Intradialytic Protein Ingestion and Exercise do Not Compromise Uremic Toxin Removal Throughout Hemodialysis

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Objective: Dietary protein and physical activity interventions are increasingly implemented during hemodialysis to support muscle maintenance in patients with end-stage renal disease (ESRD). Although muscle maintenance is important, adequate removal of uremic toxins throughout hemodialysis is the primary concern for patients. It remains to be established whether intradialytic protein ingestion and/or exercise modulate uremic toxin removal during hemodialysis.

Methods: We recruited 10 patients with ESRD (age: 65 ± 16 y, BMI: 24.2 ± 4.8 kg/m^2) on chronic hemodialysis treatment to participate in this randomized cross-over trial. During hemodialysis, patients were assigned to ingest 40 g protein or a nonprotein placebo both at rest (protein [PRO] and placebo [PLA], respectively) and following 30 min of exercise (PRO + exercise [EX] and PLA + EX, respectively). Blood and spent dialysate samples were collected throughout hemodialysis to assess reduction ratios and removal of urea, creatinine, phosphate, cystatin C, and indoxyl sulfate.

Results: The reduction ratios of urea and indoxyl sulfate were higher during PLA (76 ± 6% and 46 ± 9%, respectively) and PLA + EX interventions (77 ± 5% and 45 ± 10%, respectively) when compared to PRO (72 ± 4% and 40 ± 8%, respectively) and PRO + EX interventions (73 ± 4% and 43 ± 7%, respectively; protein effect: $P = .001$ and $P = .023$, respectively; exercise effect: $P = .25$ and $P = .52$, respectively). Nonetheless, protein ingestion resulted in greater urea removal ($P = .046$) during hemodialysis. Reduction ratios and removal of creatinine, phosphate, and cystatin C during hemodialysis did not differ following intradialytic protein ingestion or exercise (protein effect: $P > .05$; exercise effect: $P > .05$). Urea, creatinine, and phosphate removal were greater throughout the period with intradialytic exercise during PLA + EX and PRO + EX interventions when compared to the same period during PLA and PRO interventions (exercise effect: $P = .034$, $P = .039$, and $P = .022$, respectively).

Conclusion: The removal of uremic toxins is not compromised by protein feeding and/or exercise implementation during hemodialysis in patients with ESRD.

Keywords: End-stage renal disease; anabolic interventions; dialysis; physical activity; dietary protein

Introduction

METABOLIC WASTE PRODUCTS are insufficiently removed from the circulation by the kidneys of patients with renal disease. Substances that accumulate in body fluids due to reduced glomerular filtration and negatively modulate biologic functions have been named uremic toxins.[1] In end-stage renal disease (ESRD), when the glomerular filtration rate is less than 15 mL/min/1.73 m^2, uremic toxins can accumulate up to detrimental concentrations.[2,3] This can be prevented through hemodialysis treatment, which partially replaces renal solute removal. During hemodialysis,

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circulating uremic toxins diffuse through a semipermeable membrane into the dialysate and, as such, are removed from the body.[4] Small uremic toxins, such as urea and creatinine, diffuse quickly through this membrane. In contrast, compartmentalized, larger, and protein-bound uremic toxins, such as phosphate, cystatin C, and indoxyl sulfate, respectively, are removed much less efficiently during hemodialysis.[5–7]

Although the removal of uremic toxins during hemodialysis is a life-saving treatment, low muscle mass and poor physical functioning are common among patients undergoing chronic hemodialysis treatment.[8–10] Protein-energy wasting, a syndrome characterized by the progressive loss of muscle and fat mass, is present in 28%-54% of these patients.[11,12] This high prevalence can be attributed to sedentary behavior and uremic toxin accumulation between hemodialysis sessions and to the loss of nutrients, especially amino acids, during hemodialysis sessions.[13,14] As malnutrition is associated with worse clinical outcomes and a reduced quality of life in patients on chronic hemodialysis treatment,[15,16] interventions that may attenuate or prevent muscle loss in this population have received much attention over the past few years. Increasing dietary protein consumption and stimulating physical activity in patients on chronic hemodialysis treatment are key anabolic interventions to preserve muscle mass.[17] Nowadays, these interventions are often implemented during hemodialysis sessions (intradialytic) to counteract the protein deficit and sedentary behavior in these patients.[18,19]

However, it has been suggested that intradialytic dietary (protein) intake may interfere with the effective removal of uremic toxins, as smaller decreases of circulating urea concentrations during hemodialysis sessions have been reported with intradialytic food consumption.[20–22] Intradialytic protein ingestion may affect the reduction ratio of urea during hemodialysis through absorption/release of urea in splanchnic organs or through postprandial splanchnic blood pooling and/or reduced perfusion of peripheral tissues.[23,24] In contrast, intradialytic exercise increases perfusion of peripheral tissues and reduces splanchnic perfusion.[25,26] However, whether these physiological changes due to intradialytic protein ingestion and/or exercise modulate uremic toxin removal during hemodialysis remains to be determined.

