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Issue: *Barriers and Channels Formed by Tight Junction Proteins***Loss of enteral nutrition in a mouse model results in intestinal epithelial barrier dysfunction**

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**Total parenteral nutrition (TPN) administration in a mouse model leads to a local mucosal inflammatory response, resulting in a loss of epithelial barrier function (EBF). Although, the underlying mechanisms are unknown, a major contributing factor is a loss of growth factors and subsequent critical downstream signaling. An important component of these is the p-Akt pathway. An additional contributing factor to the loss of EBF with TPN is an increase in proinflammatory cytokine abundance within the mucosal epithelium, including TNF- $\alpha$  and IFN- $\gamma$ . Loss of critical nutrients, including glutamine and glutamate, may affect EBF, contributing to the loss of tight junction proteins. Finding protective modalities for the small intestine during TPN administration may have important clinical applications. Supplemental glutamine and glutamate may be examples of such agents.**

**Keywords:** small intestine; p-Akt; parenteral nutrition; epithelial barrier function

**Introduction**

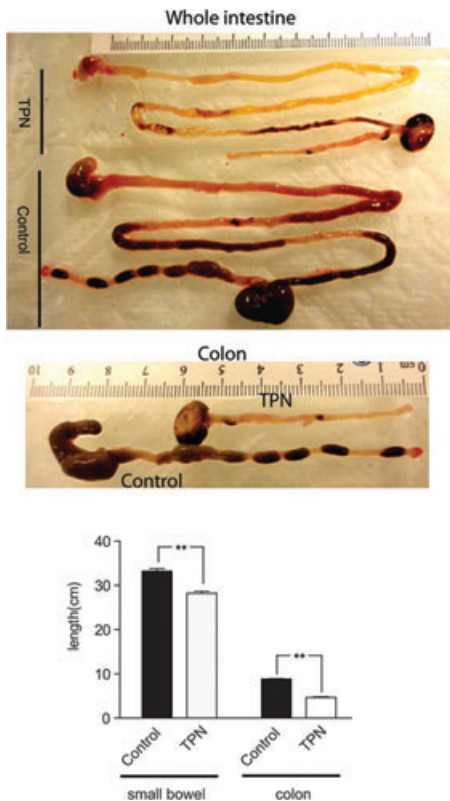
Parenteral nutrition (PN) is commonly used as treatment for many patients, ranging from short-term use in patients with gastrointestinal dysfunction,<sup>1</sup> to long-term use in patients with short bowel syndrome.<sup>2,3</sup> Although it is lifesaving for many, PN use is associated with numerous complications, including an increase in systemic infections, including pneumonia as well as infections of the urinary tract and surgical wounds.<sup>4,5</sup> Although the precise etiology of this increased rate of infections has not been fully established, it is known that organisms arising from enteric flora constitute a large percent of these infections. This suggests that a major loss of epithelial barrier function (EBF) could be a contributing factor leading to this increase in infectious complications. Additional contributing factors include a loss of local growth factors and increase in several proinflammatory mucosal cytokines. This review summarizes the work done by our laboratory and others on total parenteral nutrition (TPN)-associated loss of EBF.

**TPN administration results in a proinflammatory cytokine state within the intestinal mucosa**

The development of these inflammatory changes is complex and leads to atrophy of small bowel villi, an increase in epithelial cell (EC) apoptosis,<sup>6,7</sup> and a decrease in EC proliferation. This results in a marked reduction in the length of the small and large bowel (Fig. 1). Increases in both tumor necrosis factor-alpha (TNF- $\alpha$ ) and interferon gamma (INF- $\gamma$ ) contribute to the loss of EBF found with TPN administration. Blockade of TNF signaling with the use of TNF-receptor knockout mice demonstrate that the major mediator of mucosal atrophy is via the TNF-R1 signaling pathway, and without an intact pathway, mucosal atrophy is significantly prevented.<sup>8</sup> Blockade of IFN- $\gamma$  signaling using IFN- $\gamma$ -knockout mice has also been shown to be effective in preventing the loss of EBF.<sup>7,9</sup>

**Microbiome and TPN**

Previous studies from our laboratory and others have shown in a mouse TPN model, in which the



