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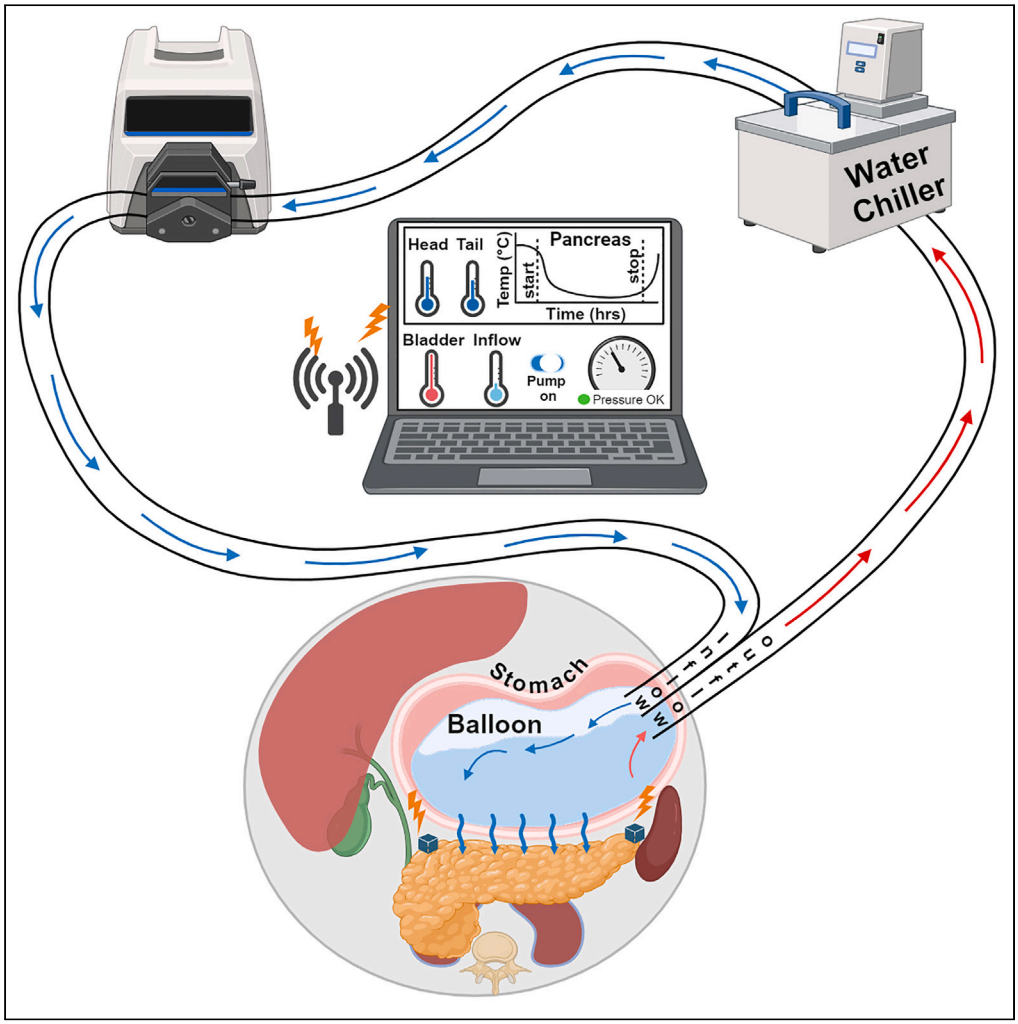
A safe method for rapid therapeutic pancreatic cooling



Megan Summers, Benjamin Russell, Prasad Rajalingamgari, ..., Naomi Gades, Nabil Wasif, Vijay P. Singh

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Highlights

A gastric cooling balloon is well tolerated in large animals

Transgastric pancreatic cooling is rapidly inducible and reversible

Transgastric cooling can achieve pancreatic temperatures required to treat pancreatitis

Pancreatic cooling can be done without generalized hypothermia

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A safe method for rapid therapeutic pancreatic cooling

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SUMMARY

Acute pancreatitis (AP) has no targeted therapy. Previously, pancreatic cooling to 31°C–33°C and 24°C–27°C, respectively, ameliorated mild and severe AP in rats. Here, Yucatan pigs (40–50Kg) whose abdominal size and anatomy are like humans underwent pancreatic cooling. This was via a gastric cooling balloon placed endoscopically with catheters exteriorized on the abdominal wall. Laparoscopically placed wireless transmitters monitored pancreas tail, head, and urinary bladder temperatures. Controls included un-perfused water filled balloons, and sedation-only groups. Tap water perfusion (375 mL/min) over 1-month was well tolerated without sedation. Perfusion with ≤19°C water achieved pancreatic temperatures ≤32°C and perfusion at ≤10°C achieved ≤26°C in <90 min in sedated supine pigs, which normalized an hour after balloon evacuation. Bladder temperatures, behavioral, biochemical, hematological, and histological parameters were similar between groups. Therefore, rapid transgastric pancreatic cooling can be achieved safely in large animals with relevant anatomy like humans, warranting future clinical studies.

INTRODUCTION

While pancreatitis is one of the most common acute GI diseases requiring hospital admission,[1] it has no targeted therapy. Previous studies have established the pancreatic temperatures that ameliorate acute pancreatitis in three mechanistically distinct models[2–5] using 200–300 gm rats. These utilized a gastric cooling balloon that transgastrically cooled the pancreas, which lies behind the stomach. Pancreatic temperatures of 31°C–33°C (normally 37°C–38°C in rats) reduced pancreatic injury and inflammation in mild acute pancreatitis induced by caerulein,[2,4] and 24°C–27°C ameliorated established severe pancreatitis,[4,5] as summarized in Table 1. The therapeutic advantage of pancreatic hypothermia over drug therapy is that hypothermia is multimodal[4,5]—i.e., it targets multiple pathways induced by different pancreatitis etiologies in parallel as shown in Table 1. We therefore hypothesized that cooling via an endoscopically placed gastric balloon would result in statistically significant localized pancreatic hypothermia.

Local pancreatic hypothermia is safer than generalized hypothermia. We aimed at avoiding generalized hypothermia since it can cause shock due to a decrease in cardiac output,[6] fluid shifts out of the vascular compartment,[7] hemoconcentration[8]; that potentially reduce pancreatic perfusion and worsen organ failure, worsen hypoxemic respiratory failure.[9] Additionally, the pancreas, due to its visceral nature, would be cooled late during external cooling. Thus taking the aforementioned into consideration, and that generalized hypothermia can potentially worsen acute pancreatitis,[10] we aimed at achieving local pancreatic hypothermia, while avoiding generalized hypothermia.

