Probiotics for kidney disease
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ABSTRACT
Diet has long been known to influence the course of chronic kidney disease (CKD) and may even result in acute kidney injury (AKI). Diet may influence kidney disease through a direct impact of specific nutrients on the human body through modulation of the gut microbiota composition or through metabolites generated by the gut microbiota from ingested nutrients. The potential for interaction between diet, microbiota and CKD has fueled research into interventions aimed at modifying the microbiota to treat CKD. These interventions may include diet, probiotics, prebiotics, fecal microbiota transplant and other interventions that modulate the microbiota and its metabolome. A recent report identified Lactobacillus casei Zhang from traditional Chinese koumiss as a probiotic that may protect mice from AKI and CKD and slow CKD progression in humans. Potential mechanisms of action include modulation of the gut microbiota and increased availability of short-chain fatty acids with anti-inflammatory properties and of nicotinamide. However, the clinical relevance needs validation in large well-designed clinical trials.

Keywords: acute kidney injury, butyrate, chronic kidney disease, fibrosis, inflammation, microbiota, probiotic, nicotinamide, short-chain fatty acids

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FIGURE 1: Interactions between diet, the microbiota and kidney disease. Dietary components are frequently modified by the gut microbiota, which in turn changes in response to the availability of specific dietary components. Thus any interaction between diet and kidney disease cannot be properly understood without understanding the impact of the diet on the gut microbiota. Specific components of the diet can directly influence the gut microbiota composition, as well as dietary molecules that are processed by the microbiota to yield both potentially kidney protective (e.g. SCFA) or kidney damaging molecules or precursors (e.g. TMA that is metabolized to TMAO in the liver). Kidney disease itself may modify the microbiota through increased availability of molecules usually excreted by the kidneys. Finally, the cause of kidney disease may influence the diet (e.g. dietary recommendations for persons with diabetes or hypertension) and the microbiota (e.g. the impact of lyso-Gb3, a metabolite accumulated in Fabry disease, on the gut microbiota).

metabolized by the gut microbiota to p-cresol, which human cells convert to the nephrotoxic compound p-cresyl sulfate (p-CS) [13]. It is also likely that CKD itself, or the cause of CKD, modifies the gut microbiota [14]. Thus lyso-Gb3, a toxic compound accumulated in Fabry disease that causes podocyte injury, also modulates the gut microbiota, resulting in decreased production of the anti-inflammatory short-chain fatty acid (SCFA) butyrate [15, 16]. The potential for interaction between diet, microbiota and CKD has fueled research into interventions aimed at modifying the microbiota to treat CKD [17]. These interventions may include diet, probiotics, prebiotics, fecal microbiota transplant and other interventions that modulate the microbiota and its metabolome.

AN ANCIENT FERMENTED DAIRY PRODUCT TO THE RESCUE

Koumiss or kumis is a traditional fermented dairy product home-made from mare’s milk or donkey’s milk by nomadic people in China and Mongolia. It has a low alcohol content. A Colombian version of kumis is a different form of fermented milk from cow’s milk [18]. In 2005, a novel Lactobacillus casei strain, L. casei Zhang was isolated from traditional koumiss in the Inner Mongolia Autonomous Region of China by Ya et al. [19]. L. casei Zhang was considered a strain of interest given its probiotic properties such as acid- and bile acid–resistance and gastrointestinal colonization ability, i.e. if administered orally, it will survive the upper gastrointestinal tract and grow in the lower gastrointestinal tract. Further research characterized in vivo antibacterial, immunomodulatory and antioxidative qualities of orally administered L. casei Zhang [19] and the complete genome was sequenced [20]. Thus L. casei Zhang increased serum interferon-γ, secretory immunoglobulin a and IgG levels and decreased serum tumor necrosis factor (TNF) levels in mice [19]. TNF has several adverse actions in kidney cells, such as promotion of necroptotic tubular cell death and reduction of the kidney production of the anti-aging protein Klotho [21, 22]. Indeed, some kidney protective drugs, such as pentoxifylline, decrease serum and urine TNF while increasing serum and urine Klotho [23]. This raises the possibility that L. casei Zhang may be nephroprotective.

