Supplementary appendix

Catabolism in critical illness: a reanalysis of the REDOXS trial

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Detailed methods

Study design

This study was not a pre-specified analysis of the REDOXS trial. The hypothesis that urea-to-creatinine ratio (UCR) is biochemical signature of catabolism and is associated with death builds on previous published work by this group.[1] The determinants of UCR were postulated and interaction with mortality decided pre analysis using a directed acyclic graph (DAG) (Figure S1). The incorporation of various methods strengthened causal investigations and allowed mechanistic inferences to be made. The REDOXS trial and protocol are published. We had access to the complete study database. We adhered to covariate adjustment sets that were predefined by the DAG in subsequent modelling.

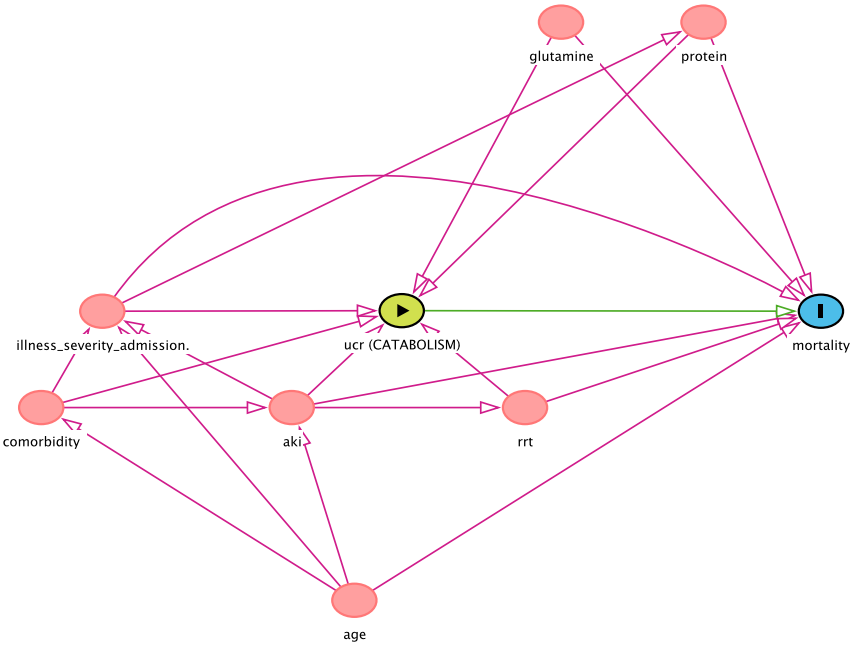
Data management first involved inspection of data completeness using base R functions and the *tidyverse* packages[2]. UCR values were inspected for missingness. Over 99% of patients had 2 or more UCR values. We expected change in UCR to be greater for the glutamine group due to the finding of increase urea generation in the original REDOXS study.

Directed Acyclic Graphs

A directed acyclic graph (DAG) for UCR and mortality (outcome) was constructed (Figure S1). Green arrows represent a causal path. Red arrows represent a confounding path. The blue oval represents the primary outcome. Red markers are ancestors of both exposures and outcomes. (http://dagitty.net)[3] We elaborated the DAG to estimate the effect of UCR on mortality in a survival analysis. Minimal sufficient adjustment sets included:

- age, acute kidney injury (AKI), glutamine, illness severity at admission, protein, renal replacement therapy (RRT)
- AKI, comorbidity, glutamine, illness severity at admission, protein, RRT

Figure S1.



The DAG was constructed using the daggity.net website and the following code:

