Supplementary data

Methods

Outcome measures- At baseline, mid-point and end of each intervention arm, venous blood was collected in the morning from all participants following a standardised evening meal (commercially prepared frozen meal adjusted by the dietitian as per the patients' energy and protein requirements) and subsequent overnight fast. Samples were stored at -80°C and then analysed in a single batch. Serum total and free concentrations of both uremic toxins, PCS and IS, were analysed by ultra-performance liquid chromatography (UPLC) using a fluorescence detection method (Waters Corporation, Milford, MA, USA).[1] The free fraction of each toxin was defined as a percentage of total concentration (free serum concentration divided by the total concentration multiplied by 100). Samples were run in duplicates and the coefficient of variation (CV) for the assays ranged from 1.8 to 2.9%.

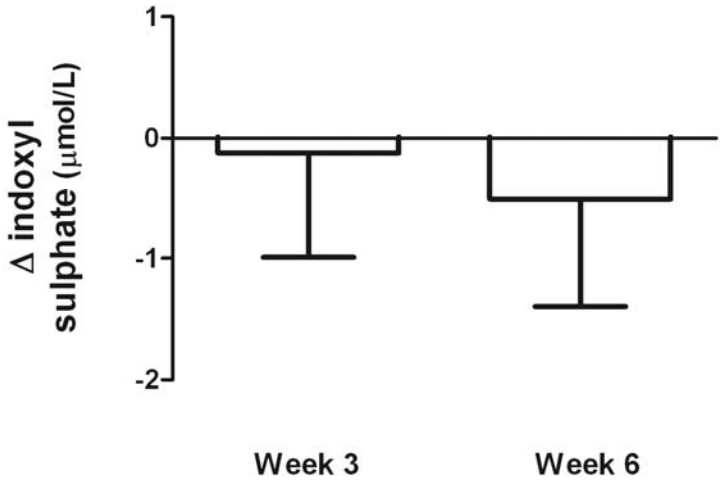
Serum creatinine, urea, albumin and phosphate were measured using automated laboratory techniques. Renal function was estimated using eGFR calculated from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[2] Kidney damage was measured using 24-hour urinary protein and albumin and mid-stream urinary kidney injury molecule-1 (Kim-1), as previously described.[3] The inflammatory markers interleukin [IL]-1β, IL-6, IL-10 and tumour necrosis factor-α (TNFα) were measured in serum samples before and after each intervention using electrochemiluminescence immunoassay techniques. The CV for the assays ranged from 0.8 to 12.4%, falling within the acceptable range (<20% CV). In addition, markers of lipid oxidation (total F_2 isoprostanes) and endogenous antioxidant activity (glutathione peroxidise [GPx]) were measured in plasma samples using validated

methods.[4, 5] Quantification of lipopolysaccharides was undertaken using a Limulus Amebocyte assay (Cambrex, Verviers, Belgium), as described previously.[6]

Changes in patient-reported health and gastrointestinal symptoms were assessed by the validated Short Form-36 (SF-36)[7, 8] and Gastrointestinal Symptom Rating Scale (GSRS)[9, 10].