L. casei Zhang and kidney disease

Writing in Cell Metabolism, Zhu et al. [24] identify a connection between L. casei Zhang and kidney protection in AKI and CKD mouse models and in an exploratory human clinical trial (Figure 2).
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FIGURE 2: Kidney protection by L. casei Zhang was observed in experimental AKI and CKD as well as for human CKD. Kidney protection by L. casei Zhang was transmissible through fecal microbiota transplantation (FMT).

Administration of L. casei Zhang or the control Lactobacillus acidophilus orally to C57BL/6 mice 4 weeks before or concurrent with ischemia–reperfusion injury (IRI) was protective at 5 (exploring AKI) and 28 and 45 days (exploring CKD). AKI results were confirmed in cisplatin and lipopolysaccharide (LPS)-induced AKI. However, L. casei Zhang was superior to L. acidophilus as shown by better kidney function and milder histological tubular injury and kidney expression of fibrosis-related genes. Reduced kidney fibrosis was also observed in the subtotal nephrectomy model in which prebiotics were started 2 weeks after surgery, i.e. after induction of kidney injury. Inflammatory infiltrates were analyzed only in a second cohort of mice treated with antibiotics before bilateral IRI and probiotic treatment, showing lower expression of macrophage-associated factors in kidneys of mice treated with L. casei Zhang. This experiment showed that the beneficial effect of L. casei Zhang was independent from the prior gut microbiota, as this was disrupted by antibiotics, a frequent occurrence in the clinic, especially in intensive care units (ICUs).

The molecular mechanisms of kidney protection by L. casei Zhang appear to be complex and multipronged (Figure 3). Zhu et al. [24] reproduced prior observations on kidney protection by administration of SCFA or nicotinamide, but since they did not interfere with these pathways in mice treated with L. casei Zhang to demonstrate loss of protection, it is unclear whether kidney protection afforded by L. casei Zhang actually involved these mediators.

L. casei Zhang improved gut microbial dysbiosis induced by IRI, as assessed by 16s sequencing, expanding SCFA-producing bacteria, such as Bacteroidetes, and increased kidney and/or serum levels of the SCFAs acetate, butyrate or propionate at 5 days after IRI. Indeed, kidney protection could be transferred through stool transplant. Previous studies assessed the beneficial effect of SCFAs in preventing AKI induced by IRI [25] and folic acid nephropathy [26]. A mixture of three SCFAs (butyrate, propionate, acetate) administered intraperitoneally 30 min...
before ischemia and at reperfusion improved IRI renal dysfunction likely through the inhibition of histone deacetylase activity [25]. The oral administration of the same SCFAs in drinking water decreased folic acid–induced tubular injury at day 2 and interstitial fibrosis and chronic inflammation at day 28. Since mice deficient in G-protein-coupled receptors GPR41 and GPR109A were not protected, SCFA activation of GPR41 and GPR109A appeared to play a major role in kidney protection [26]. Zhu et al. [24] administered acetate, butyrate or propionate or a mixture of them in drinking water from 2 weeks before IRI to the time of IRI in mice. Any of the SCFAs or the combination was associated with milder AKI, inflammation and fibrosis at 5 and 24 days, as assessed by plasma urea and histology (including Masson staining for fibrosis and quantification of neutrophils and macrophages for inflammation) and gene expression of fibrosis and inflammation markers. Propionate showed the largest benefit while the combination did not have an additive benefit. However, whether SCFA supplementation increased kidney SCFA levels was not addressed, and it remained unclear whether protection depended on activation of SCFA receptors or on epigenetic modulation through histone deacetylase inhibition or other mechanisms [27, 28].

