Creatine-loading preserves intestinal barrier function during organ preservation

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\textbf{ABSTRACT}

We have developed a novel, intraluminal preservation solution that is tailored to the metabolic requirements of the intestine. This organ-specific solution addresses many of the problems associated with low temperature organ storage including energy, oxidative and osmotic stresses. However, conservation of energy levels remains one of the most difficult obstacles to overcome due to the inherent sensitivity of the mucosa to ischemia. Creatine-loading has become a popular and scientifically proven method of augmenting energy reserves in athletes performing anaerobic burst work activities. We hypothesized that if we could develop a method that was able to augment cellular energy levels, the structure and function of the mucosa would be more effectively preserved. The purpose of this study was to determine if creatine-loading is a feasible and effective strategy for preserving the intestine.

Our data indicate that creatine loading has significant impact on energy levels during storage with corresponding improvements in mucosal structure and function. Both of our rodent models, a) continuous perfusion for 4 h and b) a single flush with our intraluminal preservation solution supplemented with 50 mM creatine, demonstrated significant improvements in creatine phosphate, ATP, Energy Charge and ATP/AMP following cold storage (P < 0.05). Notably, after 10 h creatine phosphate was 324% greater in Creatine-treated tissues compared to Controls (P < 0.05). Preferential utilization of glutathione in the Creatine group was effective at controlling oxidative injury after 10 h storage (P < 0.05). Improvements in barrier function and electrophysiology with creatine-treatment reflected superior mucosal integrity after 10 h storage; Permeability and Transepithelial resistance measurements remained at fresh tissue values. This was in stark contrast to Control tissues in which permeability rose to > 300% of fresh tissue values (P < 0.005) and transepithelial resistance dropped by 95% (P < 0.005). After 10 h storage, Park’s grading of histologic injury reflected extensive villus denudation (grade 4) in control tissues compared to healthy tissue (grade 0) in the Creatine group. This study demonstrates that a strategy of creatine supplementation of our intraluminal preservation solution facilitates the preservation of the intestinal mucosa during storage.

\section*{1. Introduction}

Intestinal failure is described as an inability of the bowel to absorb adequate nutrients, fluid and/or electrolytes, resulting in cachexia and eventual death if left untreated. This can occur by 1) loss of intestinal surface due to massive surgical resection, or 2) functional disturbances in gut motility and/or absorption \cite{4,11,14}. The small intestine has a large digestive and absorptive reserve, however, if > 75% is resected, nutrient absorption is limited and leads to a starvation-like state, Short Bowel (Gut) Syndrome \cite{4,11,39}. The mortality rate for intestinal failure is much higher than that for other digestive diseases; mortality rates are as high as 40% \cite{8,21,22,47}. The overall incidence of
intestinal failure in the pediatric population is 12/1000 live births; making it one of the most lethal conditions in infants and children [9,30,40]. First line treatment involves Total parenteral nutrition (TPN) delivering nutrition on a regular basis via intravenous infusion. Unfortunately, the long-term prognosis for those receiving TPN is poor since issues of venous access and infection can result in life-threatening consequences [11,39]. Those patients that develop complications with medical management have no other option except for small bowel transplantation. Although, morbidity and mortality of recipients are gradually improving, one of the factors limiting success with transplantation is the inability to effectively preserve the small intestine. In a recent publication from the University of Pittsburg, an overall 5-year actuarial graft survival was 50% [1]. One major obstacle in intestinal transplantation today is that of infection and sepsis. This is, in part, directly related to poor graft quality following cold storage. Ischemia-reperfusion injury initiated during storage leads to the loss of barrier function and bacterial translocation with a dramatic increase in inflammation and the potential for life-threatening infection.

