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Supporting muscle maintenance in patients undergoing hemodialysis

Citation for published version (APA):

Hendriks, F. K. (2023). *Supporting muscle maintenance in patients undergoing hemodialysis*. [Doctoral Thesis, Maastricht University]. Maastricht University. https://doi.org/10.26481/dis.20230921fh

Document status and date:
Published: 01/01/2023

DOI:
10.26481/dis.20230921fh

Document Version:
Publisher's PDF, also known as Version of record

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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The studies presented in this thesis were performed within the NUTRIM, School of Nutrition and Translational Research in Metabolism at Maastricht University and partially funded by the NUTRIM Graduate Programme.

Cover design: Rik van Kerkhoff
Layout: Floris Hendriks
Printed by: Gildeprint
ISBN: 978-94-6469-452-9

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Supporting muscle maintenance in patients undergoing hemodialysis

Dissertation

To obtain the degree of Doctor at Maastricht University,
on the authority of the Rector Magnificus, Prof. dr. Pamela Habibović
in accordance with the decision of the Board of Deans, to be defended in public
On Thursday 21 September 2023 at 13:00 hours

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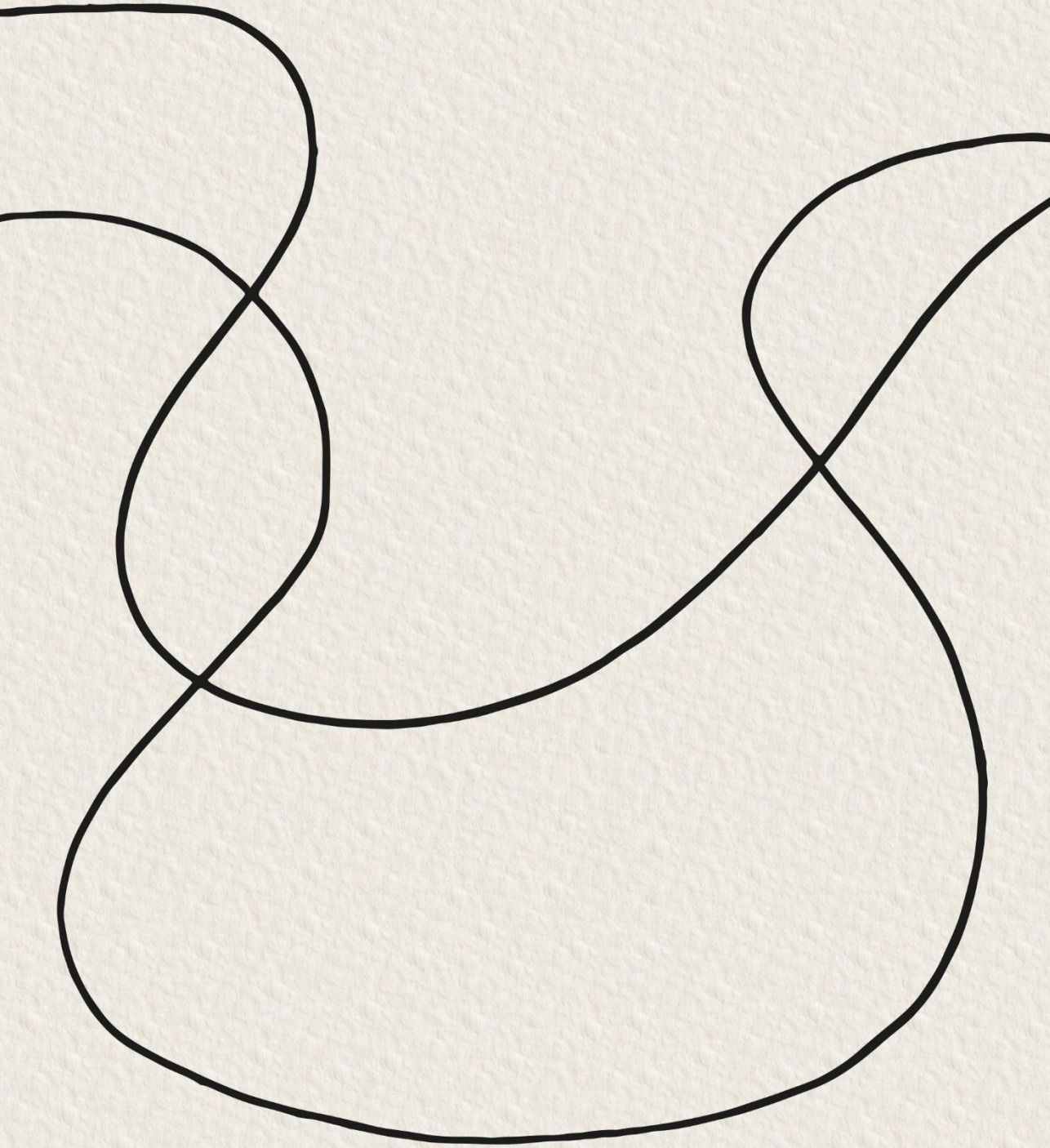
Table of Contents



Chapter 1

General introduction

Introduction

Skeletal muscle tissue is required to allow physical function and contributes substantially to whole-body metabolism. Muscle tissue is in a constant state of turnover, with skeletal muscle protein synthesis and breakdown rates ranging between 1-2% per day [1-3]. Consequently, muscle maintenance is an active process that requires constant stimuli. Food intake and physical activity are the key anabolic stimuli that support muscle maintenance. Food intake, and protein intake in particular, is followed by an increase in circulating amino acid concentrations that directly stimulates muscle protein synthesis rates (**Figure 1**) [4, 5]. Furthermore, the protein-derived amino acids are used as precursors for *de novo* muscle protein synthesis [6, 7]. Physical activity, and especially exercise, represents another potent anabolic stimulus that increases skeletal muscle protein synthesis rates [8, 9]. Exercise performed prior to protein ingestion further augments the postprandial increase in muscle protein synthesis rates [10-12]. When combined, ample protein ingestion and physical activity will act synergistically to stimulate muscle protein accretion and support maintenance or an increase in muscle mass and strength.

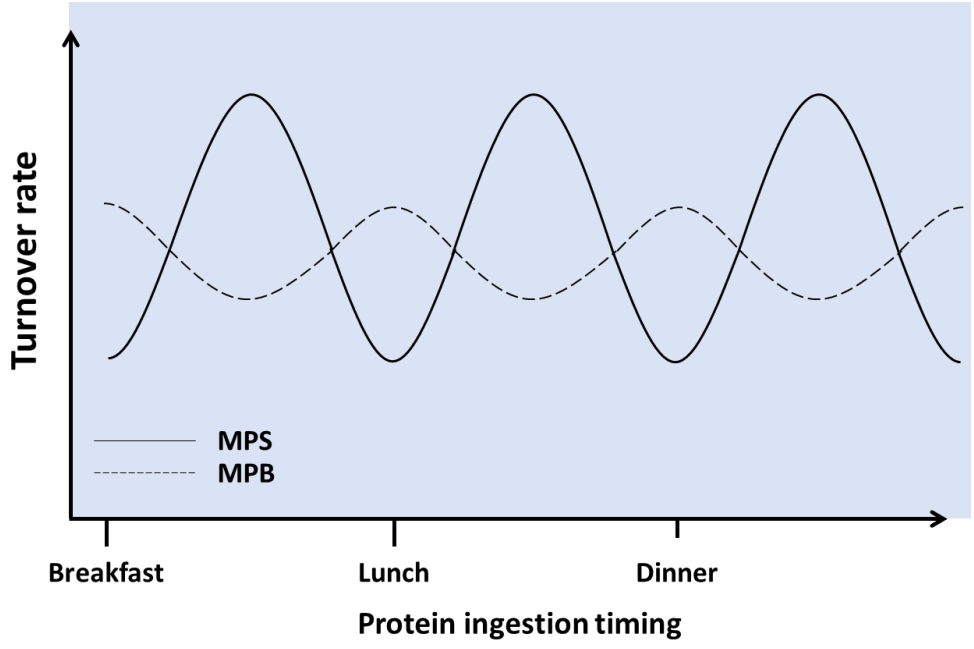


Figure 1. Effect of food intake on skeletal muscle protein synthesis and breakdown rates throughout the day. In the postabsorptive state, muscle protein breakdown rates exceed synthesis rates. Following protein ingestion muscle protein synthesis rates increase while breakdown rates decrease, allowing net muscle protein accretion during the postprandial period. MPB, muscle protein breakdown; MPS, muscle protein synthesis. Adapted from Hendriks *et al.*, Curr Opin Clin Nutr Metab Care, 2021 [13].

Demographics show that skeletal muscle mass and strength gradually decline after the age of 30 years. The age-related decline in muscle mass and strength has been estimated to occur at a rate of ∼1% and ∼3% per year, respectively [14, 15]. Low muscle mass and strength in older adults are associated with increased morbidity, higher mortality rates, and the development of chronic diseases [16, 17]. Ageing is associated with lower dietary protein intake levels and a more sedentary lifestyle, which are major contributors to the decline of muscle mass and strength in older adults [17, 18]. In addition, the age-related loss of muscle mass and function are further accelerated following the development and progression of chronic diseases, as depicted in **Figure 2** [19, 20]. When compared to healthy age-matched adults, patients with chronic diseases such as diabetes mellitus, chronic obstructive pulmonary disease, cancer, and chronic kidney disease are consistently reported to have lower muscle mass and strength [21-24]. In these populations, dietary protein intake levels are generally low due to loss of appetite, change in taste perception, early satiety, and/or specific dietary restrictions [25]. Furthermore, fatigue and exercise intolerance are highly prevalent and represent important barriers to allow ample physical activity [26, 27]. Physical inactivity lowers daily muscle protein synthesis rates and results in the net loss of muscle mass and strength in both healthy [28, 29] and clinically compromised populations [30]. In addition, muscle protein breakdown is generally upregulated in patients with chronic diseases due to disease- and treatment-related factors, such as inflammation, medication, and renal replacement therapy (dialysis) [20, 31]. In combination with insufficient protein intake levels and sedentary behavior, this results in accelerated muscle loss and poor physical functioning among patients with chronic diseases. This loss of physical function prevents many patients from performing activities of daily living, reduces their level of independence, and lowers their quality of life [32]. Interventions to maintain, or even increase, muscle mass and strength are, therefore, essential in the prevention and treatment of chronic diseases.

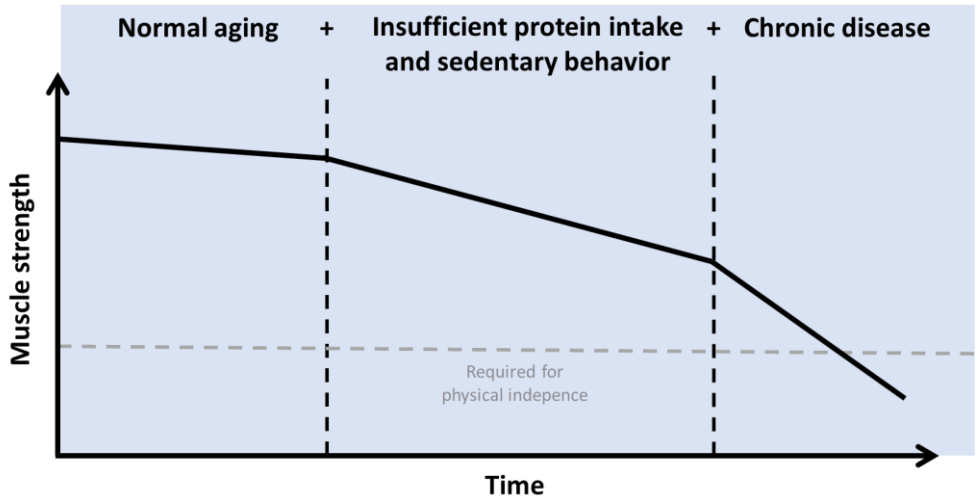


Figure 2. Schematic overview of the effects of aging, insufficient protein intake combined with a sedentary lifestyle, and the development of a chronic disease on muscle strength and the consequences for functional status.

Interventional nutritional strategies to prevent malnutrition and improve protein intake levels are increasingly implemented in clinical care to support muscle maintenance in clinically compromised populations. However, these interventions should be patient-tailored as their applicability and effectivity may vary between different patient (sub)populations. It has been shown that the muscle protein synthetic response following protein ingestion is blunted among several clinically compromised populations [33, 34]. This phenomenon, coined anabolic resistance, has also been observed in healthy older adults and seems to be aggravated by the development and progression of chronic diseases [35]. In older adults, the anabolic resistance of skeletal muscle tissue can be, at least partly, overcome through ingestion of a greater amount (high-quality) protein [36]. In accordance, the recommended daily protein intake level to maintain muscle mass and strength for older adults is greater (1.0 - 1.2 g/kg body weight) when compared to younger adults (0.8 g/kg body weight) [37]. To overcome the anabolic resistance of skeletal muscle tissue and to compensate for the upregulated muscle protein breakdown in patients with chronic diseases, a daily protein intake level of 1.2 - 1.5 g/kg body weight is generally recommended to support muscle maintenance [37]. In healthy adults, several strategies to increase protein consumption have been developed, including (pre-sleep) protein supplementation [7], food fortification [38], and focus on the consumption of more protein-rich food products [39]. However, research on the efficacy of these strategies to support muscle maintenance in clinically compromised populations remains quite limited.

Physical activity improves the anabolic sensitivity of skeletal muscle tissue to protein ingestion and is, therefore, also required to increase muscle protein accretion and achieve muscle maintenance. Structured physical activity, and resistance-type exercise training in

particular, has been proven effective to increase muscle mass and strength in older adults [40]. Furthermore, physical activity prior to protein ingestion has been shown to alleviate anabolic resistance in healthy older adults [41]. To achieve substantial health benefits, it is recommended that older adults perform at least 150 min of moderate-intensity endurance exercise and two sessions of resistance-type exercise per week [42]. Though patients with chronic diseases are likely to benefit most from an increase in physical activity, specific guidelines regarding physical activity and exercise are often lacking. Nonetheless, patient-specific exercise interventions, especially when combined with adequate dietary protein consumption, may prove to be the only effective strategy to attenuate or even prevent the accelerated loss of skeletal muscle mass and strength in clinically compromised populations. Patients with end-stage renal disease undergoing hemodialysis represent one of the most vulnerable patient populations who experience a substantially accelerated loss of skeletal muscle mass and strength [20, 43]. During hemodialysis treatments, blood is pumped through a dialyzer in which blood and dialysate fluid are separated by a semipermeable membrane. This membrane allows metabolic waste products, which are small molecules, to diffuse from the blood into the dialysate while larger molecules such as proteins remain in the circulation. However, small-sized nutrients also diffuse through this membrane and are, therefore, removed from the body during hemodialysis [44, 45]. Especially the removal of amino acids, the building blocks of tissue proteins, stimulates muscle protein breakdown during and after hemodialysis treatment [31, 46]. Patients generally receive hemodialysis treatment two or three times per week. Therefore, they are frequently exposed to hemodialysis-associated muscle catabolism. In addition, other treatment-related factors such as prescribed dietary restrictions and disease-related factors including inflammation, reduced appetite, and oxidative stress further compromise the nutritional status of patients with end-stage renal disease [47]. A recent meta-analysis reported that the protein energy wasting syndrome (i.e. reduced or loss of (lean) body mass with insufficient dietary intake and reduced biochemical parameters such as serum albumin) was present in 28-54% of patients undergoing hemodialysis treatment globally [48, 49]. As protein energy wasting is closely associated with higher mortality rates in patients with end-stage renal disease, interventions to maintain and/or improve nutritional status tailored to this clinically compromised population are urgently required [50-52].

Outline of this thesis

This thesis describes several studies that assessed the efficacy of dietary protein and exercise interventions during hemodialysis to support muscle maintenance in patients with end-stage renal disease. In **Chapter 2** we conducted a literature study on habitual dietary protein intake and physical activity in patients with end-stage renal disease on chronic hemodialysis treatment. Furthermore, we identified possible dietary protein and physical activity interventions to support muscle mass maintenance in these patients. Subsequently, we quantified amino acid removal during hemodialysis in end-stage renal disease patients who were consuming their habitual diet throughout hemodialysis treatments (**Chapter 3**). This allowed us to provide end-stage renal disease patients with an evidence-based amount of protein during hemodialysis in **Chapter 4** and assess whether this could compensate for amino acid removal. Furthermore, in this chapter we investigated the impact of performing intradialytic exercise prior to protein ingestion on circulating amino acid availability and amino acid removal throughout hemodialysis. We followed up on this work by assessing uremic toxin removal when patients performed exercise and/or ingested protein during hemodialysis in **Chapter 5**. It is clinically important that intradialytic anabolic interventions do not compromise the removal of uremic toxins during hemodialysis. Subsequently, we compared the effects of supplementing protein with and without keto-analogues of branched-chain amino acids throughout hemodialysis on circulating amino acid availability, amino acid removal, and amino acid oxidation. These data are addressed in **Chapter 6**. Lastly, the results of the studies described in this thesis are discussed in a broader context and implications for future research are provided in **Chapter 7**.

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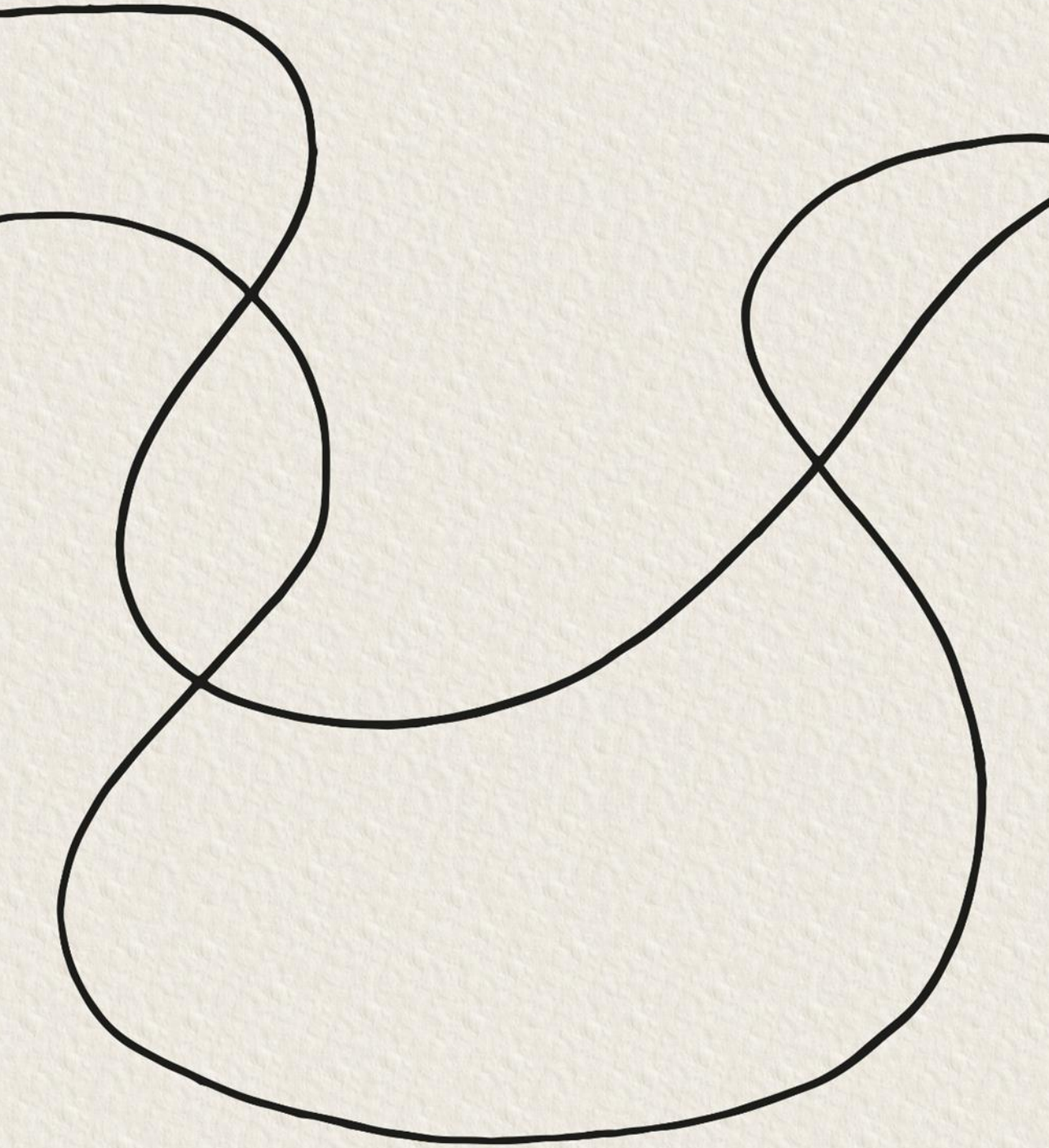

Chapter 2

Dietary protein and physical activity interventions to support muscle maintenance in end-stage renal disease patients on hemodialysis

Floris K. Hendriks, Joey S.J. Smeets, Frank M. van der Sande,

Jeroen P. Kooman, and Luc J.C. van Loon

Nutrients. 2019; 11 (12): 2972

Abstract

End-stage renal disease patients have insufficient renal clearance capacity left to adequately excrete metabolic waste products. Hemodialysis (HD) is often employed to partially replace renal clearance in these patients. However, skeletal muscle mass and strength start to decline at an accelerated rate after initiation of chronic HD therapy. An essential anabolic stimulus to allow muscle maintenance is dietary protein ingestion. Chronic HD patients generally fail to achieve recommended protein intake levels, in particular on dialysis days. Besides a low protein intake on dialysis days, the protein equivalent of a meal is extracted from the circulation during HD. Apart from protein ingestion, physical activity is essential to allow muscle maintenance. Unfortunately, most chronic HD patients have a sedentary lifestyle. Yet, physical activity and nutritional interventions to support muscle maintenance are generally not implemented in routine patient care. To support muscle maintenance in chronic HD patients, quantity and timing of protein intake should be optimized, in particular throughout dialysis days. Furthermore, implementing physical activity either during or between HD sessions may improve the muscle protein synthetic response to protein ingestion. A well-orchestrated combination of physical activity and nutritional interventions will be instrumental to preserve muscle mass in chronic HD patients.

Introduction

Chronic kidney disease (CKD) is currently a public health problem with a global prevalence of 10% and the cause of approximately 33 million disability-adjusted life-years worldwide [1,2]. Development and progression of CKD are associated with the age-related decline in renal function, especially in individuals with hypertension and diabetes mellitus [3-5]. Therefore, the rapid ageing of our population is expected to further increase prevalence of CKD and its progression to end-stage renal disease (ESRD) [6,7]. The glomerular filtration rate in ESRD patients is below 15 mL/min/1.73m2 and insufficient to adequately remove metabolic waste products and fluids from the body [8,9]. Due to the accumulation of metabolic waste products in their body, ESRD patients experience phenotypic changes that resemble the ageing process, with a progressive loss of skeletal muscle mass and strength [10].

To prevent lethal consequences of metabolic waste product accumulation in ESRD patients, hemodialysis (HD) can be used to partially replace renal solute removal [11]. Over the past decades, survival of patients undergoing HD has improved substantially [12,13]. However, prevention of the adverse effects of HD on body composition has made less progression. After initiation of chronic hemodialysis (CHD) therapy, the age-related loss of skeletal muscle mass and strength accelerates, and patients typically develop impairments in physical function [14-17]. Protein-energy wasting, a severe state of malnutrition, is observed in 28 – 54% of CHD patients [18,19]. Loss of skeletal muscle mass and strength predisposes CHD patients to frailty and substantially reduces their quality of life [20]. Furthermore, the decline in skeletal muscle mass and strength is associated with higher hospitalization and mortality rates in CHD patients [20-22]. As the duration of CHD treatment is associated with its detrimental effects on body composition, the improved survival rate of CHD patients will generate new challenges for healthcare [14]. This emphasizes the need to understand and counteract skeletal muscle mass and strength loss in CHD patients.

Muscle maintenance

Skeletal muscle mass is regulated through a dynamic balance between continuous synthesis and breakdown of muscle proteins. The muscle protein pool has shown to possess a turnover rate of 1-2%, allowing skeletal muscle tissue to adapt to circumstances such as changes in physical activity pattern (e.g. muscle hypertrophy following resistance-type exercise training) [23]. Ingesting several protein-containing meals throughout the day results in a sinusoidal pattern of subsequent increases and decreases in skeletal muscle protein synthesis and breakdown rates [24]. Skeletal muscle protein synthesis rates are high during post-prandial periods and low during post-absorptive periods, whilst skeletal muscle protein breakdown rates follow a reverse pattern. Muscle maintenance is achieved when skeletal muscle protein synthesis rates equal skeletal muscle protein breakdown rates over a given period.

Protein ingestion is an essential requirement to maintain skeletal muscle mass. After consumption, dietary protein is absorbed as amino acids in the intestine, with a large fraction being subsequently released into the circulation [25]. The release of amino acids into the circulation following protein ingestion elevates plasma amino acid concentrations for a post-prandial period of up to 5 h [26]. These circulating plasma amino acids serve as precursors for *de novo* synthesis of muscle protein [27]. However, amino acids are more than simply building blocks for muscle protein synthesis, as they can function as signaling molecules. The post-prandial increase in plasma essential amino acid concentrations, and leucine in particular, stimulates anabolic signaling through several molecular pathways, such as the mammalian target of rapamycin complex 1 (mTORC1) pathway [28,29]. This post-prandial anabolic signaling increases skeletal muscle protein synthesis rates and inhibits proteolysis, allowing net muscle protein accretion [27].

Muscle loss can be attributed both to an increase in muscle protein breakdown as well as to a decline in muscle protein synthesis rates. Previous work has reported increased muscle proteolysis in CHD patients due to inflammation, metabolic acidosis, and the dialysis procedure itself [30-33]. Furthermore, it has been suggested that the muscle protein synthetic response to feeding is impaired in patients with CKD [34]. Whereas a maximal post-prandial muscle protein synthetic response has been reported after ingesting up to 20 g of a high-quality protein in healthy young adults, a lesser response has been observed in older individuals [27,35,36]. More recently, van Vliet *et al.* were unable to detect a measurable increase in skeletal muscle protein synthesis rates in CHD patients following ingestion of a meal containing 20 g protein [37]. The latter suggests that CHD patients suffer from a blunted muscle protein synthetic response to feeding, a phenomenon that has been coined anabolic resistance. In healthy elderly individuals, it has been shown that the anabolic resistance of skeletal muscle tissue can be overcome through ingesting a greater

amount of protein (at least 30 g of a high-quality protein) [38] and/or performing a bout of resistance-type exercise prior to feeding [39]. When tailored specific to CHD patients, these anabolic strategies may prove essential to attenuate or even prevent the accelerated loss of skeletal muscle mass and strength in ESRD patients undergoing HD.

Dietary protein intake in end-stage renal disease patients on hemodialysis

For healthy young adults, the recommended dietary protein intake to achieve a net balance between muscle protein synthesis and breakdown rates has been set at 0.8 g protein/kg body weight/day by the World Health Organization [40,41]. This level of protein intake may not be sufficient to support muscle maintenance in CHD patients. According to the National Kidney Foundation K/DOQI Clinical Practice Guidelines, these patients are recommended to ingest >1.2 g protein/kg body weight/day [42-45]. However, CHD patients generally do not meet this recommended level of protein intake. Previous studies in this population have observed a dietary protein intake of 0.9 – 1.0 g protein/kg body weight/day [46-51]. Especially on dialysis days, factors such as time constraints and reduced appetite make it difficult for patients to consume ample dietary protein [52]. As a result, dietary protein intake in CHD patients has been reported to be ~0.8 g protein/kg body weight on dialysis days compared to ~1.0 g protein/kg body weight on non-dialysis days [50].

In addition to low protein intake, another factor compromises plasma amino acid availability on dialysis days. During HD, both metabolic waste products as well as circulating amino acids are able to diffuse through the semipermeable dialysis membrane [11]. The diffusion into the dialysate results in a considerable extraction of circulating amino acids throughout HD [30,53-56]. We have recently shown that during a single HD session, ~12 g amino acids are extracted from the circulation in CHD patients who ingest their habitual diet during HD [57]. This amount equals the quantity of amino acids that is released into the circulation following ingestion of a typical meal (containing 20 – 25 g protein). Loss of circulating amino acids causes a significant decline of plasma amino acid concentrations throughout HD [55,57]. Moreover, Ikizler *et al.* showed that in fasting CHD patients, plasma amino acid concentrations remain low for at least 2 hours after cessation of HD [30]. The HD-induced decline in plasma amino acid concentrations has been shown to cause substantial catabolism of skeletal muscle tissue in fasted CHD patients [58,59]. The continuous extraction of amino acids throughout HD stimulates skeletal muscle tissue to release amino acids into the circulation [60,61]. This homeostatic process attenuates the decline in plasma amino acid concentrations and may prevent subsequent detrimental effects on organs that are necessary to sustain life [62]. In addition, the decline in plasma

amino acid concentrations reduces the availability of precursors for *de novo* synthesis of muscle proteins during and following HD. To allow a muscle protein synthetic response during this period, the extraction of circulating amino acids should be compensated for through amino acid and/or protein administration.

Provision of protein-rich nutrition during HD is often recommended to increase dietary protein intake on dialysis days [63-66]. Ingestion of 40 – 60 g protein has been shown to prevent the HD-induced decline in plasma amino acid concentrations in multiple studies [58,59,67,68]. Furthermore, Pupim *et al.* demonstrated that ingestion of 57 g protein resulted in a positive forearm amino acid balance throughout HD [58]. Thus, HD-associated skeletal muscle catabolism may be prevented through ingestion of sufficient protein during HD. Several studies have also observed long-term beneficial effects of protein supplementation during HD, such as an increase in lean body mass, improvement in physical function, and decrease in mortality [69-71]. However, data from our lab [57] and others [56,67] indicate that protein ingestion during HD is also accompanied by an increase in amino acid extraction, presumably due to a higher subsequent plasma-dialysate diffusion gradient (**Figure 1**). Due to this extraction following protein ingestion during HD, less amino acids become available to stimulate muscle protein synthesis rates and serve as precursors for *de novo* synthesis of muscle protein. Considering the anabolic resistance of skeletal muscle tissue that is also present in this population, CHD patients will need to ingest well above 20 g high-quality protein during HD to allow a post-prandial increase in skeletal muscle protein synthesis and an inhibition of proteolysis.

However, high quality (animal-derived) protein is rich in phosphorous [72]. In CHD patients, an increased dietary protein intake may lead to hyperphosphatemia or the need for phosphate binders. Furthermore, it has been suggested that an increased dietary protein intake in CHD patients provides more uremic toxin precursors and leads to higher uremic solute concentrations between HD sessions [73]. Recently, our laboratory has shown that the ingestion of branched-chain ketoacids, which contain no phosphorous or nitrogen, significantly stimulates skeletal muscle protein synthesis rates in healthy elderly individuals [74]. Ketoacid supplementation in CKD disease patients has been shown to reduce the generation of toxic metabolic waste products, while maintaining a good nutritional status [75]. However, it remains to be established whether ketoacid supplementation could support muscle maintenance in CHD patients.

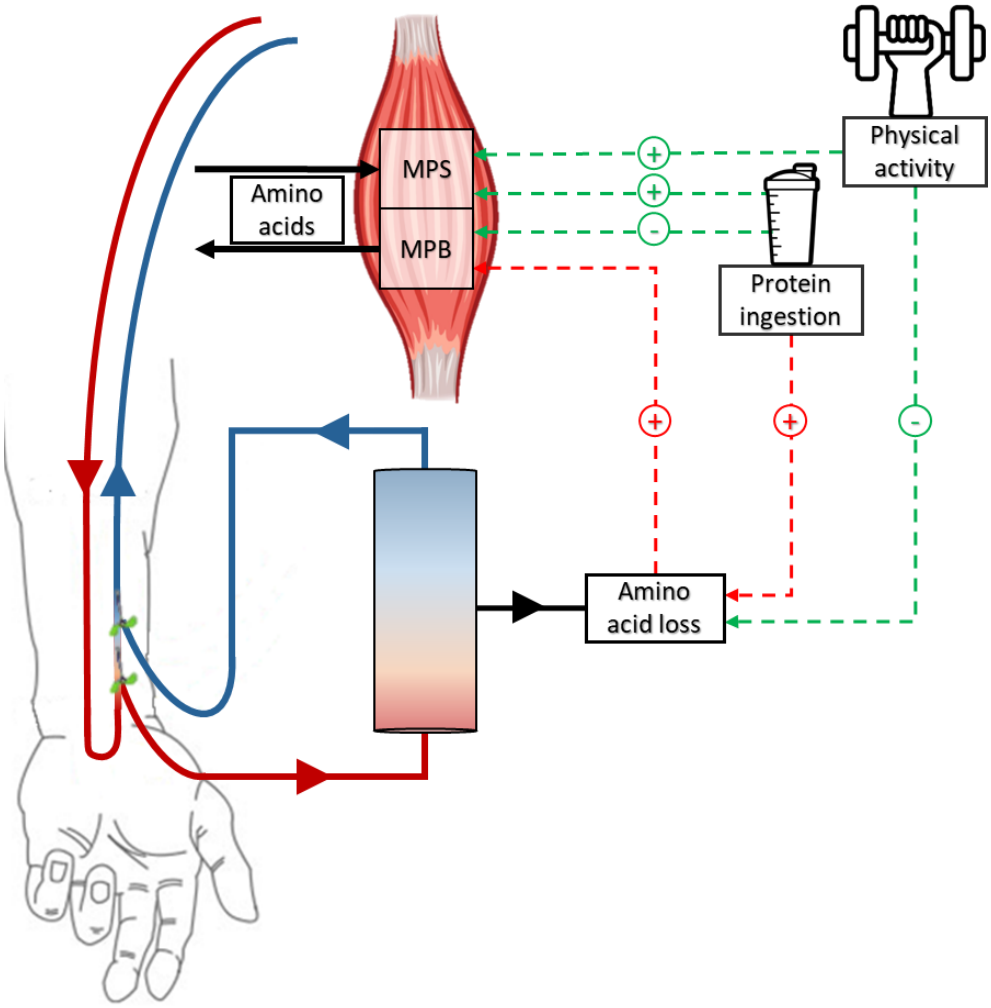

Figure 1. Conceptual overview of the effects of hemodialysis, protein ingestion, and physical activity on the muscle protein synthetic and proteolytic response. The removal of amino acids during hemodialysis (HD) stimulates muscle protein breakdown (MPB) rates due to decreased plasma amino acid concentrations. Protein ingestion can maintain, or even increase, plasma amino acid concentrations throughout HD, which increases muscle protein synthesis (MPS) rates, while it may attenuate the HD-induced increase in MPB rates. However, elevated plasma amino acid concentrations also increase amino acid removal during HD. Physical activity before or during HD may increase the use of plasma amino acids for *de novo* MPS, and thereby reduce amino acid removal from the circulation during HD. Dashed lines in green represent processes that support muscle maintenance, whereas dashed lines in red represent processes that compromise muscle maintenance.

