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ORIGINAL RESEARCH

Preoperative Short-Term Restriction of Sulfur-Containing Amino Acid Intake for Prevention of Acute Kidney Injury After Cardiac Surgery: A Randomized, Controlled, Double-Blind, Translational Trial

Thomas Osterholt, MD#; Claas Gloistein, MD#; Polina Todorova, MD; Ingrid Becker; Katja Arenskrieger, MD; Ramona Melka; Felix C. Koehler, MD; Michael Faust, MD; Thorsten Wahlers, MD; Thomas Benzing, MD#; Roman-Ulrich Müller, MD; Franziska Grundmann, MD*; Volker Burst, MD*

BACKGROUND: Acute kidney injury (AKI) is a major risk factor for chronic kidney disease and increased mortality. Until now, no compelling preventive or therapeutic strategies have been identified. Dietary interventions have been proven highly effective in organ protection from ischemia reperfusion injury in mice and restricting dietary intake of sulfur-containing amino acids (SAA) seems to be instrumental in this regard.
The UNICORN trial aimed to evaluate the protective impact of restricting SAA intake before cardiac surgery on incidence of AKI.

METHODS AND RESULTS: In this single-center, randomized, controlled, double-blind trial, 115 patients were assigned to a SAA-reduced formula diet (LowS group) or a regular formula diet (control group) in a 1:1 ratio for 7 days before scheduled cardiac surgery. The primary end point was incidence of AKI within 72 hours after surgery, secondary end points included increase of serum creatinine at 24, 48, and 72 hours as well as safety parameters. Quantitative variables were analyzed with nonparametric methods, while categorical variables were evaluated by means of Chi-square or Fisher test. SAA intake in the group with SAA reduced formula diet was successfully reduced by 77% (group with SAA reduced formula diet, 7.37[6.40–7.80] mg/kg per day versus control group, 32.33 [28.92–33.60] mg/kg per day, $P<0.001$) leading to significantly lower serum levels of methionine. No beneficial effects of SAA restriction on the rate of AKI after surgery could be observed (group with SAA reduced formula diet, 23% versus control group, 16%; $P=0.38$). Likewise, no differences were recorded with respect to secondary end points (AKI during hospitalization, creatinine at 24, 48, 72 hours after surgery) as well as in subgroup analysis focusing on age, sex, body mass index and diabetes.

CONCLUSIONS: SAA restriction was feasible in the clinical setting but was not associated with protective properties in AKI upon cardiac surgery.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique Identifier: NCT03715868.

Key Words: acute kidney injury ■ cysteine ■ dietary intervention ■ methionine ■ sulfur-containing amino acid restriction

Correspondence to: Franziska Grundmann, MD, Department II of Internal Medicine, University of Cologne, Kerpener Str. 62, 50937 Cologne, Germany. Email: franziska.grundmann@uk-koeln.de

#T. Osterholt, C. Gloistein contributed equally.

*F. Grundmann, and V. Burst contributed equally.

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CLINICAL PERSPECTIVE

What Is New?

- To our knowledge, the UNICORN trial is the first study assessing the potential benefit of restricting sulfur-containing amino acids for organ protection in humans.
- In this single-center, randomized, controlled, double-blind trial a 7-day restriction of sulfur-containing amino acids did not ameliorate acute kidney injury after cardiac surgery.

What Are the Clinical Implications?

- A deeper mechanistic insight is necessary to translate the findings elicited by cysteine and methionine restriction in animals into the human setting.
- As this study cannot report a beneficial effect of restriction of sulfur-containing amino acids on acute kidney injury in humans, further studies should rather focus on pharmacological approaches that activate underlying, evolutionary conserved mechanisms of enhanced stress resistance.

Nonstandard Abbreviations and Acronyms

AKI	acute kidney injury
CG	control group
CR	calorie restriction
IGFBP7	Insulin Like Growth Factor Binding Protein 7
SAA	sulfur-containing amino acids
TIMP-2	Tissue inhibitor of metalloproteinases 2
TSP	transsulfuration pathway

With an incidence of up to 40%, acute kidney injury (AKI) is a frequently encountered complication after cardiac surgery.[1–3] Even mild cases are associated with a longer hospital stay, higher morbidity, and increased short-term as well as long-term mortality.[4–6] Despite several clinical trials using pharmacological agents, the quest for an effective reno-protective treatment has not been successful to date.[1] In recent years, promising and innovative nonpharmacological strategies to enhance cellular resilience have emerged—remote ischemic preconditioning being the most prominent. However, the results of several trials investigating remote ischemic preconditioning in diverse clinical settings have remained equivocal.[7–11] As another nonpharmacological approach, short-term dietary restriction has been shown to induce robust cellular stress resistance in various species ranging from yeast to rhesus monkeys leading to reduced organ damage susceptibility.[12,13] Mitchell and co-workers were the first to show that a reduction of daily calorie intake by 30% over short time periods prevented AKI in a murine ischemia–reperfusion injury model.[14] Accumulating evidence suggests that restriction of macronutrients rather than calorie restriction itself may be pivotal in this context.[15–17] Mechanistically, increased hydrogen sulfide (H_2S) production via the transsulfuration pathway (TSP) by altered dietary composition is believed to be one of the central mediators.[18] The TSP can be effectively and reliably activated by reducing the intake of the sulfur-containing amino acids (SAA).[19] Dietary restriction of SAA, therefore, is an attractive target to elicit organo-protective effects, while limiting the risk of malnourishment as compared with calorie restriction (CR).[19,20]

Experimental studies examining the influence of SAArestriction demonstrated increased longevity,[21–23] increased hepatic stress resistance,[24] reduction of blood pressure,[25] prevention of neurodegeneration,[26] and inhibition of cancer cell growth as well as enhanced efficacy of chemotherapeutic agents.[27–30] Koehler et al. showed that preconditioning rodents with low-SAA diets led to significantly improved survival after ischemic AKI.[31]

Since diets containing individual amino acids or peptides are hardly palatable, a feasible way to reduce the intake of methionine and cysteine can be accomplished by using a collagen-based protein source instead of milk as it contains a different amino acid profile with predominantly low SAA concentrations.