In metabolic pathway analysis, IRI AKI resulted in lower nicotinamide metabolism (including reduced kidney NAD and nicotinic acid adenine dinucleotide levels) at day 5 and this was prevented by L. casei Zhang, which in single-cell transcriptomics analysis also increased the gene expression of enzymes in this pathway [24]. Next, intraperitoneal 400 mg/kg/day nicotinamide was administered for 4 days before IRI and 1 day after IRI, resulting in milder kidney tubular injury, kidney dysfunction and neutrophil infiltration at day 5 but unchanged macrophages. Fibrosis was not assessed. While there is a consensus that increasing kidney NAD⁺ during kidney injury is beneficial, there is a lack of consensus on the best therapeutic approach to achieve this goal [12]. Thus Piedrafita et al. [29] recently reported that intraperitoneal nicotinamide 400 mg/kg/24 h and 1 h prior to kidney IRI and 4–6 h after kidney IRI did not improve AKI and did not increase kidney NAD⁺. Most prior reports did not assess kidney NAD⁺ following nicotinamide supplementation and Zhu et al. did not assess it either.

The clinical translation of the preclinical studies was assessed in 62 young (41–46 years) patients with CKD G3–G5 estimated glomerular filtration rate (eGFR) 24–27 mL/min/1.73 m², urine albumin:creatinine ratio (UACR) 630–760 mg/g [24]. The cause of CKD and the prior use of kidney protective medication were not reported. They were randomized to either L. casei Zhang (1 × 10⁸ CFU/day) or placebo (vehicle) for 3 months. The primary endpoint was not specified. At 3 months, serum cystatin C increased by 6% in the placebo group and UACR by 27% and both were significantly different from the intervention group, which did not change, while serum creatinine was unchanged in both groups. It was then decided to extend the follow-up for up to 10 months without any further intervention, and here details become fuzzy. Cystatin C and UACR data for the extended follow-up period were not reported, while the duration of follow-up was (surprisingly) different for serum creatinine and for eGFR data. In this regard, serum creatinine increased in both groups during the longer follow-up, but significantly more in the placebo group, while (again surprisingly) the significant difference in eGFR change between the groups was mainly driven by an increase in eGFR in the L. casei Zhang group. Regarding the mechanisms of benefit, L. casei Zhang colonization of feces was demonstrated at 3 months in the intervention group, as well as differences in serum nicotinamide. However, the latter were explained by decreased nicotinamide levels in the placebo group rather than by increased levels in the L. casei Zhang group. In summary, human data are clearly exploratory and should be confirmed in a well-designed and well-reported clinical trial, which should have pre-defined primary endpoints to be assessed at predefined time points.

**WHAT’S NEXT?**

The hallmark of intestinal dysbiosis is a reduction of saccharolytic microbes that produce SCFA and, in the case of CKD, an increase in proteolytic microbes that produce different molecules possibly related to uremic toxicity. Zhu et al. [24], for the first time, uncovered the beneficial effect of L. casei Zhang in murine models and a human clinical study of kidney injury, laying the groundwork for future research about its potential role in human kidney disease. Benefit was hypothesized to depend on the production of beneficial metabolites by gut bacteria, especially SCFAs and nicotinamide, as the gut microbiota was enriched in bacteria able to provide these molecules and administration of these molecules was also beneficial. However, NAD⁺ was not directly measured in murine serum or kidney. Additionally, the hypothesis was not confirmed by assessing whether blocking the actions of SCFAs or nicotinamide prevented the
beneficial effect of *L. casei* Zhang. This highlights the need for future work to clarify the mechanism behind the observed benefit of *L. casei* Zhang supplementation.

The human CKD data were both encouraging, and surprising. They were encouraging because a relatively simple and likely safe therapeutic intervention resulted in improved kidney function, and surprising, because the small sample size would have been expected to preclude any observation of benefit on eGFR and the serum creatinine and eGFR values did not change concordantly.