At time of organ harvest and subsequent storage, intestinal blood flow necessarily becomes compromised. Cellular energy levels are quickly depleted during ischemic cold-storage because even at hypothermia there are many energy-requiring operations still in use [6,13]. The development of preservation solutions for other commonly transplanted organs has focused on an intravascular flush solution in order to remove blood and to reduce the rate of degenerative processes with little attention to tissue-specific nutrients. Fortunately, the intestine has an alternate route of access, the lumen. Our strategy of luminal administration of an effective preservation solution takes advantage of the intestine’s well-known ability for preferential substrate uptake via the mucosa. Throughout the past few years, our laboratory has developed a novel amino acid (AA)-based preservation solution called the AA solution [12,18,19,25,26,32–38,43,52]. This preservation solution has been specifically tailored to the physiologic requirements of the intestine and consists of a number of trophic agents including osmotic agents, buffer, enzyme inhibitors, and anti-oxidants, thereby facilitating energy production and control of osmotic & oxidative stress. One of our goals is to augment energy reserves prior to or in the initial phase of static cold storage. The initial 2–4 h provides a window of opportunity when augmentation of energy reserves is possible as well as one that is translatable to the clinic.

Creatine, the precursor to the high-energy phosphagen creatine phosphate, protects tissues from damage incurred during periods of low oxygen or burst work activity [15,23,45,46,50]. Creatine phosphate (CrP) is a primary energy reserve in muscle and brain; CrP replenishes ATP pools via direct phosphorylation of ADP during periods of energetic stress. Although, levels in muscle are quantitatively greater than intestine, we have determined that in relation to ATP pools, levels in the intestine are 100% of ATP (unpublished data). Mechanistically, creatine is taken up via NaCl-dependent membrane transporters located on the mucosal surface [29,42]. Furthermore, the mucosa contains relatively large amounts of the synthesizing enzyme creatine kinase (41 ± 2 IU/g in porcine mucosa; data from our lab). The goal of augmenting energy reserves is to preserve the integrity of the intestine during ischemic storage so that a return to a ‘normal’ healthy, “fully energized”, state is facilitated upon reperfusion. Since, the intestine utilizes significant pools of CrP and possesses the necessary enzyme machinery and transport mechanisms for uptake and synthesis; this could represent a novel strategy for the prophylaxis of ischemia-reperfusion injury in the intestine. The goal of the current study was to determine if a novel technique of creatine-loading is a feasible strategy to amplify energy reserves, thereby facilitating energy homeostasis and preservation of the mucosal barrier during periods of cold storage.

2. Materials and methods

2.1. Summary of experimental design

All experiments were conducted in accordance with the regulations and policies of the Canadian Council on Animal Care. Chemical agents were analytical reagent (AR)-grade and were purchased from Sigma Chemical (Oakville, Canada). Male Sprague-Dawley rats (200–250 g; n = 6) were obtained from Biosciences at the University of Alberta. They were fasted overnight in cages with raised floors to discourage coprophagy. Water was provided ad libitum. Prior to laparatomy, rats received an intraperitoneal dose of pentobarbital (65 mg/250 g; Somnotol; MTC Pharmaceuticals, Cambridge, Ontario, Canada), followed by inhalational halothane (0.5–2%) to maintain anesthesia. A midline laparotomy was performed and the supraceliac aorta was exposed and clamped, and the superior mesenteric artery was flushed with 10 ml cold modified University of Wisconsin (UW) solution via the infrarenal aorta. The suprahepatic vena cava was transected to allow outflow of blood and perfusate. The entire jejunum and ileum was then harvested. The bowel was flushed intraluminally with 40 ml an amino acid based preservation solution (with and without 50 mM creatine).

This study consisted of two parts: part A was designed to demonstrate proof of principle that isolated intestine can be loaded with creatine under aerobic conditions; part B was designed to establish relevancy to the clinic (i.e. a simple intraluminal flush prior to cold storage without requiring continuous perfusion).

2.1.1. Part A: continuous 4 h perfusion followed by static cold storage

Organs were subjected to a 4 h period of cold continuous perfusion via the lumen; perfusate was recirculated at 2 ml/g/min (equivalent to a 20 cm pressure head) and was carried out in a 4 °C temperature controlled incubator with 80 ml of the intraluminal solution. The perfusate was saturated with oxygen via bubbling the solution with 100% oxygen at a rate of 2.7 l/min. Following cannulation of the jejunal end with PE360 tubing, perfusate was allowed to freely exit the ileal end of the graft leaving it filled without turgor during perfusion. The control group (“Control”) was treated intraluminally with our nutrient-rich AA solution; the experimental group (“Creatine”) was treated with the AA Solution plus 50 mM creatine. Tissue samples were removed from the distal end of the small intestine after 1, 2 and 4 h perfusion and rapidly frozen in liquid nitrogen for subsequent analysis. After 4 h perfusion, the bowel was removed from the perfusion apparatus and both ends ligated with 3–0 silk so that the bowel remained filled without turgor. The entire bowel was then placed in an additional 40 ml AA solution in pots. Grafts were then stored on ice in a 4 °C incubator and tissues sampled after 2 h, 10 h, and 24 h storage.

