Loss of exocrine pancreatic stimulation during parenteral feeding suppresses digestive enzyme expression and induces Hsp70 expression

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Loss of exocrine pancreatic stimulation during parenteral feeding suppresses digestive enzyme expression and induces Hsp70 expression. Am J Physiol Gastrointest Liver Physiol 292: G857–G866, 2007. First published November 30, 2006; doi:10.1152/ajpgi.00467.2006.—Luminal nutrients are essential for the growth and maintenance of digestive tissue including the pancreas and small intestinal mucosa. Long-term loss of luminal nutrients such as during animal hibernation has been shown to result in mucosal atrophy and a corresponding stress response characterized by the induction of heat shock protein (Hsp)70 expression. This study was conducted to determine if the loss of luminal nutrients during total parenteral nutrition (TPN) would result in atrophy of the exocrine pancreas and small intestinal mucosa as well as an induction of Hsp70 expression in rats. In experiment 1, the treatment groups included an orally fed control, a saline-infused surgical control, or TPN treatment for 7 days. In experiment 2, the treatment groups included an orally fed control and TPN alone or coinfused with varying doses of glucagon-like peptide (GLP)-2, a mucosal proliferation agent, for 7 days. In experiment 1, TPN resulted in a 40% reduction in pancreatic mass that was associated with a dramatic reduction in digestive enzyme expression, enhanced apoptosis, and a 200% increase in Hsp70 expression. Conversely, heat shock cognate 70, Hsp27, and Hsp60 expression was not changed in the pancreas. In experiment 2, TPN resulted in a 30% reduction in jejunal mucosa mass and a similar induction of Hsp70 expression. The inclusion of GLP-2 during TPN attenuated jejunal mucosal atrophy and inhibited Hsp70 expression, suggesting that Hsp70 induction is sensitive to cell growth. These data indicate that pancreatic and intestinal mucosal atrophy caused by a loss of luminal nutrient stimulation is accompanied by a compensatory response involving Hsp70.

total parenteral nutrition; glucagon-like peptide 2; jejunal mucosa; atrophy of the pancreas; acinar cells; heat shock protein 70

PANCREATIC ACINAR CELL digestive enzyme secretion is regulated by endocrine and neural signals that are initiated in response to feeding. Secretagogues including CCK, acetylcholine, and bombesin stimulate acinar cell secretion via G protein-coupled receptors that activate PLC to ultimately increase cytosolic Ca2+ and produce diacylglycerol (45). In rodents, CCK is also known to stimulate acinar cell growth, resulting in significant increases in pancreatic weight, DNA, and protein content (28). Correspondingly, CCK is known to stimulate proliferative pathways in acini including MAPK and c-Fos activation (12, 26). In addition to proliferation, it is well established that agents that stimulate secretion also stimulate protein synthesis to replenish the digestive enzymes released in response to a meal. Indeed, secretagogues have been demonstrated to activate the p70S6K pathway involving phosphatidylinositol 3-kinase and mammalian target of rapamycin (mTOR) as well as various translational regulatory proteins including phosphorylated heat- and acid-stable protein regulated by insulin 1, eukaryotic translation initiation factor (eIF)4E, and eIF2 (4, 5).

Numerous studies on the intestinal mucosa have shown that the removal of luminal nutrients reduces mucosal DNA and protein content, and this is reversed by refeeding or by inclusion of exogenous growth factors such as IGF-1 (10) or glucagon-like peptide (GLP)-2 (8). Likewise, the removal of enteral nutrients results in a loss of pancreatic wet weight and protein that can be reversed by CCK infusion (15). A similar phenomenon of mucosal atrophy occurs in hibernating animals, which lack luminal nutrients for prolonged periods of time (6). Interestingly, despite the mucosal atrophy that occurs during torpor, the overall structure and function of epithelial cells are well preserved, as indicated by the maintenance of sucrase-isomaltase activity and sodium-dependent glucose transport (7). Additionally, intestinal epithelial cells undergo a stress response during torpor characterized by the induction of the molecular chaperones heat shock protein (Hsp)70 (or Hsp72) and glucose-regulated protein 75 (also known as mitochondrial Hsp70) and by the activation of NF-kB (7).