Physical activity in end-stage renal disease patients on hemodialysis

Another key component for muscle maintenance is physical activity. Physical activity and exercise stimulate skeletal muscle protein synthesis rates, with post-absorptive muscle protein synthesis rates being elevated for up to 24 or even 48 hours [76,77]. Furthermore, physical activity performed prior to food intake augments the post-prandial muscle protein synthetic response to feeding [78-81]. In contrast, a decline in physical activity reduces the muscle protein synthetic response to feeding [82-84]. In other words, whereas physical activity makes skeletal muscle tissue more sensitive to the anabolic properties of amino acids, muscle disuse leads to anabolic resistance of skeletal muscle tissue [85]. In support, daily exercise has been shown to increase skeletal muscle protein synthesis rates throughout the day [86], while a decline in physical activity has been shown to lower daily muscle protein synthesis rates [87]. Consequently, ample physical activity has been associated with a reduced age-related loss of muscle mass and strength [88,89], whereas a decline in the level of physical activity (e.g. during bed rest or limb immobilization) has been shown to induce a rapid decline in muscle mass and strength [90,91].

According to the Physical Activity Guidelines for Americans, patients with chronic diseases should follow the key physical activity guidelines for healthy adults to achieve substantial health benefits [92]. These guidelines recommend patients to perform at least 150 – 300 min per week of moderate-intensity aerobic exercise, 75 – 150 min of vigorous-intensity aerobic exercise per week, or an equivalent combination of both. In addition, muscle-strengthening activities that involve all major muscle groups should be performed at least twice per week. However, these guidelines do not contain specific recommendations for CHD patients. The Renal Association Clinical Practice Guideline on Hemodialysis recommends that all CHD patients without contraindication should perform at least 30 min of supervised moderate-intensity exercise during every dialysis session [93]. In addition, the guideline states that CHD patients should be encouraged to undertake physical activity on non-dialysis days. In line with this recommendation, it has recently been suggested that mortality rates are reduced in CHD patients who perform at least 4,000 steps on non-dialysis days [94].

However, CHD patients typically adopt a sedentary lifestyle and spend less time being physically active than healthy adults [95,96]. In the United States, almost 50% of CHD patients perform exercise once or less than once per week [96]. A HD session represents a long (3 – 4 h) sedentary period, which often hinders CHD patients to engage in physical activity and, as such, dialysis treatments contribute to the lower physical activity levels [97,98]. Gomes *et al.* observed that CHD patients took 4362±2084 and 7007±3437 steps on dialysis and non-dialysis days, respectively, compared to 8792±2870 steps taken by age-

matched healthy controls [98]. The low habitual physical activity level in these patients is another key factor responsible for the accelerated loss of muscle mass and strength in CHD patients [17]. Interventions in CHD patients targeted to preserve or even increase muscle mass should not only provide nutritional support but also increase physical activity levels to maximize their impact.

Interventions to support muscle maintenance in end-stage renal disease patients on hemodialysis

Physical activity interventions for CHD patients may implement exercise during HD (intradialytic) or between HD sessions (interdialytic). A recent meta-analysis by Clarckson *et al.* reported no differences in the efficacy of intradialytic when compared with interdialytic exercise on improvements of physical function in CHD patients [99]. Due to exercise intolerance, CHD patients typically show low adherence and poor compliance to long-term unsupervised physical activity intervention programs [100]. HD sessions represent an opportunity to integrate supervised physical activity in the weekly routine of CHD patients. Intradialytic physical activity is considered safe and shows greater adherence rates than interdialytic physical activity [100-102]. Furthermore, supervision of intradialytic exercise sessions provides the opportunity to prescribe a patient-specific and progressive exercise program. Physical activity during HD has some limitations compared to interdialytic physical activity, such as constraints regarding exercise intensity and upper limb exercises. On the other hand, intradialytic physical activity provides distraction for CHD patients during their treatment and has been shown to improve their quality of life [101]. Therefore, we would advocate the implementation of an intradialytic exercise routine program in lifestyle interventions designed for (sedentary) CHD patients.

In addition to timing, the type of exercise is an important determinant of its potential to support muscle maintenance. Resistance-type exercise training is considered most potent to augment muscle mass and strength. In healthy adults, resistance-type exercise training has been shown to induce a robust increase in both skeletal muscle mass as well as strength [103-105]. Furthermore, resistance-type exercise also sensitizes skeletal muscle tissue to the anabolic properties of amino acids and, as such, increases the post-prandial muscle protein synthetic response to feeding [78,79,81]. In support, it has been reported that a single bout of resistance-type exercise performed prior to HD increases amino acid uptake by muscle tissue following intradialytic protein ingestion [106]. Intradialytic resistance-type exercise programs have been shown to increase skeletal muscle strength, thereby improving physical function outcome measures such as the 6-min walk test [99,107-110]. In a systematic review of 9 trials that assessed progressive resistance-type exercise training in ESRD undergoing HD, Chan and Cheema concluded that resistance-type exercise training can effectively induce regional skeletal muscle hypertrophy [111]. However, due to inconsistent results of previous studies [69,112-118] it remains unclear whether resistance-type exercise can increase skeletal muscle mass on a whole-body level in CHD patients.

Protein ingestion during recovery from resistance-type exercise is required to achieve a positive net protein balance and, as such, to allow net muscle protein accretion [76]. Due to practical matters, the majority of studies that assessed the impact of resistance-type

exercise training in CHD patients implemented their training sessions before or during HD [119]. As circulating amino acids are extracted during HD, recovery from those exercise sessions typically occurred during conditions of reduced amino acid availability. This may have attenuated the anabolic effects of the exercise training programs. Furthermore, the combination of amino acid extraction during HD and the anabolic resistance of skeletal muscle tissue in CHD patients likely increases the amount of protein that is required following intradialytic resistance-type exercise. We suggest that at least 30 g protein should be provided to CHD patients during recovery from resistance-type exercise performed immediately prior or during HD to allow a muscle protein synthetic response.

Besides protein ingestion during recovery from exercise, it has been advocated that every main meal (breakfast, lunch, and dinner) should contain 20 g high-quality protein to optimally stimulate muscle protein synthesis rates throughout the day [120,121]. We suggest that CHD patients should ingest well above 20 g high-quality protein per main meal to compensate for the blunted muscle protein synthetic response to feeding, recognizing that additional measures to prevent hyperphosphatemia might be necessary. In addition, ingesting a protein-rich snack prior to sleep, especially on training days, may further support muscle mass maintenance [24]. Though the impact of these nutritional strategies has not been assessed in CHD patients, they would likely be supplemental in the prevention of protein malnutrition in this population. Effectiveness of any nutritional intervention largely depends on long-term adherence and compliance. However, adherence to dietary interventions in CHD patients is often poor due to barriers such as dialysis time, motivation, and lack of social support [122]. Therefore, CHD patients should be advised on protein options that are easy to prepare, convenient to consume, and have an acceptable taste.

A well-orchestrated lifestyle intervention program combining exercise and nutritional intervention for CHD patients is required to attenuate or even prevent the loss of muscle mass, strength, and functional capacity in this population. For such a multimodal interventional approach to be effective, a (more) personalized supervision of CHD patients provided by a team of healthcare specialists with physical activity and nutritional expertise is required. A close collaboration between nephrologists, physical therapists, and dietitians in both research and clinical care will be essential to improve the health and well-being of the growing number of CHD patients.

Conclusions

The gradual loss of skeletal muscle mass in CHD patients accelerates after initiation of intermittent HD treatment. Muscle protein breakdown rates in CHD patients are increased, while muscle protein synthesis rates fail to match this increase due to insufficient protein ingestion, amino acid extraction during HD, and the prevalence of anabolic resistance. Protein intake of CHD patients should be increased on dialysis days to compensate for extraction of circulating amino acids during HD and to compensate for the blunted muscle protein synthetic response to feeding in these patients. Implementing structured physical activity in the daily routine of CHD patients represents a feasible strategy to increase the skeletal muscle protein synthetic response to protein ingestion and, as such, to alleviate anabolic resistance. More insight in the impact of protein ingestion and exercise in CHD patients on both dialysis as well as non-dialysis days is required to develop more effective nutritional and exercise intervention programs that can attenuate or even prevent muscle loss in CHD patients.

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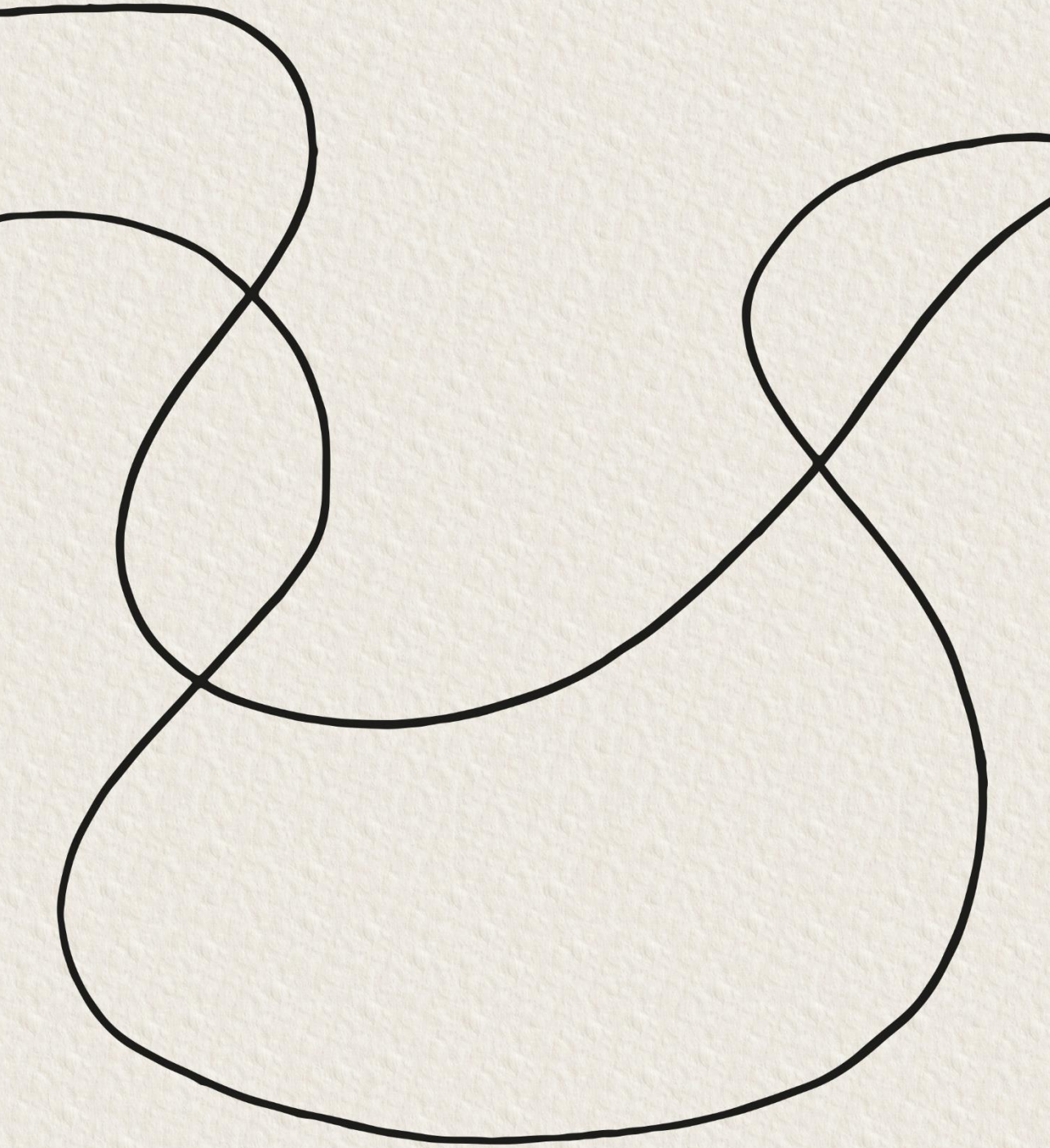

Chapter 3

End-stage renal disease patients lose a substantial amount of amino acids during hemodialysis

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The Journal of Nutrition. 2020; 150 (5): 1160-1166

Abstract

Background

Poor nutritional status is frequently observed in end-stage renal disease patients and associated with adverse clinical outcomes and increased mortality. Loss of amino acids (AAs) during hemodialysis (HD) may contribute to protein malnutrition in these patients.

Objective

We aimed to assess the extent of AA loss during HD in end-stage renal disease patients consuming their habitual diet.

Methods

Ten anuric chronic HD patients (mean±SD age: 67.9±19.3 y, BMI: 23.2±3.5 kg/m^2), undergoing HD 3 times per week, were selected to participate in this study. Spent dialysate was collected continuously and plasma samples were obtained directly before and after a single HD session in each participant. AA profiles in spent dialysate and in pre-HD and post-HD plasma were measured through ultra-performance liquid chromatography to determine AA concentrations and, as such, net loss of AAs. In addition, dietary intake before and throughout HD was assessed using a 24-h food recall questionnaire during HD. Paired-sample t tests were conducted to compare pre-HD and post-HD plasma AA concentrations.

Results

During an HD session, 11.95±0.69 g AAs were lost via the dialysate, of which 8.26±0.46 g were nonessential AAs, 3.69±0.31 g were essential AAs, and 1.64±0.17 g were branched-chain AAs. As a consequence, plasma total and essential AA concentrations declined significantly from 2.88±0.15 and 0.80±0.05 mmol/L to 2.27±0.11 and 0.66±0.05 mmol/L, respectively (P<0.05). AA profiles of pre-HD plasma and spent dialysate were similar. Moreover, AA concentrations in pre-HD plasma and spent dialysate were strongly correlated (Spearman's ρ=0.92, P<0.001).

Conclusions

During a single HD session, ∼12 g AAs are lost into the dialysate, causing a significant decline in plasma AA concentrations. AA loss during HD can contribute substantially to protein malnutrition in end-stage renal disease patients.

Introduction

End-stage renal disease patients fail to adequately remove metabolic waste products and excess fluids from the body [1]. To prevent lethal consequences of waste product accumulation, hemodialysis (HD) is employed to replace 10 – 15% of renal clearance capacity [2]. However, patients undergoing chronic hemodialysis (CHD) treatment typically develop impairments in physical function due to a decline in lean tissue mass, cardiorespiratory capacity, and muscle strength [3-5]. Though muscle and strength loss can be part of the normal ageing process, the progressive loss of skeletal muscle mass is remarkably accelerated in CHD patients [6, 7]. Skeletal muscle wasting in CHD patients can be attributed to various factors, including inflammation, malnutrition, and nutrient loss during each HD session [8-10].

Amino acids are among the nutrients lost in the dialysate during HD and of key importance for muscle maintenance [10, 11]. Previous work from our lab [12-15] as well as many others [16-21] has shown that skeletal muscle protein turnover is highly responsive to postprandial increases in plasma amino acid concentrations. In both healthy and clinical populations the postprandial rise in plasma amino acid concentrations stimulates muscle protein synthesis rates and inhibits protein breakdown, allowing net muscle protein accretion [14, 22]. In CHD patients muscle protein synthesis as well as breakdown rates are stimulated during HD [23, 24]. Previous studies have shown that loss of amino acids during HD causes a decline in plasma amino acid concentrations in fasted patients [11, 25-29]. Moreover, HD induces a negative net forearm amino acid balance in fasting patients, which may be indicative of muscle proteolysis [24].

In contrast to clinical practice in North America, most CHD patients in Europe are allowed to eat and drink during their HD treatment [30]. There is an ongoing debate on the implementation of dietary protein intake during HD to counterbalance the HD-induced decline in plasma amino acid concentrations in routine care, as some nephrologists cite concerns regarding patient safety and increased staff burden. Moreover, it remains to be established whether habitual food intake before and during HD increases the subsequent loss of amino acids in the dialysate. Previous estimates may, therefore, not accurately reflect amino acid loss in CHD patients consuming their habitual diet during HD.

Therefore, we selected ten CHD patients to participate in a study in which we obtained blood samples and spent dialysate during HD to assess the selective amino acid loss in the dialysate. Plasma and dialysate amino acid concentrations were measured to calculate individual amino acid extraction rates and to evaluate the relationship between basal plasma amino acid concentrations, food intake, and amino acid extraction during HD. This study provides insights in the amino acid extraction and nutritional requirements of CHD patients consuming their habitual diet during HD.

Methods

Subjects

Ten patients with a urine production below 100 mL/day, undergoing HD three times per week with high-flux membranes for at least 6 months, were recruited through the outpatient population visiting the HD department of Maastricht University Medical Center+, Maastricht, The Netherlands. Patients with an active infection, cognitive disorder, or missed HD session in the last month prior to the study period were excluded. Patients' characteristics are presented in **Table 1**. Patients were informed of the nature of the experimental procedures prior to obtaining written informed consent. The current study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre+ and registered at the Netherlands Trial Registry (NTR7101). The applied study design complies with the standards outlined in the most recent version of the Helsinki Declaration.

Table 1. Characteristics of included chronic hemodialysis patients

	Patients
Age, y	67.9 ± 19.3
Gender, male/female	7/3
Cause of ESRD	4 Hypertension
	2 Diabetes Mellitus
	2 Auto-immune
	2 Other
Dialysis vintage, months	46.8 ± 28.4
Height, cm	166 ± 9
Weight, kg	64.4 ± 15.9
Body mass index, kg/m^2	23.2 ± 3.5
Lean tissue index, kg/m^2	11.7 ± 1.7
Handgrip strength, kg	24.5 ± 11.7
Serum albumin, g/L	33.5 ± 2.6

All values are expressed as mean±SD, *n*=10. ESRD, end-stage renal disease.

Study design

A single test day per patient was scheduled during their second or third weekly HD session. Handgrip strength and body composition were measured before HD using a mechanical dynamometer (Jamar, Nottinghamshire, UK) and the Body Composition Monitor (Fresenius Medical Care, Bad Homburg, Germany), as described before [31].

Directly before and after a 4-hour HD session, blood was sampled from the arterial side of the arteriovenous shunt for analysis of plasma amino acid concentrations. Throughout HD, spent dialysate was continuously collected at a rate of 1.00 L/h in a container using a reversed injection pump (Alaris GW, Rolle, Switzerland). Every 30 min the container was replaced for a new one. After homogenization of the filled container, a sample of the collected dialysate was obtained.

Hemodialysis treatment

Patients' prescribed blood (300 – 400 mL/min) and dialysate flow rates (500 – 600 mL/min) were used during HD. Desired ultrafiltration volume was determined by the treating nephrologist and averaged 1.75±0.71 L. HD sessions were performed with high-flux polysulfone (*n*=7; FX-100, Fresenius Medical Care, Bad Homburg, Germany) and polynephron (*n*=3; ELISO-17H, Nipro Medical Corporation, Osaka, Japan) membranes with surface areas of 2.2 and 1.7 square meter, respectively. Dialysate composition used was equal for all HD sessions and contained sodium (138 mM), potassium (2.00 mM), calcium (1.50 mM), magnesium (0.50 mM), chloride (109 mM), bicarbonate (32.0 mM), and glucose (1.00 g/L).

Food intake

Patients were encouraged to consume their habitual diet before and during the test day. Habitual food intake during HD consisted mainly of home-made sandwiches, cookies, coffee, and tea. During the fourth hour of the HD session, dietary intake records of the participants were acquired through a 24-h food recall questionnaire. One researcher, who had received training by a licensed dietician, carefully instructed patients on how to perform the food recall questionnaire. All ingested foods and beverages were reported in household measurements or specified as portion sizes. Subsequently, energy and macronutrient intake were calculated using free available software from the Dutch Nutrition Centre (mijn.voedingscentrum.nl) based upon product specifications provided by food suppliers and the Dutch Food Consumption Database 2016 (NEVO; RIVM; Bilthoven, the Netherlands) [32]. Reported food intake was calculated for the HD session and the 24-h period.

Plasma amino acid concentrations

Blood samples were collected in EDTA-containing tubes and centrifuged at 3500*g* at 4°C for 10 min to obtain plasma. Aliquots of plasma were frozen in liquid nitrogen and stored in a freezer at -80°C until further analysis. For determination of plasma amino acid concentrations, 50 µL of blood plasma was deproteinized using 100 µL of 10% 5-sulfosalicylic acid (SSA) with 50 µM of MSK-A2 internal standard (Cambridge Isotope Laboratories, Massachusetts, USA). Subsequently, 50 µL of ultra-pure demineralized water was added and this solution was centrifuged at 14000*g* at 4°C for 15 min. After

centrifugation, 10 µL of the supernatant was added to 70 µL Borate reaction buffer (Waters, Saint-Quentin, France). In addition, 20 µL of AccQ-Tag derivatizing reagent solution (Waters, Saint-Quentin, France) was added after which the mixture was heated to 55°C for 10 min. Amino acid profiles in the derivative were determined by ultra-performance liquid chromatography mass spectrometry (UPLC-MS; ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France) as described previously [33].

Dialysate amino acid concentrations

Spent dialysate samples were collected in sterile tubes, immediately frozen in liquid nitrogen, and stored in a freezer at -80°C until further analysis. Collected dialysate was concentrated 5 times through freeze-drying 25 mL of the sample and dissolving the dried product in 5.0 mL 0.1 M hydrogen chloride. After homogenisation, 50 µL of the concentrated sample was deproteinized using 100 µL of 10% SSA with 50 µM of MSK-A2 internal standard and processed in the same manner as plasma samples. Subsequently, amino acid profiles were determined through UPLC-MS.

Calculations

Amino acid loss in the dialysate (g) was calculated by multiplying the mean total amino acid (TAA) concentration of spent dialysate (g/L) with spent dialysate and ultrafiltration volume (L). Essential amino acid (EAA) values are the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine values. Non-essential amino acid (NEAA) values equal the sum of alanine, arginine, asparagine, aspartic acid, beta alanine, cystine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine. Branched-chain amino acid (BCAA) values are the total of leucine, isoleucine, and valine values.

Statistical analysis

All data are expressed as mean±SEM unless indicated otherwise. Time-dependent variables (i.e. TAA, EAA, and individual amino acid loss per 30 min) were analysed by a one-factor repeated-measures ANOVA. If a statistically significant time-effect was found, post-hoc paired samples *t* tests were performed to locate the effects. Pre-HD and post-HD plasma amino acid concentrations were compared using paired-samples *t* test. Correlations between dialysate amino acid concentrations and pre-HD plasma amino acid concentrations, and dietary intake were assessed through determining the parametric Pearson's or the nonparametric Spearman's Rank Correlation Coefficients for normally and not normally distributed data, respectively. Statistical significance was set at $P<0.05$. All analyses were performed using SPSS Statistics software (version 24.0; IBM Corp., Armonk, NY, USA).

Results

Plasma amino acid concentrations

Pre-HD plasma TAA, NEAA, and EAA concentrations averaged 2.88±0.15, 2.08±0.11, and 0.80±0.05 mmol/L, respectively. Post-HD plasma TAA, NEAA, and EAA concentrations were significantly reduced to 2.27±0.11, 1.62±0.07, and 0.66±0.05 mmol/L, respectively (P<0.05). Pre-HD and post-HD plasma BCAA concentrations were 0.35±0.03 and 0.30±0.03 mmol/L, respectively (P=0.11). Whereas most individual amino acid concentrations decreased during HD, we observed a significant increase in plasma tryptophan concentrations (**Figure 1A**; P=0.003).

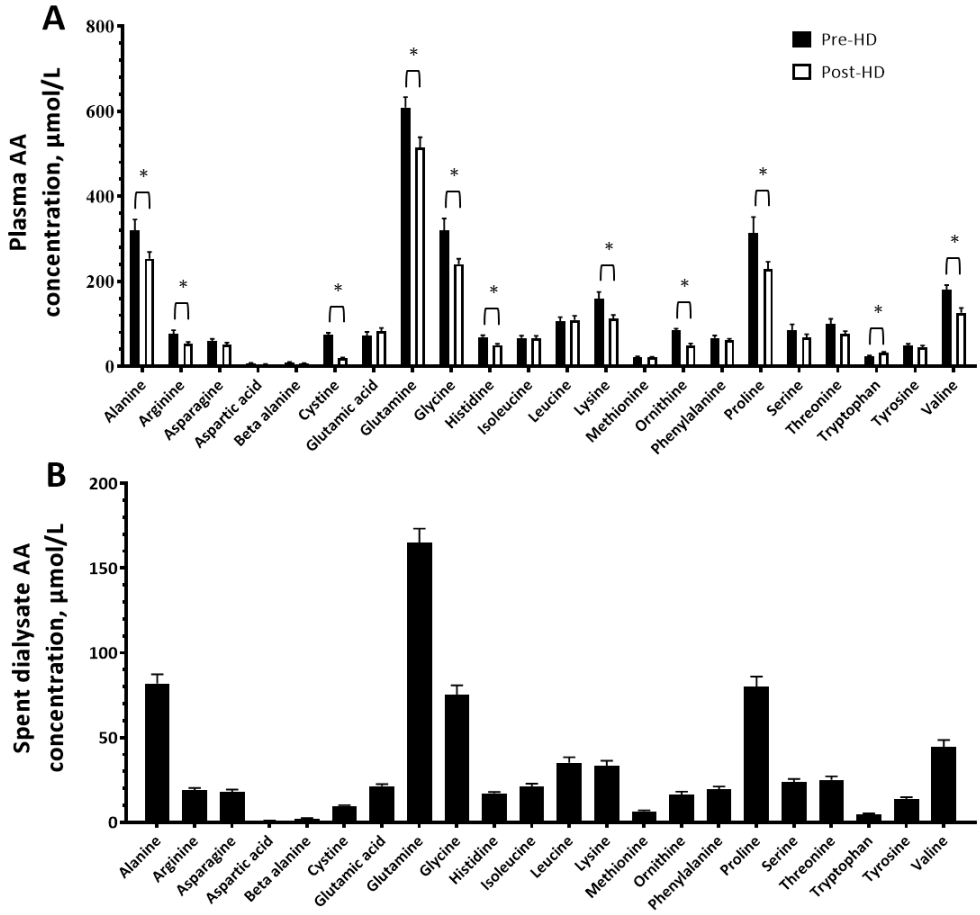

Figure 1. Amino acid concentrations in (A) pre- and post-hemodialysis plasma and (B) spent dialysate of chronic hemodialysis patients. Plasma concentrations of 22 amino acids are expressed as µmol/L**.** Values represent means+SEMs, n=10. *Post-HD plasma amino acid concentrations are significantly different from pre-HD plasma amino acid concentrations, P<0.05. AA, amino acid; HD, hemodialysis.

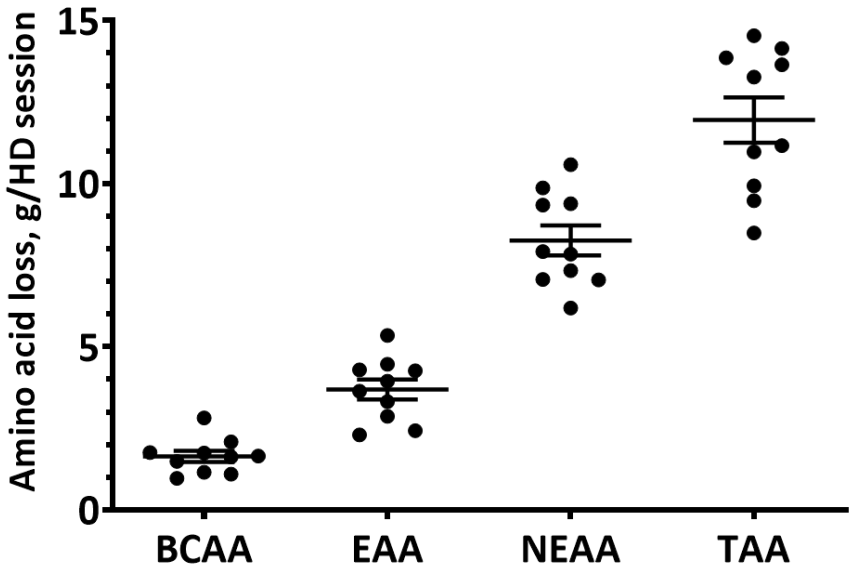

Figure 2. Amino acid loss during a single hemodialysis session in chronic hemodialysis patients. Filled circles represent individual data points and bars represent group means ± SEMs, *n*=10. BCAA, branched-chain amino acid; EAA, essential amino acid; HD, hemodialysis, NEAA, non-essential amino acid; TAA, total amino acid.

Spent dialysate amino acid concentrations

In the spent dialysate, the amino acids with the highest and lowest average concentrations were glutamine and aspartic acid, respectively (**Figure 1B)**. Spent dialysate TAA concentrations averaged 0.73±0.03 mmol/L and did not differ between the 30-min sampling periods throughout the HD session (*P*=0.94). Spent dialysate volume per HD session averaged 128±5.05 L. TAA, NEAA, EAA, and BCAA losses during a single HD session are depicted in **Figure 2**. Amino acid concentrations in spent dialysate were strongly correlated with pre-HD plasma amino acid concentrations (**Figure 3;** Spearman's ρ=0.92, *P*<0.001).

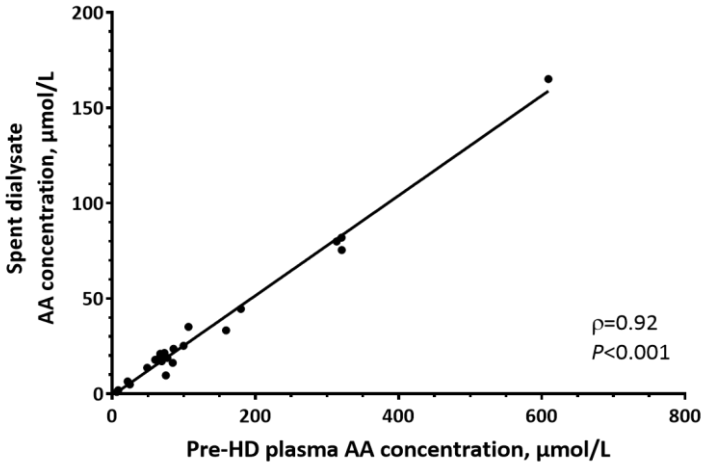

Figure 3. Correlation between amino acid profiles in spent dialysate and pre-hemodialysis plasma of chronic hemodialysis patients. Amino acid concentrations are expressed as µmol/L, *n*=10. Spearman's rank correlation coefficients were determined to assess correlations. HD, hemodialysis; TAA, total amino acid.

Dietary intake prior to and during hemodialysis

Reported 24-h dietary protein and energy intake averaged 1.03±0.13 g/kg and 28.3±2.9 kcal/kg, respectively (**Table 2)**. All included patients consumed food and beverages during HD. Patients ingested 0.33±0.05 g protein/kg and 8.9±1.0 kcal/kg during a single HD session. Protein intake during HD was not associated with an attenuated decline in plasma amino acid concentrations over the HD session (*P*=0.22). Protein intake was positively correlated with the incremental area under the curve of spent dialysate BCAA concentrations (**Figure 4A**; Pearson's *r*= 0.64, *P*=0.045). Furthermore, the correlation between of protein intake with the incremental area under the curve of spent dialysate TAA concentrations nearly reached statistical significance (**Figure 4B**; Pearson's *r*=0.62, *P*=0.055).

Table 2. Reported daily habitual energy and macronutrient intakes prior to and during hemodialysis in chronic hemodialysis patients consuming their habitual diet

	24 h intake	**During HD (4 h)**
Energy, kcal	1786 ± 189	553 ± 53
Protein, g	64.6 ± 7.5	20.1 ± 2.9
Fat, g	72.6 ± 6.8	21.5 ± 3.1
Carbohydrates, g	213 ± 26.3	67.1 ± 6.5

All values are expressed as mean ± SEM, *n*=10. HD, hemodialysis.

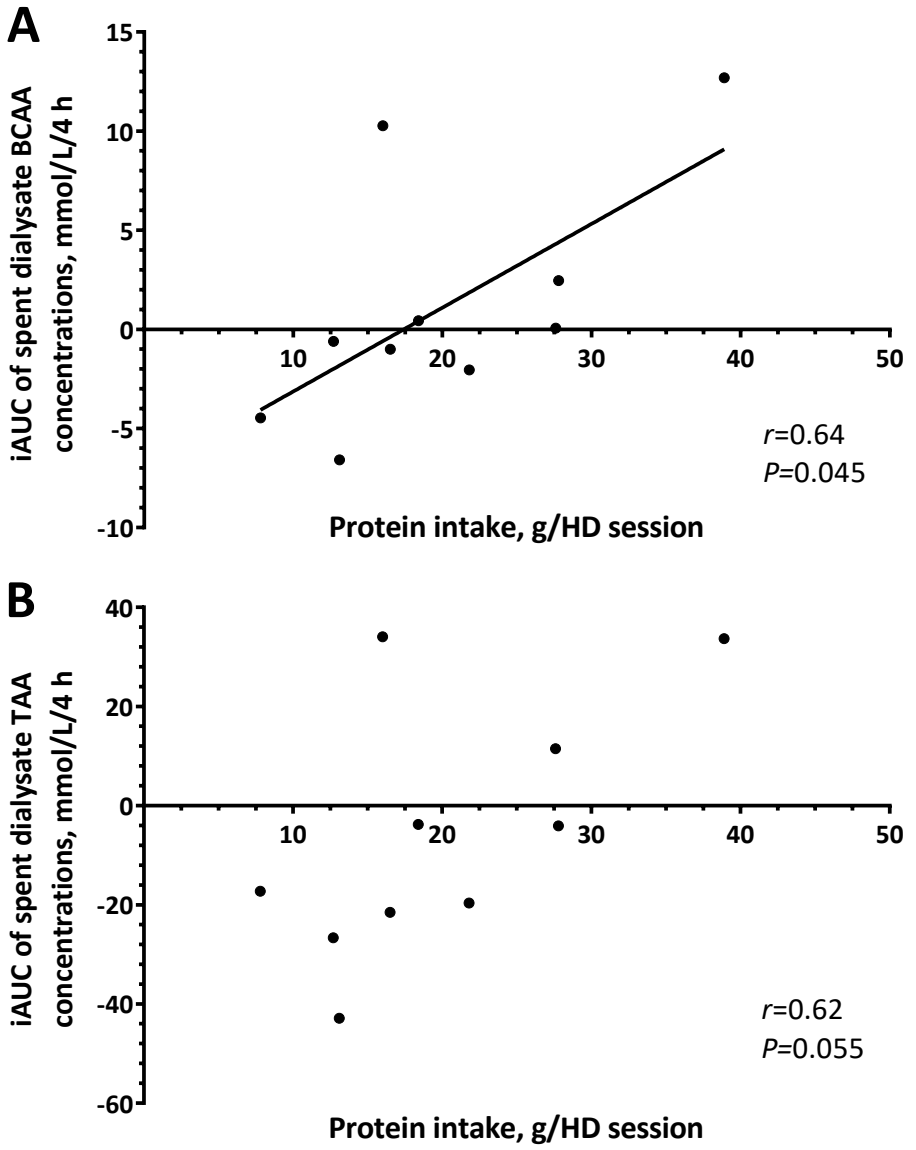

Figure 4. Correlations between protein intake during a single hemodialysis session and the incremental area's under the curve of (A) spent dialysate branched-chain amino acid concentrations and (B) spent dialysate total amino acid concentrations in chronic hemodialysis patients. Protein intake levels are expressed as g/HD session, *n*=10. Pearson's rank correlations coefficients were determined to assess correlations. BCAA, branched-chain amino acid; HD, hemodialysis; iAUC, incremental area under the curve; TAA, total amino acid.

