Dietary protein-fiber ratio associates with circulating levels of indoxyl sulfate and p-cresyl sulfate in chronic kidney disease patients

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Title page

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Dietary protein-fiber ratio associates with circulating levels of indoxyl sulphate and p-cresyl sulphate in chronic kidney disease patients

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Abstract

Background and aims: Indoxyl sulphate (IS) and p-cresyl sulphate (PCS) are uremic toxins derived solely from colonic bacterial fermentation of protein. Dietary fiber may counteract this by limiting proteolytic bacterial fermentation**.** However, the influence of dietary intake on the generation of IS and PCS has not been adequately explored in chronic kidney disease (CKD).

Methods and results: This cross-sectional study included 40 CKD participants (60% male; age 69 ±10 years; 45% diabetic) with a mean estimated glomerular filtration rate (eGFR) of 24 ±8mL/min/1.73 m^2, who enrolled in a randomized controlled trial of synbiotic therapy. Total and free serum IS and PCS were measured at baseline by ultra-performance liquid chromatography. Dietary intake was measured using in-depth diet histories collected by a dietitian. Associations between each toxin, dietary fiber (total, soluble and insoluble), dietary protein (total, and amino acids: tryptophan, tyrosine and phenylalanine), and the protein-fiber index (ratio of protein to fiber) were assessed using linear regression.

Dietary fiber was associated with free and total serum PCS ($r=-0.42$ and $r=-0.44$, both $p<0.01$), but not IS. No significant association was observed between dietary protein and either toxin. The protein-fiber index was associated with total serum IS ($r=0.40$, $p=0.012$) and PCS ($r=0.43$, $p=0.005$), independent of eGFR, sex and diabetes.

Conclusion: Dietary protein-fiber index is associated with serum IS and PCS levels. Such association, beyond fiber and protein alone, highlights the importance of the interplay between these nutrients. We speculate that dietary modification towards a lower protein-fiber index may contribute to lowering IS and PCS.

Introduction

Dietary management targeting modifiable risk factors is a cornerstone of chronic kidney disease (CKD) management. The conventional approach to dietary management in CKD, encompassing malnutrition and fluid, electrolyte and mineral balance is, however, expanding to encompass the importance of gut health. The rationale underpinning this dietary management in CKD stems from the emerging role of the gut bacterial community, termed the gut microbiota, as an important risk factor in CKD.[1]

CKD patients have a dysbiotic gut microbiota,[2] which tends to favour a higher ratio of proteolytic (protein) to saccarolytic (carbohydrate) bacterial fermentation[3]. This is owing to a number of renal specific factors including medications, urea influx, dietary restrictions, uremic pancreatopathy, high prevalence of gastroparesis, etc.[4] Furthermore, this dysbiotic gut microbiota is thought to play a role in the cardio-renal syndrome through its generation of uremic toxins.[5] Two gut-derived uremic toxins in particular, indoxyl sulphate (IS) and p-cresyl sulphate (PCS), have been extensively studied over the past decade with strong biological support for their nephrovascular toxicity.[6] Both IS and PCS are by-products of dietary protein bacterial fermentation in the colon. More specifically, certain bacterial families known to be more dominant in CKD, including Clostridiaceae and Enterobacteriaceae[3], possess the enzymatic capacity to degrade amino acids, (tryptophan, tyrosine and phenylalanine), into the precursors of IS and PCS, respectively[7]. Therefore, it appears that the quality of the diet impacts on the generation of IS and PCS both directly (through dietary protein substrate) and indirectly (through its role in shaping the colonic bacterial profile).

There have been a number of studies, particularly in the healthy population, demonstrating the impact of diet on the generation of IS and PCS.[8, 9] Furthermore, a significant difference in toxin generation rates between omnivores and vegetarians has been demonstrated in the healthy population, likely owing to their different protein and fiber profiles (ie. higher protein:fiber ratio in omnivores).[10] However, this study found no association between the toxins and individual nutrients. Moreover, a recent randomised placebo-controlled trial in the dialysis population found no significant difference between fiber supplements and placebo on total PCS serum concentrations.[11] Yet another fiber intervention study, of similar duration and fiber dose, achieved significant reductions in serum total PCS levels.[12] We speculate that the balance between protein and fiber intake may explain some of the differences in the results from these fiber studies and may be more important than the component nutrients.