This study aimed at local pancreatic cooling in a large animal, whose abdominal size and viscera are closer to humans[11,12] than rodents. We therefore chose 7–8-month-old Yucatan pigs whose weights (40–50Kg; mainly trunk weight) and abdominal dimensions (Figure 1, Table) were closer to humans.[11,12] Moreover, a pig's anatomy wherein the stomach is anterior to the pancreas is similar to humans. While nasogastric tube placement is tolerated in most humans and would be the ideal method for perfusing a gastric balloon, a nasogastric route would be very agitating in alert pigs, especially during the several weeks needed for testing device safety. The gastric balloon was therefore placed endoscopically and perfused via catheters opening on the abdominal surface. This study details the methods, tolerance, efficacy, and safety of rapidly inducible local pancreatic hypothermia in pigs using such a device. The results of this study support its use in large animals, and humans for the treatment of acute pancreatitis.

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Table 1. Table comparing the three models of pancreatitis that previously studied local pancreatic hypothermia

Model →	*Supra Stimulation*	*Lipotoxic*	*Bile Salt*
Agent	Caerulein	Triglyceride; Glyceryl trilinoleate (GTL)	5% Sodium Taurocholate
References	Mishra et al.[2] and de Oliveira et al.[4]	de Oliveira et al.[4]	de Oliveira et al.[5]
Severity of pancreatitis model	Mild	Severe	Severe
Target pancreatic temperature	31°C-33°C	24°C-27°C	24°C-27°C
Parameters improved or normalized with cooling in experimental pancreatitis model	Pancreatic apoptosis, myeloperoxidase increase.	Pancreatic necrosis, Pancreatic metabolic demand. Serum Fatty acids, Hypotension, DAMPs (dsDNA, Histone-complexed DNA), cytokines (IL-6, TNF-α, IL1β) blood urea nitrogen. Apoptosis in kidneys and lungs. Improved survival.	Reduced serum bile acids. Reduced cardiac injury, hypotension. Normalized left ventricular end diastolic volume, stroke volume. Reduced serum CK-MB, DAMPs (dsDNA, Histone-complexed DNA), cytokines (IL-6, TNF-α). Reduced lung myeloperoxidase. Improved survival.
Mechanisms affected by cooling	Reduced caerulein induced LDH leakage, propidium iodide uptake, trypsinogen activation, chemokine increase	Reduced triglyceride lipolysis into fatty acids, fatty acid monomer amounts, fatty acid uptake into cells. Reduced pancreatic metabolic demand.	Reduced breakdown of bile acid micelles into monomers. Reduced concentrations of bile acid in the systemic circulation.

Each column corresponds to a pancreatitis model. The references correspond to the respective articles describing the models in detail. The target pancreatic temperature (fourth row) mentions the temperature that protected from that model in the referenced studies.

RESULTS

The perfused gastric cooling balloon is well tolerated in upright alert pigs

Before studying tolerance to the perfused balloon, the pigs were monitored daily for 8 to 11 days after balloon and temperature sensor placement surgery. Operated pigs had excellent wound healing, with no infections and had similar activity, behavior, and food intake as controls. Baseline pancreatic temperatures were stable at 40.0 ± 0.3°C in all groups. Figure 2A shows an example of a pig's pancreatic head (red line) and tail (blue line) temperatures. The vertical line on day nine separates the days before and after perfusion with tap water (22°C–23°C). While pancreatic temperatures were similar in both static (purple line Figure 2B) and cooling groups (green line) at baseline, balloon perfusion in the cooling group reduced pancreatic temperatures by 1–4°C within the first day of cooling. These averaged at 38.3 ± 0.7°C over the 30 days in the cooling group, while the static group was unaffected over this period (39.8 ± 0.2°C; Figures 2B and 2C). Both head and tail temperatures were similarly reduced in the cooling group to 38.1 ± 1.4°C and 37.3 ± 0.8°C, respectively, ($p = 0.25$, Figure 2D). However, the bladder temperatures remained stable in both groups with means ranging from 40.0–40.3 ± 0.2°C–0.4°C. During these 30 days, food consumption was unaffected, and there was no evidence of distress or behavioral changes in the cooling or static groups. Pancreas histology at the time of necropsy was similar in all groups (Figures 2F–2H), with normal appearing islets (dashed ovals, Figures 2F–2H), and exocrine acinar cells retaining polarized cluster morphology with eosinophilic staining in the apical portion, and basophilic stain basally adjacent to the nuclei. Stomach histology in pigs with the gastric cooling balloon showed normal gastric mucosa with gastric pits arranged on top of the mucosal surface (red arrows), and normal appearance of the underlying lamina propria (black asterisks; Figure 2I). Similarly, there was no serological evidence of pancreatic injury, noted as similar serum amylase in all groups (Figure 2J), while lipases remained low, and sometimes undetectable in all groups (data not shown). There were also no effects on hematocrit, BUN (Figures 2K and 2L), WBC counts, or liver function tests (data not shown). Therefore, tap water perfusion over 30 days caused a small but significant reduction of 1.5°C–2.0°C in pancreatic temperatures without causing generalized hypothermia, or affecting pancreatic histology, enzymes, hematological, or biochemical parameters.

Transgastric pancreatic cooling is rapidly inducible and reversible without generalized hypothermia

The transgastric pancreatic cooling mentioned previously using tap water was well tolerated, but reduction in temperature while significant was small and seemed to vary with position of the pig, and food intake while they were alert and mostly upright. Since gravity likely lowered the gastric balloon in upright pigs, and distanced it from the dorsally located pancreas, and food intake could insulate the pancreas; we therefore studied pancreatic cooling in a supine position on days when food was held. As seen in Figures 1J and 1K, the supine position brought the balloon in close proximity to the pancreas. To achieve pancreatic cooling in the supine position, the pig was sedated and intubated as described in STAR Methods. This period of sedation and anesthesia extended over 8 h to allow experimental testing. We also had to use a closed cooling circuit, with a chiller and pump as shown in Figures 3A and 3B to maintain the flow rate, and balloon volumes sufficient