Discussion

This is the first study to show that CHD patients ingesting their habitual diet throughout HD lose ~12 g of amino acids from the circulation during a single HD session. This is equivalent to the amount of amino acids being released into the circulation following ingestion of a typical meal (containing 20 – 25 g protein). The loss of amino acids during HD results in a significant decline in circulating plasma amino acid concentrations.

HD is a life-saving treatment for end-stage renal disease patients with inadequate residual renal function [34]. Besides harmful substances, HD also extracts small-sized nutrients from the circulation [11]. We observed a decline in plasma concentrations of most amino acids during HD, which resulted in a ~20% decrease of plasma TAA concentrations. Individual changes in plasma TAA, NEAA, EAA, and BCAA concentrations throughout HD are depicted in **Supplemental Figure 1**. Plasma TAA concentrations after HD were ~20% lower in our patients when compared with post-absorptive plasma TAA concentrations in healthy older adults observed in our lab recently [35]. As depicted in **Figure 1**, amino acid profiles in pre-HD plasma and spent dialysate showed the same pattern. Accordingly, the correlation between amino acid concentrations in pre-HD plasma and spent dialysate was very strong (**Figure 3**). Thus, all amino acids diffused through the HD membrane without selective restriction. During a single HD session, this resulted in an extraction of 11.95 ± 0.69 g amino acids from the circulation, of which ~8 g NEAAs, ~4 g EAAs, and ~2 g BCAAs (**Figure 2**). This would be equivalent to the protein provided in a full meal containing 20 – 25 g protein, as only ~50% of ingested dietary protein derived amino acids typically reach the circulation during the first few hours after meal ingestion [22, 36].

It has been suggested that the extraction of amino acids from the circulation may be compensated for through eating during HD [37]. In the current study, patients ingested self-selected foods during HD *ad libitum*, as they would do during usual care. Despite a mean protein intake of 20 g throughout HD sessions, we observed a significant decline in plasma amino acid concentrations. An overview of individual food intake and spent dialysate TAA concentrations throughout HD is presented in **Supplemental Figure 2**. Protein ingestion has been shown to increase plasma amino acid concentrations in a dose-dependent manner [12, 16, 38], which most likely increases diffusion of amino acids into the dialysate. In agreement, Veeneman *et al.* has previously shown that ingestion of protein-enriched meals throughout HD increases spent dialysate TAA concentrations [39]. Increased amino acid extraction following food intake during HD may prevent patients consuming their habitual diet from maintaining their plasma amino acid concentrations throughout HD.

Current clinical guidelines recommend patients undergoing HD to consume at least 1.2 g protein/kg ideal body weight/day [40-42]. However, most CHD patients fail to ingest this amount of protein [43, 44]. In the current study, reported habitual dietary protein intake

was ~1.0 g/kg ideal body weight/day and only three patients reported a protein intake of at least 1.2 g/kg ideal body weight/day. Inadequate dietary protein intake predisposes to the development and progression of protein malnutrition, which is frequently observed in CHD patients [45]. Especially on dialysis days, dietary protein intake is important to compensate for the HD-induced extraction of amino acids [46]. However, throughout dialysis days habitual food ingestion patterns are typically disrupted due to time restrains and fatigue caused by the HD session [47, 48]. These barriers to food intake result in a reduced dietary protein intake on dialysis days compared to non-dialysis days [43]. In many CHD patients, habitual dietary protein intake on dialysis days may not be sufficient to compensate for the HD-induced extraction of amino acids, contributing to the depletion of body protein stores.

We would advocate that nutritional interventions to support muscle maintenance in CHD patients should aim to increase dietary protein intake on dialysis days. It has been suggested that protein intake on dialysis days can be increased through providing more protein-rich foods during HD [49]. Furthermore, previous studies have shown that supplementing 30 – 60 g protein can maintain plasma amino acid concentrations throughout HD [37-39, 50, 51]. However, our results indicate that ingestion of a large protein dose during HD will also substantially increase amino acid extraction. Consequently, CHD patients who eat during HD should consume well over 1.2 g protein/kg (ideal) body weight on dialysis days to allow compensation for (additional) HD-based amino acid extraction. It remains to be established how much protein should be ingested during HD to optimally support muscle maintenance. To allow development of individualized nutritional guidelines for CHD patients, the impact of timing and distribution of protein ingestion throughout dialysis days still needs to be assessed.

In conclusion, 8 – 15 g of amino acids are extracted from the circulation during a single HD session. Habitual food intake of Dutch CHD patients during HD cannot fully compensate for this loss, resulting in a significant decline in circulating plasma amino acid concentrations. The observed amino acid extraction contributes substantially to protein malnutrition in CHD patients and emphasizes the need to develop effective and individualized nutritional strategies to improve nutritional status in patients frequently undergoing HD.

Acknowledgments

We greatly appreciate the assistance of the whole staff of the dialysis department of the MUMC+ in the execution of the experiments. Technical expertise from Joan Senden during the sample analyses was greatly appreciated.

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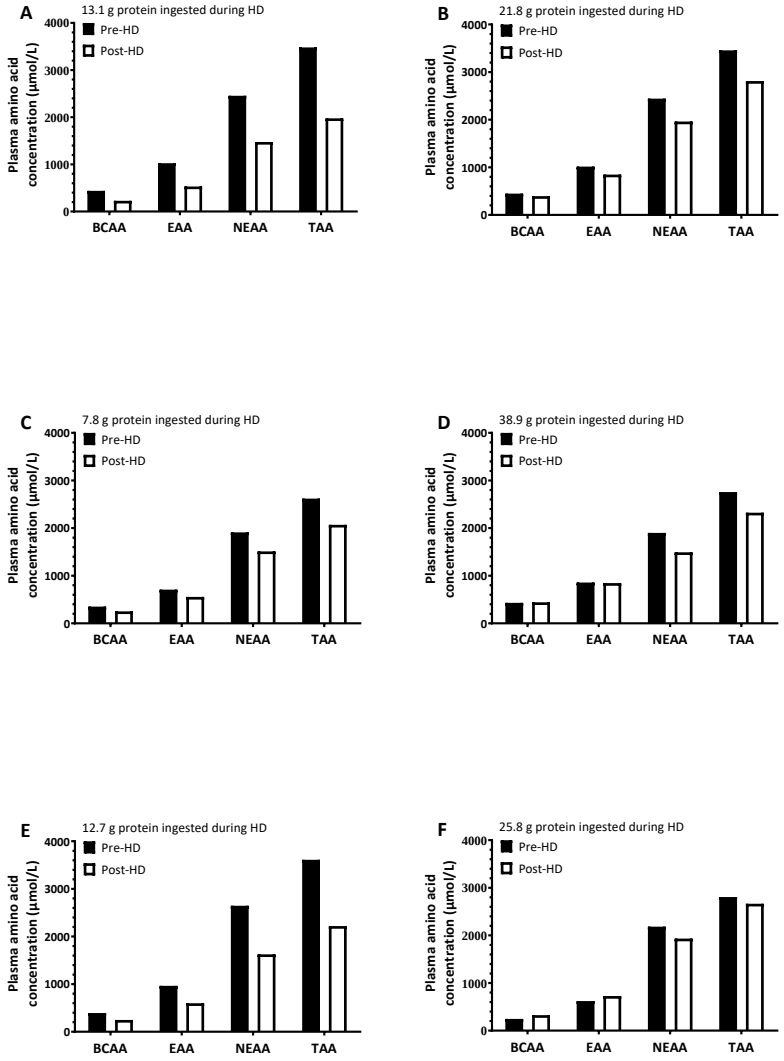
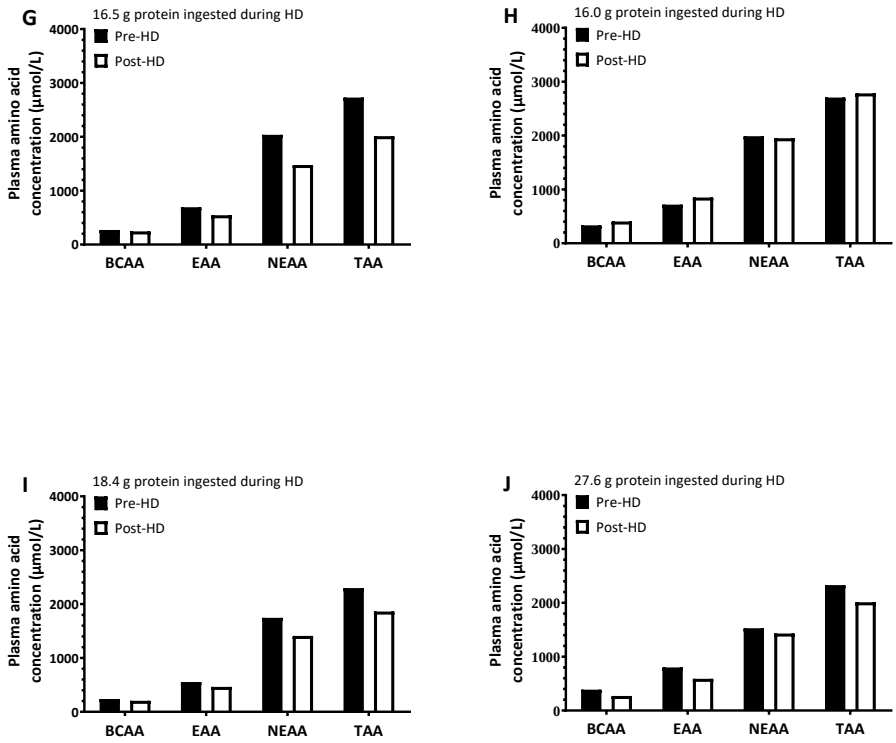
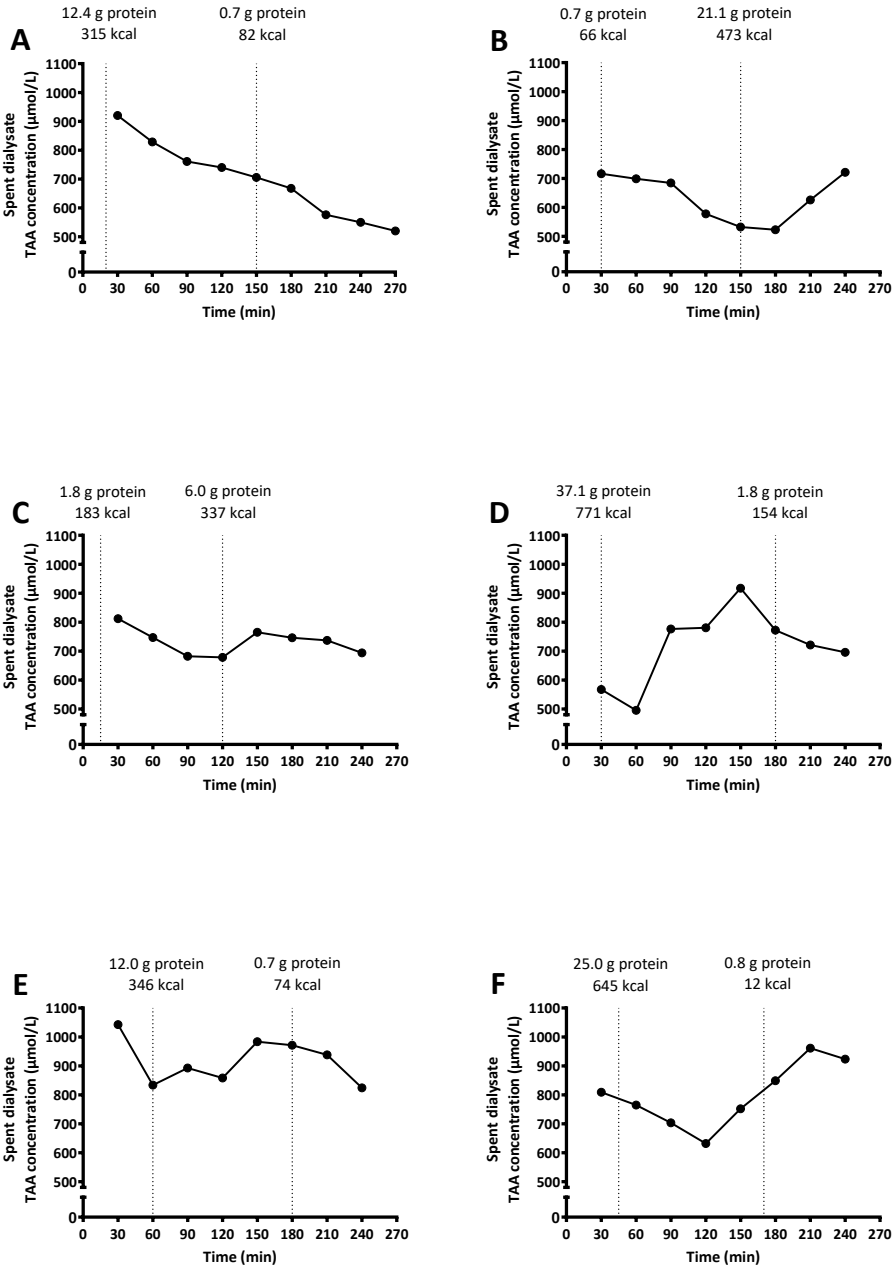
Supplementary material



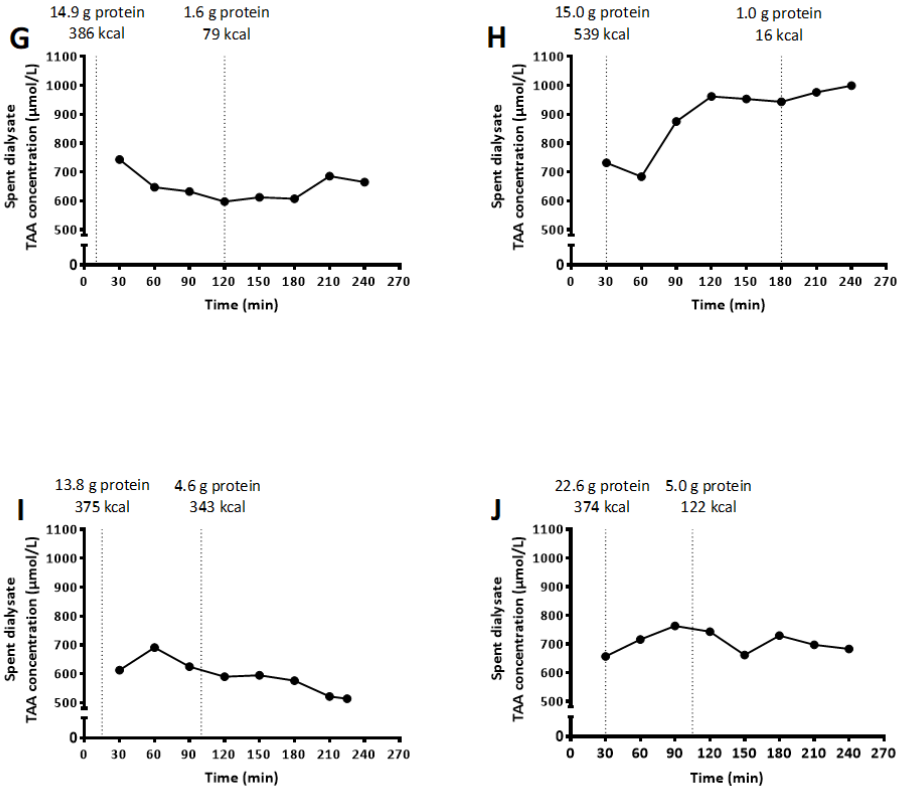
Supplementary figure 1. Individual pre-HD and post-HD plasma total amino acid concentrations and protein intake throughout a hemodialysis session in ten chronic hemodialysis patients. Protein intake was achieved through habitual dietary consumption and is expressed as g, and plasma total amino acid concentrations are expressed as µmol/L. BCAA, branched-chain amino acid; EAA, essential amino acid; HD, hemodialysis, NEAA, non-essential amino acid; TAA, total amino acid.



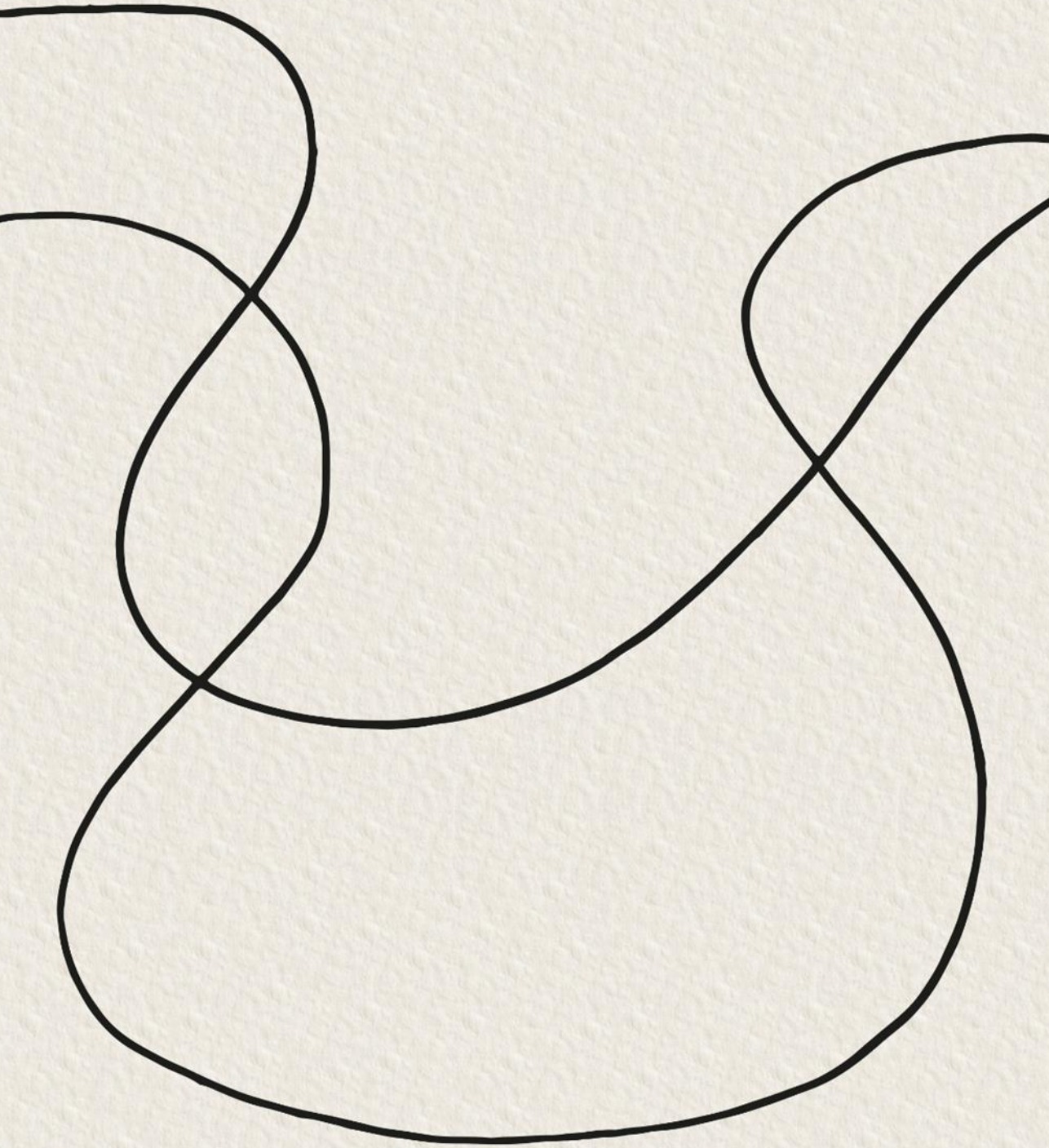
Supplementary figure 1 (continued). Individual pre-HD and post-HD plasma total amino acid concentrations and protein intake throughout a hemodialysis session in ten chronic hemodialysis patients. Protein intake was achieved through habitual dietary consumption and is expressed as g, and plasma total amino acid concentrations are expressed as µmol/L. BCAA, branched-chain amino acid; EAA, essential amino acid; HD, hemodialysis, NEAA, non-essential amino acid; TAA, total amino acid.



Supplementary figure 2. Individual pre-HD and post-HD plasma total amino acid concentrations and protein intake throughout a hemodialysis session in ten chronic hemodialysis patients. Protein intake was achieved through habitual dietary consumption and is expressed as g, and plasma total amino acid concentrations are expressed as µmol/L. BCAA, branched-chain amino acid; EAA, essential amino acid; HD, hemodialysis, NEAA, non-essential amino acid; TAA, total amino acid.



Supplementary figure 2 (continued). Individual pre-HD and post-HD plasma total amino acid concentrations and protein intake throughout a hemodialysis session in ten chronic hemodialysis patients. Protein intake was achieved through habitual dietary consumption and is expressed as g, and plasma total amino acid concentrations are expressed as µmol/L. BCAA, branched-chain amino acid; EAA, essential amino acid; HD, hemodialysis, NEAA, non-essential amino acid; TAA, total amino acid.



Chapter 4

Amino acid removal during hemodialysis can be compensated for by protein ingestion and is not compromised by intradialytic exercise: a randomized controlled cross-over trial

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The American Journal of Clinical Nutrition. 2021; 114 (6): 2074-2083

Abstract

Background

Patients with end-stage renal disease (ESRD) undergoing hemodialysis experience a rapid decline in skeletal muscle mass and strength. Hemodialysis removes amino acids (AAs) from the circulation, thereby lowering plasma AA concentrations and stimulating proteolysis.

Objectives

In the present study we evaluate the impact of intradialytic protein ingestion at rest and following exercise on AA removal and plasma AA availability in patients with ESRD.

Methods

Ten patients (age: 65±16 y, male/female: 8/2, BMI: 24.2±4.8 kg/m^2, serum albumin: 3.4±0.3 g/dL) with ESRD undergoing hemodialysis participated in this randomized controlled cross-over trial. During four hemodialysis sessions, patients were assigned to ingest 40 g protein or a placebo 60 min after initiation, both at rest (PRO and PLA, respectively) and following exercise (PRO+EX and PLA+EX, respectively). Spent dialysate and blood samples were collected every 30 min throughout hemodialysis to assess AA removal and plasma AA availability.

Results

Plasma AA concentrations declined by 26.1±4.5% within 30 min after hemodialysis initiation during all interventions (P<0.001, η^2_p>0.79). Protein ingestion, but not intradialytic exercise, increased AA removal throughout hemodialysis (9.8±2.0, 10.2±1.6, 16.7±2.2, and 17.3±2.3 g during PLA, PLA+EX, PRO, and PRO+EX interventions, respectively; protein effect P<0.001, η^2_p=0.97; exercise effect P=0.32, η^2_p=0.11). Protein ingestion increased plasma AA concentrations until the end of hemodialysis, while placebo ingestion resulted in decreased plasma AA concentrations (time effect P<0.001, η^2_p>0.84). Plasma AA availability (iAUC) was greater during PRO and PRO+EX interventions (49±87, and 70±34 mmol/L/240 min, respectively) when compared to PLA and PLA+EX interventions (-227±54 and -208±68 mmol/L/240 min, respectively; protein effect P<0.001, η^2_p=0.98; exercise effect P=0.21, η^2_p=0.16).

Conclusions

Protein ingestion during hemodialysis compensates for AA removal and increases plasma AA availability both at rest and during recovery from intradialytic exercise. Intradialytic exercise does not compromise AA removal or reduce plasma AA availability during hemodialysis in a post-absorptive or post-prandial state.

Introduction

Low muscle mass and strength are frequently observed among patients with end-stage renal disease (ESRD) undergoing hemodialysis, which leads to severe impairments in their physical function [1-4]. Hemodialysis itself is considered a key factor responsible for the accelerated loss of muscle mass and strength in patients with ESRD [5, 6]. Usually, patients undergo three 4-h hemodialysis sessions per week to remove metabolic waste products and excess fluids from their body. We [7] as well as others [8, 9] have reported that hemodialysis removes a considerable amount of amino acids (AAs) from the circulation, thereby lowering plasma AA concentrations. This decline in plasma AA availability is suggested to stimulate proteolysis, which further contributes to the loss of muscle mass in patients on chronic hemodialysis treatment [10, 11].

Recently, we have shown that ~12 g AAs are removed from the circulation during a single hemodialysis session [7]. It has been suggested that provision of protein-rich meals or supplements is warranted to compensate for AA removal during hemodialysis [8, 12, 13]. Ingested protein is digested and AAs are absorbed in the gut, with 40-70% of the protein-derived AAs being released into the circulation within the next 3 – 6 h [14-16]. However, a post-prandial increase in plasma AA concentrations during hemodialysis leads to a greater plasma-dialysate diffusion gradient and, as such, greater AA removal [7, 8]. Due to this greater AA removal, the efficacy of protein ingestion to compensate for plasma AA removal during hemodialysis remains to be determined. We hypothesize that ingestion of 40 g protein during hemodialysis will suffice to compensate for AA removal and, as such, prevent reduced plasma AA availability.

Besides protein ingestion, intradialytic exercise (exercise during hemodialysis) has been proposed as an effective strategy to improve physical function in patients on chronic hemodialysis treatment [17, 18]. Intradialytic exercise is usually performed at a low to moderate intensity using a cycle ergometer placed in front of the treatment chair or through group-based physical activity sessions [17, 19, 20]. However, the potency of intradialytic exercise to support muscle maintenance is still a matter of debate [21-23]. It has been suggested that intradialytic exercise may actually enhance hemodialysis-initiated proteolysis and, as such, could even compromise muscle conditioning [21, 24]. We hypothesize that intradialytic exercise leads to greater AA removal during hemodialysis both in a post-prandial and post-absorptive state.

The present study evaluates the impact of protein ingestion during hemodialysis at rest and during recovery from exercise on AA removal and plasma AA availability in patients with ESRD. Ten patients with ESRD on chronic hemodialysis treatment were selected to participate in a randomized cross-over design. This study provides a complete insight into the impact of both protein ingestion and intradialytic exercise on AA removal and plasma AA availability throughout hemodialysis in patients with ESRD.

Subjects

Ten patients with ESRD and well-functioning arteriovenous shunts, undergoing hemodialysis in the morning or afternoon for at least 3 months, were recruited between March 2019 and August 2020 through the outpatient population visiting the dialysis department of Maastricht University Medical Centre+, Maastricht, The Netherlands (See **Supplemental Figure 1** for the Consolidated Standards of Reporting Trials (CONSORT) flow diagram). Patients with an active infection, cognitive disorder, intolerance to food ingestion during hemodialysis, contraindication to intradialytic exercise, or missed hemodialysis session in the last month prior to the study period were excluded. After patients expressed willingness to participate to their nephrologist, they were informed by an investigator about the purpose of the study, experimental procedures, and possible risks prior to signing written informed consent. The Medical Research Ethics Committee Academic Hospital Maastricht/Maastricht University (NL65880.068.18) and the Hospital Board of the Academic Hospital Maastricht approved the current study and it was registered prospectively at the Netherlands Trial Register (NL7152). The present study design complies with the ethical standards stated in the latest version of the Helsinki Declaration of 1975 as revised in October 2013.

Methods

Pre-testing

A pre-testing session was scheduled during routine hemodialysis at least one week before the first test day to familiarize patients with intradialytic exercise and determine exercise capacity. In addition, patients' medical history, physical examinations, lab 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 adjusted until the patient was positioned properly. Blood pressure, heart rate, and an electrocardiogram were recorded and directly assessed for abnormalities by a physician throughout intradialytic exercise performance. After a 5-min warm-up, the resistance level of the dialysis cycle ergometer was increased until patients reported a score between 12 – 15 on the 6 – 20 points Borg Ratings of Perceived Exertion scale [25]. Subsequently, patients were instructed to continue cycling at the same resistance level for 10 min. When patients reported a score <12 or >15 on the 6 – 20 points Borg Ratings of Perceived Exertion scale the resistance level was adjusted accordingly. The resistance level at which patients succeeded to perform 10 min of moderate-intensity exercise was used for the exercise protocol during test days.

Dietary intake and physical activity

All patients refrained from any sort of strenuous physical activity 48 h prior to each test day. Patients who underwent hemodialysis in the morning reported in an overnight fasted state. Those who underwent hemodialysis in the afternoon consumed the same standardized breakfast at least 3 h before initiation of their hemodialysis session (providing ∼250 kcal, with carbohydrate, fat and protein providing 65, 23, and 12 En%, respectively). Thereafter, patients were instructed to remain fasted and avoid caffeine consumption until the end of the experimental protocol, but were allowed to ingest water *ad libitum*. During each test day, dietary intake records were acquired through a 24-h food recall questionnaire. Furthermore, patients filled out a food diary and wore a SenseWear pro 3 armband (Bodymedia®, Pittsburg, PA, USA) for 6 days between the first and second test day to assess habitual dietary intake and physical activity levels. A licensed dietician carefully instructed patients on how to perform the 24-h food recall questionnaires and 6-d food diary. All ingested foods and beverages were reported in household measurements or specified as portion sizes. Subsequently, energy and macronutrient intake were calculated using free available software from the Dutch Nutrition Centre (http://mijn.voedingscentrum.nl) based upon product specifications provided by food suppliers and the Dutch Food Consumption Database 2019 [26].

Study design

During four hemodialysis sessions, separated by a wash-out period of at least one week, all patients were assigned to ingest a placebo (PLA) or protein (PRO) beverage both in a rested state as well as following 30 min intradialytic exercise (PLA+EX and PRO+EX, respectively) in a randomized cross-over design. The cross-over design was chosen to minimize variability of outcome parameters in this heterogeneous population. An overview of test days, which were scheduled during patients' second or third weekly hemodialysis session, is provided in **Figure 1**. Patients were randomly assigned to an order of interventions by an independent researcher using an online randomizer (http://www.randomizer.org) and the randomization order of test beverages was not shared with investigators, study staff, 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 numbered according to participant and test day number before handing them to an investigator. The protein beverage contained 40 g of milk protein concentrate (Refit MPC 80, Friesland Campina, Amersfoort, The Netherlands) and two non-aspartame containing sweeteners (Natrena, Douwe Egberts, Amsterdam, The Netherlands) dissolved in 300 mL water. The placebo beverage contained only the two sweeteners dissolved in 300 mL water. The independent researcher shared the order of exercise performance during test days with the investigators after pre-testing was completed. Though 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 6 – 20 points Borg Ratings of Perceived Exertion 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 a cooling-down consisting of 3 min of cycling with a score between 9 – 12 and the last 2 min with a score below 9 on the 6 – 20 points Borg Ratings of Perceived Exertion scale.

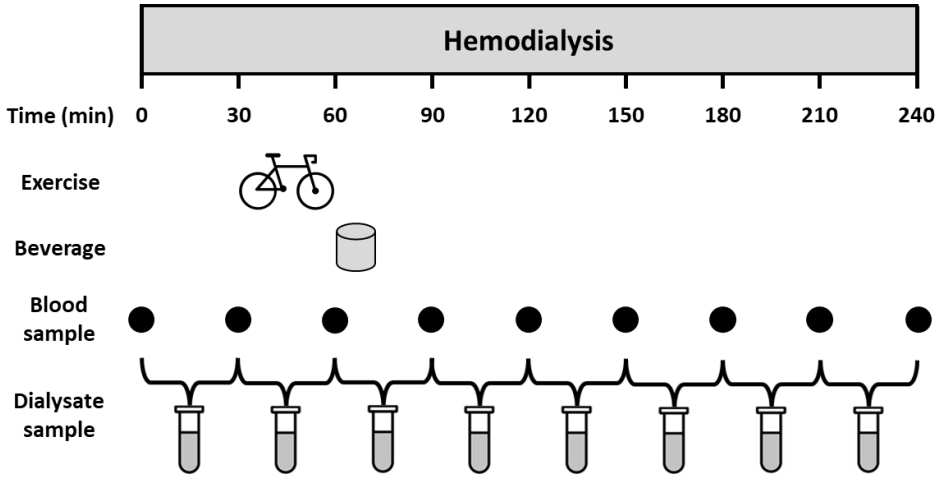

Figure 1. Schematic representation of study protocol. *t*=0 min represents the start of the hemodialysis session. During four hemodialysis sessions, patients ingested 40 g protein or placebo both at rest and during recovery from intradialytic exercise in a randomized cross-over design. This figure represents test days for the PLA+EX and PRO+EX interventions. The study protocol for PLA and PRO interventions was similar but without intradialytic exercise.