Until now, no clinical trials examining the putative organo-protective features of dietary SAA reduction have been undertaken. Here, we present the results of the UNICORN trial, a first-in-human translational study investigating the effect of SAA reduction on the rate of AKI postsurgery. In this randomized controlled trial, we test the hypothesis that a 7-day preoperative diet with low SAA content (collagen-based protein source) reduces the incidence of AKI within 72 hours after cardiac surgery compared with a commonly used formula diet with a milk-based protein source containing SAA.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Participants

This trial was designed and conducted as a randomized, controlled, double-blind, single-center study at the University Hospital Cologne. Approval was obtained

from the Institutional Review Board of the University Hospital Cologne (No. 18-262). Adult patients with age >50 years, scheduled for cardiac surgery involving cardiopulmonary bypass with a lead time of at least 9 days were enrolled after having obtained written informed consent. Exclusion criteria were chronic renal replacement therapy, kidney transplantation, vegetarian lifestyle, body mass index <18.5 kg/m^2, calorie-reduced diet within the preceding 4 weeks, underlying wasting disease, uncontrolled local or systemic infection, contraindication for enteral nutrition, known allergy to or intolerance of the ingredients of the utilized diets, pregnancy or breastfeeding, absence of safe contraceptive measures in premenopausal women, participation in other interventional trials, a dependency/employment relationship with the investigators, and accommodation in an institution by judicial or administrative order.

The study was conducted in accordance with the Declaration of Helsinki and the good clinical practice guidelines of the International Conference on Harmonization. The study protocol is registered on www.clinicaltrials.gov (NCT03715868).

Randomization and Blinding

Eligible patients were assigned to 2 arms in a 1:1 ratio, stratified by age (≤/>70 years), using a randomization list assigning blinded medication identification numbers to patients. Randomization was provided by the Institute of Medical Statistics and Computational Biology of the University of Cologne. Assignment to either group was blinded to patients as well as study personnel. In the intervention group with SAA reduction (LowS group), patients were provided with a collagen-derived protein-based formula diet (LowS diet), patients in the control group (CG) received Fresubin Energy Fiber Drink, a standard formula diet containing cow milk protein. Both formula diets were provided by Fresenius Kabi (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany), the LowS diet was developed by Fresenius Kabi exclusively for this trial. All formula diets were supplied by Fresenius Kabi as powders in standardized sachets containing 300 kcal each; sachets were labeled with corresponding identification numbers according to the randomization list.

Procedure

At enrollment, anthropometric parameters, medication, and medical history were assessed and recorded. After randomization, patients were instructed to replace their usual diet entirely by the provided formula diet from day 7 before the scheduled date of surgery (i.e., day –7) until surgery.

Participants were advised to ingest an appropriate number of sachets to consume at least 20 kcal/kg body weight but ideally ≥25 kcal/kg per day to prevent 1) malnutrition, and 2) interfering effects resulting from calorie restriction. All formula diet drinks had an energy density of 1.5 kcaL/mL and were delivered as an instant powder to be mixed with water. Details on the composition of the LowS diet are depicted in Table S1. Apart from the amino acid profile, the macronutrient composition did not differ between the t groups. In the LowS group, the total SAA intake corresponded to 55% of the daily SAA intake recommended by the World Health Organization (15 mg/kg per d) (Table 1).[32] The patients were urged to avoid consumption of food other than the formula diet. However, to allow for a better adherence, a list of acceptable beverages and snacks (ie, containing no or very little SAA) was provided (Table S2). Nutritional supplements containing any amino acids were paused for the duration of the study. Both formula diets were low in salt (~0.15 g per 100 kcal). To not interfere with steady-state salt and water homeostasis, 3 g of NaCl per day were substituted via salt tablets in both groups which resulted in an estimated overall intake of 5 to 7 g NaCl per day. Patients were provided with a diet diary for daily documentation of the amount of consumed formula diet as well as additional food and drinks. The nutritional intervention was conducted in an outpatient setting and regular telephone calls were performed to ensure protocol adherence.

On the day of admission (day –1), participants were asked to return unused sachets to the trial site for compliance check and review. The diet was continued until the usual food and fluid fasting before surgery. If, however, the scheduled date of surgery was postponed, the duration of the diet phase was extended until the day of the newly scheduled intervention.

Baseline laboratory parameters were obtained in the morning of day 0 (day of surgery). After surgery, trial-related blood samples were obtained at 24, 48, and 72 hours after the onset of intraoperative ischemia (cross-clamping). Urine samples were obtained at 4

Table 1. Characteristics of Administered Formula Diets

Amino acid	LowS group [g/100 mL]	Control group [g/100 mL]
Methionine	0.042	0.158
Cysteine	0.002	0.033
Total SAA	0.044	0.191
Calorie intake	**Total SAA intake LowS group [mg/kg per d]**	**Total SAA intake Control group [mg/kg per d]**
20 kcal/kg per d	8.3*	22.5
25 kcal/kg per d	10.3*	28.5

Amino acid concentration per ml of formula diets and calculated total daily SAA-intake depending on daily calorie intake; d indicates day; g, gram; kg, kilogram; mL, milliliter; and SAA, sulfur-containing amino acids.
*WHO recommendation SAA intake: 15 mg/kg per d.

and 24 h after cross clamping. Hourly urine output was assessed as long as a Foley catheter was in place. Biosampling of blood and urine samples for analysis of biomarkers was performed. A follow-up telephone call was performed at 6 months after discharge to evaluate mortality as well as need for renal replacement therapy within 180 days after surgery.