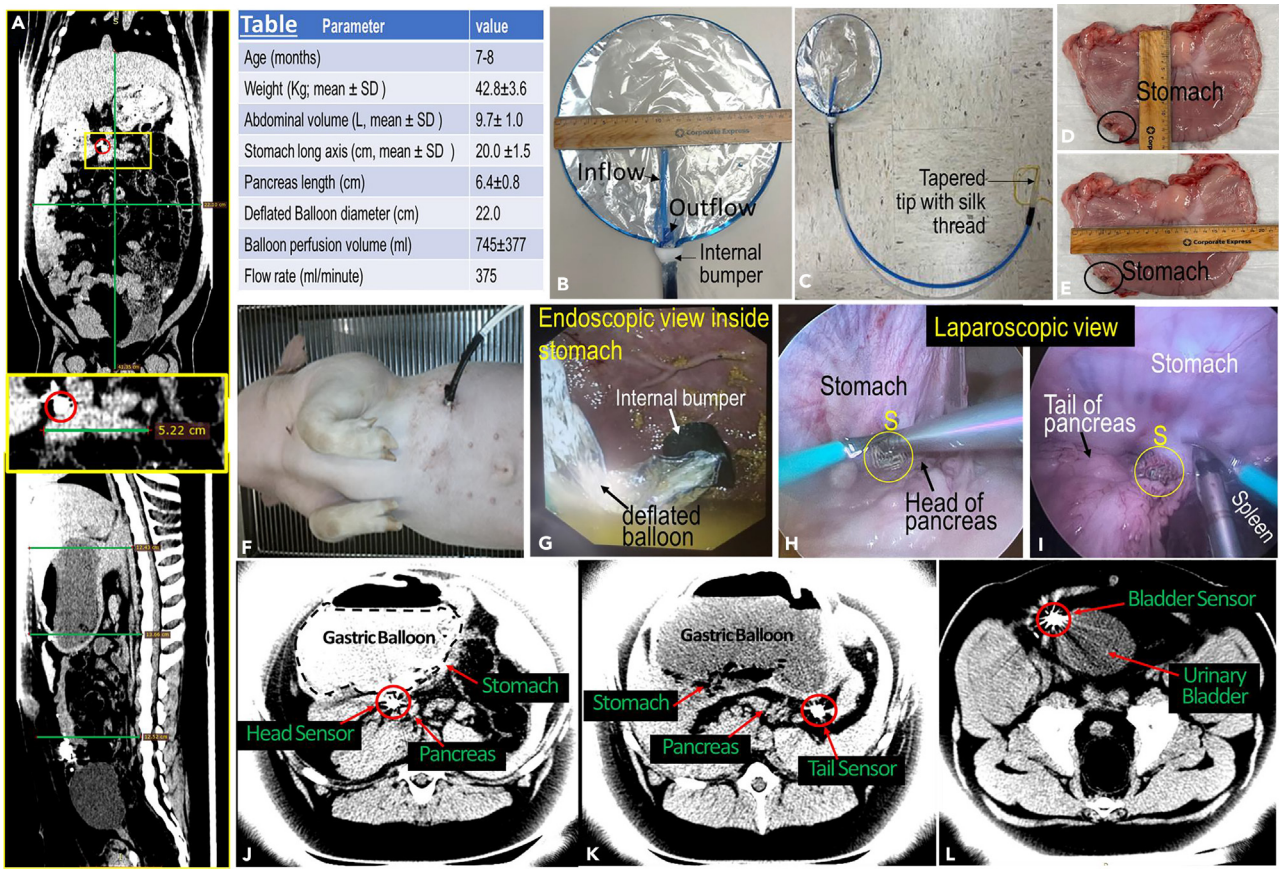

Figure 1. Experimental setup for gastric balloon and temperature sensor placement

(A) CT scan images showing coronal cuts (upper panel) in the plane of the pancreas (inset in center) with temperature sensor (red circle) in the head-neck region. The lower panel shows a sagittal CT image in the midline. Table: This mentions important pig and balloon parameters.

(B) Image of the balloon showing the inflow and outflow tubing within it, and the bumper at the entry.

(C) Image of assembled balloon, tubing, tip and thread before disinfection for placement.

(D and E) Images, and dimensions of the anterior surface of the stomach at necropsy. Black oval shows point of catheter entry.

(F) Image of inflow and outflow tubes from the gastric balloon exiting the abdomen of a Yorkshire pig.

(G) Endoscopic image of the balloon in the stomach after placement, and before filling with water.

(H and I) Laparoscopic images of temperature sensor placement adjacent to the head of the pancreas (H), and tail of the pancreas (I).

(J and K) Cross sectional abdominal CT scan images at the level of the pancreas showing the temperature sensor (red circle) near the head (J) and tail (K).

(L) CT image showing the temperature sensor on the urinary bladder.

to cover the surface of the pancreas. The flow in the inflow and outflow tubing shown in Figure 1B was reversed to prevent excessive balloon volume being required for drainage from the shorter "outflow" tube.

Figure 3C compares the effect of using this set up on the pancreatic head (orange), tail (purple), and urinary bladder temperatures (black line on top). The chiller temperatures over the 7 h of cooling are shown in the further section in black dots connected by lines. Chiller temperatures averaging between 14°C and 16°C rapidly reduced pancreatic temperature by > 10°C within 60 min. Reduction in chiller temperatures was paralleled by a similar reduction in pancreatic temperatures in both the head and tail of the pancreas, while bladder temperatures were unaffected, thus avoiding generalized hypothermia while cooling the pancreas. Cessation of balloon perfusion, and drainage of the gastric balloon at 7 h was promptly followed by normalization of both pancreatic head and tail temperatures within 1 h. Since transgastric cooling was rapidly inducible and reversible, we next studied if the pancreatic temperatures relevant to treating pancreatitis (shown in Table 1) could be achieved.

Pancreatic temperatures required for treating acute pancreatitis can be safely achieved

We next studied if 31°C–33°C, and 24°C–27°C could be achieved in the pancreas, since these are the previously established temperatures for treating acute pancreatitis, as summarized in Table 1. As seen in the blue line in Figure 4A sedation alone did not reduce pancreatic temperatures. On perfusing the balloon at 19°C (green line in chiller temperature in Figure 4A), we noted a prompt reduction of pancreatic temperatures to ≈32°C by 30–45 min (green line in mean Panc. Temps Figure 4A). Each degree reduction in chiller