**Figure 1.** (A) Representative image of harvested intestine from control (chow fed) and TPN mice. Note the significantly reduced length of both small and large intestine. (B) Mean length in centimeters of small and large bowel (colon) from control and TPN mice.  $**P < 0.01$ .

gut is devoid of all nutrients, a number of significant physical and immunologic changes develop in the intestinal mucosa.<sup>9–13</sup> Data from our laboratory demonstrates that these intestinal changes are intimately associated with alterations in the luminal microbiome.<sup>14</sup> Our laboratory identified a major shift in the microbiome, from a normally benign composition of mostly Firmacutes, to a predominantly Gram-negative, Proteobacteria population. This shift in the microbiome was strongly correlated with a proinflammatory state characterized by an upregulation in Toll-like receptor (TLR) signaling, including TLR2, TLR4, and TLR9.<sup>15</sup> As well, TPN led to a marked downstream increase in NF- $\kappa$ B activation within the lamina propria, with a resultant increase in inflammatory cytokines INF- $\gamma$  and TNF- $\alpha$ , as well as a decreased population of T regulatory cells.<sup>16</sup> Blockade of this signaling pathway using myeloid differentiation primary response gene

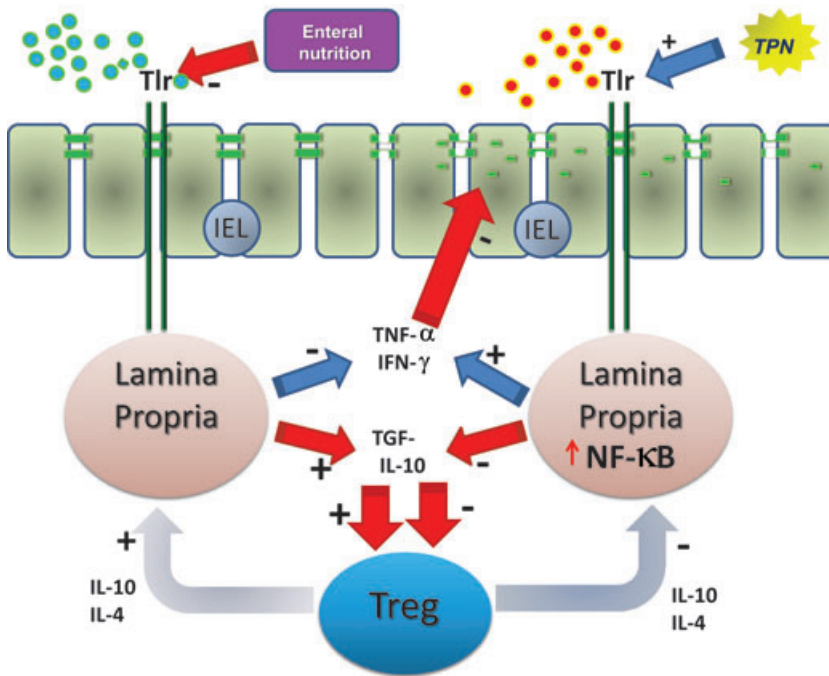
88 (MyD88) knockout mice demonstrated a significant prevention of NF- $\kappa$ B activation, prevention of a proinflammatory state, preservation of T regulatory cell population, and prevention of the loss of EBF.<sup>17</sup> Taken together, this suggests that unique strategies to prevent these major microbiome changes, or block TLR signaling, during TPN administration may prove to be a strategy to prevent the adverse effects of intestinal atrophy and loss of EBF (Fig. 2).

### TPN results in a loss of local growth factor production: implications for loss of EBF

The pathophysiology of TPN-driven EC apoptosis, decreased EC proliferation, and loss of EBF is complex. Although conventionally viewed as a mediator of cellular death, TNF- $\alpha$  signaling has also been closely linked with cell survival and regulation of proliferation. In fact, effective epidermal growth factor (EGF) signaling requires both an intact TNF- $\alpha$ <sup>18,19</sup> and ErbB1 receptor pathways. Both EGF and ErbB1 are markedly diminished with TPN administration.<sup>8</sup> The combination of a proinflammatory state, with loss of EGF signaling leads to an imbalance between TNFR1/TNFR2 and EGF signaling. Such a deranged state may well be a major contributing factor to TPN-associated mucosal atrophy and loss of EBF.

