

Preoperative Short-Term Calorie Restriction for Prevention of Acute Kidney Injury After Cardiac Surgery: A Randomized, Controlled, Open-Label, Pilot Trial

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Background—Acute kidney injury is a frequent complication after cardiac surgery and is associated with adverse outcomes. Although short-term calorie restriction (CR) has proven protective in rodent models of acute kidney injury, similar effects have not yet been demonstrated in humans.

Methods and Results—CR_KCH (Effect of a Preoperative Calorie Restriction on Renal Function After Cardiac Surgery) is a randomized controlled trial in patients scheduled for cardiac surgery. Patients were randomly assigned to receive either a formula diet containing 60% of the daily energy requirement (CR group) or ad libitum food (control group) for 7 days before surgery. In total, 82 patients were enrolled between April 16, 2012, and February 5, 2015. There was no between-group difference in the primary end point of median serum creatinine increment after 24 hours (control group: 0.0 mg/dL [−0.1 – (+0.2) mg/dL]; CR group: 0.0 mg/dL [−0.2 – (+0.2) mg/dL]; $P=0.39$). CR prevented a rise in median creatinine at 48 hours (control group: +0.1 mg/dL [0.0 – 0.3 mg/dL]; CR group: −0.1 mg/dL [−0.2 – (+0.1) mg/dL]; $P=0.03$), with most pronounced effects observed in male patients and patients with a body mass index >25. This benefit persisted until discharge: Median creatinine decreased by 0.1 mg/dL (−0.2 – 0.0 mg/dL) in the CR group, whereas it increased by 0.1 mg/dL (0.0 – 0.3 mg/dL; $P=0.0006$) in the control group. Incidence of acute kidney injury was reduced by 5.8% (41.7% in the CR group compared with 47.5% in the control group). Safety-related events did not differ between groups.

Conclusions—Despite disappointing results with respect to creatinine rise within the first 24 hours, the benefits observed at later time points and the subgroup analyses suggest the protective potential of short-term CR in patients at risk for acute kidney injury, warranting further investigation.

Clinical Trial Registration—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT01534364. (*J Am Heart Assoc.* 2018;7:e008181. DOI: 10.1161/JAHA.117.008181.)

Key Words: acute kidney injury • calorie restriction • cardiac surgery • dietary restriction • preconditioning

With incidence of up to 40%, acute kidney injury (AKI) is a frequently encountered complication after cardiac surgery.¹ Even mild cases are associated with longer hospital stay, higher morbidity, and increased short- and long-term mortality compared with those without AKI.^{2,3} Despite a

multitude of clinical trials using pharmacological agents, the quest for an effective renoprotective treatment has been unsuccessful so far.⁴ Promising and innovative nonpharmacological strategies, including remote ischemic preconditioning (RIPC), have emerged recently; however, the results of

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An accompanying Data S1 is available at <http://jaha.ahajournals.org/content/7/6/e008181/DC1/embed/inline-supplementary-material-1.pdf>

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Clinical Perspective

What Is New?

- In a 82-patient study, 7-day calorie restriction was not successful in preventing a rise in median creatinine at 24 hours, but it ameliorated renal failure at 48 hours after cardiac surgery, suggesting a protective potential of short-term calorie restriction.
- This study provides first evidence in humans that preoperative calorie restriction is safe and feasible in cardiac surgery.
- This study is the first controlled and randomized clinical trial assessing the potential benefit of calorie restriction for organ protection.

What Are the Clinical Implications?

- Dietary restriction may provide a novel and promising approach for inducing organ protection.
- The results of this study warrant further investigation to assess the value of preconditioning strategies using nutritional interventions.

several trials investigating RIPC in diverse clinical settings are equivocal.^{5–8}

Short-term dietary restriction has been shown to induce robust cellular stress resistance in various species, leading to reduced organ damage susceptibility. Mitchell and coworkers showed that a reduction in daily calorie intake by 30% over short time periods prevented AKI in a murine ischemia–reperfusion injury model.⁹ The astonishingly potent effect of calorie restriction (CR) on AKI was confirmed by a more recent study from our group.¹⁰ Similar results have been reported in other models of renal injury (eg, cisplatin-induced AKI¹¹) and in other organ systems.¹² Although a short-term diet has been proven to be feasible and safe in patients undergoing living-donor kidney transplantation,^{13,14} no prospective trials have been undertaken to investigate the possible impact of moderate CR as an organ-protective measure. We performed a prospective, single-center, randomized, open-label clinical trial to test the hypothesis that 1 week of a 40% reduction of calorie intake prevents AKI in at-risk patients undergoing cardiac surgery.

Methods

The data, analytic methods, and study materials will not be made available permanently to other researchers for purposes of reproducing the results or replicating the procedure; however, all data, analytic methods, and study materials will be provided on request.

Study Design and Participants

This pilot trial was designed and conducted as a randomized, open-label, single-center study at the University Hospital Cologne. Approval was obtained from the institutional review board of the University Hospital Cologne. Adult patients (aged >18 years) scheduled for elective cardiac surgery involving cardiopulmonary bypass who carried at least one of the following risk factors for developing AKI¹⁵ were enrolled after having obtained written informed consent: age >70 years, chronic kidney disease, diabetes mellitus, congestive heart failure of New York Heart Association class 3 or 4, reduced left ventricular ejection fraction (<50%), peripheral vascular disease, planned combined coronary artery bypass grafting and valve surgery, previous cardiac surgery, and chronic obstructive pulmonary disease. Exclusion criteria were end-stage renal disease, kidney transplantation, pregnancy, weight loss >1 kg within 2 weeks before enrollment unless due to diuretic medication, body mass index (calculated as kg/m²) of <18.5 or >35, evidence of malignancy or uncontrolled infection, or the inability to give informed consent. The study was conducted in accordance with the Declaration of Helsinki and the good clinical practice guidelines by the International Conference on Harmonization. The study protocol is provided in Data S1.

Randomization

At 8 to 12 days before the scheduled day of surgery, eligible patients were invited for a screening visit. Malnourishment was ruled out by careful physical examination, assessment of body weight and body composition using a bioimpedance scale (Tanita BC-418 segmental body composition analyzer), and confirmation of normal serum albumin. After having given written informed consent, patients were randomly assigned using a 1:1 ratio to either receive a calorie-restricted diet (CR group) or an ad libitum diet (control group). Randomization codes were generated by the Institute of Medical Statistics and Computational Biology (University of Cologne, Germany) and provided to the study center in sealed, consecutively numbered, opaque envelopes.

Procedures

Patients in the CR group were provided with a formula diet (Fresubin energy fiber drink) that contained all necessary macro- and micronutrients; however, the amount was limited to provide only 60% of the daily energy expenditure (DEE), as calculated using the Mifflin–St. Jeor equation and individually assessed activity factors. Participants were instructed not to consume extra food or calorie-containing beverages like

alcohol, fruit juices, or soft drinks. The diet commenced on preoperative day -7 and was maintained until day -1 . On the day of surgery, patients were maintained in a fasting state until surgery. In the control group, patients were allowed to ingest food at their own discretion and were encouraged to stick to their normal eating habits, thereby ensuring nonrestricted calorie intake. All participants in both groups were provided with diaries and reported their food consumption on a daily basis. In addition, regular phone calls ascertained patients' well-being and monitored adherence to the designated protocol. Reassessment of all participants was performed on the day of admittance (day -1). All blood and urine samples at baseline (ie, immediately before surgery) and within the postoperative 48-hour time frame were obtained at prespecified time points; laboratory parameters beyond day 2 after surgery as well as type of surgery, cardiopulmonary bypass time, and aortic cross-clamp time were extracted from the medical records. Baseline demographic and clinical data including comorbidities and medication were assessed at the screening visit (between days -12 and -8). Anthropometric data (eg, body weight, body composition) were recorded at the screening visit and at hospital admittance (day -1). All participants were instructed to collect their urine over a 24-hour period at day -2 , and estimation of the daily protein intake was performed by assessing urinary urea nitrogen appearance in this 24-hour sample and the equation proposed by Maroni et al.¹⁶ After surgery, hourly urine output was recorded as long as an indwelling urinary catheter was in place (at least 24 hours). All patients were followed up until their hospital discharge.

All patients received sevoflurane for anesthesia. Surgical procedures were carried out according to the local standard and were not influenced by the study protocol. Other putative measures of organ protection (eg, RIPC) were not performed throughout the study.

Outcomes

Rise in serum creatinine from baseline to 24 hours after cross-clamping was analyzed as the primary end point. As a point estimate of the effect size, we calculated the change of serum creatinine in the CR group minus the change of serum creatinine in the control group. Secondary end points were rise of serum creatinine from baseline to 48 hours after cross-clamping; maximum rise of serum creatinine from baseline within 48 hours after cross-clamping; rise in urinary NGAL (neutrophil gelatinase-associated lipocalin) from baseline to 8 hours after cross-clamping; occurrence of AKI, as defined by Kidney Disease: Improving Global Outcomes (KDIGO) criteria,¹⁷ within 72 hours after surgery; change of serum creatinine from baseline to discharge; need for renal replacement therapy; length of hospital stay; in-hospital

mortality; incidence of perioperative myocardial infarction, stroke, and atrial fibrillation; and evolution of the following biochemical parameters from baseline to 24 hours after cross-clamping: C-reactive protein, white blood cell count, creatinine kinase, troponin T, lactate dehydrogenase, NT-proBNP (N-terminal pro-brain natriuretic peptide), and lactate. The decision to initiate renal replacement therapy was made exclusively by the treating physician and was not influenced by study personnel. Safety-related events were captured throughout the study. All events that were judged by the investigator as having reasonable causal relation to the provided diet were considered to be treatment-related adverse events.

Statistical Analyses

Based on published data,^{8,18} we estimated the mean increase of serum creatinine from baseline to 24 hours after cross-clamping to be 0.4 mg/dL (SD 0.25 mg/dL). A difference in the change of 0.2 mg/dL in serum creatinine in this time frame between the 2 groups was considered to be clinically significant. Assuming a 2-tailed type I error of 5% and a dropout rate of 20%, we calculated a sample size of 41 for each group to give 80% power. Primary analysis was done with the intention-to-treat approach, excluding patients without serum creatinine values at baseline or at 24 hours. All results are given as median (interquartile range [IQR]) unless stated otherwise.

Subgroup analyses were performed with regard to sex, age, body mass index, history of diabetes mellitus, ischemia time, type of surgery (valve and/or bypass), and chronic kidney disease stage. Groups were compared using the χ^2 test with categorical data sets or the Mann-Whitney U test with numerical data sets. No race- or ethnicity-specific analyses were performed because all included patients were white. All statistical testing was 2-sided, and $P < 0.05$ was considered significant. Analysis software used was SAS 9.3 (SAS Institute) and IBM SPSS Statistics version 23.

Results

Patients were enrolled between April 16, 2012, and February 5, 2015. Overall, 278 potentially eligible patients were identified and contacted. Of these, 194 declined participation; 84 were enrolled; and, finally, 82 patients (41 in each group) were randomized. In total, 36 patients in the CR group and 40 patients in the control group were included in the intention-to-treat analysis (Figure 1). Patient demographics and clinical characteristics are shown in Table 1.

According to the patients' diary recordings and regular personal interviews, the median daily calorie intake in the CR

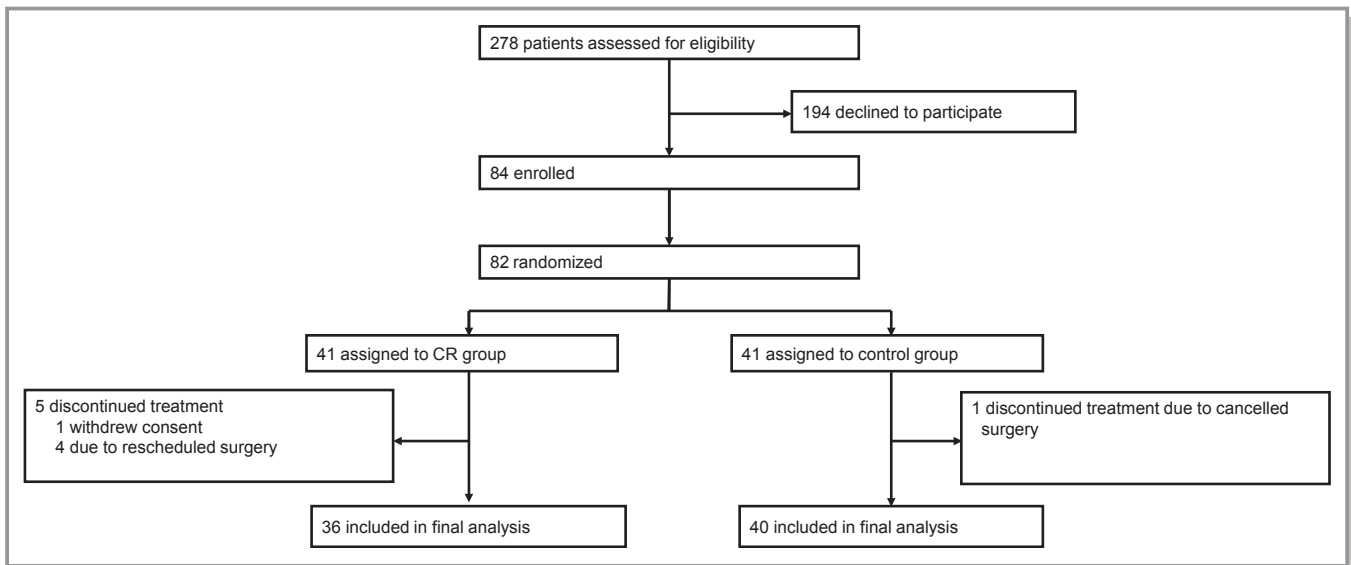


Figure 1. Patient flow and randomization. CR indicates calorie reduction.

group was 1313 kcal (IQR: 1224–1412 kcal), which is equivalent to 60% (1.5%) of the calculated DEE. Calculation of additionally ingested calories was accurate for the CR group because patients added only minor amounts of food or countable pieces (eg, fruit, piece of cake); in contrast, precise estimation of true calorie uptake in the control group was hampered by missing or uncertain reports of consumed food quantities. However, all patients in the control group reported that they had not changed their eating habits and had not conducted a diet on their own. To further assess compliance with dietary protocols, we analyzed weight changes from the time of screening to day –1 in both groups. Median weight loss in the control group was 0.1 kg (–0.8 – 0.8 kg), whereas patients in the CR group lost 3.0 kg (–3.9 – (–2.2) kg; $P<0.0001$). In the latter group, the marked weight change was largely accounted for by a high loss of total body water (–2.4 kg [–3.8 – (–1.9) kg]) compared with the control group (+0.1 kg [–1.4 – 0.9 kg]; $P<0.0001$). The calculated loss of dry body weight (ie, excluding changes in water balance) was –0.6 kg (–1.3 – 0.5 kg) in the CR group—which is in line with what can be expected with 1 week of a 40% CR—and +0.2 kg (–1.0 – 1.8 kg) in the control group ($P=0.06$), indicating a true dietary between-group difference with respect to calorie intake. The estimated daily protein intake according to the equation proposed by Maroni was not different in both groups, with 0.8 g/kg (0.7 – 1.0 g/kg) in the CR group and 0.9 g/kg (0.7 – 1.0 g/kg) in the control group ($P=0.67$), respectively. It is likely, however, that the calculation based on urinary urea nitrogen appearance is an overestimation of the true protein intake in the CR group because of the catabolic breakdown of endogenous proteins. The protein content of the actually ingested amount of the formula diet was 0.6 g/kg (0.6 – 0.7 g/kg). Consequently, there

is evidence of a protein restriction in the CR group compared with the control group; however, this difference is likely to be considerably smaller than 40%.