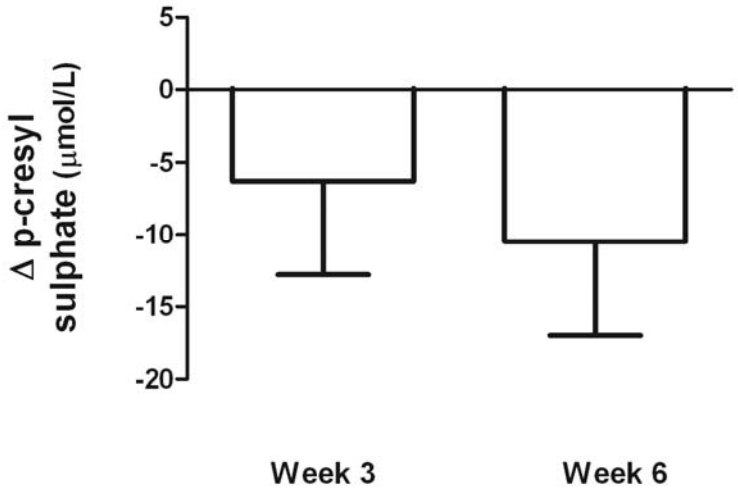
Gut microbial analysis- Samples were thawed, homogenised and subsampled (~0.15 g), then mixed with 0.6 ml of lysis buffer[11] and 0.4 g of zirconium-silica beads (0.1 to 1.0 mm diameter). The samples were homogenized using a Precelly's-24 tissue homogenizer set for 3 cycles at 60 seconds at 5,000 rpm. The samples were centrifuged at 4°C for 5 minutes at 16,300 x *g*. The supernatant was transferred to a fresh tube, 30 µl proteinase K (20 mg/ml, Promega Life Sciences) added and incubated for 20 minutes at 56C; then the DNA was extracted from this mixture using the LEV-blood DNA kit and a Maxwell 16 MDr automated DNA extraction system (Promega Life Sciences). The resulting sample was treated with RNAse A and the purity and concentration of the DNA preparations were determined using Nanodrop-Lite quantification system (Thermo Scientific). A total of 75 samples passed QC/QA specifications for PCR amplification of the V6-V9 region of the gene encoding 16S rRNA, with dual index bar-coded library construction, and sequencing using the Illumina MiSeq platform, all provided by the Australian Centre for Ecogenomics (ecogenomic.org). The resulting datafiles were analysed using the QIIME software package (v.1.8.0) in support of taxonomic assignments and alpha diversity measures.

Figure S1: Longitudinal response of serum concentrations of indoxyl sulphate to synbiotics over time[a]



[a] between weeks 3 and 6 the synbiotic dose was doubled

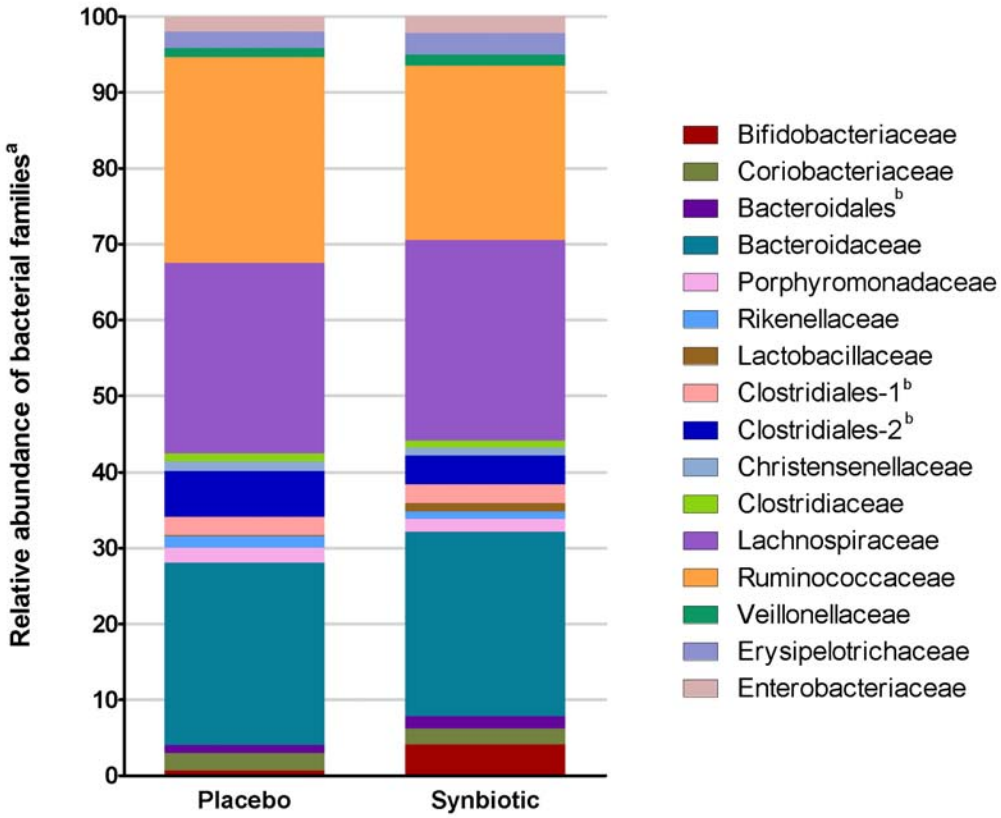
Figure S2: Longitudinal response of serum concentrations of p-cresyl sulphate to synbiotics over time[a]