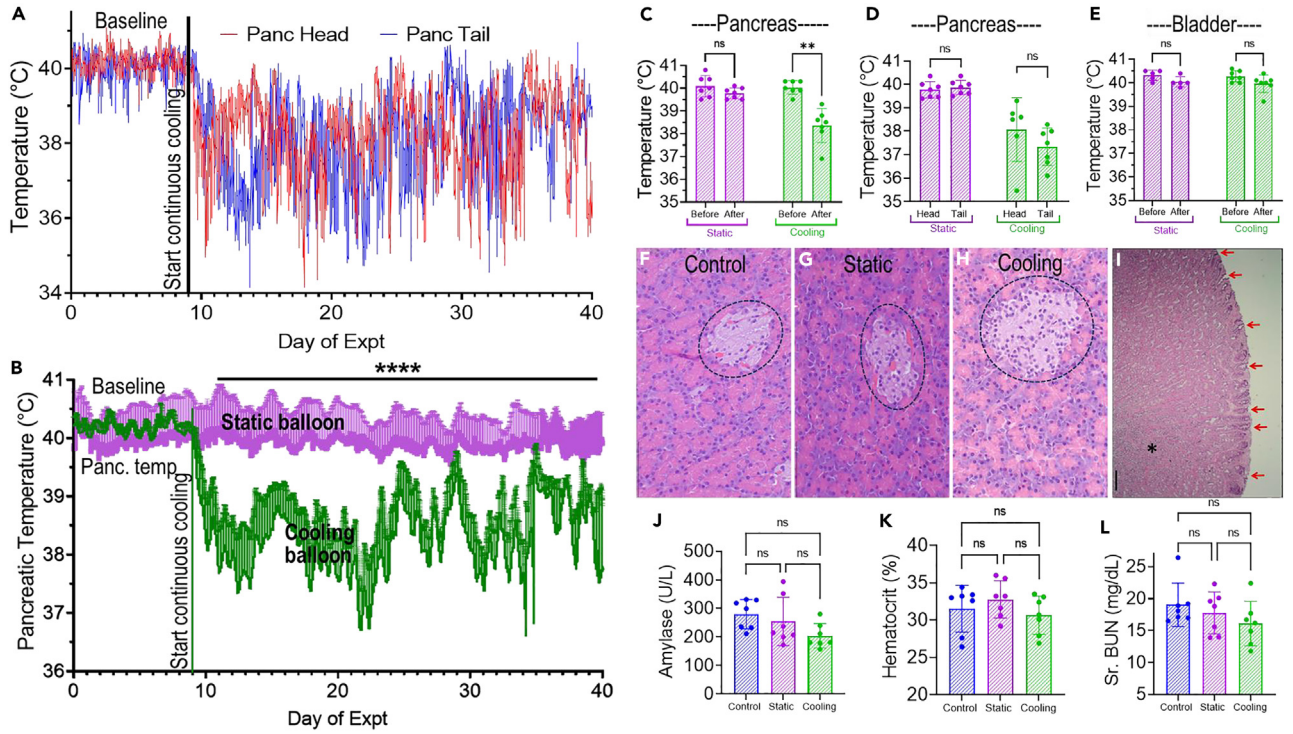

Figure 2. Gastric balloon tolerance studies done over 40 days, including 30 days of balloon perfusion in the cooling group

(A) Time course showing temperature readings of the pancreatic head (red line) and tail (blue line) sensors from a pig with a tap-water perfused gastric balloon. Perfusion was started on day 9 (vertical line). Note the lowering of pancreatic temperatures after starting perfusion.

(B) Time course of mean pancreatic temperatures ±SEM from 6 to 7 pigs with a static balloon (purple) or a perfused cooling balloon (green).

(C–E) Bar graphs (mean ± SD) comparing temperatures at baseline (before) and during the 1 month cooling (after). Individual values (dots) represent the 1-month average for a pig, with green being for the cooling, and purple being for the static balloon pigs. (C) Mean pancreatic temperatures. (D) head and tail temperatures, and (E) bladder temperatures.

(F–H) Representative H&E stained 40 x images of pancreas histological sections from the control (F), static (G) and cooling (H) groups of pigs. The dashed ovals show an islet.

(I) Low power (4x) H&E-stained image of the gastric mucosa of a pig with the cooling balloon at a site distal to the stoma. Note the normal gastric mucosa. Gastric pits palisade along the apical aspect of the section (red arrows), with the lamina propria below the pits (black asterisks), lamina muscularis is not pictured, but would be at the base of the section; Bar = 500 microns.

(J–L) Bar graphs (mean ± SD) with individual pig values (dots) at the end of the study period comparing serum amylase, blood hematocrit and blood urea nitrogen (BUN) in controls (blue), static and cooling groups by ANOVA. NS; not significant.

temperatures subsequently to 16°C over the next 2–3 h reduced pancreatic temperature to a similar magnitude, which was maintained over the next 3–4 h.

We then studied the effects of using chiller temperatures of <16°C on pancreatic cooling (black line in chiller temperature in Figure 4A). Reduction of chiller temperature to ≈10°C over 60–90 min, reduced pancreatic temperatures to ≈26°C (black lines in mean Pan. Temp, Figure 4A). Further reduction in chiller temperature to 5°C–6°C reduced pancreatic temperature to a similar extent over the next 5–6 h. Thus, pancreatic temperatures to treat acute pancreatitis were promptly achieved in large animals.

We next compared common renal, liver, and hematological parameters in the blood of the pigs at the end of the study (Figures 4B–4J). These parameters were similar in the cooled (black bars) and sedation only pigs (blue bars). The blood pressures were also similar in both groups over the course of the study (data not shown). Cessation of cooling and reversal of sedation resulted in prompt recovery of the pigs, with normal behavior and food intake. Therefore, transgastric pancreatic cooling to treat mild pancreatitis in large animals can be achieved with chiller temperatures ≈19°C, and for treating severe pancreatitis at chiller temperatures ≈10°C by using the perfusion and balloon specifications in this study.

DISCUSSION

This study shows that transgastric pancreatic cooling to temperatures required for treating acute pancreatitis is safely achievable in large animals. Here, we used 40–50Kg (88–110 lbs.) pigs whose viscera are slightly smaller than humans. Abdominal volumes in humans typically range from 10 to 25 L, gastric long axis' range from 15 to 22cms, and pancreas size averages 15–20 cm or 80–90gm.[13,14] The corresponding sizes in

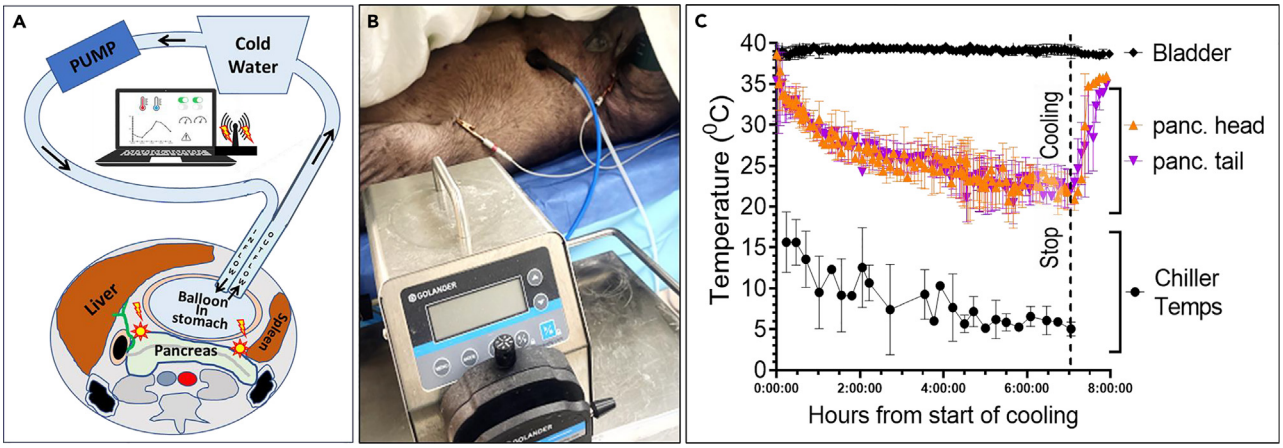

Figure 3. Sedated cooling setup, comparison of pancreatic head, tail, bladder temperatures, and effects of cessation of cooling