The aim of this study was to explore the association between the dietary protein, fiber and their ratio (protein-fiber index), and the serum concentrations of IS and PCS in individuals with CKD.

Methods

Study population

In this cross-sectional observational study, baseline data were analysed in all participants with stages 4-5 CKD enrolled in a randomised cross-over trial of synbiotic therapy at a single tertiary centre's renal outpatient department between May and December 2013.[13] Inclusion into the trial required an estimated glomerular filtration rate (eGFR) between 10-30 ml/min/1.73m^2 and aged ≥18 years. Patients were excluded if they met any of the following criteria: previous renal

transplant; current or prior radiation to the bowel or large bowel resection; consumed pre- or probiotics or antibiotic therapy within 1 month of study commencement; medically diagnosed irritable bowel syndrome, Crohn's disease or ulcerative colitis; non-English speaking or unable to give informed consent; likely to receive a transplant or progress to dialysis within 6-months, as determined by treating physician; severely malnourished (Subjective Global Assessment: C); or having had a clinically significant change to their immunosuppressant dose within 6-months (determined by the medical team).

All patients provided written consent and the study was approved by the institution's Human Research Ethics Committee and registered on the Australian New Zealand Clinical Trials Registry, ACTRN12613000493741.

Outcome measures

Venous blood was collected from all patients following an overnight fast and stored at -80°C. Serum total and free concentrations of both uremic toxins, IS and PCS, were analysed by ultra-performance liquid chromatography (UPLC) using a fluorescence detection method (Waters Corporation, Milford, MA, USA).[14] Samples were run in duplicates and the coefficient of variation (CV) for the assays ranged from 1.8 to 2.9%.

Serum creatinine was measured using automated laboratory techniques. Renal function was estimated using eGFR calculated from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[15]

Participants' dietary intake was assessed using an open-ended, structured diet history method. In order to limit recall bias, a self-administered diet history was used based on a template completed prior to the interview,[16] and verified by a single dietitian

in a face-to-face interview. Food models were utilized to increase the accuracy of estimated portions, particularly protein and fiber sources. This method is considered appropriate for capturing usual intake over a one month period.[17] Dietary data were entered into Food Works 7 (Xyris Software, version 7.0.2915) using the Australian Food, Supplement and Nutrient database (AUSNUT) 2007 (for key macronutrients) and NZ Foodfiles 2010 (to quantify fiber types and amino acids, specifically tryptophan, tyrosine and phenylalanine, not available in AUSNUT). The protein-fiber index was calculated by dividing total protein intake (grams) by total fiber intake (grams).

Statistical analysis

Summary statistics for patients' characteristics were expressed as mean (standard deviation) for normally distributed continuous data, median (inter-quartile range [IQR]) for skewed continuous data and frequencies (percentages) for categorical data. All continuous variables were assessed for normality and transformed as appropriate. Correlations between the uremic toxins and selected clinical and biochemical variables were assessed using Pearson's correlation coefficients. Multivariable linear regression was performed to identify independent associations between dietary fiber, protein, the protein-fiber index and toxins after adjustment for other known predictors of toxins including kidney function (eGFR), gender and diabetes.[18, 19] The likelihood ratio test was used to compare regression models and the Steiger's formula[20] was used to compare the strength of related correlation coefficients (r^2). The null hypothesis was rejected at the 0.05 level. All of the statistical analyses were performed using Stata (version 12, 2012, Statacorp, College Station, TX).

