Invited Review

Changes to the Intestinal Microbiome With Parenteral Nutrition: Review of a Murine Model and Potential Clinical Implications

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Abstract
Parenteral nutrition (PN) dependence, while life sustaining, carries a significant risk of septic complications associated with epithelial barrier dysfunction and translocation of gut-derived microbiota. Increasing evidence suggests that PN-associated changes in the intestinal microbiota play a central role in the breakdown of the intestinal epithelial barrier. This review outlines the clinical and experimental evidence of epithelial barrier dysfunction with PN, the role of gut inflammatory dysregulation in driving this process, and the role of the intestinal microbiome in modulating inflammation in the gut and systemically. The article summarizes the most current work of our laboratory and others and describes many of the laboratory findings behind our current understanding of the PN enteral environment. Understanding the interaction between nutrient delivery, the intestinal microbiome, and PN-associated complications may lead to the development of novel therapies to enhance safety and quality of life for patients requiring PN. (Nutr Clin Pract. 2015;30:798-806)

Keywords
parenteral nutrition; enteral nutrition; microbiome; liver disease; surgery; bacterial translocation; inflammation; microbiota; nutritional support

The nutrition management of patients facing critical illness, abdominal surgery, or intestinal failure can be particularly challenging as their pathologic state leaves the intestine as a nonviable route for nutrient delivery. Failure to provide adequate nutrition may result in diminished immune function, increased complications, and extended hospitalizations.1,2 When clinically feasible, enteral feeding provides the ideal method of nutrient delivery.2-6 Even among critically ill patients, where “bowel rest,” or the withholding of enteral feeding, has previously been practiced, the early initiation of feeds within 48 hours of intensive care unit (ICU) admission has more recently been shown to reduce mortality and costs compared with delayed feeding.7,8 These results have been codified by the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N), whose most recent recommendations from their consensus meeting in 2009 include the early initiation of enteral feeds in the postoperative setting unless specific contraindications are present.9

Parenteral Nutrition: A Necessity
When enteral feeding is not possible, the parenteral route becomes a life-sustaining method of delivering calories and nutrients. Parenteral nutrition (PN)—wherein enteral nutrient delivery is withheld and nutrition is supplied intravenously—is required for >350,000 patients per year in the United States.10 Of these, >10% require home-based, long-term PN. In critical care studies, the use of early PN resulted in reduced need for mechanical ventilation, improved quality of life, and reduced costs, benefitting patients who otherwise would not have been able to receive enteral nutrition until day 3 of ICU admission.11,12 These findings were recently confirmed in the CALORIES trial, a large randomized trial that found no significant difference in infectious complications or 30-day mortality between early PN versus enteral nutrition among the critically ill.13 These findings may be due to high rates of enteral feeding intolerance and procedural delay, which can prevent critically ill patients from attaining nutrient fulfillment when relying on the enteral route alone. No matter the cause, these results support the premise that when adequate nutrition cannot be delivered via the enteral route, PN is a necessary intervention.

Infectious Risks
Outside of the critical care setting, the adverse effects of PN become increasingly significant, with metabolic derangements, hepatic dysfunction, and systemic infection posing the...
most significant risks. A seminal Veterans Affairs study found that patients receiving PN who had mild or moderate malnutrition had an increased rate of infectious complications, including urinary tract infections, wound infections, and pneumonia. Importantly, these complications were not due to central access, a necessity for delivery of PN, but instead implicated a systemic propensity for the development of multisite infections.

As the duration of PN dependence increases, so does the associated risk of infectious complications. For example, children requiring long-term PN have up to a 27% cumulative mortality rate, with hepatic failure and systemic infections contributing significantly to this rate. The clinical management of PN-dependent patients, therefore, focuses on reevaluating the need for PN and advancing enteral feeding when tolerated.

