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A Novel Approach to Maintain Gut Mucosal Integrity Using an Oral Enzyme Supplement

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Abstract

Objective—To determine the role of intestinal alkaline phosphatase (IAP) in enteral starvation-induced gut barrier dysfunction and to study its therapeutic effect as a supplement to prevent gut-derived sepsis.

Background—Critically ill patients are at increased risk for systemic sepsis and, in some cases, multi-organ failure leading to death. Years ago, the gut was identified as a major source for this systemic sepsis syndrome. Previously, we have shown that IAP detoxifies bacterial toxins, prevents endotoxemia, and preserves intestinal microbial homeostasis.

Methods—WT and IAP- KO mice were used to examine gut barrier function and tight junction protein levels during 48 h starvation and fed states. Human ileal fluid samples were collected from 20 patients post ileostomy and IAP levels were compared between fasted and fed states. To study the effect of IAP supplementation on starvation-induced gut barrier dysfunction, WT mice were fasted for 48 h +/- IAP supplementation in the drinking water.

Results—The loss of IAP expression is associated with decreased expression of intestinal junctional proteins and impaired barrier function. For the first time, we demonstrate that IAP expression is also decreased in humans who are deprived of enteral feeding. Finally, our data

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Competing interests

The authors declare no conflicting financial interests.

Author contributions

Study concept and theory (RAH); research design (RAH, SRH, MMRM, KPE); data acquisition (SRH, MMRM, KPE, SAM, TP, TJT, SSG, MHG, QT, KK); data analyses and interpretation (RAH, SRH, MMRM, KPE, SAM); statistical analyses (RAH, SRH, MMRM, KPE, SAM); drafting of the manuscript (RAH, SRH, KPE, SAM, MSM); critical review of the manuscript for important intellectual content (all authors); obtained funding (RAH); approval of the manuscript (all authors); study supervision (RAH).

demonstrates that IAP supplementation reverses the gut barrier dysfunction and tight junction protein losses due to a lack of enteral feeding.

Conclusions—IAP is a major regulator of gut mucosal permeability and is able to ameliorate starvation-induced gut barrier dysfunction. Enteral IAP supplementation may represent a novel approach to maintain bowel integrity in critically-ill patients.

Keywords

intestinal alkaline phosphatase (IAP); intestinal permeability; tight junctions; total parenteral nutrition; enteral starvation; ICU patient

INTRODUCTION

Critically ill patients are at increased risk for systemic sepsis and, in some cases, multi-organ failure leading to death. Years ago, the gut was identified as a major source for this systemic sepsis syndrome^{1, 2} and subsequently it has become clear that a key underlying problem relates to the lack of enteral nutrition (EN). Indeed, early enteral feeding, even in the form of minimal trophic feeds, has been shown to improve outcomes and has become a standard of care in critical care settings.^{3–5} The mechanisms underlying the beneficial effects of enteral feeding are not well understood, but nutrient deprivation has been shown to disrupt the integrity of the intestinal epithelium, leading to the permeation of noxious molecules.^{6, 7} Even within a short period of enteral fasting, functional and morphological changes have been reported,^{6, 8, 9} including a disruption in the intestinal tight junctions (TJs), resulting in increased intestinal permeability^{9, 10} and the passage of luminal pro-inflammatory molecules, toxins and pathogens into the circulation.^{7, 11} TJs are composed of transmembrane and cytosolic proteins, such as claudins, occludin and zona occludens.¹² The modification of TJ proteins and paracellular permeability is dynamically regulated by various stimuli and is closely associated with a broad spectrum of diseases.¹³

Intestinal mucosal inflammation is associated with a decrease in TJ protein expression, loss of transepithelial electrical resistance and an increase in the permeability to macromolecules such as inulin.¹⁴ Miyasaka et al. showed that blockade of Myd88, a downstream signaling molecule of toll like receptors (TLRs), prevents intestinal inflammation and barrier dysfunction seen in a mouse total parenteral nutrition (TPN) model.¹⁵

The brush border enzyme intestinal alkaline phosphatase (IAP) is expressed in the small-intestinal epithelium¹⁶ and has recently been identified as a key factor at the interface between the host and the gut microbiota.^{17, 18} IAP has the ability to detoxify a variety of pro-inflammatory mediators that exist within the gut lumen, including adenosine triphosphate (ATP)¹⁹ and the TLR ligands: lipopolysaccharide (LPS), flagellin, and CpG DNA.²⁰ We have previously shown that IAP knockout mice have an impaired ability to detoxify luminal LPS and appear to be more susceptible to gut-derived inflammatory conditions.^{21, 22} Interestingly, IAP levels are known to decrease dramatically in the setting of starvation in rodents.^{23, 24} Given the known function of IAP and the dependence on enteral nutrition for its expression, we speculated that this enzyme might represent the key mediator

through which enteral feeding maintains the gut barrier and protects critically ill patients from systemic sepsis and multi-organ failure.