No difference was noted in median serum creatinine at the screening visit, with 1.1 mg/dL (0.9–1.3 mg/dL) in the CR group and 1.1 mg/dL (1.0–1.3 mg/dL) in the control group (Table 1). However, there was a 0.1-mg/dL increase in creatinine between the screening visit and day –1 to 1.2 mg/dL (1.0–1.6 mg/dL) in the CR group, and this incremental change was highly significant ($P=0.0001$) compared with the control group, in which serum creatinine was not altered (1.1 mg/dL [0.9–1.3 mg/dL]). Influences of the diet on anthropometric characteristics and biochemical parameters are summarized in Table 2.

With a median change of serum creatinine from baseline to 24 hours after cross-clamping of 0.0 mg/dL (–0.1 – (+0.2) mg/dL) for control group versus 0.0 mg/dL (–0.2 – (+0.2) mg/dL) for CR group ($P=0.39$) and an effect size of 0.04 mg/dL (95% CI, –0.14 to 0.21), there was no difference in the primary end point (Figure 2).

In accordance with the KDIGO recommendations for diagnosing AKI, we assessed the rise in serum creatinine within the first 48 hours after cross-clamping. Within this time frame, the serum creatinine decreased by 0.1 mg/dL (–0.2 – (+0.1) mg/dL) in the CR group and increased by 0.1 mg/dL (0.0 – 0.3 mg/dL) in the control group ($P=0.03$; Figure 2). Of note, in the CR group, the maximum increment was detected in the time period between baseline and 24 hours, whereas serum creatinine levels were stable or slightly decreasing between 24 and 48 hours. In contrast, in the control group, we found a steady increase of serum creatinine over the entire 48-hour interval. Subgroup analyses showed that the

Table 1. Patient Demographics and Clinical Characteristics

	CR Group (n=36)	Control Group (n=40)
Age (y), median (IQR)	72 (63–76)	75 (70–77)
Male, n (%)	29 (80.6)	31 (77.5)
Weight at screening (kg), median (IQR)	84.6 (72.1–91.4)	79.1 (75.1–92.7)
BMI at screening (kg/m ²), median (IQR)	26.9 (24.7–30.7)	26.7 (25.2–30.3)
Creatinine at screening (mg/dL), median (IQR)	1.1 (0.9–1.3)	1.1 (1.0–1.3)
Creatinine day –1 (mg/dL), median (IQR)	1.2 (1.0–1.6)	1.1 (0.9–1.3)
Creatinine day 0 (mg/dL), median (IQR)	1.1 (0.9–1.4)	1.1 (0.9–1.3)
Cleveland Clinic Foundation score, median (IQR)	2 (2–4)	3 (2–4)
Medical history, n (%)		
Chronic kidney disease	15 (41.7)	15 (37.5)
Peripheral arterial disease	4 (11.1)	7 (17.5)
Congestive heart failure	8 (22.2)	6 (15.0)
Previous heart surgery	3 (8.3)	1 (2.5)
Coronary artery disease	30 (83.3)	25 (62.5)
Left main stem disease	6 (16.7)	4 (10.0)
COPD	5 (13.9)	6 (15.0)
Hypertension	33 (91.7)	33 (82.5)
Diabetes mellitus	13 (36.1)	20 (50.0)
Medication, n (%)		
ACEI	29 (80.6)	31 (77.5)
Aldosterone antagonist	5 (13.9)	9 (22.5)
Beta blocker	33 (91.7)	31 (77.5)
Calcium antagonist	11 (30.6)	5 (12.5)
Lipid-lowering drugs	31 (86.1)	26 (65.0)
Antiplatelet therapy	26 (72.2)	26 (65.0)
Diuretics	18 (50)	31 (77.5)
Cross-clamp time (min), median (IQR)	59.0 (52–82)	58.5 (45–80)
Bypass time (min), median (IQR)	99.0 (78–123)	92.0 (68–122)
Type of operation, n (%)		
CABG	14 (38.9)	13 (32.5)
Valve	9 (25.0)	14 (35.0)
Combined or other	13 (36.1)	13 (32.5)

ACEI indicates angiotensin-converting enzyme inhibitor; BMI, body mass index; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; CR, calorie restriction; IQR, interquartile range.

significant beneficial effect of CR on change of creatinine from baseline to 48 hours shown for the entire group was largely attributable to participants who were male ($P=0.0047$), who had a body mass index >25 ($P=0.023$), and who had chronic kidney disease stage 1 ($P=0.0253$; Figure 3), whereas age, history of diabetes mellitus, ischemia time, and type of surgery had no influence. Furthermore, there was a highly significant between-group difference in creatinine evolution between baseline and discharge, with a decrease of 0.1 mg/dL ($-0.2 - 0.0$ mg/dL) in the CR group and an increase of 0.1 mg/dL ($0.0 - 0.3$ mg/dL) in the control group ($P=0.0006$).

Urinary NGAL 8 hours after cross-clamping and all other biochemical parameters at 24 hours after surgery were not different in the 2 groups (Table 3). The incidence of AKI according to the KDIGO criteria was lower in the CR group (41.7%) than in the control group (47.5%); however, this difference was not statistically significant (Table 4). No differences were observed with respect to the rate of renal replacement therapy, death, length of stay, and incidence of perioperative myocardial infarction, stroke, or atrial fibrillation.

On the basis of the patients' diary recordings, we identified those patients who had meticulously followed the assigned dietary protocol: In the CR group, $\leq 60\%$ of the calculated DEE was consumed ($n=13$); in the control group, $\geq 80\%$ of the calculated DEE was consumed ($n=11$). These individuals were included in the per-protocol analysis. As in the primary analysis, no statistically significant differences were found with regard to any of the predefined outcome parameters between the 2 groups in the per-protocol analysis; however, with $n=13$ in the CR arm and $n=11$ in the control arm, the group size was very small. As in the overall analysis, there was an increase in median serum creatinine in the CR group between the screening visit and day –1, from 0.9 mg/dL ($0.7 - 1.3$ mg/dL) to 1.3 mg/dL ($0.8 - 1.5$ mg/dL), but no change in the control group (screening: 1.2 mg/dL [$0.9 - 1.4$ mg/dL]; at day –1: 1.2 mg/dL [$0.9 - 1.5$ mg/dL]; $P=0.013$). Of note, over the first 48 hours after cross-clamping, mean serum creatinine declined steadily in the CR group, whereas there was a steady increase in the control group (Figure 4).

Patients were contacted by phone every second day between days –7 and –1, and their general condition and possible safety-related events were recorded. Throughout the diet, 45% of the patients described their general condition as good, 48% as fair, and 7% as poor. Expectedly, an increased sensation of appetite was reported in the CR group; however, only 9% of patients classified this sensation as severe, and 34% rated it as moderate; the majority (57%) had no or only minor complaints. Adverse events were assessed throughout the hospital stay. Two deaths were recorded in each group and were not deemed treatment-related: 1 case of acute respiratory distress syndrome and 1 case of carotid artery occlusion were recorded in the control group, and 1 case of

Table 2. Anthropometric Characteristics and Biochemical Parameters

	CR Group (n=36)	Control Group (n=40)	P Value
Δ Weight screening to day -1, kg	-3.0 (-3.9 to -2.2)	-0.1 (-0.8 to 0.8)	<0.0001
Δ Dry weight screening to day -1, kg	-0.6 (-1.3 to 0.5)	0.2 (-1.0 to 1.8)	0.057
Δ Body water screening to day -1, kg	-2.4 (-3.8 to -1.9)	-0.1 (-1.4 to 0.9)	<0.0001
Δ Creatinine screening to day -1, mg/dL	0.0 (-0.1 to 0.1)	0.1 (0.1-0.3)	0.0001
Calculated daily energy expenditure (DEE), kcal	2160 (1939-2334)	2153 (1909-2343)	0.75
Reported mean daily calorie intake during CR (kcal)	1313 (1224-1412)
Reported mean daily calorie intake during CR (% of DEE)	60 (60-62)
Calculated daily protein intake during CR, g/kg	0.6 (0.56-0.66)
Daily protein intake calculated from urinary urea nitrogen appearance, g/kg	0.8 (0.7-1.0)	0.9 (0.7-1.0)	0.67

Values presented as median (interquartile range). CR indicates calorie restriction; DEE, daily energy expenditure.

coronary bypass occlusion and 1 case of heart failure due to paravalvular leakage were recorded in the CR group. Regarding possible treatment-related events, 3 patients in the CR group and none in the control group experienced perioperative atrial fibrillation ($P=0.06$).

Discussion

In this study, we found that 7-day preoperative CR to 60% of the DEE in patients at risk for postsurgery AKI had no impact on the increase of serum creatinine at 24 hours after cardiac surgery but showed a significant favorable effect on creatinine kinetics at 48 hours and at later time points.

It has been known since the late 1930s that long-term dietary restriction—defined as CR without causing signs of malnutrition—leads to extension of life span by $\approx 20\%$ in rats, and this finding was repeatedly confirmed in many species, from yeast to primates.¹⁹⁻²¹ Although the underlying mechanisms are not yet fully elucidated, the current concepts suggest enhanced cellular stress resistance as the major driver of this phenomenon.^{22,23} Possible underlying mechanisms include enhanced autophagy, mitochondrial reactive oxygen species handling, induction of AMPK (AMP-activated protein kinase) signaling, inhibition of mTOR (mammalian target or rapamycin) signaling, altered hypoxia signaling, enhanced DNA damage repair, and modulation of the immune system.²⁰ The impact of a

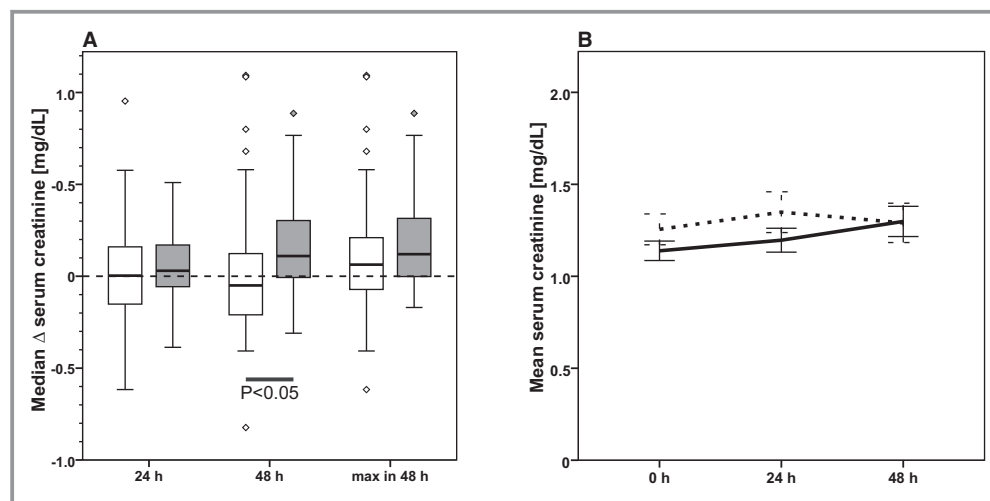


Figure 2. Intention-to-treat analysis. Evolution of serum creatinine from baseline to 24 hours after cross-clamping (primary end point) and from baseline to 48 hours after cross-clamping and maximum increase of serum creatinine within 48 hours after cross-clamping in CR patients (white boxes, dashed line; $n=36$) and control patients (gray boxes, solid line; $n=40$). A, Box plots showing the change of serum creatinine from baseline to specified time points. B, Development of mean (\pm SEM) serum creatinine from baseline (0 hour) to 48 hours after cross-clamping.

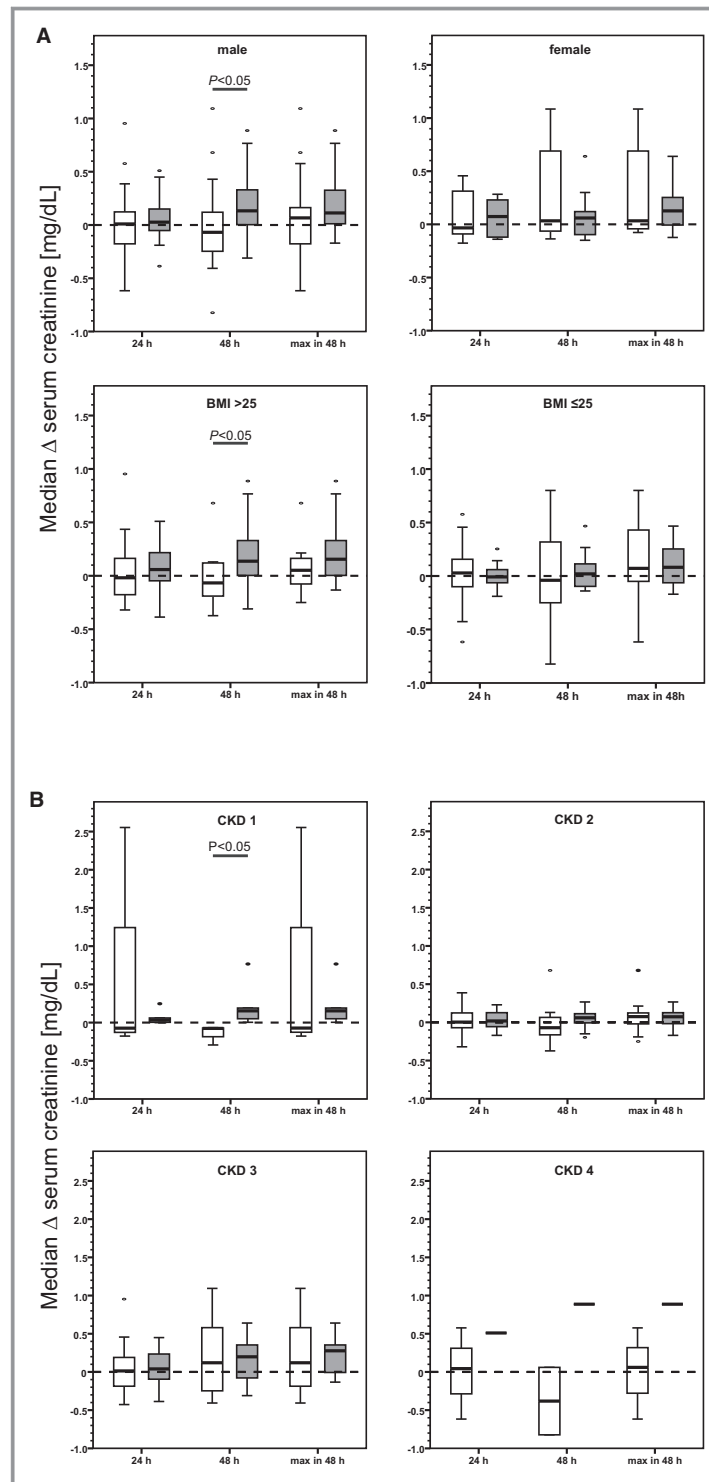


Figure 3. Subgroup analyses showing impact of sex (A, upper panel), body mass index (BMI; A, lower panel) and chronic kidney disease (CKD) stages (B). A, Male participants: calorie reduction (CR) group (white boxes), 29 of 36 participants; control group (gray boxes), 31 of 40 participants. BMI >25: CR group, 22 of 36 participants; control group, 30 of 40 participants. B, CKD 1: CR group (white boxes), n=3; control group (gray boxes), n=5. CKD 2: CR group, n=15; control group, n=13. CKD 3: CR group, n=13; control group, n=20. CKD 4: CR group, n=2; control group, n=1. Box plots showing the change of serum creatinine from baseline to specified time points.

