Supplementary Table S1. The change in plasma and effluent amino acids during RRT.

	Δ PLASMA (µmol/L)				EFFLUENT (mgs lost)				*P*-value for *effect of RRT*	
Mode of RRT…	**IHD**	**SLED-F**	**CVVH**	**s.e.d.**	**IHD**	**SLED-F**	**CVVH**	**s.e.d**	**Plasma**	**Effluent**
Alanine	-37	-129	-34	70	788	998	1586	374	0.03	0.06
Arginine	-3.2	-14.2	7.7	11.0	242	288	723	164	0.66	0.006
Asparagine	-1.5	-18.9	-13.2	14.1	249	368	571	138	0.09	0.047
Aspartic acid	-2.1	-11.4	-19.7	7.8	51	88	97	27	0.009	0.15
Cysteine	-19.4	-33.1	-44.4	14	192	235	227	39	0.67	0.43
Glutamine	-17.7	-152	-84.5	83.0	127	179	191	42	0.01	0.23
Glycine	-17.1	-54	-36.2	47	436	616	811	175	0.002	0.07
Histadine	-5.2	-22.6	-26.2	13	237	302	454	101	<.001	0.07
Isoleucine	1.9	-17.3	8.2	10.8	164	270	473	85	0.09	0.001
Leucine	6.2	-31.1	-11.0	19.1	397	645	876	152	0.01	0.005
Lysine	-11.7	-64.7	12.6	29.9	569	738	1463	312	<.001	0.009
Methionine	-4.8	-13.1	-8.3	11.9	172	228	351	80	0.002	0.058
Phenylalanine	2.2	-19.2	-20.5	16.8	363	540	752	165	<.001	0.056
Proline	-16.2	-67.3	-15.8	65.4	705	839	1419	300	0.01	0.029
Serine	-6.6	-21.5	-6.7	18.0	194	303	452	95	0.01	0.016
Threonine	-9.9	-26.7	8.1	21.4	339	508	757	185	<.001	0.057
Tryptophan	0.57	-1.08	0.38	1.4	12	30	35	10	0.11	0.058
Tyrosine	0.48	-18.2	1.28	8.1	305	401	573	128	<.001	0.099
Valine	-16	-71	-9	36	433	616	1010	174	0.001	0.003
α-aminobutyricacid	-4.7	-12.9	-7.6	5.7	70	95	111	24	<.001	0.19
aminoadipicacid	-0.10	-3.2	-5.7	2.4	61	81	85	26	0.005	0.60
aminoisobutyricacid	-18.0	-42.2	-63.6	25.1	86	160	213	66	0.11	0.12
citruline	-8.6	-13.3	-5.4	5.2	77	112	175	32	0.07	0.006
cysthionine	-3.9	-8.8	-19.4	7.1	57	65	85	33	0.004	0.60
hydroxyproline	-4.4	-10.8	-16.3	18.7	90	75	110	38	0.12	0.66
methylhistidine_1	-4.7	-14.8	-7.8	8.2	72	107	83	18	0.03	0.17
methylhistidine_3	-6.0	-14.8	-11.4	3.9	61	88	107	15	0.99	0.009
Taurine	-36.3	-40.9	-54.4	26.9	202	352	261	79	0.63	0.19

Supplementary Table 1: ***Δ Plasma losses***: Data reflect average decrease over the course of each RRT session (concentration at End minus baseline). Statistics (*P*-value, *plasma*) are for the effect of time on plasma concentration. ***Effluent losses***: Since there was no significant difference between losses estimated at mid or end RRT session, data reflect pooled average losses (for Mid and End samples). Statistics (*P*-value, *effluent*) are for the effect of RRT on net loss in effluent. 95% confidence interval for the data may be estimated as 1.96 × s.e.d. Some α-amino acids were measureable but at limits of quantification, and unreliable (anserine, betalanine, carnosine, ethanolamine, gammaaminobutyricacid, homocysteine, hydroxylysine, phosphoserine, phosphoethanolamine, sarcosine). Key: 's.e.d', standard error of the differences between means. *P*-value testing for effect of RRT mode only (as timepoints are pooled estimates).