Hemodialysis treatment

Patients' prescribed blood (300 – 400 mL/min) and dialysate flow rates (500 – 600 mL/min), dialysate composition, dialysis modality, and dialysis membranes were used during hemodialysis and kept constant throughout all test days. Desired ultrafiltration volume was determined by the treating nephrologist for each hemodialysis session. Patients were dialyzed through a well-functioning arteriovenous shunt in the arm using polysulfone (*n*=4; FX-100, Fresenius Medical Care, Bad Homburg, Germany), polynephron (*n*=3; Elisio 17H, Nipro Medical corporation, Osaka, Japan), and triacetate (*n*=2; SUREFLUX 19L and *n*=1; SURFLUX 19UX, Nipro Medical Corporation, Osaka, Japan) membranes.

Experimental protocol

After patients arrived at the dialysis department, their weight was recorded and a Body Composition Monitor (BCM®, Fresenius Medical Care, Bad Homburg, Germany) was used to assess their body composition, as described before [27]. Subsequently, the arteriovenous shunt was checked for recirculation and used to collect arterial plasma samples for AA concentrations analyses. After initiation of hemodialysis (*t*= 0 min), plasma samples were collected from the arterial line with 30-min intervals (at *t*= 30, 60, 90, 120, 150, 180, and 210 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). Every 30 min these

containers were replaced (at *t*= 30, 60, 90, 120, 150, 180, 210, and 240 min) and a homogenized sample of the spent dialysate collected over each 30-min period was obtained. Blood pressure and heart rate were measured frequently throughout hemodialysis. During the sessions including intradialytic exercise, patients started cycling 30 min after hemodialysis initiation (*t*= 30 min) and additional measurements of blood pressure and heart rate were performed during and after exercise (at *t*= 40, 50, and 70 min). In all sessions, 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 plasma sample was collected from the arterial side of the arteriovenous shunt. Following the experimental procedures, patients consumed a standard meal before leaving the dialysis department.

Plasma amino acid analysis

Plasma samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes and centrifuged at 1000*g* at 4°C for 10 min to obtain plasma. Aliquots of plasma were frozen in liquid nitrogen and stored in a freezer at -80°C until further analysis. For determination of plasma AA concentrations, 50 µL of blood plasma was deproteinized using 100 µL of 10% 5-sulfosalicylic acid with 50 µM of the metabolomics AA mix MSK-A2 internal standard (Cambridge Isotope Laboratories, Massachusetts, USA). Subsequently, 50 µL of ultra-pure demineralized water was added and the samples were centrifuged. Thereafter, 10 µL of supernatant was added to 70 µL Borate reaction buffer (Waters, Saint-Quentin, France). In addition, 20 µL of AccQ-Tag derivatizing reagent solution (Waters, Saint-Quentin, France) was added and the mixture was subsequently heated to 55°C for 10 min. AA profiles in the derivative were determined by ultra-performance liquid chromatography mass spectrometry (UPLC-MS; ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France) as described previously (28).

Dialysate amino acid analysis

Spent dialysate samples were collected in sterile tubes, immediately frozen in liquid nitrogen, and stored in a freezer at -80°C until further analysis. These samples were concentrated through freeze-drying 25 mL of the sample and dissolving the dried product in 5.0 mL 0.1 M hydrogen chloride. After homogenization, the concentrated samples were processed in the same manner as plasma samples and AA profiles were determined through UPLC-MS.

Statistical analysis

All data are expressed as means±SDs unless indicated otherwise. A power calculation was performed with differences in incremental area under the curve (iAUC) of plasma AA concentrations as the primary outcome measure. A sample size of 10 participants, including

a 20% dropout rate, was calculated using a power of 80%, a significance level of 0.025 to compensate for the cross-over design with two interventions, and a difference in iAUCs of 13% between treatments with a standard deviation of 11% based on a previous study from our lab [29]. Secondary outcome parameters include plasma and spent dialysate total amino acid (TAA), branched-chain amino acid (BCAA), non-essential amino acid (NEAA), and essential amino acid (EAA) concentrations, AA removal, correlations between AA concentrations in plasma and spent dialysate, habitual dietary energy and macronutrient intake, and habitual physical activity levels. After the randomization order of test beverages was shared with investigators, hemodialysis parameters and pre-hemodialysis weight were compared between interventions to identify possible confounders. Normal distribution of all parameters were verified by Shapiro-Wilk tests (P>0.05). No major violations for specific three-way repeated-measures ANOVA assumptions were observed and in case of non-sphericity, the Greenhouse-Geisser correction was used. Potential differences in AA concentrations over time were assessed using three-way repeated-measures ANOVA with time, protein ingestion (yes/no) and exercise (yes/no) as within-subject factors. AA removal, the iAUC of plasma AA concentrations representing the t=0-240 min period, hemodialysis parameters, and pre-hemodialysis weight were analyzed by two-way repeated-measures ANOVA with protein ingestion (yes/no) and exercise (yes/no) as within subject variables. If a statistically significant interaction was found, two-way ANOVAs, and/or subsequent paired-samples t tests, were performed. In case of significant time effects, Bonferroni post-hoc analyses were performed to locate the effects. Dietary energy and macronutrient intake and physical activity values on dialysis days and non-dialysis days were compared using paired-samples t tests. Correlations between AA concentrations in spent dialysate and the average of the two corresponding plasma samples (e.g. t= 30 and t= 60 min for spent dialysate collected between t=30 and 60 min) were assessed through determining Pearson's correlation coefficients. Effect sizes were calculated for plasma and spent dialysate AA concentrations using partial eta squared (η^2_p) for ANOVA comparisons. Statistical significance was set at P<0.05. All analyses were performed using SPSS statistics software (version 24.0; IBM Corp., Armonk, NY, USA).

Results

Patients' characteristics

All ten included patients with ESRD completed four test days. Patients' baseline characteristics are presented in **Table 1**. Six patients were anuric, one patient was oliguric, and three patients had a remaining diuresis over 400 mL/24 h. No differences were observed between PLA, PLA+EX, PRO, and PRO+EX interventions in ultrafiltration volume (1.24±1.01, 1.47±1.27, 1.23±1.08, and 1.41±1.24 L, respectively; *P*>0.05), dialysis adequacy (equilibrated Kt/V: 1.45±0.22, 1.53±0.22, 1.57±0.27, and 1.48±0.22, respectively; *P*>0.05), and pre-hemodialysis weight (71.9±14.3, 72.6±14.0, 72.2±13.9, and 71.9±14.1 kg, respectively; *P*>0.05).

Table 1. Patients' characteristics

	Patients
Age, y	65±16
Sex, male/female	8/2
Cause of end-stage renal disease	5 Glomerular
	4 Vascular
	1 Unknown
Dialysis vintage, months	36±23
Dialysis timing, morning/afternoon	5/5
Height, m	1.72±0.13
Weight, kg	71.0±13.6
Body mass index, kg/m^2	24.2±4.8
Lean tissue index, kg/m^2	13.3±2.5
Fat tissue index, kg/m^2	10.4±5.9
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.

Habitual dietary intake and physical activity

Two patients declined to fill out a food diary and two patients did not wear the SenseWear armband correctly. Reported habitual dietary energy and protein intakes averaged 25.9±6.0 kcal/kg body weight/day and 1.0±0.3 g /kg body weight/day, respectively. No statistical differences were observed in habitual energy and macronutrient intake between non-dialysis and dialysis days (**Table 2**). In contrast, activity-related energy expenditure was lower on dialysis days (7±9 kcal/kg body weight) when compared to non-dialysis days (12±13 kcal/kg body weight; *P*=0.04). However, the differences between physical activity duration and number of steps taken on non-dialysis and dialysis days were not statistically significant (Table 2).

Table 2. Habitual food intake and physical activity on dialysis and non-dialysis days

	Daily mean	DD	Non-DD	*P*
Habitual intake				
Energy, kcal	1874±605	2074±812	1763±433	0.29
Energy, kcal/kg body weight	25.9±6.0	28.1±9.5	24.8±6.3	0.24
Carbohydrate, g	217±62	240±83	205±43	0.26
Carbohydrate, g/kg body weight	3.0±0.6	3.3±1.0	2.7±0.4	0.32
Protein, g	73±29	80±37	69±24	0.33
Protein, g/kg body weight	1.0±0.3	1.1±0.4	0.9±0.3	0.19
Fat, g	71±34	81±40	66±30	0.28
Fat, g/kg body weight	1.0±0.3	1.1±0.5	0.9±0.3	0.10
Physical activity				
Number of steps	4202±3943	3575±4739	4515±3535	0.29
Activity-related energy expenditure, kcal/kg	10±12	7±9	12±13	0.04
Moderate-vigorous activity duration, min	145±162	102±127	166±174	0.12

All values are expressed as means±SDs, *n*=8. Data of dialysis days and non-dialysis days were compared using paired-samples *t* tests. Daily mean values represent the average of dialysis and non-dialysis days, measured over a 6-day period. DD, dialysis day.

Plasma amino acid concentrations

Pre-hemodialysis plasma TAA concentrations averaged 2.93±0.40 mmol/L, with no differences between interventions (**Figure 2**; $P>0.05$). A significant time × protein interaction was observed for plasma TAA concentrations throughout hemodialysis ($P<0.001$, $\eta^2_p=0.87$). Separate analyses showed that following hemodialysis initiation, plasma TAA concentrations decreased substantially during the first 30 min ($P<0.001$, $\eta^2_p>0.79$ for all interventions).

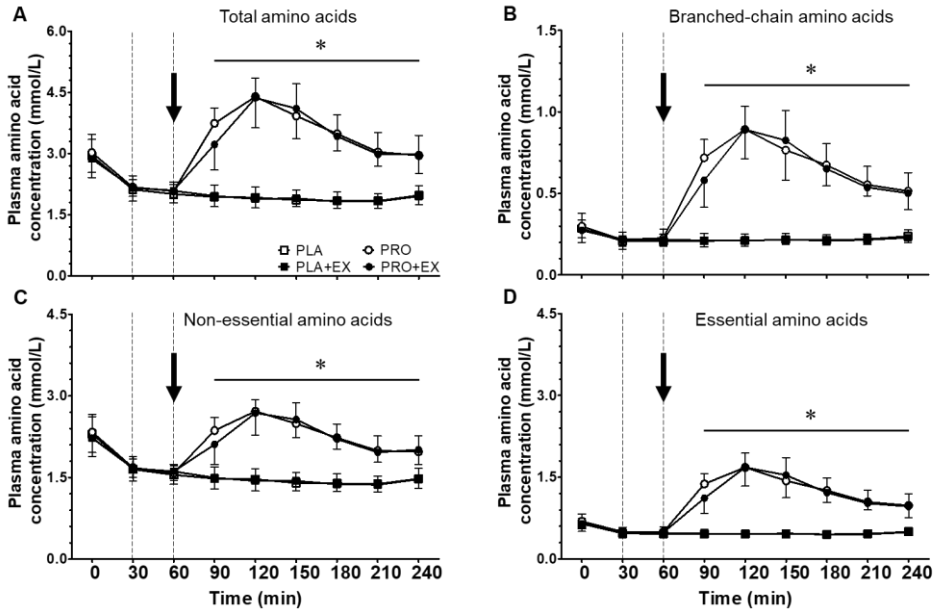

Figure 2. Plasma (A) total, (B) branched-chain, (C) non-essential and (D) essential amino acid concentrations throughout hemodialysis at rest and following exercise with and without protein ingestion. The dotted lines represent the start and end of intradialytic exercise and the arrow represents the ingestion of the test beverage. Values are expressed as means±SDs, $n=10$ for all values. 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. Time × protein interaction $P<0.05$. *, Protein interventions significantly different from placebo interventions (protein effect $P\leq0.001$); PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and exercise.

During PLA and PLA+EX interventions plasma TAA concentrations continued to decrease over time to 1.84±0.18 and 1.83±0.16 mmol/L at $t=210$ min, respectively (time effect $P<0.001$, $\eta^2_p=0.69$). Plasma TAA concentrations increased following protein ingestion during PRO and PRO+EX interventions (time effect $P<0.001$, $\eta^2_p=0.80$). Peak plasma TAA concentrations were observed 60 min after protein ingestion ($t=120$ min), with no differences between PRO and PRO+EX interventions (4.40±0.45 and 4.37±0.73 mmol/L, respectively; protein × exercise interaction $P=0.34$, $\eta^2_p=0.10$). In line with these data, an

effect of protein ingestion (protein effect P<0.001, η^2_p=0.98) but no effect of intradialytic exercise (exercise effect P=0.21, η^2_p=0.16) was observed on the iAUC of plasma TAA concentrations during PLA, PLA+EX, PRO, and PRO+EX interventions (**Figure 3**; -227±54, -208±68, 49±87, and 70±34 mmol/L/240 min, respectively). As shown in Figure 2, plasma BCAA, NEAA, and EAA concentrations throughout hemodialysis responded in the same manner as plasma TAA concentrations to protein ingestion and intradialytic exercise.

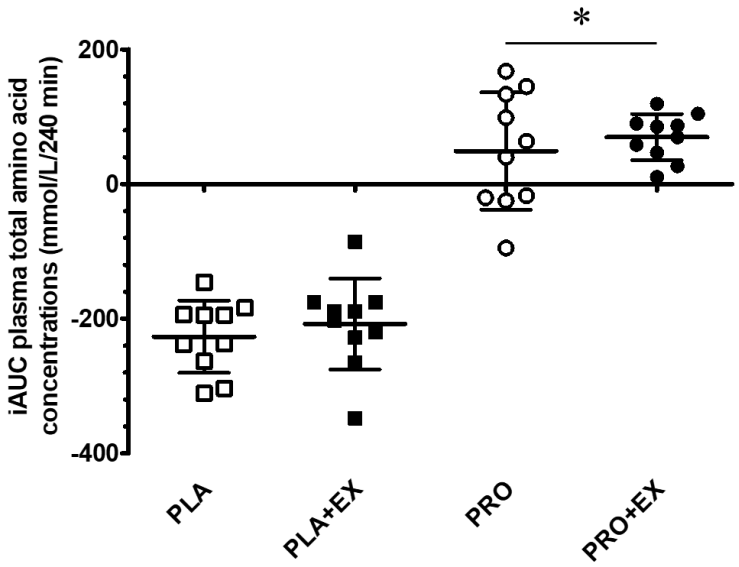

Figure 3. Incremental area under the curve of plasma total amino acid concentrations throughout hemodialysis at rest and following exercise with and without protein ingestion. The incremental are under the curve was calculated over the 240-min hemodialysis period. Squares and circles represent individual data points and bars represent group means±SDs, n=10. 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<0.001). iAUC, incremental area under the curve; PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and exercise.

Spent dialysate amino acid concentrations

AA concentrations in the spent dialysate are presented in **Figure 4**. Spent dialysate AA concentrations correlated well with circulating plasma AA concentrations (Pearson's r=0.91, P<0.001). A significant time × protein interaction was observed for spent dialysate TAA concentrations throughout hemodialysis (P<0.001, η^2_p=0.89). Spent dialysate TAA concentrations decreased over time during PLA and PLA+EX interventions towards 0.57±0.11 and 0.57±0.08 mmol/L during the last 30-min period of hemodialysis, respectively (P=0.005, η^2_p=0.77). In contrast, spent dialysate TAA concentrations significantly increased following protein ingestion during PRO and PRO+EX interventions and remained elevated until the end of hemodialysis (time effect P<0.05, η^2_p=0.87).

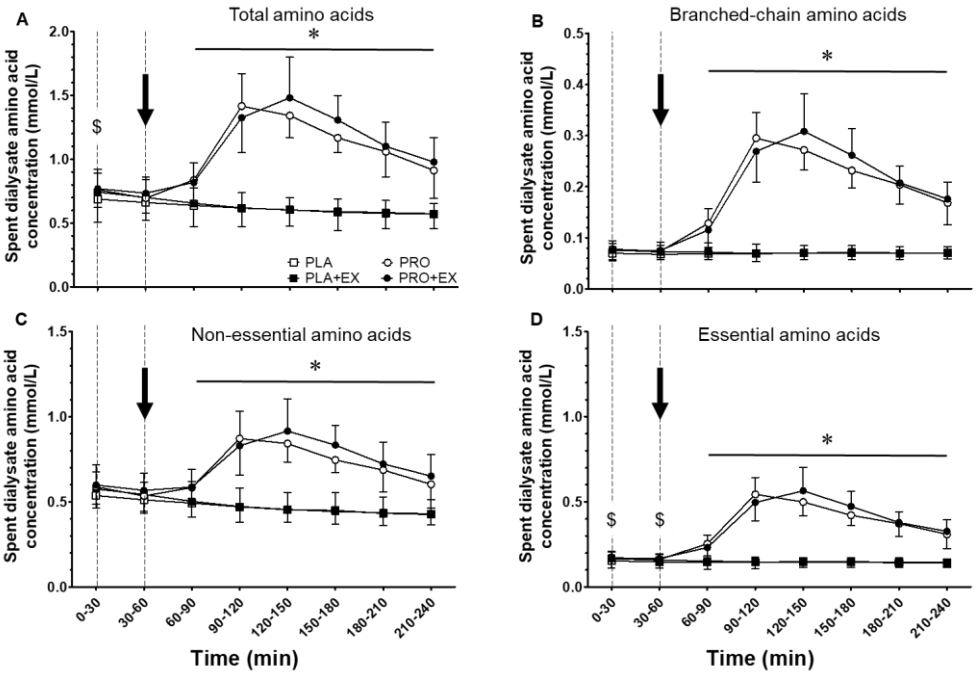

Figure 4. Spent dialysate (A) total, (B) branched-chain, (C) non-essential and (D) essential amino acid concentrations throughout hemodialysis at rest and following exercise with and without protein ingestion. The dotted lines represent the start and end of intradialytic exercise and the arrow represents the ingestion of the test beverage. Values are expressed as means±SDs, n=10 for all values. 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. Time × protein interaction P<0.05. $, Protein interventions significantly different from placebo interventions (protein effect P<0.05); *, Protein interventions significantly different from placebo interventions (protein effect P<0.001); PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and exercise.

Protein ingestion significantly increased AA removal during PRO and PRO+EX when compared with PLA and PLA+EX interventions (**Figure 5**; 16.7±2.2 and 17.3±2.3 vs. 9.8±2.0 and 10.2±1.6 g, respectively; protein effect P<0.001, η^2_p=0.97). Intradialytic exercise did not modulate AA removal (exercise effect P=0.32, η^2_p=0.11). Furthermore, spent dialysate BCAA, NEAA, and EAA concentrations showed similar perturbations throughout hemodialysis as spent dialysate TAA concentrations (Figure 4).

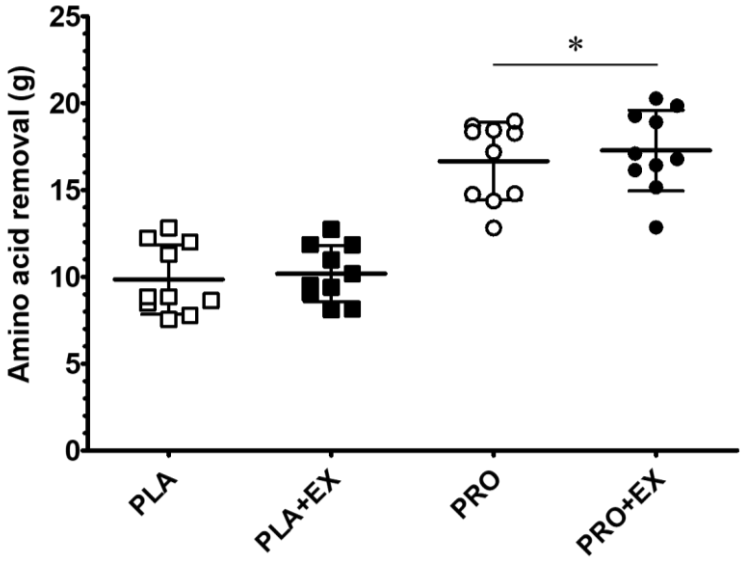

Figure 5. Total amino acid removal throughout hemodialysis at rest and following exercise with and without protein ingestion. Squares and circles represent individual data points and bars represent group means±SDs, n=10. Data were analysed with a two-way repeated-measures ANOVA with protein ingestion (yes/no) and exercise (yes/no) as within subject variables. *, Significantly different from placebo interventions (protein effect P<0.001). PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and exercise.

Discussion

In this randomized controlled cross-over study, we observed that AAs are removed from the circulation during hemodialysis, thereby lowering plasma AA concentrations in patients with ESRD. Protein ingestion during hemodialysis compensated for AA removal and prevented a decline in plasma AA availability at rest and during recovery from intradialytic exercise. Exercise performed during hemodialysis did not modulate AA removal or plasma AA availability in patients with ESRD.

Hemodialysis treatment is essential for patients with ESRD as it prevents accumulation of metabolic waste products up to lethal concentrations. However, hemodialysis also removes AAs from the circulation because they, just like metabolic waste products, diffuse through the dialysis membrane [9]. In the current study we observed a substantial decline in circulating plasma TAA concentrations from 2.93±0.40 to 2.16±0.26 mmol/L within 30 min following the initiation of hemodialysis (Figure 2). Such a decrease in plasma AA concentrations has been shown to stimulate proteolysis in peripheral tissues [10, 30-32]. Furthermore, by also measuring AA concentrations in the spent dialysate we were able to assess AA removal throughout the hemodialysis session, which ranged between 7 and 12 g during placebo interventions (Figure 5). This loss is representative of the amount of AAs being released in the circulation following ingestion of a normal meal providing approximately 20 g protein [15]. As a consequence, AA removal during hemodialysis has been proposed to represent a key factor responsible for the accelerated loss of muscle mass in patients with ESRD [6, 33, 34].

We first assessed the impact of protein ingestion during hemodialysis as a means to compensate for AA removal and, as such, to support muscle maintenance. To overcome reduced protein digestion and absorption kinetics of patients on chronic hemodialysis treatment as well as increased AA removal following protein ingestion during hemodialysis [7, 15], we provided all patients with a bolus of 40 g protein. Ingestion of 40 g protein during hemodialysis elevated plasma AA concentrations (Figure 2). This stimulated AA removal, resulting in ∼8 g more AAs being removed from the circulation when compared to placebo ingestion. Despite the greater AA removal (Figure 5), plasma AA availability was strongly elevated following protein ingestion (Figure 3). Preventing a decline in plasma AA availability throughout hemodialysis has been reported to attenuate muscle proteolysis during and after hemodialysis [8, 11, 30]. We conclude that ingestion of 40 g protein is sufficient to compensate for intradialytic AA removal, prevent a decline in plasma AA concentrations, and increase plasma AA availability. Especially the latter may be of key importance to achieve a positive muscle net protein balance during hemodialysis.

Another key strategy to support muscle maintenance in patients on chronic hemodialysis treatment is the implementation of physical activity or exercise interventions [35, 36].

Previous work has shown various benefits of lifestyle intervention in patients with chronic kidney disease, including those undergoing hemodialysis [37, 38]. However, the effectiveness of these lifestyle intervention programs for patients on chronic hemodialysis treatment are typically compromised by low adherence and compliance [39]. Exercise intolerance, fatigue, and lack of exercise knowledge often prevent these patients from increasing their physical activity levels [21, 40]. Consequently, effective physical activity intervention programs need to be individualized and performed under strict supervision. Therefore, implementation of physical activity or exercise during hemodialysis has been proposed as a practical and efficient intervention strategy as it would be more time efficient for patients and relatively easy to supervise by (para)medical staff [41]. Benefits of structured intradialytic exercise performance entail improved aerobic capacity, physical function, health-related quality of life, and better clearance of metabolic waste products during hemodialysis [17, 19, 42, 43]. However, it has been suggested that intradialytic exercise without concurrent protein ingestion may actually exacerbate muscle catabolism [44], which could result in even greater AA removal. Therefore, in the present study we assessed the impact of intradialytic exercise on AA removal and plasma AA availability both in the presence and absence of protein ingestion. Here, we observed no differences in AA removal during a hemodialysis session with (10.2±1.6 g) or without (9.8±2.0 g) intradialytic exercise (Figure 5). Furthermore, we observed no differences in plasma AA availability due to intradialytic exercise (Figure 3). This implies that intradialytic exercise performed in a post-absorptive state does not necessarily impair the net protein balance during hemodialysis. However, the muscle net protein balance will not become positive when exercise is performed without concomitant protein ingestion [45].

To facilitate the skeletal muscle adaptive response to exercise, ample availability of circulating AAs is required [46, 47]. Therefore, intradialytic exercise combined with protein ingestion to compensate for AA removal and increase plasma AA availability represents a preferred strategy. So far, there have not been any studies to assess the impact of intradialytic exercise and protein ingestion on AA removal and plasma AA availability. In line with our findings described above, we observed that ingestion of 40 g protein directly after intradialytic exercise increases plasma AA concentrations with levels remaining elevated until the end of the hemodialysis session (Figure 2). As a result, intradialytic exercise did not have any impact on plasma AA availability throughout the 4 h hemodialysis session (Figure 3). Furthermore, intradialytic exercise did not significantly increase AA removal following protein ingestion (16.6±2.2 vs 17.3±2.3 g in PRO and PRO+EX, respectively; Figure 5). Therefore, protein ingestion increases plasma AA availability during hemodialysis, which may create a setting in which hemodialysis-initiated proteolysis is inhibited and muscle conditioning after exercise performance is supported.

Combining protein ingestion and exercise during hemodialysis provides a practical interventional strategy that may help to preserve muscle mass and maintain functional

capacity in patients on chronic hemodialysis treatment. However, the design of the present study has some limitations. The included patients were volunteers, which may introduce some confounding as the less clinically compromised patients may be more likely to partake. Nonetheless, it is generally hypothesized that malnourished patients undergoing hemodialysis benefit to a greater extent from intradialytic protein supplementation with or without exercise compared to well-nourished patients [44, 48]. As we performed statistical analyses of multiple secondary outcomes in the present study, there is an increased risk of a type I error among the secondary outcome parameters. In addition, we assessed the impact of protein ingestion and exercise on plasma AA concentrations and AA removal during hemodialysis sessions, which may or may not necessarily translate to increases in muscle mass or improvements in physical function over a more prolonged treatment period.

So far, long-term intervention studies investigating the effects of intradialytic oral nutritional supplementation with or without exercise training on muscle mass and function have reported equivocal results [24, 44, 49]. This may be largely due to exercise intolerance and the low adherence of these patients to lifestyle intervention [39, 40, 50]. Furthermore, the uremic and inflammatory milieu in these patients may compromise the capacity of skeletal muscle tissue to properly respond to protein ingestion and exercise training. For example, Jeong *et al.* reported no improvements in physical function or body composition following 12 months of intradialytic protein ingestion and exercise [44]. More work will be needed to establish the various exercise modalities and adjuvant nutritional support that will effectively support muscle mass maintenance in this heterogeneous population.

In conclusion, protein ingestion during hemodialysis compensates for AA removal and increases plasma AA availability at rest and during recovery from intradialytic exercise. Intradialytic exercise should be combined with protein ingestion to compensate for AA removal during hemodialysis and, as such, allow a setting that may support muscle reconditioning in patients with ESRD.

Acknowledgments

We greatly appreciate the assistance of Wesley Hermans, Joan Senden, Therese van der Vlies, Nikki Adriaens, and the whole staff of the MUMC+ dialysis department, especially Anouk Geurts and Jaap Krohof, in the execution of the experiments.

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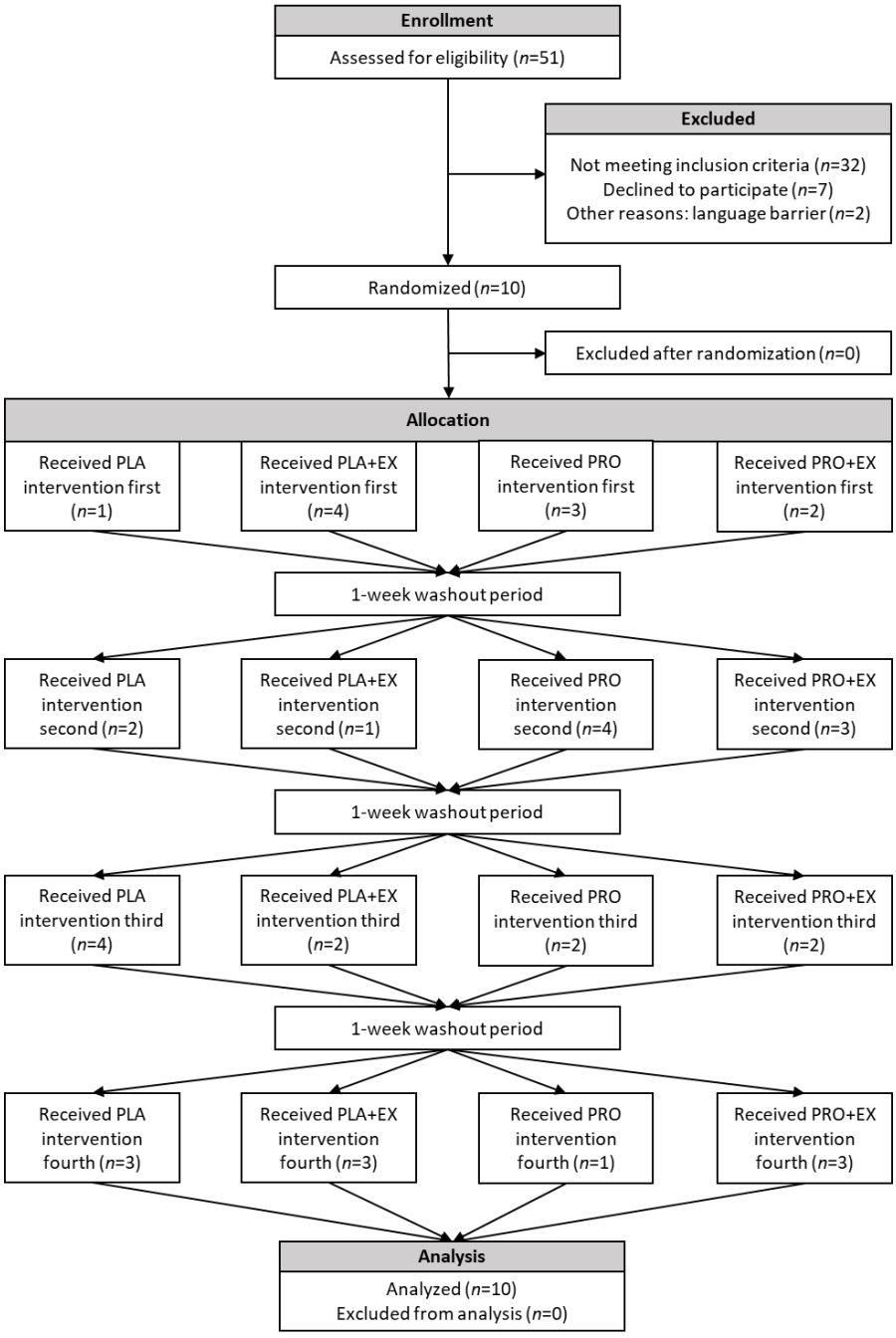
Supplementary material



Supplementary figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow chart. PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and exercise.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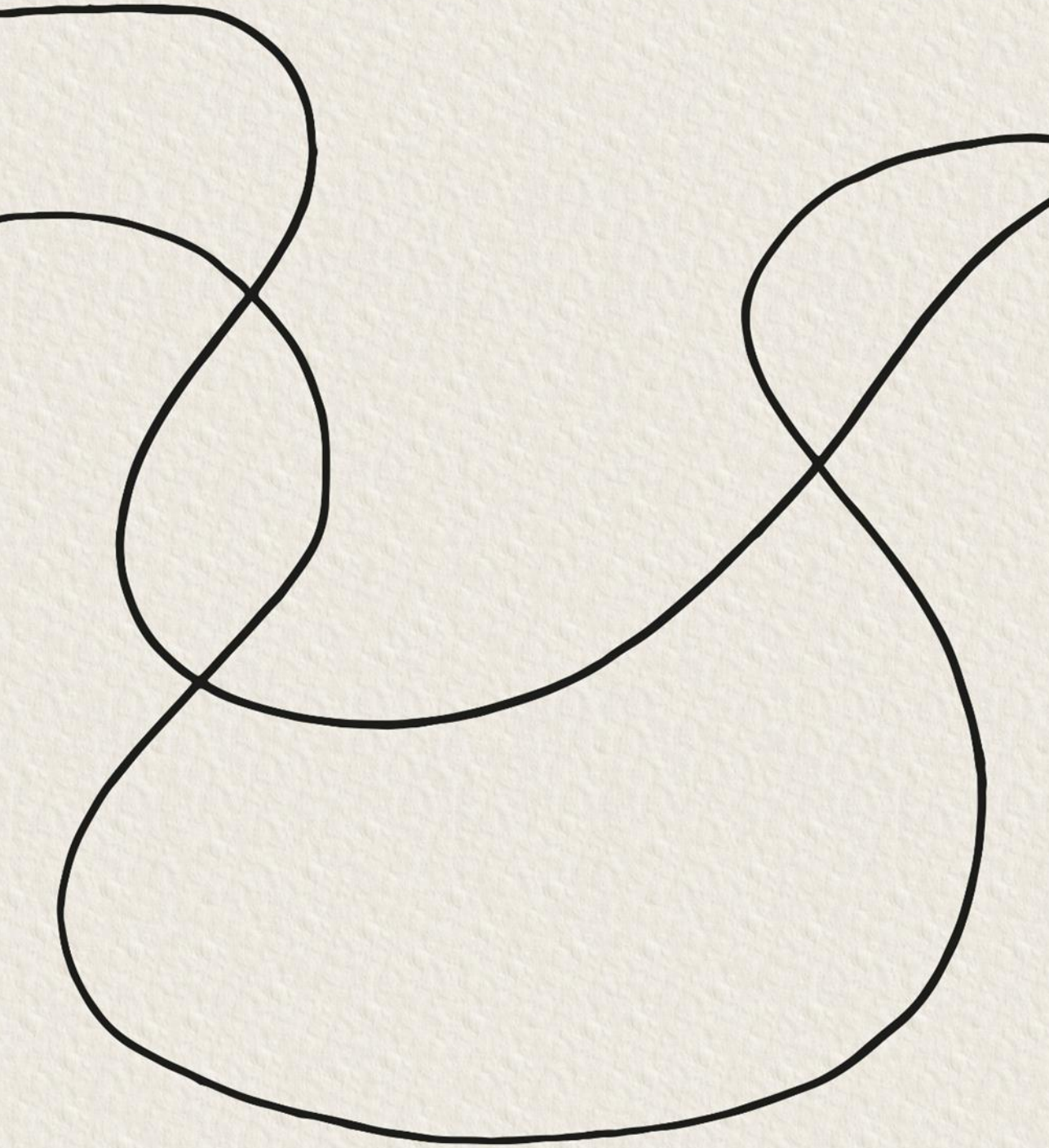

Chapter 5

Intradialytic protein ingestion and exercise do not compromise uremic toxin removal throughout hemodialysis

Floris K. Hendriks, Jeffrey H.W. Kuijpers, Janneau M.X. van Kranenburg, Joan M.G. Senden, Frank M. van der Sande, Jeroen P. Kooman, Steven J.R. Meex, and Luc J.C. van Loon

The Journal of Renal Nutrition. 2023; 33 (2): 376-385

Abstract

Background

Dietary protein and physical activity interventions are increasingly implemented during hemodialysis to support muscle maintenance in patients with end-stage renal disease. Though 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 end-stage renal disease (age: 65±16 y, BMI: 24.2±4.8 kg/m^2) on chronic hemodialysis treatment to participate in this randomized crossover trial. During hemodialysis, patients were assigned to ingest 40 g protein or a non-protein placebo both at rest (PRO and PLA, respectively) and following 30 min of exercise (PRO+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=0.001 and P=0.023, respectively; exercise effect: P=0.25 and P=0.52, respectively). Nonetheless, protein ingestion resulted in greater urea removal (P=0.046) 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>0.05; exercise effect: P>0.05). 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=0.034, P=0.039, and P=0.022, respectively).