2.1.2. Part B: single intraluminal flush followed by static cold storage

After intravascular and intraluminal flushes, both ends were ligated with 3–0 silk and the entire bowel was placed in a pot with an additional aliquot (40 ml) of AA Solution. Intestines were then stored on ice in a 4 °C incubator; tissue samples were removed after 2, 4, 10 and 24 h storage for subsequent metabolite analysis and histological assessment. Histology samples were immediately placed in 10% buffered formalin, to be processed later. Permeability studies using Ussing chambers required separate groups of animals (n = 4); after 10 h and 24 h cold storage, tissues were assessed for alterations in total permeability (paracellular and transcellular) and transepithelial resistance (TER). Freshly isolated tissue was obtained from a separate group of animals to provide an approximation of in vivo metabolite levels and electrophysiology; data is presented as t = 0 in the figures.

2.2. Composition of solutions

The Amino Acid-based solution (AA solution) contained: lactobionate (20 mM), adenosine (5 mM), allopurinol (1 mM), HES
Protein was measured according to Lowry et al. [20], ATP, ADP, and AMP. Values are reported as assays [28]. Spectrophotometric analysis was then performed to measure the absorbance of NADH at 340 nm, providing quantification of NADH activity in a scintillation counter to determine the mannitol flux across the tissue [7].

2.3. Metabolite analysis

**Adenylates:** Frozen tissue samples were extracted 1:5 w/v in perchloric acid containing 1 mM EDTA. The precipitated protein was removed by centrifugation (20 min at 20,000 × g). Acid extracts were neutralized by the addition of 3 M KOH/0.4 M Tris/0.3 M KCl and then recentrifuged (20 min at 14,000 × g). Aliquots of neutralized extracts were immediately processed via standard enzyme-linked metabolite assays [28]. Spectrophotometric analysis was then performed to measure the absorbance of NADH at 340 nm, providing quantification of ATP, ADP, and AMP. Values are reported as µmol per gram protein. Protein was measured according to Lowry et al. [20].

**Malondialdehyde (MDA):** Frozen tissue (100 mg) was homogenized 1:10 in phosphate-buffered saline. The homogenate was then processed and fluorescence was compared to standard amounts according to Ohkawa [24].

**Glutathione (GSH):** Frozen tissues (100 mg) were homogenized 1:5 w/v in 6% perchloric acid containing 1 mM EDTA. Precipitated protein was removed by centrifugation (20 min, 14,000 × g). Acid extracts were neutralized with 3 M KOH/0.3 M imidazole and recentrifuged (20 min, 14,000 × g). Extracts were analyzed based on fluorescence and compared to standard amounts of reduced glutathione as previously described [17].

2.4. Histology

Full-thickness biopsies were fixed in 10% buffered formalin solution, embedded in paraffin, cut to 5 µm, and stained with hematoxylin and eosin. Histologic damage was assessed using a modified Park’s histologic classification of intestinal tissue injury [27]; the term ‘infarction’ was replaced by ‘injury’, since this model did not contain an element of *in vivo* reperfusion.

2.5. Functional assessment - Ussing chamber analysis

Ileal segments were taken after 10 h and 24 h cold storage, stripped of their serosa and muscular layers. The mucosa was mounted in Ussing chambers exposing mucosal and submucosal surfaces to 10 mL of oxygenated Krebs’ buffer with an ionic composition of: Na+, 143 mM; K+, 5 mM; Mg²⁺, 1.1 mM; Ca²⁺, 1.25 mM; HCO₃⁻, 25 mM; Cl⁻, 123.7 mM; HPO₄²⁻, 1.95 mM; and fructose 20 mM 95% O₂ and 5% CO₂ and pH = 7.35. The spontaneous transepithelial potential difference was determined, and the tissue was clamped at zero voltage by continuously introducing an appropriate Isc with an automatic voltage clamp (DVC 1000 World Precision Instruments, New Haven, CT). Transepithelial resistance (TER) was calculated and reported. Mannitol permeability was measured by adding 10 µCi of (3H)mannitol (Dupont, NET101) to the mucosal side of the reservoir. After an equilibration period (20 min), samples were taken from the mucosal and serosal sides. Ten and 20 min after equilibration, samples were again taken from both the mucosal and serosal sides, and assessed for tritiated mannitol radioactivity in a scintillation counter to determine the mannitol flux across the tissue [7].