Supplementary Table S2. LC-MS/MS parameters and limits of detection for B-vitamins in effluent

	Mwt (g/mol)	Retention time (min)	Ion Mode	CV	SIR (m/z)	MRM	CE (eV)	LOD (ppb)
Vit B_1 (thiamine hydrochloride)	337.27	1.78 - 2.40	+	30	265.25	265>122	20	100
Vit B_2 (riboflavin)	376.36	8.83 - 9.05	+	30	377.28	377>243	30	6
Vit B_3 (nicotinic acid)	123.10	3.63 - 5.74	+	30	123.10	124>80	22	10
Vit B_6 (pyridoxal hydrochloride)	203.62	2.5 - 4.5	+	30	170.15	168>94	25	4
Vit B_9 (folic acid)	441.40	7.83 - 8.08	-	30	440.45	440>311	20	4
Vit B_{12} (cyanocobalamin)	1356.12				679.72			

Key: CE, collision energy.

Experimental Details: Standards were prepared for LC-MS/MS as described in Methods but briefly; 400ppm of standards, prepared as stock solution using mobile phase solvent A. These were diluted **serially** using mobile phase A to achieve a concentration range of 10ppm, 5ppm, 2ppm, 1ppm, 0.5ppm, 0.25ppm. The folic acid stock solution was dissolved in 0.1% NaOH before serial dilution. A standard calibration curve was plotted for each vitamin. Stock vitamins were stored at -20 C. All were purchased from Sigma-Aldrich.

LC conditions (Agilent 1100 series):
Column: Phenomenex Luna 5U C18 (2) 100A (250 x 3 mm ID; particle size-5 μm)
Mobile Phase: A. H_2O plus 0.1% Formic acid
Mobile Phase: B. Acetonitrile plus 0.1% Formic acid
Flow: 0.5 ml/min
Injection Volume: 10 μl
Between each injection the column was equilibrated for 7 mins prior to next injection.

Gradient Elution Program:			
Time (min)	**Flow Rate (mL/min)**	**A (%)**	**B (%)**
0	0.5	100	0
10		50	50
12.5		0	100
15		0	100
15.4		100	0
22		100	0

MS conditions (MicroMass Ultima MS)
Ion Mode: + ve ion electrospray for all B-vitamins, except and Vit B_9 (–ve mode)
Gas flow desolvation: 500 L/h
Cone voltage: 50V
Capillary: 3.5 kV
Source: 100°C
Dessolve gas: 450°C
Multiplier: 660
Ion energy: 0.2
Resolution setting: 12.0
Dwell time: 0.1s

Supplementary Figure S1. An *in vitro* model of micronutrient adsorption to RRT circuits.

