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Editorial Comment

EDITORIAL COMMENT

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

DIET, MICROBIOTA AND CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is set to become the fifth global cause of death by 2040 and the second cause of death by the end of the century in some countries with long life expectancy [1, 2]. Recently, sodium-glucose cotransporter 2 (SGLT2) inhibitors and the mineralocorticoid receptor blocker finerenone were shown to slow the progression of CKD, offering further hope for the future [3, 4]. However, the residual renal risk remains high, especially for more advanced stages of CKD [5]. Additional interventions that lower even more the risk of CKD progression and the associated risk of accelerated aging are needed. Diet has long been known to influence the course of CKD and may even result in acute kidney injury (AKI). Dietary components such as excess sodium, protein, phosphate or oxalate may accelerate the course of CKD and some (e.g. oxalate) may precipitate AKI [6–8]. More recently, awareness has emerged that the diet feeds both the human body and its gut microbiota. Thus diet may influence kidney disease not only through a direct impact of specific nutrients on the human body, but also through modulation of the gut microbiota composition or through metabolites generated by the gut microbiota from ingested nutrients [9, 10] (Figure 1). Among several examples, dietary choline or L-carnitine may be transformed into trimethylamine (TMA) by the gut microbiota, which is absorbed and metabolized to trimethylamine *N*-oxide (TMAO) in the liver. Gut microbiota-dependent TMAO may contribute to both CKD progression and mortality risk in CKD and may account partially for risks associated with red meat [11]. In contrast, dietary tryptophan is a precursor of nephroprotective molecules such as nicotinamide adenine dinucleotide (NAD^+) but can also be metabolized by the gut microbiota to indole, a precursor of uremic toxins with nephrotoxic potential such as indoxyl sulfate [12], while dietary tyrosine is

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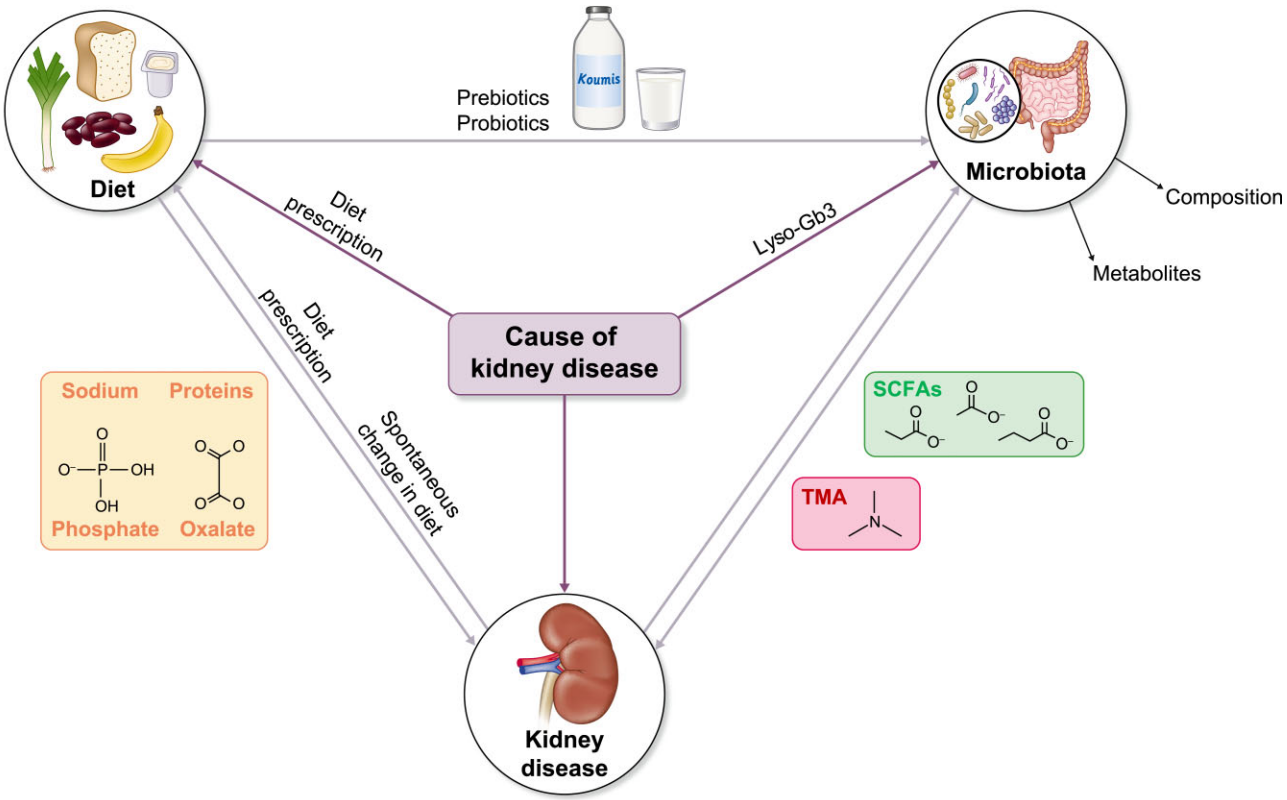