2.6. Statistical analysis

Metabolite data were reported as means ± SE for each group. Statistical differences between groups were determined using Analysis of Variance, followed by Tukey’s post hoc comparison test with *p < 0.05. Histology scores were compared using the Kruskal-Wallis test; *p < 0.05* was reported.

3. Results

3.1. Part A. Continuous 4 h perfusion followed by static cold storage

This section of the study was performed to determine if creatine loading in the intestine is a viable treatment option for organ preservation. Intestinal grafts were subjected to 4 h perfusion followed by 24 h static storage.
3.1.1. Energy levels

(Fig. 1: ATP, Creatine Phosphate, Energy Charge, ATP/AMP) Fresh tissue levels of creatine phosphate were 9.1 μmol/g protein, very similar to ATP levels of 10.0 μmol/g. Within 1 h, perfusion levels of creatine phosphate increased markedly by 51–86% above fresh tissue values in both Control and Creatine groups (P < 0.05 at all times of perfusion).

Creatine-loading was apparent in statistically significant differences between the two groups; the Creatine group was 15% and 9.5% greater than Control tissues after 2 and 4 h, respectively (P < 0.05). There was a similar jump in ATP levels and Energy Charge values after 1 h perfusion; this elevation was maintained for the full 4 h perfusion (P < 0.05). The ATP/AMP ratio was used to reflect the alteration in high to low energy adenylates as ATP was being utilized throughout the time course. Although there was no immediate increase in ATP/AMP, values in the Creatine group remained at fresh tissue levels throughout the perfusion period. This was in contrast to a reduction in ratio values in Control tissues; comparatively greater values were significantly greater in the Creatine group (P < 0.05), presumably due to increases in AMP levels (since there were no differences in ATP values).

Upon entry into static cold storage, all energetic parameters decreased significantly compared to the initial time point of storage (the 4 h time of perfusion; P < 0.05), however there were no differences between the two experimental groups after 2 h storage. The most relevant time points with respect to sustained effects of creatine loading were 10 and 24 h. All four parameters were significantly greater than Controls after 10 h (P < 0.05) and 3 of the 4 parameters, after 24 h (P < 0.05).

3.1.2. Oxidative stress

(Fig. 2: Glutathione and Malondialdehyde) Glutathione levels dropped significantly in the Creatine group during the perfusion period, whereas they remained high and constant in Controls. In creatine-treated tissues, levels continued to decline after 10 and 24 h of static storage (P < 0.05). Levels of the endogenous antioxidant also fell in the Control group throughout storage although not to the same degree (P < 0.05). Concentrations of the product of lipid peroxidation, malondialdehyde (MDA), fell dramatically immediately after 1 h perfusion and remained at approximately 30% of fresh tissue levels (P < 0.05). Despite the apparent effects of oxidative stress on glutathione levels, MDA in the Creatine group remained significantly lower than Controls after 10 h static storage. Compared to 10 h values, levels after 24 h increased significantly (P < 0.05, not shown).

3.2. Part B. Single intraluminal flush followed by static cold storage

This section of the study was performed to determine if creatine loading could be achieved with a single intraluminal flush; this would be an easier back table manipulation than continuous perfusion and would be translatable to the clinic.

3.2.1. Energy levels

(Fig. 3: ATP, Creatine Phosphate, Energy Charge, ATP/AMP) By 4 h storage, all 4 parameters had dropped significantly from fresh tissue values (P < 0.05); levels continued to decline at subsequent times. The effects of creatine loading via a single intraluminal flush were evident in both creatine phosphate and ATP/AMP levels; both parameters were significantly elevated in the Creatine group compared to Controls at all times (P < 0.05). The greatest effect of loading was apparent after 10 h, where creatine phosphate was 324% higher than Controls (P < 0.05). At the conclusion of storage, all parameters were significantly higher in Creatine tissues (P < 0.05).