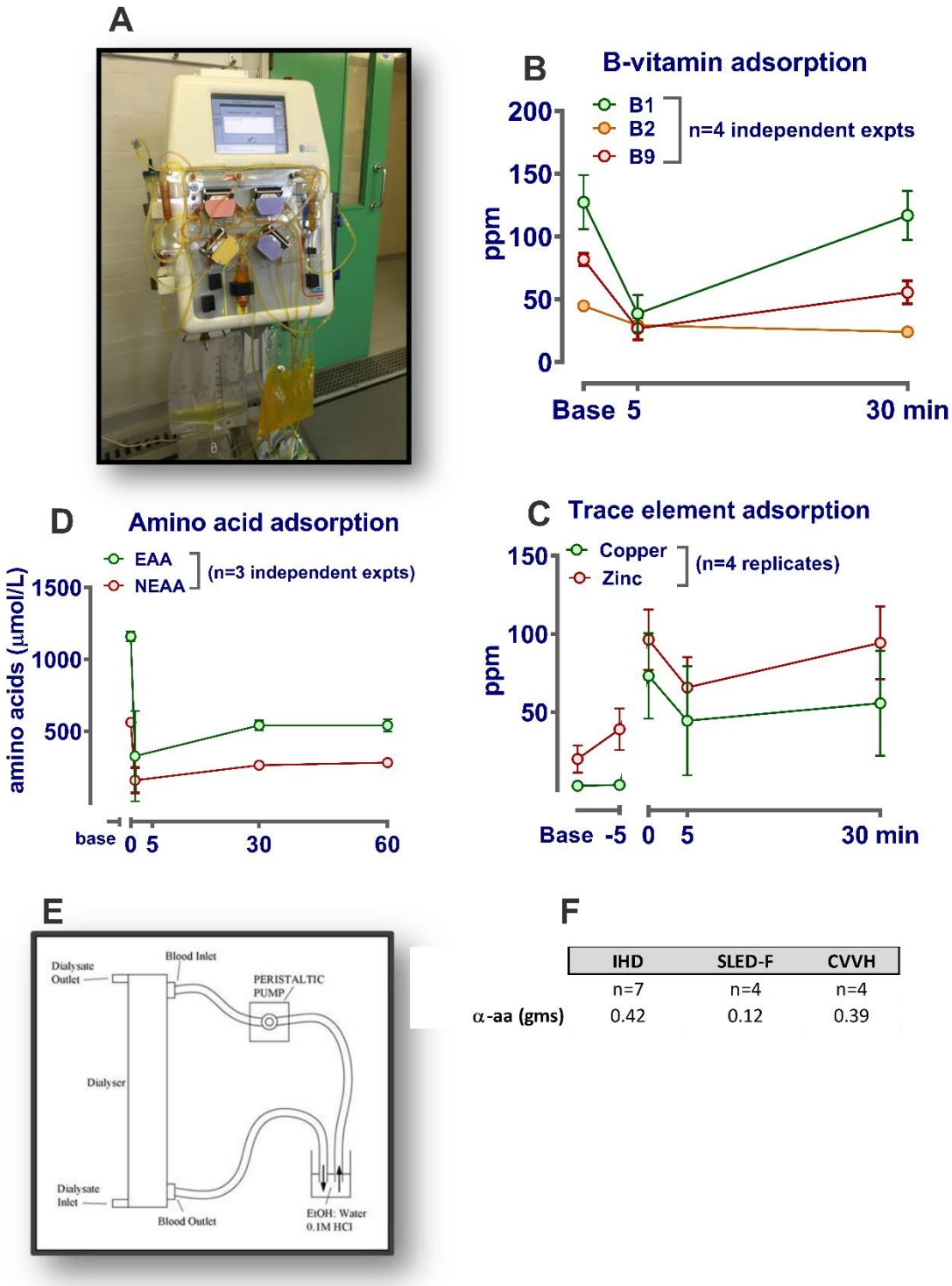

Figure S1: ***Micronutrient adsorption to RRT circuits***: **A;** An HDF440 was primed (30mins) using standard PrismaSol solution before addition of supraphysiological concentration of B-vitamins (**B**), trace elements (**C**) and amino acids (**D**, all purchased from Sigma-Aldrich). Replacement solution was sampled before (Base, -5mins) and after (time zero, 0mins) addition of micronutrients and amino acids and subsequently at 5, 30 and 60mins of 'pseudo'-RRT. Data are mean ± S.E. (standard error of the mean) of 3-4 independent experiments.

E, In a separate experiment n=15 spent filters (IHD & SLED-F, Fx60; CVVH, AN69) were rinsed of blood contamination (for 1-2h with PBS plus 3mM EDTA at 80mls/min) and flushed with an eluting fluid (100 mLs, 50:50 EtOH:Water + 0.1M HCl for 15mins at 80mls/min). The resulting elute (50-80mls) was evaporated to dryness before reconstitution in 1ml amino acid running buffer for measurement of adsorbed amino acids (**F**).