In this study, we show that the loss of IAP expression is associated with decreased intestinal junction proteins and impaired barrier function. For the first time, we demonstrate that IAP expression is also decreased in humans who are deprived of enteral feeding, similar to the previous findings shown in rodents. Finally, we provide evidence that IAP supplementation could represent a novel therapy to maintain bowel integrity, protecting the host against the development of the systemic inflammatory state seen in some critically ill patients.

MATERIALS and METHODS

For detailed information please see the on-line Supplemental Data File. (See Data File, Supplemental Digital Content, which details Materials and Methods section)

Human Subjects

Ileal fluid samples were collected from inpatients at the Massachusetts General Hospital (MGH). IAP levels from the first postoperative sample with enteric contents represented the fasted state and were compared with the last postoperative sample collected, representing the normal fed state. Demographics and clinical characteristics of patients were obtained from the medical records. All participants provided written informed consent and the study was approved by the MGH Institutional Review Board.

Animals

IAP-KO mice (Akp^{3-/-}, Mus musculus C57BL/6) construction has been described elsewhere.²⁵ IAP-KO and wild-type mice were maintained in a temperature-controlled room (22 to 24°C) with a 12-h light/12-h dark diurnal cycle with food and water ad libitum. The IACUC at MGH reviewed and approved all the animal experiments. (See Data File, Supplemental Digital Content, for more details)

Starvation Model

WT mice (n = 5 per group), each weighing approximately 25 g, were used. Mice were housed with no chow diet for 48 h to repress the expression of IAP as previously described.²⁴ Animals had free access to drinking water supplemented with IAP (calf intestinal alkaline phosphatase, Sigma Aldrich) 150 U/ml, IAP 300 U/ml, or an IAP vehicle (control group). After 48 h without food, mice were euthanized and intestinal tissues collected for further analysis.

Intestinal Loop Model

Small intestinal loops were constructed in the proximal jejunum as previously described (See Data File, Supplemental Digital Content, for detailed Materials and Methods).²⁶

Intestinal alkaline phosphatase essay

Human ileal fluid samples were homogenized in water (10 mg/ml), centrifuged (3,000 rpm) and the supernatant collected to determine protein and IAP enzyme activity as previously described (See Data File, Supplemental Digital Content, for more details).²²

In-vitro cytokine response to luminal contents

The luminal contents from the intestinal loops were centrifuged (15000 rpm for 15 min at 4°C); the supernatant was added to murine RAW 264.7 cells, and incubated overnight; TNF- α levels in the media were quantified by ELISA.

In-vivo intestinal permeability

Mice were gavaged with a phosphate buffer saline (PBS, pH 7.2) containing FITC-dextran (4 or 10kDa) at a dose of 300 mg/kg body weight to assess intestinal permeability as previously described (See Data File, Supplemental Digital Content, for more details).²²

Quantitative Real-Time PCR

The terminal ileum was isolated for RNA extraction and qRT-PCR. (See Data File, Supplemental Digital Content, for more details). The average copy number of mRNA expression in control samples was set to 1.0.

Statistical analysis

IAP levels in the fasting vs. fed state were compared using the Wilcoxon signed-rank statistical test. In animal studies, statistical significance between two groups was tested using the two-tailed Student's *t* test. Statistical significance between more than two groups was tested using one-way analysis of variance with Tukey's multiple-comparison post-hoc tests. A value of $p < 0.05$ was considered statistically significant. (See Data File, Supplemental Digital Content, for more details)