Table 3. Secondary End Points: Biochemical Parameters

	CR Group (n=36)	Control Group (n=40)	P Value
CK, day 0, U/L	98 (64–145)	83 (55–129)	0.28
Δ CK, day 0 to 24 h after cross-clamping, U/L	596 (440–779)	539 (347–839)	0.39
LDH, day 0, U/L	207 (185–238)	220 (194–230)	0.46
Δ LDH, day 0 to 24 h after cross-clamping, ng/L	107 (65–145)	100 (37–172)	0.92
NT-proBNP, day 0, ng/L	576 (268–1782)	827 (438–1708)	0.25
Δ NT-proBNP, day 0 to 24 h after cross-clamping, ng/L	924 (340–4359)	381 (–304 to 2295)	0.27
CRP, day 0, mg/L	3.0 (3.0–3.2)	3.0 (3.0–4.6)	0.44
Δ CRP, day 0 to 24 h after cross-clamping, mg/L	104 (82–130)	97 (73–124)	0.51
WBC, day 0, ×1E9/L	6.2 (4.9–6.8)	6.4 (4.9–7.4)	0.57
Δ WBC, day 0 to 24 h after cross-clamping, ×109/L	3.2 (1.5–5.2)	3.8 (1.9–5.5)	0.39
Lactate, day 0, mmol/L	1.1 (1.0–1.6)	1.2 (1.0–1.6)	0.85
Δ Lactate, day 0 to 24 h after cross-clamping, mmol/L	0.4 (0.0–0.6)	0.5 (0.1–1.5)	0.27
NSE, day 0, μmol/L	23.8 (20.1–28.3)	22.9 (19.8–29.5)	0.66
Δ NSE, day 0 to 24 h after cross-clamping, μmol/L	5.7 (–1.6 to 12.5)	10.9 (0.5–19.4)	0.26
Troponin T, day 0, μg/L	0.013 (0.010–0.024)	0.019 (0.012–0.035)	0.045
Δ Troponin T, day 0 to 24 h after cross-clamping, μg/L	0.558 (0.201–1.065)	0.458 (0.343–0.790)	0.85
NGAL in urine, day 0, μmol/L	17.8 (10.0–32.9)	13.8 (10.0–26.3)	0.41
Δ NGAL in urine, day 0 to 8 h after cross-clamping, μmol/L	17.4 (–3.9 to 48.4)	9.6 (–2.5 to 30.4)	0.56
Creatinine at discharge, mg/dL	1.0 (0.9–1.3)	1.2 (1.0–1.4)	0.24
Δ Creatinine, day 0 to discharge, mg/dL	–0.08 (–0.18 to 0.04)	0.07 (–0.02 to 0.26)	0.0006

Parameters measured in serum unless otherwise indicated. Day 0 indicates baseline value before surgery. Values presented as median (interquartile range). CK indicates creatine kinase; CR, calorie restriction; CRP, C-reactive protein; LDH, lactate dehydrogenase; NGAL, neutrophil gelatinase-associated lipocalin; NSE, neuron-specific enolase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; WBC, white blood cell count.

short-term diet on promoting stress resistance in the acute setting has been investigated in more detail only recently.²⁴ With respect to the kidney, Mitchell et al published a proof-of-principle study in a murine renal ischemia–reperfusion injury model in which short-term CR completely prevented death from AKI and dramatically ameliorated renal failure.⁹ Thus, as an analogy to ischemic preconditioning, dietary preconditioning using moderate short-term CR could be exploited as an effective, easy-to-use, and inexpensive procedure to prevent anticipated kidney injury. Moreover, although the beneficial effect of ischemic preconditioning on cardiac recovery in a model of myocardial infarction was lost with age²⁵—a possible explanation for the disappointing findings of 2 recent clinical trials investigating the impact of RIPC on cardiac outcomes in patients with an average age of 70 years—dietary preconditioning apparently is independent of age, at least in animal models. Of note, ischemic preconditioning–induced organ protection can be restored even in old animals if they are pretreated with CR.²⁶ Despite the simplicity and clinical feasibility of a short-term dietary preconditioning protocol, there is a complete lack of clinical studies investigating the value of dietary preconditioning for organ protection. In a recent

trial that analyzed the effect of a calorie-restricted enteral feeding protocol in critically ill patients on mortality, the only significant difference compared with standard enteral feeding was a lower incidence rate of renal replacement therapy, indicating that dietary restriction might be beneficial if used not only before but also after the onset of injury.²⁷ We thus set out to conduct this single-center, randomized, controlled, open-label, clinical, pilot study in AKI-prone patients scheduled for cardiac surgery.

Although the results regarding the primary outcome did not meet the expectations in the intention-to-treat analysis, there was a significant between-group difference when looking at serum creatinine change at 48 hours, the time frame used in the KDIGO AKI classification, and at discharge. Of note, analysis of the timely evolution of kidney function revealed that serum creatinine levels in the CR group were restored quickly after day 1, whereas there was a steady increase in creatinine over the first 2 days in the control group. Moreover, with 41.7% versus 47.5%, respectively, the AKI incidence was considerably lower in the CR group than in the control group. Subgroup analyses suggest that the protective effect might be most prominent in men and in obese patients,

Table 4. Secondary End Points: Clinical Parameters

	CR Group (n=36)	Control Group (n=40)	P Value
AKI, n, (%)	15 (41.7)	19 (47.5)	0.60
KDIGO stage 1, n, (%)	7 (19.4)	13 (32.5)	
KDIGO stage 2, n, (%)	6 (16.7)	5 (12.5)	
KDIGO stage 3, n, (%)	2 (5.6)	1 (2.5)	
RRT, n, (%)	2 (5.6)	0 (0.0)	0.13
Cumulative hours of urine output <0.5 mL/kg/h, mean (SD)			
0–24 h after cross-clamping (h)	2.1 (3.7)	2.3 (2.9)	0.72
24–48 h after cross-clamping (h)	1.1 (2.3)	1.8 (3.3)	0.36
48–72 h after cross-clamping (h)	0.2 (0.6)	0.9 (1.7)	0.04
Length of stay (d), median (IQR)	10 (9–11.5)	10 (8–12)	0.68
Length of stay on ICU (h), median (IQR)	38.5 (23.5–78.5)	39.5 (22–93)	0.63
Catecholamine administration (h), median (IQR)	16 (9.0–32.0)	16.5 (8.5–34.5)	0.79
Mechanical ventilation (h), median (IQR)	14.5 (12.0–25.5)	17.5 (13.0–24.5)	0.55
New onset of atrial fibrillation, n, (%)	3 (8)	0 (0)	0.06
Nonfatal perioperative myocardial infarction, n, (%)	0 (0)	0 (0)	NA
Nonfatal perioperative stroke, n, (%)	0 (0)	0 (0)	NA
Death, n, (%)	2 (5.6)	2 (5.0)	0.91

AKI indicates acute kidney injury; CR, calorie restriction; ICU, intensive care unit; IQR, interquartile range; KDIGO, Kidney Disease: Improving Global Outcomes; NA, not assessed; RRT, renal replacement therapy.

a finding that is in line with observations made in animal experiments²⁸ and in the clinical setting.^{29,30} Of note, serum creatinine decreased steadily until the day of discharge in the CR group but increased in the control group with a net

difference of 0.15 mg/dL. These findings indicate that a prophylactic short-term diet might indeed have a beneficial effect on renal recovery and restoration of kidney function in patients undergoing cardiac surgery.

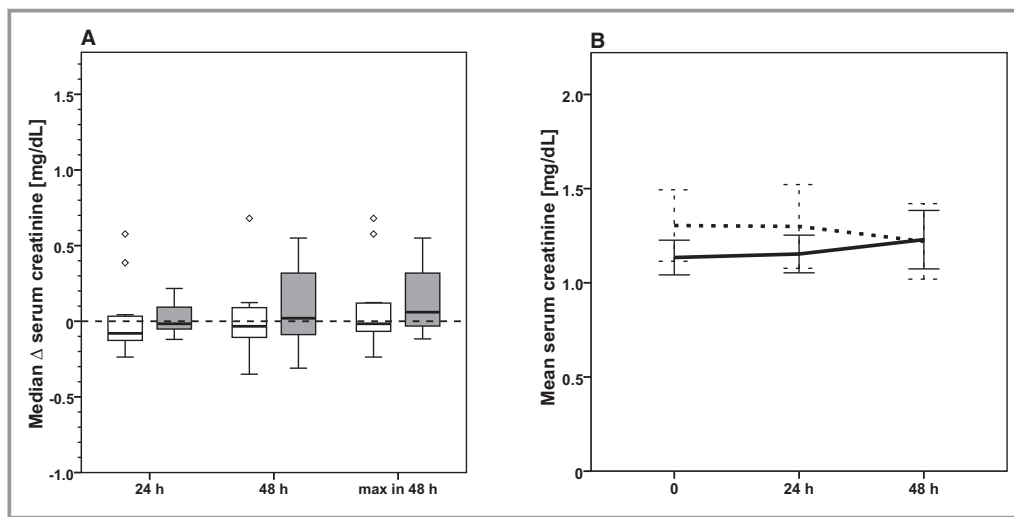


Figure 4. Per-protocol analysis. Evolution of serum creatinine from baseline to 24 hours after cross-clamping (primary end point) and from baseline to 48 hours after cross-clamping and the maximum increase of serum creatinine within 48 hours after cross-clamping in CR patients (white boxes, dashed line, n=13) and control patients (gray boxes, solid line, n=11). A, Box plots showing the change of serum creatinine from baseline to specified time points. B, Development of mean (\pm SEM) serum creatinine from baseline (0 hour) to 48 hours after cross-clamping.

Given the dramatic impact of short-term dietary restriction in animal studies, the question arises of why such small effects were observed in this trial. Several protocol-derived factors may have mitigated the protective effect of CR. First, it is unknown how long a diet must be applied and how much caloric content has to be restricted to elicit a protective effect in humans. To this end, the 7-day diet used in this pilot trial might well have been inappropriate. Moreover, we calculated DEE by using the widely accepted Mifflin–St. Jeor equation. Whether this equation (or any alternative published equation) is applicable to often very sick patients awaiting cardiac surgery is at least questionable; probably the yielded numbers overestimate the true energy demand or at least do not mirror the actually ingested amount of calories in this population. Consequently, the true calorie intake in the control group was probably lower than expected from the estimation, and the difference between the 2 groups may have been lower than anticipated. Potential unintended CR in the control group is a key confounder in CR studies, as was shown by the divergent results of the primate trials on this subject.¹⁹ In turn, although there was an excess net loss of dry body mass of 0.8 kg in the CR group over the control group, indicating a true dietary effect, the target of 40% CR compared with the control group was probably not achieved. Second, with respect to the composition of the diet itself, more recent work suggested that the reduction of protein intake, rather than the limitation of energy supply, leads to increased cellular stress resistance, which in turn promotes an organ-protective effect.^{31,32} In our trial, however, the daily protein intake was not markedly different in both groups. Third, although we enrolled only patients with at least 1 risk factor for developing postsurgery AKI, calculation using the Cleveland Clinic Foundation score³³ revealed that our patients carried only a low to intermediate risk (3 points [2–4 points] in the control and 2 points [2–4 points] in the CR group [$P=0.9$]). In 2 adequately powered studies investigating the organ-protective impact of RIPC in patients who were not preselected for an additional AKI risk—the ERICCA trial (Effect of Remote Ischemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Graft Surgery)⁵ and the RIPHeart trial (Remote Ischemic Preconditioning for Heart Surgery)⁶—no benefit was observed for any of the clinical end points, including AKI incidence. In contrast, in another study focusing on AKI after cardiac surgery as the primary end point in patients with a Cleveland Clinic Foundation score >6 , Zarbock et al reported a highly significant protective effect of RIPC.⁷ Similarly, we might speculate that preconditioning protocols in general are most likely to be effective in high-risk patients.

Despite identical levels at the time of screening in both groups (1.1 mg/dL), mean serum creatinine increased by 0.2 mg/dL (0.2 mg/dL) after 1 week of CR but did not change (0.0 mg/dL [0.2 mg/dL]) in the control group ($P=0.0001$). Why this occurred is not clear. The statistically significant

($P<0.0001$) median loss of 2.4 kg of body water in the CR group (versus a 0.1-kg gain in the control group)—most likely related to a lower sodium intake compared with the normal Western diet—may have led to a clinically relevant volume deficit that ultimately rendered the kidney even more susceptible to further damage. Consequently, the observed favorable effect of CR on creatinine kinetics would represent an underestimation of the true beneficial potential of CR.

We also analyzed biomarkers of functional and/or structural integrity of organs other than the kidney (ie, troponin T, creatinine kinase, NT-proBNP, and neuron-specific enolase), as well as general markers of inflammation (C-reactive protein, white blood cell count), systemic ischemia (lactate), and cellular integrity (lactate dehydrogenase) at 24 hours after cross-clamping. CR had no impact on any of these biomarkers, suggesting that moderately reduced energy supply before surgery is safe and does not interfere with short-term systemic recovery.

Finally, our study has several methodological limitations. The observed creatinine rise within the first 24 hours after surgery was 0.1 mg/dL and thus well below what we expected; therefore, the calculated group size in this pilot trial has to be considered too small to come to a clear estimate of the protective potential of CR. Furthermore, because a reduction in calorie intake was readily noticed by study participants, randomization in a blinded fashion was not possible. Moreover, accurate supervision of calorie intake, especially in the control group, was hampered by the ambulatory design of the trial.