Aside from a loss of ErbB ligands, TPN has also been shown to result in a loss of two other key mucosal growth factors, keratinocyte growth factor<sup>20–22</sup> and glucagon-like peptide-2 (GLP-2).<sup>23</sup> Each of these factors are contributors to maintenance of the intestinal barrier function,<sup>24,25</sup> and loss of these factors seem to be major contributors to TPN-associated intestinal atrophy.<sup>23</sup>

TPN-associated loss of EBF<sup>26,12</sup> may well result in increases in bacterial translocation.<sup>27–29</sup> The barrier function of the epithelium is the sum of several physiological processes, including the synthesis and release of mucus from goblet cells, transcytosis of dimeric secretory IgA, lumenally directed water movement, and the physical integrity of the epithelial layer itself.<sup>30</sup> Breakdown in EBF and immunologic derangement can lead to the systemic dissemination of pathogens that often lead to the development of multiorgan dysfunction.<sup>31</sup> These findings have been shown in both mouse models and in humans<sup>32</sup> on PN.



**Figure 2.** Summary of potential signaling changes with TPN administration. Note loss of nutrients with TPN administration results in a change in the microbiome (represented by the red dots). This leads to enhanced signaling via Toll-like receptors (TLR) activating lamina propria lymphoid tissue leading to NF- $\kappa$ B nuclear transcription and a proinflammatory state within the mucosa, with increases in TNF- $\alpha$  and IFN- $\gamma$ , and loss of TNF- $\beta$  and IL-10. These changes lead to a loss of T<sub>reg</sub> cells and an exacerbation in the proinflammatory state. In total this leads to a loss of epithelial barrier function.

### TPN-associated loss of p-Akt abundance and loss of tight junctional proteins

Loss of growth factor signaling may lead to a loss of downstream phosphatidylinositol 3-kinase (PI3K)/p-Akt signaling. This may have significant implications, as a critical role of PI3K/Akt signaling in resistance to apoptosis has been reported in multiple cell types.<sup>33,34</sup> Our previous data has shown that TPN administration led to decreased p-Akt activity in small bowel ECs, contributing to the TPN-associated loss of proliferation and increased apoptosis.<sup>13</sup> T-cell lymphoma-1 (TCL1) is a novel Akt-activating peptide (a head-to-tail dimer of the Akt-binding domain of T cell lymphoma-1).<sup>35</sup> We used this peptide, or an inactive mutant sequence TCL1G, conjugated to a transactivator of transcription peptide sequence (TAT), to promote intracellular uptake. This led to a significant increase in p-Akt, as well as prevention in both the loss of EC proliferation and rise in apoptosis with TPN.<sup>11</sup>

The PI3K/p-Akt signaling may well have implications on preservation of EBF. PI3K/p-Akt signaling has been reported to affect electrogenic intestinal transport.<sup>36</sup> However, this action may be partial in that these authors showed that the application of the PI3 kinase inhibitor, Wortmannin or LY294002, did not significantly alter transepithelial potential difference or resistance. Junctional proteins play a crucial role in the epithelial barrier, and these can be reflected by the measurement of the transepithelial potential difference and resistance. Zonula occludens protein 1 (ZO-1) acts as a functional cross-linker between the E-Cadherin/catenin complex and actin-based cytoskeleton.<sup>37</sup> Other critical factors that modulate EBF include the family of claudins, and these may change significantly with various disease processes, such as Crohn's disease.<sup>38,39</sup> In our TPN mice using Western immunoblots to measure junctional protein expression, we found a significant reduction in the abundance of tight junction proteins ZO-1 and

occludin, as well as the adhesion junctional protein E-Cadherin compared to sham (enterally fed) groups. TCL-1 administration (which drives p-Akt abundance) partially prevented the loss of ZO-1 and E-Cadherin expression; however, TCL-1 did not impact the loss of occludin expression. As well, transepithelial resistance was not significantly affected in the TCL-1 TPN group compared to control (non-TCL1) TPN mice. Thus, the partial prevention of the loss of p-Akt, was unable to fully prevent the loss of EBF in this TPN model. Claudins also were differentially expressed with TPN, including a loss of claudins 7 and 15.<sup>12</sup>

