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p-Cresyl sulfate serum concentrations in haemodialysis patients are reduced by the prebiotic oligofructose-enriched inulin

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Abstract

Introduction. Protein-bound uraemic retention solutes, including *p*-cresyl sulfate and indoxyl sulfate, contribute substantially to the uraemic syndrome. These and several other uraemic retention solutes originate from intestinal bacterial protein fermentation. We investigated whether the prebiotic oligofructose-enriched inulin reduced serum concentration of *p*-cresyl sulfate and indoxyl sulfate, through interference with intestinal generation.

Methods. We performed a single centre, non-randomized, open-label phase I/II study in maintenance HD patients with a 4-week, escalating dose regimen of oligofructose-enriched inulin (ORAFTI® Synergy 1, Tienen, Belgium) (www.clinicaltrials.gov NCT00695513). Changes in *p*-cresyl sulfate and indoxyl sulfate serum concentrations as well as changes in *p*-cresyl sulfate and indoxyl sulfate generation rates were analysed.

Results. Compliance with therapy was excellent. *p*-Cresyl sulfate serum concentrations at 4 weeks were significantly reduced by 20% (intention to treat, $P = 0.01$; per protocol, $P = 0.03$). Also *p*-cresyl sulfate generation rates were reduced ($P = 0.007$). In contrast, neither indoxyl sulfate generation rates ($P = 0.9$) nor serum concentrations ($P = 0.4$) were significantly changed.

Conclusion. The prebiotic oligofructose-inulin significantly reduced *p*-cresyl sulfate generation rates and serum concentrations in haemodialysis patients. Whether reduction of *p*-cresyl sulfate serum concentrations, an independent predictor of cardiovascular disease in HD patients, will result in improved cardiovascular outcomes remains to be proven.

Keywords: haemodialysis; indoxyl sulfate; intervention study; *p*-cresol; prebiotic

Introduction

Chronic kidney disease (CKD) is a disease of epidemic dimensions. According to recent data from the National Health and Nutrition Examination Survey (NHANES), the overall prevalence of CKD stages 1–4 increased from 10.0% in 1988–94 to 13.1% in 1999–2004 [1]. The physiology underlying the clinical syndrome of advanced kidney failure is only partly understood. It is assumed that uraemic illness is in large part secondary to accumulation of organic waste products that are cleared by normally functioning kidneys [2].

These organic waste products, often referred to as uraemic retention solutes, differ in their water solubility, dimensions, charge distribution, molecular mass and, importantly, in protein binding [3,4]. Several landmark studies, including the HEMO study in haemodialysis [5], and the adequacy of peritoneal dialysis in Mexico (ADEMEX) [6], failed to improve patient outcomes by increasing the clearance of water soluble uraemic retention solutes molecules and (so-called) middle molecules above current standards of care in end-stage kidney failure. These and other findings have fuelled interest in the group of protein-bound uraemic retention solutes [4,7].

The large majority of protein-bound molecules circulate bound to albumin [4]. For albumin-bound solutes, only the free fraction is able to cross an albumin-impermeable membrane [8,9], resulting in limited removal of albumin-bound uraemic retention solutes by renal replacement therapies [4]. Combination of dialysis with convection (haemodiafiltration) provides superior protein-bound solute removal compared with high-flux haemodialysis [10]. Blood clearances were further improved by adsorption-based experimental therapies, i.e. carbon particles-containing dialysate [11] and fractionated plasma separation and adsorption [12]. Although promising, neither therapy has been shown to reduce serum concentrations of the protein-bound uraemic retention solutes medium or long-term. Moreover, such therapies are not suitable to be used in the far larger patient population of patients with earlier stages of CKD.

Two of the best studied protein-bound uraemic retention solutes are indoxyl sulfate and *p*-cresyl sulfate [2,13]. Indoxyl sulfate is thought to promote CKD progression [14,15], to induce endothelial dysfunction [16,17] and to be implicated in CKD-associated bone-mineral disease

[17,18]. We recently demonstrated that free *p*-cresyl sulfate serum concentrations, indirectly quantified as *p*-cresol, are independently associated with overall mortality [19] and are an independent predictor of incident cardiovascular disease in haemodialysis patients [20]. *In vitro* studies demonstrated direct effects of *p*-cresyl sulfate on leucocytes [21] and the endothelium (Meijers *et al*., submitted). Interestingly, both molecules originate from colonic protein fermentation as unique bacterial fermentation end-products of tyrosine (*p*-cresol) and tryptophan (indol).