Outcomes

As the primary end point, rate of AKI within 72 hours after surgery was assessed. AKI was defined according to the Kidney Disease: Improving Global Outcomes guidelines[33] as an increase in serum creatinine of ≥0.3 mg/dL within 48 hours, or an increase of serum creatinine to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days, or a reduction of urine output to <0.5 mL/kg per hour for >6 hours.

Secondary end points were absolute and relative increase of serum creatinine at 24, 48, and 72 hours after cardiac surgery ("cross clamping"). Furthermore, incidence of AKI until discharge, AKI classification (I, II, III, according to Kidney Disease: Improving Global Outcomes), maximum serum creatinine detected during hospitalization, need for renal replacement therapy, in-hospital mortality, length of hospital stay, and length of stay on intensive care unit were assessed. The following biochemical markers were measured: CRP, white blood cell count, creatine kinase, troponin T, lactate dehydrogenase, NT-proBNP (N-terminal pro-brain natriuretic peptide), lactate, amino acid pattern. Additionally, urine samples were analyzed for TIMP-2 (tissue inhibitor of metalloproteinases-2) and IGFBP7 (insulin-like growth factor-binding protein 7) using NEPHROCHECK Test (Astute medical, San Diego) before surgery and 4 hours after cross clamping. The tubular markers IGFBP7 and TIMP-2 are involved in G1 cell cycle arrest and facilitate the identification of patients at high risk for AKI.[34] The product of TIMP-2 and IGFBP7 has been successfully used in predicting cardiac surgery-associated AKI.[34,35,36]

Statistical Analysis

With a targeted absolute reduction of AKI rate by 20%, a published incidence of AKI postsurgery of 40%,[1–3] an estimated dropout rate of 15%, an α error of 0.05, and a power of 80%, the calculated sample size for each group was 97. All group comparisons were 2-sided with a significance level of α=0.05. Primary analysis was conducted according to the intention-to-treat principle. Furthermore, per protocol analysis included all patients in the LowS group with an average daily intake of <12 mg SAA per kg body weight and all patients in the CG with an average daily intake of at least 25 mg SAA per kg body weight. The quantitative parameters were analyzed by nonparametric Mann–Whitney *U* tests. The categorical variables were evaluated using χ^2 test or Fisher exact test. Subgroup analyses were performed for sex, age (≤70 versus >70 years), body mass index (≥25 versus <25 kg/m^2), and diabetes (yes/no). Additional analyses were performed for chronic kidney disease and coronary artery disease as well as for type of conducted surgery. All numbers are given as median (interquartile range) unless stated otherwise. SAS 9.4 was used as statistical software. An adjusted analysis was performed for the primary end point AKI, i.e., a logistic regression with factor treatment adjusted for sex, age, and comorbidities as listed in Table 2 of the manuscript (chronic kidney disease, peripheral artery disease, congestive heart failure, CAD, chronic obstructive pulmonary disease, arterial hypertension, diabetes).

RESULTS

The study commenced on January 1, 2019. Because of the COVID-19 pandemic, recruitment eventually stalled in early 2020 when trial activities had to be reduced to a minimum for safety reasons. Therefore, we decided to stop the trial on August 31, 2020. Until then, 392 patients had been contacted, of which 273 patients had declined to participate; 119 patients had been screened; and 115 patients had met the inclusion criteria and were randomized to the intervention group (56 patients) and CG (59 patients). Details are shown in Figure 1.

Information on demographics, patient characteristics, and comorbidities are depicted in Table 2. Surgery-related characteristics such as surgical procedure, cardiopulmonary bypass time, ischemia time, transfusion rate, and fluid balance were equal in both groups. Also, the mandatory machine-driven laminar blood pressure between 55 and 70 mm Hg was equal in both groups. No differences were found with regard to renal function parameters (i.e., serum creatinine, estimated glomerular filtration rate, and chronic kidney disease stage) at screening as well as at baseline (d 0). Furthermore, diet-induced changes in weight or changes in total body water—which could lead to unintended hemodynamic effects on renal perfusion and, thus, render kidneys more susceptible to AKI per se—were not different in the 2 groups (see Table 3).

In the intervention and the CG, calorie intake was within the anticipated range to prevent malnutrition and did not differ between both groups (P=0.42). Considering all ingested food including permitted additional snacks, a highly significant reduction of SAA (methionine and cysteine) intake by 77% was achieved in the LowS group as compared with the CG (P<0.001). To further analyze quantitative aspects