Expanded materials and methods

Clinical study: Regional ethics committee approval and sponsorship from Nottingham University Hospitals NHS Trust were obtained. Patients were eligible for recruitment once a diagnosis of AKI requiring RRT was made. Participants were recruited into the study prior to their first RRT session. Inclusion and exclusion criteria and general study protocol are illustrated in Figure 6. Patients were able to withdraw from the study after consent had initially been given though none did. Signed consent was obtained from each participant after a full explanation has been given, an information leaflet offered and time allowed for consideration. We had ethics committee permission to recruit patients who did not have capacity to consent, in which case we discussed the study with a consultee. The first venous blood sample was drawn from the dialysis catheter prior to RRT commencement, spun at 800g for 5min at 4°C and plasma obtained and stored at -80°C until analysis (baseline plasma). Subsequent venous samples were obtained hourly during IHD, every 1.5 to 2.0 hours during SLEDf session and every 6 hours during CVVH. The 'mid' and 'end' sample were designated as those samples exactly mid-way through treatment (e.g. at 2h for a 4h IHD treatment; at 3h for a 6h SLED-F treatment; at 12h for a 24h CVVH treatment) or the final sample before end of treatment. The first two RRT sessions were included in the study. If the participant required only a single session, no further samples were taken upon completion of the study. Similar to plasma samples, spot-samples of effluent were obtained at regular intervals during the first two RRT sessions from the effluent port of the RRT machine. 'Baseline' effluent was considered as the first sampled dialysate after machine priming had occurred, prior to patient attachment. Further spot-samples of effluent were obtained according to the plasma sampling schedule and were designated as 'mid' or 'end' as appropriate. For CVVH, a sample of the effluent was taken from each discarded bag. Thus the number of samples obtained depended on the number of effluent bags that were changed in one treatment session.

Analysis of free amino acids in plasma and effluent: Plasma (490 µl) was mixed with 10µl internal standard (nor-leucine) and deproteinised by adding 60mg 5-sulfosalicylic acid. After standing at 4 °C for 30 min, samples were centrifuged for 10 min at 16 000 g (4°C). The supernatant was passed through a PVDF Millipore syringe-driven filter unit with 0.22 µm pore size and amino acids measured using a Biochrom 20 amino acid analyser (Pharmacia LKB, Biochrom Ltd, Cambridge, UK) with ninhydrin detection. Peak integration was performed using EZChrom Elite Software (Scientific Software International Ltd, Duston, Northampton, UK). Internal standards were run every 10 samples to check for instrument drift. Values are determined as µmoles/L but for quantification of absolute losses were mass-corrected to µg/L.

Analysis of major and trace elements in plasma and effluent: Elemental analysis was conducted on 500 µl of plasma or effluent after nitric-acid digestion (i.e. biofluid added to 3.0 mL of 70% Trace Analysis Grade (TAG) HNO_3, 2.0 mL H_2O_2 and 3.0 mL milli-Q water [18.2 MΩ cm]; Fisher Scientific UK Ltd, Loughborough, UK). Parameters for inductively coupled plasma-mass spectrometry (ICP-MS; iCAP^{TM} Q, Thermo Fisher Scientific Inc., Waltham, MA, USA) were: using a He collision cell with 'kinetic energy discrimination' to reduce polyatomic interference, we were able to discriminate Ag, Al, As, B, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, S, Sr, Tl, U, V and Zn. Lithium, Be and P were determined in standard (vacuum) mode and Se in 'hydrogen-cell' mode, with in-sample switching. Internal standards were Ge, Rh and Ir. Certified reference materials (CRMs) were included in duplicate

for each ICP-MS batch run and were SeroNorm^{TM} L-2 (REF203105, LOT0903107) and SeroNorm^{TM} L-2 (REF210705, LOT1011645; LGC, Middlesex, UK) for plasma and urine, respectively. Recovery was 93-110% for the majority of major and trace elements. Operational variation was <10% after n=12 independent analyses. Blanks (n = 10) were used to determine operational limits of detection (LOD = 3 × SD for each element). Limits of quantification (LOQ) was determined as 10 × SD. Intra-assay variability for all elements was <2%. For reference and comparison, LOD, LOQ and measured values of ultrapure water, tap-water and filtrate (PrismaSol) are presented in Table 4.