Various therapies have been developed to regulate the complex bacterial fermentation processes [22]. Younes and coworkers elegantly demonstrated that fermentable carbohydrates alter colonic bacterial fermentation in CKD [23,24]. We recently demonstrated that the prebiotic oligofructose-enriched inulin (OF–IN, ORAFTI® Synergy 1, Tienen, Belgium) reduced urinary *p*-cresol excretion (including its sulfate conjugate) in healthy volunteers [25]. Whether fermentable carbohydrates, such as oligofructose and inulin, affect serum concentrations of protein-bound uraemic retention solutes in patients with CKD is not known.

The aims of the current study (Clinicaltrials.gov NCT00695513) were (1) to investigate whether serum concentrations of the protein-bound uraemic retention solutes *p*-cresol and indoxyl sulfate were altered by the intake of oligofructose-enriched inulin and (2) to investigate the safety and tolerability profile of the orally administered prebiotic oligofructose-enriched inulin in patients with end-stage renal disease treated with HD.

Subjects and methods

Study population

Patients, treated with maintenance haemodialysis for at least 3 months at the nephrology department of the University Hospital Gasthuisberg (Leuven, Belgium), were enrolled in this study. Eligible patients were 18 years or older and able to give written informed consent. Exclusion criteria were the use of pre-, pro-, syn- or antibiotics during 4 weeks preceding the study. The study was performed according to the World Medical Association Declaration of Helsinki and approved by the local ethics committee. All patients provided written informed consent prior to enrolment.

Study design

This was a single centre, non-randomized, open-label phase I/II study with an escalating dose regimen of oligofructose-enriched inulin in maintenance HD patients to investigate the safety, tolerability and effects on *p*-cresyl l sulfate serum concentrations and generation rates (Figure 1).

After the patient provided informed consent, a baseline evaluation was made during the midweek dialysis session. The patients were started on oligofructose-enriched inulin 10 g once daily during the first week. At Day 8, the dose of oligofructose-enriched inulin was escalated to 10 g BID (twice daily) until study end. Study treatment was stopped at Day 28, after the midweek dialysis session. The patients were followed for another 4-week run-out period.

To assess patient compliance, empty and unopened study recipients were collected for each individual patient in the study. To assess tolerability, patients completed five-level Likert item questionnaires. To assess safety, the patients were followed clinically during the study period. Biochemical safety assessment included leukocyte count and C-reactive protein levels.

The primary efficacy endpoint was change in *p*-cresyl sulfate serum concentrations at 4 weeks from baseline. Secondary endpoints included change in *p*-cresol generation rate, change in indoxyl sulfate serum concentrations and change in blood urea concentrations and serum creatinine concentrations.

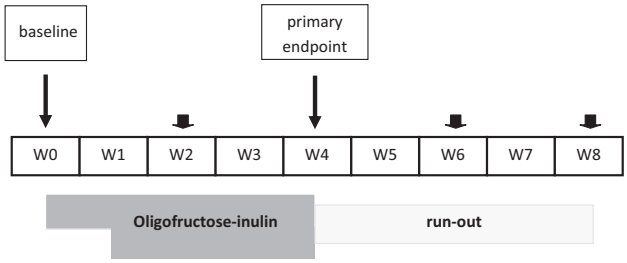

Fig. 1. Schematic representation of the study design. The study was conducted over an 8-week period. At Week 0, the patients were started on oligofructose-enriched inulin (ORAFTI® Synergy 1, Tienen, Belgium) 10 g daily. After the midweek dialysis session of the second week, oligofructose inulin dosage was escalated to 10 g BID. Study therapy was stopped after the midweek dialysis session of Week 4. Primary endpoint evaluation at the midweek dialysis session of Week 0 and Week 4 (long arrow) included blood sampling, spent dialysate collections and 44 h inter-dialytic urinary collections. Additional blood samples were taken at regular intervals during the study (short arrows).

Study treatment

ORAFTI® Synergy 1 is a 50/50 wt/wt mixture of oligofructose and Raftiline HP (ORAFTI, Tienen, Belgium). Raftiline HP is a mixture of $\beta(2-1)$ linear fructans obtained from chicory root with a degree of polymerization (DP) ranging between 10 and 60 (average DP: 12). Oligofructose (DP: 2–8, average DP: 4) is obtained by partial enzymatic hydrolysis of inulin. Study treatment was distributed in unlabelled sachets, each containing 10 g of ORAFTI® Synergy 1, a white powder with a slightly sweet taste.