Table 2. Patient Characteristics

	LowS group (n=56)	Control group (n=59)
Age (y), median (IQR)	67 (61–64)	69 (63–76)
Men, n (%)	43 (77%)	32 (54%)
Weight at screening (kg), median (IQR)	88 (78.4–99.8)	78 (66.6–87.0)
BMI at screening (kg/m^2), median (IQR)	28 (26.1–32.6)	28 (24.1–30.3)
Creatinine at screening (mg/dL), median (IQR)	0.99 (0.90–1.17)	0.98 (0.79–1.28)
Creatinine at day−1 (mg/dL), median (IQR)	1.02 (0.88–1.21)	0.97 (0.82–1.25)
Creatinine at baseline (mg/dL), median (IQR)	1.0 (0.84–1.19)	0.95 (0.83–1.25)
Cleveland Clinic Foundation score, median (IQR)	2 (1–3)	2(1–3)
Comorbidities, n (%)		
Chronic kidney disease	9 (16%)	11 (19%)
Peripheral artery disease	2 (4%)	7 (12%)
Congestive heart failure	8 (14%)	9 (15%)
Coronary artery disease	36 (64%)	27 (46%)
COPD	8 (14%)	7 (12%)
Arterial hypertension	37 (66%)	33 (56%)
Diabetes	12 (21%)	17 (29%)
Medication, n (%)		
RAAS-I	41 (73%)	45 (76%)
Beta blockers	32 (57%)	40 (68%)
Calcium antagonists	14 (25%)	14 (24%)
Lipid-lowering drugs	37 (66%)	36 (61%)
Oral antidiabetics	11 (20%)	12 (20%)
Insulin	1 (2%)	10 (17%)
Surgery characteristics		
Duration of ischemia (min), median (IQR)	67.0 (47.0–78.5)	67.5 (56.5–91.0)
Bypass time (min), median (IQR)	107 (89–119)	112 (88–137)
Fluid balance during surgery, median (IQR)	2432 (1833–3583)	2533 (2045–3331)
Type of surgery, n (%)		
CABG only	7 (16.3%)	5 (10%)
Valve only	8 (18.6%)	18 (36%)
Combined or other	28 (65.1%)	27 (54%)

Patient characteristics: anthropometrics, comorbidities, baseline renal function, medications, and surgery characteristics. BMI indicates body mass index; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; LowS, sulfur-containing amino acid-reduced formula diet; and RAAS-I, renin-angiotensin-aldosterone system inhibitors.

of SAA uptake, we measured serum methionine. As expected, median serum methionine levels after 7 days of diet were significantly lower in the LowS group (P<0.001) than in the CG. Diet-specific details are depicted in Table 3.

Primary End Point

The primary end point of AKI during the first 72 hours after cardiac surgery occurred in 23% in the LowS group and in 16% of the CG; this effect was not significant. There was no difference with respect to Kidney Disease: Improving Global Outcomes AKI classification. During the whole hospitalization period, the fraction of patients with AKI increased in both groups without significant between-group differences. Comparisons of serum creatinine levels before diet, 24, 48, and 72 hours after surgery did not reveal any effect of the LowS diet as compared with the control diet. Creatinine at discharge and time to reaching baseline creatinine levels were similar in both groups (see Figure 2). The product [TIMP-2]×[IGFBP7] (NEPHROCHECK) did not differ between both groups. Renal replacement was necessary in neither group, 2 deaths occurred in the CG, but were of no statistical significance. The length of intensive care unit stay was significantly lower in the LowS group (P<0.04) but the overall duration of hospitalization did not differ (Table 4). Other biomarkers reflecting organ damage or systemic inflammation, i.e., CRP, white blood cell count, creatine kinase, troponin T, lactate dehydrogenase, NT-proBNP,