(A) Schematic for sedated cooling, showing the chiller and pump bringing in water to the gastric cooling balloon via the inflow tubing, while the outflow tubing returns water to the chiller for cooling. The flow rates were 375 mL/min, and water in the gastric balloon varied from 300 to 1100 mL during various studies.

(B) Image of the inflow and outflow tube exiting the left upper abdomen of a Yucatan pig. The pig is covered by a Bair Hugger blanket (white), and the pump is seen in the front.

(C) Time course of changes in pancreatic head (orange), tail (purple) and urinary bladder temperatures (black line on top) after starting cooling (chiller temperatures at the bottom). Individual points are average, and error bars show SEM. Cooling was stopped at 7 h. Note the similar reduction in head and tail temperatures with cooling, that promptly normalize after cessation of cooling.

our pigs were 40–100% of humans (Table in Figure 1), though the pancreas length in our pigs was measured in a single coronal CT cut and is probably longer. The pancreatic cooling temperatures targeted were those established in the 3 mechanistically different pancreatitis models shown in Table 1. Keeping the hypothermia localized to the pancreas, and avoiding generalized hypothermia was an important part of this study. Thus, we chose pigs to test pancreatic cooling here due to the closeness of pigs' size and anatomy to humans. We did not test pig models of acute pancreatitis, since these require a laparotomy[15,16] and cause operative stress that would confound interpreting the effects of cooling.

The device was an endoscopically placed gastric balloon perfused with water via two catheters that exited onto the abdominal wall (Figure 1F). This route was necessary to evaluate tolerance to the perfused balloon over 1-month in alert upright pigs, since nasogastric placement would have been intolerable for the animals. The perfused balloon volumes ranged from 300 to 1100mL which are <70% of the calculated stomach volume. The pigs with perfused balloons exhibited similar behavior, activity, dietary intake, laboratory parameters, and pancreatic histology as control groups.

A supine position achieved pancreatic temperatures for treating both mild (31°C–33°C) and severe (24°C–27°C) acute pancreatitis within 30–90 min. This required anesthesia and was done with chiller water temperatures ranging from 5–20°C perfused at 375 mL/min and required a Bair hugger external warmer to maintain core temperature. Such cooling resulted in similar pancreatic head and tail temperature, avoided generalized hypothermia, and was maintained for 6 h, as per the approved anesthetic protocol. Pancreatic cooling was rapidly reversible after emptying the balloon, with return of the pig to normal activity, behavior, and food intake after recovery from anesthesia.

Pancreatic cooling in the tolerance studies done in upright pigs had a slower onset (over 1 day) and was of a lesser magnitude than in the supine position. In the upright pig, gravity increased the distance between the dependent balloon and the pancreas (which was higher and fixed in the retroperitoneal space), thus reducing cooling efficiency. Perfusion in upright pigs was done while they were active, ambulating, and turning around in the pen on the jacket swivel. This necessitated using external drainage of the balloon outflow to avoid kinking of the inflow tubing. This in turn required large volumes of water, i.e., 540 L per day. Therefore, tap water (~22°C) was used for the tolerance studies. Tap water was ~18°C cooler than baseline pig pancreatic temperatures, but only cooled the pancreas by ~1.7°C over a day. Conversely, perfusing at 19°C in the supine position (~21°C cooler than baseline) reduced pancreatic temperatures to <32°C, i.e., ~8°C below baseline within 30–60 min. Thus, pancreatic cooling in the supine position is more efficient than upright cooling. The supine position is also relevant to acute pancreatitis patients who typically lie supine. It should be noted that the balloon typically contained 25–75% of its full volume (1600 mL) while being perfused with water. These volumes allowed maximal contact with the posterior gastric wall overlying the pancreas (Figures 1J and 1K), while keeping pressures within the balloon low. The volume of water in the perfused balloon was about 1–3 times the perfusion rate (375 mL/min), thus allowing a reasonable contact time of the cooler balloon water with the warmer stomach before it exited the balloon.

As shown in Table 1, reducing pancreatic temperatures to 24°C–27°C provided protection in the GTL model. Such cooling reduced severe pancreatic necrosis, hypotension, serum fatty acids, cytokines (like serum IL-6, TNF-α), damage associated molecular patterns (DAMPs) like ds-DNA, and histone DNA complexes, lung and renal injury, thus protecting from local and systemic injury.[4] Similarly, reducing pancreatic