Sample collection

Blood was sampled at the start of midweek dialysis sessions at baseline, at the end of study treatment, at the end of the run-out period and at regular intervals during the study period. To assess changes in *p*-cresol generation rates, total spent dialysate collections were performed during the midweek dialysis session at baseline and at the end of study treatment, together with inter-dialytic urinary collections. Assuming steady-state conditions, total solute removal equals total solute generation. To calculate indoxyl sulfate and *p*-cresyl total solute removals, spent dialysate collections were weighed, vigorously stirred and sampled. Given that dialysate relative density is near to 1, dialysate total solute removals were calculated as dialysate concentrations times dialysate weights. Inter-dialytic urinary solute removals were calculated as urinary volumes multiplied with urinary solute concentrations.

Biochemical measurements

Biochemical parameters including urea, creatinine, leukocyte counts and C-reactive protein concentrations were measured using standard laboratory techniques. We quantified *p*-cresyl sulfate and indoxyl sulfate using high-performance liquid chromatography (HPLC) as described previously [26]. Total, within-run, between-run and between-day imprecision for indoxyl sulfate and *p*-cresyl sulfate were below 6%. The limit of quantification was 3.2 μmol l^{-1} for both analytes. Recovery, tested in haemodialysis patients, was 102% for indoxyl sulfate and 105% for *p*-cresyl sulfate.

Statistics

Continuous variables were expressed as mean [standard deviation (SD)] for normally distributed variables or median (interquartile range), otherwise. Normality was tested according to Shapiro–Wilk. Comparisons between start and end of the study period were tested by paired *t*-test or by Wilcoxon-signed rank (WSR) as appropriate.

The following prespecified outcomes were analysed: changes in *p*-cresyl sulfate serum concentrations (primary endpoint), in *p*-cresol generation rates, in serum creatinine concentrations, in serum indoxyl sulfate concentrations and in blood urea nitrogen concentrations. The following prespecified safety outcomes were analysed: differences in C-reactive