While the risk of septic complications is well-established with PN, the origin of these infections is incompletely understood. Increasing evidence implicates the intestinal microbiota as a possible source. The largest source of bacteria in the human body, the intestinal microbiome exists normally as a symbiotic community. When this symbiotic relationship is disturbed, however, altered gut microbiota may drive systemic pathological responses, including inflammatory bowel disease, obesity, cardiovascular disease, and sepsis. In surgical patients, decreased diversity of the microbiome has been associated with postoperative complications, including anastomotic leaks.

**Loss of Barrier Function**

A common finding in PN-dependent animal models involves cultured organisms from the blood that are normally found within the gut. This finding has implied an enteric origin of these bacteria, which has been supported by animal studies for nearly 30 years. Alverdy et al. described the translocation of gut-derived bacteria to mesenteric lymph nodes in up to two-thirds of PN-dependent rats, compared with one-third of those receiving both enteral nutrition and PN. Rats receiving only enteral nutrition had no such evidence of translocation. This classic study has since been repeated in other animal models, indicating a PN-associated loss of epithelial barrier function (EBF) as a key factor in the development of septic complications from PN dependence. This loss of EBF has been quantified using ex vivo measurements of intestinal transepithelial resistance — findings that have recently been replicated in unfed human intestinal tissue.

In cell-culture models, this translocation has been observed more directly. The exposure of cultured intestinal epithelial cells (IECs) to bacteria induces epithelial breakdown and bacterial translocation in a dose- and species-dependent manner, with gram-negative enteric bacteria (dominant bacteria in PN dependence) exhibiting high rates of translocation. Supplemanting the enteral microenvironment with dietary fiber decreases the rate of bacterial translocation from the gut. These findings suggest that it is the lack of enteral nutrition that drives the loss of EBF in the PN-dependent state, as opposed to the delivery of PN itself. Certain enteric microbiota also contribute to bacterial translocation by directly injuring the epithelial barrier. Enteric microbiota and bacteria-derived toxins can then translocate across this dysfunctional epithelial barrier, manifesting clinically as endotoxemia, bacteremia, and potentially sepsis.

Understanding of how PN-associated epithelial barrier dysfunction occurs, particularly the evidence that changes in the bacterial population drive this process, has been gained through a combination of animal and human studies. Our laboratory has used a mouse model with which PN is delivered intravenously and all enteral nutrition is withheld. These animals are compared with controls that receive intravenous saline and are allowed an oral diet. Importantly, the total nutrient and calorie delivery to all animals is equal; therefore, any deleterious effects are due to the method of nutrition delivery: PN versus enteral nutrition. This model, and others similar to it, have provided insight into the loss of EBF, inflammatory dysregulation, and changes in the mucosa-associated microbiome that occur with PN — findings that are increasingly confirmed in humans.

**Intestinal epithelial barrier** is a functional term, describing the role of the epithelium in protecting the host against the invasion of gut-dwelling bacteria and their products. The structural components of the epithelial barrier include a continuously renewing single layer of epithelial cells; tight junction proteins, which regulate the movement of ions and small molecules through the paracellular space; a layer of mucus comprised of glycoproteins and produced by goblet cells; Paneth cell–derived antimicrobial factors, including lysozyme and α-defensins, and secretory immunoglobulin A produced by gut-associated lymphoid tissue. Each of these factors plays a role in maintaining intraluminal homeostasis through interactions with the microbiome, influencing the relative abundance of individual bacterial strains.

An example of one such interaction is the intestinal mucus layer, which serves as a medium for bacterial colonization, with several bacterial species using mucin-derived oligosaccharides as a carbon source. In turn, intestinal bacteria stimulate the epithelium to secrete additional mucus sugars. In this manner, mucus function as epithelium-derived “prebiotics” by providing metabolic substrate for certain commensal bacteria and therefore influencing the makeup of the microbiome. Disruption of this relationship between microbiota and mucin degradation allows pathogenic bacteria to expand.