RESULTS

Endogenous IAP plays an important role in the gut barrier function

Disruption in gut barrier function results in translocation of luminal pathogens and toxins into the blood stream. We have showed earlier that IAP-KO mice are prone to endotoxemia and sepsis.^{22, 24} In order to study the effect of endogenous IAP on gut barrier function we compared the intestinal permeability in IAP-KO and WT mice. Eight-week-old male mice were orally gavaged with 300 mg/kg body weight FITC labeled dextran (4,000 MW) to assess the paracellular permeability. Ninety minutes later, FITC-dextran concentration in the serum was measured using a fluorospectrophotometer. We found that IAP-KO mice had a 2-fold increase in intestinal permeability to FITC-Dextran 4-kDa compared to WT mice (Fig. 1A). To better assess the paracellular permeability to macromolecules, we measured the intestinal permeability to higher molecular weight FITC-dextran (10-kDa). The absence of endogenous IAP also resulted in significantly higher 10-kDa FITC-dextran in the serum of IAP-KO mice compared to WT (Fig. 1B).

To understand the mechanism by which IAP deletion increased intestinal permeability in mice, we assessed TJ protein expression in the intestinal tissue of IAP-KO compared to WT mice. Altering the abundance of junctional proteins claudin1 and occludin is known to increase intestinal paracellular permeability.^{13, 27, 28} Furthermore, the depletion in ZO-1 protein levels has been shown to disrupt gut barrier function.¹⁴ We found that the absence of IAP resulted in significant decreases in the expression of the junctional proteins, claudin1, occludin, ZO-1 and ZO-2 in the intestinal tissue (Fig. 1C). These results suggest that the endogenous IAP plays a crucial role in maintaining gut barrier function through regulation of junctional protein expression.

Reduction in endogenous anti-inflammatory effects in the jejunum of IAP-KO mice

The above data indicate that the absence of IAP has a damaging effect on barrier function. In the gut, elevated levels of mucosal inflammatory cytokines such as TNF- α have been shown to induce intestinal permeability to macromolecules.^{29, 30} Previous studies have shown that bacterial endotoxins induce mucosal inflammation and play a pivotal role in gut barrier dysfunction.³¹ Given the known function of IAP as an anti-inflammatory factor, we studied the effect of endogenous IAP on bacterial pro-inflammatory mediators in a physiologic environment. We incubated bacterial toxins in an isolated jejunal loop of intestine in WT and IAP-KO mice. Two hours later, the luminal contents were collected, centrifuged to eliminate bacteria and food debris, the supernatant applied to mouse RAW264.7 macrophage cells, and after an overnight incubation, TNF- α levels were assayed in the cell media. Figure 2 shows that luminal contents from the intestinal loop of IAP-KO mice instilled with LPS caused more TNF- α release compared to their WT counterparts ($p=0.006$). We observed the same effect in regard to two other bacterial inflammatory mediators, flagellin and CpG DNA, ($1,117.9 \pm 21.6$ vs. 662.0 ± 60.0 pg/ml cell media; $p<0.001$) and (949.6 ± 57.3 vs. 638.7 ± 61.8 pg/ml cell media; $p=0.006$), respectively (Fig. 2). These data demonstrate that the endogenous IAP enzyme functions as an anti-inflammatory factor, inhibiting the pro-inflammatory effects of a variety of bacterially-derived mediators.

Lack of enteral feeding disrupts intestinal barrier function

To further elucidate the impact of the endogenous IAP enzyme on gut barrier function, we examined the effects of silencing IAP expression by starvation. We have previously demonstrated that starvation (water only diet for 48 hours) results in a dramatic decrease in endogenous IAP levels.^{23, 24} Accordingly, we fasted mice on a water only diet for up to 48 h and compared the impact on intestinal permeability and junctional protein expression compared to mice on a standard chow diet. As shown in Figure 3A, there was a 3-fold increase in paracellular permeability to 4-kDa FITC-dextran in starved compared to normally fed mice ($p=0.04$). Furthermore, starvation resulted in significantly higher passage of larger molecular weight FITC-dextran 10-kDa ($p=0.01$) (Fig. 3B).

We next quantified the mRNA levels of junctional proteins in the intestinal tissue of starved and fed mice. Our results showed that starvation significantly lowered the expression of occludin and ZO-3 (Fig. 3C).