Therefore, we recruited 10 patients with ESRD to participate in a cross-over study of 4 hemodialysis sessions during which these patients ingested a protein or a nonprotein placebo beverage both at rest and following exercise. Throughout hemodialysis, we measured the concentrations of urea, creatinine, phosphate, cystatin C, and indoxyl sulfate in blood and spent dialysate to provide a detailed insight into the impact of exercise and protein ingestion on uremic toxin removal.

Methods

Study Population

A total of 10 patients with ESRD undergoing hemodialysis in the morning or afternoon through a well-functioning arteriovenous shunt for at least 3 months were recruited between March 2019 and August 2020 at the dialysis department of Maastricht University Medical Center+, Maastricht, The Netherlands (Figure S1 provides the Consolidated Standards of Reporting Trials flow diagram). Patients with an active infection, cognitive disorder, intolerance to food ingestion during hemodialysis, missed hemodialysis session in the last month prior to the study period, or contraindication to intradialytic exercise were excluded. Patients were informed about the purpose of the study, experimental procedures, and possible risks prior to signing a written informed consent. This study is part of a greater project investigating the impact of exercise and protein ingestion during hemodialysis, parts of which have already been published.[27] For this project, a sample size of 10 participants was calculated a priori based on differences in incremental area under the curve of plasma amino acid concentrations.[27] All available samples from these patients were used for the present study. Spent dialysate urea, creatinine, phosphate, and cystatin C concentrations could only be assessed in n = 9 due to an insufficient amount of spent dialysate available for analysis. The study was approved by the Medical Research Ethics Committee Academic Hospital Maastricht/Maastricht University (NL65880.068.18), conformed to standards for the use of human subjects in research as outlined in the latest version of the Helsinki Declaration of 1975 and was registered at the Netherlands Trial Register (NTR7152).

Pretesting

At least 1 week before the first test day, a pretesting session was scheduled during routine hemodialysis to familiarize patients with intradialytic exercise. In addition, patient's medical history, physical examinations, laboratory analysis results, and hemodialysis regimen were registered. A dialysis cycle ergometer (Thera Riser, Medica Medizintechnik GmbH, Hochdorf, Germany) was placed in front of the treatment chair and after a 5-min warm-up, the resistance level of the dialysis cycle ergometer was increased until patients reported a score between 12 and 15 on the Borg Ratings of Perceived Exertion (RPE) scale.[28] If patients reported a score <12 or >15 on the Borg RPE scale within this period, the resistance level was adjusted accordingly. When patients succeeded to perform 10 min of moderate-intensity exercise, the resistance level was noted and used for the exercise intervention.

Study Design

During 4 hemodialysis sessions, separated by at least 1 week, all patients were assigned to ingest a placebo or protein beverage both in a rested state (placebo [PLA] and

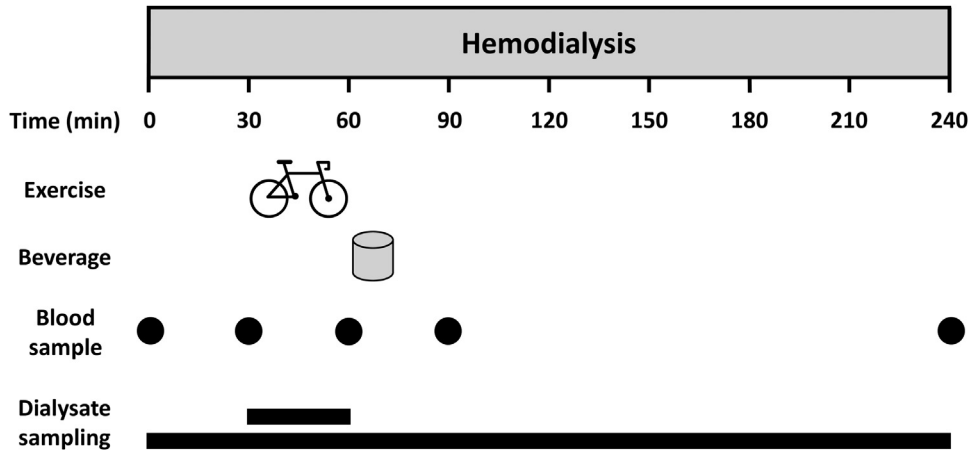

Figure 1. Schematic representation of study protocol. t = 0 min represents the start of the hemodialysis session. During four hemodialysis sessions, patients ingested 40 g protein or a nonprotein placebo both at rest and during recovery from intradialytic exercise in a randomized cross-over design.