Similarly, secretory immunoglobulin A (sIgA) plays a key role in shaping intestinal microbial ecology. Antigen-specific production of sIgA occurs via T-cell-dependent pathways, and it is secreted into the luminal environment, where it interacts with gut microbiota. sIgA acts by binding to certain bacteria and microbial products, preventing enteroinvasion. Like mucin secretion, sIgA is influenced by the presence of intestinal microbiota, with germ-free mice demonstrating low levels in the gut until they are colonized with intestinal bacteria.
Even at the tight junction level, a complex interaction between microbiota and host function exists (Figure 1). Tight junction integrity is preserved through interactions of multiple different barrier proteins and IECs. Tight junction permeability appears to be dependent on microbial signaling—bacteria and their products modify permeability and ion selectivity. This manifests electrophysiologically as altered transepithelial resistance. This phenomenon has been demonstrated by inoculating cultured intraepithelial cells with bacterial metabolites, which leads to increased expression of junctional-associated protein occludin bolstering the tight junction function. Conversely, incubation of human intestinal organoids (tissue grown through pluripotent stem cells) with *Clostridium difficile* leads to a marked deterioration of EBF. Therefore, an interesting relationship exists in which tight junctions limit the translocation of small molecules, including bacteria-derived products, yet their expression and function are altered by these same products and their source bacteria.

In the setting of enteral deprivation with PN, multiple elements of the intestinal epithelial barrier are compromised. PN-dependent mice demonstrate diminished secretion of key antimicrobial factors phospholipase and lysozyme, leading to reduced bactericidal activity and increased susceptibility to bacterial enteroinvasion. Perhaps reflecting a compensatory defensive response by the epithelium, PN dependence has been shown to lead to an increase in goblet cell (immune modulators) numbers. Tight junction integrity is reduced in the PN-dependent setting, with loss of multiple components of the junctional complex resulting in decreased barrier function. Finally, the epithelial cell layer itself is compromised during the administration of PN, with decreased intraepithelial cell proliferation coupled with cell atrophy and death. These
structural changes in the intestinal epithelial barrier with PN dependence result in the functional outcome of increased bowel wall permeability and manifest clinically as the bacterial translocation associated with PN.\textsuperscript{53} As previously mentioned, current data suggest that the loss of EBF seen with PN dependence is the result of enteral deprivation as opposed to the PN solution. This has been corroborated by administering “trophic” feeding in a rodent model, consisting of 25% of caloric needs. This minimal enteral nutrition restored tight junction integrity and preserved EBF compared with full enteral deprivation.\textsuperscript{54}

Adding to the loss of EBF is the up-regulation of proinflammatory cytokines tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interferon-\(\gamma\) (IFN-\(\gamma\)), which each independently induce loss of tight junctions and increased bowel wall permeability.\textsuperscript{55} Their expression is increased 3-fold in the PN-dependent mucosa. The subsequent epithelial barrier dysfunction is mitigated in IFN-\(\gamma\)-knockout mice.\textsuperscript{32,56} Similarly, TNF-\(\alpha\) expression is increased in the PN-dependent setting.\textsuperscript{57} Expression of this cytokine is associated with apoptosis and mucosal atrophy via activation of a Toll-like receptor-4 (TLR-4) signaling pathway.\textsuperscript{57,58} Activation of this inflammatory cascade recruits immunocytes, further contributing to immune dysregulation.\textsuperscript{59} This proinflammatory state has been observed in unfed human intestine as well, which demonstrated significant increases in TNF-\(\alpha\) and TLR-4 compared with matched fed intestine from the same patient.\textsuperscript{59}