Luminal fluid from starved mice has lower anti-inflammatory effect

Previous reports have shown elevated levels of TNF- α in the intestinal epithelia of fasted animals.³² Furthermore, TPN administration in mice has been shown to result in increased TLR expression in the gut leading to hyper-response to bacterially derived ligands³³. We employed the intestinal loop model to delineate the impact of suppression of endogenous IAP on luminal pro-inflammatory molecules during enteral starvation. In this study, mice were fasted for 48 h and a jejunal loop instilled with 100 μ l of LPS (100 ng/ml). Two hours later the abdominal incision was reopened and the loop contents collected and applied to the RAW264.7 cells. Figure 4 shows that the luminal contents of jejunal loops from starved animals instilled with LPS resulted in a significantly higher TNF- α response from macrophage cells when compared to the contents from fed mice ($p=0.01$).

Perioperative fasting reduces IAP levels in human ileal fluid

Although IAP silencing has been demonstrated in both rats and mice in response to starvation,^{23, 24} it is unknown whether a similar regulation occurs in humans. We therefore sought to study the effect of enteral nutrient deprivation on human IAP levels. We enrolled 20 patients who had undergone ileostomy creation and collected effluents under fasted and fed conditions. The first enteric contents seen from the ileostomy after surgery was taken to represent the fasted condition and these IAP levels were compared to the enteric fluid obtained when the patient was eating normally. The demographic and clinical characteristics of the patients are shown in Table 1. We found that IAP was significantly reduced in the fasting compared with the fed ileal fluid samples (176.5 ± 32.2 vs. 381.9 ± 42.2 pmole pNPP hydrolyzed/min/ μ g of protein; $p=0.001$) (Fig. 5). Age, BMI, ASA physical status, gender, type of diagnosis, history of diabetes and history of hypercholesterolemia had no effect on the levels of IAP.

Oral supplementation with IAP up-regulates junctional protein expression

Based on our results above, we sought to study the effect of exogenous IAP on the TJ protein levels. WT mice were given free access to normal chow diet and drinking water. In one of the groups ($n=5$), IAP was added to drinking water at a concentration of 300 U/ml. The water was changed daily with new addition of IAP. After 10 days of treatment, the mice were euthanized and the terminal ilea were collected for RNA extraction. Figure 6 shows that oral supplementation with IAP increased the expression of claudin1 and the cytosolic junctional proteins ZO-1 and ZO-3, (6.9 and 10.3 mRNA relative expression, respectively; $p=0.03$ and $p=0.02$, respectively).

Oral supplementation with IAP prevents enteral starvation-induced gut barrier dysfunction and junctional protein losses in mice

Inflammatory pathways are known to exert a major impact on gut barrier dysfunction related to fasting.^{34, 35} Furthermore, we have shown the beneficial effect of exogenous IAP in the context of a variety of gut-derived inflammatory conditions.²² We therefore sought to determine the effect of exogenous IAP supplementation on gut barrier function under conditions of starvation. In this study, WT mice were placed on a water only diet $\pm$ different concentrations of IAP (150 U/ml and 300 U/ml). After 48 h of food deprivation, mice were

orally gavaged with FITC-dextran and ninety minutes later the animals were euthanized and blood and intestinal tissue collected. Figure 7A shows that oral supplementation with 300 U/ml IAP reversed the effect of starvation on intestinal permeation of 4-kDa FITC-Dextran ($p=0.04$). In regard to serum levels of the higher molecular weight FITC-dextran 10-kDa, both doses of IAP (150 U/ml and 300 U/ml) significantly reduced the permeability to this macromolecule, (0.16 ± 0.03 vs 0.41 ± 0.08 10-kDa FITC-dextran $\mu\text{g/ml}$; $p=0.036$) and (0.16 ± 0.01 vs 0.41 ± 0.08 10-kDa FITC-dextran $\mu\text{g/ml}$; $p=0.032$) (Figure 7B), respectively. To further elucidate the therapeutic effect of IAP on starvation-induced barrier dysfunction, we quantified the expression of junctional proteins in the intestine from the starved mice $\pm$ IAP treatments. Figure 7C shows that oral treatment with 300 U/ml IAP in the drinking water significantly increased the levels of the tight junction gene products, claudin1, occludin, ZO-1, ZO-2 and ZO-3.

DISCUSSION

The lack of enteral feeding in critically ill patients has been linked to an increased risk of systemic sepsis and multi-organ failure.²⁻⁴ Administration of parenteral nutrition in ICU patients is associated with increased intestinal permeability and endotoxemia,⁷ while enteral feeding has been clearly demonstrated to improve outcomes in patients suffering from severe trauma.⁵ It is thought that nutrient deprivation in the gut leads to a disruption in intestinal mucosal integrity through a reduction in tight junction protein expression. An impaired gut mucosal barrier results in the systemic absorption of luminal inflammatory mediators, and perhaps other bacterially-derived toxins. Interestingly, the precise mechanisms responsible for the beneficial effects of enteral nutrition remain largely unknown.