protein [PRO], respectively) and following 30 min intradialytic exercise (PLA + exercise [EX] and PRO + EX, respectively) in a randomized cross-over design. Patients were randomly assigned to an order of interventions using an online randomizer (http://www.randomizer.org/) and the randomization order of test beverages was not shared with investigators or participants until all procedures and statistical analyses of the primary and secondary outcomes were complete. The independent researcher was responsible for the preparation of test beverages, which were labeled as per participant and test day number before handing them to an investigator. The protein beverage contained 40 g milk protein concentrate (Refit MPC 80, Friesland Campina, Amersfoort, The Netherlands) and 2 sweeteners (Natrena, Douwe Egberts, Amsterdam, The Netherlands) dissolved in 300 mL water. The placebo beverage consisted of only the 2 sweeteners dissolved in 300 mL water. The independent researcher shared the order of exercise performance during test days with the investigators after pretesting was completed. Although patients were blinded to the order of exercise performance, it was not possible to conceal the intervention during test days due to the nature of the exercise intervention. Patients started the intradialytic exercise by performing a 5-min warming-up on the dialysis cycle ergometer, during which they were instructed not to surpass a score of 9 on the Borg RPE scale. Subsequently, the resistance level was increased to the previously determined value and patients continued cycling for 20 min. At the end of the intradialytic exercise, patients performed 3 min of cycling with a score between 9 and 12 and the last 2 min with a score less than 9 on the Borg RPE scale as a cooling-down. Between the first and second test day, patients filled out a food diary for 6 days to assess habitual dietary intake. A licensed dietician carefully instructed patients on how to perform the 6-day food intake diary. All ingested foods and beverages were reported in household measurements or specified as portion sizes.

Experimental Protocol

An overview of test days, which were scheduled during patients' second or third weekly hemodialysis session, is provided in Figure 1. All patients refrained from any sort of strenuous physical activity 48 h prior to each test day. Patients who underwent hemodialysis in the morning fasted overnight. Those who underwent hemodialysis in the afternoon consumed the same standardized breakfast (~250 kcal, with carbohydrate, fat, and protein providing 65, 23, and 12 En%, respectively) at least 3 h before initiation of their hemodialysis session. Thereafter, patients were instructed to remain fasted until the end of the experimental protocol but were allowed to ingest water. After patients arrived at the dialysis department, their prehemodialysis weight was recorded and a Body Composition Monitor (Fresenius Medical Care, Bad Homburg, Germany) was used to assess their body composition, as described previously.[29] Subsequently, the arteriovenous shunt was checked for recirculation and used to collect an arterial blood sample for uremic toxin analyses. After hemodialysis initiation (t = 0 min), blood samples were collected from the arterial blood line with 30-min intervals (at t = 30, 60, and 90 min) and spent dialysate was collected continuously in a container at a rate of 1.0 L/h using a reversed injection pump (Alaris GW, Rolle, Switzerland). An additional spent dialysate sample was collected throughout intradialytic exercise or the corresponding period (t = 30-60 min) during nonexercise interventions to assess the effect of intradialytic exercise on uremic toxin removal. After collection, the spent dialysate was homogenized and thereafter sampled. During all interventions, patients ingested the test beverage 1 h after hemodialysis

initiation (t = 60 min) and remained in a rested state thereafter. Directly after hemodialysis (t = 240 min), a final blood sample was collected from the arterial side of the arteriovenous shunt.

Uremic Toxins Analysis

Blood samples were collected in serum (t = 0 and 240 min) and ethylenediaminetetraacetic acid-containing (t = 30, 60, and 90 min) tubes. Blood samples were centrifuged at 1000 G for 15 min at 20°C or 10 min at 4°C to obtain serum or plasma, respectively. Aliquots of serum and plasma were frozen in liquid nitrogen and stored in a freezer at -80°C until further analysis. Spent dialysate samples were collected in sterile tubes, frozen in liquid nitrogen, and stored in a freezer at −80°C until further analysis. For determination of urea concentrations, urea was hydrolyzed to ammonium using urease. After adding 2-oxoglutarate, nicotinamide adenine dinucleotide + hydrogen, and glutamate dehydrogenase, urea concentrations were determined photometrically on a Cobas 8000 (Roche Diagnostics, Basel, Switzerland). Creatinine was enzymatically converted so that quinone imine chromogen was formed, which was measured on a Cobas 8000 (Roche Diagnostics, Basel, Switzerland) to determine creatinine concentrations. Phosphate concentrations were assessed through conversion of phosphate to an ammonium phosphomolybdate complex, which was measured photometrically on a Cobas 8000 (Roche Diagnostics, Basel, Switzerland). Cystatin C concentrations were determined via turbidimetry on a Cobas 8000 (Roche Diagnostics, Basel, Switzerland) after latex particles coated with anti-cystatin C antibodies were added to the samples. Indoxyl sulfate concentrations were determined by ultraperformance liquid chromatography mass spectrometry (UPLC-MS; ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France).