In addition to the increase in proinflammatory signaling driven by TNF-\(\alpha\) and IFN-\(\gamma\), a reduction in the counterinflammatory cytokine interleukin-10 (IL-10) occurs in the PN-dependent setting. IL-10 expression is necessary to maintain EBF,\textsuperscript{60} with IL-10 knockout mice demonstrating barrier breakdown and gut inflammation.\textsuperscript{61} PN dependence results in a decline in mucosal IL-10, with a subsequent increased EBF with administration of exogenous IL-10.\textsuperscript{59} Compounding the inflammatory cytokine imbalance, PN dependence results in a loss of mucosal growth factors. Epidermal growth factor, keratinocyte growth factor, and glucagon-like growth factor are all down-regulated in PN dependence. Extensive study has revealed an imbalance between proinflammatory and anti-inflammatory cytokines and loss of growth hormone signaling in the PN model to which the loss of growth factors is attributed.\textsuperscript{62,63} The above interactions are a brief overview of the very complex changes seen in PN dependence, the intricacies of which are beyond the scope of the current review. We encourage referral to above studies referenced for the mechanistic descriptions of changes involved.

**Microbial Population Changes**

The PN-associated proinflammatory state within the intestinal epithelium and resulting loss of EBF increase the susceptibility of the epithelial barrier to entero invasion and translocation of microbiota-derived products. This mechanism accounts for the microbiota as the final actor in PN-associated bacteremia, as bacteria remain passive within the lumen until epithelial barrier loss occurs. Increasing evidence suggests, however, that the PN-dependent state leads to a primary, significant shift in the makeup of the intestinal microbiome. While a causal relationship has not been definitively demonstrated, it is possible that the expansion of pathogenic microbial populations drives the proinflammatory epithelial state and subsequent loss of barrier function, subsequently allowing for translocation and further inflammatory insult.

In a mouse model, 6 days of PN dependence results in an altered small bowel mucosa-associated microbiome. This is characterized, at the phylum level, by an expansion of Proteobacteria and Bacteroidetes while there is a relative loss of Firmicutes.\textsuperscript{58} Similar changes were observed in PN-dependent rats, with a shift in the ratio of Firmicutes to Bacteroidetes in favor of Bacteroidetes after 14 days of PN compared with fed control animals.\textsuperscript{54} A neonatal pig model has also showed a significant change in the gut microbiome with PN, characterized by a reduction in bacterial concentration throughout the intestine, as well as loss of bacterial diversity at the genus level.\textsuperscript{65} Interestingly, the PN-dependent pigs demonstrated higher levels of colonization with toxin-expressing *Clostridium difficile*. These various animal models consistently demonstrate reduced bacterial diversity with a relative expansion of pathogenic microbiota in the PN-dependent setting.

To date, limited data exist on the impact of PN on the human intestinal microbiome. Most human studies have evaluated fecal microbiota, which represent a distinct community from the small bowel mucosa-associated microbiota.\textsuperscript{66,67} Interesting trends still result and parallel those of animal studies. In a study of patients with Crohn’s disease, Shiga et al\textsuperscript{68} found reduced species diversity of the fecal microbiome after initiation of PN and a significant increase in the population of *Enterococcus* species. Engstrand Lilja et al\textsuperscript{69} evaluated the fecal microbiome of PN-dependent children with short bowel syndrome—a significant cause of PN dependence in the pediatric population—and compared these with healthy siblings. Interestingly, this study found a marked reduction in bacterial diversity in PN-dependent children compared with both healthy siblings and those with short bowel syndrome who had transitioned to an enteral diet. Along with a loss of diversity, the PN-dependent children were found to have a marked increase in the abundance of *Enterobacteriaceae*. This family of gram-negative Proteobacteria includes pathogens such as *Salmonella*, *Shigella*, and *Proteus*, which have been previously found in mouse models.\textsuperscript{58}