The gut mucosal barrier is a complex system comprised of a mucus layer and other luminal components, the epithelium, the tight junctions between the epithelial cells, and the immune system.^{12, 13} The two major pathways by which luminal molecules cross the barrier are the transcellular and paracellular routes. A key component of the gut mucosal barrier is comprised of the paracellular pathway, which is considered to be the major route for diffusion of hydrophilic molecules across the intestinal epithelia. This pathway is regulated by tight junctions that require the assembly of several proteins with linkage to the actin-based cytoskeleton, including occludin, the claudins, junction adhesion molecule, and the zonula occludens.¹²⁻¹⁴

IAP is a brush-border enzyme that is exclusively produced by the enterocytes of the proximal small intestine.¹⁶ This enzyme is secreted into the intestinal lumen, especially in response to luminal fats and other nutrients.³⁷ IAP has a myriad of functions that include detoxification of LPS and other bacterial products,²⁰ dephosphorylation of ATP,¹⁹ prevention of gut inflammation, and preservation of intestinal microbiota homeostasis.¹⁸ The pharmacological properties of exogenous IAP include the prevention of sepsis,³⁵ inflammatory bowel disease,²¹ and the metabolic syndrome.²²

Based on the known functions of IAP and the fact that its expression is lost with starvation in rodents,^{23, 24} we sought to explore a potential role for this enzyme in the gut barrier dysfunction that is associated with the lack of enteral nutrition. Our findings suggest that the

endogenous IAP enzyme plays a major role in maintaining intestinal barrier function, likely through its ability to block a variety of inflammatory pathways and through its regulation of the expression of tight junction proteins. A strong link exists between starvation, mucosal inflammation and intestinal barrier dysfunction. Inflammatory cytokines such as TNF- α and IL-1 β are associated with down-regulation of the junctional proteins, occludin and ZO-1¹⁴ and deletion of toll like receptors (TLRs) or the inhibition of their downstream signaling in the intestinal epithelium has been shown to prevent inflammation and improve intestinal barrier function in many mouse disease models.³⁸ Furthermore, a lack of enteral feeding increases intestinal inflammation and results in an increased sensitivity to luminal pro-inflammatory mediators.^{32, 33} Miyasaka et al. showed that the blockade of Myd88, a downstream signaling molecule of TLRs, prevents intestinal inflammation and barrier dysfunction seen in a mouse TPN model.¹⁵ Similarly, inhibition of TNF- α signaling was shown to ameliorate the impairment in intestinal permeability due to enteral starvation.^{29, 34} Since IAP blocks the inflammatory effects of a number of mediators, including the TLR ligands LPS, flagellin, and CpG DNA,²⁰ it is possible that the improvement in gut barrier function induced by this enzyme is related to its inhibition of inflammatory pathways in the gut. The increase in tight junction protein expression could be a direct effect of the IAP enzyme on these gene products, or an indirect effect through blockade of one or more inflammatory pathways. Further work will be needed to delineate the precise mechanism by which IAP maintains the intestinal tight junction barrier.

Our data are consistent with other reports linking starvation to gut barrier dysfunction. We demonstrated that starved animals are more sensitive to luminal pro-inflammatory mediators, based on the effects of the enteric contents on target cells *in vitro*. The changes seen with starvation compared to the fed state were similar to what we saw in the IAP-KO mice. Taken together, our data suggest that the decline in endogenous IAP due to enteral starvation plays a critical role in the development of barrier dysfunction and increased paracellular permeability. Further studies will be needed to more fully understand the impact of IAP deficiency in regard to gut mucosal dysfunction.

Although the focus of the present study is on the acute barrier dysfunction that accompanies starvation and critical illness, it is thought that a more chronic impairment in the gut barrier (leaky gut) may lead to a variety of human diseases, including obesity, rheumatoid arthritis, asthma, and others. Indeed, we have recently reported on the beneficial effects of oral IAP in preventing the metabolic syndrome in mice.²² Further studies will be needed to determine the precise role that the gut and perhaps IAP play in these chronic systemic inflammatory conditions.