Results

Patient characteristics

The 40 participants had an average age of 69±10 years and a mean eGFR of 24 ±8 ml/min/1.73m^2. The leading cause of CKD was diabetes followed by hypertension, similar to the prevalence reported in other developed countries.[21] Participant baseline demographics, dietary intake and biochemical characteristics are outlined in Table 1.

Uremic toxins and diet

Total dietary fiber was significantly associated with free and total serum PCS (r=-0.42 and r=-0.44, both p<0.01), but not IS. No significant association was observed between total protein intake and either toxin (all p>0.05). Similar associations were reflected with the soluble, insoluble and amino acids of interest outlined in Table 2. The protein-fiber index was significantly correlated with both IS and PCS (Figure 1).The protein-fiber index explained more variance in PCS levels compared to fiber alone (r^2=0.29 vs. 0.20, both p<0.005), although the difference between the two models was not statistically significant (p=0.67). Further, the protein-fiber index was directly associated with serum IS concentrations (p=0.015), despite no significant association with either nutrient in isolation.

The associations between IS and PCS and the protein-fiber index were independent of other known predictors of toxin levels, including kidney function (eGFR), gender and diabetes (p=0.007 [IS] and p<0.001 [PCS]). In addition, prediction of serum toxin levels was significantly improved by adding the protein-fibre index to the regression model with eGFR (both p<0.005); the two variables predicting 35% and 48% of the

total serum IS and PCS concentrations, respectively (both $p<0.001$).

Discussion

This study showed that the protein-fiber dietary index was significantly associated with serum IS and PCS levels, beyond its well-known association with kidney function. Furthermore, the significant association between this index and the toxins, beyond that observed for fiber and protein alone, highlights the plausible importance of the interplay between these nutrients.

Both dietary intake (particularly fiber[22] and protein[23]) and uremic toxins (IS[24] and PCS[25]) have previously been demonstrated to be independently associated with mortality in the CKD population. This study demonstrated an association between the serum concentrations of these toxins and these nutrients, particularly their ratio, which may shed light on the mechanism underpinning the association between diet and mortality in CKD as reported by large cohort studies.

The association identified in this study between the protein-fiber index and serum IS and PCS (an indirect measure of their generation) supports the interaction between the two nutrients and the gut microbiota. Nutrient availability is a key regulator of bacterial metabolism, particularly the ratio of nitrogen to carbohydrate.[26] Nitrogen sources that enter the colon include dietary protein that has escaped digestion, which is influenced by a number of factors including dietary protein intake. Carbohydrate that reaches the colon is generally indigestible in the small intestine; this is typically dietary fiber. The availability of these two nutrients influences the amount of saccharolytic versus proteolytic fermentation that occurs in the colon.[27] Similar to

human cell metabolism, the main source of energy for bacterial cells is carbohydrate. However, when this is limited, such as occurs in the case of either low dietary fiber intakes or proportionally greater availability of protein, proteolytic bacterial fermentation predominates. Therefore, a colonic environment with a high nitrogen:carbohydrate ratio, which is likely to reflect a diet with a high protein-fiber index, promotes amino acid fermentation and therefore increased production of IS and PCS. Consequently, proteolytic:saccharolytic fermentation is the likely link between the protein-fiber index and IS and PCS serum concentrations reported in this study.

The association between serum IS and PCS and kidney function described in this study is well defined in the literature.[28] However, this study was the first to show the protein-fiber index was a determinant of IS and PCS, independent of kidney function. When considered together, kidney function and the protein-fiber index explained less than 50% of variance in the toxins, suggesting that there are a number of other factors influencing the toxin levels. The impact of modifying the protein-fiber intake on the toxins, in addition to uncovering other potentially modifiable predictors, is important to identify future therapeutic opportunities.