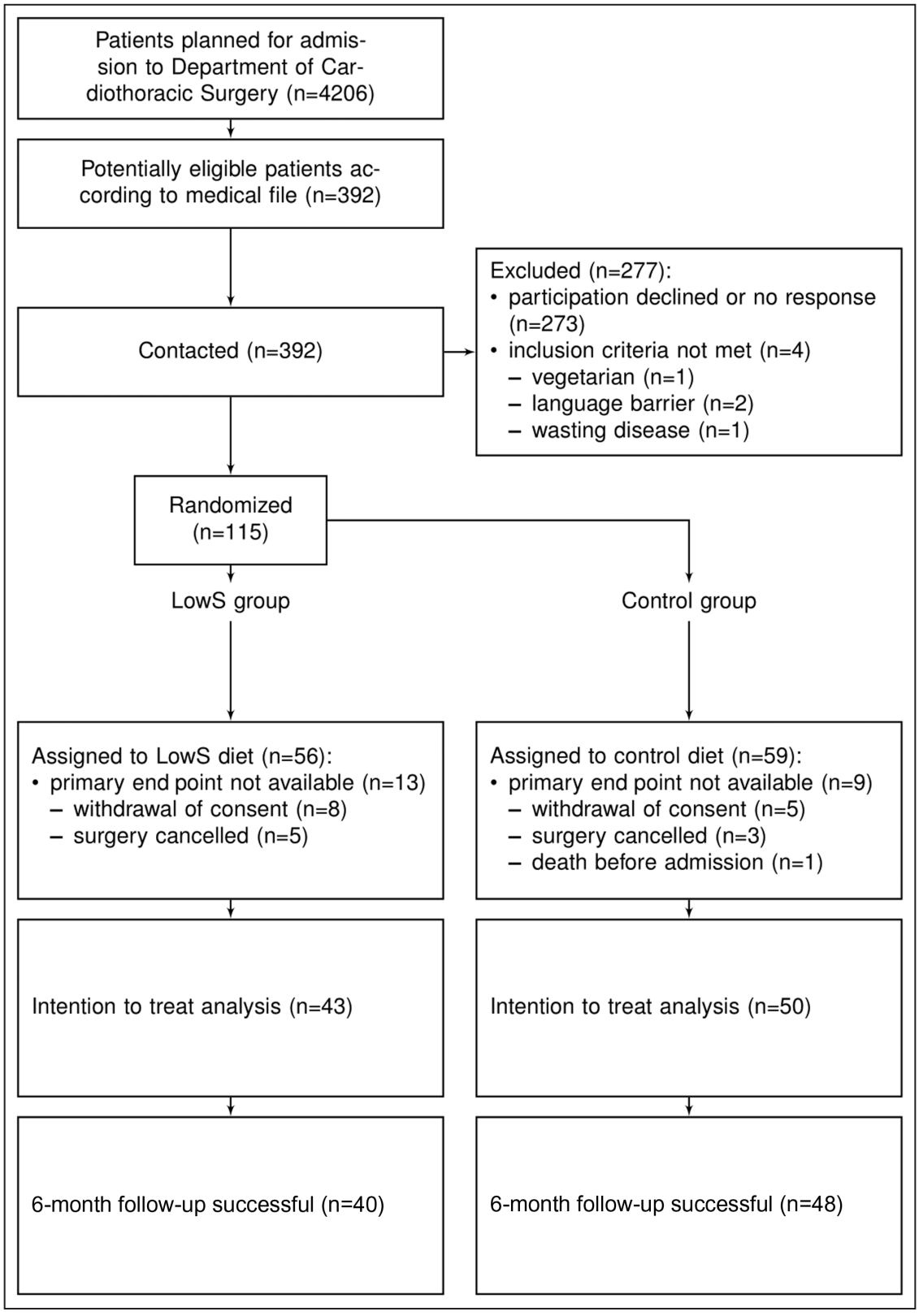

Figure 1. Consort-Flow-Diagram.
LowS indicates sulfur-containing amino acid-reduced formula diet.

Table 3. Diet-Specific Characteristics

	LowS group	Control group	*P* value
Δ Lean body mass screening to day –1, kg, median (IQR)	–1.5 (–2.7 to (–0.4))	–0.8 (–1.8 to 0)	0.13
Δ Total body water screening to day –1, kg, median (IQR)	–1.1 (–2.0 to (–0.5))	–0.6 (–1.3 to 0)	0.06
Δ Creatinine screening to day –1, mg/dL, median (IQR)	0.02 (–0.09 to 0.08)	0.02 (–0.07 to 0.06)	0.875
Median calorie intake during diet, kcal/kg, median (IQR)	25 (22–27)	25 (23–27)	0.422
Median SAA-intake during diet, mg/kg, median (IQR)	7.37 (6.4–7.8)	32.33 (28.9–33.6)	<0.001
Serum methionine at day –1, μmol/L, median (IQR)	21 (19–27)	29 (24–36)	<0.001

With regard to diet-specific characteristics, no effect of diet was seen with respect to lean body mass, serum creatinine or calorie intake. There was a significant reduction of median SAA-intake during diet and a significant lower serum methionine level the day before surgery. LowS indicates sulfur-containing amino acid-reduced formula diet; and IQR, interquartile range.

and lactate were similar in both groups 24 hours after cross clamping (see Table 5).

A per-protocol analysis consisting of 33 patients in the LowS group with daily intake of SAA of <12 mg/kg body weight and 36 patients in the CG with an average daily intake of ≥25 mg/kg body weight was performed. With respect to the primary end point, no significant difference could be shown for the occurrence of AKI within 72 hours after surgery, likewise no effect was seen on the entire hospitalization period. Serum creatinine at all timepoints did not differ between LowS group and CG and renal replacement therapy and death did not occur in both groups. In contrast to the intention-to-treat analysis, patients in the per-protocol analysis did not show different lengths of stay in the intensive care unit (see Tables S3 and S4).

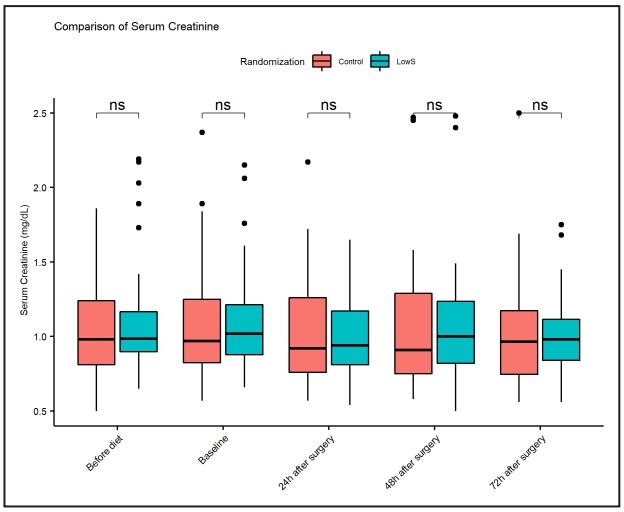

Figure 2. Comparison of serum creatinine at different time points in mg/dL.
No difference of serum creatinine could be shown at any timepoint during diet and after surgery. LowS indicates sulfur-containing amino acid-reduced formula diet.

We also performed subgroup analyses of patient groups according to age, sex, body mass index, and presence of diabetes as well as additional analyses of the subset of patients with chronic kidney disease, coronary artery disease, and type of operation (valve only, coronary artery bypass graft only, combined/other). In none of these subsets, a statistically significant effect of SAA reduction on the primary end point was observed (see Table S3).

Also, an adjusted analysis was performed for the primary end point AKI, considering sex, age, and comorbidities, as listed in Table 2 of the manuscript. In line with the unadjusted analysis no differences between the LowS group and the CG were revealed (intention-to- treat, *P*=0.098, per-protocol, *P*=0.119).

There were no serious adverse events related to the intervention. Patients reported the occurrence of diarrhea in 35.7% of cases in the LowS group and in 18.6% in the CG (*P*<0.05).

In the follow-up assessment after 6 months, none of the patients were on or had been on renal replacement therapy, and no difference in mortality was observed.

DISCUSSION

No beneficial effects of a 77% reduction of dietary intake of methionine and cysteine over 7 days before cardiothoracic surgery on the incidence of AKI post-surgery could be observed in this first-in-human trial, neither in the intention-to-treat nor in the per protocol population. Likewise, no effects of the dietary intervention were detected on secondary end points or in subgroup analyses. Long-term effects could not be observed at 6 months after surgery.