Conclusion

The removal of uremic toxins is not compromised by protein feeding and/or exercise implementation during hemodialysis in patients with end-stage renal disease.

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 below 15 mL/min/1.73m^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, 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].

Though 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 [11,12]. This high prevalence can be attributed to sedentary behavior and uremic toxin accumulation between hemodialysis sessions as well as 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 four hemodialysis sessions during which these patients ingested a protein or a non-protein placebo beverage both at rest as well as 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 Centre+, Maastricht, The Netherlands (See **Supplemental Figure 1** for the Consolidated Standards of Reporting Trials (CONSORT) 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 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).

Pre-testing

At least one week before the first test day a pre-testing session was scheduled during routine hemodialysis to familiarize patients with intradialytic exercise. In addition, patient's medical history, physical examinations, lab 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 – 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 four hemodialysis sessions, separated by at least one week, all patients were assigned to ingest a placebo or protein beverage both in a rested state (PLA and PRO, respectively) as well as following 30 min intradialytic exercise (PLA+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 labelled according to 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 two sweeteners (Natrena, Douwe Egberts, Amsterdam, The Netherlands) dissolved in 300 mL water. The placebo beverage consisted of only the two sweeteners dissolved in 300 mL water. The independent researcher shared the order of exercise performance during test days with the investigators after pre-testing was completed. Though 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 – 12 and the last 2 min with a score below 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 pre-hemodialysis weight was recorded and a Body Composition Monitor® (Fresenius Medical Care, Bad Homburg, Germany) was used to assess their body

composition, as described before [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). Additional spent dialysate was collected throughout intradialytic exercise or the corresponding period (t=30 – 60 min) during non-exercise 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.

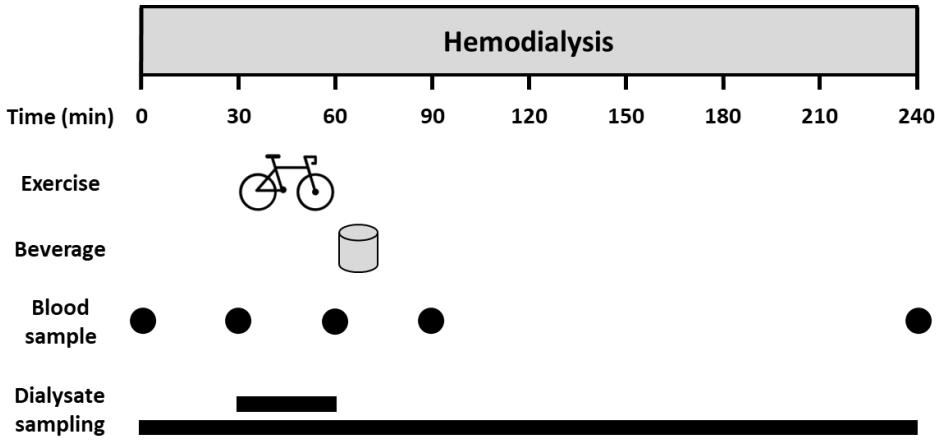

Figure 1. Schematic representation of study protocol. *t*=0 min represents the start of the hemodialysis session. During four hemodialysis sessions, patients ingested 40 g protein or a non-protein placebo both at rest and during recovery from intradialytic exercise in a randomized cross-over design.

Uremic toxins analysis

Blood samples were collected in serum (t=0 and 240 min) and EDTA-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, NADH, 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 ultra-performance 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 two time points (i.e., $RR_{0\text{-}240}$, $RR_{30\text{-}60}$, and $RR_{60\text{-}90}$) were calculated using the following equation:

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

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

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

Statistical analysis

All data are expressed as means±SDs 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 were verified by Shapiro-Wilk tests. No major violations for repeated-measures ANOVA assumptions were observed and in case of non-sphericity, the Greenhouse-Geisser correction was applied. Potential differences in removal and reduction ratios of uremic toxins, hemodialysis parameters, and pre-hemodialysis 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 $P<0.05$. All analyses were performed using SPSS statistics software (version 24.0; IBM Corp., Armonk, NY, USA).

Results

Patients' characteristics

Patients' baseline characteristics are presented in **Table 1.** All included patients with ESRD completed four test days. No differences were observed between the test days with PLA, PLA+EX, PRO, and PRO+EX interventions in pre-hemodialysis weight (71.9±14.3, 72.6±14.0, 72.2±13.9, and 71.9±14.1 kg, respectively; protein *P*=0.49; exercise *P*=0.51), urea distribution volume (34.7±4.6, 35.3±5.1, 35.7±4.8, and 35.2±5.0 L, respectively; protein *P*=0.16; exercise *P*=0.91), and ultrafiltration volume (1.24±1.01, 1.47±1.27, 1.23±1.08, and 1.41±1.24 L, respectively; protein *P*=0.78; exercise *P*=0.26). 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

	Patients
Age, y	65±16
Sex, 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 $P<0.001$ for all). Circulating uremic toxin concentrations declined between each timepoint ($P<0.05$ for all) except for circulating phosphate concentrations, which did not further decrease during the last 2.5 h of hemodialysis (t= 90 – 240 min; $P=0.70$). Protein ingestion resulted in higher circulating indoxyl sulfate concentrations throughout hemodialysis (protein effect $P=0.024$; exercise effect $P=0.35$). Circulating urea, phosphate, and cystatin C concentrations were not affected by protein ingestion or intradialytic exercise (protein effect $P=0.35$, $P=0.59$, and $P=0.67$, respectively; exercise effect $P=0.46$, $P=0.66$, and $P=0.20$, respectively). A significant time × exercise interaction ($P=0.007$) was observed for circulating creatinine concentrations throughout hemodialysis. Separate analyses showed that circulating creatinine concentrations decreased substantially during hemodialysis (time effect $P<0.001$) but were not influenced by intradialytic exercise (exercise effects $P>0.05$).

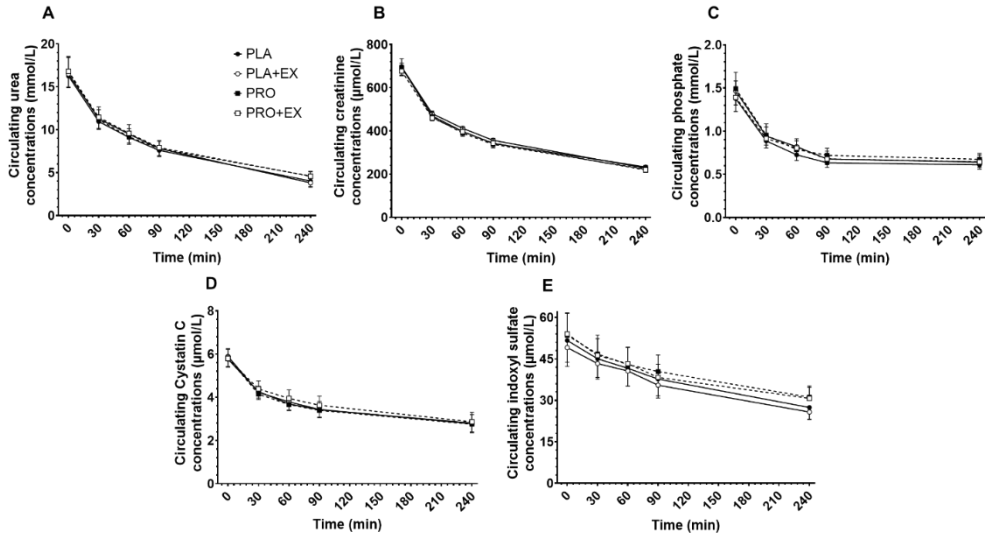

Figure 2. Circulating urea (A), creatinine (B), phosphate (C), Cystatin C (D), and indoxyl sulfate (E) concentrations throughout hemodialysis at rest and following exercise with and without protein ingestion. Values are expressed as means±SEMs, n=10 for all values. The dotted lines represent the interventions 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 ($P<0.05$) was observed for circulating creatinine concentrations. Circulating indoxyl sulfate concentrations throughout protein interventions were significantly different from placebo interventions (protein effect $P<0.05$). PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and exercise.

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_{30-60}), the 30-min period following ingestion of the test beverage (RR_{60-90}), and the 4-h hemodialysis session are presented in **Table 2**. No protein × exercise interaction was observed ($P>0.05$ for all). Protein ingestion reduced the reduction ratios of urea and indoxyl sulfate over the entire hemodialysis session (protein effect $P=0.001$ and $P=0.023$, 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 1.49±0.17, respectively; protein effect $P<0.001$; exercise effect $P=0.179$). Following protein ingestion only the RR_{60-90} of indoxyl sulfate was reduced (protein effect $P=0.029$). However, the RR_{60-90} of urea following protein ingestion did not differ from placebo ingestion (protein effect $P=0.14$). Intradialytic exercise resulted in lower RR_{30-60} of urea, creatinine, and phosphate when compared to the non-exercise interventions (exercise effect $P=0.046$, $P=0.033$, and $P=0.007$, respectively). In contrast, intradialytic exercise resulted in a higher RR_{60-90} of phosphate (exercise effect $P=0.010$).

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_{30-60} (%)	17±3	16±3	18±2	16±2	0.458	0.046	0.673
	RR_{60-90} (%)	17±3	17±4	15±2	17±3	0.127	0.136	0.178
	RR_{0-240} (%)	76±6	77±5	72±4	73±4	0.001	0.254	0.226
Creatinine	RR_{30-60} (%)	16±3	15±3	16±2	14±2	0.914	0.033	0.185
	RR_{60-90} (%)	14±2	14±3	13±2	14±3	0.546	0.892	0.658
	RR_{0-240} (%)	67±6	68±4	68±4	68±4	0.270	0.671	0.348
Phosphate	RR_{30-60} (%)	18±5	12±6	17±5	10±8	0.203	0.007	0.483
	RR_{60-90} (%)	12±5	17±7	7±8	16±6	0.070	0.010	0.096
	RR_{0-240} (%)	53±11	54±10	53±11	52±12	0.535	1.000	0.300
Cystatin C	RR_{30-60} (%)	14±5	11±7	12±7	11±7	0.254	0.053	0.713
	RR_{60-90} (%)	8±7	10±5	8±5	9±5	0.754	0.392	0.587
	RR_{0-240} (%)	51±20	53±19	53±20	52±18	0.808	0.809	0.308
Indoxyl sulfate	RR_{30-60} (%)	8±8	6±9	8±4	7±6	0.796	0.485	0.846
	RR_{60-90} (%)	10±4	13±5	6±5	10±7	0.029	0.103	0.750
	RR_{0-240} (%)	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_{30-60}, reduction ratio between 30 and 60 min after hemodialysis initiation (intradialytic exercise or non-exercise period); RR_{60-90}, reduction ratio between 60 and 90 min after hemodialysis initiation (directly after test beverage ingestion); RR_{0-240}, reduction ratio over the 4-h hemodialysis session.

Uremic toxin reduction ratios

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*=0.046; exercise effect *P*=0.337). 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*=0.62, *P*=1.00, *P*=0.36, and *P*=0.69, respectively; exercise effect *P*=0.25, *P*=0.22, *P*=0.16, and *P*=0.21, 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*=0.034), creatinine (0.29±0.04 and 0.28±0.04 vs 0.28±0.03 and 0.28±0.04 g; exercise effect *P*=0.039), and phosphate (0.40±0.16 and 0.39±0.14 vs 0.33±0.10 and 0.37±14 g; exercise effect *P*=0.022) were removed during intradialytic exercise.

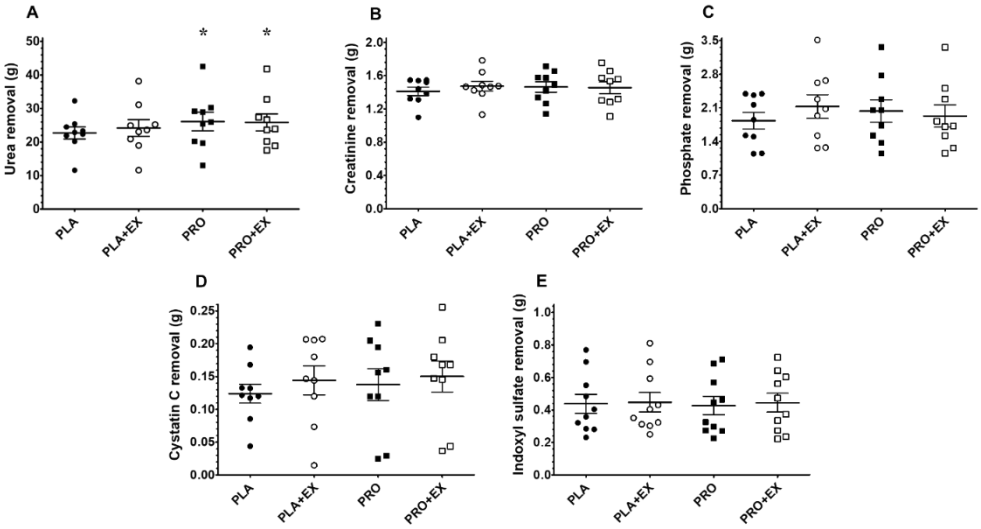

Figure 3. Total urea (A), creatinine (B), phosphate (C), Cystatin C (D), and indoxyl sulfate (E) removal throughout hemodialysis at rest and following exercise with and without protein ingestion. Squares and circles represent individual data points and bars represent group means±SEMs, *n*=10 for indoxyl 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*<0.05). PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and 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 according to KDOQI 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 as well as 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 current 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 suggests 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]. Though 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 non-exercise 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. Though 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 current study include the combination of both the placebo and protein beverage with as well as 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.

Acknowledgments

We greatly appreciate the assistance of Wesley Hermans, Joan Senden, Therese van der Vlies, Nikki Adriaens, and the whole staff of the MUMC+ dialysis department, especially Anouk Geurts and Jaap Krohof, in the execution of the experiments.

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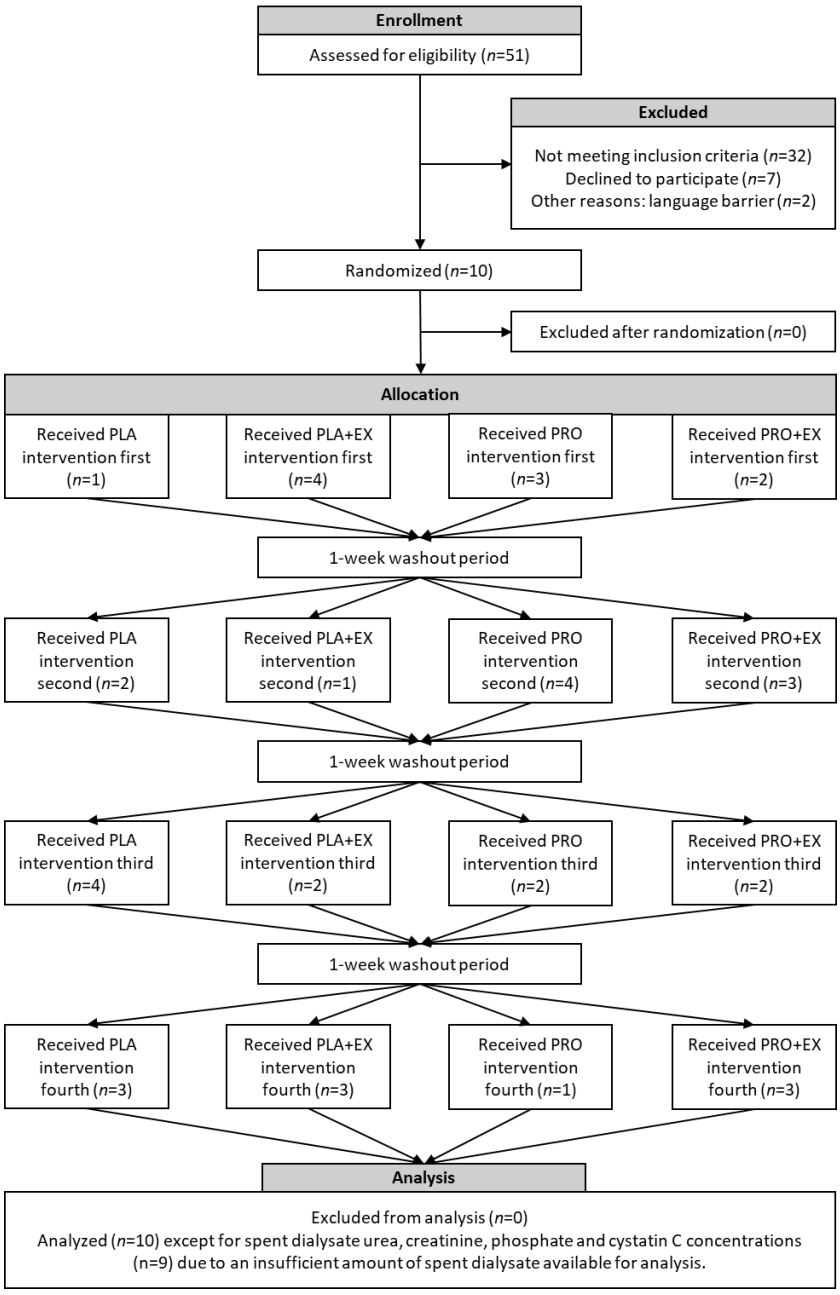
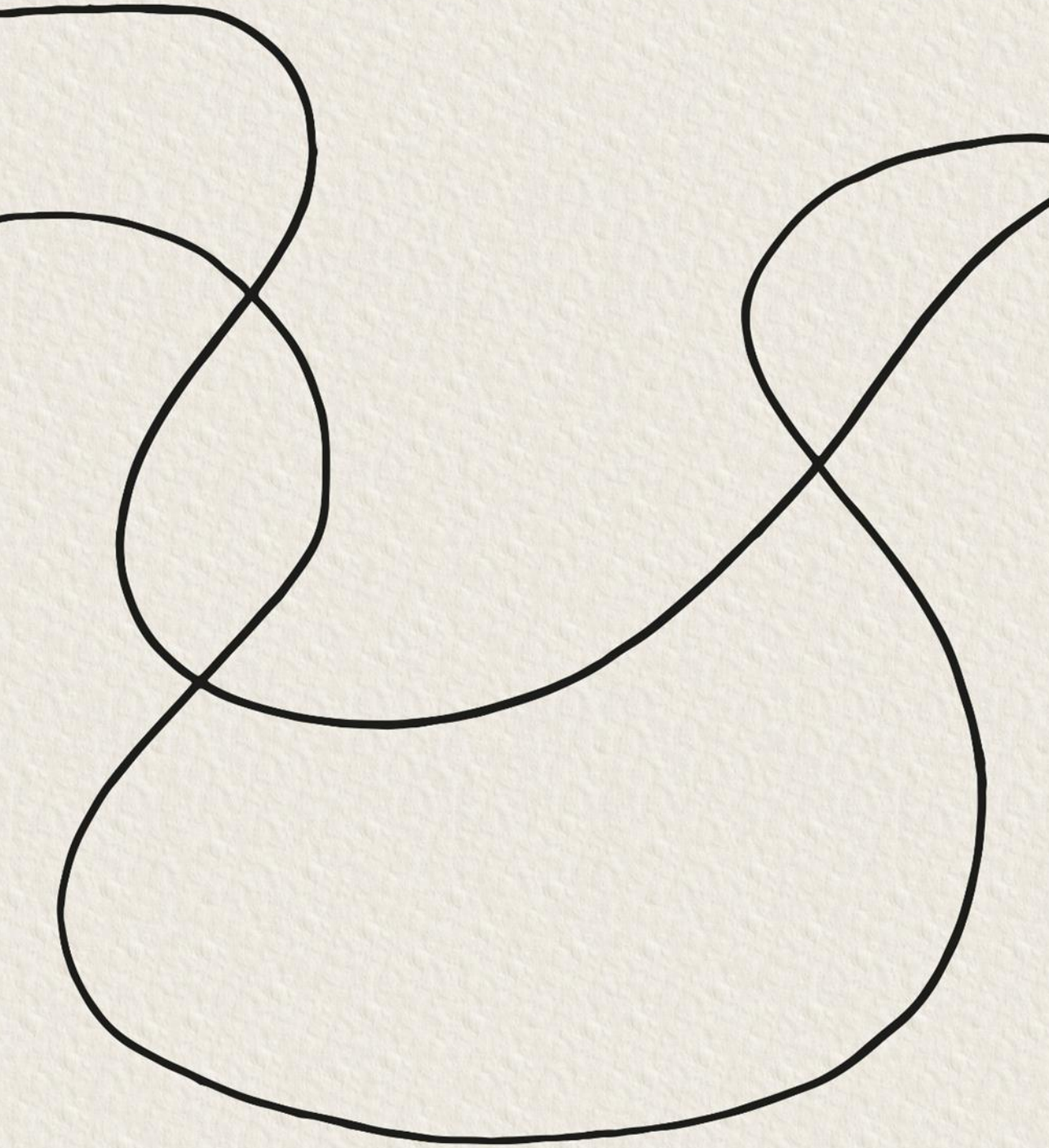
Supplementary material



Supplementary figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow chart. PLA, placebo; PLA+EX, placebo and exercise; PRO, protein; PRO+EX, protein and exercise.



Chapter 6

Branched-chain ketoacid co-ingestion with protein lowers amino acid oxidation during hemodialysis

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Clinical Nutrition. 2023; 42 (8): 1436-1444

Abstract

Background

Hemodialysis removes amino acids from the circulation, thereby stimulating muscle proteolysis. Protein ingestion during hemodialysis can compensate for amino acid removal but may also increase uremic toxin production. Branched-chain ketoacid (BCKA) co-ingestion may provide an additional anabolic stimulus without adding to uremic toxin accumulation. In the present study we assessed the impact of BCKA co-ingestion with protein on forearm amino acid balance and amino acid oxidation during hemodialysis.

Methods

Nine patients (age: 73±10 y) on chronic hemodialysis participated in this crossover trial. During two 4-h hemodialysis sessions, patients ingested 18 g protein with (PRO+BCKA) or without (PRO) 9 g BCKAs in a randomized order. Test beverages were labeled with L-[ring-$^{13}C_6$]-phenylalanine and provided throughout the last 3 h of hemodialysis as 18 equal sips consumed with 10-min intervals. Arterial and venous plasma as well as breath samples were collected frequently throughout hemodialysis.

Results

Arterial plasma total amino acid (TAA) concentrations during PRO and PRO+BCKA treatments were significantly lower after 1 h of hemodialysis (2.6±0.3 and 2.6±0.3 mmol/L, respectively) when compared to pre-hemodialysis concentrations (4.2±1.0 and 4.0±0.5 mmol/L, respectively; time effect: $P<0.001$). Arterial plasma TAA concentrations increased throughout test beverage ingestion (time effect: $P=0.027$) without differences between treatments (time × treatment: $P=0.62$). Forearm arteriovenous TAA balance during test beverage ingestion did not differ between timepoints (time effect: $P=0.31$) or treatments (time × treatment: $P=0.34$). Whole-body phenylalanine oxidation was 33±16% lower during PRO+BCKA when compared to PRO treatments ($P<0.001$).

Conclusion

BCKA co-ingestion with protein during hemodialysis does not improve forearm net protein balance but lowers amino acid oxidation.

Introduction

Hemodialysis is a lifesaving treatment for patients with end-stage renal disease, as it removes uremic toxins and excess fluid from the body [1]. During hemodialysis, uremic toxins diffuse from the blood through a semipermeable membrane into dialysate fluid and, as such, are removed from the body. Patients on chronic hemodialysis therapy typically undergo two or three hemodialysis sessions per week to allow sufficient removal of uremic toxins and excess fluid [2].

Poor nutritional status is prevalent among patients undergoing chronic hemodialysis treatment [3, 4]. This is further aggravated by the observation that hemodialysis does not only removes uremic toxins, but also circulating amino acids (AAs) [5, 6]. We have recently shown that approximately 12 g AAs are removed from the circulation during a single hemodialysis treatment [7]. The subsequent decline in circulating AA concentrations stimulates muscle proteolysis during hemodialysis and likely for some period thereafter [7-12]. The intermittent exposure to the catabolic properties of hemodialysis contributes to the etiology of low muscle mass and strength observed in patients undergoing chronic hemodialysis [13].

Recently, we have shown that ingestion of 40 g protein during hemodialysis can compensate for AA removal and, as such, may improve net muscle protein balance during hemodialysis [14]. However, we also observed that intradialytic protein ingestion attenuated uremic toxin reductions during hemodialysis, likely because dietary protein contains nitrogen, which is known to stimulate production of several uremic toxins [15, 16]. Higher uremic toxin concentrations between hemodialysis sessions may further stimulate muscle proteolysis and, as such, offset the potential anabolic properties of protein ingestion during hemodialysis [17].

Co-ingestion of branched-chain amino acids (BCAAs), and leucine in particular, has been suggested as a strategy to further enhance the anabolic properties of dietary protein ingestion [18, 19]. The ketoanalogues of these BCAAs, the branched-chain ketoacids (BCKAs), do not contain nitrogen and are transaminated to their corresponding AA using an amino group (from urea) [20]. BCKA infusion has been reported to attenuate urea generation, which indicates an improved nitrogen balance due to lower AA oxidation [21]. In addition, we have shown that ingestion of BCKAs increases muscle protein synthesis rates in healthy volunteers [22]. We hypothesize that BCKA co-ingestion with dietary protein may represent an effective strategy to augment the anabolic potential of protein ingestion during hemodialysis without increasing nitrogen load.

In the present study, we assessed the impact of supplementing protein with and without BCKAs during hemodialysis. Test beverages were enriched with L-[ring-$^{13}C_6$]-phenylalanine and provided as frequent sips throughout hemodialysis to achieve a constant rate of tracer appearance in the circulation, which allowed us to compare AA oxidation rates between treatments by measuring $^{13}CO_2$ appearance in the expired breath.

Methods

Participants

A total of 10 patients with end-stage renal disease undergoing hemodialysis through a well-functioning arteriovenous shunt for at least 3 months were recruited between June 2021 and December 2021 at the dialysis department of Maastricht University Medical Centre+, Maastricht, The Netherlands (See **Supplementary Figure 1** for the Consolidated Standards of Reporting Trials (CONSORT) flow diagram). Patients with an active inflammatory disease, malignancy, cognitive disorder, intolerance to food ingestion during hemodialysis, uncontrolled hypertension, or a hospitalization/missed hemodialysis session in the last month prior to the study period were excluded. Patients were informed about the purpose of the study, experimental procedures, and possible risks prior to signing written informed consent. The study was approved by the Medical Research Ethics Committee Academic Hospital Maastricht/Maastricht University (NL76362.068.20), conformed to standards for the use of human subjects in research as outlined in the latest version of the Helsinki Declaration, and was registered prospectively at the Netherlands Trial Register (NTR9296). The study was independently monitored by the Clinical Trial Center Maastricht.

Dietary intake and physical activity

All patients refrained from any sort of strenuous physical activity 48 h prior to each test day. Patients who underwent hemodialysis in the morning arrived in an overnight fasted state. Those who underwent hemodialysis in the afternoon or evening consumed a standardized meal 3 h before initiation of their hemodialysis session (providing ~500 kcal, with carbohydrate, fat and protein providing ∼60, 30, and 10 En%, respectively) and were instructed to remain fasted thereafter until the start of the experimental protocol. During test days, patients were allowed to ingest water *ad libitum*.

Study design

During two hemodialysis sessions, separated by a wash-out period of at least one week, all patients were assigned in a randomized cross-over design to ingest a protein beverage with (PRO+BCKA) or without (PRO) 9 g BCKAs. The cross-over design was chosen to minimize variability of outcome parameters in this heterogeneous population. An overview of test days, which were scheduled during patients' second or third weekly hemodialysis session, is provided in **Figure 1**. Patients were randomly assigned to an order of interventions by an independent researcher using an online randomizer (http://www.randomizer.org) and the randomization order of test beverages was not shared with investigators, study staff, or participants until all procedures and statistical analyses of the primary and secondary outcomes were complete.

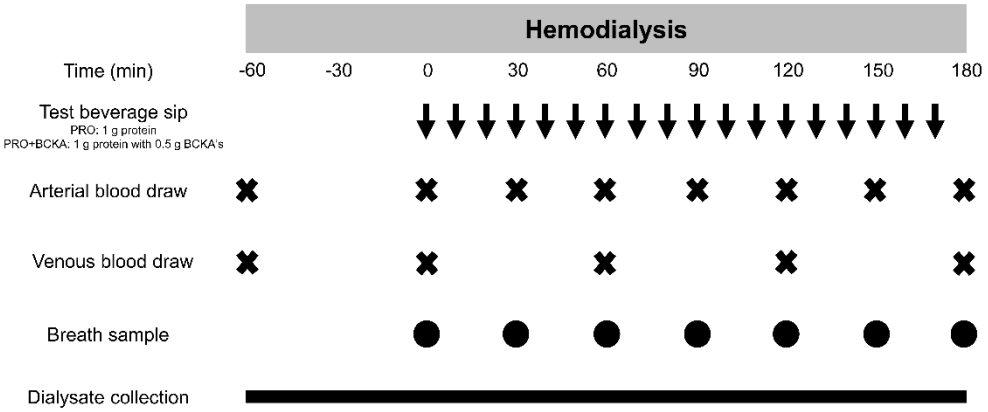

Figure 1. *t*= -60 min represents the start and *t*= 180 min the end of the hemodialysis session. In a randomized cross-over manner, patients were provided with 18 test beverage sips containing either 1 g protein (PRO) or 1 g protein with 0.5 branched-chain ketoacids (PRO+BCKA) per sip.

Hemodialysis treatment

Patients' prescribed blood (350 – 400 mL/min) and dialysate flow rates (500 – 700 mL/min), dialysate composition, and dialysis membranes were not altered between test days. Desired ultrafiltration volume was determined by the treating nephrologist for each hemodialysis session. Patients were dialyzed through a well-functioning arteriovenous shunt in the arm using polysulfone (*n*=4; FX-800, Fresenius Medical Care, Bad Homburg, Germany), polynephron (*n*=2; Elisio 17H, Nipro Medical corporation, Osaka, Japan), and triacetate (*n*=3; SUREFLUX 19L, Nipro Medical Corporation, Osaka, Japan) membranes.

Experimental protocol

After patients arrived at the dialysis department, their weight was recorded and a Body Composition Monitor (BCM®, Fresenius Medical Care, Bad Homburg, Germany) was used to assess their body composition, as described previously [23]. A catheter was inserted into an antecubital vein of the non-shunted arm and the arteriovenous shunt was checked for recirculation. Prior to the start of hemodialysis (*t*= -60 min), a venous and arterial plasma sample were collected from the catheter and the shunt, respectively. Just before the first sip of the test beverage (*t*= 0 min), a venous plasma sample was collected using the catheter, an arterial plasma sample was collected from the arterial line, and a breath sample was collected in a 10 mL Exetainer tube (Labco Limited, Lampeter, UK) using an EasySampler® system (Quintron, Milwaukee, USA). Thereafter, sips of the test beverage were consumed with 10-min intervals (at *t*= 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, and 170 min) to achieve a constant rate of tracer appearance in the circulation, which

allowed us to compare AA oxidation rates between treatments by measuring $^{13}CO_2$ appearance in the expired breath. In addition, arterial plasma and breath samples were collected with 30-min intervals (at *t*= 30, 60, 90, 120, 150, and 180 min), and venous plasma was sampled with 60-min intervals (at *t*= 60, 120, and 180 min). Spent dialysate was collected continuously throughout hemodialysis in a container at a rate of 1.0 L/h using a reversed injection pump (Alaris GW, Rolle, Switzerland). Every 2 h these containers were replaced (at *t*= 60 and 180 min) and a homogenized sample of the spent dialysate collected over each 2-h period was obtained. Following the experimental procedures, patients were offered a meal before leaving the dialysis department.

Test beverages

The independent researcher who randomized the order of test beverages was responsible for their preparation. The protein beverage contained 18.0 g milk protein concentrate (Refit TMP 90, Friesland Campina, Amersfoort, The Netherlands; containing 1.7 g leucine, 0.9 g isoleucine, and 1.2 g valine), 0.36 g L-[ring-$^{13}C_6$]-phenylalanine, and a non-aspartame containing sweetener (Natrena, Douwe Egberts, Amsterdam, The Netherlands) dissolved in 270 mL water. During PRO+BCKA treatments, 9.0 g BCKAs (i.e. 4.5 g keto-leucine, 2.3 g keto-isoleucine, and 2.3 g keto-valine) were added to the protein beverage. Subsequently, the test beverages were homogenized, weighted, and divided in 18 equal servings (with each serving containing 1/18 of total beverage weight), which were numbered according to participant and test day number before handing them to an investigator.