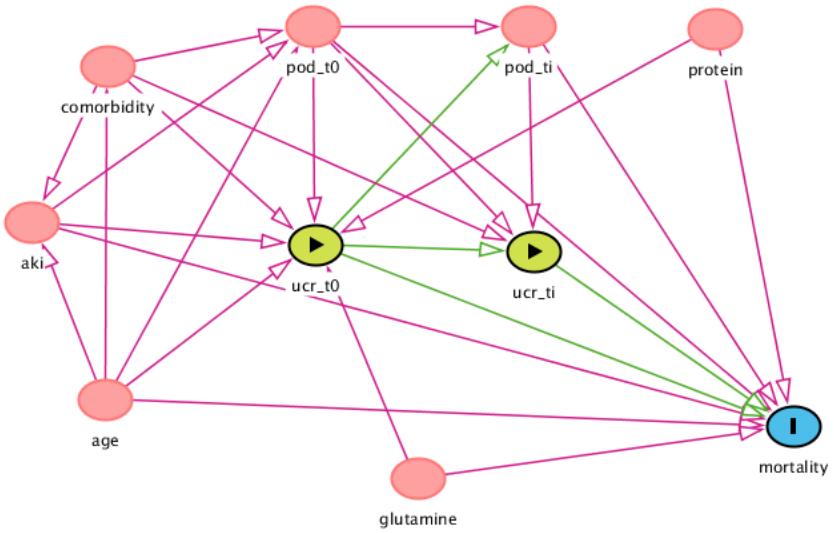
```
dag {
age [pos="-0.567,-0.047"]
aki [pos="-0.607,-0.049"]
comorbidity [pos="-0.767,-0.049"]
glutamine [pos="-0.435,-0.053"]
illness_severity_admission. [pos="-0.728,-0.050"]
mortality [outcome,pos="-0.269,-0.050"]
protein [pos="-0.344,-0.053"]
```

```
rrt [pos="-0.458,-0.049"]
ucr [exposure,pos="-0.460,-0.050"]
age -> aki
age -> comorbidity
age -> illness_severity_admission.
age -> mortality
aki -> illness_severity_admission.
aki -> mortality
aki -> rrt
aki -> ucr
comorbidity -> aki
comorbidity -> illness_severity_admission.
glutamine -> mortality
glutamine -> ucr
illness_severity_admission. -> mortality [pos="-0.595,-0.053"]
illness_severity_admission. -> protein
protein -> mortality
protein -> ucr
rrt -> mortality
rrt -> ucr
ucr -> mortality
}
```

A second DAG was constructed for the marginal structural model to incorporate changing organ failure of the time course of critical illness (Fig S2). Illness severity at admission and RRT are replaced by persistent organ dysfunction (POD). Both UCR and POD are considered at baseline and then across multiple time-points (ti). Minimal adjustment set included:

- Age, AKI, glutamine, persistent organ dysfunction, protein

Figure S2.




```
dag {
age [pos="-0.698,-0.050"]
aki [pos="-0.731,-0.051"]
comorbidity [pos="-0.697,-0.052"]
glutamine [pos="-0.559,-0.050"]
mortality [outcome,pos="-0.392,-0.050"]
pod_t0 [pos="-0.606,-0.053"]
pod_ti [pos="-0.510,-0.053"]
protein [pos="-0.427,-0.053"]
```

```
ucr_t0 [exposure,pos="-0.605,-0.051"]
ucr_ti [exposure,pos="-0.508,-0.051"]
age -> aki
age -> comorbidity
age -> mortality
age -> pod_t0
age -> ucr_t0
aki -> mortality
aki -> pod_t0
aki -> ucr_t0
comorbidity -> aki
comorbidity -> pod_t0
comorbidity -> ucr_t0
comorbidity -> ucr_ti
glutamine -> mortality
glutamine -> ucr_t0
pod_t0 -> mortality
pod_t0 -> pod_ti
pod_t0 -> ucr_t0
pod_t0 -> ucr_ti
pod_ti -> mortality
pod_ti -> ucr_ti
protein -> mortality
protein -> ucr_t0
ucr_t0 -> mortality
ucr_t0 -> pod_ti
ucr_t0 -> ucr_ti
ucr_ti -> mortality
}
```

Variable selection

The DAG was constructed from all authors input and based on previous work[1 4] and credible biological mechanisms. UCR is a potential biochemical signature of catabolism but is influenced by several biological pathways in critical illness that have potential or proven associations with mortality. Protein delivery results in an increase in intravenous amino acids with a rise in ureagenesis.[5] The amount of protein delivered to critically ill patients is under investigation in large RCTs with observational data suggesting increase protein may improve patient outcomes.[6 7] We calculated the mean daily protein delivered during the ICU stay in g/kg. The change in both urea and creatinine concentrations can be affected by changes in kidney function as excretion of both is decreased by kidney dysfunction, thus lowering the UCR. For patients receiving RRT, the rise in UCR is dampened due to the equimolar removal of creatinine and urea. Use of RRT at any point during the ICU stay was defined as a binary variable. We used the REDOXS definition of kidney dysfunction at admission. Poor premorbid health is associated with low serum creatinine at ICU admission and poor outcomes. In addition, increasing age is associated with reduced muscle mass and worse outcomes[8]. Age and the Charlson comorbidity index were included in the DAG due to the association of both with low muscle mass. Finally, severity of illness was included due to its association with increased muscle catabolism and is impact on ICU survival.[9]

