Does Regular Physical Activity During Hemodialysis Affect the Levels of FoxO Proteins and Myostatin Concentration?

Keywords
hemodialysis, renal disease, virtual reality, intradialytic exercise

Abstract

Introduction

The implementation of virtual reality (VR) technology in the context of hemodialysis represents a groundbreaking approach that has the potential to greatly enhance patients' motivation to engage in physical exercise. Physical exercise during hemodialysis has a positive impact on chronic inflammation, well-being, muscle mass and strength, and the risk of cardiovascular diseases. The aim of this study was to comprehensively evaluate the impact of regular physical activity using virtual reality during hemodialysis sessions on plasma concentrations of myostatin and FOXO proteins, with particular emphasis on enhancing training attractiveness and patient motivation.

Material and methods

The study was conducted at the Department of Nephrology, Transplantology, and Internal Diseases in Szczecin (Polnad). The study group was tasked with engaging in training sessions utilizing the NefroVR system prototype. In contrast, patients in the control group did not participate in any intervention. To assess the concentration of FOXO1, FOXO2, FOXO3 and myostatin parameters, blood was collected from the subjects twice: on the day of the study and after 3 months of its duration.

Results

A total of 102 patients were selected for participation in the study. The study group showed lower FOXO-1 values compared to the control group at measurement 2 ($p = 0.041$).
In the study group, a statistically significant correlation was observed between measurements 1 and 2 in the FOXO1 measurement ($p = 0.045$).

Conclusions

The findings of the study suggest that that training performed 3 times a week for 3 months through virtual reality (VR) could reduce FOXO-1 protein levels.

Title: The effect of Regular Physical Activity During Hemodialysis on Levels of FoxO Proteins and Myostatin Concentration

Abstract

Background: The implementation of virtual reality (VR) technology in the context of hemodialysis represents a groundbreaking approach that has the potential to greatly enhance patients' motivation to engage in physical exercise. Physical exercise during hemodialysis has a positive impact on chronic inflammation, well-being, muscle mass and strength, and the risk of cardiovascular diseases. The aim of this study was to comprehensively evaluate the impact of regular physical activity using virtual reality during hemodialysis sessions on plasma concentrations of myostatin and FOXO proteins, with particular emphasis on enhancing training attractiveness and patient motivation.

Methods: The study was conducted at the Department of Nephrology, Transplantology, and Internal Diseases in Szczecin (Polnad). The study and the control groups consisted of end-stage renal disease patients who underwent haemodialysis as a renal replacement treatment. The study group was tasked with engaging in training sessions utilizing the NefroVR system prototype. In contrast, patients in the control group did not participate in any intervention. To assess the concentration of FOXO1, FOXO2, FOXO3 and myostatin parameters, blood was collected from the subjects twice: on the day of the study and after 3 months of its duration.

Results: A total of 102 patients were selected for participation in the study. The study group showed lower FOXO-1 values compared to the control group at measurement 2 ($p = 0.041$). In the study group, a statistically significant correlation was observed between measurements 1 and 2 in the FOXO1 measurement ($p = 0.045$).

Conclusion: The findings of the study suggest that that training performed 3 times a week for 3 months through virtual reality (VR) could reduce FOXO-1 protein levels.

Keywords: hemodialysis, intradialytic exercise, renal disease, virtual reality

1. Introduction

Chronic kidney disease (CKD) is an increasingly common clinical and public health problem worldwide. It affects approximately 8% to 16% of the general adult population [1]. It is estimated that by 2040, CKD will become the fifth most common cause of death in the world [2]. Treatment of CKD is complex. It requires treatment of the pathophysiology of CKD itself and a multidisciplinary approach to reduce CKD risk factors and treat common complications and comorbidities [3]. In addition to pharmacological interventions, regular physical activity and appropriately selected exercises are considered a basic element of the lifestyle modification strategy in the treatment of CKD [4]. Oxidative stress plays a key role in the development and progression of CKD, causing kidney cell damage, inflammation, and fibrosis [5]. There is a lack of effective therapeutic interventions to slow the progression of CKD [6,7].

Chronic kidney disease is disabling control of muscle protein metabolism, which leads to muscle atrophy [6]. Myostatin is a protein belonging to the TGF-β (transforming growth factor beta) family. It is a myokine that is mainly expressed in skeletal muscle [8]. It inhibits the growth of muscle mass and also plays a key role as a mediator of muscle atrophy in chronic kidney disease. Moreover, it is a muscle growth inhibitor [9]. In an animal model, a 2- to 3-fold increase in muscle myostatin expression has been observed accompanying chronic kidney disease. Sarcopenia is a serious complication that significantly reduces the quality of patients' life [10]. Therefore, it has been suggested that inhibiting myostatin expression may be a strategy for the treatment of muscle wasting in chronic kidney disease [8, 11]. Studies indicate that plasma myostatin levels correlate with kidney function, highlighting its potential as a biomarker for assessing kidney health and muscle mass in patients with CKD. MSTN

expression is increased in skeletal muscles of patients with chronic kidney disease (CKD), which may play a role in the pathogenesis of sarcopenia or protein-energy wasting (PEW). The observation suggests that plasma MSTN levels might be correlated with renal function [12]. Low physical activity, persistent chronic inflammation and the accumulation of uremic toxins observed in patients with chronic kidney disease are factors that may contribute to the increase in myostatin production [13, 14].