3.2.2. Oxidative stress

(Fig. 4: Glutathione and Malondialdehyde) Patterns of change in both glutathione and MDA were similar to those of part A. Although glutathione levels declined in both groups over the storage time course (P < 0.05), levels were consistently lower in Creatine tissues, except for 10 h where they were equivalent. Interestingly, MDA levels dropped in both groups compared to fresh tissue values, however, after 10 h levels were significantly lower in the Creatine group (P < 0.05). In Control tissues, MDA rose gradually over storage, indicative of oxidative stress occurring during cold storage.

3.2.3. Mucosal barrier function

(Fig. 5: Permeability and Transepithelial Resistance) Fresh tissue values for both parameters were set to 100%. Permeability in control tissues rose to 174% after 10 h storage and by 24 h, permeability has risen to 331% of fresh tissue values. In contrast, creatine-treatment resulted in no change from fresh tissue at both 10 h and 24 h time points. Transepithelial Resistance values for control tissues dropped markedly to 29% by 10 h and subsequently fell to 4.5% of fresh tissue after 24 h. In contrast, creatine treatment resulted in no significant change from fresh tissue following both 10 h and 24 h storage. Mucosal barrier function as assessed by electrophysiology was indicative of a preservation of function when treated with creatine.

3.2.4. Histology

(Table 1: Park's grading of intestinal injury; Fig. 6: Photographs of representative injury) After 10 h, Parks' grading of injury reached a median grade of 4 (range of 3–8) for control tissues compared to 0 (range of 0–4) for creatine-treated specimens; these grades were significantly different (P < 0.05) and corresponded to denudation of villi and a normal mucosa, respectively. After 24 h, histology grades in both groups increased, with complete disintegration of the regenerative cryptal regions (commonly referred to the Crypts of Lieberkühn) in 5/6 Control specimens and frank necrosis of the tissue in 50% of samples. This was in contrast to Creatine-treated tissues exhibiting only a moderate degree of subepithelial clefting (median grade 1.5); significance reached P < 0.01. Fig. 6 shows representative injury and median grades following 10 h.
4. Discussion

Preservation solutions play a key role in minimizing the detrimental effects of ischemia and maximizing the safe storage time of an organ. This was highlighted by introduction of the University of Wisconsin (UW) solution in the late 1980’s. Developed by Belzer and Southard, this solution more than doubled the safe storage times of liver, kidney, and pancreas [2,6,41] Although this solution was not widely adopted for cardiac preservation, as it was with intra-abdominal organs, it served to demonstrate that the key to successful development of preservation solutions is to understand the pathophysiological changes that occur during ischemia, and then to formulate a solution to counteract these changes. Although the problems associated with low-temperature organ preservation are multifaceted, one important issue is the exhaustion of high-energy adenylates cold storage. Dynamic, energy-

![Energy Levels during Static Cold Storage](image1)

**Fig. 3.** Energy Levels during Static Cold Storage. Data are means ± SE; n = 6. Values for creatine phosphate and ATP were μmol/g protein. * - denotes significantly different from freshly isolated tissue (t = 0); P < 0.05. c – denotes significantly different from Control group; P < 0.05.

![Oxidative Stress during Static Cold Storage](image2)

**Fig. 4.** Oxidative Stress during Static Cold Storage. Data are means ± SE; n = 6. Values for glutathione (reduced form) were μmol/g protein and for malondialdehyde, nmol/g protein. * - denotes significantly different from freshly isolated tissue (t = 0); P < 0.05. c – denotes significantly different from Control group; P < 0.05.

![Barrier Integrity during Static Cold Storage](image3)

**Fig. 5.** Barrier Integrity during Static Cold Storage. Data are means ± SE; n = 4 with duplicates. Permeability and Transepithelial Resistance were reported relative to fresh tissue values. * - denotes significantly different from freshly isolated tissue (t = 0); P < 0.05. c – denotes significantly different from Control group; P < 0.05.