For the second DAG, persistent organ dysfunction was incorporated as a time-dependent variable. For each day of the REDOXS patients were allocated a point for each organ failure (vasopressor requirement, mechanical ventilation, kidney replacement therapy).

Linear mixed effects modelling

After data processing we inspected the relationship between logUCR and time using restricted cubic splines which revealed a higher rate of increase of UCR from day 1 to 7 compared to the subsequent days 8 to 30. In addition, change over the first 7 days was greatest for the glutamine group. To accommodate this relationship an interaction was included between days and glutamine. Predicted values of logUCR were produced from the final model to understand the effects of candidate variables in determining logUCR including; glutamine, RRT, and protein delivered. We used the *nlme*[10] and *ggeffects*[11] packages to build models for the effects plots.

Joint modelling

Firstly, a Cox model was constructed. The elaborated DAG specified variables that tested the effect of UCR on mortality were included in the Cox analysis. 30-day mortality was chosen as the covariate history of UCR measurements were only available for days 1 to 30. Secondly, the linear mixed effects model was specified for the joint model. The JMbayes package uses JAGS version 4.3.0 engine for estimation of posterior means using the default settings (iterations: 28000; adapt: 3000; burn-in: 3000; thinning: 50). Model diagnostics were done by visual inspection of the diagnostic plots. The results are presented as hazard ratios (HR) with corresponding two-sided 95% credible intervals (CrIs).

Missing data

There were no missing data for baseline covariates in cox and joint modelling. 13 659 urea-to-creatinine observations were made over 30-days and were modelled as a continuous variable after log-transformation. Of the 13 659 study days with UCR measurements, there were 3353 missing daily protein measurements. We performed a sensitivity analysis (table S7) to ensure there were no systematic changes in joint model estimations when calculating average daily protein calculated from the first 7 days of the REDOXS study (639 missing protein measurements) compared to the 30-day study period.

Marginal structural modelling

We used the R package *IPW*[12] for the marginal structural analysis. The adjustment set was based on the modified DAG in figure S2. We calculated stabilised patient specific weights (inversed probability weights) that represent the cumulative risk of UCR exposure for each patient. Figure S3 shows two hypothetical patients with different organ failure profiles over time adapted from Klein et al[13].

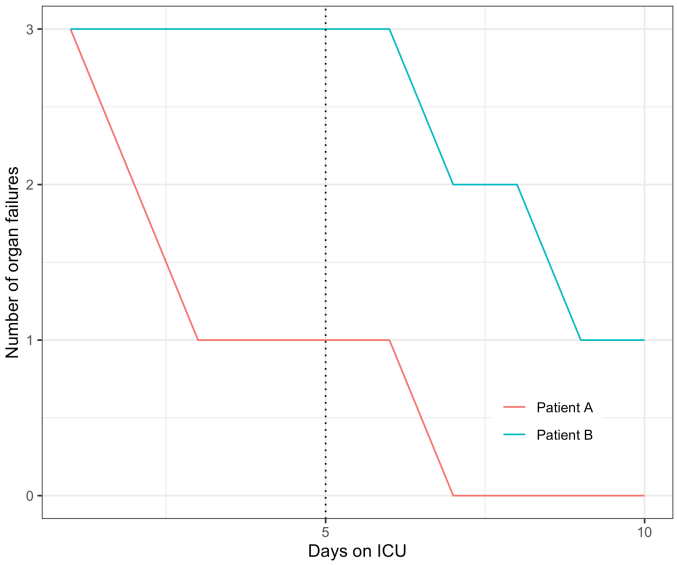


Figure S3. Evolution of organ failure before exposure to high UCR in two hypothetical patients admitted to the intensive care unit. Both patients have the same number of organ failures at admission, but patient A improves with less organ failures over time, patient B remains in multi-organ failure. As catabolism (and raised UCR) is more likely to develop in patients with ongoing organ failure, confounding may occur when organ failure after baseline is not adjusted for. Our joint models adjust for baseline variables only. Our marginal structural model adjusts for changes in organ failure status until the exposure to a high urea-to-creatinine ratio (vertical dotted line).