Analysis of B-vitamins in effluent: Samples of effluent fluid were analysed for B-vitamin concentration using liquid chromatography-mass spectrometry (LC-MS). Unknown samples were interpolated from standard curves of each B-vitamin (0.25 to 10 ppm [0.25-10 mg/L). Vitamin B9 (folic acid) was first solubilized in 0.1% NaOH. All B-vitamins were purchased from Sigma-Aldrich (Cheshire, UK). ***Chromatography***: Conditions for liquid chromatography were; using an Agilent 1100 series fitted with a Phenomenex Luna 5U C18 (2) 100A (250 x 3 mm ID; particle size-5 µm) column. Mobile phase A was (H_2O plus 0.1% Formic acid) and mobile phase B was (Acetonitrile plus 0.1% Formic acid). Sample injection volume was 10µl, with a mobile phase flow rate of 0.5 ml/min. Chromatography was run using a pre-programmed linear gradient of mobile phase A: mobile phase B i.e. from 0-10 min, A: B 100:0 (v/v) to A: B 50:50 (v/v); Linear gradient from 10-12.5 min, A: B 50:50 (v/v) to A: B 0:100 (v/v); the gradient was kept constant from 12.5-15 min, at A: B 0:100 (v/v); linear gradient from 15-15.4 min, A: B 0:100 (v/v) to A: B 100:0 (v/v); kept constant from 15.4 min to 22 min, at A: B 100:0 (v/v). This was to equilibrate the column between samples. ***Mass spectrometry:*** Samples were analysed using a Micromass Ultima, (Manchester, UK). Conditions were operated in positive ion electrospray mode for all B-vitamins with the exception of B9, which was run in negative ion mode. The capillary voltage was set to 3.5 kV, cone voltage was 50V and multiplier at 660V. The source temperature and dissolvation gas temperature was set to 100°C and 450°C, respectively. Single-ion recording (SIR) of mass-to-charge ratio (m/z) were obtained for B1 (thiamine hydrochloride, m/z 265.25, molecular weight 337.27 g/mol), B2 (riboflavin, m/z 377.28, molecular weight 376.36 g/mol), B3 (nicotinic acid, m/z 123.10, molecular weight 123.109 g/mol), B6 (pyridoxal hydrochloride, m/z 170.15, molecular weight 203.62 g/mol, B9 (folic acid, m/z 440.45, molecular weight 441.4 g/mol) and B12 (cyanocobalamin, m/z 679.72, molecular weight 1356.123 g/mol). Dwell time for each ion was 0.20s. Further information is given in Supplementary Table S2.

Statistical Analysis

Spot comparisons between patients receiving different types of dialysis were by analysis of variance (ANOVA) for normally distributed continuous data (e.g. plasma creatinine at admission) or by Kruskall-Wallis ANOVA for skewed data (e.g. C-reactive protein at admission). Continuous data are presented as means with standard deviation (1 SD) or standard error of the mean (1 SE) or estimated standard error of the differences between means (s.e.d.) where appropriate, as indicated in the text. 95% confidence intervals may be approximated from the data as the mean ± 2 s.e.d. Comparison of categorical or binomial data between treatment groups (e.g. proportion of patients with diabetes at admission) was by logistic regression, assuming a binomial (yes or no) error distribution. Categorical data are presented as mean proportions (i.e. number [percent of total]). Comparison of treatment groups in which the data naturally followed an ordinal scale (e.g. measures of nutritional status;