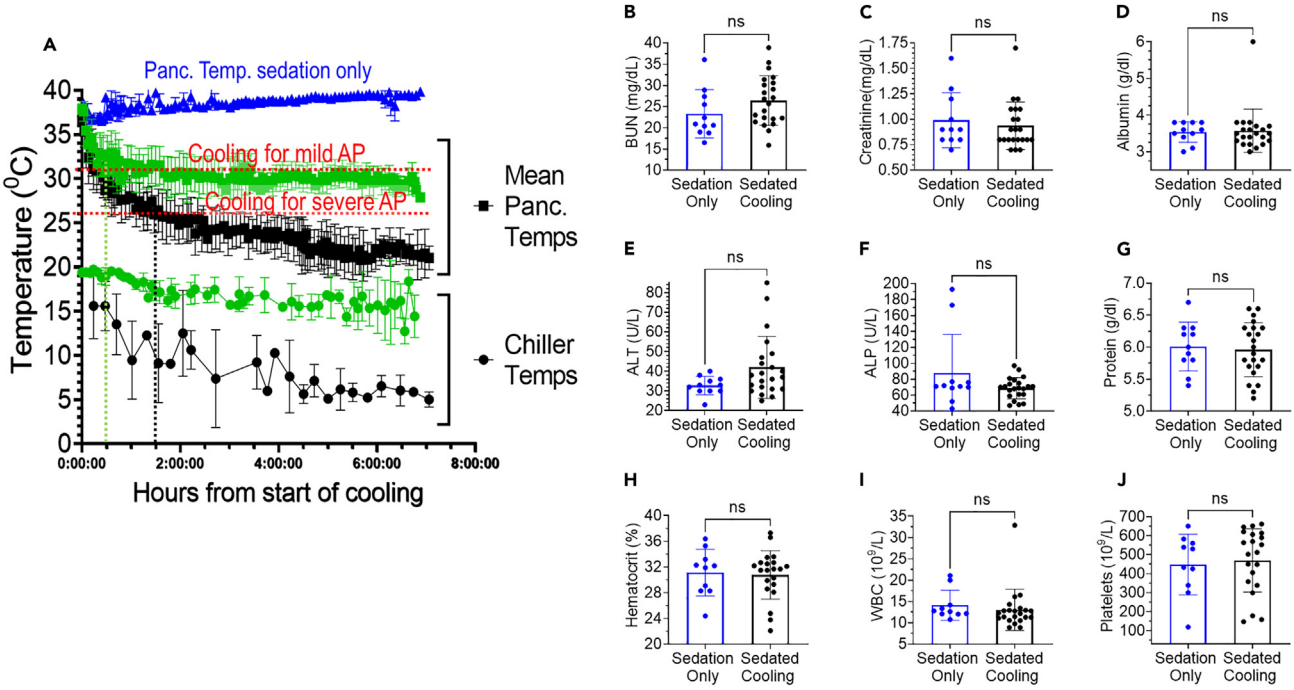

Figure 4. Efficacy of sedated cooling, and its effect on common blood parameters

(A) Graph showing relationship (as mean ± SEM) of mean pancreatic temperatures in pigs (upper part) and corresponding chiller water temperatures (lower part). The black lines show the effects of using chiller water temperatures ≤16°C with a goal to achieve pancreatic temperatures <26°C, that target severe AP (lower dashed red line). The green lines show the effects of chiller water temperatures of 16°C–20°C that target pancreatic temperatures of ≈31°C for treating mild pancreatitis (upper dashed red line). The blue line shows pancreatic temperatures of pigs undergoing sedation only.

(B–J) Biochemical serum (B–G) and hematological (H–J) parameters (mentioned on y axis) at the end of the study period in pigs with sedation alone (blue) vs. those with pancreatic cooling and sedation (black). There were no significant (ns) differences between the groups on a Mann-Whitney test.

temperatures to 24°C–27°C reduced systemic injury in necrotizing pancreatitis induced by sodium taurocholate. Here cooling reduced hypotension, cardiac injury, lung inflammation, DAMP, and cytokine increases.[5] These two previous studies defined the pancreatic temperatures required to protect from acute severe pancreatitis, however, it remained unknown if these temperatures could be achieved in large animals before doing human trials.

The multimodal effects of cooling aforementioned are important in pancreatitis since multiple mechanisms of acinar cell death like phosphatidyl inositol 3-kinases,[17,18] Src activation,[19] trypsinogen activation[17,18] can be triggered in pancreatic acini, with the same stimuli also activating pro-inflammatory transcription factors, such as NF-κB and AP-1,[20,21] and generating numerous cytokines and chemokines.[22] Additionally, cooling can slow the lipolytic lipotoxic pathways in severe pancreatitis.[2,4,5,23,24] No single drug therapy has been effective in treating established acute pancreatitis. For example, targeting serine proteases with gabexate mesylate,[25] reactive oxygen species (ROS) with allopurinol,[26] and platelet-activating factor with lexipafant,[27] showed no benefit in clinical trials. Similarly, while NSAIDs prevent endoscopic retrograde cholangiopancreatography (ERCP) induced pancreatitis, they do not reduce the severity of established pancreatitis.[28,29] These observations support a multi-pronged modality like local hypothermia, the efficacy of which was evaluated in previous studies,[4,5] and the large animal feasibility was assessed here.

In summary, the current studies show that transgastric pancreatic cooling is feasible in a supine large animal of a size similar to humans with a gastric balloon that covers most of the posterior stomach while being perfused with cool fluid. This ensures uniform cooling of the pancreas from head to tail. The previously established pancreatic temperatures required to treat pancreatitis, i.e., 24°C–33°C can be achieved in this manner. Generalized hypothermia can be avoided by combining this approach with external heating while monitoring core (e.g., bladder) temperature. In the absence of established drug therapy for severe acute pancreatitis, we believe that regional pancreatic hypothermia, as demonstrated in this proof of principle study, can be achieved safely, with the potential to improve clinical outcomes. Future studies are required to translate this approach to treating human acute pancreatitis.

Limitations of the study

While our study showed the efficacy and feasibility of localized pancreatic hypothermia in large animals, it is yet to be tested in humans to understand its clinical applications. Moreover, while the active alert pigs tolerated the gastric cooling balloon perfusion for a whole month, in humans, such a device would ideally be placed nasogastrically, and therefore our findings using the percutaneous endoscopic

gastrostomy route would need to be validated. Since humans tolerate similar balloon volumes (500–900mls) over several months for weight loss,[30] this should not be an issue. Cooling the pancreas in supine pigs required sedation, and external heating to prevent generalized hypothermia. Gastric cryotherapy (e.g., with dry ice at −80°C) is currently used in several US medical centers and worldwide.[31,32] There have been no reported complications of generalized hypothermia, visceral ischemia, or cardiorespiratory or renal compromise due to such cooling. Therefore, the mild perfusion temperatures of 10°C–20°C required to therapeutically cool the pancreas are unlikely to cause complications. Lastly, we could not test pancreatic cooling to treat acute pancreatitis in pigs due to the operative stress and confounding effects of laparotomy[15,16] required in these surgical models. However, previous rodent studies have shown the therapeutic benefits of cooling in three mechanistically distinct models ranging from mild to severe pancreatitis. These support further studies of this technology in humans.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Vijay P. Singh (singh.vijay@mayo.edu).

Material availability

This study did not generate any new materials or reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Acquisition of data were facilitated and carried out by M.S., B.R., P.R., S.J., A.G., N.W., N.G., and V.P.S. Analysis and interpretation were done by M.S., B.R., A.G., N.W., N.G., and V.P.S., who also helped in the critical evaluation of the manuscript. The manuscript was drafted by M.S. and V.P.S. Statistical analysis was done by M.S. and V.P.S. V.P.S., N.W., and N.G. supervised the study and V.P.S. designed and conceptualized the study.