Zhu et al.’s [24] intriguing findings will drive further research aimed at addressing the clinical translation of the potential health-promoting effects of *L. casei* Zhang in kidney disease (Figure 4). Given the available preclinical data, the most plausible scenario for clinical validation is *L. casei* Zhang supplementation before a programmed intervention known to result in a high incidence of AKI, such as cardiovascular surgery, cisplatin chemotherapy for cancer or patients at high risk of AKI at hospital admission [30, 31]. Also, a large-scale randomized trial is required to evaluate the clinical efficacy of *L. casei* Zhang for CKD. This new trial should overcome some of the deficiencies of the clinical study reported by Zhu et al. [24]. Additionally, the fact that kidney protection could be transmitted in mice by stool transplant and was observed in mice treated with antibiotics opens the door to trials of kidney protection in ICUs with the aim of providing herd protection. In ICUs, the widespread use of antibiotics, debilitated nature of patients and frequent use of emergency procedures favors cross-contamination with pathogens such as *Clostridium difficile* [32], implying fecal–oral transmission of microbiota between patients. The hypothesis that *L. casei* Zhang may provide herd protection from AKI in ICUs through cross-transmission between patients may be addressed by comparing ICUs from different hospitals or different ICUs in the same hospital, some of which may provide the standard of care and others the standard of care plus oral *L. casei* Zhang supplementation to all patients and personnel in the unit that agree to participate, having primary endpoints of AKI and severe AKI.

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**CONFLICT OF INTEREST STATEMENT**