Transcription factors belonging to the forkhead box class O (FOXO) family are integral to numerous cellular functions, thereby contributing significantly to the maintenance of cellular homeostasis [15, 16, 17] The group includes four molecules-FOXO1 (FKHR), FOXO3A (FKHRL1), FOXO4 (AFX1), and FOXO6—that affect various gene expression mechanisms. [18]. There are few studies in the human population, and existing studies on animal models based on the inhibition of the action of individual FOXO proteins emphasize their great importance in physiology [15, 19, 20]. FOXO transcription factors, due to their distribution in tissues and multidirectional actions, may constitute a therapeutic target in many diseases. Further research is necessary on the physiological effects of FOXO proteins and their participation in the pathogenesis of civilization diseases [21, 22, 23]. Studies have shown that FOXO3 is involved in the transition from acute to chronic kidney disease by regulating autophagy and the stress response. Activation of FOXO3 during hypoxia leads to its accumulation in the cell nucleus, where it acts as a transcription factor, promoting the expression of genes related to stress response and cell repair [24]. In turn, FOXO1 is crucial for the regulation of muscle energy homeostasis, playing an important role as a regulator of glucose metabolism in skeletal muscles [25]. In addition, FOXO1 and FOXO2 regulate the transcription of cell cycle genes that control cell proliferation and are involved in cancer, obesity, diabetes, autoimmune diseases, and aging [26].

Patients undergoing renal replacement therapy often have a negative attitude towards

physical exercise, which makes cooperation with them in this area extremely difficult. Modern technologies such as virtual reality are increasingly being incorporated into the rehabilitation process [27, 28]. The incorporation of VR technology into the hemodialysis process represents a groundbreaking approach that has the potential to enhance patients' motivation for physical activity. By integrating VR into rehabilitation protocols, patients can benefit from real-time feedback and engage in repetitive, functional exercises. Integrating these elements is zparticularly advantageous during hemodialysis, as it maximizes the efficiency of treatment time, alleviates the tedium associated with the procedure, boosts exercise motivation, and guarantees ongoing medical oversight [29-34]. More and more evidence indicates that physical exercise reduces levels of inflammatory markers (e.g. IL-6, CRP), improves psychological parameters (e.g. reduced anxiety and stress), increases muscle mass and strength, and reduces cardiovascular risk (e.g. reduced blood pressure, improved lipid profile) [13, 14, 35, 36, 37].

Further studies, including functional analysis and correlation of myostatin and FOXO protein expression levels with other clinical and metabolic parameters, may contribute to a better understanding of these mechanisms and enable the development of new therapeutic strategies.

The aim of this study was to comprehensively evaluate the impact of regular physical activity using virtual reality during hemodialysis sessions on plasma concentrations of myostatin and FOXO proteins, with particular emphasis on enhancing training attractiveness and patient motivation.

2. Material and methods

2.1 Qualification to participate in a research project

The study was conducted at the Department of Nephrology, Transplantology, and Internal Diseases at Pomeranian Medical University in Szczecin (Poland) between February 2021 and December 2021. The research focused on individuals diagnosed with the fifth stage of chronic kidney disease who were undergoing renal replacement therapy through hemodialysis at the dialysis facility within the Department of Nephrology, Transplantology, and Internal Diseases at Pomeranian Medical University in Szczecin. Participants in the research program were recruited by a nephrology specialist taking into account the inclusion and exclusion criteria.

The criteria for qualifying individuals to participate in the research project included: written consent to participate in the study, complete lack of diuresis, undergoing hemodialysis as renal replacement therapy for at least 3 months (three sessions per week) and age over 18 years. The exclusion criteria included: lack of written consent to participate in the study, musculoskeletal disease preventing participation in the study, serious cardiovascular diseases (NYHA III or IV heart failure), acute coronary syndrome in the last three months, uncontrolled arterial hypertension, uncorrected visual impairment, poorly controlled diabetes (HbA1c above 8% for 3 months), senile dementia, other neurological or mental disorders preventing consent to the study or understanding the nature of the study and the conditions of participation, malignant tumors, surgeries performed in the last month or amputation of the lower limb preventing the study or epilepsy.

