size from our model, which showed a diminution in zymogen pool size but not turnover.

We are not aware of other attempts to measure trypsin synthesis in humans with acute pancreatitis, but a number of investigators have examined the question in animal models and have come to opposing conclusions. With the use of acinar cells derived from animals with caerulein-induced acute pancreatitis, Steer’s group pulse-labeled newly synthesized enzymes with [3H]phenylalanine and studied the passage of the labeled proteins through the cell (22). They concluded that the synthesis of enzymes was no different between cells derived from animals with acute pancreatitis and from healthy animals but that the subsequent secretion of enzymes was impaired. On the other hand, Sans et al. (20), using a “flooding dose” technique with [3H]phenylalanine in mice with acute pancreatitis induced by repetitive caerulein injections, demonstrated a decrease in the labeling rate of pancreatic proteins associated with a decrease in the activity of protein translocation regulatory mechanisms. This suggested that the suppressed synthesis was due to inhibition of translation initiation. It should be noted that both of the above groups of workers measured the isotope incorporation into “pancreatic protein” and did not specifically examine uptake into trypsin or enzyme protein. Second, a lower rate of protein labeling does not necessarily equate with lower synthesis because it could equally be explained by a greater degree of retention of previously synthesized, nonlabeled enzymes due to the failure of secretion described above. We need to emphasize that our measurement of synthesis time represents the total production time for trypsin, which would include the times taken for the process of amino acid uptake, ribosomal synthesis, posttranslational enzyme modification, transport through the cell, and secretion into the duodenum. In other words, the time represents the minimum time for newly labeled enzymes to take part in the digestion of food. From the results of experimental pulse-chase labeling studies (8), ribosomal synthesis will only contribute a small fraction of this time (~3–5 min), and it remains possible that rate changes may have occurred at the ribosomal level that were masked by changes of greater magnitude in the remaining enzyme production pathway. However, it is doubtful whether rate changes in this compartment would be of physiological significance, unless there was synthesis arrest.

Loss of secretions in transit to the duodenum in acute pancreatitis is certainly part of the explanation of our findings, because all the patients studied had massive elevations of blood levels of lipase and amylase, indicating breakdown of the normal secretory process and sequestration into the systemic circulation. The focus of the present study was to examine the isotope labeling and activity of duodenal trypsin rather than that of sequestered trypsin in the circulation, and therefore, we can only assume that some of the newly synthesized trypsin was “lost” into the inflammatory mass and bloodstream. Activated trypsin cannot usually be detected in the bloodstream because it is rapidly inactivated by α1-antitrypsin and α2-macroglobulin, but trypsinogen and trypsin-antiprotease complex levels have been shown, similar to serum lipase concentrations, to correlate closely with the severity of the pancreatitis (10). It is even more difficult to measure the loss of trypsin into the inflammatory “phlegmon” in patients, but we have recently reported CT scan evidence of increase in the mass following stimulation of pancreatic secretion by enteral feeding in a patient with necrotizing pancreatitis (16).

An unexpected finding was the significant early appearance of labeled enzyme in patients with necrotizing pancreatitis. Although this finding needs confirmation with larger numbers, it is intriguing to speculate on potential mechanisms. Examination of the sequence of enzyme synthesis and intracellular trafficking originally outlined by Jamieson and Palade (see Fig. 1) shows that at 30 min, namely the time-labeled enzymes were first identified in duodenal secretions in the patients with necrotizing pancreatitis, most of the pulse-chase-labeled enzyme would be passing from the condensing vacuoles to the zymogen pool. Consequently, our finding could be explained by divergence of enzyme traffic from the normal secretory pathway at a posttranslational stage, with some of the enzymes exiting the cell before condensation into the zymogen pool. As discussed above, this would also be supported by our measurements of depleted zymogen stores with no change in zymogen turnover, suggesting that the enzymes that do not diverge out are handled normally. This suggestion fits well with the evidence presented by Arvin and Castle (3) that the earliest branching point for newly synthesized enzymes is at the level of the trans-Golgi network of the Golgi. From this point, enzymes can be channeled into distinct trafficking pathways. They have proposed that under normal physiological conditions, enzymes are channeled into two secretory pathways: one a low capacity (“constitutive”) pathway for resting secretion, the other a high capacity (“regulated”) pathway that responds to cholinergic stimuli to provide the massive discharge of enzymes needed to cover digestive needs (7). Consequently, our findings in patients with severe necrotizing pancreatitis could be explained by amplification of the constitutive pathway, or alternatively, and more likely in our view, simple leakage form the acinar cell into the surrounding structures, which include the pancreatic ducts, bloodstream, and parenchyma.

In summary, our investigations have shown that even in patients with the most severe form of acute pancreatitis, dietary amino acids can be synthesized into trypsin, raising the possibility that feeding could perpetuate the inflammatory process. However, our methodology was unable to measure the fraction of newly synthesized trypsin lost into the inflammatory mass and circulation, and therefore, we were unable to determine how total enzyme synthesis was affected. Further studies are needed to address this issue and to determine whether the injured pancreas is capable of responding to dietary change and pancreatic rest with intravenous feeding.
REFERENCES