Hsp70 is a member of the super family of Hsps characterized by their expression in response to various cellular stresses including hyperthermia and ischemia (41). Hsp70 is a cytosolic protein that targets to substrates by binding to short hydrophobic sequences to mediate protein folding and prevent aggregation (17). In addition to Hsp70, heat shock cognate (Hsc)70 is a constitutively expressed chaperone under normal conditions that likely shares similar protein substrates (34). Interestingly, the prophylactic induction of Hsp70 was shown to have a protective effect against the onset of cerulein-induced pancreatitis (44) and arginine-induced pancreatitis (43) in rodents. Supporting this, inhibition of Hsp70 expression using antisense oligonucleotides in rat pancreatic fragments abolished its protective effects against the onset of trypsinogen activation in response to cerulein hyperstimulation, which is believed to cause pancreatitis (3).

The present study utilized a rat model of total parenteral nutrition (TPN) to examine the role of exogenous luminal nutrients in the maintenance of exocrine pancreatic structural and functional integrity and in the expression of Hsps in the pancreas and jejunal mucosa. The results indicated that a loss of pancreatic stimulation by luminal nutrients leads to pronounced atrophy of acinar cells characterized by a large reduction in digestive enzyme content within the gland. Concomitant
with acinar atrophy was a striking induction of Hsp70 expression. Similar findings were also detected in the intestinal mucosa, and infusion of the intestinotrophic hormone GLP-2 fully prevented atrophy and Hsp70 induction. As resting the digestive tract by TPN is a common practice to resolve gastrointestinal disorders, evidence for the induction of Hsp70 expression may have therapeutic implications.

**MATERIALS AND METHODS**

**Materials**

Isoflurane was purchased from Abbott Laboratories (North Chicago, IL), the BCA Kit was from Pierce Chemical (Rockford, IL), and catheters were from Access Technologies (Skokie, IL). Anti-Hsp70, anti-Hsc70, anti-Hsp25, and anti-Hsp60 antibodies were purchased from StressGen (Victoria, BC, Canada), anti-IκB-α polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), anti-amylose was purchased from Chemicon (Temecula, CA), and the anti-CRHSP24 polyclonal antibody was as characterized previously (16). Anti-lipase antibody was a generous gift from Mark Lowe (25). The Phadebas Amylase Test was purchased from Magle (Lund, Sweden). GelCode Blue Stain Reagent was purchased from Pierce Chemical. Hoechst reagent was purchased from Calbiochem (La Jolla, CA), and GLP-2 was purchased from CA Peptide (Napa, CA). The in situ Cell Death Detection Kit was purchased from Roche Diagnostics (Indianapolis, IN). R software (version 1.8.0) was from the Department of Statistics, University of Auckland (Auckland, NJ); SAS software was from the SAS Institute (Carey, NC). Sprague-Dawley rats were purchased from Harlan (Madison, WI); amino acid and electrolyte mix, 8.5% traversal, and dextrose were from Baxter (Deerfield, IL). Long-chain diacylglycerol lipid emulsion (Intralipid) was purchased from Pharmacia (Clayton, NC). Vitamins were purchased from Astra USA (Westborough, MA), and trace elements (Multitrace-4) were from American Regent Laboratories (Shirley, NY).

**Animals**

The University of Wisconsin (Madison, WI) Institutional Animal Care and Use Committee approved the animal facilities and protocols. Male Sprague-Dawley rats initially weighing 200–230 g (8–10 wk old) were housed in stainless steel cages and acclimated to the facility at 22°C and a 12:12-h light-dark cycle for 5 days prior to the start of the experiment. Animals had ad libitum access to semipurified powdered diet and water during this acclimation period. Animals were fasted overnight before the placement of jugular catheters on day 0 as described previously (22). The infusion of TPN solution began on day 0 and was increased gradually to provide 100% of nutritional needs (see below). The volume of TPN infused was recorded daily. Urine output was collected daily in a final concentration of 0.01% boric acid, the volume was recorded, and aliquots were stored at −20°C for nitrogen analysis. The nitrogen balance was calculated as the difference between nitrogen infused and nitrogen excreted. The nitrogen concentration in urine was determined by microkeldjahl analysis as previously described (46). On day 7, rats were anesthetized by isoflurane inhalation, and tissues were collected.