2.2 Organization and schedule of the study

The study was conducted after obtaining the consent of the Bioethics Committee (number KB-0012/144/2020), issued on October 5, 2020. Each patient gave written informed

consent to participate in this study. Every effort has been made to protect the privacy and anonymity of patients. The study was conducted in accordance with the current version of the Declaration of Helsinki. Financing for the research was provided by the European Regional Development Fund under the grant number RPZP.01.01.00-32-0010/19-10. The costs of publication were covered by the Pomeranian Medical University in Szczecin.

Patients were randomly assigned to a study group or a control group. Researchers and study participants were aware of group assignment. The study group was tasked with engaging in training sessions utilizing the NefroVR system prototype, which occurred three times weekly for a duration of 20 minutes during hemodialysis (HD) over a period of three months. The sessions were conducted during the initial two hours of HD treatment or until a target ultrafiltration (UF) of 2.5 was reached. In contrast, patients in the control group received standard dialysis care, without additional exercise intervention.

2.3 Research tools

The research employed a prototype of the NefroVR system, which comprised various components assembled on a mobile platform equipped with ballast. Key elements of the system included: a central unit that integrates all components and operates specialized software; a rehabilitation rotor featuring a flywheel that enables load adjustment during exercises conducted during hemodialysis; virtual reality goggles designed for the patient to provide an immersive experience; a panoramic display for the patient; a touchscreen interface for medical personnel (such as doctors, nurses, or physiotherapists); and a patient control kit that consists of a digital joystick and buttons. The study had three identical NefroVR system sets available and were used in rotation by the study group participants.

The system operated based on the concept of audiovisual stimulation, motivating the patient to partake in physical activity through a virtual game that utilized a rehabilitation rotor

for movement. The rotor's speed influenced the game's tempo. The rotor was attached to the hemodialysis chair, thanks to which patients could easily perform the exercise during hemodialysis. Physical activity consisted of performing movements of the lower limbs on the rotor. Healthcare professionals, including physicians and physiotherapists, oversaw the rotor's revolutions and the flywheel's resistance, adjusting these parameters according to the patient's health status to prevent undue stress, such as elevated heart rates or hazardous fluctuations in blood pressure.

In the course of clinical trials, participants were presented with a selection of five mini-games, each with an approximate duration of 20 minutes. The selection of five mini games was made by a research team consisting of specialists in nephrology, physiotherapy and new technologies in medicine. The selection criteria included safety of dialysis patients, the possibility of performing exercises in a sitting position, the level of involvement and intuitive use. Alongside the use of the rotor, patients were able to engage with the game through a joystick and buttons. The extent of interactivity was deliberately constrained to facilitate the introduction of patients to the NefroVR system. Before the study began, all patients received training to use the VR system. When needed, medical staff assisted participants in using the device. The correctness of the exercises was assessed by a physiotherapist. His task was to supervise the session, correct any errors in technique and ensure the safety of patients during the exercises. Both the VR device and the games were dedicated to hemodialysis patients. Their selection took into account the specific needs of this group of patients, such as the ability to perform exercises in a sitting position, safety and intuitive operation.

The NefroVR research tool has also been used in other studies, where its detailed description is also available [29, 30, 40].

At the outset of the research (E0), participants were requested to fill out a tailored survey questionnaire specifically developed to address the objectives of this study. The

questionnaire included inquiries about participants' demographic characteristics, health conditions, lifestyle choices, and routine behaviors.

2.4 Laboratory tests

Two ml of blood was collected from study participants. Blood was collected twice: on the day of the study (E0) and after 3 months of its duration (E3). In both cases, samples were collected before the hemodialysis procedure. In the study participants, venous blood samples were obtained from the dialysis fistula prior to the initiation of the hemodialysis procedure. The samples were collected into test tubes containing EDTA at the dialysis facility located within the Department of Nephrology, Transplantology, and Internal Diseases at the Medical University of Szczecin, specifically on the designated days for cyclical evaluations (E0, E3). Following collection, the blood samples underwent centrifugation at 4000 rpm for a duration of 10 minutes at a temperature of 4°C, utilizing an MPW-350R centrifuge. The resulting blood plasma was aliquoted into two separate Eppendorf Safe-Lock Tubes (1.0 ml, Eppendorf Quality™, colorless), each containing 1.0 ml, and was promptly frozen. The samples were subsequently preserved at -70°C until they were ready for analysis. For laboratory testing, the samples were thawed at room temperature immediately prior to use.