Calculations

Uremic toxin removal was calculated by multiplying their mean concentration (g per L) in the spent dialysate with spent dialysate and ultrafiltration volume (L). Reduction ratios of uremic toxins between 2 time points (i.e., $RR_{0\text{-}240}$, $RR_{30\text{-}60}$, and $RR_{60\text{-}90}$) were calculated using the following equation:

$$\textit{Reduction ratio}\ (\%) = \left(1 - \frac{UTC_{t2}}{UTC_{t1}}\right) \times 100$$

In which, UTC_{t2} is the concentration of circulating uremic toxins at the second time point (t_2) and UTC_{t1} represents the concentration of circulating uremic toxins at the first time point (t_1). Dialysis adequacy (single pool Kt/V) was calculated using the prehemodialysis circulating urea concentrations (U_{pre}), posthemodialysis circulating urea concentrations (U_{post}), hemodialysis duration (t), ultrafiltration volume (UF), and posthemodialysis weight (W) using the following equation[30]:

$$\textit{Single pool Kt} \Big/ V = \ln\left(\frac{U_{post}}{U_{pre}}\right) - (0.008 \times t) + \left(4 - 3.5 \times \frac{U_{post}}{U_{pre}}\right) \times \frac{UF}{W}$$

Statistical Analysis

All data are expressed as means ± standard deviations unless indicated otherwise. The primary outcome of the present study was urea removal throughout a 4-h hemodialysis session. Secondary outcome parameters include the removal, circulating concentrations, and reduction ratios of creatinine, phosphate, cystatin C, and indoxyl sulfate. Normal distribution of all parameters was verified by Shapiro-Wilk tests. No major violations for repeated-measures analysis of variance (ANOVA) assumptions were observed and in case of nonsphericity, the Greenhouse-Geisser correction was applied. Potential differences in removal and reduction ratios of uremic toxins, hemodialysis parameters, and prehemodialysis weight were analyzed by two-way repeated-measures ANOVA with protein ingestion (yes/no) and exercise (yes/no) as within subject variables. Circulating uremic toxin concentrations throughout hemodialysis were assessed using three-way repeated measures ANOVA with protein ingestion (yes/no), exercise (yes/no), and time as within subject variables. If a statistically significant interaction was found, two-way ANOVAs and/or paired-samples t-tests were performed. In case of significant time effects, Bonferroni post-hoc analyses were performed to locate the effects. Statistical significance was set at $P < .05$. All analyses were performed using SPSS statistics software (version 24.0; IBM Corp., Armonk, New York).

Results

Patients' Characteristics

Patients' baseline characteristics are presented in Table 1. All included patients with ESRD completed 4 test days. No differences were observed between the test days with PLA, PLA + EX, PRO, and PRO + EX interventions in prehemodialysis weight (71.9 ± 14.3, 72.6 ± 14.0, 72.2 ± 13.9, and 71.9 ± 14.1 kg, respectively; protein $P = .49$; exercise $P = .51$), urea distribution volume (34.7 ± 4.6, 35.3 ± 5.1, 35.7 ± 4.8, and 35.2 ± 5.0 L, respectively; protein $P = .16$; exercise $P = .91$), and ultrafiltration volume (1.24 ± 1.01, 1.47 ± 1.27, 1.23 ± 1.08, and 1.41 ± 1.24 L, respectively; protein $P = .78$; exercise $P = .26$). Two patients declined to fill out the 6-day food intake diary. Reported habitual dietary energy and protein intakes of the other 8 patients averaged 25.9 ± 6.0 kcal/kg body weight/day and 1.0 ± 0.3 g protein/kg body weight/day, respectively.

Table 1. Patients' Characteristics

Characteristic	Patients
Age, y	65 ± 16
Gender, male/female	8/2
Cause of end-stage renal disease	
Glomerular	5
Vascular	4
Unknown	1
Remaining diuresis	
<100 mL/24 h	6
100-500 mL/24 h	1
500-2000 mL/24 h	3
Dialysis vintage, months	36 ± 23
Dialysis timing, morning/afternoon	5/5
Height, m	1.72 ± 0.13
Weight, kg	71.0 ± 13.6
BMI, kg/m^2	24.2 ± 4.8
Serum albumin, g/dL	3.4 ± 0.3
C-reactive protein, mg/L	7 ± 6

Continuous and categorical values are expressed as means ± SDs and counts, respectively, n = 10.

Circulating Uremic Toxin Concentrations

As depicted in Figure 2, circulating urea, phosphate, cystatin C, and indoxyl sulfate concentrations decreased substantially throughout hemodialysis (time effect $P < .001$ for all). Circulating uremic toxin concentrations declined between each time point ($P < .05$ for all) except for circulating phosphate concentrations, which did not further decrease during the last 2.5 h of hemodialysis (t = 90-240 min; $P = .70$). Protein ingestion resulted in higher circulating indoxyl sulfate concentrations throughout hemodialysis (protein effect $P = .024$; exercise effect $P = .35$). Circulating urea, phosphate, and cystatin C concentrations were not affected by protein ingestion or intradialytic exercise (protein effect $P = .35$, $P = .59$, and $P = .67$, respectively; exercise effect $P = .46$, $P = .66$, and $P = .20$, respectively). A significant time × exercise interaction ($P = .007$) was observed for circulating creatinine concentrations throughout hemodialysis. Separate analyses showed that circulating creatinine concentrations decreased substantially during hemodialysis (time effect $P < .001$) but were not influenced by intradialytic exercise (exercise effects $P > .05$).

