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#### **Permalink**

<https://escholarship.org/uc/item/3n80c9nx>

#### **Journal**

Journal of Renal Nutrition, 27(6)

#### **ISSN**

1051-2276

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#### **Publication Date**

2017-11-01

#### **DOI**

10.1053/j.jrn.2017.02.010

Peer reviewed

# The Leaky Gut and Altered Microbiome in Chronic Kidney Disease



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Chronic kidney disease results in disruption of the intestinal epithelial barrier as well as profound changes in the gut microbial flora. These events are largely mediated by (1) heavy influx of circulating urea to the gut lumen and (2) dietary restrictions of foods containing high fiber (such as fruits and vegetable) and symbiotic organisms (such as yogurt and cheese) imposed to mitigate hyperkalemia and hyperphosphatemia. Collectively, these factors promote systemic inflammation and cardiovascular morbidity by mediating microbial dysbiosis, disruption of the intestinal epithelial barrier, and translocation of endotoxin, bacterial fragments, and uremic toxins across the “leaky gut” into the bloodstream. Strategies aimed at increasing dietary fiber and lowering urea burden may help to attenuate uremia-induced microbial dysbiosis and epithelial barrier breakdown, and thereby improve systemic inflammation.

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## Introduction

CHRONIC KIDNEY DISEASE (CKD) results in systemic inflammation and oxidative stress which play a role in the progression of kidney disease and the associated complications including cardiovascular disease, cachexia, and anemia.<sup>1</sup> The gastrointestinal tract is now recognized as a major source of this systemic inflammation and oxidative stress. Endotoxin and gut bacterial DNA fragments are found in the bloodstream of CKD patients.<sup>2,3</sup> Levels of circulating endotoxin, which is derived from the cell wall of Gram-negative bacteria, increase with severity of CKD stage and are most elevated in end-stage renal disease (ESRD) patients on maintenance dialysis.<sup>3</sup> In a cohort of 306 hemodialysis patients, blood endotoxin levels correlated with severity of systemic inflammation in the absence of clinically detectable infection.<sup>4</sup>

Indoxyl sulfate and p-cresyl sulfate are well-characterized uremic toxins that induce inflammation and leukocyte activation and have been associated with increased systemic inflammation and mortality in CKD patients.<sup>5</sup> Aronov et al. confirmed the colonic origin of several known uremic toxins (including p-cresyl sulfate and indoxyl sulfate) and many as yet unidentified products by comparing plasma solutes from ESRD patients who had undergone total colectomy with data from ESRD and

control subjects with intact colons.<sup>6</sup> Over 30 mass spectroscopy-detected solutes that were present in the plasma from ESRD patients with intact colons were absent or present in significantly lower concentration in ESRD subjects without colons.<sup>6</sup> Nearly, all these compounds were significantly lower in healthy individuals, suggesting that they represented uremic solutes.

Trimethylamine-N-oxide (TMAO) is a toxin generated by conversion of choline to trimethylamine by the gut microbial flora; trimethylamine is subsequently absorbed and converted to TMAO by monooxygenases in the liver. Dietary TMAO supplementation in animal models leads to tubulointerstitial fibrosis and progressive renal dysfunction.<sup>7</sup> Elevated levels of TMAO have been linked to increased 5-year mortality risk in CKD subjects after multivariate adjustment.<sup>7</sup>

## Gut Epithelial Barrier Breakdown: The Uremic Leaky Gut

Breakdown of the gut epithelial barrier is both a consequence of CKD and a cause of CKD progression. Chronic inflammation throughout the gastrointestinal tract of dialysis patients, extending from esophagus to large bowel, was demonstrated on autopsy studies in the 1980s.<sup>8</sup> These inflammatory changes sometimes co-existed with peptic ulcer disease and ischemic lesions.<sup>8</sup> Using oral polyethylene glycols of various molecular weights and their detection in the urine, Magnusson et al. demonstrated increased permeability of the intestinal wall in CKD patients and uremic rats.<sup>9,10</sup>

More recently, we examined the integrity of protein constituents of the tight junction complex in the intestinal tissue of rats with CKD caused by subtotal nephrectomy or adenine-induced chronic tubulointerstitial nephritis.<sup>11,12</sup> The study revealed marked depletion of the occludin, claudin, and zona-occludens (the key components of epithelial tight junction), in the intestinal tract of CKD

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Support: See Acknowledgments on page 460.

Financial Disclosure: The authors declare that they have no relevant financial interests.

