Nephrol Dial Transplant (2021) 36: 1357–1360
doi: 10.1093/ndt/gfab033
Advance Access publication 12 February 2021

A call for a better understanding of the role of dietary amino acids and post-translational protein modifications of the microbiome in the progression of CKD

Laetitia Koppe[1,2], Srinivasan Beddhu[3,4], Philippe Chauveau [5], Csaba P. Kovesdy[6], Denise Mafra [7], Shivam Joshi[8,9], Kamyar Kalantar-Zadeh [9] and Denis Fouque [1,2]

[1]Department of Nephrology, Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, Pierre-Bénite, F-69495, France, [2]University of Lyon, CarMeN lab, INSA-Lyon, INSERM U1060, INRA, Université Claude Bernard Lyon 1, F-69621 Villeurbanne, France, [3]Division of Nephrology and Hypertension, University of Utah School of Medicine, Salt Lake City, UT, USA, [4]Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA, [5]Association Pour l'Utilisation Du Rein Artificiel A Domicile, F-33110 Gradignan, France, [6]Division of Nephrology, University of Tennessee Health Science Center, Memphis, TN, USA, [7]Federal University Fluminense, Niteroi, Rio de Janeiro, Brazil, [8]Department of Medicine, New York University School of Medicine, New York, NY, USA, [9]Harold Simmons Center for Kidney Disease Research and Epidemiology, Division of Nephrology and Hypertension, University of California, Irvine, School of Medicine, Orange, CA, USA

Correspondence to: Laetitia Koppe; E-mail: laetitia.koppe@chu-lyon.fr

Randomized clinical trials and systematic reviews strongly support a beneficial role of a low protein diet (LPD) in slowing kidney disease progression and to mitigate uraemic symptoms in patients with chronic kidney disease (CKD) Stages 3B–5 [1–3]. Hence, the 2020 Kidney Disease Outcome Quality Initiative (KDOQI) Clinical Practice Guideline for Nutrition in CKD recommended an LPD to patients with CKD Stages 3B–5 as evidence grade level 1 A evidence [4].

However, apart from the amount of total protein, dietary source (plant versus animal) might also have an impact on uraemic toxic production and CKD progression [5]. The costs of dietetic counselling [6], social constraints and the potential for protein-energy wasting are commonly cited concerns regarding increasing plant protein and decreasing animal protein intake. In addition, lack of clear understanding of the LPD mechanisms on CKD progression is also presumably another brake on its implementation.

Among the potential pathways involved during the reduction in protein intake, the lower intake of nitrogenous compounds leading to less production of uraemic toxins has been particularly explored [7]. A number of investigations, especially in animals, have highlighted the key role of intestinal microbiota in the production of uraemic toxins from amino acids precursors such as *p*-cresyl sulphate (PCS) (from tyrosine), indoxyl sulphate (IS) (from tryptophan) and trimethylamine *N*-oxide (TMAO) (from leucine) [7, 8]. The discovery of the 'intestine–kidney axis' and the impact of diet in the modulation of the intestinal microbiota open up innovative research perspectives in the field of nutrition. The historical concept of globally decreasing amino acids (i.e. LPD) to limit uraemic syndrome can be challenged by understanding the role of each amino acid in the production of uraemic toxins, since gut dysbiosis is a common feature of CKD. Therefore, recent studies have focused more on the quality and diversity of proteins rather than simply their quantity for the prevention and management of CKD. The first approach focuses on the source of proteins. The risk and benefits of different sources of proteins have been widely demonstrated in numerous studies, meta-analyses and systematic reviews in normal health [9, 10]. Plant-based or plant-dominant diets have been associated with a delay in dialysis initiation [1, 2], but large randomized controlled trials are warranted to confirm the efficacy and safety of this diet strategy [11]. In contrast, higher consumption of animal-based proteins has been linked to an increased risk of Type 2 diabetes, cardiovascular disease and mortality [9, 10]. A high amount of red meat in the diet has been shown to increase leucine intake and production of deleterious uraemic toxins such as TMAO [12]. In contrast, an LPD (with low red meat) prescribed for 6 months was associated with reduction in the PCS levels in non-dialysis patients with CKD [13]. A short-term (12-week) randomized crossover trial reported that patients with CKD who followed a very-low, vegetarian protein diet exhibited a decrease in major uraemic toxins such as PCS and IS, and experienced a modification in intestinal microbiota composition [3, 14]. Indeed, the increased intake of fibers in plant-based diets allows for a modification of colic microbiota towards bacteria that decrease inflammation, bacterial translocation and the production of gut-derived uraemic toxins [15–19].