There were several limitations to this study. First, the index was based on the ratio of nutrients and not absolute intakes defined by the evidence based guidelines.[15] Therefore, the index does not inform protein requirements for nutrition recommendations. Second, the small size of the study restricted the multivariable regression analysis to four predictor variables. Nevertheless, the model included the predictor variables with the greatest body of evidence and therefore was thought to be sufficient for the purposes of this study. Third, the Australian food database, similar to

most other countries, analyses the fiber content of foods using the Prosky method[29] (AOAC 985.29), which excludes a number of important types of resistant starch (including chemically modified starch) as well as low molecular weight type fibers like inulin and fructooligosaccharides. Given foods are becoming more frequently fortified with a number of fiber types in addition to a number of foods naturally containing some of these fibers excluded by the Prosky method, this study may underestimate total fiber intakes.[30] Finally, measurement error in dietary recalls is unavoidable although efforts were made to minimise this with diet history verification undertaken by a qualified renal dietitian using food models.

In summary the protein-fiber index is a novel dietary measure that may provide important insight into the role of gut health in CKD. The significant association between key nephrovascular uremic toxins and the dietary protein-fiber index, beyond the nutrients in isolation, highlights the potential role of diet quality in mediating this risk and warrants further exploration as a potential target for future dietary interventions in this patient population.

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Tables

Table 1: Participant characteristics (n=40)

Characteristic	Value
Age (years)	69 ±10
range	42-82
Male (%)	24 (60)
White Caucasian (%)	38 (95)
Cause of kidney disease (%)	
Glomerulonephritis	5 (13)
Hypertension/vascular	8 (20)
Diabetic nephropathy	16 (40)
BMI (kg/m2)	29 ± 6
Co-morbidities (treated)	
Hypertension	40 (100)
Hyperlipidemia	31 (78)
Diabetes mellitus	18 (45)
Smoking history (%)	22 (58)
eGFR (ml/min/1.73m^2)	24 ± 8
Uremic toxins (μmol/L)	
Total indoxyl sulphate	19 (12-28)
Free indoxyl sulphate	0.7 (0.4-1.0)
Total p-cresyl sulphate	111 (69-133)
Free p-cresyl sulphate	2.9 (1.8-4.1)
Dietary intake	
Energy (MJ)	7.4 ±2.1
Protein	
Total (g)	79 ±24
grams/ kg body weight	1.0 ± 0.3
Phenylalanine (g)	3.3±1.1
Tyrosine (g)	2.9±0.9
Tryptophan (g)	1.2±0.4
Fiber (g)	
Total (g)	23±8
Soluble (g)	7 (6-9)
Insoluble (g)	11±4
Protein-Fiber index	3.6 (2.8-4.2)

Data is presented as the mean ±SD, median (inter-quartile range) or number (%)
BMI, body mass index; eGFR, estimated glomerular filtration rate

Table 2: Correlation between indoxyl sulphate and p-cresyl sulphate and dietary components of protein and fiber intake

	Indoxyl sulphate (µmol/L)*				P-cresyl sulphate (µmol/L)*			
	Free		Total		Free		Total	
	r	P value	r	P value	r	P value	r	P value
Protein (g)								
Total	0.03	0.869	0.12	0.466	-0.19	0.234	-0.14	0.381
Phenylalanine	0.03	0.849	0.11	0.498	-0.17	0.306	-0.13	0.420
Tyrosine	-0.01	0.951	0.07	0.677	-0.17	0.305	-0.13	0.450
Tryptophan	-0.03	0.858	0.06	0.740	-0.15	0.360	-0.11	0.528
Fiber (g)								
Total	-0.27	0.089	-0.19	0.240	-0.42	0.007	-0.44	0.004
Soluble*	-0.19	0.253	-0.16	0.312	-0.36	0.024	-0.42	0.007
Insoluble	-0.24	0.140	-0.16	0.325	-0.41	0.009	-0.42	0.007
Protein-Fiber index*	0.34	0.031	0.40	0.012	0.29	0.068	0.43	0.005

* Data square root transformed prior to Pearson's correlation
Protein-fiber index is the ratio of total protein to total fiber

Figures

Figure 1: Correlation between uremic toxins and dietary components of protein and fiber intake

* Data square root transformed prior to pearson's correlation Protein:fibre index is the ratio of total protein to total fibre