In the present study, we demonstrate for the first time that IAP expression is decreased in humans who are deprived of enteral feeding, similar to what has previously been shown in rodents.^{23, 24} Given this apparent nutrient regulation of human IAP expression, we speculate that critically ill patients who are not being enterally fed will experience a decline in endogenous IAP activity and that this will predispose them to gut barrier dysfunction. It is interesting to note that decades ago the gut was identified as a major source for the systemic sepsis syndrome seen in some critically ill patients. Although the underlying cause of the gut dysfunction has remained a mystery, it has become clear that aggressive enteral nutrition is

of great benefit. Indeed, early enteral feeding, even in the form of minimal trophic feeds, has been shown to improve outcomes and has become a standard of care in critical care settings.^{3–5} Given the functions of the endogenous IAP enzyme in regard to the preservation of gut mucosal integrity and the fact the levels of this enzyme are low in starved humans suggested to us that IAP could be used in a therapeutic paradigm in clinical settings where enteral nutrition may not be feasible. We therefore tested this idea in starved mice, providing supplemental IAP in their drinking water. Indeed, our results suggest that oral supplementation with IAP improves gut barrier function. IAP treatment up-regulated TJ protein mRNA levels under normal fed conditions, but more importantly, when the animals were starved. IAP also improved gut barrier function as determined by macromolecular permeability. It is likely that IAP ameliorates the effects of enteral starvation on intestinal permeability by preventing the decline in junctional protein expression, although other mechanisms may also be at play, i.e., detoxification of pro-inflammatory mediators in the intestinal lumen and prevention of gut wall inflammation (Figure 8). IAP is a naturally occurring gut enzyme and has been safely administered to some patients without any adverse effects.³⁹ We therefore suggest that IAP may represent a novel clinical intervention to improve outcomes in the setting of critical illness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Lower level of endogenous IAP disrupts gut barrier function. WT and IAP-KO mice (n=5) were orally gavaged with FITC-dextran to assess intestinal permeability. Ninety minutes later, mice were euthanized, blood was collected via heart puncture and serum assayed for FITC-dextran concentration. Serum levels of FITC-dextran of IAP-KO mice were compared to those of WT: (A) Serum 4-kDa FITC-Dextran ($\mu\text{g/ml}$). (B) Serum 10-kDa FITC-Dextran ($\mu\text{g/ml}$). Upon euthanasia in the same mice groups (n=5), terminal ileum was harvested to extract tissue RNA. qRT-PCR was performed to determine the levels of tight junction protein expression. (C) Junctional protein *mRNA* expression in the terminal ileum of IAP-KO mice compared to WT. Values are expressed as mean $\pm$ SEM. Statistics: Statistical significance between two groups was tested using the two-tailed Student's *t* test.; *p < 0.05, **p < 0.01.

Figure 2.

Lower level of endogenous IAP in intestinal loops results in higher inflammatory response of mice macrophages to bacterial proinflammatory mediators. Laparotomy was performed on IAP-KO and WT mice (n = 5 per group) under general anesthesia and a 5-cm jejunal loop was constructed. 100 µl of either endotoxin-free water, LPS (100 ng/ml), Flagellin (100 ng/ml) or CpG-DNA (10 µg/ml) were instilled by injection into the loop. After 2 hours, while still under anesthesia, the loop was harvested, and the supernatants of the luminal contents were added to murine RAW 264.7 cells, incubated overnight, and TNF-α levels in the media quantified by ELISA. Values are expressed as mean ± SEM. Statistics: Statistical significance between two groups was tested using the two-tailed Student's *t* test.; *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 3.

Starvation disrupts gut barrier function. Groups of mice (n=5) were food starved for 48h with free access to drinking water. The control groups (n=5) were housed with free access to regular chow diet and drinking water. Mice were orally gavaged with FITC-dextran to assess intestinal permeability. Ninety minutes later, mice were euthanized, blood was collected via heart puncture and serum assayed for FITC-dextran concentration. Serum levels of FITC-dextran of starved mice were compared to those of well-fed mice: (A) Serum 4-kDa FITC-Dextran (µg/ml). (B) Serum 10-kDa FITC-Dextran (µg/ml). Upon euthanasia in the same mice groups (n=5), terminal ileum was harvested to extract tissue RNA. qRT-PCR was performed to determine the levels of tight junction protein expression. (C) Junctional protein *mRNA* expression in the terminal ileum of starved mice compared to well-fed mice. Values are expressed as mean $\pm$ SEM. Statistics: Statistical significance between two groups was tested using the two-tailed Student's *t* test.; *p < 0.05.