Trend p=0.002

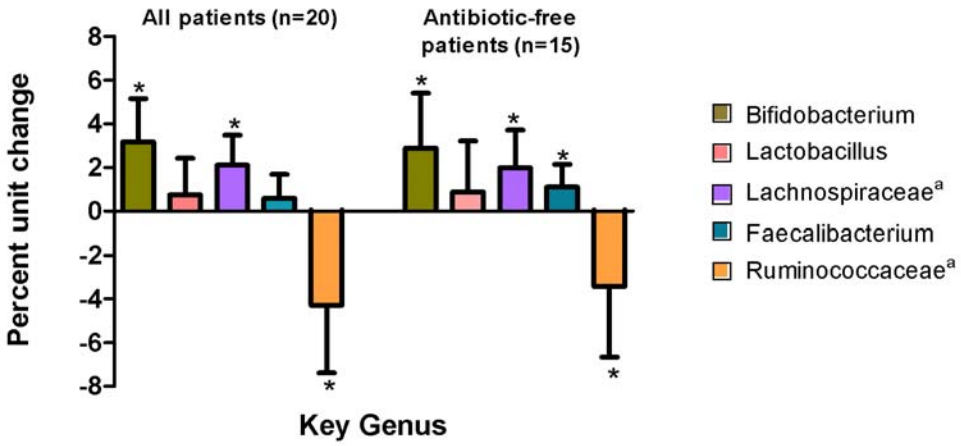
[a] between weeks 3 and 6 the synbiotic dose was doubled

Figure S3: Effect of the synbiotics on relative abundance of bacterial families



[a] Families with an abundance of ≥1%
[b] Unspecified members with the order

Figure S4: Effect of the synbiotics on key genus in all analysed patients (n=20) and in antibiotic-free patients (n=15)



Treatment effect (95% CI) derived from regression modelling accounting for period effect

[a] Unspecified members with the family

* $p<0.01$

Table S1: Baseline characteristics of SYNERGY participants who completed the study by antibiotic use during either intervention (n=31)

Characteristic	All patients who completed the study (n=31)	Antibiotic use (n=10)	No antibiotic use (n=21)	P-value
Age (years)	69 ±9	70 ±10	69 ±9	0.65
range	50-82	54-82	50-82	
Male (%)	19 (61)	6(60)	13 (62)	0.92
White Caucasian (%)	29 (94)	10	19 (90)	0.31
Cause of kidney disease (%)				0.65
Glomerulonephritis	4 (13)	0 (0)	4 (19)	
Hypertension/vascular	7 (23)	3 (30)	4 (19)	
Diabetic nephropathy	11(39)	5 (50)	7 (33)	
BMI (kg/m2)	28 ±6	29±5	28±6	0.69
Co-morbidities (treated)				
Hypertension	31 (100)	10(100)	21(100)	>0.99
Hyperlipidemia	26 (84)	9 (90)	17(81)	0.52
Number of antihypertensive medications	2.3 ±1.1	2.5 ±1.1	2.2 ±1.1	0.55
Angiotensin converting enzyme inhibitor	7 (23)	2 (20)	5 (24)	0.81
Angiotensin receptor II blocker	19 (61)	6(60)	13(63)	0.92
Diuretics	8 (26)	1 (10)	7 (33)	0.17
Smoking history (%)	17 (55)	6 (60)	11(52)	0.68
EPI GFR (ml/min/1.73m2)	25 ± 8	28 ±9	24 ±8	0.19
Proteinuria (mg/24hr)	296 (168-1100)	160(119-431)	838(263-1800)	0.01
Albuminuria (mg/24hr)	97(21-677)	21(7-74)	361(97-1200)	0.01
Uremic toxins				
Total indoxyl sulphate	20 ±11	19 ±9	21±13	0.66
Total p-cresyl sulphate	108 ± 52	112 ±51	106 ±53	0.76
Free indoxyl sulphate	0.8±0.4	0.8 ±0.4	0.7 ±0.5	0.87
Free p-cresyl sulphate	3.3±2.5	4.1 ±3.4	2.9 ±1.9	0.21
IS:PCS ratio	0.24 ±0.18	0.19 ±0.09	0.26 ±0.21	0.37
Percent free fraction				
Indoxyl sulphate	3.9 ± 1.3	4.3 ±1.8	3.7 ±1.0	0.25
P-cresyl sulphate	2.8 ± 0.9	3.3 ±1.3	2.6± 0.7	0.05