In conclusion, the findings of this trial do not reflect the remarkable effects of CR seen in animal experiments; however, the intervention itself is safe and feasible, and the analysis of secondary end points revealed small but promising beneficial signals. Given the mounting experimental evidence that dietary interventions are powerful tools for promoting enhanced cellular stress resistance with far-ranging impact for disease prevention and treatment, further clinical investigations are warranted.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

University of Cologne



Protocol

Influence of preoperative calorie-reduced diet on renal function after heart surgery interventions in at-risk patients

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Protocol code: 001

Version dated 12.08.2014, version V001-30

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I. Synopsis

National Coordinator of the clinical trial (NC)	Dr. med. Volker Burst Clinic II for Internal Medicine Cologne University Hospital Kerpener Str. 62 D-50937 Cologne
Title of clinical trial:	Influence of preoperative calorie-reduced diet on renal function after heart surgery interventions in at-risk patients
Indication	Optional therapy options for nephroprotection during heart surgery
Phase:	Other trial non-AMG/non-MPG trial
Type of trial:	Monocentric clinical trial
Trial design/methodology:	Controlled, prospective, unblinded, two-arm trial
Number of patients:	Randomized: 82 (treatment group 41, control group 41) PP population: 52 (treatment group 26, control group 26)
Primary endpoint	By way of a “proof of concept”, it is to be investigated whether a short-term calorie reduction before cardiac surgery favorably influences the development of acute renal failure (ARF).
Target variables:	Primary target variable: <ul style="list-style-type: none"> • ¹Increase in serum creatinine in mg/dl 24 h after the onset of ischemia (“cross clamping”) Secondary target variables: <ul style="list-style-type: none"> • Neutrophil gelatinase-associated lipocalin (NGAL in µg/l) in urine 8h after the onset of ischemia

	<p>Other target variables:</p> <ul style="list-style-type: none"> • ¹Maximum increase in serum creatinine within the first 48 h after the onset of ischemia • Occurrence of an ARF according to KDIGO I, II, III • ¹Maximum serum creatinine value postoperatively during hospitalization • Need for renal replacement treatment during hospitalization • In-hospital mortality • Duration until possibility of discharge • Length of hospital stay • ¹CRP 24 h after onset of ischemia • ¹Leukocyte count 24 h after onset of ischemia • ¹Creatine kinase (CK) 24 h after onset of ischemia • ¹Troponin T 24 h after onset of ischemia • ¹Lactate dehydrogenase 24 h after onset of ischemia • ¹N-terminal Pro brain natriuretic peptide (NT-ProBNP) 24h after onset of ischemia • ¹Lactate 24 h after onset of ischemia • ²Left ventricular pumping function in echocardiography according to record, unless done postoperatively <p>¹ <i>Evaluation of the target parameters compared to the day of surgery (Day 0) in the morning before the surgery initial value</i></p> <p>² <i>Evaluation compared to the most recent preoperative findings</i></p>
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<p>Diagnosis and main inclusion criteria:</p>	<p>Diagnosis: Cardiac surgery need in patients with chronic kidney disease as a risk factor for the development of postoperative renal failure</p> <p>Main inclusion criteria:</p> <ol style="list-style-type: none">1. Men and women aged over 18 years2. Caucasian ethnicity3. Planned cardiac surgery with use of the heart-lung machine with a lead time of at least 8 days4. the indication for cardiac surgery is made by the supervising referring physicians, and the Department of Cardiac and Thoracic Surgery of the University of Cologne5. Written consent in the case of existing legal competence6. At least one of the following risk factors (according to records):<ul style="list-style-type: none">• Serum creatinine > 1.1 mg/dl in men or serum creatinine > 0.9 mg/dl in women• Diabetes mellitus• pAOD• Cardiac insufficiency with NYHA 3-4 or EF ≤ 50%• Combined CABG+valve surgery• Re-operation in status post CABG or status post valve surgery• Age ≥ 70 years• COPD• > 70% stenosis of the main stem of the left coronary artery
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	<p>Main exclusion criteria:</p> <ol style="list-style-type: none"> 1. Terminal renal insufficiency (compulsory dialysis) 2. Status post kidney transplantation 3. Malnutrition (BMI < 18.5 kg/m²) 4. Body weight: < 46 kg for men < 51 kg for women 5. BMI > 35 kg/m² or body weight > 120 kg 6. Catabolic metabolism (serum albumin <25 g/l) 7. Calorie-reduced diet within the previous 4 weeks 8. Loss of appetite 9. Weight loss > 1 kg in the past 2 weeks, unless explained by diuretics 10. Underlying wasting disease 11. Uncontrolled local or systemic infection 12. Contraindication for enteral nutrition 13. Known allergy to or intolerance of the ingredients of the formula diet used 14. Pregnancy or breastfeeding 15. Participation in other interventional trials 16. Absence of safe contraceptive measures or non-occurrence of menopause (in women) 17. Persons who are in a dependency/employment relationship with the investigators 18. Accommodation in an institution by judicial or administrative order.
Name of the measure:	<p>Formula diet (Fresubin[®] energy fiber Drink, Fresenius, Kabi Deutschland GmbH)</p> <p>Caloric reduction to 60% of the daily energy metabolism</p>

Comparison with:	Diet ad libitum according to the habits of the patients.								
Duration of therapy:	Diet: Day -7 up to and including Day -1 preoperatively (Day 0 is the day of surgery)								
Schedule:	<table> <tr> <td>First patient first visit (FPFV):</td> <td>01.12.2011</td> </tr> <tr> <td>Last patient first visit (LPFV):</td> <td>30.04.2015</td> </tr> <tr> <td>Last patient last visit (LPLV):</td> <td>30.06.2015</td> </tr> <tr> <td>Integrated final report:</td> <td>29.02.2016</td> </tr> </table>	First patient first visit (FPFV):	01.12.2011	Last patient first visit (LPFV):	30.04.2015	Last patient last visit (LPLV):	30.06.2015	Integrated final report:	29.02.2016
First patient first visit (FPFV):	01.12.2011								
Last patient first visit (LPFV):	30.04.2015								
Last patient last visit (LPLV):	30.06.2015								
Integrated final report:	29.02.2016								
Statistician	<p>Dipl.-Math. Ingrid Becker. Institute for Medical Statistics, Computer Science and Epidemiology University of Cologne Kerpener Str. 62 D-50937 Cologne</p>								
Statistical methods:	<p><u>Primary endpoint</u></p> <p>The difference between serum creatinine 24h after the onset of ischemia and serum creatinine on Day 0 before the intervention is compared in the two trial groups by means of a t-test in the ITT population. A PP analysis is also performed, as well as subgroup analyses for gender, age class, BMI class (normal/overweight), diabetic (yes/no), ischemia time, type of surgery, and CKD stage before surgery.</p> <p><u>Secondary endpoints</u></p> <p>The quantitative characteristics are analyzed in 2-group comparisons with t-tests or nonparametric methods. The categorical variables are evaluated by means of chi-squared or Fisher tests. Subgroup analyses are performed on 2-group comparisons with regression models if necessary.</p>								
GCP conformity:	This trial is conducted according to the current version of the protocol and the internationally recognized guidelines for Good Clinical Practice (ICH-GCP) including the archiving of essential documents for 10 years (according to §13, para. 10 GCP-V).								

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1. Introduction

Acute renal failure (ARF) constitutes a significant complication in hospitalized patients with an incidence of about 30%. Depending on existing comorbidities, a mortality of up to 60% occurs in critically ill or postoperative patients. In addition, according to recent findings, the long-term life expectancy of patients is significantly reduced in those who have once experienced ARF[1]. The development of chronic renal failure up to the need for chronic dialysis treatment after surviving ARF is also of increasing importance[2]. These prognostically unfavorable developments must be recorded not only with the most severe manifestation of ARF with an essential need for dialysis, but even with only a slight renal function impairment[3]. The overall increasing incidence of ARF in itself means that acute renal failure therefore represents an increasing medical and significant health economic problem.

In the current classifications of ARF (RIFLE criteria and AKIN criteria), a slight increase in creatinine is therefore already considered to be ARF. In the KDIGO (Kidney Disease: Improving Global Outcome) guidelines for ARF, ARF is similarly defined as a serum creatinine increase of ≥ 0.3 mg/dL or 50% of the baseline value within 48 h.

Unlike in community acquired, isolated ARF, in hospitalized patients with ARF glomerular, vascular or interstitial changes are causes of secondary importance. In the majority of cases, however, there is functional (prerenal ARF) or structural (renal ARF) damage to the tubulus apparatus. Etiologically, renal hypoperfusion with subsequent ischemia is almost always responsible, in particular of hypoxia-sensitive regions such as the S3 segment of the proximal tubulus and the ascending limb of Henle's loop. In addition, there is often toxic tubulus damage, usually combined with ischemia. The triggers of renal hypoperfusion, in addition to sepsis and conditions with reduced cardiac output, include in particular cardiac surgeries, especially when using a heart-lung machine. In the literature, the incidence of ARF after such interventions is indicated as 45%. The development of severe ARF requiring dialysis is 4% [4]. An effective treatment of ARF could not be established despite intensive research in the last 40 years.

For the pathophysiological description of ARF, the multiphase concept was introduced [5]. After a more or less long-running prerenal phase, a so-called initial phase occurs, and finally

an extension phase in which the first structural changes to the tubular epithelium occur. At the same time, there is an inflammatory co-reaction and congestion of the peritubular capillaries. These developments are initially reversible, but with increasing duration the potential for an early cure decreases. At the end of this development, there is complete dedifferentiation and loss of tubular epithelium up to tubular necrosis (maintenance phase of ARF). Only through proliferation and redifferentiation of the remaining epithelial cells (or organ-derived stem cells) is repair of the tubular apparatus then possible (repair phase).

A therapeutic intervention is useful according to these processes, in particular in the early stages that last from several hours to one or two days, in the sense of prevention or protection of tubular epithelium. Although pharmacological renal protection is possible in this context, at least in animal models, translational approaches failed without exception in clinical trials to date. The most likely reason for this is the late diagnosis of ARF in clinical practice, since the increase in serum creatinine does not indicate the tubular lesion itself, but rather the loss of function of glomerular filtration that often follows the lesion only with a considerable time lag. Therefore, the initial and extension phase, and thus the relevant therapeutic window, are often missed.

A new preventive approach is to increase the ischemia resistance before the actual lesion leading to the ARF. This approach would be feasible in particular for the prevention of postoperative ARF after cardiac surgery. In this case, we speak of ischemic preconditioning [6]. In classic animal experiments, rats are exposed in several short episodes to renal ischemia-reperfusion (by clamping the renal artery). As a consequence, a significantly increased tolerance of the kidneys over a longer ischemia interval is then developed. A similar approach in humans, however, is not possible.

Another way to achieve increased ischemia resistance is short-term caloric restriction. It has long been known that a diet with moderate caloric restriction leads to a prolongation of life in both animals and humans. Among other mechanisms, this effect is mainly attributed to the antioxidant characteristics of a reduced calorie diet. In recent years, in addition, it was shown in a number of animal trials that even a short-term diet results in direct biochemical and cellular adaptation processes, leading to a significantly increased resistance to ischemic organ damage. Several working groups have demonstrated this, firstly in a rat model of liver transplantation [7]. If the donor animals were left fasting for 3 days before the surgery, then

89% of recipient animals survived after liver transplantation, whereas all of the recipient animals that received an organ from donor animals in the control group without a previous diet died as a result of ischemia. In other trials, similar observations were made in models of cerebral and cardiac ischemia[8][9]. Although the mechanisms are not completely understood, it is assumed that caloric restriction leads to a reduction in oxidative stress levels per se and a strengthening of the body's own antioxidant defense mechanisms.

Mitchell et al. demonstrated that a four-week caloric reduction by 30% in mice leads to a dramatic reduction in renal ischemia reperfusion damage [10]. In the control group, ARF-associated mortality of 60% was observed after 40 minutes of clamping of the renal arteries. In the diet group, on the other hand, 100% of the animals survived with markedly less pronounced renal dysfunction. A similar result was found when the animals, instead of several weeks of dieting, fasted only in the last 3 days before surgery (with free fluid intake).

Similar trials in humans do not exist to date (PubMed search, no ongoing investigation at clinicaltrials.gov). The aim of the proposed clinical trial is to clarify the question of whether the impressive results of the above animal experiments can be transferred to humans and short-term caloric restriction is an effective prophylaxis of ARF.

Approximately 85 to 90% of all cardiac procedures (bypass, valve replacement) are now done with planning, usually with a forward or waiting period of about four weeks. This interval can be used in principle for "dietary preconditioning".

2. Endpoints of the clinical trial

2.1. Rationale for the clinical trial

Cardiosurgical interventions are associated with a high risk of postoperative acute renal failure (ARF) with significant morbidity and mortality. To date, no preventive or therapeutic measures exist to prevent this. According to this data from animal trials, a preoperative reduced-calorie diet may be a new preventive measure in this context.

This trial will investigate whether this also applies to humans. Patients with an increased risk of developing postoperative renal failure are randomized into two arms. In a diet arm, patients receive a restrictive diet of 60% of the individual's daily energy metabolism in the form of a formula diet during the last 7 days prior to cardiac surgery. In the control arm, patients eat as usual and without caloric or other restrictions.

2.2. Primary endpoint

The primary endpoint of the clinical trial is to investigate whether preoperative caloric reduction in the sense of a preventive measure leads to a reduction of the postoperative loss of renal function. It is known that even a small postoperative renal dysfunction is associated with increased mortality and morbidity, so it can be assumed that patients with a high risk of developing ARF benefit most from an effective nephroprotective measure. The renal function impairment is measured by the absolute increase in serum creatinine in mg/dl 24 h after the onset of ischemia time (cross-clamping). This corresponds to the standard procedure in comparable intervention trials and, by recording serum creatinine as a steady variable, is the most sensitive indicator of renal function impairment immediately after surgically induced renal damage.

Hypothesis: A seven-day, calorie-reduced diet reduces the increase in serum creatinine after cardiosurgery in patients with pre-existing risk factors for postoperative renal function disorder.

2.3. Secondary and other endpoints

The determination of neutrophil gelatinase-associated lipocalin in urine (uNGAL in $\mu\text{g/l}$) is performed as a sensitive marker of renal tubule damage.- As a secondary target variable, the uNGAL concentration is determined 8 hours after the onset of the ischemia time[11-14].

The classification of acute renal failure is based on the KDIGO criteria (Table 1) with 3 degrees of severity KDIGO I, II and III. The central laboratory parameters are the maximum absolute increase in serum creatinine within the first 48 h after surgery and the restriction of urine production. Creatinine measurements are done 24 h and 48 h after the onset of ischemia (cross-clamping), in addition to more routine creatinine recording in the context of inpatient treatment. Urine excretion is recorded hourly. Other target parameters of this trial, therefore, are the maximum increase in serum creatinine within the first 48 h after surgery (recorded based on all creatinine values during this period; see above) as a steady variable and the categorical KDIGO classification.