These encouraging data support the hypothesis that in CKD, high-protein intake and particularly animal proteins are deleterious to intestinal microbiota composition and may be key players in the progression of CKD. However, this has been challenged by a recent publication in *Science* [20] Lobel *et al.* [20] showed that some dietary-specific amino acids (e.g.

methionine and cysteine) can improve the uraemic syndrome and its complications in an animal model. Indeed, they observed that sulphide from bacterial metabolism of dietary sulphur-containing amino acids (SAAS, mainly methionine and cysteine) can regulate *Escherichia coli* indole, ammonia and urea production. This occurs through an inhibition of tryptophanase by S-sulphydration, a post-translational protein modification (PTM) of the cysteine residues presents in this enzyme. Methionine and cysteine are found in highest concentrations in animal proteins (e.g. muscle, organ meat and eggs), implying that red meat could protect from CKD complications. However, this study must be interpreted carefully. First, SAAS are also present but at lower levels in dairy (e.g. casein), cereal grains and pulses (e.g. legumes). Secondly, the authors only focused on one uraemic toxin of the indol group and not on other important ones such as PCS or TMAO. Indeed, it has been shown that proteins in meat and eggs are precursors of TMAO [12]. Thirdly, the transposition of animal studies to human trials must be done, even if authors have reported an increase of *E. coli* in the feces of CKD patients [20].

The Lobel *et al.* study should not be used to once again discredit LPD prescription, but on the contrary, as a call for understanding the role of each amino acid in uraemic conditions. The ambivalent role of each amino acid is not novel and has been described for half a century [21]. Early mechanistic studies suggested that not all proteins or amino acids equally induce glomerular hyperfiltration, and sometimes displayed seemingly opposite effects. For example, branched-chain amino acids (BCAAs) infusion caused a moderate renal vasoconstriction and a slight increase in glomerular filtration rate (GFR), whereas an infusion of a mixture including all amino acids induced a significantly higher increase in GFR [22]. In 5/6 nephrectomy rats, diets containing 8% casein supplemented with 10% of a mix of BCAAs increased kidney fibrosis compared with a diet with 10% aromatic amino acids (AAAs), which stimulated renal plasma flow and to a lesser extent GFR [23]. Also, preliminary data suggest that reduction of specific AAAs in CKD mice decreases PCS and IS production [24]. The role of SAAS on renal function must also be clarified. On one hand, Lobel *et al.* and others reported that a high dietary SAAS intake could delay CKD progression in patients and experimental disease models [25, 26]. On the other hand, high SAAS consumption has been associated with an increased cardiovascular risk [27] and reduced kidney function in the general population, which are undesired outcomes in CKD management [28].