Plasma and spent dialysate analysis

Plasma samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes and centrifuged at 1000*g* at 4°C for 10 min to obtain plasma. Homogenized spent dialysate samples were collected in sterile tubes, immediately frozen in liquid nitrogen, and stored in a freezer at -80°C until further analysis. These samples were concentrated through freeze-drying 25.0 mL of the sample and dissolving the dried product in 5.0 mL 0.1 M hydrogen chloride. AA and BCKA concentrations and enrichments in plasma and spent dialysate were determined by ultra-performance liquid chromatography mass spectrometry (ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France) as described previously [14, 24].

Breath analysis

Breath CO_2 samples were analyzed for $^{13}C/^{12}C$ ratio by continuous flow isotope ratio mass spectrometry (CF-IRMS; Finnigan, Bremen, Germany) using a GasBench II (Thermo Scientific, Waltham, USA). Standard regression curves were applied from a series of known standard enrichment values to assess the linearity of the mass spectrometer and to account for any isotope fraction that may have occurred during the analysis.

Calculations

The net forearm protein balance was calculated by subtracting venous plasma AA concentrations from arterial plasma AA concentrations. AA removal during hemodialysis and reduction ratios of urea were calculated described before [16, 25]. The percentage of protein-derived/exogenous AAs within the total amount of removed AAs during the second half of hemodialysis (i.e. *t*= 60 – 180 min) was calculated using the following formula:

$$AA_{ex}(\%) = \left(\frac{E_{dia}}{E_{drink}}\right) \cdot 100$$

In which AA_{ex} is the percentage of exogenous AAs, E_{dia} is the L-[ring-$^{13}C_6$]-phenylalanine enrichment of the spent dialysate, and E_{drink} is the L-[ring-$^{13}C_6$]-phenylalanine enrichment of the test beverage, with the phenylalanine content of the milk protein concentrate corrected for its digestibility (95%) [26]. Breath isotopic enrichment was expressed as δ per mil difference between the $^{13}C/^{12}C$ ratio of the sample and an international standard (Vienna PDB) according to the following formula [27]:

$$\delta^{13}\mathrm{C} = \left(\left(\frac{^{13}\mathrm{C} \,/\, ^{12}\mathrm{C}\ \text{sample}}{^{13}\mathrm{C} \,/\, ^{12}\mathrm{C}\ \text{standard}}\right) - 1\right) \cdot 10^3$$

Phenylalanine oxidation (Phe_{ox}) throughout the tracer steady state period in µmol/h was estimated using a modification of the formula described by Bandyopadhyay *et al*. [28]:

$$Phe_{ox} = \frac{E_{breath} \cdot VCO_2 \cdot 44.6 \cdot 60}{E_{plasma} \cdot 0.82 \cdot {}^{13}C_n}$$

where E_{breath} is the ^{13}C enrichment in expired breath, VCO_2 was the amount of carbon dioxide exhaled from the body in mL/min, which was assumed to be similar to the previously reported value during high-efficiency hemodialysis of 214 mL/min [29], E_{plasma} is plasma L-[ring-$^{13}C_6$]-phenylalanine enrichment in MPE expressed as fraction and $^{13}C_n$ is the number of labelled carbon atoms per phenylalanine molecule (i.e. 6).

Statistical analysis

All data are expressed as means±SDs unless indicated otherwise. A power calculation was performed with forearm arteriovenous AA balance as the primary outcome measure. A required sample size of 10 participants including a 10% dropout rate was calculated using a power of 80%, a significance level of 0.05, and the difference and standard deviation in forearm AA balance following ingestion of one and two protein supplements during hemodialysis as reported by Sundell *et al.* [8]. After the randomization order of test beverages was shared with investigators, hemodialysis parameters and pre-hemodialysis weight and body composition were compared between treatments to identify possible confounders. Normal distribution of all parameters was verified by Shapiro-Wilk tests (P>0.05). No major violations for specific 2-factor repeated-measures ANOVA assumptions were observed and in case of non-sphericity, the Greenhouse-Geisser correction was used. Potential differences in forearm arteriovenous AA balance, plasma AA and BCKA concentrations, arterial plasma L-[ring-$^{13}C_6$]-phenylalanine enrichment, breath $^{13}CO_2$, and plasma urea concentrations were assessed using 2-factor repeated-measures ANOVAs with time and BCKA ingestion (yes/no) as within-subject factors. If a statistically significant interaction was found, subsequent paired-samples *t* tests were performed. In case of significant time effects, Bonferroni post-hoc analyses were performed to locate the effects. Other outcome parameters were compared between treatments by paired-samples *t* tests. All analyses were performed using SPSS statistics software (version 27.0; IBM Corp., Armonk, NY, USA).

Results

Patients' characteristics

Nine patients completed both test days (**Table 1**). One patient got nauseous during the second test day and was unable to complete the test. The period for which patients were undergoing chronic hemodialysis treatment prior to the first test day ranged from 3 – 89 months. No differences were observed in pre-hemodialysis weight (80.5±10.9 and 80.9±11.4 kg, *P*=0.09), lean tissue index (14.5±2.7 and 14.4±3.5 kg/m^2, *P*=0.84), ultrafiltration volume (1.52±0.81 and 1.67±0.86 L, *P*=0.27), and hemodialysis adequacy (equilibrated Kt/V: 1.46±0.12 and 1.45±0.15, *P*=0.80) between the PRO and PRO+BCKA treatments, respectively.

Table 1. Patients' characteristics

	Patients
Age, y	73±10
Sex, male/female	7/2
Cause of end-stage renal disease	4 Glomerular
	3 Malignancy
	2 Vascular
Dialysis vintage, months	27±26
Dialysis timing, morning/afternoon/evening	4/3/2
Height, m	1.73±0.06
Weight, kg	79.4±11.3
Body mass index, kg/m^2	26.5±4.1
Lean tissue index, kg/m^2	14.0±2.9
Fat tissue index, kg/m^2	11.9±5.3
Mean systolic blood pressure, mm Hg	136±27
Mean diastolic blood pressure, mm Hg	61±17
Serum albumin, g/dL	3.3±0.4
C-reactive protein, mg/L	6±4

Continuous and categorical values are expressed as mean±SD and counts, respectively, *n*=9.

Arterial plasma amino acid concentrations

Arterial plasma total amino acid (TAA) concentrations during PRO and PRO+BCKA treatments were significantly lower at *t*= 0 min (**Figure 2A**; 2.6±0.3 and 2.6±0.3 mmol/L, respectively) when compared to pre-hemodialysis concentrations (*t*= -60 min; 4.2±1.0 and 4.0±0.5 mmol/L, respectively; time effect: *P*<0.001) with a similar decline in both sessions (time × treatment: *P*=0.37). Similarly, arterial plasma BCAA, essential amino acid (EAA) and non-essential amino acid (NEAA) concentrations declined during the first 60 min of hemodialysis (**Figure 2**; time effect: *P*<0.001 for all) with no differences between treatments (time × treatment: *P*=0.35, 0.21, and 0.37, respectively).

Throughout test beverage ingestion, arterial plasma TAA, BCAA, and EAA concentrations increased over time (Figure 2A; time effect: *P*=0.027, <0.001, and <0.001, respectively) without differences between treatments (time × treatment: *P*=0.62, 0.53, and 0.71 respectively). Arterial plasma NEAA concentrations during test beverage ingestion did not differ over time (time effect: *P*=0.10) or between treatments (time × treatment: *P*=0.52). Plasma concentration fold changes of individual AAs throughout hemodialysis per treatment are displayed as a heat map in **Supplementary Figure 2**.

The iAUCs of arterial plasma TAA and NEAA concentrations were greater during PRO (2.06±1.02 and 1.38±0.84 mmol/3 h, respectively) when compared to PRO+BCKA treatments (1.23±0.71 and 0.51±0.56 mmol/3 h, respectively; *P*=0.041 and 0.020, respectively). The iAUCs of arterial plasma BCAA and EAA concentrations did not differ between PRO and PRO+BCKA treatments (*P*=0.25 and *P*=0.32, respectively).

Plasma BCKA concentrations

Pre-hemodialysis arterial plasma BCKA concentrations averaged 0.02±0.01 mmol/L, with no differences between treatments (*P*=0.14). Arterial plasma BCKA concentrations were higher during the PRO+BCKA when compared to the PRO treatment from *t*= 30 min until the end of the hemodialysis session (**Figure 2B**; time × treatment *P*< 0.001). In accordance, the iAUC of arterial plasma BCKA concentrations was significantly greater during PRO+BCKA (0.26±0.08 mmol/3 h) when compared to the PRO treatment (0.04±0.02 mmol/3 h; *P*<0.001).

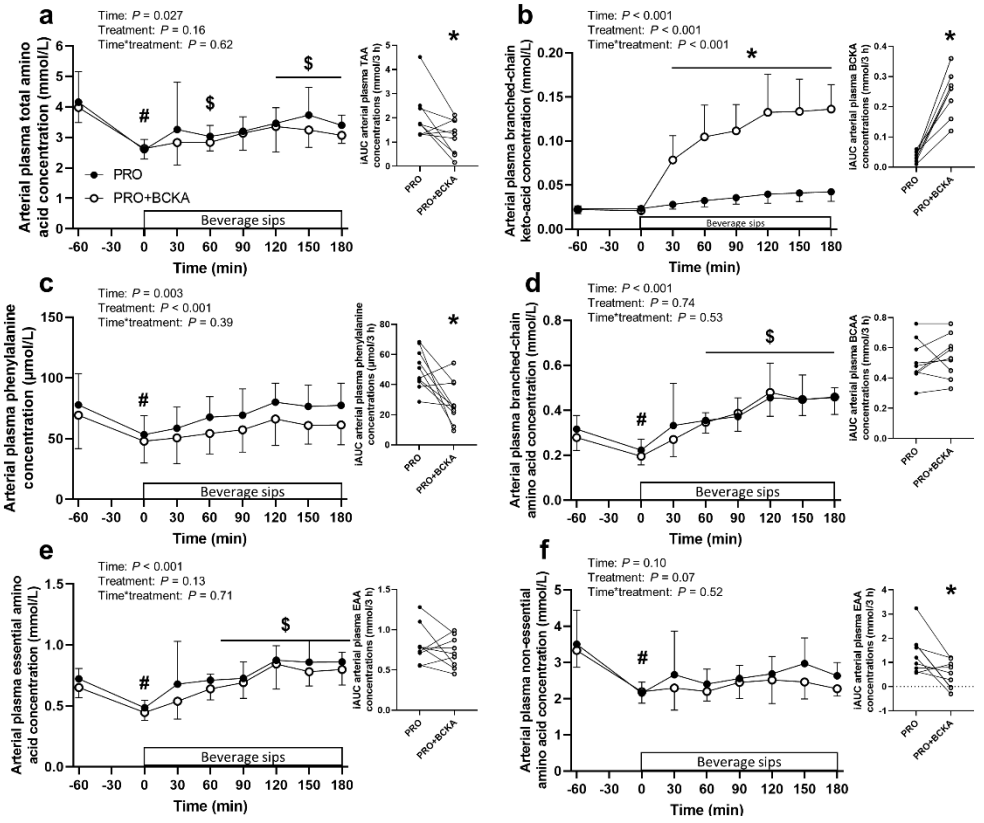

Figure 2. Arterial plasma total amino acid (A), branched-chain ketoacid (B), phenylalanine (C), branched-chain (D), essential (E), and non-essential (F) amino acid concentrations throughout hemodialysis. Test beverages were ingested as sips consumed with 10-min intervals between *t*= 0 and 180 min. Values are expressed as means±SDs, *n*=9 for all values. Data were analyzed with a two-way repeated-measures ANOVA with time and treatment as within subject variables. *, PRO significantly different from PRO+BCKA treatment (*P*<0.05); #, *t*= 0 min concentrations significantly lower when compared to *t*= -60 min concentrations (*P*<0.001); $, concentrations significantly higher when compared to *t*= 0 min concentrations (*P*<0.05). PRO, protein; PRO+BCKA protein with branched-chain ketoacids.

Forearm arteriovenous amino acid BCKA balance

During PRO and PRO+BCKA treatments, forearm arteriovenous TAA, BCAA, EAA, and NEAA balances were significantly lower at t= 0 min when compared to pre-hemodialysis (time effect: *P*=0.021, 0.032, 0.014, and 0.020, respectively), with no differences between treatments (time × treatment: *P*=0.97, 0.66, 0.37, and 0.95, respectively). Throughout test beverage ingestion, forearm arteriovenous TAA and NEAA balance did not differ between timepoints (**Figure 3**; time effect: *P*=0.31 and 0.59, respectively) or between treatments (time × treatment: *P*=0.34 and 0.28, respectively). Forearm arteriovenous BCAA and EAA balance improved significantly during test beverage ingestion (time effect: *P*=0.002 and <0.001, respectively) with no differences between treatments (time × treatment: *P*=0.62 and 0.84, respectively). Forearm arteriovenous BCKA balance throughout test beverage ingestion was significantly higher during the PRO+BCKA when compared to the PRO treatment from *t*= 60 min until the end of the hemodialysis session (time × treatment: *P*<0.001). The plasma arterial/venous ratios of individual AAs throughout hemodialysis per treatment are displayed as a heat map in **Supplementary Figure 3**.

No differences were observed in iAUCs of the forearm arteriovenous TAA, BCAA, EAA, and NEAA balance throughout the test beverage ingestion (*P*=0.92, 0.18, 0.77, and 0.75, respectively), while the iAUC of the forearm arteriovenous BCKA balance during this period was significantly greater during the PRO+BCKA (0.09±0.38 mmol/3 h) when compared to the PRO treatment (0.00±0.12 mmol/3 h; *P*<0.001).

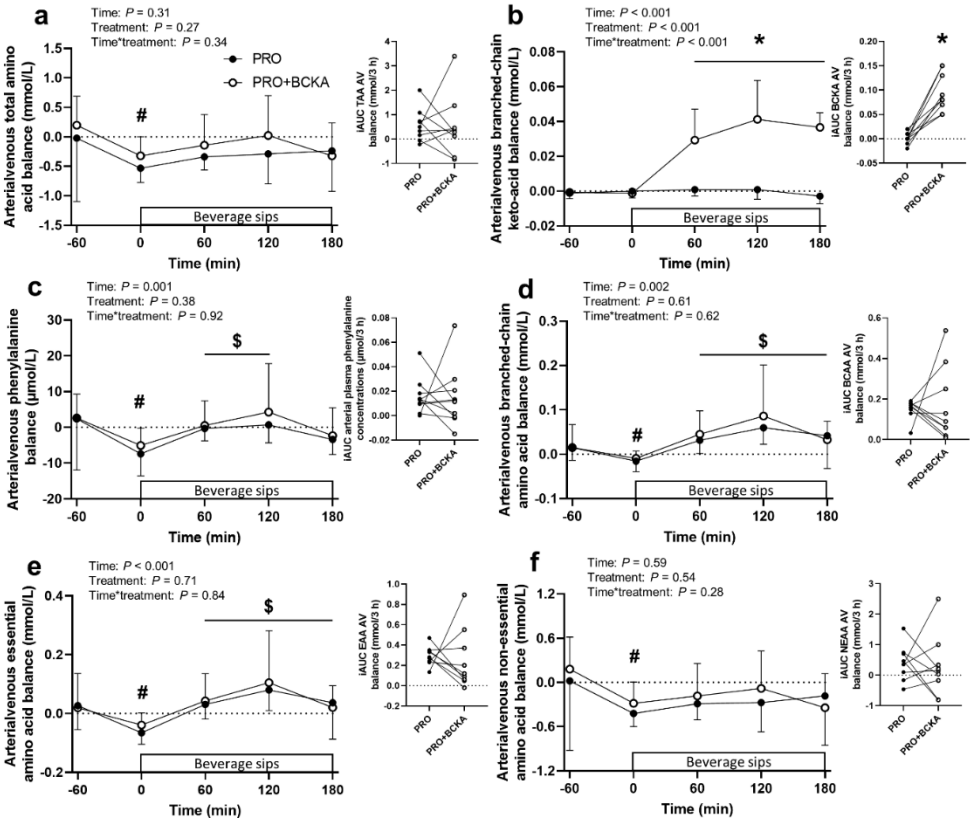

Figure 3. Forearm arteriovenous total amino acid (A), branched-chain ketoacid (B), phenylalanine (C), branched-chain (D), essential (E), and non-essential amino acid (F) balance throughout hemodialysis. Test beverages were ingested as sips consumed with10-min intervals between *t*= 0 and 180 min. Values are expressed as means±SDs, *n*=9 for all values. Data were analyzed with a two-way repeated-measures ANOVA with time and treatment as within subject variables. *, PRO significantly different from PRO+BCKA treatment (*P*<0.05); #, *t*= 0 min concentrations significantly lower when compared to *t*= -60 min concentrations (*P*<0.05); $, concentrations significantly higher when compared to *t*= 0 min concentrations (*P*<0.05). PRO, protein; PRO+BCKA protein with branched-chain ketoacids.

Stable isotope enrichments

Arterial plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments increased throughout the first 60 min of test beverage ingestion ($P \leq 0.001$) after which they remained in a steady state between $t = 60 – 180$ min period with no differences between treatments (time × treatment: $P = 0.07$; **Figure 4**). During the steady state period, arterial plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments averaged 11.2±1.9 and 11.1±2.2 MPE during PRO and PRO+BCKA treatments, respectively. In accordance, average spent dialysate L-[ring-$^{13}C_6$]-phenylalanine enrichments throughout the $t = 60 – 180$ min period were not different during the PRO (8.1±1.5 MPE) when compared to the PRO+BCKA treatment (7.8±2.3 MPE; $P = 0.78$).

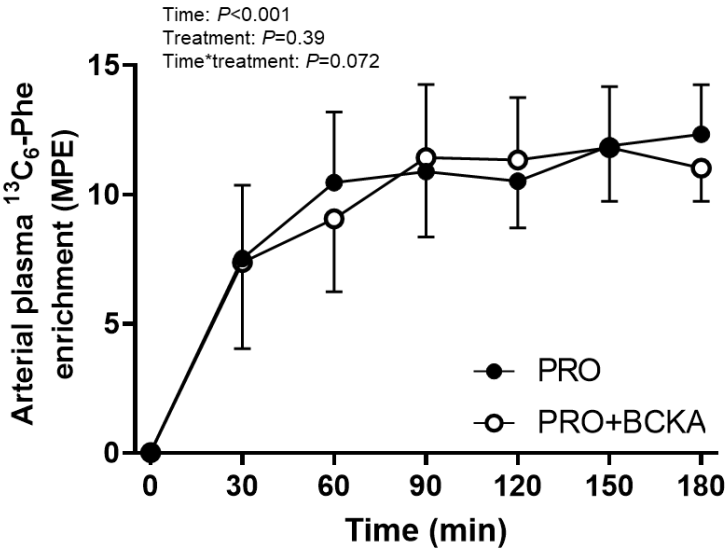

Figure 4. Arterial plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments throughout test beverage ingestion protocol during hemodialysis. Values are expressed as means±SDs, $n = 9$ for all values. Data were analyzed with a two-way repeated-measures ANOVA with time and treatment as within subject variables. MPE, mole % excess; PRO, protein; PRO+BCKA protein with branched-chain ketoacids.

Amino acid oxidation

Breath $\delta^{13}C$ enrichments following the start of test beverage ingestion are depicted in **Figure 5** and showed significant time × treatment interaction (P<0.001). Breath $\delta^{13}C$ increased between every timepoint (P<0.01 for all) except for the last 30-min interval (P=0.15). During PRO+BCKA treatments, breath $\delta^{13}C$ enrichments were lower when compared to the PRO treatment from t= 60 min until the end of the hemodialysis session (P<0.01 for all). Furthermore, peak AA oxidation was significantly lower during PRO+BCKA (breath $^{13}CO_2$ enrichment: 7.1±8.3 δ per mil vs VPBD) when compared to PRO treatments (breath $^{13}CO_2$ enrichment: -1.4±6.5 δ per mil vs VPBD; P<0.001). Consequently, estimated phenylalanine oxidation rates were lower during PRO+BCKA (0.029±0.008 g/h) when compared to PRO treatments (0.041±0.007 g/h; P=0.001).

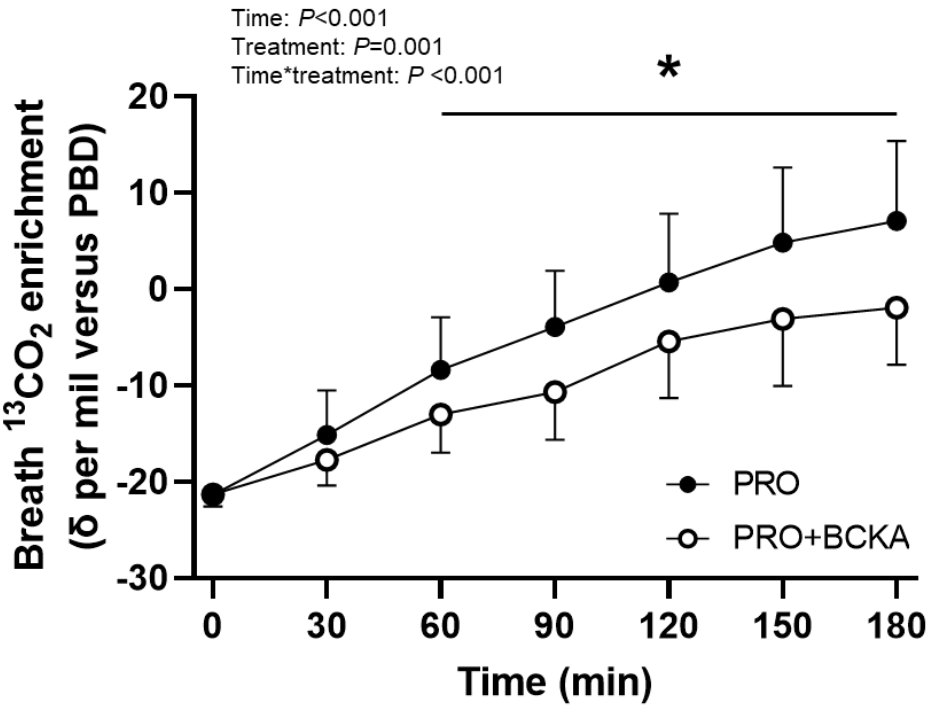

Figure 5. Breath $^{13}CO_2$ enrichments throughout test beverage ingestion during hemodialysis. Values are expressed as means±SDs, n=9 for all values. Data were analyzed with a two-way repeated-measures ANOVA with time and treatment as within subject variables. *, PRO significantly different from PRO+BCKA treatments (P<0.01). PRO, protein; PRO+BCKA protein with branched-chain ketoacids.

Amino acid and BCKA removal

AA removal throughout the whole hemodialysis session (24.0±7.6 vs 22.8±9.3 g, respectively; *P*=0.62) and the second half of the hemodialysis session (i.e. *t*= 60 – 180 min; 11.8±3.4 vs 11.2±4.5 g, respectively; *P*=0.55) did not differ between the PRO and PRO+BCKA treatment, respectively. Total AA removal of individual participants during the PRO and PRO+BCKA treatment are shown in **Figure 6A**. During the *t*= 60 – 180 min period during PRO and PRO+BCKA treatment, test beverage protein-derived AA removal averaged 3.3±1.2 and 3.0±1.5 g (**Figure 6B**; *P*=0.46), respectively, while endogenous-derived AA removal was 8.5±2.5 and 8.2±3.3 g (*P*=0.67), respectively.

BCKA removal was greater during PRO+BCKA when compared with PRO treatment over the whole hemodialysis session (0.34±0.10 vs 0.10±0.02 g; *P*<0.001) and the second half of the hemodialysis session (i.e. *t*= 60 – 180 min; 0.23±0.07 vs 0.06±0.02 g; *P*<0.001).

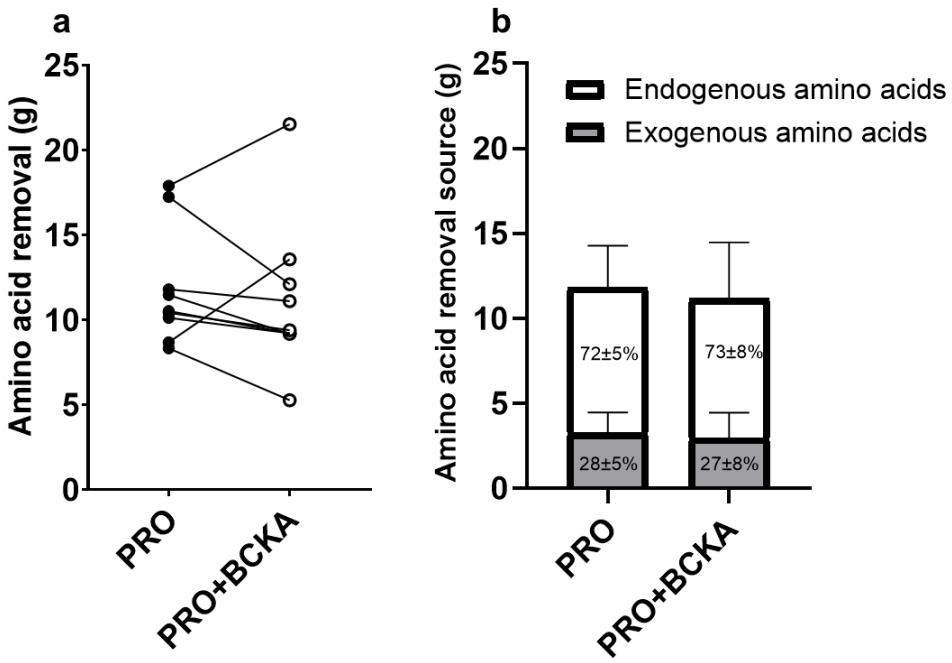

Figure 6. Amino acid removal (A) and the source of removed amino acids (B) during the second half of hemodialysis. Squares and circles represent individual data points and bars represent treatments as means+SDs, *n*=9. Data were analyzed between treatments with paired-samples *t* tests. PRO, protein; PRO+BCKA protein with branched-chain ketoacids.

Urea

Arterial plasma urea concentrations declined significantly throughout the hemodialysis session (time effect: $P<0.001$) with no differences between treatments (time × treatment: $P=0.51$). In addition, no differences were observed in the reduction ratio of urea (76.8±4.1 vs 77.3±3.9%; $P=0.46$) and urea removal (19.5±7.0 vs 18.8±6.5 g; $P=0.33$) throughout hemodialysis between the PRO and PRO+BCKA treatment, respectively.

Discussion

In the present study, we observed that hemodialysis reduces circulating AA availability and lowers the forearm arteriovenous net protein balance. Subsequently, protein provided as frequent sips during hemodialysis increased circulating AA concentrations and improved the forearm arteriovenous essential AA balance. In addition, we assessed the impact of co-ingesting BCKAs with protein during hemodialysis as a means to enhance the anabolic potential of dietary protein ingestion. Co-ingestion of BCKAs with protein had no impact on the forearm arteriovenous balance throughout hemodialysis, but significantly reduced AA oxidation rates when compared to the ingestion of protein.

Hemodialysis has been developed to remove uremic toxins from the body of patients with end-stage renal disease, but also removes AAs [5, 7]. Throughout hemodialysis this substantially reduces plasma AA concentrations, which has been shown to stimulate muscle protein breakdown. [10, 30] In the present study, we observed that during the first hour of hemodialysis plasma TAA concentrations declined by as much as ~35% (Figure 2), making the forearm arteriovenous AA balance become negative (Figure 3). Recently, we have shown that ingestion of 40 g protein during hemodialysis can compensate for the removal of AAs [14]. Here, we show that ingestion of 18 g protein increased plasma AA concentrations from 2.6±0.3 mmol/L at *t*= 0 min to 3.4±0.3 mmol/L at the end of hemodialysis session (Figure 2A). In addition, the average iAUC of forearm arteriovenous TAA balance was positive (0.58±0.67 mmol/L/3 h), which indicates no further deterioration but rather an improvement in the forearm net protein balance following intradialytic protein ingestion. These findings are in line with previous studies that reported beneficial effects of protein ingestion during hemodialysis on protein homeostasis [8, 9, 30, 31]. Therefore, protein ingestion during hemodialysis is advocated as a means to support muscle maintenance [32, 33]. However, protein intake also stimulates the production of protein-derived uremic toxins such as urea and indoxyl sulfate [16].

To enhance the beneficial impact of protein ingestion during hemodialysis without increasing total protein intake, we added BCKAs to the supplemented protein. BCKAs are the ketoanalogues of BCAAs and can be reversibly transaminated to their corresponding AA in skeletal muscle, liver, and kidney tissue [34-37]. BCKA ingestion has been shown to stimulate muscle protein synthesis rates *in vivo* and to suppress protein breakdown *in vitro* [22, 37]. In the present study, we assessed whether co-ingesting protein with BCKAs during hemodialysis could further augment the anabolic properties of dietary protein ingestion. Following the ingestion of the PRO+BCKA beverages, arterial plasma BCKA concentrations increased significantly more than following ingestion of the PRO beverages. Arterial plasma TAA, BCAA, and EAA concentrations increased following protein ingestion with no differences between PRO+BCKA and PRO treatments (Figure 2). In accordance, while the

forearm arteriovenous BCKA balance became significantly more positive during PRO+BCKA when compared to the PRO treatment, the improvements in forearm arteriovenous TAA, BCAA, and EAA balance did not differ between treatments (Figure 3). This implies that though BCKAs are effectively taken up by peripheral tissues, their co-ingestion with dietary protein does not further improve the forearm arteriovenous net protein balance.

In the present study, we applied a frequent sip protocol with test beverages containing L-[ring-$^{13}C_6$]-phenylalanine. Using this protocol, we were able to reach a tracer steady state throughout the *t*= 60 – 180 min period during both PRO and PRO+BCKA treatment (Figure 4). This allowed us to compare AA oxidation rates between PRO and PRO+BCKA treatments by measuring $^{13}CO_2$ enrichment from the oxidation of the ingested $^{13}C_6$-phenylalanine in expired breath. Following test beverage ingestion, phenylalanine oxidation rates remained significantly lower during PRO+BCKA when compared to the PRO treatment (Figure 5). With the assumption that relative oxidation rates of other AAs are comparable to phenylalanine, we estimated that approximately 6.0 and 4.7 g AAs were oxidized throughout the 3 h period during which patients ingested PRO and PRO+BCKA beverages, respectively. Because the bicarbonate in dialysate fluid represents additional CO_2 buffer capacity and $^{13}CO_2$ enrichment in expired breath was still increasing at *t*= 180 min, these values should be regarded as a minimal estimate of AA oxidation rates [29]. Nonetheless, even the observed effect of BCKA supplementation (i.e. 1.3 g reduction in total AA oxidation) may be clinically relevant over a longer period due to the high frequency of hemodialysis (generally 3 sessions/week) and a possible sustained effect beyond the end of hemodialysis. However, whether intradialytic ketoacid supplementation has beneficial long-term effects on patients' nutritional status remains to be determined.

Throughout all hemodialysis sessions, we continuously collected spent dialysate to quantify AA removal. As shown in Figure 6A, AA removal over the whole hemodialysis session did not significantly differ between PRO and PRO+BCKA treatments. During the *t*= 60 – 180 min period, AA removal during both PRO and PRO+BCKA treatment did not differ and averaged 11.8±3.4 and 11.2±4.5 g, respectively. This amount was similar to the total amount of protein provided during this period (12.0 g). Using the spent dialysate $^{13}C_6$-phenylalanine enrichment throughout the *t*= 60 – 180 min period, we were able to calculate removal of dietary protein-derived AAs during the PRO (3.3±1.2 g; 28±5% of total AA removal) and PRO+BCKA (3.0±1.5 g; 27±8% of total AA removal) treatments. These percentages are in line with estimations from our previous study, in which ingestion of a single bolus containing 40 g protein resulted in ~8 g (~25%) additional AA removal [14]. Interestingly, only 4±1% (0.23±0.08 g) of the BCKAs provided during the *t*= 60 – 180 min period were removed, suggesting that BCKAs are mostly retained in the body, oxidated, or transaminated into their corresponding AAs.

BCKAs use an excess amino group (containing nitrogen) during transamination towards their corresponding AA and have been suggested to reduce urea production [20, 21, 37]. In

contrast, protein ingestion during hemodialysis results in greater urea accumulation [16]. In the present study, we observed no differences in urea reduction or urea removal during hemodialysis between PRO and PRO+BCKA treatments. However, throughout hemodialysis we did observe a substantial decline in AA oxidation, which has been linked to urea production [38]. Therefore, future studies should determine whether BCKA ingestion during hemodialysis can modulate urea accumulation during and after hemodialysis.

Acknowledgments

We greatly appreciate the whole staff of the MUMC+ dialysis department, especially Thijs de Graaf, and Antoine Zorenc in the preparation and execution of the experiments. We also extend our gratitude to all study participants for their time and commitment. Friesland Campina supplied the Refit TMP 90 protein powder that was used in this study.