FIGURE 1: Interactions between diet, the microbiota and kidney disease. Dietary components are frequently modified by the gut microbiota, which in turn changes in response to the availability of specific dietary components. Thus any interaction between diet and kidney disease cannot be properly understood without understanding the impact of the diet on the gut microbiota. Specific components of the diet can directly influence the course of kidney disease. Kidney disease, in turn, may lead to spontaneous changes of the diet (e.g. spontaneous decrease in protein ingestion as glomerular filtration decreases) or to prescription of kidney disease–adapted diets. However, the diet may contain or lack prebiotics or probiotics that 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 IG 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).

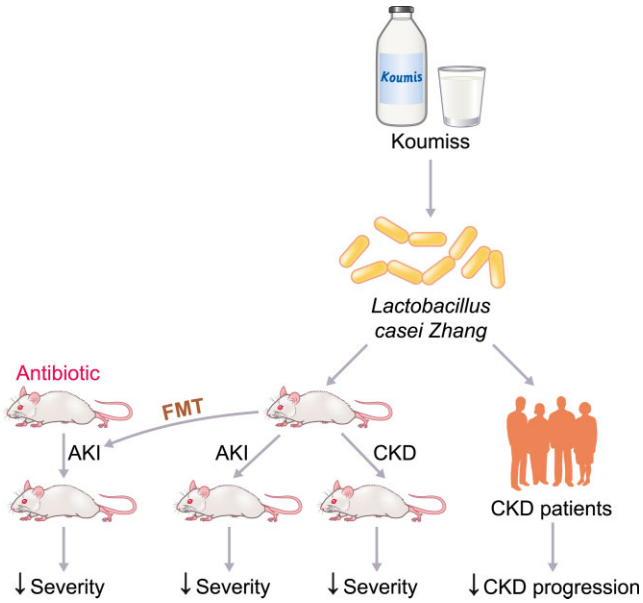

FIGURE 2: Kidney protection by *L. casei* Zhang was observed in experimental AKI and CKD as well as for human CKD. Kidney protection by *L. casei* Zhang was transmissible through fecal microbiota transplantation (FMT).