**Experiments**

**Experiment 1: effect of TPN on Hsp70 expression in pancreas and jejunal mucosa.** This study included three treatment groups: orally fed nonsurgical controls (n = 6), orally fed surgical controls (0.9% saline infused through catheter with ad libitum access to semipurified powdered diet, n = 7), and TPN-fed rats with no access to an oral diet (n = 8). All groups had ad libitum access to water. Saline and TPN treatment groups underwent surgery on day 0 for jugular catheter placement in preparation for the TPN infusion. Body weight and food intake were measured daily. Rats were maintained with TPN for 7 days, after which time they were anesthetized and the pancreas, mucosa, and blood were collected.

**Experiment 2: effect of GLP-2 on intestinal and pancreatic adaptation.** This study included six treatment groups: orally fed rats (n = 6) and five TPN groups with 0, 50, 100, 170, or 850 μg·kg⁻¹·day⁻¹ GLP-2 coinfused with TPN solution (n = 5–8 rats/group). GLP-2 was diluted in sterile phosphate-buffered 0.9% saline and added to fresh TPN solution each morning. TPN rats were maintained with exclusive TPN for 7 days following catheter placement. After 7 days, rats were anesthetized with isoflurane for tissue collection and analysis.

**Composition of the TPN Solution and the Oral Diet**

The TPN solution was prepared aseptically using commercial preparations of amino acids and electrolytes, 60% dextrose, 20% long-chain triacylglycerol lipid emulsion, and all essential vitamins and trace elements as detailed previously (10). The solution consisted of (in g/l) 44 amino acids, 180 dextrose, and 28 lipid. The TPN infusion began gradually, with 1 ml/h on day 0 to 1.67 ml/h on day 1 and increased to a final rate of 2.5 ml/h (64 kcal/day and 2.64 g protein/day) for days 2–7. The saline (0.9%) infusion rate was identical to the TPN infusion rate.

Orally fed animals, including the saline group, had free access to a nutritionally complete, semipurified powdered diet with a macronutrient composition comparable with the TPN solution (10). The average intake of the oral group over 7 days was 30 ± 1 g/day; the average intake of the saline group over 7 days was 15 ± 1 g/day.

**Pancreatic and Jejunal Mass and Cellularity**

The pancreas and jejunum (defined as from the ligament of Treitz to 25 cm proximal to the cecum) were excised from the rats, flushed with ice-cold 0.9% saline, blotted dry, and weighed intact. The first 5-cm section of the jejunum was cut longitudinally and scraped with a glass slide to separate the mucosa from muscularis for the determination of wet and dry mass. A portion of each pancreas and the next 1 cm of the jejunum were fixed in 10% buffered formalin and paraffin embedded, and 5-μm sections were stained with hematoxylin and eosin (H&E) for histological analysis. The majority of the pancreas and the next 5 cm of the jejunal mucosa were immediately frozen in liquid nitrogen and stored at −80°C until they were ready for analysis. The pancreas was homogenized in 7 volumes and the jejunal mucosa in 1 volume of ice-cold 12 mM Tris buffer (pH 7.1), 300 mM mannitol, and 5 mM EGTA using a Polytron homogenizer. In both the pancreas and jejunum, the protein concentration was determined by a BCA assay and the DNA concentration by a fluorometric method (21).

**Protein Expression and Amylase Activity**

Homogenized pancreatic and jejunal tissue samples were prepared for electrophoresis by boiling in SDS sample buffer. Hsp70, Hsc70, Hsp60, Hsp27, IκB-α, lipase, and CRHSP24 expression were measured by immunoblot analysis. Pancreatic or jejunal proteins (200 μg) were separated in polyacrylamide gels and transferred onto nitrocellulose membranes. Membranes were blocked in Tris-buffered saline containing 5% nonfat dry milk and 3% Tween 20 and then incubated with the indicated antibodies for 90 min at room temperature. Immunoreactivity was detected using horseradish peroxidase-conjugated secondary antibodies and detected by enhanced chemiluminescence. Protein expression was quantified by scanning densitometry using a PDI model DNA 35 scanner interfaced with the protein and DNA image system.

Amylase activity was determined using the Phadebas blue starch test. Total pancreatic amylase expression was examined by SDS-PAGE (10 μg protein) and staining with GelCode Blue Stain Reagent. Amylase band location was confirmed by immunoblot analysis with an anti-amylase monoclonal antibody (not shown).
Pancreatic Histology and TUNEL Staining

Conventional light microscopy of H&E-stained samples was used to identify pyknotic acinar cells based on characteristic morphological changes, including condensed nuclei. Quantitating apoptosis using H&E-stained histological sections is considered to be the reference standard by Potten (33). Pancreatic sections were examined in a blinded manner under the supervision of an experienced animal pathologist (Dr. A. Gendron, Research Animals Resource Center, University of Wisconsin, Madison, WI). Pyknotic cells were quantified in all animals from each treatment group by analyzing five randomly chosen frames of the same size for each specimen under ×40 magnification and averaged to derive the mean numbers of pyknotic cells per field.