Figure 4.

Starvation diminishes the endogenous anti-inflammatory factors in the gut. Groups of mice (n=5) were fed or food starved for 48 hours with free access to drinking water, followed by construction of the loop in the proximal jejunum. 100 μ l of either endotoxin-free water or LPS (100 ng/ml) were instilled into the loops using a 28-gauge needle. After 2 hours, while still under anesthesia, the loop was harvested, and the supernatants of the luminal contents were added to murine RAW 264.7 cells, incubated overnight, and TNF- α levels in the media quantified by ELISA. Values are expressed as mean $\pm$ SEM. Statistics: Statistical significance between two groups was tested using the two-tailed Student's *t* test.; *p < 0.05.

Figure 5.

Enteral fasting reduces IAP levels in human. Ileal fluid were collected from 20 patients undergoing ileostomy construction. IAP levels were compared from the first postoperative sample of enteric contents (fasted state) with their last postoperative sample collected (fed state). Values are expressed as mean $\pm$ SEM. Statistics: Statistical significance between fast and fed status was tested using the Wilcoxon signed rank statistical test.; *** $p < 0.001$.

Figure 6.

Exogenous IAP supplementation improves tight junction protein abundance. WT mice (n=5) were housed in MGH animal facility with free access to normal chew diet and drinking water. In one of the groups (n=5), IAP was added to drinking water at a concentration of 300 U/ml. After 10 days of treatment, the mice were euthanized and intestinal tissues were collected for RNA extraction. Values are expressed as mean $\pm$ SEM. Statistics: Statistical significance between IAP treated and no treatment groups was tested using the two-tailed Student's *t* test.; *p < 0.05.

Figure 7.

Exogenous IAP supplementation reverses the effect of starvation on gut barrier function. WT mice (n = 5 per group) were housed with no chow diet for 48 h and had free access to drinking water supplemented with IAP 150 U/ml, IAP 300 U/ml or IAP vehicle as a control group. After 48 h of no food, were orally gavaged with FITC-dextran to assess intestinal permeability. Ninety minutes later, mice were euthanized, blood was collected and serum assayed for FITC-dextran concentration. Serum levels of FITC-dextran of IAP treated mice were compared to those of vehicle treated group: (A) Serum 4-kDa FITC-Dextran (μg/ml). (B) Serum 10-kDa FITC-Dextran (μg/ml). Upon euthanasia in the same mice groups (n=5), terminal ileum was harvested to extract tissue RNA. qRT-PCR was performed to determine the levels of tight junction protein expression. (C) Junctional protein *mRNA* expression in the terminal ileum of IAP treated mice compared to no treatment. Values are expressed as mean ± SEM. Statistics: Statistical significance between the groups was tested using one-way analysis of variance with Tukey's multiple comparison posttests.; *p < 0.05, **p < 0.01.

Figure 8.

Gut homeostasis during enteral nutrition. The brush border enzyme IAP appears to play a crucial role in regulating the gut barrier function through a mechanism that involves the dephosphorylation of bacterial proinflammatory mediators in the intestinal lumen. By dephosphorylating bacterial toxins, IAP blocks their binding to TLR receptors, preventing inflammation in the gut. Inflammatory cytokines exerts an inhibitory effect on the expression of tight junction proteins, resulting in disrupted gut barrier function. Lack of enteral feeding diminishes IAP levels in the intestine, resulting in hypersensitivity to bacterial toxins and elevated levels of inflammatory cytokines. Depletion of IAP leads to increased intestinal permeability.

Table 1

Demographic and clinical characteristics of patients' status post ileostomy included in this study.

	Patients
Number of patients	20
Male, n (%)	9 (45.0)
Mean age $\pm$ SE	51.4 $\pm$ 4.3 years
Age range	20 – 89 years
Mean BMI $\pm$ SE	24.3 $\pm$ 1.1 kg/m ²
Type of diagnosis, n (%)	
Cancer	10 (50.0)
Ulcerative colitis	7 (35.0)
Crohn's disease	3 (15.0)
Comorbidities, n (%)	
Diabetes	1 (5.0)
Hypercholesterolemia	2 (10.0)
Mean ASA status $\pm$ SE	2.2 $\pm$ 0.1