Table 1: Classification of ARF according to KDIGO

Stage	S-creatinine increase	Diuresis
1	≥ 0.3 mg/dl within 48h or ≥ 1.0 – 1.9-fold increase (48h)	< 0.5 ml/kg/h over 6 – 12h
2	≥ 2.0 – 2.9-fold increase (48h)	< 0.5 ml/kg/h over ≥ 12 h
3	≥ 3.0 -fold increase or Increase to ≥ 4.0 mg/dl or Acute dialysis	< 0.3 ml/kg/h over ≥ 24 h or anuria over ≥ 12 h

In addition, the maximum serum creatinine value detected postoperatively during hospitalization (based on the routinely performed blood sampling), the need for renal replacement treatment, the mortality during hospitalization, the duration until discharge readiness, and the length of hospital stay are recorded as other target parameters.

Since the caloric restriction in animal experiments also has similarly pronounced protective effects on other organ systems, subsequent further examinations must be performed. To

assess the general inflammatory response, we measure the C-reactive protein (CRP) in $\mu\text{g/l}$, the leukocyte count per μl and, for the evaluation of cardiac function, troponin T in $\mu\text{g/l}$, creatine kinase in U/l, lactate dehydrogenase in U/l and N-terminal Pro brain natriuretic peptide (NT-ProBNP) in ng/l , and as a surrogate parameter for tissue ischemia lactate in mmol/l .- All measurements are taken 24 h after the onset of ischemia. All other measurements of the named parameters, which are collected and documented in the context of the medical care until discharge from inpatient care are also recorded for the trial. A possible postoperative echocardiographic measurement of left ventricular function is also recorded in the trial if preoperative findings in the recent past are available for comparative purposes.

3. Organizational structure

3.1. Sponsor

There is no need for a sponsor because this clinical trial is not subject to AMG or MPG. Since the clinical trial is to be performed for quality assurance according to the internationally recognized guidelines of Good Clinical Practice (ICH-GCP), the National Coordinator assumes the corresponding responsibilities.

3.2. National Coordinator of the clinical trial

National Coordinator of the clinical

trial (NC): Dr. Volker Burst
 Clinic IV for Internal Medicine
 Cologne University Hospital
 Kerpener Str. 62
 D-50937 Cologne

3.3. Statistics

Statistician: Dipl.-Math. Ingrid Becker,
 Institute for Medical Statistics, Computer Science and
 Epidemiology University of Cologne
 Kerpener Str. 62
 D-50937 Cologne

3.4. Data Monitoring Committee

A Data Monitoring Committee, consisting of an independent expert, PD Dr. Müller-Ehmsen (Department III for Internal Medicine, Cologne University Hospital), is set up. The task of the DMC is to monitor the safety of the participants in the clinical trial, by the DMC assessing the

safety and efficacy of the trial measures and continuously monitoring the integrity and validity of the recorded data and the conduct of a clinical trial. The DMC receives access to all necessary data for this.

3.5. Other Committees

3.5.1. Steering Committee

Not established

3.5.2. Advisory Committee

Not established

3.5.3. Review Board

Not established

3.6. Test laboratories and other technical facilities

The laboratory parameters are measured by the Institute for Clinical Chemistry of the Cologne University Hospital.

The medical pre- and postoperative care, and the surgical procedure are performed by the Department of Cardiac and Thoracic Surgery at the University Hospital.

The recording of anthropometric parameters (body weight, survey of body composition by means of bioimpedance measurement, waist circumference) and the care of the trial participants from a nutrition physiology viewpoint before, during and after the diet is under the supervision of Dr. Michael Faust, lead senior physician, Center for Endocrinology, Diabetology and Preventive Medicine.

This also includes medical monitoring, specialist diabetological monitoring and adaptation of a possible antidiabetic therapy.

3.7. Central organization units

Project management: Dr. Franziska Grundmann

Dr. Volker Burst

Dr. Torsten Kubacki

Monitoring: Center for Clinical Studies Cologne (CCS)

Gleuelerstr. 269

D-50935 Cologne

Data management: NC

3.8. Investigators and trial sites

The clinical trial is performed at a single trial site.

Requirements for investigators and trial sites

The National Coordinator has several years of experience in clinical trials and can demonstrate his expertise in GCP through successful participation in a recognized investigator course and a trial director course.

The investigators have methodological and practical key knowledge about the conduct of clinical trials and knowledge of the GCP Guideline and can prove this by a certificate from a recognized investigator course.

3.9. Financing

The financing for the trial is provided by Fresenius Kabi GmbH (see contract).

4. Trial implementation

4.1. General trial design

Patients with an increased risk of developing postoperative renal failure are randomized into two arms. In a diet arm, patients receive a standardized calorie-restricted diet of 60% of the individual's daily energy metabolism in the form of a formula diet during the last 7 days prior to planned cardiac surgery (with use of an HLM). In addition, you are allowed to drink calorie-free drinks, e.g. in the form of unsweetened tea or water. In the control arm, patients eat as usual and without caloric or other restrictions.

The diet formula used (Fresubin® energy fiber drink, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) is approved as a food according to §14 of the German Diet Regulation (amended version of the Diet Regulation, 2005); it is not a medicinal product. In addition, no promise of a cure in the sense of an AMG or MPG trial is linked to this trial with the formula diet used. Rather, it is purely a reduction of the calorie intake with a physiological nutritional composition for exclusively preventive purposes. This trial therefore corresponds to a non-AMG, non-MPG trial.

This trial is designed as a monocentric, randomized trial.

4.1.1. Schedule

The recruitment and inclusion of patients are planned from 01.12.2011 to 30.04.2015 (Table 2).

All of the following times refer to Day 0, which is the date of the cardiac surgery.

After inspecting the files and identifying the possible trial participants, the trial site is contacted and scheduling takes place no later than on the ninth day before the planned surgery (Day -9). The transport costs for these individual trial-related appointment dates in the trial outpatient clinic are reimbursed to the patient and to one companion. An insurance policy is concluded to cover the risk of transport and accidents. Another insurance policy covers the risk of the trial-related blood sampling and the associated venepunctures. As far as possible, blood is preserved via inserted venous catheters in the course of the trial. After

obtaining the informed consent, a blood sample is first taken with measurement of serum potassium in mmol/l and serum albumin in g/l. A screening visit with a review of the inclusion and exclusion criteria takes place. If the patient can be included in the trial, the randomization is performed based on an envelope system (IMSIE).

This is followed by the 1st visit with recording of anthropometric parameters in the period from Day -12 to Day -8.

The formula diet (Fresubin® energy fiber drink, Fresenius Kabi Deutschland GmbH) is made available to the patient. Patients in the control group continue eating according to their usual habits. In addition, the patients are given a diet journal or a food journal for the daily documentation of the food consumed, and a urine collection container with detailed instructions for correct storage of a 24-hour urine sample. Patients in the diet group receive an additional substitution with a total of 4 g sodium chloride/day to ensure an appropriate electrolyte absorption according to the guidelines (see also 4.6.1). These are handed out in the form of salt tablets and their administration is explained. If hypokalemia is detected in the screening, an additional substitution with 24 mmol potassium/day is performed.

The patients are advised of the need for appropriate fluid intake.

The dietary measure begins on the morning of Day -7 before the surgery date. The trial participants will be reminded by phone of the start of the diet on Day -8. The diet participants are urged to collect the emptied bottles of the formula diet and bring them to the trial site on the day of hospitalization.

On Day -1 before the surgery, the patients are hospitalized. During the hospitalization, a routine preoperative blood collection is performed, which includes the measurement of serum creatinine, CRP, leukocyte count, creatine kinase, troponin T and lactate dehydrogenase.

On the morning of the same day, the patients attend the trial site after fasting for the 2nd visit. The presentation occurs between 8:00 and 10:00 a.m. After remeasuring the body weight, body composition and waist circumference, and receiving the 24h urine sample, interviewing the patients about diet compliance during the previous 6 days and collecting the diet journals, as well as the receipt of the emptied bottles of the formula diet, the patients undergo the usual preoperative examinations according to cardiosurgery. The diet is

continued until Day -1 with inpatient monitoring until the anesthesiology-required food and fluid fasting in the department.

Between 6:00 and 8:00 a.m. on the morning of the surgery day, a blood sample is taken for measurement of serum creatinine (mg/dl), CRP (mg/l), leukocyte count (/ μ l), creatine kinase (U/l), troponin T (μ g/l), lactate dehydrogenase (U/l), NT-ProBNP (ng/l) lactate (mmol/l) and a urine collection for uNGAL measurement (μ g/l).

After the surgery, in addition to the routine blood sampling, trial-related samplings are performed 24 h after the onset of intraoperative ischemia (cross clamping) for measurement of serum creatinine (mg/dl), CRP (mg/l), leukocyte count (/ μ l), creatine kinase (U/l), troponin T (μ g/l), lactate dehydrogenase (U/l), NT-ProBNP (ng/l), lactate (mmol/l), 48 h after the onset of ischemia to determine the serum creatinine (mg/dl) and 8 h after the onset of ischemia for urine collection for measuring NGAL (μ g/l) in urine (Figure 1).

A deviation of 120 minutes in the postoperative approval of the blood values, and 60 minutes in the postoperative approval of the uNGAL is considered tolerable.

If these laboratory parameters are determined at a time within these tolerances in the context of inpatient care for a medical indication, these findings can be included in the trial and a new trial-related acceptance is then unnecessary.

After the sampling 48 h after the onset of ischemia, a follow-up is performed as long as the patient is hospitalized in the Cologne University Hospital. During this period, no samples are taken according to the trial protocol; however, the results of certain laboratory parameters in the care routine are documented, as well as the need for renal replacement treatment, other complications, death, time to discharge readiness and duration of hospitalization. Any postoperative echocardiographic findings are also recorded for the trial, in particular the left ventricular function.

Since corresponding key scientific trials are being performed at the present time on the mechanism of action of the protective properties of caloric restriction at our nephrology research laboratory, serum samples from all patients (7.5 ml) are collected at the time points 0 (Day 0, the morning before the surgery) and 24h after the ischemia and stored at -80°C to check new findings in the trial population. The patients are informed and explained about this storage of patient material. This measure can only be performed if the patients sign a

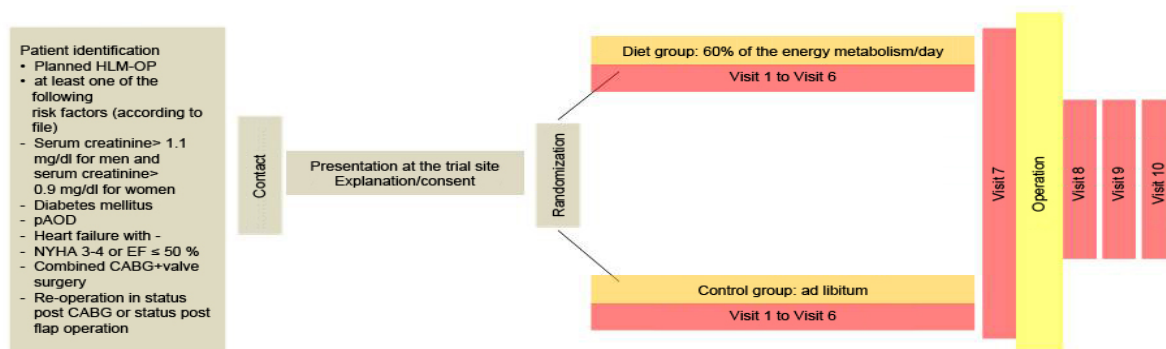
separate informed consent declaration. A transfer to third parties is excluded, and the samples are stored pseudonymized so that the samples can only be assigned to the investigators. A genomic analysis of these samples is excluded per se. All samples must be destroyed no later than 5 years after the end of the trial.

End of trial: 30.06.2015 is set as the end of the trial.

Table 2: Schedule of the trial

First patient first visit (FPFV):	01.12.2011
Last patient first visit (LPFV):	30.04.2015
Last patient last visit (LPLV):	30.06.2015
Integrated final report:	29.02.2016

Figure 1: Flow chart of the clinical trial



4.2. Discussion of the trial design

This is a randomized and controlled trial of the influence of a preoperative caloric reduction on the development of a postoperative ARF. Randomization is used to minimize possible selection bias and is performed with numbered and sealed envelopes created in the Institute for Medical Statistics, Computer Science and Epidemiology (IMSIE). Blinding is not possible due to the obviousness of the group membership.

Concerning the laboratory parameters to be determined, measurements are performed by the Institute for Clinical Chemistry of the Cologne University Hospital.

Regarding the case number planning (see 6.1), based on data available in the literature for the primary target variable, the increase of serum creatinine in mg/dl 24 h after onset of ischemia is compared to the serum creatinine in mg/dl on Day 0, and a difference of 0.2 mg/dl is adopted as the expected and clinically relevant effect size.[15, 16].

According to an evaluation by the Controlling of the Cologne University Hospital, during the period from 01.01.2010 to 31.12.2010, elective cardiac surgeries were performed on 217 patients with advanced renal failure. They included 26 dialysis patients who are not eligible for the trial. We therefore expect that, in principle, around 190 patients per year will be recruited for the trial.

Due to the possible benefits for patients, with simultaneously low invasiveness of the measures provided for in the trial (diet), and due to the current lack of alternative secure nephroprotective measures before cardiac surgery, we assume that 70% will agree to participate in the trial.

The drop-out rate after randomization depends primarily on the compliance of the patients in the diet group. Information on compliance within the first week of a calorie-reduced diet for the purpose of weight reduction is not available, because such trials usually focus on an observation period of months or years. In corresponding diet programs that have been and are being performed in the Cologne University Hospital (e.g. Optifast program, Nestlé), according to the attending physician Dr. M. Faust, an almost 90% diet compliance is regularly observed during the first week.* Due to the different motivation of the patients in this trial compared to patients in trials with the goal of weight loss, we here assume a dropout rate of 30% in the diet group and 10% in the control group.

4.3. Selection of the trial population

The trial population is Caucasian men and women over the age of 18 years capable of giving consent, in whom cardiac surgery with the use of a heart-lung machine (HLM) is planned with a lead time of at least 8 days, and in whom one of the following risk factors is already present: Renal function impairment with creatinine greater than 0.9 mg/dl (women) or 1.1 mg/dl (men), diabetes mellitus, peripheral arterial occlusive disease, cardiac insufficiency (EF \leq 50%), combined CABG+valve surgery, resurgery in status post CABG or status post valve surgery. Through this limitation, it is possible to obtain information about the efficacy of diets in the form of a “proof of concept” with a relatively small number of cases, because the above-described factors are considered important risk factors for the development of postoperative acute renal failure and thus a high-risk group is investigated that should benefit in particular from the preventive strategy of the approach studied here.

According to an evaluation by the Controlling, at least 190 patients can be recruited annually (see 4.2).