BMI, Body mass index; EPI GFR, Chronic Kidney Disease Epidemiology Collaboration Glomerular Filtration Rate

Data presented as mean ±SD, median (inter-quartile range), number (%)

[a] Differences between antiobiotic use determined using t-test with normally distributed data and Wilcoxon-Mann-Whitney with non-normal data

Table S2: Sensitivity and secondary analysis of treatment effect on uremic toxins (µmol/L)

	All completers (n=31)		Antibiotic-free completers (n=21)	
	Treatment effect[a] (95% CI)	**P-value**	**Treatment effect[a] (95% CI)**	**P- value**
PRIMARY ANALYSIS				
Total IS	-2 (-5 to 1)	0.12	-5 (-8 to-1)	0.03
Total PCS	-14 (-27 to -2)	0.03	-25 (-38 to -12)	0.001
SENSITIVITY ANALYSIS				
Baseline toxins				
Total IS	-2 (-5 to 1)	0.22	-4 (-7 to 0)	0.04
Total PCS	-14 (-27 to -1)	0.04	-24 (-36 to -11)	0.001
Δ Dietary fibre intake				
Total IS	-2 (-5 to 1)	0.18	-4 (-9 to 0)	0.05
Total PCS	-15 (-28 to -6)	0.02	-25 (-39 to -10)	0.01
Δ Dietary Protein intake[b]				
Total IS	-2 (-6 to 1)	0.12	-5 (9-9 to 0)	0.03
Total PCS	-14 (-26 to -2)	0.03	-25 (-38 to -11)	0.001
Δ Kidney function (eGFR)[c]				
Total IS	-2 (-5 to 1)	0.13	-5 (-9 to -1)	0.02
Total PCS	-14 (-26 to -1)	0.03	-25 (-39 to -11)	0.001
Analysis using paired t-test[d]				
Total IS	-3 (-5 to 0)	0.10	-4 (-8 to -1)	0.03
Total PCS	-16 (-29 to -3)	0.01	-25 (-37 to -13)	<0.001
SECONDARY ANALYSIS	(n=37)		(n=23)	
Mixed model				
Total IS	-1(-3 to 0)	0.09	-2 (-4 to 0)	0.02
Total PCS	-9 (-14 to -3)	0.01	-13 (-18 to -7)	<0.001

[a] Treatment effect derived from regression modelling accounting for period effect

[b] Same conclusion when adjusted for estimated protein intake based on 24-hr urinary urea nitrogen equation

[c] Same conclusion when adjusted for serum change in serum creatinine

[d] Treatment effect without adjusting for period effect

Table S3: Summary of antibiotic use during intervention (n=10)

Pt ID	Time in trial	Indication	AB	Duration (total days)	Daily dose
11	V2, V3	Urinary Tract Infection	Trimethoprim	6	900mg
14	V2	Chest Infection	Amoxycillin + Clavulanic acid	10	1750/250mg
14	V5	Chest Infection	Amoxil	7	1500mg
14	V6	Not disclosed	Cephalexin	7	1500mg
20	V2, V3, V4	Chest Infection	Amoxycillin + Clavulanic acid	16	1750/250mg
22	V6	Infected wound	Cephalexin	10	1000mg
24	V3	Not disclosed	Trimethoprim	14	300mg
33	V2, V3	Infected wound	Cephlaexin	20	500mg
36	V2	Suspected infected wound	Staphlex	7	1000mg
36	V5	Suspected Cellulitis	Cephalexin	5	1000mg
38	V5	Not disclosed	Cephalexin	5	1000mg
39	V6	Lung consolidation	Amoxycillin + Clavulanic acid	5	1750/500mg
41	V6	Ear Infection	Cephalexin	5	1000mg

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