mild-moderate-severe) then ordinal logistic regression was used. Repeated measurements from the same individual (e.g. plasma or effluent amino acids at the start and end of RRT) were analyzed with time as a within-subject fixed effect by RM-ANOVA, including appropriate correction (i.e. included as a co-variate in the analysis) for dialysis dose (e.g. urea reduction ratio, [URR] or solute removal index [SRI]) and baseline plasma concentration of micronutrient. Kt/V is widely used as a measure of dialysis dose for patients with CKD, receiving dialysis regularly but is inappropriate for AKI patients. As appropriate, unbalanced or skewed data were $\log_{10}$ transformed prior to analysis. Any time-series analyses in which there were a few missing data points were analysed by restricted maximum likelihood (REML) with the subject as a nested, random effect. To explore any potential relationships between fixed effects and ordinal categorical data (e.g. nutritional status) logistic generalised estimating equations were used, adjusted for potential covariates (e.g. body weight, initial blood pressure and/or serum creatinine). Appropriateness of each statistical comparison was assessed by visualising histograms of residuals and further residual (on y-axis) plots of 1) fitted-values and 2) expected normal quantiles. We considered $P \leq 0.050$ as indicating statistical significance. The appropriate experimental *n* for each comparison and statistical method used is indicated in each Table and Figure legend. All data were analysed using Genstat v17 (VSNi, Rothampsted, UK).

Estimation of dose of dialysis: Kt/V is presented in Table 3 but is considered an inappropriate correction factor for patients with AKI, as oppose to patients with CKD receiving dialysis regularly, often x3/week, which is built into the equations for Kt/V. Hence for estimation for blood-based kinetics we used the urea reduction ratio (Eq 1 below) and for efluent-based kinetics we used the solute removal index (Eq 2 below)

Equation 1:

$$URR = \frac{[U_{pre}] - [Upost]}{[Upr_e]} \times 100\% \quad \text{e.g.} \frac{[55.1] - [22.1]}{[55.1]} \times 100\% = 60\%$$

Equation 2:

$$SRI = \frac{[U_{effluent}]}{[Ubo_{dy}]} \times 100\% \quad \text{e.g.} \frac{[1449]}{[2870]} \times 100\% = 51\%$$

$U_{effluent}$ = effluent volume [L] ×effluent urea [mmol/L]
U_{body} = total body water [TBW, L] ×plasma urea [mmol/L]
TBW = body weight (kg) ×0.60 [♂] or 0.55 [♀]

A priori power calculation for sample size: The clinical study was powered using data from a pilot study involving n=5 patients per type of RRT, measuring 21 different amino acids in plasma and effluent. Corrected for baseline plasma concentration (IHD, 1513 ± 403; SLEDf, 1894 ± 471; CVVH, 3141 ± 267 µmol/L; P=0.06) and RRT dose using SRI (IHD, 0.28 ± 0.04; SLEDf, 0.30 ± 0.04; CVVH, 0.45 ± 0.09 units; P=0.06) we calculated mean amino acid losses (IHD, 6746; SLEDf, 9420; CVVH, 10792 mgs), between-individual S.E.M. = 3848; within-individual S.E.M. = 861. Setting α at 0.05 and experimental power at 90% required n=9 patients per treatment (size of the difference to be detected, 2674 [9420-6746], between subject variance = 107900000; within subject variance = 3227000). However, accounting for multiple comparisons (e.g. separate analysis of 20 amino acids) and patients with varied co-morbidities,

we adjusted α to 0.0025. This increased our sample size to n=17 patients per treatment. Allowing for unforeseen increased variance in outcomes and possible attrition we aimed to recruit n=24 patients per group (n=72 patients in total). Full data were available for analysis on n=27 IHD, n=12 SLEDf and n=21 CVVH patients. Correct n-numbers are indicated on each figure and table.