List of Investigators and Participating Sites

Number in parentheses refers to number of patients enrolled at each site.

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Asklepios Clinics Hamburg-Altona, Department of Anesthesiology and Intensive Care Medicine, Hamburg (6): Hanswerner Bause, Philip Gabriel, Axel Prause, Cornelia Wolf

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Supplementary tables

Table S1. Baseline characteristics and demographics stratified by 30-day mortality.

	Stratification by 30-day mortality	
	Alive at day 30 (n=855)	Dead by day 30 (n=166)
Characteristics		
Arm (%)		
Glutamine	403 (47.1)	89 (53.6)
Age (mean (sd))	61.6 (14.4)	67.78 (14.00)
Female - no. (%)	346 (40.4)	62 (37.3)
BMI (mean (sd))	30.2 (8.6)	28.4 (8.0)
APACHE II score (median [IQR])	25.0 [21.0, 30.0]	27.0 [22.0, 32.0]
Charlson Comorbidty index (median [IQR])	1.0 [0.0, 2.0]	1.0 [1.0, 3.0]
Cause of shock (%)		
Cardiogenic	166 (19.4)	29 (17.5)
Septic	570 (66.7)	123 (74.1)
Neurogenic	8 (0.9)	1 (0.6)
Anaphylactic	2 (0.2)	0 (0.0)
Not in shock	23 (2.7)	3 (1.8)
Other	11 (1.3)	0 (0.0)
Uncertain origin	35 (4.1)	8 (4.8)
Haemorrhagic	40 (4.7)	2 (1.2)
Baseline SOFA score (median [IQR])	8.0 [7.0, 10.0]	8.0 [7.0, 10.0]
Maximum SOFA score (median [IQR])	10.0 [9.0, 13.0]	12.0 [10.0, 14.0]
REDOXS inclusion criteria no. (%)		
Pao2:fio2 ratio ≤300 (respiratory failure)	804 (94.0)	157 (94.6)
Clinical evidence of hypoperfusion	789 (92.3)	157 (94.6)
Kidney dysfunction	295 (34.5)	67 (40.4)
Platelets ≤50 x10^9/L	36 (4.2)	7 (4.2)

Data are median [IQR] or n (%). ICU intensive care unit. Body mass index (BMI) is the weight in kilograms divided by the square of the height in metres.
The Acute Physiology and Chronic Health Evaluation II (APACHE II) score ranges from 0-71. The Sequential Organ Failure Assessment (SOFA) score ranges from 0-24.

Table S2. For the longitudinal sub-model predicting the log of urea-to-creatinine at time t (log(UCR)t), posterior mean of parameters (ß) and related 95% credible intervals (95% CrI). Natural cubic spline with 3 knots specified for day number and interaction term with glutamine.

Covariates	ß (95% CrI)
(Intercept)	3.825 (3.722, 3.928)
ns(Day number), 1	0.259 (0.174, 0.352)
ns(Day number), 2	0.613 (0.514, 0.717)
ns(Day number), 3	0.139 (0.022, 0.251)
Glutamine	0.021 (-0.351, 0.081)
Age, years	0.007 (0.005, 0.008)
Kidney replacement therapy	-0.423 (-0.473, -0.374)
Protein, g/kg/day	0.167 (0.119,0.215)
Kidney dysfunction	-0.066 (-0.114, -0.019)
ns(Day number), 1:Glutamine	0.024 (-0.103, 0.158)
ns(Day number), 2:Glutamine	1.127 (0.986, 1.260)
ns(Day number), 3:Glutamine	0.384 (0.213, 0.562)