TUNEL staining was performed on paraffin sections of pancreas (5 μm) using the in situ Cell Death Detection Kit according to the manufacturer’s instructions. Briefly, sections were dewaxed in xylene and rehydrated in a graded series of ethanol and double-distilled water. Sections were incubated with proteinase K (0.2 mg/ml) for 15 min at 37°C, rinsed with PBS, and incubated with terminal deoxynucleotidyl transferase and a label solution of a nucleotide mixture in reaction buffer. Staining was visualized by an incubation with anti-fluorescein antibody conjugated to horseradish peroxidase and diaminobenzidine as a substrate. Sections were counterstained with hematoxylin and a bluing agent of 1% lithium carbonate.

Statistical Analysis

Unless otherwise stated, treatment groups in each experiment were compared by one-way ANOVA with linear models in R or SAS. Unless otherwise stated, data are presented as means ± SE. P values are given when significant.

RESULTS

Animal Growth Parameters

In both experiments, all animal groups gained weight following surgery (data not shown). In experiment 1, animals were fed orally or instrumented for parenteral feeding and infused with either saline (and allowed oral food ad libitum) or TPN. No significant differences in daily body weights among treatment groups were noted until the 5th day postsurgery, when animals in the TPN group weighed significantly less than those in the oral group but not in the saline group. The reduction in body weights of the TPN group continued to the 7th day postsurgery, when tissues were collected. Likewise, cumulative weight gains for the 7 days of treatment were not significantly different between the saline and oral groups, but the TPN group gained significantly less weight. Final body weights were 299 ± 6 g in the oral group, 290 ± 6 g in the saline group, and 262 ± 7 g in the TPN group (means ± SE, n = 6–8 rats/group). In experiment 2, animals were fed orally or received TPN alone or coinfused with various doses of GLP-2. Although final body weights of the oral control group were significantly higher than those in the TPN groups, no significant differences in body weights among TPN groups receiving GLP-2 were noted. As previously reported using this well-characterized model of TPN (46), nitrogen retention increased each day in all TPN groups (data not shown).

Pancreatic Mass Is Reduced in Response to TPN

In experiment 1, pancreatic weights were reduced by 40% in the TPN group compared with the both orally fed and saline-infused treatment groups, indicating significant atrophy of the organ in response to a loss of luminal nutrient stimulation (Fig. 1A). When expressed as a percentage of the total body weight, pancreatic weights were 0.37 ± 0.01 in the orally fed group, 0.38 ± 0.01 in the saline-infused group, and 0.25 ± 0.01 in the TPN group (means ± SE, n = 6–8 rats/group), clearly demonstrating a significant decrease in the pancreatic mass of TPN-treated rats compared with controls (P < 0.001 for both). Animals on TPN showed
a significant 18% decrease in pancreatic protein concentration but no significant change in DNA concentration compared with orally fed rats (Fig. 1, B and C). The pancreatic protein and DNA concentration were not significantly different between orally fed and saline treated controls; however, DNA concentration in the saline-infused group was significantly decreased compared with the TPN-treated group. As a consequence, the ratio of pancreatic protein to DNA in the TPN group was significantly less than that in saline-infused rats but not orally fed rats. The total pancreatic DNA content was 18 ± 1 mg in the orally fed control, 13 ± 2 mg in the saline control, and 14 ± 2 mg in the TPN-treated group (means ± SE, n = 6–8 rats/group), with a trend toward a reduction when saline-infused control and TPN-treated rats were compared with orally fed controls (P = 0.05 and 0.07, respectively). Collectively, the reduction in pancreatic weight seen in TPN-treated rats was primarily due to a loss of protein, as DNA content and concentration were not significantly different compared with those in orally fed rats.