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**REFERENCES**

1. Foreman KJ, Marquez N, Dolgert A et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016–40 for 195 countries and territories. Lancet 2018; 392: 2052–2090
2. Ortiz A, Sanchez-Niño MD, Crespo-Barrio M et al. The Spanish Society of Nephrology (SNEFRO) commentary to the Spain GBD 2018 report: keeping chronic kidney disease out of sight of health authorities will only magnify the problem. Nefrologia 2019; 39: 29–34
3. Fernandez-Fernandez B, Sarafidis P, Kanbay M et al. SGLT2 inhibitors for non-diabetic kidney disease: drugs to treat CKD that also improve glycaemia. Clin Kidney J 2020; 13: 728–733
4. Ortiz A, Ferro CJ, Balafa O et al. Mineralocorticoid receptor antagonists for nephroprotection and cardioprotection in patients with diabetes mellitus and chronic kidney disease. Nephrol Dial Transplant 2021; doi: 10.1093/ndt/gfab167
5. Ortiz A, Fernandez-Fernandez B. Atrasentan: the difficult task of integrating endothelin a receptor antagonists into current treatment paradigm for diabetic kidney disease. Clin J Am Soc Nephrol 2021; 16: 1775–1778
6. Rovin BH, Adler SG, Barratt J et al. Executive summary of the KDIGO 2021 guideline for the management of glomerular diseases. Kidney Int 2021; 100: 753–779
7. Carriazo S, Perez-Gomez MV, Cordido A et al. Dietary care for ADPKD patients: current status and future directions. Nutrients 2019; 11: 1576
8. Makkapati S, D’Agati VD, Balsam L. “Green smoothie cleanse” causing acute oxalate nephropathy. Am J Kidney Dis 2018; 71: 281–286
9. Fernandez-Prado R, Esteras P, Perez-Gomez MV et al. Nutrients entered into toxins: microbiota modulation of nutrient properties in chronic kidney disease. Nutrients 2017; 9: 489
10. Favero C, Carriazo S, Cuarental L et al. Phosphate, microbiota and CKD. Nutrients 2021; 13: 1273
11. Tang WHW, Wang Z, Kennedy DJ et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. Circ Res 2015; 116: 448–455
12. Fontecha-Barriuso M, Lopez-Diaz AM, Carriazo S et al. Nicotinamide and acute kidney injury. Clin Kidney J 2021; 14: 2453–2462
13. Poveda J, Sanchez-Niño MD, Glorieux G et al. P-Cresyl sulphate has pro-inflammatory and cytotoxic actions on human proximal tubular epithelial cells. Nephrol Dial Transplant 2014; 29: 56–64
14. Vanholder R, Glorieux G. The intestine and the kidneys: a bad marriage can be hazardous. Clin Kidney J 2015; 8: 168–179
15. Aguilera-Correa J-J, Madrazo-Clemente P, Martinez-Cuesta MDC et al. Lyso-Gb3 modulates the gut microbiota and decreases butyrate production. Sci Rep 2019; 9: 12010
16. Sanchez-Niño MD, Carpio D, Sanz AB et al. Lyso-Gb3 activates Notch1 in human podocytes. Hum Mol Genet 2015; 24: 5720–5732
17. Castillo-Rodriguez E, Fernandez-Prado R, Esteras R et al. Impact of altered intestinal microbiota on chronic kidney disease progression. Toxins 2018; 10: 300
18. Wikipedia. Kumis. https://es.wikipedia.org/wiki/Kumis (5 February 2022, date last accessed)
19. Ya T, Zhang Q, Chu F et al. Immunological evaluation of Lactobacillus casei Zhang: a newly isolated strain from koumiss in Inner Mongolia, China. BMC Immunol 2008; 9: 68
20. Zhang W, Yu D, Sun Z et al. Complete genome sequence of Lactobacillus casei Zhang, a new probiotic strain isolated from traditional homemade koumiss in Inner Mongolia, China. J Bacteriol 2010; 192: 5268–5269
21. Moreno JA, Izquierdo MC, Sanchez-Niño MD et al. The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFκB. J Am Soc Nephrol 2011; 22: 1315–1325
22. Martin-Sanchez D, Fontecha-Barriuso M, Carrasco S et al. TWEAK and RIPK1 mediate a second wave of cell death during AKI. Proc Natl Acad Sci 2018; 115: 4182–4187
23. Navarro-González JF, Sanchez-Nino MD, Donate-Correa J et al. Effects of pentoxifylline on soluble klotho concentrations and renal tubular cell expression in diabetic kidney disease. Diabetes Care 2018; 41: 1817–1820
24. Zhu H, Cao C, Wu Z et al. The probiotic L. casei Zhang slows the progression of acute and chronic kidney disease. Cell Metab 2021; 33: 1926–1942.e8
25. Andrade-Oliveira V, Amano MT, Correa-Costa M et al. Gut bacteria products prevent AKI induced by ischemia-reperfusion. J Am Soc Nephrol 2015; 26: 1877–1888
26. Liu Y, Li YJ, LohYW et al. Fiber derived microbial metabolites prevent acute kidney injury through G-protein coupled receptors and HDAC inhibition. Front Cell Dev Biol 2021; 9: 648639
27. Kimura I, Miyamoto J, Ohue-Kitano R et al. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. Science 2020; 367: eaaw8429
28. Sanchez-Niño MD, Aguilera-Correa JJ, Politei J et al. Unraveling the drivers and consequences of gut microbiota disruption in Fabry disease: the lyso-Gb3 link. Future Microbiol 2020; 15: 227–231
29. Piedrafita A, Balayssac S, Mayeur N et al. The tryptophan pathway and nicotinamide supplementation in ischaemic acute kidney injury. Clin Kidney J 2021; 14: 2490–2496
30. Martin-Cleary C, Molinero-Casares LM, Ortiz A et al. Development and internal validation of a prediction model for hospital-acquired acute kidney injury. Clin Kidney J 2021; 14: 309–316
31. Del Carpio J, Marco MP, Martin ML et al. External validation of the Madrid Acute Kidney Injury Prediction Score. Clin Kidney J 2021; 14: 2377–2382
32. Lessa FC, Mu Y, Bamberg WM et al. Burden of Clostridium difficile infection in the United States. N Engl J Med 2015; 372: 825–834