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1051-2276/\$36.00

<http://dx.doi.org/10.1053/j.jrn.2017.02.010>

compared with healthy control animals. However, the messenger RNA expression was not significantly changed, pointing to a posttranscriptional or posttranslational mechanism. Disruption of the colonic epithelial tight junction apparatus in the CKD animals was associated with gut wall edema and heavy infiltration of mononuclear leukocytes.<sup>11</sup> These findings revealed the underlying mechanism responsible for impaired intestinal barrier function and endotoxemia in humans and animals with advanced CKD.

Retention of urea in CKD is 1 of the mechanisms that lead to epithelial tight junction breakdown. Urea diffuses from the blood into the gut lumen and is metabolized by gut bacterial urease to ammonia ( $\text{CO}(\text{NH}_2)_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3$ ); the latter is converted into caustic ammonium hydroxide ( $\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4\text{OH}$ ) which is capable of disrupting proteins that seal the gap between epithelial cells.<sup>13,14</sup> *In vitro*, human colonocytes exposed to media containing urea at clinically relevant concentrations (42 or 74 mg/dL) showed a concentration-dependent fall in transepithelial electrical resistance and loss of tight junction proteins.<sup>14</sup> When urease was added to the culture media to simulate the presence of microbial flora, there was extensive loss of tight junction proteins with subsequent detachment of the cell monolayer.<sup>14</sup> Once urea-induced tight junction breakdown is initiated, this triggers influx of leukocytes and local cytokine production with the resultant retraction and endocytosis of the transcellular tight junction proteins (claudins and occludin). The net result is a “leaky gut” with paracellular movement of endotoxin and bacterial fragments into the bloodstream, thus promoting chronic systemic inflammation.

### Gut Microbiome Alterations in CKD

The symbiotic intestinal microbiome plays an integral role in the host's health. The enormity of the gut flora is evidenced by the fact that the number of the microorganisms residing in the gut far exceeds the total number of cells in the human body.<sup>15</sup> The intestinal microbiota maintains the micronutrient environment for enterocytes by providing short-chain fatty acids, amino acids, and vitamins to enterocytes. In addition, gut bacteria play a major role in shaping and maintaining the immune system, by colonizing the intestinal tract after birth and modulating antigen responsiveness of the lymphoid tissues.<sup>15</sup> While a healthy microbiome is defined by the diversity in microbial species that provide a varied repertoire of metabolic functions, there is remarkable commonality between individuals such that 3 discernable enterotypes of the human microbiome have been described.<sup>16</sup>

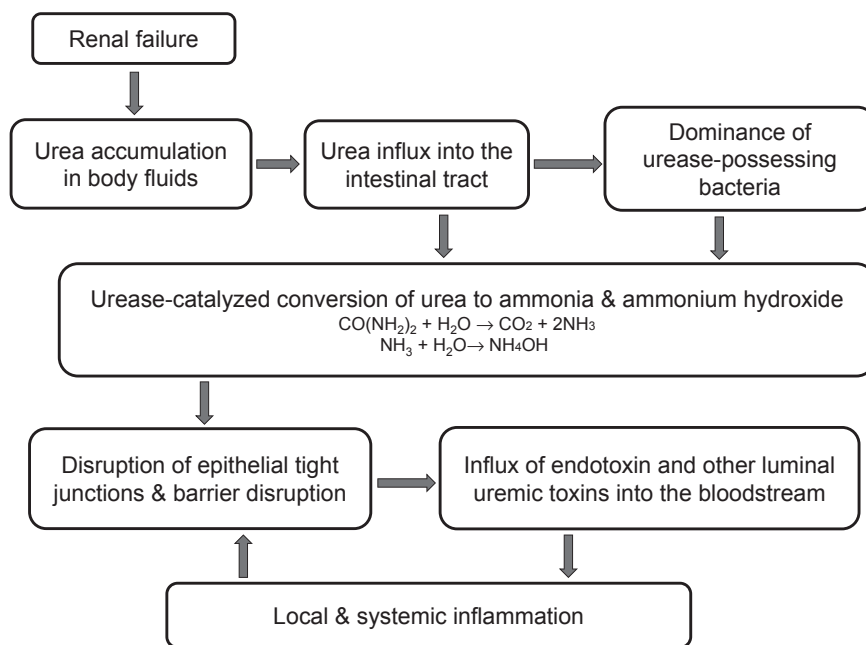
Alterations in the gut microbiota have been implicated in the pathophysiology of a variety of chronic diseases including cancer, asthma, obesity, diabetes mellitus, heart failure, and mood disorders. The field is rapidly expanding; a PubMed search for “microbiome” and “chronic disease” yields >1500 articles as of July 2016, and the majority of these were published in the recent decade.