**TPN Results in a Decrease in Zymogen Content and an Increase in Acinar Apoptosis**

Consistent with the reduction in pancreatic protein, histological analysis of H&E-stained pancreatic sections revealed a dramatic loss of acidic granular staining within apical aspects of acini following TPN treatment (Fig. 2, A and B). Acinar zymogen staining was unchanged in saline-infused rats (not shown). Although the total pancreatic size was obviously decreased following dissection, microscopic examination revealed that pancreatic islets, nerves, and blood vessels were largely normal. Additionally, no obvious signs of necrosis or inflammation including white blood cell infiltration, edema, or fibrosis were detected in any of the groups. As tissue atrophy is commonly associated with an enhancement of apoptosis, quantification of pyknotic cells in multiple sections in each treatment group was conducted, demonstrating a >100% increase in pyknotic nuclei in TPN-treated groups compared with orally fed and saline-treated controls (Fig. 2E). In agreement with these findings, TUNEL staining of nuclei was greatly

![Fig. 2. Pancreatic atrophy induces acinar cell apoptosis and a loss of zymogen granule staining. Microscopic examination of pancreas sections from rats either orally fed (A and C) or maintained exclusively with TPN (B and D) are shown. Sections were stained with hematoxylin (purple, nuclear) and eosin (pink, granular). Note the reduced granular staining in the TPN-treated pancreas sections compared with the controls. C and D: TUNEL staining (brown) was used to detect apoptotic nuclei. E: pyknotic nuclei in the hematoxylin and eosin-stained sections were quantified in 5 fields from the pancreas in each experimental group by a blinded investigator. Field magnification: ×40. Data are means ± SE; n = 6–8 animals/treatment group. P values are shown when statistically significant (P < 0.05).](http://ajpgi.physiology.org/)
increased in the pancreas following TPN treatment compared with control (Fig. 2, C and D). Because the total pancreatic DNA content in TPN-treated rats was not significantly different compared with that in orally fed rats, these findings suggest that the enhancement of total acinar apoptosis had not yet resulted in a significant loss of acinar cells within the gland.

Lack of luminal stimulation causes a significant loss in acinar digestive enzyme content. To determine if the loss of acidic granular staining in acini correlated with a loss in digestive enzyme content, amylase activity was measured in pancreatic homogenates, revealing a striking 80–84% decrease in activity as a result of TPN treatment compared with the oral and saline counterparts (Fig. 3A). In accordance with enzyme activity, the examination of amylase expression by SDS-PAGE revealed a large decrease in amylase protein in the TPN group compared with the orally fed and saline-infused controls (Fig. 3B). Amylase is a major 55-kDa acinar protein that is easily identified by SDS-PAGE and Coomassie staining (40). To determine if this effect was specific to amylase, lipase expression was also measured by immunoblot analysis, indicating a 54% and 40% reduction in TPN-treated rats compared with orally fed and saline-infused surgical controls, respectively (Fig. 3C). There was also a small but significant decrease in lipase expression in saline-infused rats compared with controls. Taken together, these data indicate that the loss of luminal nutrient stimulation during TPN resulted in a major loss of digestive enzyme content within the pancreas.

Pancreatic Atrophy Induces Hsp70 Expression

Based on previous reports demonstrating that the resting of digestive tissues during torpor may lead to an induction of Hsp70 expression, we examined pancreatic Hsc70 and Hsp70 levels in control and TPN-treated rats (Fig. 4A). Strikingly, Hsp70 expression increased >200% following 7 days of TPN treatment compared with the orally fed and saline groups, whereas Hsc70 expression was unchanged in all treatment groups. As Hsp27 and Hsp60 have been shown to be expressed under basal conditions in acinar cells and to undergo enhanced induction with various forms of cellular stress (18, 39, 43), these molecules were also evaluated (Fig. 4B). Both Hsp27 and Hsp60 expression showed no significant changes among treatment groups. For comparison, the calcium-sensitive signaling protein CRHSP24 was also analyzed and likewise showed no significant alterations in expression among treatment groups. Although microscopic examination revealed no evidence of immune cell infiltration or inflammation within the gland of TPN-treated animals, IκB-α expression was also evaluated as an indirect measure of NF-κB activity, which is a highly characterized early-immediate signaling molecule involved in acinar inflammation (18, 35). Consistent with the morphology, no changes in IκB-α were detected between any treatment groups. To further establish if pancreatic damage had occurred during atrophy, plasma amylase activity was quantified (Table 1). Interestingly, a small but significant reduction in plasma amylase activity was noted in plasma from saline- and TPN-treated rats compared with oral controls. These data demonstrate that pancreatic atrophy caused by a lack of luminal nutrient stimulation highly and selectively induces pancreatic Hsp70 expression and further suggest that this response is not secondary to inflammation or damage of the organ.