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

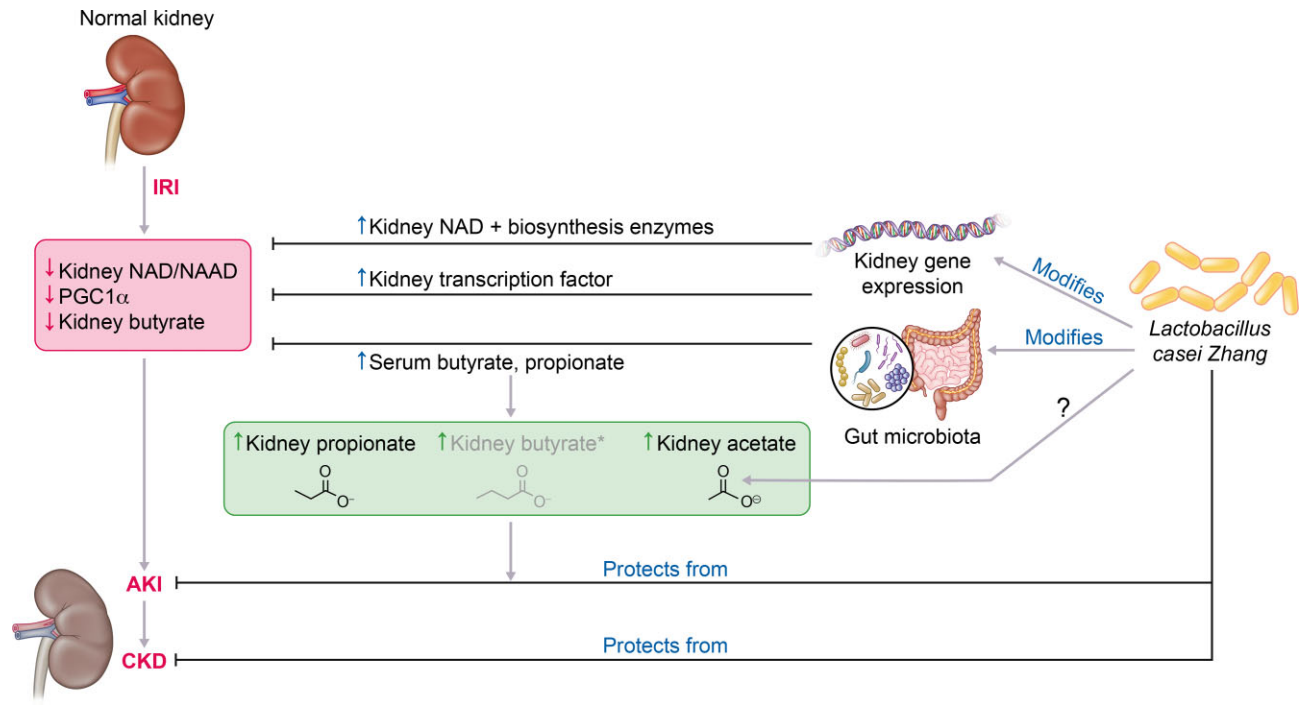

FIGURE 3: Molecular mechanisms of kidney protection by *L. casei* Zhang. *L. casei* Zhang administration to mice with kidney IRI increased the expression of enzymes related to NAD^+ biosynthesis and the expression of *Ppargc1a*, the gene encoding for the master regulator of mitochondrial biogenesis and nephroprotective molecule PGC1α. *L. casei* Zhang modified the gut microbiota, increasing serum SCFA (butyrate, propionate, acetate) and consequently increasing kidney propionate. Additionally, there was a trend toward increased kidney butyrate* and an increase in kidney acetate not explained by higher serum acetate levels. Overall, gut microbiota SCFA may represent the molecular link between *L. casei* Zhang and protection from AKI and CKD. *There was a tendency towards an increase in kidney butyrate in the IRI group with *L. casei* Zhang compared with the group that did not receive probiotics. However, the difference was not significant.

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 m^2, 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 (1×10^9 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 is (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.

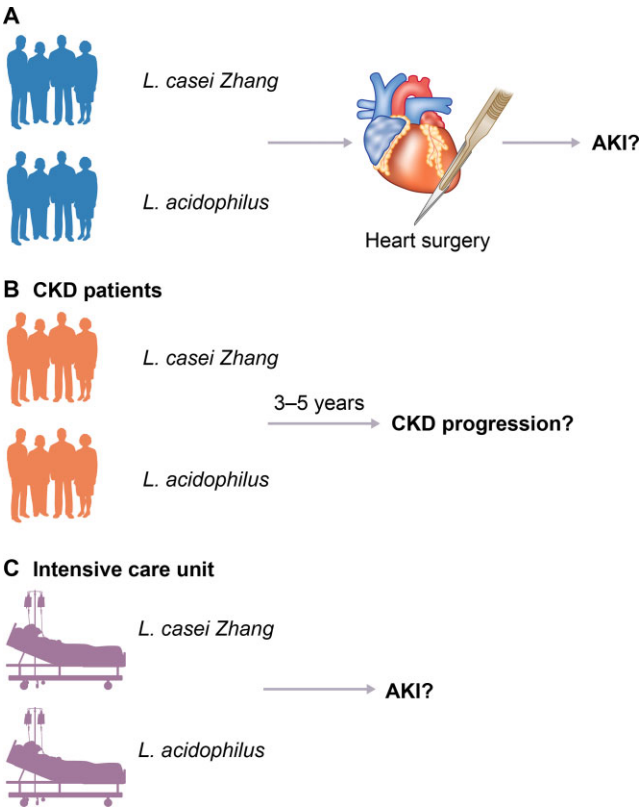

FIGURE 4: Clinical research roadmap for *L. casei* Zhang kidney protection. Based on preclinical studies, three settings may be used to probe the clinical translation of kidney protection by *L. casei* Zhang. **(A)** Prevention of AKI in high-risk settings. Individuals at high risk of AKI may be randomized to *L. casei* Zhang administration or control. **(B)** Prevention of CKD progression. **(C)** 'Herd' kidney protection in ICUs. ICUs will be randomized to administration of *L. casei* Zhang or control to all new admissions and healthcare personal who agree to participate. This would be expected to result in 'cross-contamination' with kidney-protective bacteria on top of the ongoing cross-contamination with microbes such as *C. difficile* and antibiotic-resistant bacteria.

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 SCFA 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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