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A modified Park’s classification for intestinal injury was used to assess mucosal morphology as follows: Grade 0 = Normal mucosa; grade 1 = Subepithelial space at villus tip; grade 2 = Moderate subepithelial space; grade 3 = Epithelial lifting along villus sides; grade 4 = Denuded villi; grade 5 = Loss of villus tissue; grade 6 = Crypt layer injury; grade 7 = Transmucosal injury; grade 8 = Transmural injury.
acids. While components of the AA solution address many of the same issues as the UW solution, the AA solution is specifically tailored to the metabolic requirements of the intestine. Our research over the past several years focusing on amino acids and intestinal preservation supports the involvement of enhanced ATP production (from gln, glu, asp) [48,49]; antioxidant augmentation (due to the presence of precursor amino acids, gly, cys, glu/gln for glutathione and additional antioxidant, Trolox) during storage; xanthine oxidase inhibitor (allopurinol), DNA repair enzyme, PARP inhibitor (3-aminobenzamide), adenosine (purine substrate), precursors of growth-promoting polyamines (gln, arg, pro), nitric oxide donor (arginine). Although the requirement of each component has not been established on an individual basis, we have attempted to build on the work of others in the field to develop an effective intestinal-specific preservation solution. The results of the current study suggest that we are closer to the end goal of effective preserving small bowel for transplantation. Studies from our lab utilizing both in vivo and in vitro models of organ storage and ischemia-reperfusion have established that the AA solution confers substantial protection of energy levels and mucosal morphology after the intraluminal infusion prior to ischemic insult [12,34,36]. Of direct clinical relevance, a small animal transplant study from our lab established that intraluminal treatment with AA solution resulted in superior graft function and animal survival compared to UW solution; post-transplant survival after 14 days was 80% with complete mucosal restoration for grafts stored in our AA solution, compared to 0% survival after 12 h post-transplant for grafts treated intravascularly and intraluminally due to hemorrhagic shock and fluid loss [37].

In the current communication, we targeted the direct augmentation of energy levels via a unique strategy of delivering an immediate precursor of the high energy phosphagen, creatine phosphate. Athletes have been using oral creatine supplementation for years. A number of well-designed studies document increases in muscle power during intense exercise following a creatine-loading regimen [15,23]. Furthermore, there is an increase in muscle mass and strength performing an anaerobic activity such as weight lifting [15,23]. Although there have been concerns about the safety of ingesting large doses of creatine on a daily basis, no studies have established any serious side-effects in healthy individuals [10,31]; creatine loading is widely accepted as a valid supplement for athletes participating in burst-work type exercise. Interestingly in recent years, research has discovered some new exciting properties of high levels of exogenously supplied creatine in a number of pathologies: a) Several creatine analogs possess potent anticancer properties; b) creatine phosphate and the creatine analog cyclocreatine prevent ischemic tissue damage; c) and there are cases of patients with neurological disorders that improve following oral creatine supplementation [51].

In this study, Part A was performed to establish the principle of creatine loading via continuous intraluminal perfusion supplemented with supra-physiologic creatine concentrations (50 mM). The time of perfusion (4 h) was selected as a clinically possible cold ischemic time required for the transport and storage of the intestine prior to implantation; it is during this period that perfusion would potentially be utilized. Our data indicates that creatine loading and its impact on tissue high energy phosphagens (Creatine phosphate) and adenylylate (ATP) under the conditions of this study (ie. temperature, concentration of supplemented creatine, flow rate) reached maximum effect after 2 h perfusion. Subsequent static storage following the 4 h loading phase revealed that the effect on high energy compound was sustained throughout 24 h. According to the canonical view, creatine phosphate buffers alterations in ATP and ADP levels by serving as storage for high-energy phosphate. The more recent “shuttle” hypothesis suggests that creatine serves to carry high-energy phosphates to and from regions of adenylates within cells [51]. Specific creatine kinase isoenzymes are closely associated with sites of ATP synthesis and consumption, transporting high-energy phosphates between the two sites. Whether humans obtain creatine by consuming meat products or through

Fig. 6. Morphologic integrity after 10 h cold storage. Photos are of representative median grades of histologic injury after 10 h storage according to a modified Park’s classification of intestinal injury. Park’s grades for control section was 4 and 0 for creatine sections. Freshly isolated tissue had Parks’ grade of 0. Magnification with 10× objective. H&E staining, 5 μm sections.

dependent tight junctions, located near the apical surface of intestinal epithelial cells, comprise a physical barrier that keeps enteric contents isolated in the external environment [16,44]. At times of stress when cellular energy reserves decline, the ability of epithelial cells form the mucosal barrier becomes compromised [44]. As the tight junctions continue to dilate, increasing amounts of macromolecules and enteric bacteria traverse the epithelial barrier. If hypoxic conditions persist, structural and functional integrity are lost, and the translocation of bacteria increases the risk of sepsis; sepsis is by far the most common cause of mortality in intestinal transplant recipients [3].