As mentioned earlier, our laboratory's data also showed that downregulation of E-Cadherin expression in TPN mice is tightly related to a decrease in p-Akt activity.<sup>13</sup> In addition, we showed an association linking the loss of EC proliferation and increase of EC apoptosis, to a decline in the downstream signaling of the p-Akt pathway via GSK3- $\beta$  and mTOR pathways.<sup>11</sup> Interestingly, supplementation of TPN mice with intravenous glutamine (GLN) prevented the loss of p-Akt abundance, and resulted in a recovery of EC proliferation as well as barrier function.<sup>40</sup> (see next section on nutrient supplementation). These findings are similar to the McKay group, which recently reported that PI3K and protein kinase C were mediators of IFN- $\gamma$ -induced increases in epithelial paracellular permeability and the transcytosis of noninvasive, non-pathogenic *Escherichia coli* passage across monolayers of human gut derived ECs. Although Akt is an important mediator of many PI3K-dependent events, this event was Akt independent.<sup>41,42</sup> This may explain our lack of impacting EBF by driving p-Akt abundance.

Recently, it has been reported that TLR2-induced tight junction (TJ) modulation strongly interrelates with promotion of intestinal EC survival through the PI3K/Akt pathway.<sup>43</sup> An *ex vivo* culture model of primary intestinal epithelial cells showed functionally that TLR2 activation promotes cell survival by inhibiting apoptosis. This effect requires the adaptor molecule MyD88, which contains a p85-binding site,<sup>44</sup> as a putative mechanism to recruit PI3K downstream on ligand stimulation. TLR2 stimulation efficiently preserves ZO-1-associated barrier integrity against stress-induced damage, which is controlled by the PI3K/Akt-pathway via MyD88. As well, intestinal EC TLR2 functions via PI3K/Akt

to attenuate the MAPK/NF- $\kappa$ B-signaling cascade. Therefore, the complex interactions of an upregulation of TLR2 in our TPN model may well interrelate with the loss of EC proliferation and EBF. Because a major signaling mechanism for TLR4 is via lipopolysaccharide (LPS), much attention has been focused on how to block this signal. Intestinal alkaline phosphatase (IAP), which is released by the brush border of ECs can neutralize LPS and normalize the intestinal microbiota.<sup>45</sup> It will be fascinating to explore whether nutrient deprivation, with TPN, can influence IAP production.

### **Loss of critical nutrients may be a contributing factor to the development of EBF loss**

With the comprehensive clinical application of TPN, it would seem that clinicians have achieved consensus about the optimal TPN formula. However, even with the prerequisite of enough caloric intake, there still are two problems not well handled in the current approach to clinical TPN administration: loss of critical nutrients in the TPN solution and the lost luminal sensing for nutrients in the gut. Each of these may contribute to TPN-associated EBF disruption. These two issues are an emerging research focus in this field.

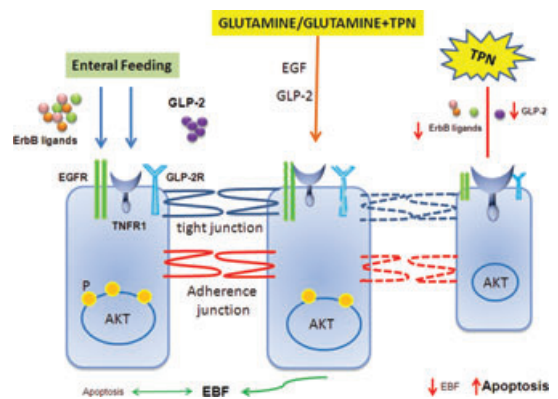
To address the first issue, we compared the difference between TPN versus TPN plus partial (25%) oral feeding on the intestinal immune system.<sup>46</sup> This small amount of enteral feeding significantly prevented atrophy of the intestinal epithelium and changes in intraepithelial lymphocytes (IEL) phenotypes seen with TPN. As there was a prevention in the upregulation of proinflammatory cytokines (which downregulated normal tight junction function) with partial enteral feeding, the results suggest that the major factor responsible for TPN-associated loss of EBF, bacterial translocation, and IEL-changes, is the lack of enteral feeding and not the administration of the TPN solution itself.