The second interesting point of the Lobel *et al.* study is to highlight the importance of PTMs in causing CKD complications [20]. PTMs such as carbamylation, glycation, sulphatation and oxidation of proteins and peptides have recently emerged as mechanisms explaining the accelerated ageing of CKD patients [29]. New studies have demonstrated that epigenetic events are highly dynamic, changing in response to nutrient availability, and can be a cross-talk between microbiota and host metabolism. Lobel *et al.* demonstrated for the first time the direct role of methionine and cysteine by modifying intestinal microbiota function in CKD. The role of diet and in particular proteins in the induction of PTMs deserves to be explored in uraemia [30]. Indeed, methionine could influence methylation by providing methyl groups. These epigenetic alterations, for instance DNA methylation, could potentially provide explanations for altered gene expressions in CKD and in bacterial metabolism [31, 32]. The nitrogenous waste content resulting from protein intake is degraded into urea, which since it is not excreted in the urine, is slowly metabolized into cyanate [33]. Urea can additionally contribute to PTMs of proteins via increased O-glycosylation and could potentially modify bacterial activity [34]. For example, increased O-glycosylation of mucins strongly and negatively affects the mucus layer cohesive properties in the gut lumen [35]. However, the consequence of O-glycosylation of key enzymes in gut microbiota and functional consequences in CKD is unknown. Cyanate, an electrophile, attacks nucleophilic groups such as amino groups in proteins, and these results in carbamylation, which is associated with an increased mortality in CKD patients [36]. Carbamylation can alter the gut microbiome and can favour expansion of bacterial families that produce uraemic toxins during CKD [37]. Finally, PTMs must be interpreted according to their exposition time since their functional impact may be different. For instance, much of our understanding of the role of O-glycosylation on cellular function is based on chronic disease processes. However, there is increasing evidence that O-glycosylation occurs in response to stress and that an acute increase of this biological event is cytoprotective, at least in the short term [34].

The third point that Lobel *et al.* highlighted is the behaviour of intestinal microbiota. Several studies have reported that the composition of gut microbiota is modified and its diversity is reduced during CKD, and could contribute to CKD progression [38]. The cause of dysbiosis in unhealthy individuals across age and geography has mainly been correlated with dietary habits but is still debated in the context of CKD. On one hand, an LPD or a Mediterranean diet could mitigate dysbiosis [3, 14] in patients with CKD. Poesen *et al.* also observed that if patients with CKD have a distinct colonic microbial metabolism, the effect of renal function loss *per se* may be inferior to the effects of diet and other CKD-related factors [39]. On the other hand, in a large study of more than 200 haemodialysis patients, a multivariate analysis did not observe a significant impact of food categories on the metabolome and intestinal microbiome. These discordant results can be explained by the technical level of analysis of the gut microbiota. Until now, the intestinal microbiota has been analysed by 16S ribosomal RNA gene amplification or by a metagenomic approach. Lobel *et al.* did not observe a difference in the taxonomic abundance of the gut microbiota in mice between the low- and high-SAAS diets. The only difference was an increase of S-sulphydration proteins. These results call for additional metaproteomic approaches focusing on the characterization of expressed proteins alteration like PTMs in order to provide a better understanding of the microbial species.

In conclusion, there is a growing scientific interest in better analysing the role of individual amino acids and their interaction with the intestinal microbiota and the host during CKD. This will reinforce the role of a dietary approach to better control uraemia. The historical and empirical approach through a global reduction in protein intake can only be