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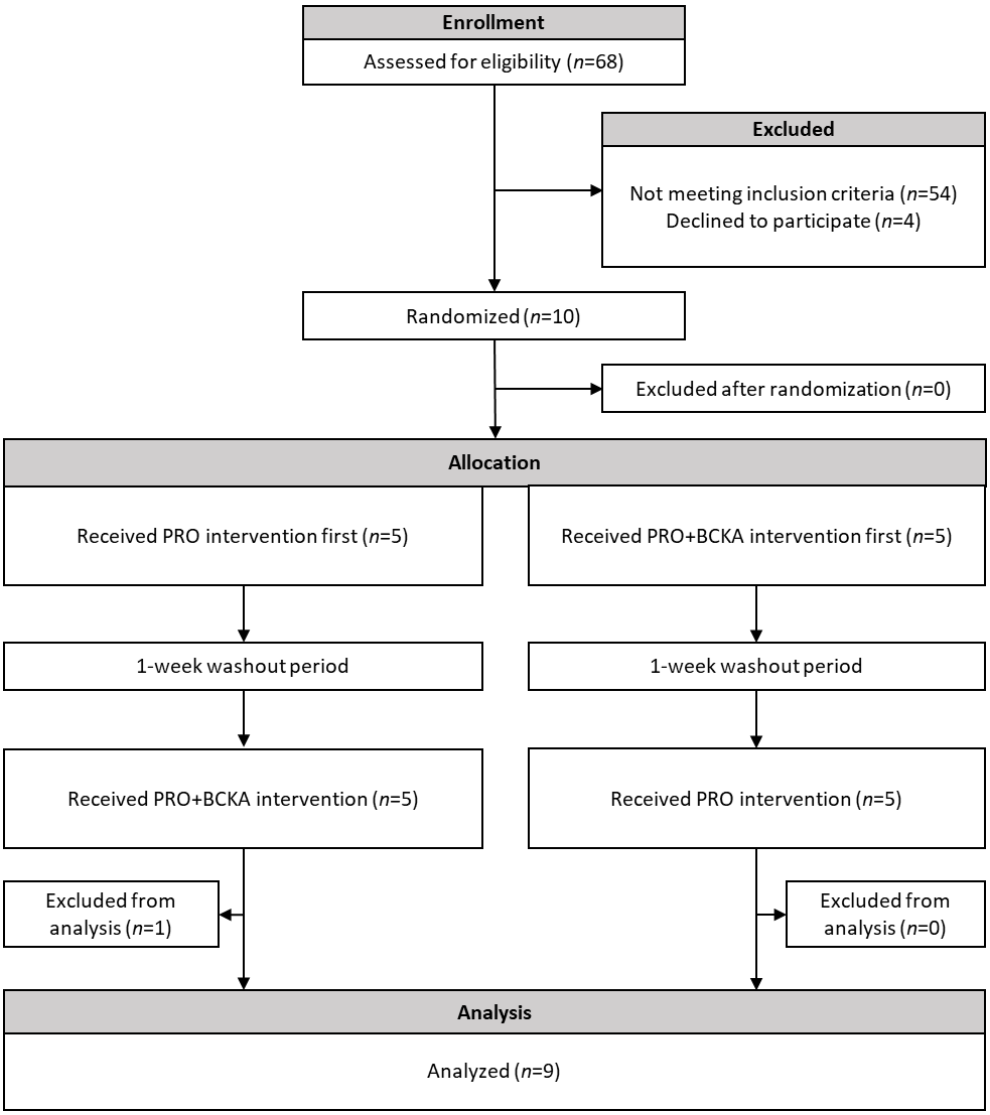
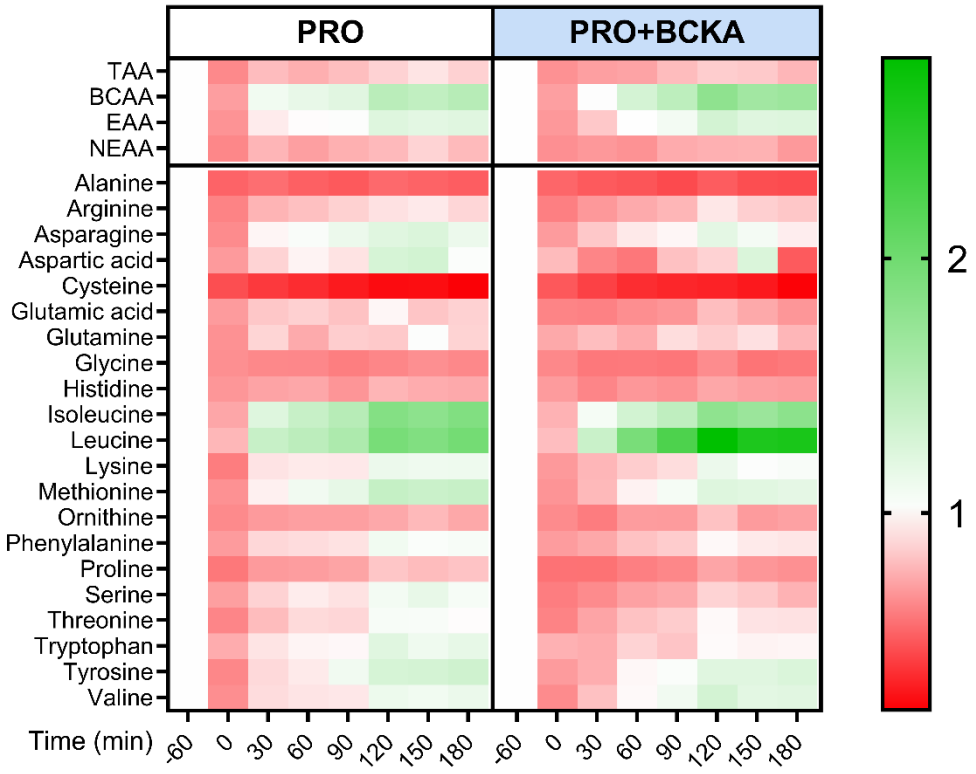
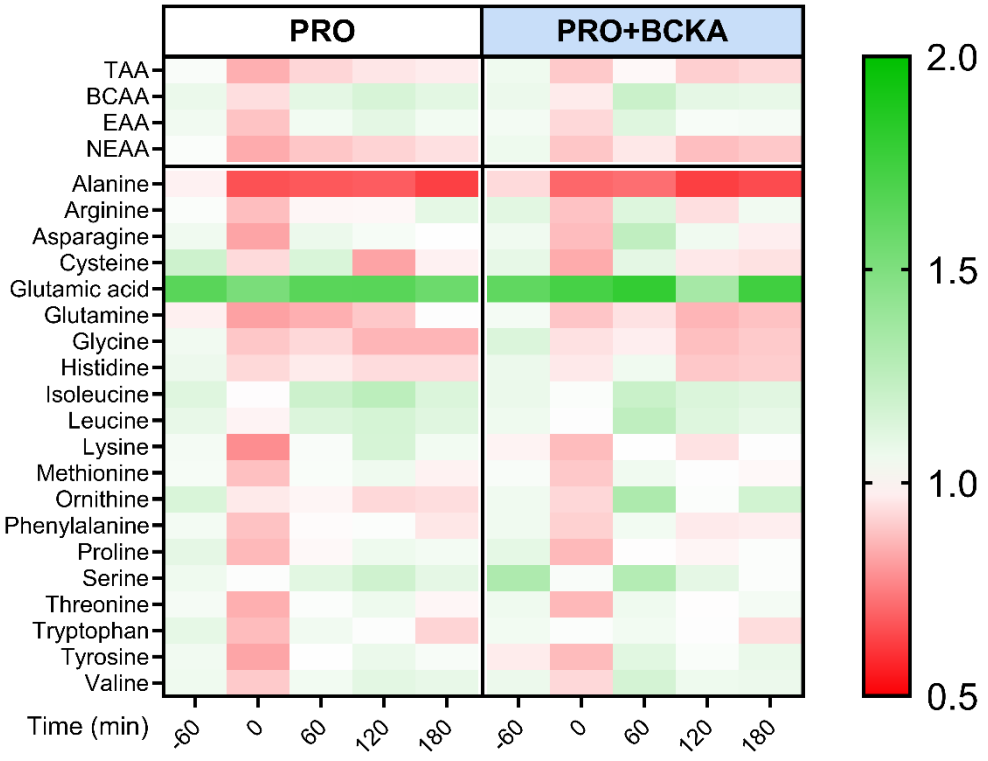
Supplementary material



Supplementary figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow chart. PRO, protein; PRO+BCKA, protein with branched-chain ketoacids.

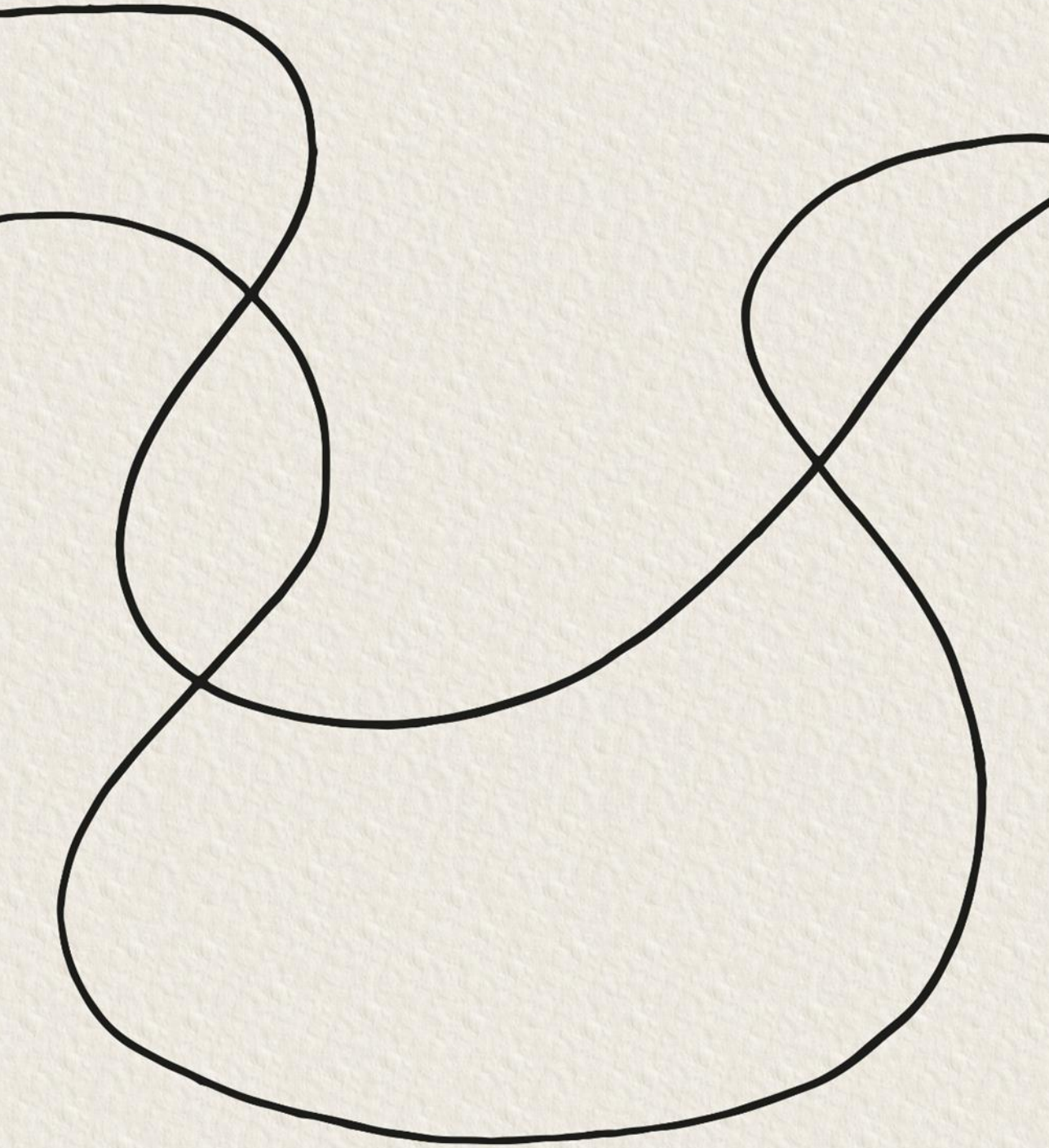


Supplemental figure 2. Heat map of plasma amino acid concentration fold changes from baseline throughout hemodialysis per treatment. *t*= -60 min represents the start (baseline) and *t*= 180 min the end of hemodialysis. BCAA, branched-chain amino acids; EAA, essential amino acids; NEAA, non-essential amino acids; TAA, total amino acids.



Supplemental figure 3. Heat map of plasma arterial/venous ratios throughout hemodialysis per treatment. *t*= -60 min represents the start (baseline) and *t*= 180 min the end of hemodialysis. BCAA, branched-chain amino acids; EAA, essential amino acids; NEAA, non-essential amino acids; TAA, total amino acids.


6



Chapter 7

General discussion

General discussion

In this thesis, we have described several studies performed in patients with end-stage renal disease on chronic hemodialysis treatment. This population is characterized by an accelerated loss of skeletal muscle mass and strength, which generally results in poor physical functioning and high morbidity [1-4]. Nutritional and physical activity interventions that increase muscle mass and strength have the potential to increase the quality of life in patients on chronic hemodialysis treatment. In **Chapter 2 – 6**, we have evaluated the application of multiple nutritional and physical activity interventions during hemodialysis to support muscle maintenance. First, we observed that a substantial amount (~12 g) of amino acids is removed during a single hemodialysis session. Subsequently, we showed that ingestion of 40 g protein during hemodialysis (intradialytic) with and without prior exercise can compensate for amino acid removal, thereby elevating plasma amino acid concentrations throughout hemodialysis. In addition, we demonstrated that intradialytic protein ingestion and exercise do not compromise uremic toxin removal during hemodialysis, which disproves previous assumptions [5]. We also studied the impact of co-ingesting branched-chain ketoacids as a strategy to further enhance the anabolic potential of intradialytic protein supplementation. Ketoacid co-ingestion lowered amino acid oxidation rates and may further support a more positive net protein balance throughout dialysis. Together, our results indicate that intradialytic protein ingestion combined with exercise should become standard clinical practice to support muscle maintenance for patients undergoing hemodialysis. However, long-term adherence and compliance of patients on chronic hemodialysis treatment to lifestyle interventions is often low due to the high disease burden and time restraints that these patients experience [6]. In addition, exercise intolerance limits the intensity that can be achieved during physical activity interventions to such a modest intensity, that beneficial effects of exercise may be limited [7]. In accordance, recent work suggests that nutritional and physical activity interventions may help to attenuate the loss of physical function, but that these interventions are not effective enough to increase muscle mass and strength in patients on chronic hemodialysis treatment [8, 9].

A different strategy to effectively improve physical functioning of patients on chronic hemodialysis treatment would be to implement interventions prior to hemodialysis initiation. Low physical functioning and frailty are already common among patients with advanced chronic kidney disease (CKD) and are strongly correlated with higher morbidity and reduced quality of life [4, 10-13]. When compared to healthy adults, the loss of muscle mass and strength in patients with advanced CKD is accelerated (**Figure 1**) [14]. Patients with advanced CKD are often identified by a healthcare professional long before hemodialysis is required. In addition, they generally visit their healthcare provider regularly to monitor their disease progression [15]. These visits to healthcare professionals provide a great opportunity to implement lifestyle interventions to improve nutritional and physical activity status prior to the development of end-stage renal disease. Due to the lesser disease burden in patients who do not yet require dialysis treatment, lifestyle interventions implemented at this stage may be more effective and, as such, could improve physical functioning. In addition, such a pre-habilitation program could increase patients' muscle mass and strength prior to the start of chronic hemodialysis treatment, helping them to remain physically active and (more) independent (Figure 1).

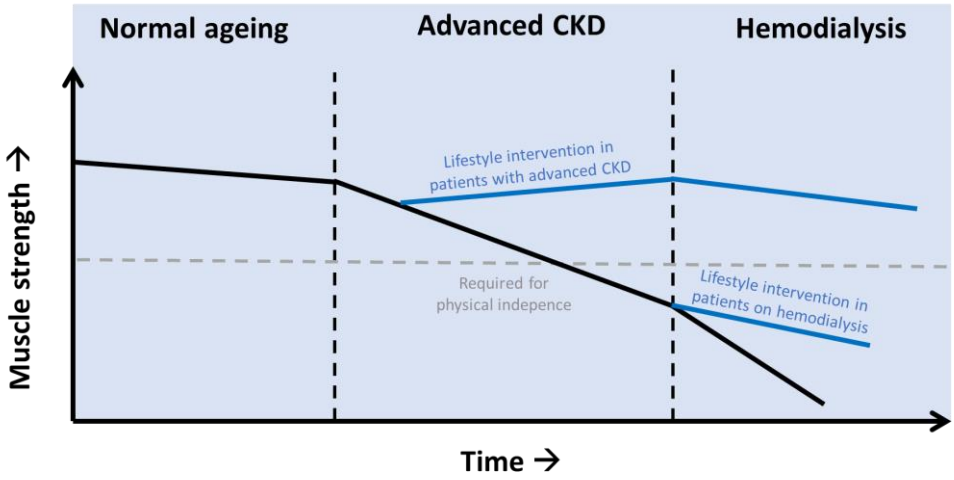


Figure 1. Theoretical different effects of implementing a lifestyle intervention in patients with advanced CKD and patients undergoing hemodialysis on muscle strength and the consequences for functional status.

Dietary protein consumption and physical activity are both essential to maintain or increase muscle mass and strength. Dietary protein provides amino acids, which can be used as precursors for *de novo* muscle protein synthesis [16]. Furthermore, protein derived amino acids, and leucine in particular, directly stimulate muscle protein synthesis through activation of translation initiation via the mammalian target of rapamycin complex 1 (mTORC1) pathway [17, 18]. Physical activity and exercise improve skeletal muscle strength

and endurance. Dietary protein consumption of patients with CKD is often modest due to dietary restrictions as well as reduced appetite caused by systemic inflammation and the accumulation of metabolic waste products [19-21]. In addition, these patients generally have a sedentary lifestyle characterized by low physical activity levels and infrequent exercise participation [22]. In addition, the lack of ample protein ingestion and physical activity in patients with advanced CKD are important contributors to the accelerated loss of muscle mass and function observed within this population [19, 23]. Therefore, a great potential exists to improve the lifestyle of patients with advanced CKD through nutritional and physical activity interventions tailored to this population.

Nutritional requirements and recommendations vary widely throughout the CKD trajectory. Dietary interventions are currently extensively implemented in clinical care for patients with advanced CKD [24]. However, nutritional prescriptions (i.e., low-protein and -salt diets) mainly aim to attenuate the progression of CKD, while maintenance of muscle mass and physical function are not of primary concern [24-26]. It has been suggested that a high-protein diet induces renal hyperfiltration and increases the production and accumulation of uremic toxins in patients with advanced CKD [27, 28]. Current clinical practice guidelines therefore recommend patients with advanced CKD without diabetes to ingest 25 – 35 kcal and 0.55 – 0.60 g protein/kg body weight/day to reduce the risk of kidney failure and delay the requirement for dialysis treatment [29]. Hence, strategies that allow the limited amount of ingested protein to be optimally used for protein synthesis in skeletal muscles and reduce the breakdown of amino acids to uremic toxins are promising in this population. Such interventions could further delay the need for dialysis treatment while simultaneously supporting muscle maintenance in patients with advanced CKD. A more equal distribution of protein between breakfast, lunch, and dinner could allow ingested protein to be used more effectively. Protein distribution between meals is often skewed with the meals later in the day containing more protein [30, 31]. In healthy older adults, the amount of protein required to stimulate muscle protein synthesis rates (20 – 30 g) is generally not consumed during breakfast and lunch [30, 32, 33]. Furthermore, dinner generally contains a large amount of protein, which may stimulate protein derived amino acid oxidation and increase uremic toxin production [34, 35]. A more equal distribution of protein over all meals could result in a stimulation of muscle protein synthesis with every meal, while reducing amino acid oxidation throughout the day [36]. In addition, ingestion of higher quality proteins (i.e. protein with a high essential amino acid content) may be more effective to achieve a proper anabolic response when compared to the same amount of protein with a lower essential amino acid content [37]. Recently, Holwerda *et al.* have shown that 15 g protein can effectively stimulate (post-exercise) muscle protein synthesis rates in older adults when 1.5 g leucine was co-ingested [38]. In support, several studies have reported that supplementing keto-analogues of essential amino acids can stimulate muscle protein synthesis rates and, as such, support muscle maintenance [39-42]. In contrast to dietary

protein, keto-analogues do not contain phosphate or nitrogen and can therefore induce an anabolic stimulus without increasing uremic toxin production in patients with advanced CKD [43]. Future studies that assess the muscle anabolic response following nutritional interventions in patients with advanced CKD should be conducted to determine which interventions could help to effectively support muscle maintenance.

Physical activity is an essential stimulus for skeletal muscle maintenance in health and disease. When this stimulus is absent (i.e., during bedrest or limb immobilization) both post-absorptive and post-prandial muscle protein synthesis rates are lowered, resulting in net muscle loss [44, 45]. Furthermore, low levels of physical activity (i.e. sedentary behavior), which are common among patients with advanced CKD, also results in a decline in muscle protein synthesis rate when compared to levels observed in people adopting a more healthy, active lifestyle [46, 47]. In contrast, it has been shown that a single exercise bout can stimulate skeletal muscle protein synthesis rates [48, 49]. Furthermore, exercise has been shown to improve the sensitivity of skeletal muscle to the anabolic properties of dietary protein for a period up to 24 h [50]. Abolishing the sedentary behavior often seen in patients with advanced CKD will likely result in substantial health benefits. A recent study by Sheshadri *et al*. showed that low-intensity exercise (i.e., walking) integrated in daily living routine can preserve muscle mass in patients with advanced CKD [51]. Though walking and endurance-type exercise have been shown to increase aerobic capacity in patients with CKD [52], progressive resistance-type exercise is considered to be a more potent exercise modality to increase muscle mass and strength [53]. In healthy adults, progressive resistance-type exercise training has been shown to effectively stimulate muscle protein accretion, muscle strength, and physical functioning [54]. In support, it has been shown that progressive resistance-type exercise training can increase muscle mass and physical functioning in patients with CKD [55-57]. However, whether the muscle protein synthetic response to resistance-type exercise training in patients with advanced CKD is similar when compared to adults without CKD remains to be determined. It has been suggested that low-grade inflammation and metabolic acidosis, which are common among patients with advanced CKD, could influence muscle protein metabolism and compromise the skeletal muscle adaptive response to exercise training [58, 59]. Nevertheless, patients with CKD patients should be counseled and guided to incorporate more habitual physical activity and structured exercise training in their daily living routines due to the wide range of documented benefits.

Structured and sustained physical activity interventions can be applied to alleviate anabolic resistance and, as such, will likely further complement and augment the anabolic effects of dietary protein consumption [60]. In addition, sufficient dietary protein ingestion is essential to allow muscle net protein accretion during exercise training [61]. In frail older adults with low dietary protein intake levels, exercise training only was able to increase muscle strength, but induced no increase in muscle mass unless it was combined with

protein supplementation [31]. To optimally increase muscle mass and strength, lifestyle interventions should include the synergistic effect of combined exercise training and dietary protein consumption. However, due to the restraint of providing excess protein to patients with advanced CKD, the impact of protein ingestion following exercise training has not yet been evaluated in this population. Nonetheless, ingesting a single dose of (high-quality) protein directly following exercise, when the muscle is more sensitive to the anabolic properties of dietary protein, may prove to be instrumental to further support muscle maintenance while adhering to recommended low daily protein intake levels. However, patient-tailored lifestyle interventions that include both exercise training and well-timed protein consumption are not yet developed for patients with advanced CKD.

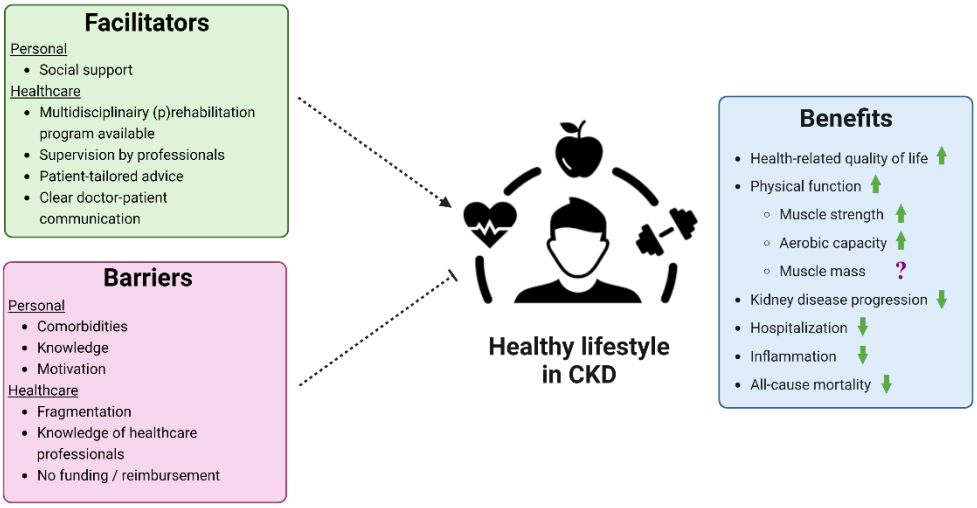


Figure 2. General overview of facilitators, barriers, and benefits of healthy lifestyle behavior in patients with chronic kidney disease.

Physical activity and nutritional interventions in patients with advanced CKD have been shown to have benefits beyond increases in muscle mass and function (**Figure 2**). Higher levels of physical activity have been shown to be associated with a slower progression of CKD, reduced inflammation, increased health-related quality of life, and lower all-cause mortality rates [1, 55, 62-64]. In addition, dietary interventions may slow the progression of CKD, delay dialysis initiation, and increase health-related quality of life in patients with advanced CKD [41, 42, 65]. However, adherence and compliance to lifestyle interventions are generally problematic in patients with chronic diseases. It has been reported that patients with CKD experience the lack of support from healthcare providers and family, fatigue, and anxiety as important barriers to engage in exercise training (Figure 2) [66].

Nonetheless, several well-designed exercise programs that incorporate training supervision by exercise professionals have successfully achieved high training adherence in patients with advanced CKD [67]. In addition, adherence to exercise training and dietary interventions in research settings among patients with advanced CKD is often high. This indicates that, at least for a motivated subgroup within this population, long-term lifestyle changes are feasible. Unfortunately, several barriers including funding and fragmentation of health care providers often prevent the effective implementation of lifestyle intervention programs into standard clinical care for patients with CKD [68]. As nephrologists and primary healthcare providers regularly, and over a longer period, guide patients throughout their CKD trajectory, it is important that they are involved in lifestyle intervention programs [15]. However, they often do not feel confident to provide advice regarding lifestyle interventions. This can, at least partially, be explained by the lack of a clear consensus on the proper implementation of lifestyle interventions in the advanced CKD population. Therefore, future endeavors should focus on the development of evidence-base lifestyle interventions and a clinical care network that allows a more effective translation and integration of lifestyle intervention programs in the clinical care for patients with advanced CKD.

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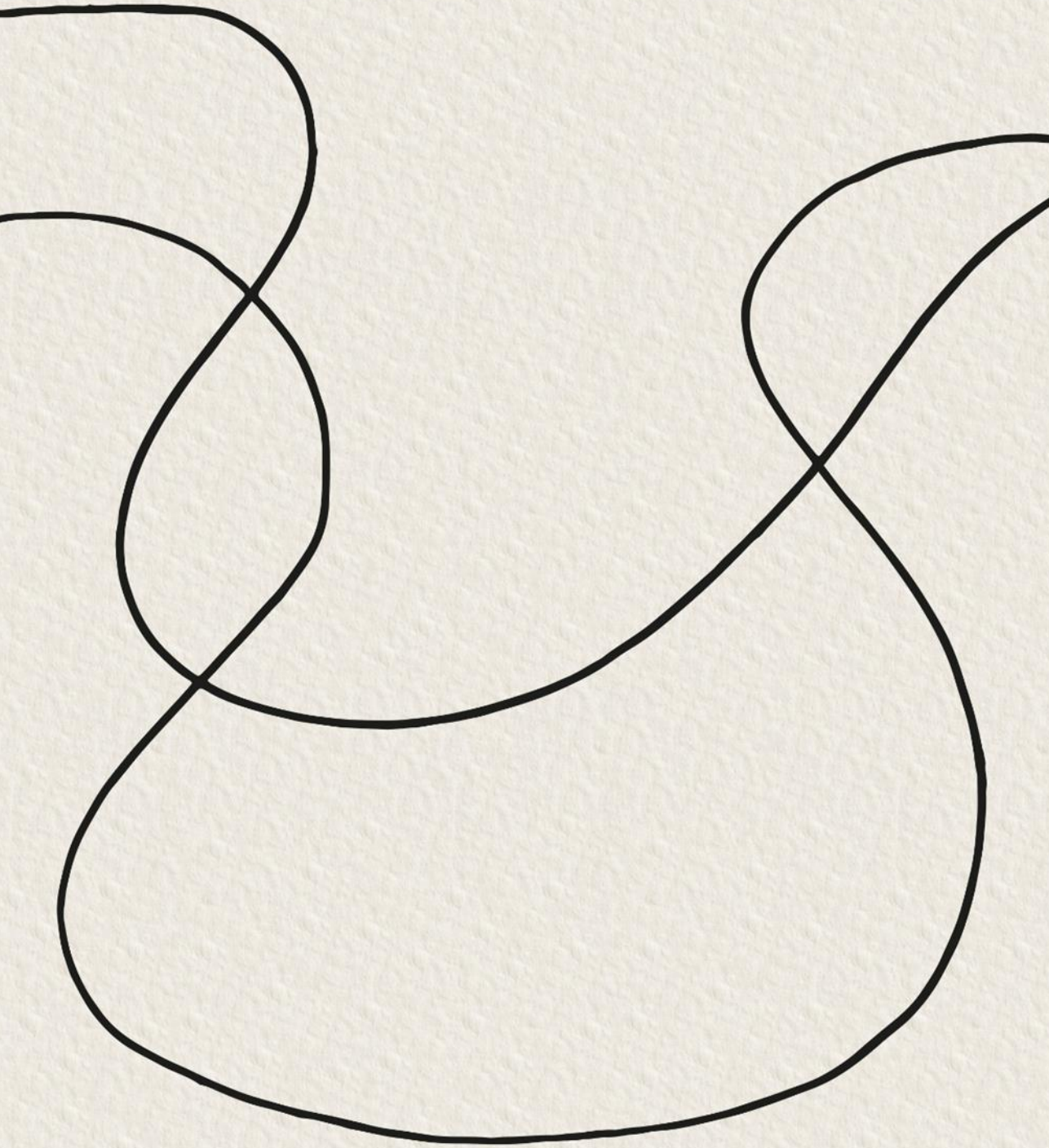

Chapter 8

Addendum

Summary

Maintenance of skeletal muscle mass and function over time is essential for physical function and to maintain quality of life. Low muscle mass and strength in older adults are associated with increased morbidity, higher mortality rates, and the development of chronic diseases. Among clinically compromised populations, such as patients on chronic hemodialysis treatment, loss of muscle mass and strength is generally accelerated and strategies to support muscle maintenance are therefore of vital importance. Though adequate dietary protein ingestion and ample physical activity are crucial to allow muscle maintenance, few lifestyle interventions are currently applied in clinical care to prevent or attenuate muscle wasting in patients on chronic hemodialysis treatment.

In **Chapter 2**, we have performed a literature review in which we describe that patients on chronic hemodialysis treatment generally do not ingest the recommended amount of protein (1.0 – 1.2 g protein/kg body weight/day) and have a sedentary lifestyle. Therefore, we proposed that interventions aiming to preserve or even increase muscle mass in this population should incorporate nutritional support as well as strategies to increase physical activity levels. Furthermore, we suggested that the hemodialysis period represents a time-efficient opportunity to implement nutritional and physical activity interventions in the lifestyle of these patients. During hemodialysis, metabolic waste products are removed from the body by diffusion through a semipermeable dialysis membrane. However, small nutrients, such as amino acids, are also able to diffuse through this membrane. In **Chapter 3**, we quantified amino acid removal in patients ingesting their habitual diet throughout hemodialysis. Here, we showed that 8 – 15 g amino acids were removed during a single hemodialysis session. Furthermore, patients' plasma amino acid concentrations declined significantly throughout hemodialysis, indicating that habitual dietary protein intake during hemodialysis (~20 g) was not sufficient to compensate for amino acid removal. These findings emphasize the need for additional nutritional support during hemodialysis.

In **Chapter 4**, we investigated the impact of intradialytic protein ingestion at rest and following exercise on amino acid removal and plasma amino acid availability throughout hemodialysis. Though intradialytic ingestion of 40 g protein resulted in additional amino acid removal when compared to placebo ingestion, it significantly increased circulating amino acid availability until the end of hemodialysis. In addition, we observed that intradialytic exercise, performed as 30 min moderate intensity cycling, did not influence amino acid removal or plasma amino acid availability throughout hemodialysis. Though it is important to know the anabolic potential of intradialytic protein ingestion and exercise, adequate removal of uremic toxins is the main goal of hemodialysis and should not be compromised by intradialytic interventions. Therefore, we assessed whether protein ingestion and exercise modulate uremic toxin removal during hemodialysis. In **Chapter 5**, we showed that intradialytic protein ingestion slightly reduced the reduction ratio of

protein-derived uremic toxins (i.e. urea and indoxyl sulfate), but also resulted in greater urea removal during hemodialysis. Furthermore, we showed that removal of small uremic toxins (i.e. urea, creatinine, and phosphate) was greater throughout intradialytic exercise performance. Yet, removal of these toxins over the whole hemodialysis session did not differ between exercise performance and sedentary hemodialysis sessions. Therefore, exercise and protein ingestion can be implemented during hemodialysis to support muscle mass and strength maintenance without attenuating hemodialysis efficiency.

Apart from exercise, other strategies can be applied to enhance the anabolic properties of dietary protein. One of these strategies is the co-ingestion of branched-chain amino acids, and leucine in particular, with dietary protein. Recently, it has been shown that the ketoanalogues of branched-chain amino acids, branched-chain ketoacids (BCKAs), induce a muscle anabolic response. The application of BCKAs to support muscle maintenance in patients with kidney disease is promising, since BCKAs do not contain nitrogen or phosphate and, as such, do not result in the production of protein-derived uremic toxins. In **Chapter 6**, we used stable isotope methodology to assess whether the co-ingestion of BCKAs with protein during hemodialysis resulted in a greater anabolic response when compared to protein ingestion only. In this study, we extended on our previous findings by showing that protein ingestion improves the forearm arteriovenous net protein balance during hemodialysis. Though we did not observe a further improvement in the forearm arteriovenous net protein balance when compared to protein ingestion only, BCKA co-ingestion substantially reduced amino acid oxidation rates. From these results it can be speculated that co-ingestion of BCKAs with protein during hemodialysis does not further improve the net protein balance of peripheral tissues but may improve the whole-body net protein balance by reducing amino acid oxidation.

Patients on chronic hemodialysis treatment are generally in such a deconditioned state that appropriate exercise prescription is highly problematic. In **Chapter 7**, we discuss the implementation of exercise and nutritional interventions in patients with advanced chronic kidney disease prior to initiation of chronic hemodialysis treatment. Such pre-habilitation programs may improve physical functioning and nutritional status of patients starting hemodialysis, thereby supporting them to remain physically active and increasing their quality of life. In this chapter, we provide an overview of habitual dietary protein intake and physical activity levels of patients with advanced chronic kidney disease as well as nutritional and physical activity interventions to support muscle maintenance in this population. Future research in close collaboration with nephrologists, exercise professionals, and dietitians should be performed to establish evidence-based lifestyle interventions and improve the health, nutritional status, and quality of life of patients throughout all chronic kidney disease stages.