It is hypothesized that the reduced bacterial diversity and relative abundance of *Proteobacteria* in the PN-dependent mucosa-associated intestinal microbiome may be a result of a marked change in available nutrients for bacterial utilization. The gut microbiome is highly sensitive to nutrient availability, which is determined largely by the host diet.\textsuperscript{70} Changes in host enteral nutrition may change the nutrition selection pressures
within the gut, leading to an altered microbiome and influencing host pathological processes. 71,72 Presumably, the lack of enteral feeding in the setting of complete intravenous nutrition results in sufficient nutrient delivery to the host while creating a nutrient-deprived environment for the bacteria within the gut. This hostile environment may select for Proteobacteria, which have been shown to survive in states of relative starvation, versus Firmicutes, which dominate in the nutrient-rich environment. 73,74 Aggressive microbiota such as Proteobacteria may then cause or exacerbate PN-associated inflammation by capitalizing on the reduced EBF that occurs with PN. Deplancke et al explored this concept in the context of mucolysis, wherein bacteria metabolize mucin oligosaccharides. Using culture-dependent methods, the investigators found that the ileal mucosa-associated microbiome from PN-dependent piglets was enriched for mucolytic bacteria, with opportunistic pathogen Clostridium perfringens significantly increased compared with enterally fed animals. 75

Microbial Population Shifts, Immune Regulatory Changes

The mechanism by which the altered microbiome drives gut inflammatory dysregulation may be dependent on the myeloid differential primary response gene 88 (MyD88). A key function of the gut microbiota is to modulate the intestinal immune system by interacting with lamina propria (LP) cells, including dendritic cells and macrophages. 76,77 This interaction is mediated by Toll-like receptor binding. This pathway is dependent on MyD88 and leads to increased expression of proinflammatory cytokines TNF-α and IFN-γ, as previously discussed. There is also a loss of T-regulatory cells from the LP. 78,79 When MyD88 knockout mice were used, a similar PN-associated population shift occurred in the mucosa-associated gut microbiome, characterized by a loss of Firmicutes and a predominance of Proteobacteria. PN-dependent MyD88 knockout mice, however, exhibited preservation of EBF, increased epithelial cell proliferation, preserved regulatory cells population, and reduced proinflammatory cytokine signaling compared with wild-type PN-dependent mice. This suggests that PN-associated shifts in the microbiome lead to downstream inflammatory effects and loss of EBF in a MyD88-dependent manner. 58

Hepatic Complications

Given the wide-ranging pathological processes associated with the intestinal microbiome, it is not surprising that other PN-associated complications are also being attributed to specific gut microbial changes. Intestinal failure–associated liver disease (IFALD) is one of the principal end-stage complications of chronic PN dependence and remains the primary indication for intestinal transplantation. 80 Characterized by cholestasis progressing to fibrosis and liver failure, the pathogenesis of IFALD is poorly understood. 81 Several contributing factors have been implicated, including recurrent sepsis, excess substrate or toxin delivery via PN, loss of gastrointestinal peptide production, increased bile viscosity, and bacterial translocation (Figure 2). Recent animal and human evidence suggests a role for the microbiome. In a mouse model of IFALD, the administration of PN and induction of small bowel permeability led to expansion of the family Erysipelotrichaceae and subsequent TLR-4-mediated liver injury. 82,83 Administration of antibiotics with reduction in this bacterial family attenuated the severity of liver injury.

Korpela et al recently evaluated the intestinal microbiota (from ileostomy, jejunostomy, or feces) as well as liver histologic results of children with intestinal failure and compared these with healthy children. The abundance of Proteobacteria was associated with prolonged PN dependence and liver inflammation, whereas Lactobacilli were associated with children who had been weaned off of PN and displayed liver steatosis. Interestingly, the composition of the microbiota better predicted liver steatosis than did the duration of PN dependence or the remaining intestinal length. This initial investigation reveals an intricate potential mechanism wherein an abundant Proteobacteria population, as in the PN-dependent state, results in liver inflammation, whereas Lactobacilli, which recover following weaning from PN, then mediate steatosis. The use of antibiotic prophylaxis to lessen the bacterial burden for these patients has been suggested. Use of metronidazole was retrospectively studied by Kubota et al in 21 patients receiving prolonged PN. The authors reported a