Uremic Toxin Reduction Ratios

Reduction ratios of urea, creatinine, phosphate, cystatin C, and indoxyl sulfate throughout intradialytic exercise during PLA + EX and PRO + EX interventions or the corresponding 30-min period during PLA and PRO interventions ($RR_{30\text{-}60}$), the 30-min period following ingestion of the test beverage ($RR_{60\text{-}90}$), and the 4-h hemodialysis session are presented in Table 2. No protein × exercise interaction was observed ($P > .05$ for all). Protein ingestion

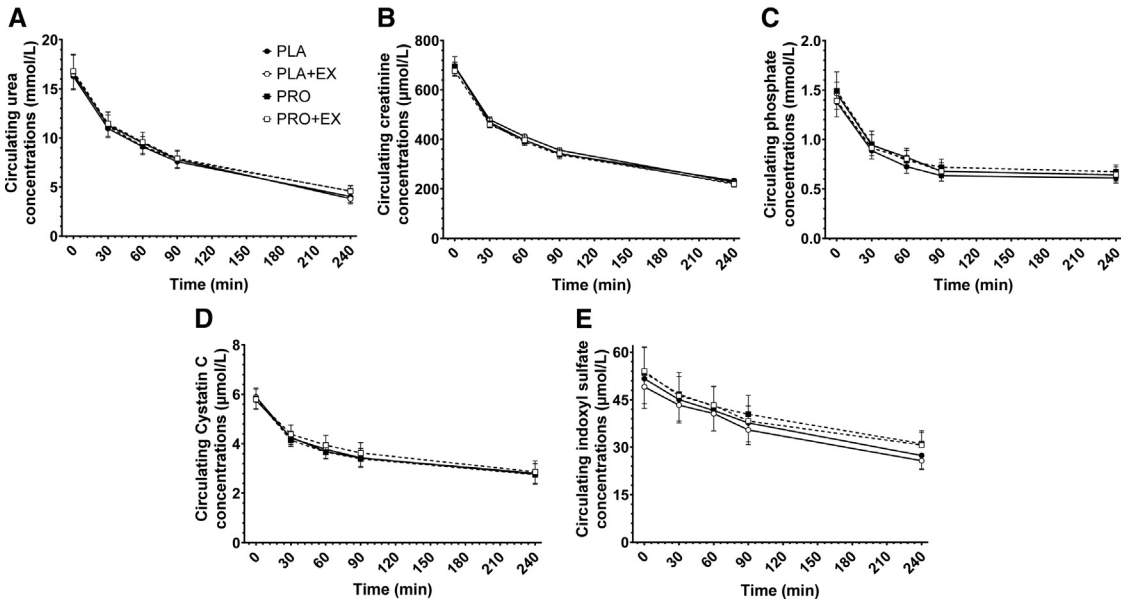

Figure 2. Circulating urea (A), creatinine (B), phosphate (C), cystatin C (D), and indoxyl sulfate (E) concentrations throughout hemodialysis at rest and following exercise with and without protein ingestion. Values are expressed as means ± SEMs, n = 10 for all values. The dotted lines represent the interventions during which the protein beverage was ingested, while the continuous lines represent the interventions during which the placebo was ingested. Data were analysed with a three-way repeated-measures ANOVA with time, protein ingestion (yes/no), and exercise (yes/no) as within subject variables and separate analysis were performed when a significant interaction was detected. A time × exercise interaction ($P < .05$) was observed for circulating creatinine concentrations. Circulating indoxyl sulfate concentrations throughout protein interventions were significantly different from placebo interventions (protein effect $P < .05$). PLA, placebo; PLA + EX, placebo and exercise; PRO, protein; PRO + EX, protein and exercise.

Table 2. Reduction Ratios of Uremic Toxins Throughout Hemodialysis

Uremic Toxin	PLA	PLA + EX	PRO	PRO + EX	Protein Effect *P*	Exercise Effect *P*	Protein × Exercise Interaction *P*
Urea							
$RR_{30\text{-}60}$ (%)	17 ± 3	16 ± 3	18 ± 2	16 ± 2	0.458	0.046	0.673
$RR_{60\text{-}90}$ (%)	17 ± 3	17 ± 4	15 ± 2	17 ± 3	0.127	0.136	0.178
$RR_{0\text{-}240}$ (%)	76 ± 6	77 ± 5	72 ± 4	73 ± 4	0.001	0.254	0.226
Creatinine							
$RR_{30\text{-}60}$ (%)	16 ± 3	15 ± 3	16 ± 2	14 ± 2	0.914	0.033	0.185
$RR_{60\text{-}90}$ (%)	14 ± 2	14 ± 3	13 ± 2	14 ± 3	0.546	0.892	0.658
$RR_{0\text{-}240}$ (%)	67 ± 6	68 ± 4	68 ± 4	68 ± 4	0.270	0.671	0.348
Phosphate							
$RR_{30\text{-}60}$ (%)	18 ± 5	12 ± 6	17 ± 5	10 ± 8	0.203	0.007	0.483
$RR_{60\text{-}90}$ (%)	12 ± 5	17 ± 7	7 ± 8	16 ± 6	0.070	0.010	0.096
$RR_{0\text{-}240}$ (%)	53 ± 11	54 ± 10	53 ± 11	52 ± 12	0.535	1.000	0.300
Cystatin C							
$RR_{30\text{-}60}$ (%)	14 ± 5	11 ± 7	12 ± 7	11 ± 7	0.254	0.053	0.713
$RR_{60\text{-}90}$ (%)	8 ± 7	10 ± 5	8 ± 5	9 ± 5	0.754	0.392	0.587
$RR_{0\text{-}240}$ (%)	51 ± 20	53 ± 19	53 ± 20	52 ± 18	0.808	0.809	0.308
Indoxyl sulfate							
$RR_{30\text{-}60}$ (%)	8 ± 8	6 ± 9	8 ± 4	7 ± 6	0.796	0.485	0.846
$RR_{60\text{-}90}$ (%)	10 ± 4	13 ± 5	6 ± 5	10 ± 7	0.029	0.103	0.750
$RR_{0\text{-}240}$ (%)	46 ± 9	45 ± 10	40 ± 8	43 ± 7	0.023	0.521	0.314