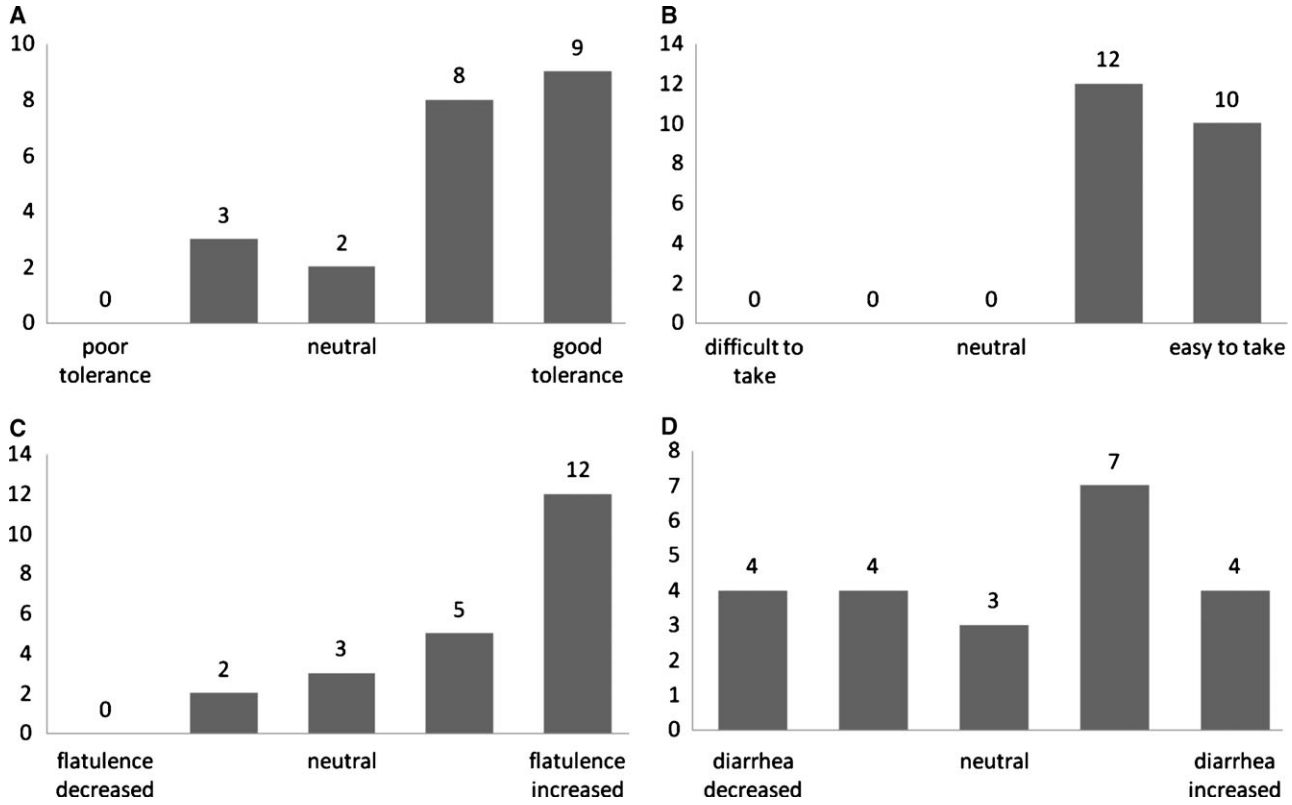

Fig. 2. Histogram plots of the number of patient responses to individual five-level Likert items of (**A**) tolerance, (**B**) study treatment intake, (**C**) flatulence and (**D**) diarrhoea. Likert item questionnaires were scored at the midweek dialysis session of Week 4, after 4 weeks of the intake of study treatment.

protein and leukocyte counts. *Post hoc* analyses included total solute removal of urea. For intention to treat analysis, data of all patients were included. For per protocol analysis, patients who received pro-, pre-, syn- or antibiotics during the study period or who missed at least 25% of the scheduled doses were excluded from analyses. Two-sided *P*-values of <0.05, unadjusted for multiple comparisons, were considered statistically significant.

Based on preliminary experiments, we estimated that at least 20 patients needed to be included to have a power of 0.8 to detect a significant difference in *p*-cresyl sulfate serum concentrations, assuming a two-sided type I error of 0.05. Taking into account an expected drop-out rate of 10%, we planned to include 22 patients.

Results

Study population

Between February 2006 and April 2008, 22 maintenance haemodialysis patients followed up at the nephrology department of the University Hospital Gasthuisberg, Leuven, Belgium, were found eligible to be enrolled in the study and were started on study therapy. Table 1 represents the demographic and baseline characteristics of the study population.

Compliance, tolerability and safety evaluation

Overall, adherence to the study treatment was excellent. Of 1078 distributed doses, 1015 (94.2%) doses were reported to be consumed and the empty sachets returned. One patient stopped the study treatment after 12 days due to diarrhoea. One patient did not escalate the treatment after the first week due to flatulence that he considered socially unacceptable, but maintained treatment at half the dose until study end. Based on Likert item questionnaires, the study treatment was easy to take and, overall, well tolerated. The majority ($n = 17$; 77%) of patients reported an increase of flatulence. Four (14%) patients reported diarrhoea (Figure 2).

During the study period, leukocyte counts ($P = 0.4$) and C-reactive protein concentrations ($P = 0.4$) were not significantly changed. During the run-out period, one patient deceased due to sudden cardiac death. After case review, this was considered unrelated to the intake of the study treatment, which had been stopped three weeks earlier.

Table 1. Baseline demographic and laboratory data

Variable	
Age (years)	62.2 (12.8)
Sex (male/female) (%)	15/7 (68/32%)
Diabetes (yes/no) (%)	1/21 (5/95%)
Residual renal function (yes/no) (%)	10/12 (45/55%)
Blood pressure (systolic/diastolic mmHg)	138 (22)/78 (12)
Cause of renal failure, *n* (%)	
ADPKD	2 (9%)
Diabetes	1 (5%)
Tubulointerstitial disease	2 (9%)
Glomerulonephritis	5 (23%)
Vascular	1 (5%)
Other/unknown	11 (50%)

ADPKD, autosomal dominant polycystic kidney disease.