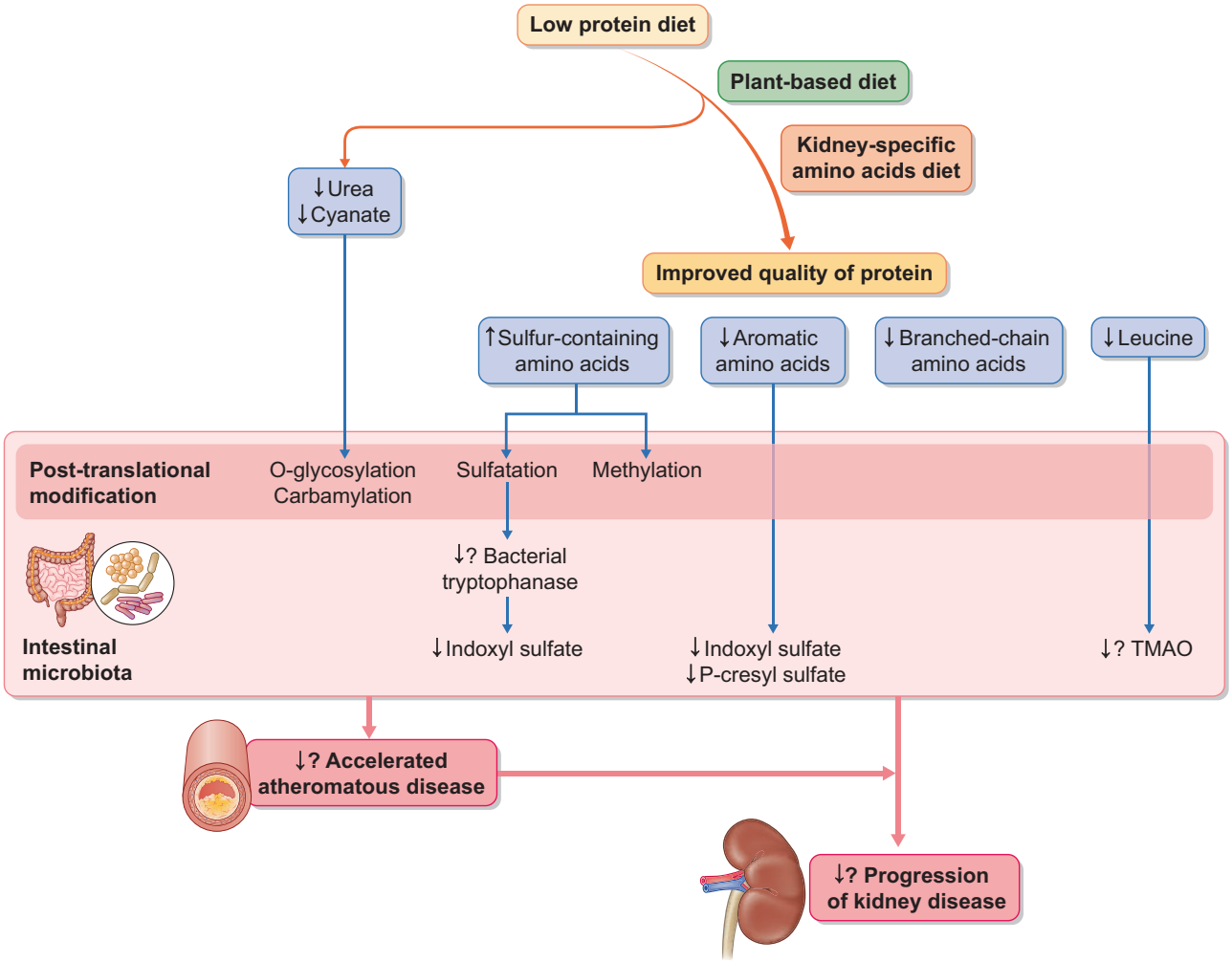

FIGURE 1: From LPD to a specific amino acid diet during CKD. Nutrition by different strategies can decrease uraemic toxins production and posttranslational modification of protein. All of this contributes to reduce cardiovascular complications and CKD progression.

reinforced by new studies confirming how to modulate amino acids intake. This should be viewed as a continuum in dietary strategies (Figure 1). Finally, gaining knowledge on the 'microbiota–nutrient metabolism–epigenetics–kidney' axis could facilitate the stratification of patients towards a personalized-diet care to more efficiently slow down CKD progression. So far, this call stands at the research level, and meanwhile we still have LPDs to help patients (KDOQI 2020) while awaiting additional data [4].

FUNDING

This study was supported by Hospices Civils de Lyon and University of Lyon.

CONFLICT OF INTEREST STATEMENT

All the authors have no conflict of interest to disclose.