The concept of dietary interventions for organ protection has a long history in research, beginning with the 1935 seminal work by McCay who reported that chronic calorie restriction leads to extended life span

Table 4. Outcome Parameters

	LowS group (n=56)	Control group (n=59)	*P* value
AKI within the first 72 h, n (%)*	10 (23%)	8 (16%)	0.377
AKI during hospitalization, n (%)*	16 (37%)	14 (28%)	0.344
Δ Serum creatinine (mg/dL), median (IQR)*			
−24 h—baseline	−0.010 (−0.170 to 0.100)	−0.030 (−0.130 to 0.040)	0.956
−48 h—baseline	−0.005 (−0.115–0.130)	−0.050 (−0.160 to 0.060)	0.343
−72 h—baseline	−0.025 (−0.115–0.095)	−0.030 (−0.140–0.085)	0.763
Max serum creatinine (mg/dL), median (IQR)*	1.24 (1–1.52)	1.15 (0.93–1.52)	0.463
AKI Risk Score (NEPHROCHECK) after surgery, median (IQR)*	0.12 (0.04–0.41)	0.17 (0.04–0.38)	0.895
KDIGO AKI stages*			0.135
Stage 0	33 (77%)	42 (84%)	
Stage 1	4 (9%)	6 (12%)	
Stage 2	6 (14%)	1 (2%)	
Stage 3	0 (0%)	1 (2%)	
Renal replacement therapy, n (%)	0 (0.0%)	0 (0.0%)	
In-hospital mortality, n (%)	0 (0.0%)	2 (3.4%)	0.496
Total mortality within 6 mo, n (%)	0 (0.0%)	4 (6.8%)	0.110
ICU length of stay, h; median (IQR)	45.0 (26.0–71.0)	53.5 (32.5–105.5)	0.041
Length of hospitalization, days; median (IQR)	11.5 (10–15)	13.0 (12–15)	0.070

AKI indicates acute kidney injury; ICU, intensive care unit; IQR, interquartile range; KDIGO, Kidney Disease Improving Global Outcome; and LowS, sulfur-containing amino acid-reduced formula diet.

*Available for analysis: LowS group: n=43, Control group: n=50.

in rats.[37] Since then, this evolutionary conserved effect has been repeatedly shown in a wide range of species including rhesus monkeys.[12,13] Enhancement of cellular stress resistance—the likely mechanism for this phenomenon—can already by elicited with short periods of dietary restriction which could provide a preventive strategy if organ damage is anticipated. Extensive renal protection by short-term calorie restriction for only several days has been described in a murine AKI model by Mitchell et al.[14] Similar effects on kidney protection were reported not only using calorie restriction,[38–41] but also dietary protein restriction.[42] However, efforts to translate these findings into the clinical setting were less satisfactory, so far. Only minimal or no beneficial effects on rate of or recovery from AKI were found in patients undergoing cardiac surgery after a 7-day period of calorie restriction to 60% of their calculated energy demand.[43] A discrete beneficial impact was observed in men as well as obese patients. Likewise, a 4-day calorie-restricted diet in patients that were subject to scheduled coronary angiography did not prevent contrast-induced AKI; again, small positive effects were seen only in specific subgroups.[44] In contrast, in the setting of living donor kidney transplantation, a combination of 30% calorie and 80% protein restriction applied for 5 days before transplantation led to a significantly improved recovery of kidney function in both, the organ donor and the recipient. Although the effects on creatinine were small and maybe clinically irrelevant, this finding is still of importance since living donor kidney transplantation is usually associated with rather good immediate renal outcomes *per se*. Furthermore, lower incidences of slow graft function and acute rejection were also reported in the kidney recipients.[45] A major drawback of such dietary interventions might be that—while being harmless for healthy laboratory animals—the general restriction of calories or proteins could have detrimental effects on recovery in the clinical setting. Moreover, although the trials successfully demonstrated that dietary restriction protocols were feasible and safe, a marked depletion of energy and protein supply could still interfere with possible beneficial effects on cellular stress resistance blunting putative protective effects.[46,47] Hence, more specific interventions without the risk for malnourishment are desirable.

Similar to CR, chronic reduction of intake of SAA, in particular methionine, has been shown to extend healthy life span in mice,[48] and to be effective as adjunct cancer therapy.[27,28–30,] There is also an association of lower SAA-consumption with lower cardiac risk scores[49] in the human population.

In 2015, Hine and colleagues showed that CR led to increased production of H_2S via activation of the TSP which was pivotal for the CR-mediated protective effect seen in a rodent ischemic liver failure model. The TSP, in turn, is activated by depletion of SAA, methionine and cysteine. In line with this notion, Hine *et*

Table 5. Biomarkers of Organ Damage and Inflammation after 24 hours

	LowS group (n=56)	Control group (n=59)	*P* value
CRP (mg/dL), median (IQR)* reference range: <5 mg/L	126.2 (94.5–146.6)	117.4 (89.7–139.8)	0.539
White blood cell count ×1E9/L, median (IQR)* reference range: 4.4–11.3×10^9/l	10.0 (8.0–13.4)	11.2 (9.2–13.7)	0.122
CK (IU/L), median (IQR)* reference range for men: <190 IU/L, for women: <170 IU/L	612 (499–1159)	564 (412–874)	0.119
High-sensitivity troponin T (µg/L), median (IQR)* reference range: ≤0.014 µg/L	0.387 (0.29–0.68)	0.463 (0.27–0.94)	0.794
LDH (IU/L), median (IQR)* reference range: <250 IU/L	336 (294–376)	326 (287–379)	0.852
NT-proBNP (ng/L), median (IQR)* reference range for men: 35–44 y: ≤115 ng/L 45–54 y: ≤173 ng/L 55–64 y: ≤386 ng/L 65–74 y: ≤879 ng/L reference range for women: 35–44 y: ≤237 ng/L 45–54 y: ≤284 ng/L 55–64 y: ≤352 ng/L 65–74 y: ≤623 ng/L	1026 (651–2180)	1297 (625–2323)	0.515
Lactate (mmol/L), median (IQR)* reference range: 0.5–2.2 mmoL/L	2.1 (1.4–2.6)	1.7 (1.4–2.3)	0.216

No difference between both diets could be observed with respect to the incidence and the severity of acute kidney injury, inflammatory and organ damage parameters, renal replacement therapy and mortality. CK indicates creatinine kinase; CRP, C-reactive protein; IQR, interquartile range; LowS, sulfur-containing amino acid-reduced formula diet; LDH, lactate dehydrogenase; and NT-proBNP, N-terminal pro-brain natriuretic peptide.