Children or persons incapable of consent are not included in the clinical trial.

Justification of the gender distribution

The above-mentioned 217 patients in 2010 included 159 male and 58 female patients. This also corresponds to the gender distribution in the overall population of patients having cardiac surgery. A subanalysis of the gender-specific data is performed.

4.3.1. Inclusion criteria

1. Men and women aged over 18 years
2. Caucasian ethnicity
3. Planned cardiac surgery with use of a conventional heart-lung machine with a lead time of at least 8 days
4. the indication for cardiac surgery is made by the supervising referring physicians, and the Department of Cardiac and Thoracic Surgery of the University of Cologne
5. Written consent in the case of existing legal competence

6. At least one of the following risk factors (according to records):
- Serum creatinine > 1.1 mg/dl in men or serum creatinine > 0.9 mg/dl in women
 - Diabetes mellitus
 - pAOD
 - Cardiac insufficiency with NYHA 3-4 or EF ≤ 50%
 - Combined CABG+valve surgery
 - Re-operation in status post CABG or status post valve surgery
 - Age ≥ 70 years
 - COPD
 - > 70% stenosis of the main stem of the left coronary artery

The existing risk factors must be able to be tracked based on the documented diagnoses or parameters, which are found in the information transmitted about the patients in the application.

4.3.2. Exclusion criteria

1. Terminal renal insufficiency (compulsory dialysis)
2. Status post kidney transplantation
3. Body weight < 46 kg for men, < 51 kg for women
4. Malnutrition (BMI < 18.5 kg/m²)
5. BMI > 35 kg/m² or body weight > 120 kg
6. Catabolic metabolism (serum albumin <25 g/l)
7. Calorie-reduced diet within the previous 4 weeks
8. Loss of appetite
9. Weight loss > 1 kg in the past 2 weeks, unless explained by diuretics
10. Underlying wasting disease

11. Uncontrolled local or systemic infection
12. Contraindication for enteral nutrition
13. Known allergy to or intolerance of the ingredients of the formula diet used
14. Pregnancy or breastfeeding
15. Participation in other interventional trials
16. Absence of safe contraceptive measures *or* non-occurrence of menopause (in women)

Safe contraceptive measures include procedures with a Pearl Index of less than 1%:

- a. Oral hormonal contraception (“the pill”)
- b. Dermal hormonal contraception,
- c. Vaginal hormonal contraception (NuvaRing®),
- d. Contraceptive plaster,
- e. Long-term effective, injectable contraceptives,
- f. Progesterone-releasing implant (Implanon®),
- g. Tubal ligation (female sterilization),
- h. Hormone-releasing intrauterine device,
- i. Double-barrier methods.

The unreliable methods therefore include: Condom plus spermicide, single-barrier methods (vaginal pessary, condom, female condom), copper coils, rhythm method, basal body temperature method, coitus interruptus.

17. Persons who are in a dependency/employment relationship with the investigators
18. Accommodation in an institution by judicial or administrative order.

4.4. Subsequent exclusion of clinical trial subjects

It is not intended to subsequently exclude trial participants from the trial. Exceptions are patients who end the trial early at their own request or cannot continue taking the formula diet due to an incompatibility or newly discovered allergy to the ingredients, and those patients whose surgery cannot take place at the scheduled time for non-trial-related reasons (Day 0). According to an “intention to treat” approach, the acquired data of all persons participating in the trial is included in the evaluation.

4.4.1. Procedure for premature end of treatment in the clinical trial

After excluding a patient due to incompatibility or newly discovered allergy to the ingredients of the formula diet, a follow-up observation of the complete disappearance of symptoms is performed. In the case of exclusion for any other reason, a follow-up observation is performed until the end of the diet phase (Day -1). Since this trial is not an investigation according to AMG or MPG, there is no need for a follow-up observation in the case of the premature end of the treatment.

4.5. Closure of trial sites / Premature termination of the clinical trial

4.5.1. Closure of trial sites

See 4.5.2.

4.5.2. Discontinuation of the entire trial

The National Coordinator is entitled to terminate the trial prematurely due to relevant medical or ethical concerns or the lack of feasibility of the trial. In such a case, the reasons for the early termination of the trial are documented in detail. If an investigator has ethical concerns regarding the continuation of the trial, these must be indicated to the National Coordinator immediately.

The premature termination of the trial is considered when

- the risk-benefit ratio for the patients has changed considerably,
- the termination of the clinical trial is considered necessary for safety reasons,
- the clinical trial proves to be unfeasible.

The National Coordinator decides about the termination of the trial.

4.6. Treatments

4.6.1. Treatments used

The patients randomized to the diet group receive a caloric reduction of 60% of the individually calculated total daily energy metabolism. The calculation of the individual energy metabolism is performed after randomization in the trial outpatient department.

Calculation of the daily energy metabolism:

With the Mifflin-St.Jeor formula, the daily basal metabolism of humans is approximately calculated as:

Men: $G_m = 9.99 \times \text{weight [kg]} + 6.25 \times \text{height [cm]} - 4.92 \times \text{age [years]} + 5$

Women: $G_w = 9.99 \times \text{weight [kg]} + 6.25 \times \text{height [cm]} - 4.92 \times \text{age [years]} - 161$

The total daily metabolism as the sum of the basal metabolic rate and active metabolic rate is calculated by multiplying the basal metabolic rate by the activity factor (AF):

AF 1.2 → no or only minimal physical load (sitting, lying)

AF 1.375 → light physical load (corresponding to walking for 2 h/day)

AF 1.550 → moderate physical load (corresponding to walking for 3 h/day)

AF 1.725 → high physical load (corresponding to walking for 4 h/day)

The patients receive a diet corresponding to: $60/100 \times G \times AF$

Diet form used

To standardize the calorie-reduced diet, the diet will consist of a formula diet as a nutrition drink on Days -7 to -1. Usually, formula diets for weight loss or in the initial phase of a change in diet are used. The recommended energy intake according to the guidelines (German Society for Nutritional Medicine, 2007) [17] must therefore be between 800 and 1200 kcal/day (in exceptional cases even below 800 kcal) and can be performed for up to 12 weeks. An intake of 800 kcal corresponds to a calculated daily metabolism of 1,333 kcal (100%) when following a reduction diet at 60% of energy metabolism. According to the simplified Mifflin St.Jeor formula:

$$E_m = 1.2 \times 24 \text{ kcal} \times \text{KG in kg} = 29 \text{ kcal} \times \text{KG in kg} \text{ for a man}$$

$$E_w = 1.2 \times 0.9 \times 24 \text{ kcal} \times \text{KG in kg} = 26 \text{ kcal} \times \text{KG in kg} \text{ for a woman}$$

thus results in the following lower safety margin for the body weight:

46 kg for men

51 kg for women

These limit values are therefore defined in this trial as the exclusion criteria. In addition, the caloric reduction is performed only for one week and under medical supervision.

In summary, there is therefore a very low expected risk to the patient due to the caloric restriction conducted here. In fact, in most of the patients investigated here according to the recommendations of professional societies, a caloric restriction according to the diet following here is indicated to reduce the cardiovascular risk.

The following are used: Fresubin[®] energy fiber drink (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). Fresubin[®] energy fiber drink is approved as a food according to §14 of the German Diet Regulation (amended version of the Diet Regulation, 2005); it is not a medicinal product. Fresubin[®] energy fiber drink has an energy density of 1.5 kcal/ml and is administered in 200 ml disposable plastic bottles (EasyBottle).

The composition of the basic nutrients is as follows:

Proteins	15%	(5.6g/100ml)
Fats	35%	(5.8g/100ml)
Carbohydrates	50%	(18.8 g/100ml)

In addition, the following ingredients are included: 2 g/100ml fiber.

The formula diet is rich in monounsaturated fatty acids, contains sufficient polyunsaturated fatty acids and is low in saturated fatty acids and cholesterol. This composition corresponds to the recommendations regarding the cardiovascular risk reduction diet of the American Heart Association, the European Society of Cardiology and the German Society of Cardiology. Coverage of the daily requirements of vitamins and trace elements is guaranteed. The diet is gluten-free and available in 6 flavors.

In summary, it is a balanced, high-fiber formula diet with a physiological composition of basic nutrients as recommended by the cardiology guidelines.[18-20]

Special notes for chronic renal disease patients

For patients with chronic renal insufficiency in stage CKD 1 to 4 (pre-dialysis), a low-protein diet of about 0.6 g/kg body weight is recommended[21]. With estimation of the average energy metabolism at an activity factor of 1.2 and with the simplified Mifflin St.Jeor formula:

$$E_m = 1.2 \times 24 \text{ kcal} \times \text{KG in kg} = 29 \text{ kcal} \times \text{KG in kg} \text{ for a man}$$

$$E_w = 1.2 \times 0.9 \times 24 \text{ kcal} \times \text{KG in kg} = 26 \text{ kcal} \times \text{KG in kg} \text{ for a woman}$$

the caloric restriction to 60% results in an average protein intake of 0.64 g/kg body weight for men and 0.58 g/kg body weight for women, which thus constitutes supplementation according to the guidelines.

For phosphorus intake with renal insufficiency, a target value of around 1000 mg is recommended, [21] which equates to a consumption of around 1,250 ml Fresubin® energy fiber drink. The upper limits of the recommended sodium and potassium intake are achieved with 3,000 ml and 1,400 ml Fresubin® energy fiber drink respectively. The prescription of 1,400 ml Fresubin® energy fiber drink or more is not planned in the diet group with 40% caloric reduction, because according to the above formulas this would mean a body weight of around 120 kg for men or 137 kg for women, but a body weight of ≥ 120 kg was formulated as an exclusion criterion.

In order to ensure adequate supplementation with sodium according to the ESPEN criteria for patients with chronic renal disease [22] (1.8 - 2.5 g/day), at a molecular weight of 23 grams and a dietary sodium intake of 480-680 mg/day (for 900-1200 kcal), an additional substitution of 1500 mg/day is provided. This corresponds to about 65 mmol. The substitution is provided as sodium chloride (corresponding to 4g per day). This is a sufficient intake comparable to the control group, in order to exclude a possibly hemodynamically relevant influence of salt.

With respect to trace elements and vitamins, the diet provides sufficient supplementation.

In order to avoid hypokalemia, particularly in the cardiac pre-loaded patient population, the patients receive a potassium check within the scope of Visit 1. If the potassium value in the plasma is below the normal range, supplementation with 24 mmol potassium per day is provided in the form of sustained-release capsules. The risk of hyperkalemia does not exist at this low dose.

Contraindications

According to the SPC, Fresubin® energy fiber is not used in disease states with fundamental contraindication of enteral nutrition, such as jejunal atonia, ileus, severe organ-specific disorders such as liver failure or acute pancreatitis and congenital metabolic disorders of the nutrients contained in Fresubin® energy fiber. In addition, Fresubin® energy fiber is not used in the case of known allergies or intolerance to the ingredients.

Progress of the preoperative phase

In Visit 1 after randomization into the diet arm, the patients, according to their energy metabolism, receive an individually calculated amount of Fresubin® energy fiber drink from Day -7 up to Day -1. The liquid food is given to the patient and stored at home at room temperature or in the refrigerator. Patients receive a tabular weekly schedule of the exact consumption. The possible need to consume only a portion of the individual drink bottles to achieve the calculated number of calories is discussed with the patient in detail. For this purpose, the patients receive a measuring cup at Visit 1. The consumption occurs under the

patient's own responsibility and independently at home. By means of regular phone calls, the patients are asked about their well-being and diet compliance. The patients are encouraged to write down all the food they have eaten and to keep the empty bottles and bring them on the day of hospitalization. For standardization purposes, the patients receive a diet journal in Visit 1. Any non-consumed drink bottles are disposed of by the patient after completion of the trial.

Patients are advised that only the intake of non-caloric beverages is allowed, e.g. in the form of unsweetened tea or water.

In patients with diabetes mellitus, careful monitoring is performed by the trial doctors under the supervision of Dr. M. Faust (diabetologist) and an adjustment of the antidiabetic treatment (insulin, oral medications).

The control group will follow the diet according to their normal habits, i.e. ad libitum without caloric or other restrictions on Day -7 until Day -1. Following a formula diet does not take place. The patients are encouraged to record all food consumed in writing. For standardization purposes, the patients receive a food journal in Visit 1.

Before the surgery, the period of food and fluid fasting set by the anesthesiology department is followed. After the operation, all treatment steps are determined by the department for cardiac and thoracic surgery according to the prevailing clinical standards.

The patients are followed up until the end of the hospitalization (see 4.1.1).

4.6.2. Description of the IMP

Not applicable, because no medicinal products are administered.

4.6.2.1. Production of the IMP

Not applicable

4.6.2.2. Labeling of the trial medication

Not applicable

4.6.2.3. Storage of the investigational medicinal product

Not applicable

4.6.3. Compliance with treatment/issuance and return of trial medication

Not applicable

4.6.4. Method for assignment of patients to treatment groups

The patients are randomized to the two treatment groups by means of consecutively numbered envelopes. The envelopes are created in IMSIE.

4.6.5. Selection of dosage of the IMP

Not applicable

4.6.6. Determination of the dosage and timing of the IMP administration for each participant

Not applicable

4.6.7. Blinding

Blinding of the patients is not possible due to the design and the associated obviousness of the group membership (delivery of the formula diet vs. instruction for free food intake) and is therefore omitted.

4.6.7.1. Unblinding

Omitted

4.6.8. Prior treatment and concomitant treatment

Any concomitant treatment initiated by a local physician or clinician is permitted. In diabetics, an adjustment of the medicinal treatment and monitoring of daily blood glucose profiles is performed under the supervision of Dr. Faust (diabetologist DDG).

4.6.8.1. *Escape treatment in emergencies*

Not applicable

4.6.9. **Further treatment after the end of the clinical trial**

The calorie-reduced diet of the patient ends with the start of the preoperative fasting established by the anesthesiology department.

In diabetics, the adjustment of the antidiabetic medication is again performed in the postintervention phase by the cardiology colleagues according to the medical condition under the supervision of Dr. Faust.

4.7. **Efficacy and safety parameters**

4.7.1. **Measurement of the efficacy and safety parameters**

4.7.1.1. *Primary target variable*

The primary target variable was set as the increase in serum creatinine in mg/dl 24 h after the onset of the ischemia time (cross-clamping) compared to the serum creatinine on Day 0 in the morning at 8 a.m. before the surgery. The start time of the ischemia can be seen in the surgery documentation. The measurements are made at the Institute for Clinical Chemistry of the University of Cologne as a three-time determination.