GLP-2 Prevents Jejunal but not Pancreatic Atrophy

To examine if the tissue atrophy leading to Hsp70 induction in the pancreas also occurred in mucosal tissue, experiment 2...
was conducted to compare orally fed rats with rats receiving TPN supplemented with GLP-2, which is known to induce mucosal growth (14). As previously shown with this model (32), TPN caused significant reductions in mucosal weight, protein, and DNA content that were reversed by treatment with as little as 50 μg·kg⁻¹·day⁻¹ GLP-2 (Fig. 5, A–C). The effects of GLP-2 on mucosal cellularity were dependent on concentration and induced a growth response that was significantly greater than that in orally fed controls. As seen in experiment 1, pancreatic weights were also significantly reduced in TPN-treated rats; however, GLP-2 had no effects on the pancreas at concentrations as high as 850 μg·kg⁻¹·day⁻¹ (Fig. 5D).

**GLP-2 Prevents Atrophy and Hsp70 Induction in the Jejunal Mucosa**

Similar to the large induction of Hsp70 expression seen in the pancreas with TPN treatment, Hsp70 expression was induced by >200% in the jejunal mucosa. Significantly, treatment with GLP-2 to prevent mucosal atrophy strongly inhibited Hsp70 induction in the mucosa. The effects of GLP-2 on Hsp70 expression were evident at a concentration as low as 50 μg·kg⁻¹·day⁻¹ (Fig. 6A). In contrast, GLP-2 at concentrations as high as 850 μg·kg⁻¹·day⁻¹ had no effect on Hsp70 expression caused by pancreatic atrophy (Fig. 6B). Because GLP-2 did not stimulate pancreatic growth in TPN-treated animals, these data suggest that the induction of Hsp70 in mucosal and pancreatic epithelial cells occurs as a result of tissue atrophy.

**DISCUSSION**

The present study demonstrates that a loss of luminal nutrient stimulation results in significant atrophy of pancreatic acini and the small intestinal mucosa and, moreover, that this phenomenon correlates with a striking induction of Hsp70 expression. These data are in agreement with those of other studies demonstrating that luminal nutrients are necessary for stimulating hormonal and/or neural regulation of digestive tissue maintenance and protein synthesis (1, 4, 11, 15, 19). The saline-infused orally fed group, which served as a surgical control, accumulated weight over the 7-day experiment; however, this was not to the extent of the nonsurgical control group. Despite this lower weight gain, there were no significant differences in pancreatic mass or total protein content between the saline-infused surgical and orally fed nonsurgical controls, indicating sufficient luminal nutrient stimulation in both groups. Compared with controls, administration of TPN for 7 days caused a 40% loss of pancreatic mass, which was accounted for mainly by a decrease in tissue protein rather than DNA content. There was a significantly greater concentration of pancreatic DNA in the TPN-treated group compared with the saline-infused surgical and orally fed nonsurgical controls, indicating sufficient luminal nutrient stimulation in both groups.

![Heat shock protein (Hsp)70 expression is strongly induced in TPN-fed rats. Pancreatic Hsp70 and Hsc70 expression were measured by immunoblot analysis in orally fed controls, saline-infused orally fed surgical controls, and TPN-fed rats. A: anti-Hsp70 (1:1,000) and anti-Hsc70 (1:500) expression were determined in pancreatic homogenates (200 μg). The band intensity was determined by densitometry and expressed as the Hsp70-to-Hsc70 ratio as Hsp70 expression was not changed among treatment groups. The gels at the top are a single representative sample for each treatment group. B: for comparison, Hsp27, Hsp60, IxBα, and CRHSP24 protein expression were measured using specific antibodies (all at 1:1,000 except for Hsp60, which was used at 1:5,000) in 200 μg protein except for IxBα, which was determined in 100 μg pancreatic protein. Data in A are means ± SE; n = 4–8 rats/treatment group. P values are shown when statistically significant (P < 0.05).](image)