All values are expressed as means ± SDs, n = 10. Data were compared using two-way repeated-measures ANOVAs with protein ingestion (yes/no) and exercise (yes/no) as within subject variables.

PLA, placebo; PLA + EX, placebo and exercise; PRO, protein; PRO + EX, protein and exercise; $RR_{30\text{-}60}$, reduction ratio between 30 and 60 min after hemodialysis initiation (intradialytic exercise or nonexercise period); $RR_{60\text{-}90}$, reduction ratio between 60 and 90 min after hemodialysis initiation (directly after test beverage ingestion); $RR_{0\text{-}240}$, reduction ratio over the 4-h hemodialysis session.

reduced the reduction ratios of urea and indoxyl sulfate over the entire hemodialysis session (protein effect $P = .001$ and $P = .023$, respectively). In addition, single pool Kt/V was higher during PLA and PLA + EX interventions when compared to PRO and PRO + EX interventions (1.64 ± 0.22 and 1.71 ± 0.24 vs. 1.48 ± 0.20 and

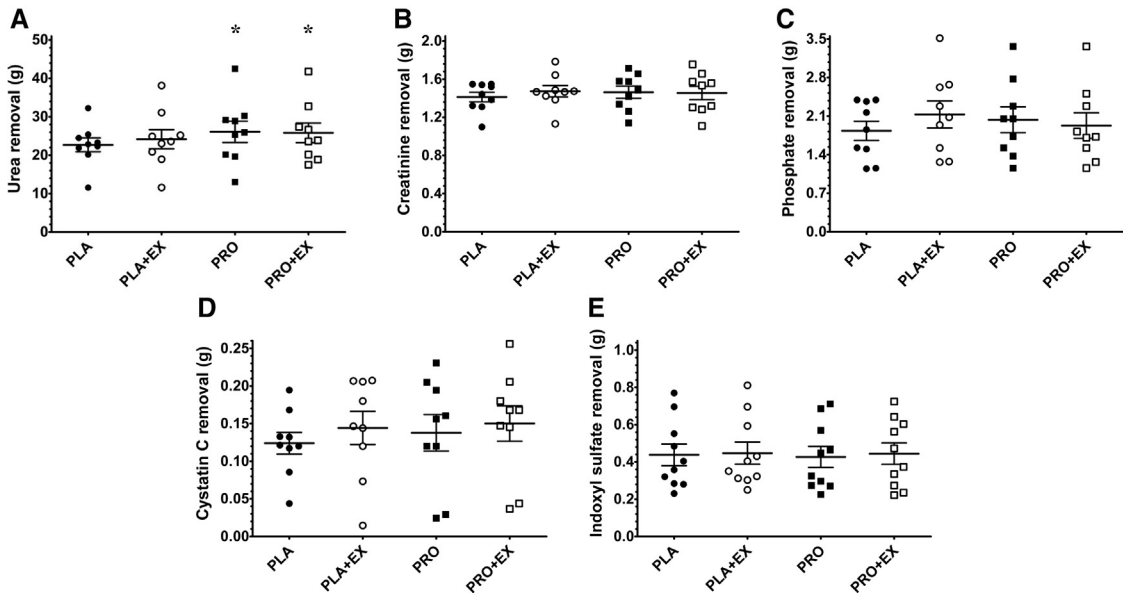

Figure 3. Total urea (A), creatinine (B), phosphate (C), cystatin C (D), and indoxyl sulfate (E) removal throughout hemodialysis at rest and following exercise with and without protein ingestion. Squares and circles represent individual data points and bars represent group means ± SEMs, n = 10 for indoxyl sulfate and n = 9 for urea, creatinine, phosphate, and cystatin C removal. Data were analysed with a two-way repeated-measures ANOVA with protein ingestion (yes/no) and exercise (yes/no) as within subject variables. *, Significantly different from placebo interventions (protein effect $P < .05$). PLA, placebo; PLA + EX, placebo and exercise; PRO, protein; PRO + EX, protein and exercise.