4.7.1.2. *Secondary and other target variables*

- Neutrophil gelatinase-associated lipocalin (NGAL) in µg/l in urine 8 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- Maximum increase of serum creatinine in mg/dl within the first 48h after surgery compared with the value on Day 0, in the morning at 8:00 a.m. before the operation.
- Categorical evaluation of the occurrence of ARF according to the KDIGO severity grades

- Maximum serum creatinine value in mg/dl during hospitalization, postoperative absolute value and compared to Day 0, in the morning at 8:00 a.m. before surgery.
- Creatine kinase in U/l 24 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- Leukocytes per μ l 24 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- C-reactive protein (CRP) in mg/l 24 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- Troponin T in μ g/l 24 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- NT-ProBNP in ng/l 24 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- Lactate dehydrogenase in U/l 24 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- Lactate in mmol/l 24 h after the surgery compared to Day 0, in the morning at 8:00 a.m. before the surgery.
- Need for renal replacement treatment during hospitalization

The need for the renal replacement procedure does not always correspond to KDIGO stage III, since in the postoperative environment the control of the volume balance is justified for the use of a renal replacement procedure.

- In-hospital mortality
- Duration of hospitalization in days
- Duration until possibility of discharge in days
- Echocardiographically determined postoperative left ventricular pump function (according to Simpson) compared to the echocardiographically determined preoperative left ventricular pump function (according to Simpson), if an

echocardiography during the postoperative hospitalization and an echocardiography in the period ≤ 30 days is preoperatively documented

4.7.1.3. *Safety analysis*

The formula diet used is approved as a food product with a physiological composition of carbohydrates, proteins and fats (balanced diet). In order to meet the recommendations according to the ESPEN Guidelines for chronic renal disease, additional salt and possibly potassium are substituted. The formula diet is not a medicinal product. The health risk due to the preparation was therefore estimated to be very low. A moderate caloric reduction, as provided for in this trial, does not have any harmful side-effects. Vice versa, a comparable diet for the reduction of cardiovascular risk in the studied population would even be recommended with the goal of weight loss. Patients in whom weight loss or a reduction in the diet are undesirable for medical reasons are not provided for due to the exclusion criteria in this trial.

To prevent possible blood glucose fluctuations in patients with diabetes due to the change of food intake and to make any necessary changes to the administered insulin dose or oral antidiabetic treatment, these patients are given diabetology care in the trial by a diabetologist (Dr. M. Faust). Postoperatively, the patients are treated according to standards in force and the requirements of the supervising physicians in cardiac and thoracic surgery. Patients are routinely given monitoring after the surgery, initially routinely in intensive care, and the transfer to the normal ward and discharge is according to the treating department.

Safety-related and perioperatively unexpected events are documented and made available to the ethics committee as well as the manufacturer of the formula diet. (for details see also 7.1.).

4.7.1.4. Description of the individual visits

Visit 1:

Visit 1 takes place between Day -12 and Day -8, in the morning between 8:00 and 10:00 a.m. after fasting, and includes the following points:

- Recording of anthropometric parameters: Height (cm), body weight (kg) (after emptying the bladder), body composition (bioimpedance method), waist circumference
- Calculation of the daily energy metabolism by: $G \times AF$ (see 4.6.1)
- Distribution of a diet journal and training in use
- Taking a urine sample (spot urine) for performing a urine sediment and a proteinuria determination.
- Distribution of a 24-hour urine container and detailed instructions for proper taking of a 24-hour urine collection

Patients who were randomized into the diet arm receive Fresubin® energy fiber drink bottles for 6 days (Day -7 to Day -2). The amount provided includes the number of bottles of 200 ml (equivalent to 300 kcal) containing 60% of the calculated daily energy metabolism. To ensure an accurate caloric intake in the amount of 60% of the energy metabolism, according to the calculated values, a certain proportion of a bottle per day must be discarded if necessary. For compliance reasons, this must always be done with the first bottle of the day. The patients receive a calibrated measuring cup and detailed instructions of how much volume must be discarded. In addition, you are encouraged to keep empty bottles and bring them with you on the day of hospitalization.

In patients with diabetes mellitus, an evaluation and possible change of the administered dose of insulin or oral antidiabetic treatment by a diabetologist (Dr. M. Faust) to prevent possible blood sugar fluctuations due to the change in the food intake.

Patients in the control group eat according to their usual habits, but document their food intake in a journal.

Visit 2 on Day -8:

The dietary measure begins on the morning of Day -7 before the surgery date. The trial participants will be reminded by phone of the start of the diet on Day -8.

Visit 3 on Day -7:

Telephone interview for evaluation of the general condition. The clarification of the newly discovered questions is performed. The patients in the diet group are questioned about their diet compliance and all patients are reminded of the importance of accurate documentation of the food intake in the diet journal provided.

Visit 4 on Day -5:

Telephone interview for evaluation of the general condition. The clarification of the newly discovered questions is performed. The patients in the diet group are questioned about their diet compliance and all patients are reminded of the importance of accurate documentation of the food intake in the diet journal provided.

Visit 5 on Day -3:

Telephone interview for evaluation of the general condition. The clarification of the newly discovered questions is performed. The patients in the diet group are questioned about their diet compliance and all patients are reminded of the importance of accurate documentation of the food intake in the diet journal provided. The patients are reminded of the urine collection on the following day (Day -2). The procedure is explained to them again.

Visits 2 to 5 are not compulsory, but are used to maximize compliance and ensure the most accurate recording of nutritional data. Since they can only be performed by phone, full compliance with the visits at the indicated times cannot always be safely guaranteed. Unsuccessful telephone contact attempts are repeated on Days -6, -4 and -2.

Visit 6 on Day -1:

In the morning, the patients attend the trial site after fasting. The presentation occurs between 8:00 and 10:00 a.m. After another measurement of body weight (after emptying the bladder), body composition and waist circumference and acquisition of the diet journals, emptied drinks bottles, and the 24-hour urine collection, the patients are hospitalized in the department of cardiac and thoracic surgery of Cologne University Hospital. During the hospitalization, a routine preoperative blood collection is performed, which includes the measurement of serum creatinine, CRP, leukocyte count, creatine kinase, troponin T and lactate dehydrogenase. These values are documented for the trial. Thereafter, the patients undergo the usual preoperative examinations according to the cardiosurgery department. The diet is continued until Day -1 with inpatient monitoring until the anesthesiology-required food and fluid fasting in the department.

Visit 7 on Day 0:

Between 6:00 and 8:00 a.m. on the morning of the surgery day, a blood sample is taken for measurement of serum creatinine (mg/dl), CRP (mg/l), leukocyte count (/ μ l), creatine kinase (U/l), troponin T (μ g/l), lactate dehydrogenase (U/l), NT-ProBNP (ng/l) lactate (mmol/l) and a urine collection for uNGAL measurement (μ g/l). In addition, a serum sample is taken (7.5 ml), with storage for later analysis at -80°C in the rooms of the nephrology lab (Department II for Internal Medicine).

Visit 8 on Day 0:

Study-related urine collection for the determination of NGAL in urine 8h after the start of intraoperative ischemia (cross-clamping, according to the operation report)

Visit 9 on Day +1:

Trial-related blood collection 24 h after the onset of intraoperative ischemia (cross-clamping, according to the surgery report) to determine: Serum creatinine, CRP, leukocyte count, creatine kinase, troponin T, lactate dehydrogenase, NT-ProBNP, lactate. Taking of a serum sample (7.5 ml) 24 h after the onset of ischemia, with storage for later analysis at -80°C in the rooms of the nephrology lab (Department II for Internal Medicine).

Visit 10 on Day +2:

Trial-related blood collection 48 h after the onset of intraoperative ischemia (cross-clamping, according to the surgery report) to determine the serum creatinine.

A difference of 120 minutes in the postoperative blood samples for determination of serum creatinine, CRP, leukocyte count, creatine kinase, troponin T, lactate dehydrogenase, NT-ProBNP, lactate, and a difference of 60 minutes in the postoperative measurement of uNGAL are considered tolerable.

If the laboratory parameters for serum creatinine, CRP, leukocyte count, LDH, creatine kinase, troponin T, lactate dehydrogenase, NT-ProBNP, lactate are determined at a time within this tolerance in the context of the inpatient stay for a medical indication, these findings can be included in the trial and a new acceptance is then unnecessary.

After the sampling 48 h after the operation, a follow-up is performed as long as the patient is hospitalized in the Cologne University Hospital. During this period, no samples are taken according to the trial protocol; however, the results of certain laboratory parameters in the care routine are documented, as well as the need for renal replacement treatment, other complications, death, duration of inpatient treatment and duration until actual discharge readiness. Possible postoperative echocardiographic findings, especially the left ventricular

pump function, are also recorded for the trial if a preoperative echocardiographic examination is documented within the period ≤ 30 days before surgery. The end of the trial participation is recorded in the patient file.

Duration of the clinical trial in the individual patient:

From Day -7 until the date of the dismissal

4.7.2. Adequacy of the measurement methods

As a measure of renal function impairment after the surgery in the sense of an acute renal failure (ARF), the increase in serum creatinine is recognized as a continuous variable. The maximum increase in serum creatinine within 48 h is used for the definition (KDIGO) of acute renal failure and the corresponding classification into the different degrees of severity. For better comparability, studies generally investigate the increase in serum creatinine after 24 or 48 h. This is reflected in the primary and secondary target variables selected.

As an expression of tubular damage, the determination of the tubular expressed neutrophil gelatinase-associated lipocalin in urine is established for the preclinical testing. Here, the detection of NGAL in urine very sensitively reflects the damage to the tubular system affected by ARF and thus represents a significantly more direct biomarker, in contrast to serum creatinine which is a measure of the glomerular function deterioration as a result of tubular damage. The NGAL determination in the urine was evaluated in previous examinations for the quantification of ARF after cardiac surgery and is used analogously to these trials.

As a measure of a postoperative inflammatory action, the variables C-reactive protein and leukocyte count introduced in the clinical routine and established in a variety of trials are appropriate.

Creatine kinase, troponin T and LDH are clinically well-evaluated parameters for the estimation of structural heart muscle damage. In everyday clinical practice, they are an integral part of cardiac diagnostics, especially with regard to ischemic heart damage.

N-terminal Pro-Brain natriuretic peptide (NT-ProBNP) is established as a marker of cardiac function in preclinical research as well as in everyday clinical practice. An increase in the peptide is correlated closely with a cardiac dysfunction.

Lactate is a systemic marker for ischemia but is nonspecific with regard to the genesis of ischemia.

4.7.3. Pharmacokinetics/determination of medicinal product levels

Not applicable

4.8. Data quality assurance

4.8.1. Monitoring

For quality assurance in the trial, monitoring is performed at the trial site. The aim of the monitoring is to review the assurance and protection of the rights and safety of the trial participants, the validity, verifiability and completeness of the trial data and the compliance of the trial conduct with the protocol, GCP and the applicable regulatory requirements.

All investigators agree that the monitor will periodically visit the trial site and is supported by the trial site appropriately. A corresponding passage is included in the declaration of consent (see chapter 5.4), which gives the monitor the right, taking into account the data protection act, to compare the case report forms (CRFs) with the original documents (medical records, findings, laboratory printouts etc.). The investigators allow the monitor to have direct access to all necessary documents for the trial-related monitoring. A monitor report is issued for every visit, documenting the progress of the trial and reporting any difficulties encountered (e.g. refusal of access).

4.8.2. Audits / inspections

Not expected in this trial.

4.9. Documentation

All audit-related data is collected by the responsible investigator promptly in the documentation forms provided in paper format. The documentation can be delegated to other members of the audit team. Exceptions are made for the notification of the patient, which must be done and documented by a trial doctor according to GCP. The recording forms are signed by the investigator personally.

The documentation in the CRF must be complete. For corrections to the documentation, the original entry must be readable and signed according to ICH-GCP E6.

4.9.1. Data management

The data management and evaluation are performed in IBM SPSS® Statistics. The national coordinator is responsible for the IT infrastructure and the development of the trial database in SPSS®. IMSIE helps to create the SPSS® database. The transfer of the collected data takes place by means of double data input by two independent data input clerks with subsequent data comparison. The national coordinator manages the access rights for the SPSS® database. Apart from the national coordinator and the two employees responsible for the data input, no other persons have access rights. The national coordinator creates a data management manual for the correct entry of the data in SPSS®. There is no need for an audit trail.

The database is integrated into a general IT infrastructure and security concept with a firewall and backup system. The data is backed up daily. After completing and cleaning the data, the database is closed and the data is exported for the statistical analysis.

4.9.2. Archiving

All documentation forms, consent forms and other important trial documents are stored according to §13 para. 10 GCP-V for at least 10 years. The patient identification list is stored separately from the documentation components.

5. Ethical and regulatory aspects

5.1. Independent ethics committees

The clinical trial is started only after the presence of a favorable opinion of the Ethics Committee.

5.2. Ethical conduct of the clinical trial

The present protocol and possible subsequent amendments to the protocol were or are written according to the Declaration of Helsinki, as amended in October 1996 (48th General Assembly of the World Medical Association, Somerset West, Republic of South Africa).

5.2.1. Legal provisions and guidelines considered

This clinical trial is conducted in accordance with the published principles of Good Clinical Practice (ICH-GCP) guidelines. These principles relate inter alia to Ethics Committee operations, patient clarification and informed consent, compliance with the protocol, administrative documents, documentation of the IMP, data collection, patient record (source documents), recording and reporting of safety-relevant events, and archiving of documents. All investigators and other staff directly involved in the trial were informed that staff authorized by the national coordinator are entitled to inspect the trial documents and medical records at any time.

5.3. Approval of the Ethics Committee and registration

The trial is submitted to the competent Ethics Committee for approval. Before the start of the clinical trial, the registration of the clinical trial is performed at Current Controlled Trials (www.controlled-trials.com) or a trial registry recognized by the WHO.

5.4. Informed consent of the trial participants

Patients can only be included in the clinical trial, if they have granted consent to participate in it, after having been informed by an auditor/investigator verbally and in writing about the nature, significance and implications of the clinical trial in an appropriate and comprehensible manner. They must have declared at the same time as the consent that they are in agreement with the data recording established in the context of the trial and its verification by people commissioned by the national coordinator (e.g. monitor). It must be clear that they can withdraw their consent at any time, without stating any reasons or suffering any adverse consequences.

The original of the written consent form is stored in the trial folder at the trial site. The patient must be given a copy of the written patient information, including a copy of the insurance certificate along with the conditions and the declaration of consent. In addition, both documents are stored in a copy in the patient record.

The patient information and informed consent form, all other documents that the participants receive and possible recruitment notices are submitted for approval to the competent Ethics Committee prior to use. In the context of the monitoring, it is checked whether the respective current consent was personally dated and signed by the patients concerned before the start of the clinical trial.

5.5. Trial participants insurance

A subjects insurance policy is taken out for all patients included under the group insurance contract of the Cologne University Hospital. The location, policy no., telephone and fax number of the insurance company are included in the patient information. In addition, travel accident insurance was taken out for all included patients to safeguard the visits to our trial site.

5.6. Data protection

The provisions of the data protection laws are observed. It is ensured that all trial materials and data are pseudonymised appropriately according to the data protection provisions before scientific use.