**Table 1. Plasma amylase activity is not increased following TPN**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma Amylase Activity, U/l plasma</th>
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<tr>
<td>Oral</td>
<td>63.70±14.21</td>
</tr>
<tr>
<td>Saline</td>
<td>47.41±4.17*</td>
</tr>
<tr>
<td>TPN</td>
<td>36.85±8.07*</td>
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Data are means ± SD; n = 5 rats/group in the orally fed group, 8 rats/group in the saline-infused group, and 5 rats/group in the total parenteral nutrition (TPN)-treated group. *Significantly different from the orally fed group (P ≤ 0.01) based on Student’s t-test.
control group, whereas we found a trend toward a reduction that was not statistically significant (P = 0.07). Our study utilized a smaller sample size (n = 6–8 rats/group) compared with the Ekelund group (n = 25 rats/group), which may explain why our DNA measures were not significantly decreased. These authors also demonstrated a decrease in amylase content following TPN treatment, whereas they found lipase and colipase to be unchanged. Our observation that pancreatic amylase activity and expression decreased following TPN treatment is in agreement with this previously shown data; however, in contrast with their lipase observation, we found a significant reduction in lipase expression following TPN treatment. The divergence of our observations on lipase as a consequence of TPN treatment could be attributed to the different parameters that were measured; Fan et al. measured lipase activity, whereas we measured lipase protein expression. These data suggest that the loss of zymogen expression may account for a significant portion of the reduction in pancreatic protein concentration in the TPN-treated rats. Moreover, these data clearly underscore the significance of luminal nutrients in maintaining exocrine pancreatic integrity as well as the pivotal role of luminal nutrients in regulating digestive enzyme synthesis.

Although pancreatic amylase activity was not significantly different between oral and saline control groups, there was a small reduction in lipase expression in the saline control group compared with the oral control group (see Fig. 2B). Likewise, basal serum amylase activity was also decreased in the saline control group (see Table 1). These findings may be attributable to the reduced food intake and weight gain noted in the saline control group. The trend toward reduced pancreatic DNA content, a reduction in lipase expression, and reduced plasma amylase activity in the saline-infused surgical control group compared with the orally fed nonsurgical control group suggests that these rats as a control did undergo changes that can be attributed to surgery rather than a lack of luminal nutrient stimulation.

In addition to a loss of pancreatic protein and digestive enzyme content, the lack of luminal nutrients in TPN-treated animals also resulted in a significant increase in pyknotic nuclei in acinar cells. Based on TUNEL staining, the increase in pyknotic cells strongly correlated with enhanced apoptosis. Examination of neutrophil infiltration, IκB-α expression, and serum amylase activity was not indicative of necrosis or inflammation, further suggesting that pyknotic cells were undergoing apoptosis. Because there was no significant reduction in DNA content at day 7 in TPN-treated animals, these data suggest that acinar cell numbers had not changed. It is possible that a significant reduction in acinar cell number and/or DNA had not yet occurred at day 7 but would be evident at later time points or that the trend toward lower DNA content would be significant with a larger sample size. Alternatively, acinar cell proliferation may have increased in TPN-treated animals to compensate for enhanced apoptosis. Similar to that seen in the pancreas, administration of TPN also resulted in a 30% reduction in jejunal mucosal mass and a dramatic induction of Hsp70 expression. Interestingly, GLP-2 both enhanced mucosal growth and mitigated the Hsp70 response in a concentration-dependent manner. Unlike the jejunal mucosa, however, GLP-2 did not prevent atrophy or the induction of Hsp70 expression in the pancreas, demonstrating that the exocrine pancreas is likely not responsive to GLP-2. The time course for TPN-induced Hsp70 expression was not evaluated, although in preliminary experiments Hsp70 was not induced following a 48-h fast, suggesting that a prolonged lack of stimulus and/or more significant atrophy is required to induce the Hsp70 response.

Fig. 5. TPN results in jejunum mucosal atrophy and is prevented by glucagon-like peptide (GLP)-2 treatment. Mass, protein, and DNA content were measured in the jejunal mucosa in 6 groups of rats including orally fed control, TPN alone, and TPN with increasing doses of GLP-2 (50, 100, 170, and 850 µg·kg−1·day−1). A: 5 cm of the jejunal mucosa were scraped and dried to determine dry weight (in mg/cm). B: protein concentration was determined by BCA assay. C: DNA concentration of the jejunal mucosa was determined using Hoescht reagent. D: pancreatic atrophy during TPN was not prevented by GLP-2 treatment. Data are means ± SE; n = 5–8 animals/group. *P < 0.05 and **P ≤ 0.01 compared with the control group.