Table S3. Mediation analysis. Decomposition of glutamine effect on mortality by day 7 urea-to-creatinine ratio (mediator). Models were adjusted for mediator-outcome confounders (age, kidney replacement therapy, baseline SOFA, protein received [g/kg/day], and kidney dysfunction). HR, hazard ratio; CI, confidence interval. Analysis was performed using the *regmedint* R package. https://CRAN.R-project.org/package=regmedint

	HR estimate (95% CI)	% (95% CI)	P value
Total natural indirect effect	1.20 (1.04–1.38)	-	0.014
Total natural direct effect	0.90 (0.62–1.30)	-	0.566
Total effect	1.16 (0.85–1.58)	-	0.331

Table S4. Results of sensitivity analysis using median protein over first 7 days of admission in the joint model for effect of changes in longitudinal urea-to-creatinine ratio on hazard ratios.

Variables	Joint model (UCR value)
Adjustment factors:	
Baseline covariables	Yes
Time-varying UCR	Yes
Evolution of organ failure over time	No
Effect estimate*	2.19 (1.70–2.84)

* Effect estimate is for two-fold increase in time-varying urea-to-creatinine ratio with 95% credible intervals.

Supplementary figures

Figure S4. Patient flow and joint model analysis summary.

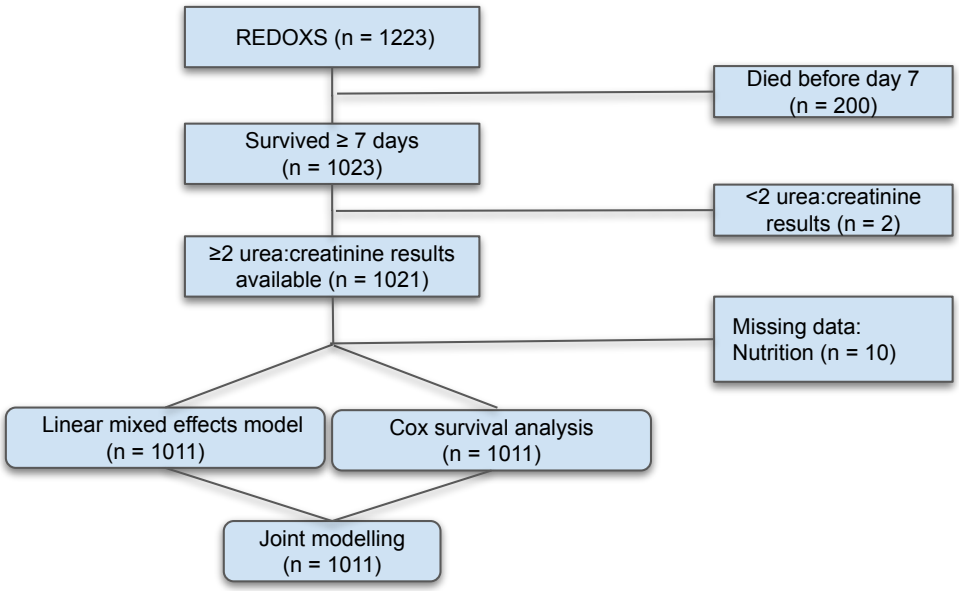


Figure S5. Effects plots of predicted log urea-to-creatinine ration for (A); the whole cohort over the time course of the REDOXS study, (B); glutamine and no glutamine groups, (C); renal replacement therapy, and (D); a range of values for mean protein g/kg/day received. Bands represent prediction intervals. Dots represent original raw data points.