Unlike the gold standard practice of flushing the vasculature with UW solution, we have proposed an additional back-table intraluminal infusion with a nutrient-rich preservation solution that our lab has been developing over the past few years. We refer to our novel solution as the AA solution to denote the high concentration of amino acids among other important components. In addition to delivering nutrients, buffers, antioxidants, and osmotic agents in the AA solution to the direct site of mucosal injury, one significant advantage of an intraluminal flush is the removal of enteric contents prior to storage. These components include bacterial endotoxins, proteolytic enzymes, and bile...
endogeneous biosynthesis, creatine is transported to body tissues through the bloodstream. Once creatine is at the tissue, it enters the cells via a Solute Carrier (SLC) membrane transport protein, specifically SLC6 [5]. The transporter SLC6 uses energy from the co-transport of Na+ and Cl-down their concentration gradients to concentrate solutes within the cell [52].

In this study, part B was performed to determine if similar effects on creatine loading and subsequent augmentation of energy levels during organ storage are attainable during a single simple intraluminal table flux. A single intraluminal flux is most clinically relevant and would require minimal modification of current practice. In this study, after 10 h storage, creatine-treatment afforded protection of barrier function; mucosal permeability and electrophysiology parameters were superior to untreated controls (P < 0.05). The overall interpretation of the functional data suggests that ion transport was changing in both groups; however creatine-treated tissues were able to maintain permeability and transepithelial resistance as a result of better transport function across the epithelium. Similarly, mucosal morphology confirmed superior integrity with an intact epithelial layer as a result of creatine-loading; a median Park's grade of 0 was indicative of no apparent injury. It must be noted however, there were two specimens with non-zero grades; these tissues had grades of 3 and 4, indicating extended epithelial clefting and villus denudation. In contrast extensive injury to all 6 specimens was apparent in grades of 3–8 (including extended clefting, villus denudation and transmucosal/transmural injury).

One aspect of this study that is noteworthy relates to the evidence of oxidative stress. In this study, GSH and MDA levels exhibited patterns that are counter-intuitive to the effects of oxidative stress on cellular metabolism. Metabolites in the control group during static storage (with no initial perfusion) showed a progressive decline in GSH in combination with a gradual increase in MDA; this is consistent with the effects of a period of oxidative stress. However in the creatine supplemented group, low MDA levels implies reduced oxidative stress, while declining GSH levels indicate a period of elevated oxidative stress. These apparently contradictory events cannot be explained according to our current understanding of oxidative stress and its effect on tissue during cold storage. These events may be confounded by the presence of a compound with strong antioxidant properties, notably Trolox.

The strategy outlined in the current communication would need to be refined in order to establish optimal loading conditions for use with human tissue. However, the administration of creatine in an effective intraluminal flush solution is entirely feasible and could easily be performed in the isolated organ at the time of back table manipulation following procurement. For any modification of current clinical practice to occur two factors must be satisfied: 1) the modification must be safe; and 2) a significant and clinically relevant improvement must be demonstrated. In our experimental model, the improvement in barrier structure and function was marked. Additionally, the method of preservation solution delivery (i.e. via the lumen) is much safer than changes to the intravascular preservation solution would be to implement.

The field of intestinal transplantation is in its fruition and improvements in immunosuppression, surgeon/physician experience and patient management have translated to an increase in graft and patient survival. A strategy of an intraluminal flush with a solution rich in nutrients, antioxidants, buffer, osmotic agents has been shown to be an effective method of preserving the integrity of the mucosa. We propose that the option of supplementing the AA solution with creatine should be considered as a viable, organ-specific method of effecting preservation of the intestine.

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