Samenvatting

Het behoud van spiermassa en spierkracht is belangrijk voor het in stand houden van fysiek functioneren en behoud van kwaliteit van leven. Lage spiermassa en verminderde spierkracht bij ouderen verhogen het risico op ziekenhuisopnames, verminderd fysiek functioneren en het ontwikkelen van chronische ziekten. Daarnaast verloopt het verlies van spiermassa en spierkracht vaak sneller bij patiënten met een chronische ziekte, zoals patiënten met nierfalen die hemodialyse ondergaan. Daarom zijn leefstijlinterventies met als doel spierbehoud essentieel in deze populaties. Echter, er worden nu weinig van zulke leefstijlinterventies geïmplementeerd in de zorg voor patiënten die langdurig hemodialyse ondergaan.

In **Hoofdstuk 2** hebben we een literatuurstudie uitgevoerd waarin we beschrijven dat patiënten die langdurig hemodialyse ondergaan meestal de aanbevolen hoeveelheid eiwitinname (1.0 – 1.2 g eiwit/kg lichaamsgewicht/dag) niet halen en een inactieve leefstijl hebben. Daarom suggereren wij dat interventies met als doel spierbehoud in deze populatie zowel een voedings- als een bewegings-component zouden moeten bevatten. Daarnaast zijn hemodialysebehandelingen een tijds-efficiënte periode waarbinnen interventies ter verbetering van de voedingstoestand en het fysiek functioneren geïmplementeerd kunnen worden. Tijdens hemodialyse worden uremische afvalstoffen verwijderd uit het lichaam met behulp van een dialysemembraan. Voedingsstoffen zoals aminozuren diffunderen echter ook door dit membraan heen. In **Hoofdstuk 3** hebben we in patiënten met nierfalen de mate van verwijdering van aminozuren uit het bloed gemeten tijdens hemodialyse. In dit onderzoek hebben we aangetoond dat er maar liefst 8-15 g aminozuren worden verwijderd tijdens een enkele hemodialyse behandeling, hetgeen leidde tot een significante daling van de aminozuurconcentraties in het bloed. Deze resultaten benadrukken dat voedings-interventies nodig zijn om aminozuurverlies tijdens hemodialyse te compenseren.

In **Hoofdstuk 4** hebben we het effect van eiwitinname in rust en na fysieke inspanning tijdens hemodialyse onderzocht. Hoewel intradialytische eiwitinname (eiwitinname tijdens hemodialyse) resulteerde in meer verwijdering van aminozuren, voorkwam het de daling van aminozuurconcentraties in het bloed gedurende de hemodialysebehandeling. Intradialytische fysieke inspanning had geen invloed op de verwijdering van aminozuren of de aminozuurconcentraties in het bloed tijdens hemodialyse. Ondanks dat intradialytische eiwitinname en fysieke inspanning een positief effect hebben op spierbehoud, is het verwijderen van uremische afvalstoffen de belangrijkste functie van hemodialyse. Deze functie mag dan ook niet gecompromitteerd worden door intradialytische interventies. Hierom hebben we de invloed van intradialytische eiwitinname en fysieke inspanning op de verwijdering van uremische afvalstoffen tijdens hemodialyse onderzocht. In **Hoofdstuk 5** hebben we aangetoond dat door intradialytische eiwitinname de concentraties van

uremische afvalstoffen (zoals ureum) in het bloed iets minder dalen tijdens hemodialyse. We zagen echter ook dat intradialytische eiwitinname ervoor zorgt dat er meer ureum uit het lichaam verwijderd wordt tijdens hemodialyse. Dit suggereert dat de inname van eiwit de verwijdering van uremische afvalstoffen tijdens hemodialyse niet verminderd, maar wel de aanmaak van ureum in het lichaam stimuleert. Fysieke inspanning had geen invloed op de verwijdering van uremische afvalstoffen gedurende de hemodialysebehandeling. Zodoende concluderen we dat eiwitinname en fysieke inspanning tijdens hemodialyse kan bijdragen aan een beter behoud van spiermassa zonder daarbij de effectiviteit van de hemodialyse-behandeling te verminderen.

Naast het combineren van eiwitinname met fysieke activiteit zijn er ook andere strategieën die het anabole effect van eiwitinname kunnen versterken. Het innemen van vertakte-keten aminozuren, vooral leucine, met eiwit kan de spiereiwit aanmaak versterken. Recentelijk is aangetoond dat inname van de ketoanalogen van vertakte-keten aminozuren, ook wel ketozuren genoemd, de spieraanmaak kan stimuleren. Gecombineerde inname van eiwit met ketozuren kan een veelbelovende strategie vormen voor spierbehoud bij patiënten met nierziekte aangezien ketozuren geen stikstof bevatten en hierdoor niet leiden tot de aanmaak van uremische afvalstoffen. In **Hoofdstuk 6** hebben we middels het gebruik van stabiele isotopen onderzocht of de eiwitbalans van het lichaam positiever is tijdens het innemen van eiwit met ketozuren ten opzichte van het innemen van enkel eiwit. In lijn met onze eerdere resultaten zagen we dat eiwitinname de arterioveneuze eiwitbalans van de onderarm tijdens hemodialyse verbeterd. De inname van ketozuren met eiwit leidde niet tot een verdere verbetering van de arterioveneuze eiwitbalans van de onderarm. Wel zagen we dat inname van ketozuren de oxidatie van aminozuren verminderde. Op basis van deze resultaten speculeren we dat het innemen van ketozuren (met eiwit) tijdens hemodialyse de eiwitbalans kan verbeteren.

Patiënten die chronisch hemodialyse ondergaan hebben over het algemeen zo'n slechte conditie en gezondheid dat het adequaat toepassen van leefstijlinterventies problematisch is. In **Hoofdstuk 7** bespreken we de implementatie van voedings- en bewegingsinterventies bij patiënten met gevorderde nierziekte die nog niet middels hemodialyse behandeld hoeven te worden. Zulke (p)rehabilitatie interventies zijn van belang om de voedingsstatus en het fysiek functioneren van patiënten te verbeteren voordat ze beginnen met hemodialyse. Hierdoor zullen deze patiënten mogelijk meer fysiek actief blijven en hun kwaliteit van leven (grotendeels) behouden wanneer gestart wordt met hemodialyse. In dit hoofdstuk geven we een overzicht van de habituele eiwitinname en fysieke activiteit van patiënten met gevorderde nierziekte. Ook beschrijven we mogelijke voedings- en bewegingsinterventies met als doel het opbouwen van spiermassa en spierkracht voor deze populatie. Samenwerking tussen nefrologen, fysiotherapeuten en diëtisten is essentieel om praktische en effectieve leefstijlinterventies te ontwikkelen om de gezondheid, voedingsstatus en kwaliteit van leven te verbeteren bij patiënten met nierziekte.

Impact

The goal of clinical research is to establish new facts, reach new conclusions, and improve the quality of clinical care for patients. In this paragraph, we will address how the work described in this thesis can improve clinical care for patients on chronic hemodialysis treatment.

Results and relevance of this thesis

Chronic kidney disease (CKD) is currently a public health problem with a global prevalence of 10% [1]. Yet, it is expected that its prevalence will further increase over the upcoming decades since risk factors for the development and progression of CKD, such as diabetes mellitus and hypertension, are becoming increasingly prevalent [1-3]. Consequently, the number of patients with the final stage of CKD, coined end-stage renal disease, who will require renal replacement therapy (dialysis) is also expected to increase. Hemodialysis is globally the most applied chronic renal replacement therapy when kidney transplantation is not (yet) possible. Over the past decades, the life expectancy of patients on hemodialysis and, as such, the period that they undergo this treatment, has increased substantially due to advances in hemodialysis techniques and management of comorbidities [4]. However, poor nutritional status has proven to be a persistent problem in patients on chronic hemodialysis treatment [5, 6]. A recent meta-analysis reported that protein-energy wasting (a state of malnutrition with insufficient dietary intake) is present in 28-54% of patients on dialysis treatment [7]. In addition, patients undergoing hemodialysis are generally frail and/or have severely reduced levels of physical functioning [8, 9]. Protein energy wasting, poor nutritional status, and frailty are closely associated with a reduced quality of life, increased morbidity, greater healthcare costs due to more hospitalizations, and higher mortality rates in patients on chronic hemodialysis treatment [10-13]. Therefore, it is essential to understand why poor nutritional status is so highly prevalent among this population and to develop effective interventions that can preserve muscle mass and function.

In this thesis, we report that a substantial amount of amino acids is removed from the body during hemodialysis, which has been shown to stimulate skeletal muscle protein breakdown [14]. For healthcare professionals involved in the clinical care for patients with CKD, it is relevant to understand that hemodialysis is a catabolic procedure and that anabolic interventions to counterbalance its effects should be part of their treatment plan. Interventions during hemodialysis (intradialytic) are time-efficient for patients and easy to supervise for healthcare professionals as patients are already present in the healthcare center. We showed that ample protein ingestion during hemodialysis can compensate for the removal of amino acids from the circulation. Furthermore, we showed that exercise performed prior to protein ingestion does not further enhance amino acid removal.

Previously, it had been suggested that intradialytic protein ingestion could reduce uremic toxin removal due to splanchnic blood pooling during hemodialysis [15]. In this thesis, we quantified uremic toxin removal throughout hemodialysis with protein ingestion for the first time and showed that protein feeding with or without prior exercise during hemodialysis did not compromise uremic toxin removal and actually increased urea removal. Therefore, intradialytic exercise and protein ingestion can be implemented to support muscle maintenance in this vulnerable population without compromising uremic toxin removal during hemodialysis.

Furthermore, we assessed the impact of co-ingestion of branched-chain ketoacids with protein during hemodialysis as a strategy to augment the anabolic properties of dietary protein without providing additional phosphate. Our results indicate that co-ingestion of ketoacids with protein during hemodialysis significantly reduces amino acid oxidation when compared to protein ingestion only, thereby likely improving the net protein balance. Therefore, adding ketoacids to protein supplementation during hemodialysis may represent an alternative strategy for additional protein ingestion to counteract the catabolic effects of hemodialysis.

Stakeholders of this thesis

This thesis contributes to the scientific field of clinical nutrition and nephrology, as it provides insight in the impact of nutritional and physical activity interventions during hemodialysis. Furthermore, the scientific community may benefit from research methods that we applied during hemodialysis for the first time, such as sip feeding of test beverages containing a stable isotope amino acid tracer and quantifying the removal of protein-derived amino acids in the spent dialysate. This thesis will be of use for nephrologists and nurses working with kidney patients through providing a better understanding why patients on chronic hemodialysis treatment generally have poor nutritional status. In addition, it allows them to provide evidence-based recommendations (i.e. to supplement protein ingestion during hemodialysis and to stimulate physical activity) to patients. To implement nutritional and physical activity interventions in the clinical care for patients, involvement of dietitians and exercise professionals will be crucial. These healthcare professionals can also use the results of this thesis to provide evidence-based recommendations to patients on hemodialysis. Furthermore, the presented results are relevant to health care policy makers. Our work demonstrates the importance of patient-specific nutritional and physical activity interventions to support muscle maintenance patients with end-stage renal disease. In addition, such interventions to prevent frailty could reduce the high morbidity and hospitalization rates in this population and, as such, lower healthcare costs. At present, lifestyle interventions to support muscle maintenance are currently not incorporated in standard clinical guidelines for patients with CKD. For patients with end-stage renal disease, it is important to know and understand the side-effects of their hemodialysis treatment.

The results from this thesis provide them with tools to maintain, or even improve, their physical functioning and prevent frailty. It is important that patients are aware of the importance of proper dietary (protein) intake and sufficient physical activity to maintain their nutritional status, physical function, and general health. This will allow them to maintain functional independence and experience less adverse outcomes of their disease and its treatment and, as such, improve their quality of life.

The results presented have been communicated towards stakeholders through various forums. The research work described in this thesis has been established through a continuous collaboration of biomedical researchers, nephrologists, dietitians, exercise professionals, and patients. Five chapters of this thesis are openly accessible as published articles in peer-reviewed scientific journals and this thesis will be distributed to interested shareholders. In addition, we have presented our findings at (inter)national scientific conferences, workshops for healthcare professionals, and patient meetings. To simplify the translation of our results to clinical practice, we collaborated with seven other hospitals and the Knowledge Centre for Sport & Physical Activity to create a fact sheet about implementing physical activity interventions for patients on hemodialysis. Parts of this thesis were also summarized in layman language and published in a journal for dialysis nurses and the magazine of the Maastricht Organization for Patients with Kidney Disease (NvM).

General impact and future goals

Based on the results of the presented findings regarding protein ingestion during hemodialysis, the dialysis department of the Maastricht University Medical Center+ has changed their nutritional strategy as of February 2021 and now offers protein-rich foods to patients during hemodialysis. Furthermore, the findings regarding intradialytic physical activity were used to start a Dutch Kidney Foundation-funded project that resulted in the implementation of intradialytic cycling into routine care at the dialysis department of the Maastricht University Medical Center+. Thereafter, the Maastricht Organization for Patients with Kidney Disease (NvM) requested to expand this program to the dialysis department in Valkenburg. As a result, patients now have the opportunity to perform physical activity during their hemodialysis treatments.

The chapters of this thesis provide proof-of-principle evidence regarding nutritional and physical activity interventions during hemodialysis to support muscle maintenance. However, future research to establish evidence-based lifestyle interventions for all CKD stages is still required. To optimize the anabolic potential of intradialytic protein ingestion, the impact of protein dose, timing, and type should be further assessed. In addition, long-term effects of the nutritional and physical activity interventions described in this thesis remain to be evaluated. Lifestyle interventions to support muscle maintenance should preferably be already implemented prior to initiation of chronic hemodialysis treatment. In an earlier stage of CKD, it will be less complicated for patients to adopt lifestyle changes, which also may be more effective to increase muscle mass and function due to lower disease burden. Such a pre-habilitation strategy is not yet part of routine clinical care for patients with advanced CKD as evidence for its efficacy remains to be established. However, this population is underrepresented in product-development by companies and scientific research focusing on muscle maintenance, especially when compared to the work conducted in hemodialysis patients. Future studies should aim to provide insight in the etiology of accelerated muscle loss and the efficacy of lifestyle interventions to support muscle maintenance in patients at different stages of CKD.

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Dankwoord

Eind goed, al goed. Maar dit proefschrift had niet geschreven kunnen worden zonder de hulp van vele collega's, vrienden, familieleden, proefpersonen en geliefden. In dit dankwoord wil ik mijn dank voor een aantal personen uitlichten. Daarnaast ben ik ook de onbenoemde namen ben ik meer dan dankbaar.

Allereerst wil ik alle proefpersonen die mee hebben gedaan aan mijn onderzoeken hartelijk bedanken. Jullie hebben mij de mogelijkheid geboden om onderzoek te doen in levensfases waarin jullie het moeilijk hadden. Dank ook voor alle gastvrijheid, gezelligheid en jullie meeslepende doorzettingsvermogen en optimisme.

Luc, toen ik voor het eerst jouw kantoor binnenliep had ik geen idee dat ik jaren later de deur nog niet zou dichttrekken. Jouw oprechte interesse, enthousiasme en liefde voor onderzoek heeft aanstekelijk gewerkt. Ondanks dat mijn promotie niet altijd even makkelijk verliep, heb je toch vertrouwen in mij gehouden en me altijd gesteund. Dankzij jou heb ik een promotie- en postdoc-traject kunnen opzetten die 100% van mij zijn. Dankjewel daarvoor.

Jeroen, ik was voor jou geen doorsnee promovendus aangezien je mijn begeleiding moest delen met Luc. Wat ik erg heb gewaardeerd is dat ik altijd bij je kon binnenlopen, je altijd tijd voor me maakte voor wat dan ook en jouw klinische kijk op het project.

Frank, voor jou geen woorden maar daden. Je was altijd bereid om mee te denken en mee te helpen waar nodig. Ondanks dat je zelf door een hele moeilijke periode bent gegaan tijdens mijn promotietraject, ben je er toch altijd voor me geweest.

I would also like to thank the members of the assessment committee, Ellen Blaak, Yves Boirie, Fred Hartgens, Marc Hemmelder, and Annemie Schols. After reading much of your work and listening to many of your presentations on the big stages, I'm honored that you were willing to assess my thesis.

Joey, Ik heb het *** toch maar mooi geflikt. Van jou heb ik zowel geleerd om zeer secuur te werken in het lab (dank voor je geduld met deze geneeskundestudent die nog nooit een pipet had vastgehouden) als ook om praktisch onderzoek uit te voeren rondom de reguliere zorg. Ook heb ik veel opgestoken van onze niet-werk gerelateerde gesprekken met kop koffie of glas bier, je oprechtheid, en natuurlijk ook je heerlijke scheldpartijen als het even niet zo liep zoals gewenst.

Milan, dankjewel dat je altijd klaar stond om te helpen bij mijn onderzoeken als ik een paar handen tekort kwam. Het is mooi om te zien hoeveel passie jij voor jouw onderzoek hebt, dat straalt af op iedereen in ons kantoor. Ook alle avondjes kapsalon of iets minders culinairs (als we met de meiden aten) hebben mij mooie momenten opgeleverd afgelopen jaar.

Ook zonder mijn andere kantoorgenootjes had mijn boekje niet zijn huidige vorm gekregen. Dion, het is mooi om te zien hoe je je in twee jaar hebt opgewerkt van een nog wat timide student tot iemand waarvan ik weet dat alle stappen van onze projecten in goede handen zijn. Zonder jouw werk afgelopen jaar had ik nooit de tijd gevonden om dit boekje af te schrijven en/of de projecten zo ver te krijgen als ze nu zijn. Ale, you have been a great colleague and friend. During the winter, you always make me laugh at least once a day with your layers of clothes. I also enjoyed our talk over some (good) coffee and your party-energy.

Michelle - Weijzen, die andere moet nog even wachten – jouw aanstekelijke vrolijkheid en behulpzaamheid hebben het laatste stukje van mijn promotietraject een stuk leuker gemaakt. Samen in (ongeveer) hetzelfde schuitje zitten schept in ons geval zeker een band. Ook mooi om te zien hoe je na jouw promotie hindernissen overwint en nu een hele mooie positie hebt verdiend!

Anouk, zonder jou waren de studies op de dialyse-afdeling nooit zo goed gelopen zoals ze zijn gegaan. Ik bewonder hoe je omgaat met patiënten en je niet aflatende vrolijkheid. En als er eens iets minder ging, dan zette jij jouw sterke (judo)schouders eronder en was het zo opgelost! Thijs en Piet, jullie hebben mij ook veel geholpen met het oplossen van alle (technische) problemen tijdens de studies: van de CE-certificatie van de dialysefiets tot de accu's van infuuspompen uit de steentijd. Toch bijzonder dat er met al die tegenvallers geen enkel probleem was dat jullie niet op konden lossen.

Lieve dames van het secretariaat, lieve Cleo, Claudia, Desiree en Rachelle, dank voor alles wat jullie voor mij hebben geregeld. Maar nog meer herinner ik me alle gesprekken op het secretariaat over koetjes en kalfjes, wat er speelde op de afdeling en jullie interesse in mij. Het is fijn dat jullie deur altijd open staat.

Janneau, je hebt ontiegelijk veel samples voor mij geanalyseerd met de UPLC en was onmisbaar voor de mooie resultaten in dit proefschrift. Antoine, bedankt voor het inzetten van je geluk op onmogelijke tijden bij onmogelijke aders. Joan, dankjewel voor al het meedenken in het lab als ik weer iets nieuws wilde uitproberen. Joy en Annemie, al leverde ik mijn studies steeds in allerlei verschillende batches in, bedankt voor jullie geduld en vlotte analyses.

Steven en Jeffrey, dankjewel voor het analyseren van mijn samples bij het CDL en realiseren van en geloven in mijn extra metingen. Het was fijn samenwerken en we hebben er heel wat moois uit gehaald!

Al mijn andere collega's, bedankt voor alle mooie congressen, afdelingsuitjes, borrels, en het vormen van een hecht team als onderzoeksgroep. Wesley, dankjewel voor je hulp bij mijn studies en je gezelligheid/geblaat zonder pauze om loze momenten te vullen. Philippe, dankjewel voor het organiseren van o.a. de M3-ritten en dat je altijd klaarstaat om te helpen zelfs al heb je het eigenlijk zelf al te druk. Jorn, toch mooi dat ik jou het klinisch onderzoek in heb gekregen met je eerste dag op de dialyse-afdeling. Dankjewel voor je grappen en

sfeermaken. Cas, work hard, play hard, dankjewel voor al je vrolijkheid en de mooie trip naar Krakow. Andy, thanks for teaching me how to work with deuterated water and for your catchy bursts of laughter. Glenn, dankjewel voor alle gezelligheid, vooral de avond na het kerstdiner in de Twee Heeren en de dagen met Juweeltje. Thorben, thanks for stretching the boundaries of my biopsy limits. Heather, it was nice to welcome you in the Netherlands, thanks for all your enthusiastic stories on biking, running, and science. Lisanne, dankjewel voor het delen van je ervaringen van en met klinisch onderzoek (scheelde mij zeker een paar valkuilen). Tof om te zien dat je nu de stappen maakt die je graag wilde maken. Lisa, dankjewel voor het overnemen van (bijna) alle biopten en de feestavondjes. Kenneth, dankjewel voor je praatjes en interesse. Ook jij mag trots op jezelf zijn. Tim en Lex, bedankt dat jullie deuren (tegenwoordig deur) altijd open stonden voor mijn 'korte' vragen. Milou, Jean, Maarten, Luuk, Imre en Stefan, bedankt voor alle gezelligheid en adviezen in het begin van mijn promotietraject. Julia, dankjewel voor het verlichten van mijn taak als biopteur en de mooie avonden, onder andere in Wenen.

Ook wil ik alle nefrologen, met name Elife, Elisabeth en Bianca, bedanken voor hun hulp met het rekruteren en screenen van proefpersonen. Mijn dank gaat ook uit naar alle verpleegkundigen van de dialyseafdeling, onder andere naar Jaap en Patricia, voor hun hulp. Ik heb veel extra van jullie gevraagd en toch stonden jullie altijd klaar om te helpen. De dialysegroepen zijn in goede handen bij jullie. Anke, bedankt voor de inhoudelijke gesprekken over voeding bij dialysepatiënten.

Naast collega's hebben mijn vrienden mijn promotietraject ook een stuk leuker gemaakt. Aike, je hebt me veel rust gebracht afgelopen jaren met onze avondjes voetbal kijken, middagen fietsen, ochtenden koffie drinken en zondagen tennissen. Alleen het bijhouden van alle vrouwen leverde soms wel wat stress op. Feit is dat ik altijd met je kan relaxen, lachen en ook serieus kan praten. Respect voor dat je je steeds weer terug knokt na al je fysieke tegenslagen.

Ook met andere leden van mijn tennisteam, Menno en Roma, heb ik veel mooie herinneringen van de afgelopen jaren. Competitiewedstrijden in verre oorden, toernooien tot Groningen en wedstrijden verliezen tegen jochies van elf. Maar de beste herinneringen zijn wel de verhalen op zondagmiddagen en -avonden met Witte Trappist op tafel. En invaller Joep, verstrooide promovendus, het was mooi mijn struggles tijdens de promotie te vergelijken met de jouwe.

Dames van mijn oude Stennis bestuur, Sadaf, Nadine, Laura, Esmee, bedankt voor het blijven organiseren van activiteiten die ons samenbrengen. Van de diners en weekendjes met de andere Oude Hanen, Emiel, Heleen, Jurjen, Phillip en Sven heb ik nog vele mooie herinneringen (aan Chocoprins). Ik kijk ook uit naar het moois wat de babyboom gaat brengen.

Rik, 4 jaar lang in mijn studententijd stond mijn bed nog geen meter van die van jou af met een dun muurtje ertussen. Ik denk nog steeds met veel nostalgie terug aan die mooie tijd. Maar het is nog mooier om gezien te hebben dat 'papa Rik' nu echt vader is geworden, wat al heel vroeg terug te zien was in je autokeuze. Ik vind het fantastisch dat de omslag van dit boekje door zo'n goede vriend is gemaakt. Linda, dankjewel voor je interesse, dat je lekker zegt wat je denkt en dat ik Rik met nog wat extra werk op mocht zadelen met de twee kleintjes thuis. Ook andere (half)huisgenoten van de SS188, Terry, Ruth, Steven, Coen, Marjolein en Carline hebben veel voor me betekend.

Tom, ik vind het mooi dat we elkaar zoveel jaren na ons eindexamen nog spreken en dat het dan altijd meteen weer zo vertrouwd als vroeger voelt. Emma, Daan en de andere wajo's, ik geniet van elke keer als wij elkaar weer zien, op 5 mei of op andere data.

Mam en pap, jullie verdienen een bijzonder woord van dank. Meer dan 30 jaar lang hebben jullie mij onvoorwaardelijk lief gehad, gesteund en het beste uit mij naar boven gehaald. Ik ben daar erg bevoorrecht mee, al leek het daar misschien niet op toen ik per se zo ver mogelijk weg wilde gaan studeren (Groningen en toen toch Maastricht). Dit boekje is af doordat jullie mij hebben gestimuleerd te doen wat ik leuk vind en mij de vrijheid hebben gegeven om mij te ontwikkelen.

Sara, ik ben blij met mijn kleine zusje. Al was ik vroeger niet altijd de voorbeeldige grote broer en hadden we daardoor soms ruzie, ik ben gelukkig met de band die we nu hebben. Het is mooi om te zien hoe jij afgelopen jaren langzaam volwassen aan het worden bent en dat je Daniël hebt gevonden. Daniël, ik vond het leuk en leerzaam afgelopen jaren het onderzoeks- lief en leed te delen met een promovendus op het technische gebied.

Joyce en John, ik vind het erg fijn dat ik jullie bij Michelle heb gekregen. Ik geniet van de avondjes Bunde met altijd lekker eten en gezelligheid. Ik waardeer ook dat jullie kritisch met mij en Michelle meedenken, ons stimuleren om het beste uit situaties te halen en ook de serieuze gesprekken aangaan. En dan allemaal naast jullie eigen bedrijven en de zorg voor Bomma, heel knap!

Michelle, vanaf dag één heb jij mij geholpen met mijn promotietraject. Je hebt me gesteund als het moeizaam ging en hebt samen met mij de successen gevierd als het beter ging. En er is niemand waarmee ik dit liever had willen doen. Ik vind het knap hoe jij jouw promotietraject combineert met de opleiding tot huisarts. Ik ben zielsgelukkig dat ik met jou goed kan praten over wat er echt in me omgaat en dat we kunnen reflecteren op keuzes die we maken binnen onze promotie (een ander advies geven is toch net wat makkelijker dan het zelf doen). Van onze reizen naar Canada en Nieuw-Zeeland heb ik enorm genoten, maar eigenlijk is elke dag met jou genieten. Ik hou van jou tot aan de sterren en terug.

Curriculum Vitae

Floris Kees Hendriks was born on July 6, 1993 in Bennekom, the Netherlands. In 2011, he graduated from secondary school at Pantarijn in Wageningen. Thereafter, he studied Medicine at Maastricht University and graduated in 2017.

During the last year of his Medicine studies, his self-designed PhD-project was selected for the NUTRIM Graduate Programme. This allowed him to start as a PhD-candidate at the department of Human Biology of Maastricht University and the department of Internal Medicine, division of Nephrology of the Maastricht University Medical Centre+ under the supervision of Professor Luc van Loon and Professor Jeroen Kooman. Throughout his PhD-project, Floris focused on nutritional and physical activity interventions to support muscle maintenance in patients undergoing hemodialysis.

In November 2021, Floris was awarded with a Kootstra Talent Fellowship. This grant allowed him to continue performing research at the department of Human Biology and the department of Internal Medicine as a postdoctoral researcher. Currently, Floris is performing research on the anabolic effects of lifestyle interventions in clinically compromised populations.

List of publications

FK Hendriks, J Trommelen, FM van der Sande, JMX van Kranenburg, JHW Kuijpers, DCJ Houtvast, GHJ Jetten, JPB Goessens, SJR Meex, JP Kooman, LJC van Loon. *Branched-chain ketoacid co-ingestion with protein lowers amino acid oxidation during hemodialysis: A randomized controlled cross-over trial.* Clinical Nutrition. 2023 Jul 6;42(8):1436-1444.
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J Trommelen, GAA van Lieshout, P Pabla, J Nyakayiru, **FK Hendriks**, JM Senden, JPB Goessens, JMX van Kranenburg, AP Gijsen, LB Verdijk, LCPGM de Groot, LJC van Loon. *Pre-sleep Protein Ingestion Increases Mitochondrial Protein Synthesis Rates During Overnight Recovery from Endurance Exercise: A Randomized Controlled Trial*. Sports Medicine. 2023 Jul; 53(7):1445-1455.
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FK Hendriks, JHW Kuijpers, JMX van Kranenburg, JMG Senden, FM van der Sande, JP Kooman, SJR Meex, LJC van Loon. *Intradialytic Protein Ingestion and Exercise do Not Compromise Uremic Toxin Removal Throughout Hemodialysis*. Journal of Renal Nutrition. 2023 Mar;33(2): 376-385.
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FK Hendriks, JSJ Smeets, NJH Broers, JMX van Kranenburg, FM van der Sande, LB Verdijk, JP Kooman, LJC van Loon. *Amino acid removal during hemodialysis can be compensated for by protein ingestion and is not compromised by intradialytic exercise: a randomized controlled crossover trial.* The American Journal of Clinical Nutrition. 2021;114(6):2074-2083. https://doi.org/10.1093/ajcn/nqab274

PJM Pinckaers, IWK Kouw, **FK Hendriks**, JMX van Kranenburg, LCPGM de Groot, LB Verdijk, T Snijders, LJC van Loon. *No differences in muscle protein synthesis rates following ingestion of wheat protein, milk protein, and their protein blend in healthy, young males.* British Journal of Nutrition. 2021 Feb 18;1-38. https://doi.org/10.1017/S0007114521000635

FK Hendriks, JP Kooman, LJC van Loon. *Dietary protein interventions to improve nutritional status in end-stage renal disease patients undergoing hemodialysis*. Current Opinion in Clinical Nutrition & Metabolic Care. 2021;24(1):79-87. https://doi.org/10.1097/MCO.0000000000000703

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FK Hendriks, JSJ Smeets, FM van der Sande, JP Kooman, LJC van Loon. *Dietary Protein and Physical Activity Interventions to Support Muscle Maintenance in End-Stage Renal Disease Patients on Hemodialysis.* Nutrients. 2019;11(12):2972. https://doi.org/10.3390/nu11122972

CF McKenna, AF Salvador, **FK Hendriks**, APY Harris, LJC van Loon, NA Burd. *Exercising to offset muscle mass loss in hemodialysis patients: The disconnect between intention and intervention.* Seminars in Dialysis. 2019;32(4):379-85. https://doi.org/10.1111/sdi.12805

Awarded Grants

Kootstra Talent Fellowship for Postdocs Fall 2021
This grant provides the opportunity for talented researchers to develop and conduct their own research ideas after obtaining their PhD degree. The Kootstra Talent Fellowship for postdocs Fall 2021 provides 75% of the 21-month salary for the applicant (~€ 100,000).

Dutch Kidney Foundation Challenge 2018
A grant for innovative ideas to improve the quality of care for patients with renal diseases. This grants provides €10,000 for the development of the proposed idea.

NUTRIM NWO GP Grant 2017
To apply for this grant, the PhD-candidate selected his own topic and designed the entailed studies using the available infrastructure and expertise within NUTRIM. The NUTRIM NWO GP Grant 2017 provides 3 years of salary for the PhD-candidate (~€ 140,000).

Awards

1st Presentation prize NUTRIM Symposium 2022 (€250)
1st place Nutrition Interest Award at the 2021 ACSM Annual Meeting ($500)
2nd place Pélerin Symposium pitches 2021.

Presentations

2023
Dutch Nephrology Days, Veldhoven, The Netherlands.
Oral presentation entitled: 'Branched-chain ketoacid co-ingestion with protein lowers amino acid oxidation during hemodialysis'.

2022
European Society of Tissue Regeneration in Orthopaedics and Traumatology (ESTROT) Congress, Maastricht, The Netherlands.
Oral presentation entitled: 'Trabecular, but not cortical, bone protein synthesis rates are lower in the femoral head compared to the femoral shaft following an intracapsular hip fracture'.

The International Biochemistry of Exercise Conference (IBEC), Toronto, Canada. Poster presentation entitled: 'Protein ingestion and exercise counteract amino acid loss during hemodialysis without compromising toxin removal'.

International Society of Renal Nutrition and Metabolism (ISRNM) Congress, hybrid format. Invited oral presentation entitled: 'Managing muscle wasting in advanced CKD through exercise and nutritional interventions'.

2021
European Society for Clinical Nutrition and Metabolism (ESPEN) Congress, online format.
Selected oral 'Best in theme' presentation entitled: 'Uremic toxin removal during hemodialysis is not compromised by protein ingestion or intradialytic exercise'.

American College of Sports Medicine (ACSM) Annual Meeting, online format.
Poster presentation entitled: 'Amino acid removal during hemodialysis can be compensated for by protein ingestion and is not affected by intradialytic exercise in end-stage renal disease patients'.

Pélerin Symposium, Maastricht, The Netherlands.
Selected pitch presentation (top 10 abstracts) entitled: 'Amino acid removal during hemodialysis can be compensated for by protein ingestion and is not compromised by intradialytic exercise: a randomized controlled cross-over trial'.
https://m.youtube.com/watch?v=8U3XO7CljGw&t=58s

2019
Dutch Nephrology Days, Veldhoven, The Netherlands.
Invited oral presentation entitled: 'Nutrition and physical activity in hemodialysis patients'.

Dialysis Initiatives Nephrology, Vaals, The Netherlands.
Invited oral presentation entitled: 'Removal of amino acids in patients who eat during hemodialysis'.

2018
European Society for Clinical Nutrition and Metabolism (ESPEN) Congress, Madrid, Spain.
Poster presentation entitled: 'Amino acid loss during hemodialysis in end-stage renal disease patients'.

Dutch federation for nephrology (NFN) Scientific Fall Symposium, Leiden, The Netherlands
Selected oral presentation (top 10 abstracts) entitled: 'Amino acid loss during hemodialysis in end-stage renal disease patients'.

Pélerin Symposium, Maastricht, The Netherlands.
Selected pitch presentation (top 10 abstracts) entitled: 'Amino Acid Loss During Hemodialysis In End-Stage Renal Disease Patients'

Financial support for printing this thesis was kindly provided by: