Nutrition, Metabolism & Cardiovascular Diseases (2015) **25**, 860–865

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

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

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Received 29 January 2015; received in revised form 25 March 2015; accepted 30 March 2015
Available online 9 April 2015

KEYWORDS
Indoxyl sulfate;
p-Cresyl sulfate;
Protein-fiber index;
Uremic toxins

Abstract *Background and aims:* Indoxyl sulfate (IS) and p-cresyl sulfate (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 ±8 mL/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

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http://dx.doi.org/10.1016/j.numecd.2015.03.015
0939-4753/

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 favor 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 sulfate (IS) and p-cresyl sulfate (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 (i.e. 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 and 30 ml/min/1.73 m^2 and aged $\geq$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.