1.49 ± 0.17, respectively; protein effect $P < .001$; exercise effect $P = .179$). Following protein ingestion, only the $RR_{60\text{-}90}$ of indoxyl sulfate was reduced (protein effect $P = .029$). However, the $RR_{60\text{-}90}$ of urea following protein ingestion did not differ from placebo ingestion (protein effect $P = .14$). Intradialytic exercise resulted in lower $RR_{30\text{-}60}$ of urea, creatinine, and phosphate when compared to the nonexercise interventions (exercise effect $P = .046$, $P = .033$, and $P = .007$, respectively). In contrast, intradialytic exercise resulted in a higher $RR_{60\text{-}90}$ of phosphate (exercise effect $P = .010$).

Uremic Toxin Removal

Urea, creatinine, phosphate, cystatin C, and indoxyl sulfate removal throughout the hemodialysis sessions are shown in Figure 3. Urea removal was greater throughout PRO and PRO + EX interventions when compared to PLA and PLA + EX interventions (protein effect $P = .046$; exercise effect $P = .337$). Protein ingestion and intradialytic exercise did not affect the removal of creatinine, phosphate, cystatin C, and indoxyl sulfate over the 4-h hemodialysis sessions (protein effect $P = .62$, $P = 1.00$, $P = .36$, and $P = .69$, respectively; exercise effect $P = .25$, $P = .22$, $P = .16$, and $P = .21$, respectively). When comparing the intradialytic exercise period during PLA + EX and PRO + EX interventions to the same 30-min period during PLA and PRO interventions, greater amounts of urea (4.8 ± 1.5 and 4.9 ± 1.2 vs. 4.4 ± 0.9 and 4.7 ± 1.4 g, respectively; exercise effect $P = .034$), creatinine (0.29 ± 0.04 and 0.28 ± 0.04 vs. 0.28 ± 0.03 and 0.28 ± 0.04 g; exercise effect $P = .039$), and phosphate (0.40 ± 0.16 and 0.39 ± 0.14 vs. 0.33 ± 0.10 and 0.37 ± 14 g; exercise effect $P = .022$) were removed during intradialytic exercise.

Discussion

In this randomized controlled cross-over study, hemodialysis effectively removed small uremic toxins from the circulation during all interventions (Kt/V > 1.2, creatinine reduction ratio > 65%). We observed that intradialytic protein ingestion resulted in lower reduction ratios of urea and indoxyl sulfate throughout the entire hemodialysis session. However, protein ingestion also resulted in greater urea removal throughout hemodialysis. Furthermore, we showed that intradialytic exercise did not modulate uremic toxin removal during hemodialysis.

Adequate removal of uremic toxins is the main purpose of hemodialysis treatment, as it is essential for patients with ESRD that circulating metabolic waste products do not reach harmful concentrations. In the present study, we measured circulating concentrations and removal of small, compartmentalized, and protein-bound uremic toxins throughout hemodialysis. When no interventions were applied (PLA sessions), reduction ratios of uremic toxins during hemodialysis varied between 45% and 75% (Table 2). Furthermore, single-pool Kt/V during these sessions was 1.64 ± 0.22, which indicates that hemodialysis treatment was delivered effectively as per Kidney Disease Outcomes Quality Initiative clinical practice guidelines.[31] Nonetheless, even when effective hemodialysis treatment is delivered, the level of physical functioning among patients with ESRD generally remains poor and limits patients' quality of life.[32] To improve the low physical functioning of patients undergoing chronic hemodialysis treatment, anabolic stimuli (i.e., protein and exercise interventions) are increasingly implemented during hemodialysis.[33-35] However, studies investigating the effects of such interventions on the removal of uremic toxins during hemodialysis have reported equivocal results.[21-23,36-38] Therefore, we comprehensively assessed the impact of intradialytic exercise and protein ingestion on uremic toxin removal throughout hemodialysis.

Protein ingestion can be implemented during hemodialysis to compensate for amino acid removal and, as such, to support muscle maintenance in patients with ESRD.[27,34,39,40] However, it has been suggested that postprandial splanchnic blood pooling following food consumption during hemodialysis interferes with dialysis adequacy.[20] Several studies have observed lower reduction ratios of circulating protein-derived uremic toxins and dialysis adequacy (as measured by Kt/V) when patients consumed food during hemodialysis.[21-23] Our findings support this suggestion, as the reduction ratios of urea and indoxyl sulfate were significantly lower when patients ingested protein compared to placebo ingestion (Table 2). Furthermore, in the present study intradialytic protein ingestion reduced single-pool Kt/V by ~10%. However, the reduction ratios of creatinine, phosphate, and cystatin C throughout hemodialysis were not affected by protein ingestion (Table 2). In addition, during the 30-min period following protein ingestion, the decline in circulating urea concentrations was similar to the 30 min following placebo ingestion. Through quantification of uremic toxin removal in the spent dialysate, we observed that intradialytic protein ingestion actually resulted in an additional ~2 g urea being removed during hemodialysis when compared to placebo ingestion (Figure 3). These findings suggest that the lower reduction ratio of urea throughout hemodialysis is not caused by hemodynamic changes but can rather be attributed to a postprandial increase in urea production.[41] Similarly, protein ingestion is known to increase indoxyl sulfate production by colon microbes, which results in higher concentrations in the circulation.[42] Although intradialytic protein ingestion increased urea removal, it did not result in greater indoxyl sulfate removal throughout hemodialysis (Figure 3). This difference may be explained by the fact that > 90% of circulating indoxyl sulfate is protein-bound and, as such, is not available for diffusion through the dialysis membrane.[43] Thus, intradialytic protein ingestion does not compromise uremic toxin removal during