The gut microbiome is markedly altered in CKD as demonstrated in a study where microbial DNA was isolated from the stool samples of ESRD patients maintained on hemodialysis and from age-, sex- and ethnicity-matched healthy individuals.<sup>17</sup> Phylogenetic microarray analysis showed highly significant differences in the abundance of over 200 bacterial operational taxonomic units belonging to 23 bacterial families between ESRD and the healthy control subjects. To isolate the effects of uremia from those of comorbid conditions and inter-individual variations including dietary and medication factors, the gut microbiome was further studied in CKD rats 8 weeks after 5/6 nephrectomy.<sup>17</sup> Compared with sham-operated control rats, the CKD rats showed significant differences in the abundance of 175 bacterial operational taxonomic units, thus confirming the effect of uremia per se on gut bacterial composition.

In a subsequent study, microbial genomic analyses of stool samples from ESRD and healthy controls confirmed dominance of bacterial families possessing urease, uricase, and p-cresol- and indole-forming enzymes.<sup>18</sup> This may be related to the influx of circulating urea and other toxins into the gut lumen, which applies a selection pressure favoring growth of bacteria that express urease, uricase, indol, and p-cresol forming enzymes. In turn, influx of bacterial-derived toxins into the bloodstream occurs across the weakened gut epithelial barrier as discussed previously.

The adverse effects of the toxic byproducts by dysbiotic microbiota are compounded by the reduction of numerous useful micronutrients made by the normal microbiota. The aforementioned study noted depletion of bacteria that produce short-chain fatty acids<sup>18</sup> which are essential for the integrity of colonocytes and the growth of anti-inflammatory regulatory T lymphocytes.<sup>15</sup> In addition to production of numerous solutes, fermentation of nutrients by the gut bacteria generates a variety of organic and inorganic gases that can be detected in the expired breath, as well as in blood and feces. Marked elevation of breath ammonia content has long been recognized as a marker of renal failure. Gas chromatography studies have shown significantly altered exhaled breath gases in dialysis patients compared with healthy controls.<sup>19</sup> A study in CKD rats revealed significant increase in isoprene and a significant reduction in pentanal, hexanal and heptanal in exhaled breath compared with control animals.<sup>20</sup>

CKD patients are often advised to adhere to low-potassium and low-phosphorus diets to avoid the adverse effects associated with hyperkalemia and hyperphosphatemia. This translates into a diet low in fermentable plant fiber (to avoid potassium-rich fruits and vegetables) and poor in symbiont-rich cheese/yogurt (which have high phosphorus content). This change in food substrate could further alter the bacterial composition and jeopardize microbial nutrient production.<sup>15</sup> In fact, recent studies have demonstrated significant amelioration of systemic inflammation and CKD progression, and partial restoration of



**Figure 1.** Relationship between the leaky gut, altered microbiome, and systemic inflammation in chronic kidney disease. Urea accumulates in renal failure and diffuses into the gut lumen where it drives expansion of bacterial species that express the urease enzyme. Luminal urea is converted by bacterial urease to ammonia which is hydrolyzed to ammonium hydroxide. The latter causes breakdown of the epithelial barrier with subsequent translocation of bacterial toxins into the circulation. The chronic local and systemic inflammation promotes further disruption of the gut epithelial barrier, thus forming a vicious cycle that impacts progression of renal failure and its associated adverse outcomes.

blood, urine, and intestinal fluid metabolome, as well as the gut microbiome with consumption of a diet rich in indigestible fermentable complex carbohydrate.<sup>21,22</sup> The underlying mechanisms of the uremia-induced gut microbial dysbiosis, disruption of the intestinal epithelial barrier, and their contribution to systemic inflammation are summarized in Figure 1.

Potential strategies aimed at minimizing pathologic alterations in the uremic gut such as lowering urea, avoiding volume depletion to prevent gut ischemia, and use of probiotics, oral adsorbents and high-fiber diet are discussed in a recent review.<sup>15</sup>

## Conclusions

Changes in the biochemical environment of the gut in CKD affect the integrity of the intestinal epithelial barrier and the composition and function of the microbiome. Bacterial-derived fragments and toxins enter the bloodstream through the leaky gut, contributing to systemic inflammation, malnutrition, cardiovascular morbidity, and other complications in patients with advanced CKD.

## Acknowledgments

Support: W.L.L. received research funds from the Division of Nephrology and Hypertension, University of California, Irvine, derived from a philanthropic endowment grant by Dr Joseph Lee.

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