Table 2. Uremic retention solute concentrations and generation rates

	Baseline ($n = 22$)	Intervention ($n = 22$)[a]	*P*-value
Serum/plasma			
Creatinine[b] (mg dl^{-1})	8.80 (2.05)	8.48 (1.87)	0.2
Urea[b] (mg dl^{-1})	126.5 (36.0)	119.3 (37.7)	0.03
p-cresyl sulfate (μM)	204.6 (157.3–333.3)	170.0 (126.0–280.1)	0.01
Indoxyl sulfate (μM)	111.1 (71.3–172.1)	105.2 (77.5–167.3)	0.4
Total solute removal			
Urea[b] (g week^{-1})	107.4 (31.5)	97.3 (30.1)	0.1
p-cresyl sulfate (μmol week^{-1})	2000.9 (1317.9–2558.9)	1282.7 (992.8–2501.8)	0.007
Indoxyl sulfate (μmol week^{-1})	1247.8 (911.6–1970.1)	1296.3 (837.7–1970.1)	0.9

[a]Means and medians of the intention to treat cohort are given.
[b]Data are presented as mean (standard deviation) for normally distributed variables and as median (25th–75th percentile) otherwise.

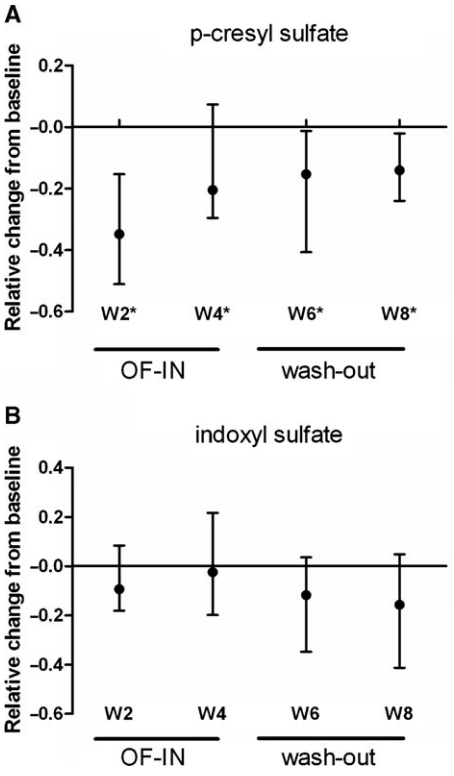

Fig. 3. Relative change of serum concentrations, compared to baseline of (**A**) *p*-cresyl sulfate and (**B**) indoxyl sulfate during the intake of the study treatment and during the 4-week wash-out period. Data are presented as median (interquartile range). *Reduction rate significantly different from 0 at $P < 0.01$ level.

Efficacy evaluation

Intention to treat analysis of changes in *p*-cresyl sulfate serum concentrations at 4 weeks (primary endpoint) demonstrated a median 20% reduction (WSR, $P = 0.01$) (Figure 3A). Representative chromatograms are shown in Figure 4. During the study period, one patient was prescribed antibiotics for a lower respiratory tract infection. Per protocol analysis yielded qualitatively identical results ($P = 0.03$). Individual responses varied substantially, and for some patients, *p*-cresyl sulfate serum concentrations were nearly identical after the study period (<10% change from baseline *p*-cresyl sulfate serum concentrations, $n = 6$). About half ($n = 10$) of the patients, based on an arbitrary cut-off of at least 20% change in *p*-cresyl sulfate serum concentrations, were considered therapy responders. During the 4-week wash-out, *p*-cresyl sulfate serum concentrations remained lower as compared to baseline (Figure 3A). In contrast, serum indoxyl sulfate concentrations were not significantly changed (Figure 3B).

Secondary endpoint analyses (Table 2) demonstrated that *p*-cresol generation rates were significantly reduced by on average 20% (WSR, ITT $P = 0.007$; PP $P = 0.007$). Serum creatinine concentrations did not change during the study period. In contrast, blood urea nitrogen concentrations were significantly reduced [59.1 (16.8) mg dl^{-1} versus 55.8 (17.6) mg dl^{-1}, *t*-test $P = 0.02$].

Discussion

In this prospective phase I/II study, 4 weeks of oligofructose inulin (ORAFTI® Synergy 1) significantly reduced *p*-cresol generation rates and *p*-cresyl sulfate serum concentrations. This effect lasted at least 4 weeks after cessation of study treatment.