The trial subjects receive an explanation about the disclosure of their pseudonymous data as part of the documentation and notification requirements according to § 12 and § 13 GCP-V. Persons who do not consent to the disclosure are not included in the clinical trial.

6. Statistical methods and determination of the case number

6.1. Statistical and analytical plan

All tests are performed 2-sided at a significance level $\alpha = 0.05$. The primary analysis is performed in the ITT population; secondarily, the PP population is analyzed.

6.1.1. Trial populations

Intention to treat

This data set contains all patients who were randomized and in whom the planned surgery was performed. For these patients, at least 1 serum creatinine value must be present before the operation (measured on Day 0 or alternatively on Day -1).

Per protocol

This data set contains all randomized patients who were treated according to the protocol and whose serum creatinine values are available on Day 0 prior to the surgery and 24 h after the onset of ischemia. In addition, patients in the control group must have at least one caloric intake according to their calculated daily energy metabolism; patients of the diet group must have achieved a calorie restriction of at least 30% of the daily energy metabolism. This assessment is done according to the documentation in the diet journals.

Safety

The tertiary evaluation data set (safety population) contains all patients who have received the trial treatment.

6.1.2. Description of the patient population

The demographic data of the patients is evaluated descriptively for the overall population and the individual treatment groups.

6.1.3. Primary target variable

The primary target parameter is the difference in serum creatinine 24 h after the onset of ischemia for serum creatinine on Day 0 prior to surgery. The primary analysis is the comparison of the two trial groups using the t-test in the ITT population. The PP population is also analyzed as a sensitivity analysis. Subgroup analyzes are performed for gender, BMI class (normal/overweight) and CKD stage before surgery (see also 6.1.5.).

For the ITT evaluation, missing serum creatinine values 24 h after the onset of ischemia are replaced by the last value measured during the trial period up to 24h after the onset of ischemia in the context of the standard or trial treatment. As a rule, the measurement is performed by default once in the first 24h after surgery.

6.1.4. Secondary target variables

The quantitative characteristics are analyzed in 2-group comparisons with t-tests or nonparametric methods (U-Mann-Whitney). The categorical variables are evaluated by means of chi-squared or Fisher tests. Subgroup analyses are performed on 2-group comparisons with regression models if necessary.

6.1.5. Subgroup analyses

Subgroup analyses are performed for the gender, age class, nutritional status (BMI < 25kg/m², BMI > 25kg/m²), diabetes, ischemia time, type of surgery and CKD stage before surgery with respect to the primary target variable.

6.1.6. Intermediate evaluation

None

6.2. Determination of the number of cases

From the literature, [15] [16] an average increase in serum creatinine 24h after cardiac surgery of 1.7 +/- 0.3 mg/dl to 2.1 +/- 0.2 mg/dl can be expected. This results in a change

(estimated for variance correlation of 0.5) of 0.4 +/- 0.25 mg/dl. A treatment effect of 0.2 mg/dl is considered to be detectable and clinically relevant.

A dropout rate (or noncompliance) of 30% is assumed in the diet arm and 10% in the control arm; the dropout rate is calculated according to Donner [23], to balance out a possible distortion of the therapy effect due to the expected high dropout rate in the ITT population. For an alpha error of 0.05, a power of 80%, an average dropout rate of 20%, and the use of a two-sided test, the following case numbers are obtained:

Evaluation (PP): 26 per group = 52 patients

Plus dropouts:

41 per group

Randomization (ITT): 82 patients

7. Safety

7.1. Definitions of adverse events and side-effects

Since this is not an AMG/MPG trial and no drug is used or even tested, no adverse events (AEs), severe adverse events (SAEs) or side-effects in the narrow sense are observed. Due to the severity of the operation performed on the patients, numerous complications are possible in the perioperative course. In addition, the underlying disease of the participants may be worsened. These complications are not documented separately.

However, if safety-relevant and perioperative events occur which are not explainable or expected during the course of the underlying disease or cardiac surgery, they are documented and tested for a possible connection with the diet. In addition, these events are made available to the Ethics Committee and the manufacturer of the nutrition drink.

The obligation to document safety-relevant events begins at the start of the diet phase (Day - 7, 8:00 a.m.) and ends on the date of discharge.

7.1.1. Adverse event

See 7.1

7.1.2. Adverse drug reaction

The formula diet used is not a medicinal product. ADRs within the meaning of AMG/MPG therefore do not occur. Incompatibilities are nausea, vomiting or diarrhea. These are documented and reported at the request of the Ethics Committee.

It is expected that the seven-day calorie reduction will lead to a subjective feeling of hunger and weight loss of about 0.3-1 kg. These are not side-effects.

7.1.3. Serious adverse event or serious adverse drug reaction

See 7.1. Serious ADRs within the meaning of AMG/MPG therefore do not occur.

7.1.4. Unexpected adverse reaction

A suspected unexpected adverse reaction (SUSAR) in the sense of AMG/MPG is not observed in this trial.

7.1.5. Case of a suspected unexpected serious adverse reaction

A severe suspected unexpected adverse reaction (SUSAR) in the sense of AMG/MPG is not observed in this trial.

7.1.6. Other possible trial-specific complications and/or risks

Not applicable

7.2. Control of adverse events

The national coordinator ensures that all persons involved in the clinical trial are adequately informed of their responsibilities in the case of safety-related events that are not expected in the context of the underlying disease or perioperatively. At each visit, the patients are asked, if possible from a medical point of view, whether any safety-relevant events have occurred.

7.2.1. Documentation of safety-relevant events and side-effects

All safety-relevant events (see also 7.1) are documented in the CRF, including the parameters listed below.

- Date and time of start and end,
- Intensity,
- Relationship to the diet,

- **Serious or non-serious,**
Serious events are considered to be those that:
 - lead to the death of a subject
 - are immediately life-threatening
 - make an unforeseen hospitalization or the extension of a hospitalization necessary
 - cause a congenital anomaly or birth defect
 - cause a permanent or serious disability or invalidity
 - another clinically relevant event according to medical judgment.
- Interruption or discontinuation of the diet or other measures taken.

If a safety-relevant and perioperatively unexpected event occurs, the trial participants in question must be observed in any case until the symptoms have subsided, pathological laboratory values have declined to baseline values, a plausible explanation for the adverse event is found, until the death of the trial subject or until the end of the clinical trial for the patients concerned.

7.2.2. Intensity of the safety-relevant event

The investigator will classify the safety-relevant events that have occurred with regard to their intensity as follows:

- Mild: Clinical symptom or sign which is well tolerated.
- Moderate: Clinical symptom or sign which is sufficient to impair normal activities.
- Severe: Clinical symptom or sign which leads to severe impairment or disability or the inability to perform everyday tasks.

7.2.3. Relationship of the safety-relevant event with the formula diet

For each safety-relevant and perioperatively unpredictable event, it is assessed by the investigator whether or not a relationship with the formula diet is suspected. The type and pattern of the reaction, the clinical status of the patient, the concomitant medications and other relevant clinical parameters must be considered.

Similar to the AMG, this study defines a causality evaluation with regard to the formula diet (WHO Causality Assessment of Suspected Adverse Reactions):

- Certain: An event that follows a comprehensible time sequence after the administration of the formula diet, disappears after discontinuation or dose reduction and occurs again on renewed exposure.
- Probable: An event that follows a comprehensible time sequence after administration of the formula diet, disappears after discontinuation or dose reduction and cannot be explained by the known characteristics of the clinical condition of the subject/patient.
- Possible: An event that follows a comprehensible time sequence after administration of the formula diet, but that could easily have been caused by a range of other factors.
- Unlikely: An event in which sufficient information exists to assume that there is no connection with the formula diet.
- Not evaluated: An event that was reported as an adverse event in which an evaluation of the relationship was not made at the time of reporting, because additional data is necessary or is being collected.

Not evaluable: An evaluation of the relationship is not possible.

A suspected case occurs when the causal relationship is assessed as being at least “possible” or “not evaluable” or “not estimated”. Events that are classified with regard to a causal relationship as “unlikely” are not considered to be suspected cases.

7.3. Notification of serious adverse events, pregnancies and changes in the risk-benefit ratio

Serious adverse events in the narrower sense are not observed in this trial (see 7.1). A pregnancy is documented in a separate pregnancy form and safety-relevant and perioperatively unexpected events are reported to the Ethics Committee and the manufacturer of the nutrition drink. All safety-relevant and perioperatively unexpected events

are entered in a tracking list which can be viewed by the national coordinator at all times. They are also made available to the DMC (see 3.4.).

7.3.1. Reporting by the investigator to the national coordinator

The investigators inform the national coordinator as soon as possible of the occurrence of a safety-relevant and perioperatively unexpected event. The investigators also inform the national coordinator immediately about a pregnancy that has occurred during the clinical trial. This is documented in a separate pregnancy form. For the follow-up of the outcome of the pregnancy, a separate consent declaration by the pregnant woman is necessary.

7.3.2. Second opinion by the Sponsor

Not applicable. The national coordinator must be informed promptly of all safety-relevant and perioperatively unexpected events and will evaluate them.

7.3.3. Unblinding for blinded IMPs

Not applicable

7.3.4. Reporting to the Ethics Committee

During the clinical trial, a notification is made in the case of suspected safety-relevant and perioperative events or unexpected events in the context of the underlying disease.

Fatal or life-threatening SUSARs

Not applicable.

Non-fatal and non-life-threatening SUSARs

Not applicable.

7.3.5. Verification and reporting of changes in the risk-benefit ratio

The National Coordinator must immediately, but no later than 15 days after acquiring the knowledge, must inform the competent Ethics Committee about any situation that requires a new review of the risk-benefit evaluation of the IMP. This includes, in particular, events relating to the conduct of the trial which may possibly affect the safety of the persons involved.

7.3.6. Information of the data monitoring committee

The DMC is informed by the national coordinator of all safety-related events. (see also 3.4)

7.3.7. Information of the investigators

If new information becomes known which differs from the scientific information given to the investigator, the national coordinator informs all investigators about it.

7.3.8. Information of the authorization holder

Fresenius Kabi is informed about all safety-related events in parallel with the Ethics Committee and the national coordinator. This information is provided via a safety fax with the fax no. +49-6172-608-390224.

7.4. Annual report on the safety of the trial participants

At the request of the competent Ethics Committee, the national coordinator submits a report on the safety of the trial participants in accordance with ENTR/CT 3, which takes account of all available relevant information during the reporting period.

- Report on the safety of the participants in the clinical trial in question,
- List of all suspected cases of safety-related and perioperatively unexpected events that have occurred in the clinical trial in question,

The national coordinator submits the report within 60 days after the deadline (data closing date).

8. Use of the data and publication

8.1. Reports

8.1.1. Interim reports

Only at the request of the Ethics Committee (see 7.4.)

8.1.2. Final report

The competent Ethics Committee must be informed of the completion of the clinical trial within 90 days.

Within one year of completion of the clinical trial, the Ethics Committee must be sent the summary (synopsis) of the final report on the clinical trial, which covers all significant events in the trial.

8.2. Publication

It is intended to present the results of the clinical trial in due course and after mutual agreement with the National Coordinator in a scientific journal and/or at German and international congresses. In principle, the joint publication of the clinical trial must be prioritized. The “Uniform requirements for manuscripts submitted to biomedical journals. International Committee of Medical Journal Editors” (ICMJE) [24] are considered.

The registration of the clinical trial in a public register is also performed according to the recommendations of the ICMJE (see also 5.3).

For all publications, the data protection must be respected both for all data from the persons concerned and for the data of the participating investigators.

The publication or presentation of the results of this clinical trial require the prior notification and prior comment and approval of the national coordinator.

By signing the registration form, the investigator agrees that the results of this clinical trial may be submitted to the national approval and surveillance authorities, the German Medical Association, the Accredited Physicians' Association and the health insurance companies. At the same time, the investigators agree that their name, address, qualification details and the extent of their participation in the clinical trial will be notified in this context.

9. Amendments to the protocol

An amendment to the agreed trial conditions set out in the protocol is not provided. In exceptional cases, however, amendments to the trial conditions are possible. These occur only after mutual agreement between the national coordinator and the biometrician as well as all signatories of this protocol. Any amendment to the trial procedures provided in the protocol must be made in writing, stating the appropriate justification, and must be signed by all authorized signatories of this protocol (amendment).

According to § 10 para. 1 and 4 GCP-V. subsequent amendments requiring approval are submitted to the Ethics Committee and only implemented when approval is granted. Amendments that are necessary to avert imminent danger are not affected by this.

10. Literature

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11. Annexes

11.1 Trial site

11.2 Test laboratories and other technical facilities

11.3 Data Monitoring Committee

11.4 Consent of the Department Director

11.5 Protocol Agreement Form

11.6 Patient information and informed consent

11.7 Scientific Product Rationale

11.8 Confirmation of insurance

11.9 Conditions of insurance

11.1. Trial site

Cologne University Hospital
Clinic II for Internal Medicine
Nephrology and General and Internal Medicine
Kerpener Straße 62
D-50937 Cologne

11.2. Test laboratories and other technical facilities

Cologne University Hospital
Institute for Clinical Chemistry
Kerpener Straße 62
D-50937 Cologne

Cologne University Hospital
Clinic II for Internal Medicine
Nephrology, Rheumatology, Diabetology and General Internal Medicine
Nephrology Research Laboratory
Kerpener Straße 62
D-50937 Cologne

Cologne University Hospital
Clinic and Polyclinic for Cardiac and Thoracic Surgery
Heart Center
Kerpener Straße 62
D-50937 Cologne

Cologne University Hospital
Center for Endocrinology, Diabetology and Preventive Medicine
Kerpener Straße 62
D-50937 Cologne

11.3. Data Monitoring Committee

PD Dr. med. Müller-Ehmsen

Clinic III for Internal Medicine

Clinic and Polyclinic for Cardiology, Pneumology, Angiology and Intensive Care Medicine

Heart Center

Kerpener Straße 62

D-50937 Cologne

11.4. Consent of the Department Director

Influence of preoperative calorie-reduced diet on renal function after heart surgery interventions in at-risk patients

Protocol code: 001

Declaration of the Clinic Director

Organization: Clinic II for Internal Medicine
Cologne University Hospital

Address: Kerpener Straße 62
D-50937 Cologne

Head of Clinic: Prof. Dr. T. Benzing

I hereby declare that I am in agreement with the conduct of the above-mentioned clinical trial under the leadership of Dr. med. V. Burst.

Place, date

Signature of head of clinic / stamp

11.5. Protocol Agreement Form

I guarantee that I will conduct the clinical trial according to the protocol “Influence of preoperative calorie-reduced diet on renal function after cardiac surgeries in at-risk patients” according to the guidelines and all applicable legal requirements, the principles of the “International Conference on Harmonization (ICH) Guideline on Good Clinical Practice (GCP)” and the “World Medical Association Declaration of Helsinki (2008)”. I have read and understood all clinical and administrative sections of this protocol.

National Coordinator of the clinical trial (block capitals)

National Coordinator of the clinical trial (signature)

Date