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The induction of Hsp70 expression following TPN administration appears to be quite selective as other Hsps including Hsc70, Hsp27, and Hsp60 were unchanged with acinar atrophy. Hsp70 is known to be induced in the pancreas in response to a variety of stresses including heat, ischemia, pancreatitis, and catecholamines (39); however, this is the first report of induction related to acinar atrophy. Likewise, Hsp27 and Hsp60, which were unchanged by TPN treatment, were previously shown to be induced by hyperthermia and water immersion, respectively (18, 43). Hsp70 is part of the cellular protein folding machinery, and its role in preventing protein aggregation and protein misfolding during periods of stress is well characterized (30). The physiological significance of Hsp70 induction with acinar atrophy is unclear, although it is possible that Hsp70 may serve to maintain tissue integrity during fasting until normal growth stimuli return. Overexpression of inducible Hsp70 has been shown to protect cells against apoptosis in several studies (24, 27, 39), potentially by suppressing caspase activation downstream of the mitochondrial release of cytochrome c and upstream of the activation of caspase 3 (2, 23, 37). In our study, there was a positive correlation between Hsp70 expression and increased apoptosis; however, the role of Hsp70 in modulating this process is unclear. Additional experimentation inhibiting Hsp70 expression with acinar atrophy will be necessary to elucidate the role of Hsp70 in this process.

A recent study (42) demonstrated that signaling pathways resulting in pancreatic growth in response to the synthetic trypsin inhibitor camostat are dependent on the Ca2+ - activated phosphatase calcineurin. Camostat is known to cause the release of endogenous CCK, which stimulates signaling cascades including MAPK and ERKs, JNKs, and the mTOR pathway. Interestingly, treatment of mice with FK506, a calcineurin inhibitor that is known to inhibit camostat-induced pancreatic growth, fully ablated the activation of JNK and significantly reduced the phosphorylation of S6 and eIF4E binding protein 1, two downstream targets of the mTOR pathway. Another study (9) further confirmed that pancreatic growth in response to camostat is dependent on the mTOR signaling pathway by utilizing the mTOR inhibitor rapamycin. mTOR inhibition had no effect on the MAPK cascade or mRNA levels of c-Jun and c-Fos but significantly reduced camostat-induced increases in 4E binding protein 1 and S6 phosphorylation. Whether or not Hsp70 expression plays a role in the calcineurin and/or the mTOR signaling cascade is unclear but warrants further investigation.

The induction of Hsp70 expression prior to the onset of experimental pancreatitis in rodents protects against the zymogen activation and acinar cell damage characteristic of this clinical condition (3, 43, 44). In humans, pancreatitis is often resolved by preventing pancreatic stimulation via fasting, TPN, or enteral feeding (31). Although Hsp70 induction appears most beneficial prior to the onset of pancreatitis, Hsp70 induction during pancreatic rest could potentially inhibit a recurrence or relapse of acinar damage upon refeeding. As resting the digestive tract by TPN is a common practice in the treatment of gastrointestinal disorders, and taking into account the considerable focus on the protective effects of Hsp70 in inflammatory disease of the pancreas and intestine (36), these data suggest that the induction of Hsp70 during gastrointestinal rest may have therapeutic implications. Recent studies have suggested that enteral feeding during pancreatitis reduces the risk of bacterial translocation across the intestinal mucosa that potentially occurs following TPN-induced mucosal atrophy.

A meta-analysis (29) demonstrated that treating pancreatitis patients with enteral feeding rather than TPN significantly reduced the risk of infections, surgical interventions, and length of hospital stay, although there were no differences in mortality. Furthermore, it was shown in pancreatitis patients that enteral feeding via a nasogastric tube rather than a nasojugal tube did not stimulate the pancreas or exacerbate the disease, indicating that the location of enteral infusion may not be as important as was once considered (13). Thus, further examination addressing whether pancreatic atrophy and Hsp70 expression is induced as a result of enteral feeding will be helpful in evaluating the therapeutic potential of this phenomenon.
In summary, the data presented demonstrate that atrophy of the pancreas and small intestinal mucosa as a result of the loss of luminal nutrients strongly induces Hsp70 expression. Hsp70 induction occurs in conjunction with tissue atrophy and is prevented with the maintenance of mucosal proliferation. Further studies utilizing molecular approaches to prevent Hsp70 induction will be necessary to determine the physiological implications of this phenomenon (20, 38).

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REFERENCES