Bacterial protein fermentation in the large intestine is the predominant source of several uraemic retention solutes, implicated in uraemia [2,14,19]. It is generally accepted that the most important regulator of bacterial metabolism is nutrient availability and especially the ratio of available carbohydrates to nitrogen [27]. Higher colonic availability of carbohydrates drives this process towards lower production of toxic metabolites. Small intestinal α-glucosidase inhibitors like Acarbose (Glucobay®, Germany) enhance the amount of undigested carbohydrates reaching the colon. In a cohort of healthy volunteers, serum concentrations of *p*-cresol declined significantly after Acarbose treatment [28]. Other fermentation regulators include colonic transit times [29] and composition of the bacterial microbiota. Pre- and/or pro-biotics induce changes in the ecological balance of intestinal microbiota, thus affecting microbial metabolic activities [22,25]. We recently demonstrated that both the prebiotic oligofructose-enriched inulin as well as the probiotics *Lactobacillus casei* Shirota and *Bifidobacterium breve* Yakult® (Almere, The Netherlands) significantly reduced urinary *p*-cresol excretion in healthy volunteers [25]. In HD patients, however, an oral preparation of lactic acid bacteria containing

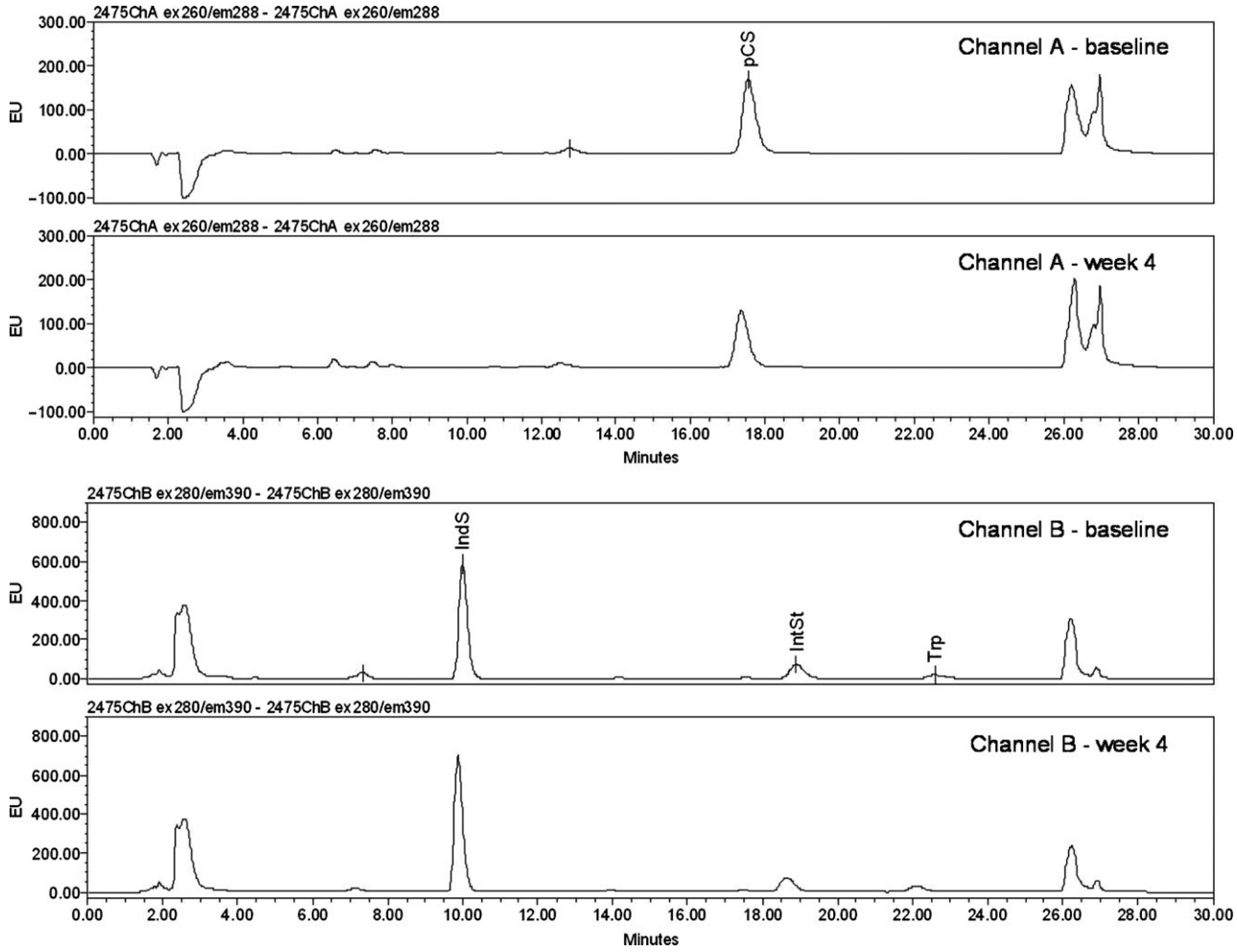

Fig. 4. Representative chromatograms of a patient at start and after 4 weeks of treatment with oligofructose-enriched inulin. Solutes are quantified by using the analyte to standard peak area ratio. Detector settings were λ_{ex} 260 nm/λ_{em} 288 nm for *p*-cresyl sulfate (channel A) and λ_{ex} 280 nm/λ_{em} 390 nm for indoxyl sulfate and internal standard (channel B).

Bifidobacterium infantis, *Lactobacillus acidophilus* and *Enterococcus faecalis*) did not reduce *p*-cresol serum concentrations [30].

This is the first study to describe the use of prebiotics to reduce serum concentrations of uraemic retention solutes in patients with end-stage renal disease. Oligofructose-enriched inulin decreased *p*-cresol generation, resulting in a reduction of *p*-cresyl sulfate serum concentrations by on average 20%. Remarkably, although indoxyl sulfate is also generated by colonic bacteria, serum concentrations were not systematically reduced, nor was the indoxyl sulfate generation rate. These findings suggest that indoxyl sulfate and *p*-cresyl sulfate are end-products of unrelated bacterial metabolic pathways. This might also explain why baseline serum indoxyl sulfate and *p*-cresyl sulfate concentrations were completely unrelated.