*Available for analysis: LowS group: n=43, Control group: n=50.

al. were able to demonstrate that the protective effect of CR or dietary restriction in general could be completely abolished by supplementing methionine and cysteine.[19] Methionine restriction led to increased H_2S-production[23] and might delay senescence,[50] ameliorate myocardial ischemia–reperfusion injury[51] and renal damage in animal models.[52] In the human population a lower SAA-consumption—reflecting more plant-derived protein sources—has also been associated with lower cardiac risk scores[49] .

In recent experiments, our group compared the reno-protective properties of several dietary interventions—SAA-reduced diets were among the most effective strategies and led to a significant reduction of AKI.[31] In light of these findings, dietary SAA restriction may be the long-sought-for strategy providing effective organ protection without malnourishment.

First human trials showed that dietary SAA reduction is feasible and safe[20] which prompted us to launch the UNICORN trial as a randomized, controlled and double-blind study. With an actual reduction of dietary SAA by 77%, the interventional diet was comparable to the SAA-reduced diets administered in our animal experiments and biochemical analyses proved that the LowS diet indeed led to markedly reduced serum levels of methionine. Still, the results of the UNICORN trial are—with regard to effective organ protection—largely disappointing. Various reasons may account for this discrepancy. In the murine model, unilateral nephrectomy plus prolonged contralateral ischemia led to robust and pronounced renal failure while patients in UNICORN were at relatively low risk for renal replacement therapy mirrored by a low Cleveland Clinic Foundation score[53] and rarely developed advanced renal failure. The incidence of AKI within 72 hours post-surgery in our cohort was considerably lower than expected. Furthermore, in contrast to the experimental setting in which mice were subject to SAA restriction for 2 weeks, only 1 week of dietary intervention was feasible in the clinical trial. Of note, using a collagen-based protein source does not only reduce SAA content but leads to quantitative alterations in other amino acids as well. Hence, the applied LowS formula diet cannot be considered equivalent to the diets used in animal studies.

Whether there might still be some benefit in clinical circumstances associated with more severe AKI remains elusive. However, many if not most of such cases are found in the acute setting which renders time-consuming preventive strategies largely unsuccessful. Further studies should therefore rather focus on pharmacological approaches that activate molecular pathways associated with organ protection including TSP and that carry the advantage of rapid action as opposed to dietary interventions that need to be carefully controlled and conducted for days or maybe weeks. To this end, a deeper mechanistic insight is necessary.

Lastly, patients reported a higher incidence of intestinal discomfort in the LowS group which might be attributable to change in amino acid composition. Higher occurrence of diarrhea might lead to a higher vulnerability to prerenal AKI and therefore might influence the outcome of this study and mask the protective effect. However, the difference of body water between randomization and baseline visit was not significantly different (P=0.06).

Our study has several limitations. Most importantly, the trial had to be terminated early because scheduled cardiac operations with the appropriate lead time—a prerequisite for enrolment—were largely canceled or postponed during the COVID-19 pandemic. At the same time, access to intensive care unit was restricted for safety reasons which impeded structured data acquisition by study personnel. This led to a major decrease in the statistical power. However, given the tendency towards a higher rate of AKI in the LowS group (23%) as compared with the CG (16%), it seems unlikely that greater numbers would have led to a result in favor of the interventional diet. Statistical power was further reduced by an overall AKI rate that was considerably lower than anticipated. Additionally, although the group assignment was performed via randomization, imbalances in demographic and clinical characteristics are evident. The LowS group consisted of more male patients than CG and several comorbidities were distributed unequally, but analyses of the primary end point adjusted for these factors confirmed the result of a lack of significant influence of the intervention on AKI.

Despite these shortcomings the major strength of the UNICORN trial is its design as one of the few double-blind, randomized, controlled diet studies to date.

In conclusion, this study cannot show a renoprotective effect of LowS diet. Whether the underlying, evolutionary conserved mechanisms of enhanced cellular stress resistance elicited by dietary interventions in animals can be exploited in humans (eg, through pharmacologic interventions directly targeting TSP) should be investigated in future clinical trials.

ARTICLE INFORMATION

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Affiliations

Department II of Internal Medicine and Center for Molecular Medicine Cologne, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany (T.O., C.G., P.T., K.A., R.M., F.C.K., T.B., R.M., F.G., V.B.); Institute of Medical Statistics and Computational Biology (I.B.); and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD) (F.C.K., T.B., R.M.), Institute of Medical Statistics and Computational Biology, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany; Polyclinic for Endocrinology, Diabetes and Preventive Medicine, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany (M.F.); and Department of Cardiothoracic Surgery, University of Cologne, Faculty of Medicine and University Hospital Cologne Germany, (T.W.).

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Disclosures

None.

Supplemental Material

Tables S1–S4

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Supplemental Material

Table S1. Amino acid profile in LowS formula diet.