Of great interest is the identification of critical nutrients that could be involved in EBF protective mechanisms, thereby helping us greatly improve the formula of TPN administration. Because GLN has been shown to be an important amino acid in the modulation of EBF under various luminal threats, such as endotoxin lipopolysaccharide, we investigated the

benefits of GLN supplementation on TPN mice.<sup>40</sup> Intravenous GLN supplementation significantly prevented the loss of EBF and mucosal atrophy in TPN mice. This was shown with an accompanied decreased intestinal permeability, increased expression of tight junction proteins (including ZO-1, occluding, and junctional adhesion molecule-1), as well as prevention of changes in IEL-derived cytokine profile. It seems that GLN showed this EBF protective effect in TPN mice by regulating the phenotype and function of IEL, potentially via an immunomodulatory effect of GLN.<sup>40</sup>

The GLN studies described earlier were supplemented intravenously. It is also possible that the lack of luminal nutrients with TPN administration not only leads to some certain nutritional deficiencies, but may also result in a loss of gut-nutrient sensing. An increasing literature has shown gut taste receptors (TRs), located throughout the gastrointestinal tract, to be involved in gastrointestinal mucosal defense mechanisms.<sup>47</sup> It is clear that a variety of TRs are present throughout the gastrointestinal tract, and that these are predominately located in enteroendocrine cells and ECs on the luminal side of the mucosa, facing a variety of nutritive and nonnutritive stimuli signals.<sup>48</sup> Interestingly, growing evidence has demonstrated that certain sweet receptors and umami receptors may be under dynamic regulation under different metabolic (glucose/glutamate [GLM]) and luminal stimuli (acid injury/luminal microbiota); and may have particular relevance in disease conditions such as diabetes.<sup>49</sup> This suggests that a compensatory mechanism may exist, which balances nutrient sensing and absorption.<sup>50</sup> Furthermore, through activating TRs, taste stimulants supplementation could enhance the function of the intestinal immune system.<sup>51</sup> Potentially, this stimulation could enhance EBF.

Recently, the umami receptor stimulant, GLM has been shown to enhance duodenal mucosal defense mechanisms through the TRs T1R1/T1R3 and GLM receptors.<sup>52</sup> The Wang group reported that perfusion of L-Glu/IMP (inosine 5'-monophosphate) increased portal venous concentrations of GLP-2 and GLP-1 and increases duodenal bicarbonate secretion.<sup>53</sup> Another recent *in vitro* study demonstrated that similar to GLN, GLM treatment reduced the permeability of IEC monolayers,<sup>54</sup> thereby enhancing EBF. Although there are no publications examining the action of GLM on EBF during TPN ad-



**Figure 3.** Summary of the potential mechanisms, which may be involved in sustaining epithelial barrier function via the p-Akt signaling pathway. Note loss of local growth factors, including EGF and GLP2, along with respective receptors results in loss of EBF. Restitution of EBF, along with epithelial cell proliferation and prevention of apoptosis, occurs in TPN mice with supplementation of glutamine or glutamate, as well as with exogenous growth factors.

ministration, it is quite possible that stimulation of these TRs may act in this way, and thus, restoring gut nutritional sensing could be a potential strategy to improve EBF in TPN patients. A summary of these findings is given in Figure 3.

## Conclusions

Although providing the essential macro- and micronutrients required during a prolonged fasting state, the administration of TPN also leads to small bowel atrophy, increase in EC apoptosis, and increases in proinflammatory mediators. The resultant effect is a significant loss of EBF, predominantly via a loss of critical tight junction proteins. The mechanisms for these actions are complex, but decreases in the p-Akt signaling pathway, and an increase in proinflammatory cytokines, likely play important roles. The additional administration of GLN, the principal energy source for enterocytes, helps prevent these deleterious effects TPN has on the small bowel. Conceivably directed enteral stimulates, such as GLM, may also demonstrate intestinal protection via signaling of intestinal TRs. By continuing to examine the mechanisms of TPN related complications in the small bowel, and potential protective modalities, the harmful effects of this life-prolonging therapy may be eliminated.

## Acknowledgment

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## Conflicts of interest

The authors declare no conflicts of interest.

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