An interesting finding of this study is that, besides *p*-cresyl sulfate, blood urea concentrations also significantly declined during the intake of oligofructose inulin by on average 11.0% ($P = 0.03$). This observation corroborates previous experimental and clinical data. Younes *et al*. demonstrated that fermentable carbohydrates exert urea-lowering effects in normal and nephrectomized rats through interference with bacterial metabolism [23]. The same authors confirmed this observation in a clinical trial in nine patients with CKD not yet on dialysis [24]. Using the stable-isotope labelled lactose-[^{15}N, ^{15}N′]-ureide assay, we demonstrated that oligofructose-enriched inulin induces a shift from urinary to faecal ^{15}N-excretion in healthy individuals [31].

It is of note that reduced *p*-cresol generation rates and *p*-cresyl sulfate serum concentrations persisted at least 4 weeks after cessation of intake, suggesting that bacterial metabolism was reset by the intake of oligofructose-enriched inulin. This has several consequences. In future cross-over trials, wash-out periods need to be sufficiently long to prevent carry-over effects between treatment arms. Secondly, this leaves open the possibility of intermittent treatment.

Overall, the intake of oligofructose-enriched inulin was well tolerated and, as a powdered formulation, was considered easy to take, in agreement with previous reports [24]. Although not perceived as a major side effect, most study participants reported substantially increased flatulence. Whether this reflects a temporary effect secondary

to changes in bacterial fermentation, or that flatulence is persistent, needs to be studied longer-term. Persistent flatulence might prove relevant to therapy compliance. We observed a clear inter-individual variation in *p*-cresyl sulfate reduction rates in response to oligofructose-enriched inulin intake. Prediction of response to therapy would help to reduce the number of side effects. We were not able to predict response to therapy based on biochemical variables, including baseline *p*-cresyl sulfate concentrations and total *p*-cresyl sulfate removals (data not shown).

A potential limitation of the current study is that nutrient intakes were not recorded. As we aimed to study the effect of oligofructose-enriched inulin in normal daily circumstances, study participants were maintained on their regular diet. In a previous study, nutrient intakes did not decrease during supplementation with fermentable carbohydrates [24].

In conclusion, the oral intake of the prebiotic oligofructose-enriched inulin was well tolerated and significantly reduced *p*-cresol generation and *p*-cresyl sulfate serum concentrations in haemodialysis patients. Whether reduction of *p*-cresyl sulfate serum concentrations, an independent predictor of cardiovascular disease in HD patients [20], will result in improved cardiovascular outcomes remains to be proven.

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