hemodialysis but increases the postprandial production of protein-derived uremic toxins.

In contrast to protein ingestion, intradialytic exercise has been suggested to improve uremic toxin removal throughout hemodialysis.[44,45] In the latest Clinical Practice Guidelines on Hemodialysis, the Renal Association recommends that patients on chronic hemodialysis treatment without contraindications should perform ≥ 30 min of intradialytic exercise during every hemodialysis session.[46] In the present study, 30 min of intradialytic cycling did not influence the reduction ratios (Table 2) or removal (Figure 3) of any uremic toxin during hemodialysis. This is in line with previous work from De Vos et al., who showed that intradialytic exercise did not change serum concentrations of small and protein-bound uremic toxins throughout hemodialysis.[37] Nevertheless, we found that urea, creatinine, and phosphate removal were greater during performance of intradialytic exercise when compared to the same 30-min period during the nonexercise interventions. Intradialytic cycling increases perfusion of muscle tissue in the legs, an area which would otherwise receive relatively little blood flow during hemodialysis.[47,48] Increased perfusion of leg muscles allows uremic toxins to diffuse from this compartment into the circulation more efficiently, which may increase uremic toxin removal during hemodialysis.[44] However, over the 4-h hemodialysis period intradialytic cycling did not significantly modulate uremic toxin removal. It remains to be established whether a longer period or higher intensity of cycling would be able to further increase uremic toxin removal throughout hemodialysis.

The combination of protein ingestion and physical activity creates a synergistic benefit to preserve, or even increase, muscle mass and function and are, therefore, combined in lifestyle interventions.[49-51] Implementation of protein ingestion together with intradialytic exercise during hemodialysis provides a supervised and time-efficient interventional strategy that is instrumental to maintain muscle mass and functional capacity in patients on chronic hemodialysis treatment.[17] In addition to the separate interventions, the present study also shows that the combination of intradialytic protein ingestion and cycling does not compromise uremic toxin removal during hemodialysis (Figure 3). Therefore, exercise combined with protein ingestion can be implemented during hemodialysis to support muscle mass and strength preservation without attenuating hemodialysis efficiency.

The present study has several limitations. First, the sample size is relatively small with merely 10 patients included. However, to minimize variability and increase the power of our measures, we have employed a randomized cross-over study design and standardized food intake prior to the hemodialysis sessions. In accordance, we were able to show a difference in urea removal throughout hemodialysis between interventions. Second, we provided patients with 40 g of milk protein concentrate during hemodialysis. Although this allowed us to isolate the impact of protein ingestion on uremic toxin removal, patients generally ingest whole foods during hemodialysis. Ingestion of whole foods may influence uremic toxin removal differently than ingestion of a protein isolate or concentrate. Major strengths of the present study include the combination of both the placebo and protein beverage with and without intradialytic exercise. Furthermore, uremic toxin concentrations throughout hemodialysis were not only measured in blood but also in spent dialysate to quantify uremic toxin removal. In conclusion, intradialytic protein ingestion lowers the reduction ratios of protein-derived uremic toxins but increases urea removal throughout hemodialysis. Intradialytic exercise does not compromise uremic toxin removal throughout hemodialysis in patients with ESRD.

Practical Application

In the present study, we show that intradialytic protein ingestion lowers the reduction ratios of protein-derived uremic toxins but does not compromise uremic toxin removal during hemodialysis. In addition, the combination of intradialytic protein ingestion and exercise does not compromise the removal of uremic toxins during hemodialysis. Therefore, exercise combined with protein ingestion can be implemented during hemodialysis to support muscle mass and strength preservation without attenuating hemodialysis efficiency.

CRediT Authorship Contribution Statement

Floris K. Hendriks: Conceptualization, Investigation, Writing – original draft. **Jeffrey H.W. Kuijpers:** Investigation, Writing – review & editing. **Janneau M.X. van Kranenburg:** Investigation. **Joan M.G. Senden:** Investigation. **Frank M. van der Sande:** Conceptualization, Investigation. **Jeroen P. Kooman:** Conceptualization, Writing – review & editing. **Steven J.R. Meex:** Conceptualization, Resources, Writing – review & editing. **Luc J.C. van Loon:** Conceptualization, Resources, Writing – review & editing, Supervision.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1053/j.jrn.2022.07.006.

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