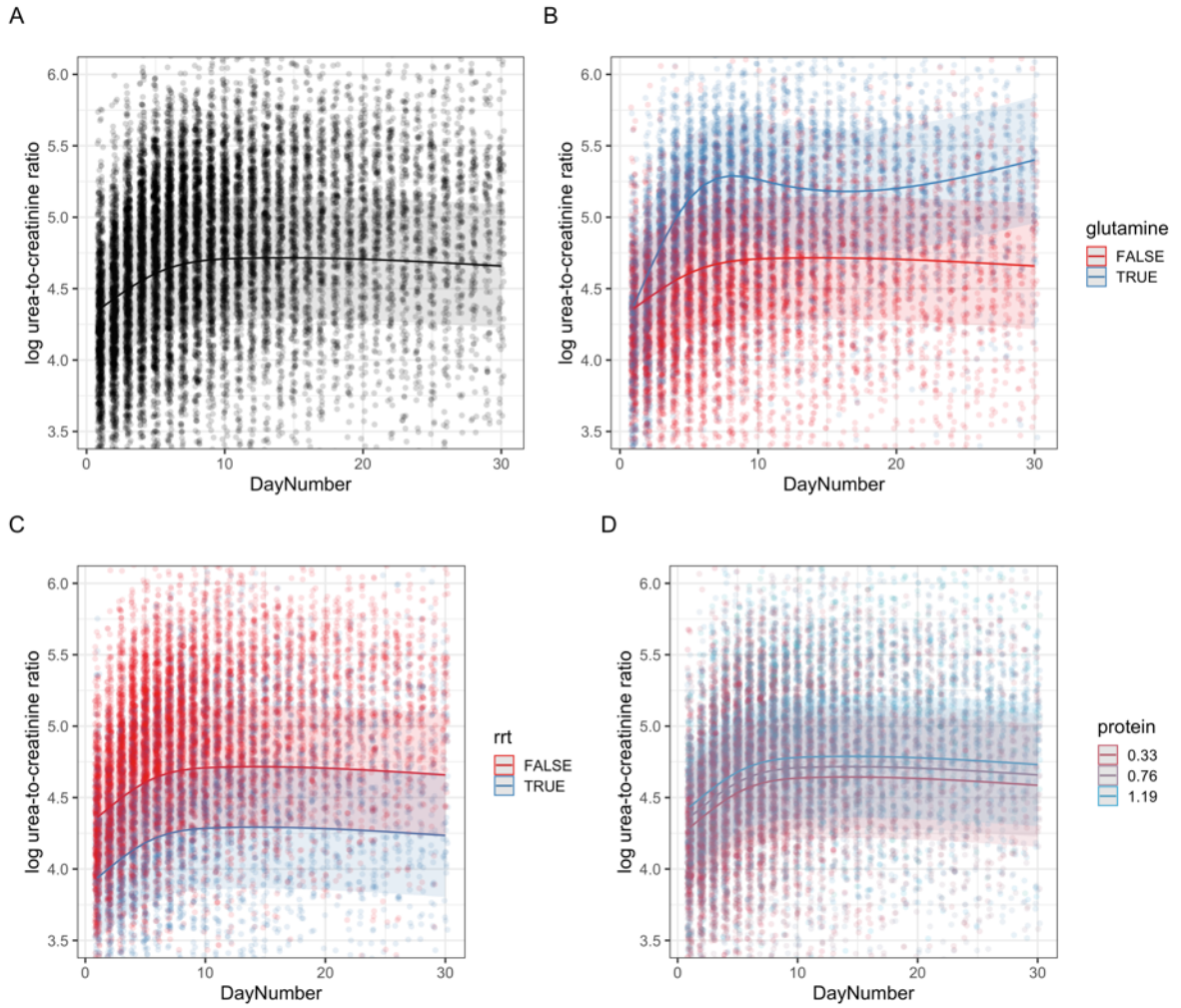
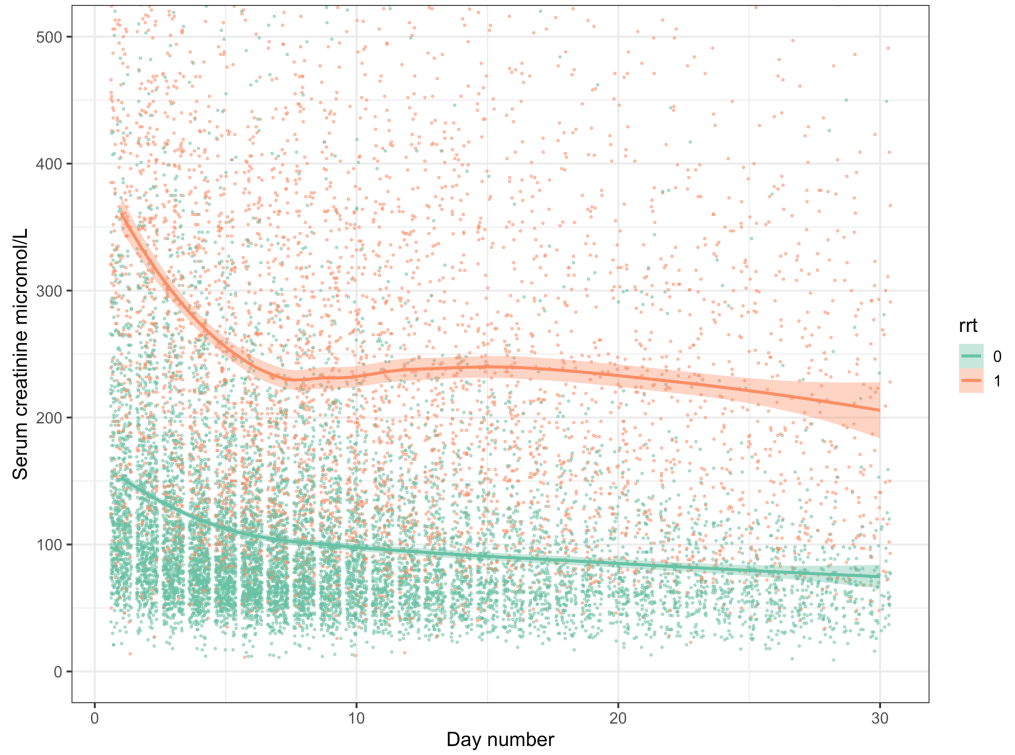
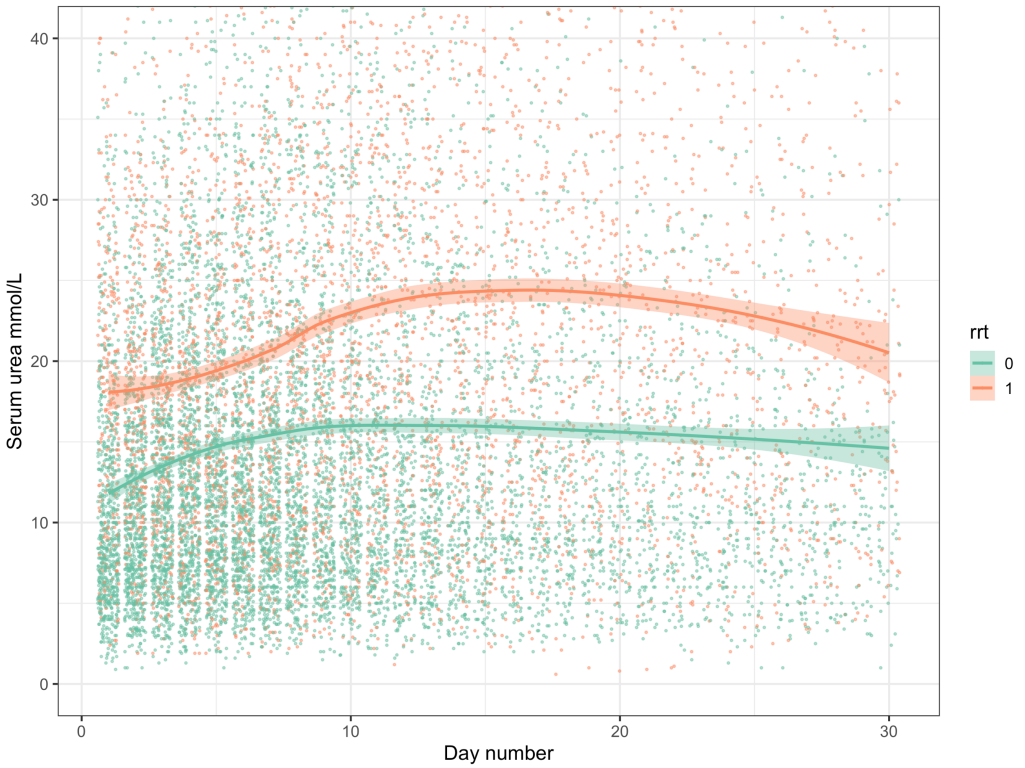


Figure S6. Trajectories of creatinine (A) and urea (B). Trend line and confidence intervals using loess smoother.

A



B



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