Figure 2. Intestinal failure–associated liver disease (IFALD). The pathogenesis of IFALD is likely multifactorial, with systemic factors, parenteral nutrition (PN)–associated factors, and gut-derived factors contributing to IFALD development. Increasing evidence suggests an altered microbiome predicts the development of IFALD. SMA, superior mesenteric artery.
resultant decrease in transaminases; however, given the lack of other significant findings, antibiotics currently are used on a case-by-case basis depending on the clinical scenario at hand. The use of probiotics to support a healthy microbiome and decrease pathologic bacteria is the interest of other IFALD therapeutic studies.86 Despite evidence showing the prevention of bacterial translocation with probiotic administration in animal models87,88 and clinical improvement in small case studies,89,90 further human studies are necessary before routine use can be recommended. Given the association between enteric bacteria and liver injury in the setting of PN dependence, further study to ascertain the exact nature of this relationship is warranted.

Conclusions and Future Studies

PN plays a critical role in fulfilling the nutrition needs of patients who cannot receive enteral nutrition. However, PN is associated with significant risks, culminating in potentially life-threatening complications such as sepsis and liver failure. The reduced EBF seen with PN dependence is driven by proinflammatory signaling derived from an altered microbial community, as summarized in Figure 3. The consequences of PN-associated changes in the microbiome include reduced EBF, systemic inflammatory responses, and liver injury. Better understanding of the factors contributing to these microbial shifts might reveal strategies to prevent PN-associated complications.

Future work should investigate the causal role of microbiota in generating PN-induced disease. Like early investigations of other microbiome-driven diseases, studies to date largely provide associations between PN-associated microbial community changes and host pathophysiological features. Further studies should examine whether these microbial changes are sufficient to produce the PN-associated mucosal phenotype of epithelial barrier loss.

Questions remain regarding the role of the intestinal metabolome (ie, the metabolic composition of the intestinal lumen) in driving changes in the microbiome. In the PN-dependent state, no enteral nutrition is provided, significantly altering the availability of metabolic substrate for intestinal bacteria. The metabolic milieu present in the enterally deprived state has yet to be characterized, although this is likely a central factor in determining the makeup of the microbiota. It will be important to understand the available nutrients in the PN-dependent intestinal lumen, how these shape the bacterial community, and how bacteria in turn forage for nutrients both in the short term and with long-term PN administration.

Figure 3. Parenteral nutrition–associated changes in the microbiome and epithelial barrier dysfunction. (A) In the enterally fed state, intestinal epithelial barrier function is maintained by the interaction between Firmicutes-dominated intestinal microbiota and the host immune system. (B) With dependence on parenteral nutrition, the lack of enteral nutrients leads to an altered microbiome, characterized by increased Proteobacteria, which in turn drives a proinflammatory state in the intestinal mucosa, resulting in epithelial barrier dysfunction. EBF, epithelial barrier function; EGF, epidermal growth factor; IEL, intraepithelial lymphocyte; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; sIgA, secretory immunoglobulin A; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor; Treg, T-regulatory cell.
The provision of adequate intraluminal nutrients for use by intestinal microbiota may attenuate microbiome-associated conditions. Using ever-improving methods of characterizing composition and function of the gut microbiota will likely reveal further insight into how bacteria are able to attain nutrients in the state of enteral deprivation and what mechanisms these strains use to cause host disease. With continued study, improvements or adjuncts to PN therapy might allow for optimization of the luminal environment, prevention of harmful shifts in the microbiota, and preservation of host barrier function and health.

Statement of Authorship
F. R. Demehri, M. Barrett, and D. H. Teitelbaum contributed to conception and design of the work; contributed to acquisition, analysis, and interpretation of the data; drafted the manuscript; critically revised the manuscript; and agree to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

References