DECLARATION OF INTERESTS

V.P.S. has a related patent issued (US 10,842,668 B2) entitled GASTRODUODENAL BALLOON TUBES AND METHODS FOR USE IN LOCALIZED HYPOTHERMIA.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Yucatan pancreas tissue	Pigs in study	
Chemicals, peptides, and recombinant proteins		
Xylazine	Biomeda-MTC	
Telazol	Zoetis	
Glycopyrrolate	Hikma Faramaeutica	
Isoflurane USP	Piramal Critical Care	
Cefazolin	Hikma Faramaeutica	
Omeprazole	Sandoz; Lek Pharmaceuticals	
Banamine	Merck & Co	
Cidex	Johnson & Johnson	
Critical commercial assays		
Hematological parameters panel	HESKA	N/A
Biochemical parameters Fuji Dri-Chem Comprehensive S-panel	HESKA	CAT 6330-COMP/EWRAP
Deposited data		
Raw and analyzed data	This paper	
Experimental models: Organisms/strains		
Pig: Sus scrofa Yorkshire	Premier Biosource	
Pig: Sus scrofa Yucatan	Premier Biosource	
Software and algorithms		
Temperature sensors CubiSens	Cubeworks Inc	TS100s
GraphPad Prism 9	Graphpad Software	
Radiant DICOM Viewer	RadiAnt	

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animal studies

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Mayo Clinic foundation, and the Animal Care and Use Review Office (ACURO) of the Department of Army. There were two strain of pigs. Preliminary feasibility studies were done in 35-80kg castrated male Yorkshire pigs (*Sus scrofa Yorkshire*) due to the larger range of sizes in this strain. After optimizing feasibility and compatibility, definite studies were done in the more obese Yucatan strain (*Sus scrofa Yucatan*) using male barrows, (35–40 Kg). Animals were 6–7 months old. Both strains were procured from Premier BioSource (Ramona, CA). Pigs were acclimated for at least 7 days before any procedures were done, and 7 days after procedures for recovery. At the time of completing studies, the pigs weighed 40–50 Kg i.e., 88–110 pounds. The pigs were housed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition and the USDA Animal Welfare Regulations. The temperature in the animal room was maintained between 21°C and 24°C and relative humidity of 30–70%. The pig housing room light cycle was 12 h of light (0600–1800) and 12 h of dark (1800–0600). Toys, including balls, chains, and/or food enrichment were provided to the pigs daily. These were housed as a group until the day before the experiment, and thereafter individually in pens (at least 32.0 sq. ft./pig). The pigs were provided *ad libitum* access to chlorinated water via automatic watering devices and were given 2.3 kg Purina Laboratory Porcine Grower Diet 5084 (4% fat, Lab Diet, St. Louis, MO) between 6 and 7 AM daily. Each pig was given 20 mg omeprazole by mouth in a treat (grape, banana, marshmallow, etc.) 30 min before being given their standard diet to avoid gastric ulcers from balloons.[13]

METHOD DETAILS

Balloon design

A 22 cm diameter foil balloon (Anagram International Inc., Minneapolis, MN) was used (Figures 1B and 1C). The goal was to have maximal surface area of the balloon to contact the interior of the stomach under low pressure when the partly filled balloon was being perfused with water. The size is suitable for the pig stomach size[13] (Table in Figures 1D and 1E), the long axis of which was 19–22 cm, similar to adult humans.[11] The inflow, outflow polyethylene tubing (John Guest, Parsippany, NJ) was sealed at the balloon orifice using adhesive, and heat-shrink tubing (black cuff; Figures 1B and 1C) and was covered by soft silicone internally. (Figure 1B). The internal rubber retaining bumper (white in image 1B, C and black in image 1G) was then implanted and a tapered 5mL pipette tip with an adherent 20cm loop 0-0 silk was superglued to the free end of the tubes. The balloons and catheters were disinfected overnight (Cidex; ortho-pthaldehyde; Johnson and Johnson) and implanted into the pig endoscopically the next morning.

Pre-operative care

Endoscopic and laparoscopic surgery were performed after the 7-day acclimatization period. Pigs were fasted overnight before the procedure. These were sedated with Tiletamine and Zolazepam [Telazol®; Zoetis, Kalamazoo, MI] (5 mg/kg), Xylazine [XylaMed®; Biomeda-MTC, Cambridge, ON, Canada] (2 mg/kg), Glycopyrrolate [Hikma faramaeutica, Portugal, S.A.] (0.01 mg/kg) intramuscularly. At the time of balloon placement, these were transferred to the endoscopy suite and endotracheally intubated, ventilated with airway protection and maintained on Isoflurane, USP [Piramal Critical Care, Bethlehem, PA anesthesia] (1.5–3.0%) for the surgery starting with endoscopy till the end of the laparoscopic procedure mentioned below. Cefazolin [Hikma faramaeutica, Portugal, S.A.], an antibiotic, was given at a dose of (15–25 mg/kg) intravenous every 90 min during the surgical procedure. Pre-operative analgesia included Banamine-S [flunixin meglumine injection, Merck & Co., Inc Madison, NJ), 2.2 mg/kg, intramuscularly.

Endoscopic balloon placement in the pig

A GIF-2TH180 dual channel endoscope (Olympus) was passed into the stomach *trans*-orally, and the balloon was placed like a percutaneous endoscopic gastrostomy (PEG) tube using the "pull" method.[14] Briefly, the stomach was insufflated with air, *trans*-illuminated, and a trocar needle was introduced into the stomach from the anterior abdominal wall using sterile precautions. A 0-0 silk suture was passed through the cannula, grasped with a snare, and taken out though the mouth along with the endoscope. The silk thread at the tapered end of the tubes (Figure 1C) was tied to the suture and pulled though the mouth, stomach, and the anterior abdominal wall (Figure 1F) until resistance was felt. The endoscope was then passed into the stomach and the internal bumpers confirmed to be near the anterior gastric wall (black bumper, Figure 1G). The external bumpers were slid over and superglued to the tubes. In case of the static balloon group, the balloon was inflated with water and the tubes were sealed with silicone adhesive.

Laparoscopic temperature sensor placement

Using sterile technique and general anesthesia, the abdominal cavity was accessed via a cut-down technique in the midline just above the genital area. Initial placement of a 10-12mm laparoscopic trocar was followed by insufflation of the abdomen to 12-15mm Hg using carbon dioxide to create the working space needed. Two additional 5 mm trocars were placed under direct vision in the left lateral and left lower quadrants of the abdomen. Non-traumatic laparoscopic graspers were used to lift the stomach and the left lobe of the liver to expose the pancreatic head and tail. The wireless temperature sensor devices (S; Figures 1H and 1I; CubiSens TS100s; Cubeworks, Ann Arbor, MI) were introduced through the midline trocar and placed adjacent to the head or tail of the pancreas as shown. The sensor was encased in a Vicryl mesh bag (Ethicon Style 12 Ref# VM220K). The sensors were secured with either with 3-0 Vicryl sutures or 5mm clip appliers (Covidien Endo Clip III Ref# 176630). Similarly, a third temperature sensor was placed over the dome of the bladder and secured using 5mm clips. The location of these sensors remained stable until the time of necropsy as shown in the cross-sectional CT images (Figures 1J–1L). At the end of the procedure the trocars were removed and the abdomen desufflated. The midline trocar site fascia was closed with interrupted 3-0 Vicryl sutures and the skin incisions with 4-0 Monocryl suture followed by sterile dressing. There was one complication of urethral injury at the time of trocar placement, therefore, the pig was euthanized and excluded from the study.