REFERENCES

1. Hahn D, Hodson EM, Fouque D. Low protein diets for non-diabetic adults with chronic kidney disease. *Cochrane Database Syst Rev* 2018; 10: CD001892
2. Garneata L, Stancu A, Dragomir D *et al.* Ketoanalogue-supplemented vegetarian very low-protein diet and CKD progression. *J Am Soc Nephrol* 2016; 27: 2164–2176
3. Di IB, Rocchetti MT, De AM *et al.* Nutritional therapy modulates intestinal microbiota and reduces serum levels of total and free indoxyl sulfate and P-cresyl sulfate in chronic kidney disease (Medika Study). *J Clin Med* 2019; 8: 1424
4. Ikizler TA, Burrowes JD, Byham-Gray LD *et al.* KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis* 2020; 76: S1–S107
5. Kalantar-Zadeh K, Joshi S, Schlueter R *et al.* Plant-dominant low-protein diet for conservative management of chronic kidney disease. *Nutrients* 2020; 12: 1931
6. Kalantar-Zadeh K, Mattix-Kramer HJ, Moore LW. Culinary medicine as a core component of the medical nutrition therapy for kidney health and disease. *J Ren Nutr* 2021; 31: 1–4
7. Koppe L, Fouque D, Soulage CO. The role of gut microbiota and diet on uremic retention solutes production in the context of chronic kidney disease. *Toxins* 2018; 10: 155
8. Gryp T, De Paepe K, Vanholder R *et al.* Gut microbiota generation of protein-bound uremic toxins and related metabolites is not altered at different stages of chronic kidney disease. *Kidney Int* 2020; 97: 1230–1242
9. Song M, Fung TT, Hu FB *et al.* Association of animal and plant protein intake with all-cause and cause-specific mortality. *JAMA Intern Med* 2016; 176: 1453–1463