Amino acid	LowS group [g/100 ml]
Lysine	0.314
Threonine	0.132
Methionine	0.042
Phenylalanine	0.127
Tryptophan	0.080
Valine	0.155
Leucine	0.186
Isoleucine	0.100
Tyrosine	0.060
Cysteine	0.002
Histidine	0.059
Arginine	0.421
Glutamine	0.023
Glycine	1.278
Alanine	0.500
Proline	1.443
Serine	0.204
Glutamic acid	0.594
Aspartic acid and Asparagine	0.356

g - gram, ml – milliliter

Table S2. Additional allowed food items and beverages.

Maximum 2 units per day from category A or maximum 1 unit per day from category B (instead of category A)
Additionally: maximum 3 units per day from category C

A **Fruits/vegetables**	**Serving size**
Apple	1 piece 150 g
Apple sauce	1 serving 250 g
Orange	1 piece, 150 g
Banana	1 piece, 150 g
Pear	1 piece, 150 g
Strawberry	1 handful, 150 g
Grapefruit	½ piece, 150 g
Blueberries	1 handful, 125 g
Cherry, sweet	1 handful, 125 g
Kiwi	1 piece, 100 g
Tangerine	1 piece, 100 g
Peaches (tinned)	1 serving (125 g)
Cucumber	½ piece, 150 g
Carrot	2 pieces, 125 g
Tomato	2 pieces (ca. 150 g) or 1 handful cherry tomatoes
Field salad*	1 unit, 50 g
Miscellaneous	**Serving size**
Crisps	25 g
Hard candy	2-3 pieces

B **Fruits/vegetables**	**Serving size**
Raspberry	1 handful, 125 g
Peaches	1 piece, 125 g
Avocado	1 piece, 125 g
Chinese cabbage*	1 serving max. 100 g
Kohlrabi	½ piece, 150 g
Lettuce*	1 serving, 100 g
Bell Pepper	½ piece, 100 g
Miscellaneous	**Serving size**
Sorbet	1 piece, 40-75 g
Dark chocolate	20 g

One piece refers to a medium-sized fruit or vegetable.
*Concerning salads: Oil-vinegar or citrus dressings can be used, no dressing containing yoghurt or cream.

C: Beverage	**amount**
Coffee (no milk, no cream)	1 cup, 150 ml
Tea (green, black) (no milk)	1 cup, 150 ml
Apple juice, orange juice	1 cup, 200 ml
Coke, lemonade	1 cup, 200 ml
Red wine, white wine	1 cup, 125 ml
Sparkling wine	1 cup, 100 ml
Beer (pilsner, non-alcoholic,	1 cup, 200-330 ml
Water	No limitation

Table S2: List provided to the patients containing additionally allowed food items and beverages
Patient could choose up to 2 items of category A or 1 item of category B. Additionally up to 3 items of category C were allowed.
g - gram, ml – milliliter

Table S3. Per protocol and subgroup analyses: primary endpoint.

	LowS group	Control Group	p-value
	n=33	**n=36**	
Per Protocol, n (%)	9 (27%)	6 (17%)	0.286
	n=35	**n=29**	
Sex male, n(%)	6 (17%)	5 (17%)	1
	n=8	**n=21**	
Sex female, n(%)	4 (50%)	3 (14%)	0.068
	n=25	**n=29**	
Age ≤ 70 y, n(%)	6 (24%)	5 (17%)	0.539
	n=18	**n=21**	
Age >70 y, n(%)	4 (22%)	3 (14%)	0.682
	n=6	**n=14**	
BMI <25, n(%)	2 (33%)	2 (14%)	0.549
	n=37	**n=34**	
BMI ≥ 25, n(%)	8 (22%)	6 (18%)	0.674
	n=8	**n=14**	
Diabetes (%)	5 (63%)	2 (14%)	0.052
	n=35	**n=36**	
No Diabetes, n(%)	5 (14%)	6 (17%)	0.782

	n= 9	**n=11**	
CKD, n(%)	2 (22%)	3 (27%)	0.949
	n = 47	**n=48**	
No CKD, n(%)	8 (17%)	5 (10%)	0.261
	n= 36	**n=27**	
CAD, n(%)	7 (19%)	3 (11%)	0.252
	n=20	**n=32**	
No CAD, n(%)	3 (15%)	5(16%)	0.916
	n=7	**n=5**	
CABG only, n(%)	1 (14%)	0 (0%)	1
	n=8	**n=18**	
Valve only, n(%)	2 (25%)	2 (11%)	0.56
	n=28	**n=27**	
Combined or other, n(%)	7 (25%)	6 (22%)	1

Table S3 showing the primary endpoints of the subgroup analyses

Abbreviations: % percent, n = number, CKD = chronic kidney disease, CAD = coronary artery disease, CABG = coronary artery bypass graft

Table S4. Secondary Endpoints of the Per Protocol Analyses.

	LowS group (n=33)	**Control Group (n=36)**	**p-value**
AKI during hospitalization, n (%)	14 (42%)	13 (36%)	0.591
Δ serum creatinine (mg/dl), median (IQR)			
-24 hours - baseline	-0.010 (-0.190-0.080)	-0.025 (-0.115- 0.065)	0.665
-48 hours - baseline	-0.030 (-0.150-0.100)	-0.045 (-0.150-0.085)	0.710 0.652
-72 hours - baseline	-0.050 (-0.120-0.090)	-0.015 (-0.120-0.105)	
ICU length of stay in hours, median (IQR)	46.0 (27.0-71.0)	52.0 (28.0-96.0)	0.390
Length of hospitalization in days, median (IQR)	11.5 (10-16)	13.0 (11-14)	0.791

AKI - acute kidney injury, Δ - delta, dL - deciliter, ICU - intensive care unit, IQR - interquartile range, mg – milligram