Post-operative care

For the tolerance studies described below, the pig was then placed in a medium sized jacket (Sai-infusion technologies, Lake Villa, IL) with a tether in the mid-back, through which the inflow tubing was routed. A grommet was placed below the tether to prevent slipping of the inflow tubing, and thus avoid pressure on the balloon if the inflow tubing were under tension. The pig was then allowed to recover and given food in the evening. Analgesia was continued up to 3 days post-operatively using Banamine-S. Animals were monitored closely for adverse events like listlessness, vocalization, isolation, and lack of appetite, agitation, tooth grinding, apathy, grunting, vomiting as signs indicating unwellness, discomfort or need for analgesia. There were no complications at the initial balloon placement, and recovery after each balloon placement was uneventful. After balloon placement, the PEG site was inspected daily. Balloon perfusion studies or sedated cooling studies (described below) were initiated 8–11 days late to ensure healing and confirming that there was no infection or leakage at the PEG site. The connection between the CubiSens TS100s and the wireless receiver placed within 20 feet of the pig was confirmed before use and provided temperature data every 5 or 10 min depending on the software setting.

Tolerance studies in alert pigs

These studies were done over 30 days to study balloon tolerance while continuously perfusing water at room temperature. There were three animal groups in these studies, with 7 pigs in each group that went till the completion of the studies without major complications. These were: 1) a control group with no intervention. 2) the static balloon which was filled up with water and sealed and not perfused. 3) the cooling group, which had the inflow tubing to the balloon attached to running tap water with continuous perfusion at 375 ml/min and the outflow tubing drained to gravity. The flow rate was chosen based on preliminary studies of the balloon on the bench, simulating appropriate locations of the inflow and outflow tubing, and a half-filled balloon, with low pressures. The perfusion was regulated by a flowmeter. The pig had the freedom to move anywhere in the pen during the perfusion via the pulley and swivel installed in the inflow tubing. Pig behavior including normal activity, playing with toys and food intake were monitored. Adverse events such as those mentioned above were looked for daily, and not noted. The presence of particulate matter or turbidity in the outflow was monitored twice day, and if present taken as balloon perforation and leakage. A perforation without any adverse signs was taken as a minor complication. Perfusion and food intake was withheld immediately, and the balloon replaced the next day. There was one such case of minor rupture. A major balloon rupture overnight because the pig laid on and kinked the outflow tubing was excluded from the studies.

Targeted pancreatic cooling in sedated supine pigs

The overnight fasted pigs were sedated and intubated prior to ballon perfusion as described above under the section on pre-operative care. During this period, the pigs here were supine as would be typical of a pancreatitis patient lying on their back, with the balloon in the stomach just above the pancreas, as shown in Figures 1J and 1K. There were two groups in these studies: 1) sedation alone, and 2) sedation with cooling, both of which were similarly ventilated, and received intravenous saline. In the sedation with cooling group, a Bair hugger® blanket (3M, Saint Paul, MN) was placed over the pig to provide external heating and prevent generalized hypothermia. The inflow tubing was connected to a pump that brought in water at 375 mL/min from a chiller whose temperature could be regulated. The water from the outflow tubing was returned to the chiller as in a closed circuit. Blood samples were collected at the end of the procedure via a cranial vena cava puncture. At the end of the studies, terminal blood draws were done, and the animals were euthanized with intravenous Euthasol® (390 mg pentobarbital sodium & 50 mg phenytoin sodium) [Virbac AH, Inc., Fort Worth, TX]. CT scans were done postmortem on a Ceretom 8-slice helical CT scanner and were analyzed using RadiAnt DICOM Viewer software.

QUANTIFICATION AND STATISTICAL ANALYSIS

Parameters measured

Pancreatic and bladder temperatures were recorded. Weights for each animal were measured in Kilograms (Kg). Stomach size was calculated by measuring its length (Figure 1D). Abdominal volumes were calculated by using CT measurements shown in Figure 1A. The vertical height (D1) and transverse diameter (D2) were measured on coronal cuts. The anteroposterior diameters were measured at the lower end of the sternum, mid abdomen, and the pelvic brim and averaged to a mean AP diameter (D3). The 3 radii (r1, r2, r3) were calculated from these diameters respectively, and the abdominal volume calculated using the equation for an ellipsoid as $4/3\ \pi$ r1.r2.r3. The longest pancreas length was directly measured from CT scans (e.g., Inset Figure 1A) in a single plane. Blood was collected at the end of each study in EDTA vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) for hematologic parameters, and was allowed to clot in a plastic vacutainer for serum parameters. Hematologic parameters were measured within 2 h of collection on a HESKA veterinary hematology system, (HESKA, Loveland, CO). Serum was immediately separated after clotting and processed for routine chemistry on a FUJI DRI-CHEM FDC4000 (FUJIFILM corporation, Tokyo, Japan), including creatinine, blood urea nitrogen, liver function tests. Remaining serum was aliquoted on ice and stored at −80°C within 4–5 h of blood collection and analyzed for pancreatic enzymes including serum amylase using the biochemical kit from Pointe Scientific (Canton, MI).

Statistics

Temperature readings were depicted either over the time-course of the study, with time (hours or days) on the x axis, and temperature on the Y axis, or as bar graphs depicting average temperature for each sensor. For the month-long tolerance study, the data points from individual pigs were averaged over an hour, since the number of data points exceeded 5000. Data was compiled in an Excel sheet and graphed using GraphPad Prism (Boston, MA). Data from multiple pigs are shown as mean ± SD. Lab values at the end of the study are compared between various groups. Comparison between two groups (e.g., sedation alone vs. sedation+ cooling) were done using a Student's t test or a Mann-Whitney test for abnormally distributed data, and by using ANOVA between 3 groups (control, static and cooling) in the tolerance studies. A p value < 0.05 was regarded as significant, and shown as asterisk notation (*), or $p < 0.01$ (**), $p < 0.001$(***), and $p < 0.0001$ (****).