10. Budhathoki S, Sawada N, Iwasaki M et al.; for the Japan Public Health Center–based Prospective Study Group. Association of animal and plant protein intake with all-cause and cause-specific mortality in a Japanese cohort. *JAMA Intern Med* 2019; 179: 1509–1518
11. Carrero JJ, González-Ortiz A, Avesani CM *et al.* Plant-based diets to manage the risks and complications of chronic kidney disease. *Nat Rev Nephrol* 2020; 16: 525–542
12. Koeth RA, Lam-Galvez BR, Kirsop J *et al.* l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *J Clin Invest* 2018; 129: 373–387
13. Black AP, Anjos JS, Cardozo L *et al.* Does low-protein diet influence the uremic toxin serum levels from the gut microbiota in nondialysis chronic kidney disease patients? *J Ren Nutr* 2018; 28: 208–214
14. Di Iorio BR, Marzocco S, Bellasi A *et al.* Nutritional therapy reduces protein carbamylation through urea lowering in chronic kidney disease. *Nephrol Dial Transplant* 2018; 33: 804–813
15. Sirich TL, Plummer NS, Gardner CD *et al.* Effect of increasing dietary fiber on plasma levels of colon-derived solutes in hemodialysis patients. *Clin J Am Soc Nephrol* 2014; 9: 1603–1610
16. Xie L-M, Ge Y-Y, Huang X *et al.* Effects of fermentable dietary fiber supplementation on oxidative and inflammatory status in hemodialysis patients. *Int J Clin Exp Med* 2015; 8: 1363–1369
17. Xu H, Rossi M, Campbell KL *et al.* Excess protein intake relative to fiber and cardiovascular events in elderly men with chronic kidney disease. *Nutr Metab Cardiovasc Dis* 2016; 26: 597–602
18. Rossi M, Johnson DW, Xu H *et al.* Dietary protein-fiber ratio associates with circulating levels of indoxyl sulfate and p-cresyl sulfate in chronic kidney disease patients. *Nutr Metab Cardiovasc Dis* 2015; 25: 860–865
19. Wu GD, Chen J, Hoffmann C *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; 334: 105–108
20. Lobel L, Cao YG, Fenn K *et al.* Diet posttranslationally modifies the mouse gut microbial proteome to modulate renal function. *Science* 2020; 369: 1518–1524
21. Lee KE, Summerill RA. Glomerular filtration rate following administration of individual amino acids in conscious dogs. *Exp Physiol* 1982; 67: 459–465
22. Claris-Appiani A, Assael BM, Tirelli AS *et al.* Lack of glomerular hemodynamic stimulation after infusion of branched-chain amino acids. *Kidney Int* 1988; 33: 91–94
23. Pillai SM, Herzog B, Seebeck P *et al.* Differential impact of dietary branched chain and aromatic amino acids on chronic kidney disease progression in rats. *Front Physiol* 2019; 10: 1460
24. Barba C, Soulage C, Glorieux G *et al.* P0922A low aromatic amino-acid diet improves renal function and prevents kidney fibrosis in mice with chronic kidney disease. *Nephrol Dial Transplant* 2020; 35
25. Thielemann LE, Oberhauser EW, Rosenblut G *et al.* Sulfur-containing amino acids that increase renal glutathione protect the kidney against papillary necrosis induced by 2-bromoethylamine. *Cell Biochem Funct* 1990; 8: 19–24
26. Trachtman H, Sturman JA. Taurine: A therapeutic agent in experimental kidney disease. *Amino Acids* 1996; 11: 1–13
27. Dong Z, Gao X, Chinchilli VM *et al.* Association of sulfur amino acid consumption with cardiometabolic risk factors: Cross-sectional findings from NHANES III. *EClinicalMedicine* 2020; 19: 100248
28. Banerjee T, Tucker K, Griswold M *et al.* Dietary potential renal acid load and risk of albuminuria and reduced kidney function in the Jackson Heart Study. *J Ren Nutr* 2018; 28: 251–258
29. Gajjala PR, Fliser D, Speer T *et al.* Emerging role of post-translational modifications in chronic kidney disease and cardiovascular disease. *Nephrol Dial Transplant* 2015; 30: 1814–1824
30. Chattopadhyay T, Maniyadath B, Bagul HP *et al.* Spatiotemporal gating of SIRT1 functions by O-GlcNAcylation is essential for liver metabolic switching and prevents hyperglycemia. *Proc Natl Acad Sci USA* 2020; 117: 6890–6900
31. Mafra D, Borges NA, Lindholm B *et al.* Food as medicine: Targeting the uraemic phenotype in chronic kidney disease. *Nat Rev Nephrol* 2020; doi: 10.1038/s41581-020-00345-8
32. Fellows R, Denizot J, Stellato C *et al.* Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. *Nat Commun* 2018; 9: 105
33. Poesen R, Mutsaers HAM, Windey K *et al.* The influence of dietary protein intake on mammalian tryptophan and phenolic metabolites. *Plos One* 2015; 10: e0140820
34. Koppe L, Nyam E, Vivot K *et al.* Urea impairs β cell glycolysis and insulin secretion in chronic kidney disease. *J Clin Invest* 2016; 126: 3598–3612
35. Desai MS, Seekatz AM, Koropatkin NM *et al.* A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016; 167: 1339–1353
36. Koeth RA, Kalantar-Zadeh K, Wang Z *et al.* Protein carbamylation predicts mortality in ESRD. *J Am Soc Nephrol* 2013; 24: 853–861
37. Wang F, Jiang H, Shi K *et al.* Gut bacterial translocation is associated with microinflammation in end-stage renal disease patients. *Nephrology (Carlton)* 2012; 17: 733–738
38. Wang X, Yang S, Li S *et al.* Aberrant gut microbiota alters host metabolome and impacts renal failure in humans and rodents. *Gut* 2020; 69: 2131–2142
39. Poesen R, Windey K, Neven E *et al.* The influence of CKD on colonic microbial metabolism. *J Am Soc Nephrol* 2016; 27: 1389–1399

Received: 19.1.2021; Editorial decision: 25.1.2021