Prior to the commencement of the study, standard plates were prepared in accordance with the guidelines provided by Sun Red Biotechnology Company. Subsequently, biotin-labeled antibodies, the test samples, and streptavidin were introduced to the plates. The volumes of the materials and reagents utilized were contingent upon the specific parameter being assessed. The plates underwent incubation for a duration of 60 minutes at a temperature of 37°C, followed by five washes with a washing buffer. Afterward, Chromogen A and B were added, and the mixture was incubated for an additional 10 minutes at 37°C before the application of the stopping solution. Absorbance readings were taken at a wavelength of 450

nm, and data analysis was conducted using the Envision® program, which was based on a linear calibration curve.

The evaluation of myostatin and FOXO protein levels in plasma was conducted utilizing the commercially available Human MSTN test and the FOXO Elisa Kit from SunRed Biotechnology Company. Myostatin concentrations were reported in nanograms per liter (Ng/L), with a sensitivity threshold of 5.113 Ng/L and a linearity range extending from 7 to 2000 ng/L. Additionally, myostatin levels were expressed in ng/ml, featuring a sensitivity range of 0.175 ng/ml and a linearity range of 0.2 to 60 ng/ml. The concentration values for FOXO proteins were measured in Ng/L, with a sensitivity range of 2.827 ng/L and a linearity range from 3 to 900 ng/L.

2.5 Statistical analysis

Statistical analyzes were performed using Statistica 13 (StatSoft, Inc., Tulsa, OK, USA). All data regarding continuous variables were presented as means ± standard deviation (±SD) and medians, while qualitative variables were presented as numbers and percentages. The normality of the distribution was tested using the Shapiro-Wilk test. The data used in the study did not meet the assumptions of a normal distribution. The chi-squared test or chi-squared test with Yates' correction was used to analyze qualitative data. The Mann-Whitney U test was employed to analyze continuous variables across different groups. The evaluation of laboratory data collected prior to and following the intervention in both the study and control groups was conducted utilizing the Wilcoxon test. A p-value of less than 0.05 was deemed indicative of statistically significant differences.

3. Results

Table 1 presents the characteristics of the study and control groups. The analysis of data regarding demographics, comorbidities and habitual behaviors did not reveal statistically significant differences between these groups.

Table 1. The distinguishing features of the study and control groups.

		Study group (n=39)		**Control group** (n=46)		P
Age, mean±SD; Me		57.56±17.61; 63.0		62.63±15.47; 64.0		0.266 [a]
Sex, n (%)	males	29	74.36%	29	63.04%	0.264 [b]
	females	10	25.64%	17	36.96%	
BMI (kg/m2)		28.23±5.75; 28.28		27.89±5.79; 27.57		0.760 [a]
Professional activity, n (%)	no	26	72.22%	40	88.89%	0.103 [b]
	yes	10	27.78%	5	11.11%	
Professional activity before the start of haemodialysis, n (%)	no	10	27.78%	21	46.67%	0.082 [b]
	yes	26	72.22%	24	53.33%	
Type of job, n (%)	blue-collar	18	47.37%	18	54.55%	0.829 [b]
	white-collar	11	28.95%	7	21.21%	
	no job	9	23.68%	8	24.24%	
Currently smoking cigarettes, n (%)	no	30	76.92%	35	76.09%	0.868 [b]
	yes	9	23.08%	11	23.91%	
Number of cigarettes per day, mean±SD; Me		14.44±6.13; 15.0		14.09±7.41; 10.0		0.676 [a]
How many years ago quit smoking, mean±SD; Me		9.71±10.95; 5.0		16.67±16.17; 13.0		0.520 [a]

Number of HD per week, mean±SD; Me	2.95±0.23; 3.0		2.98±0.15; 3.0		0.805 a
Duration of dialysis [min], mean±SD; Me	223.85±20.47; 240.0		216.52±28.92; 210.0		0.110 a
Concomitant diseases					
Diabetes, n (%)	5	14.71%	13	28.89%	0.224 b
Arterial hypertension, n (%)	25	73.53%	32	71.11%	0.812 b
Epilepsy, n (%)	4	12%	3	7%	0.697 b
Ophthalmic, n (%)	8	24%	15	33%	0.484 b
Neurological, n (%)	2	6%	3	3	0.745 b
Treatment with another renal replacement therapy, n (%)	7	20.59%	8	17.78%	0.979 b

Legend: n - number of patients, SD - standard deviation, Me - median, HD - hemodialysis, p - level of statistical significance, * statistical significance; a-Mann-Whitney U test; b - chi-squared test

A total of 102 patients, comprising 65 males and 37 females, were selected for participation in the study. Of these, 85 patients who underwent renal replacement therapy via hemodialysis were included, consisting of 58 males and 27 females, as illustrated in Figure 1. The participants were randomly assigned to two distinct groups: the experimental group, which included 39 participants (mean 57.56, SD=17.61), and the control group, consisting of 46 patients (mean 62.63; SD=15.47). All patients reported a level of physical fatigue during the exercise, which was assessed at 8-14 on the Borg scale.

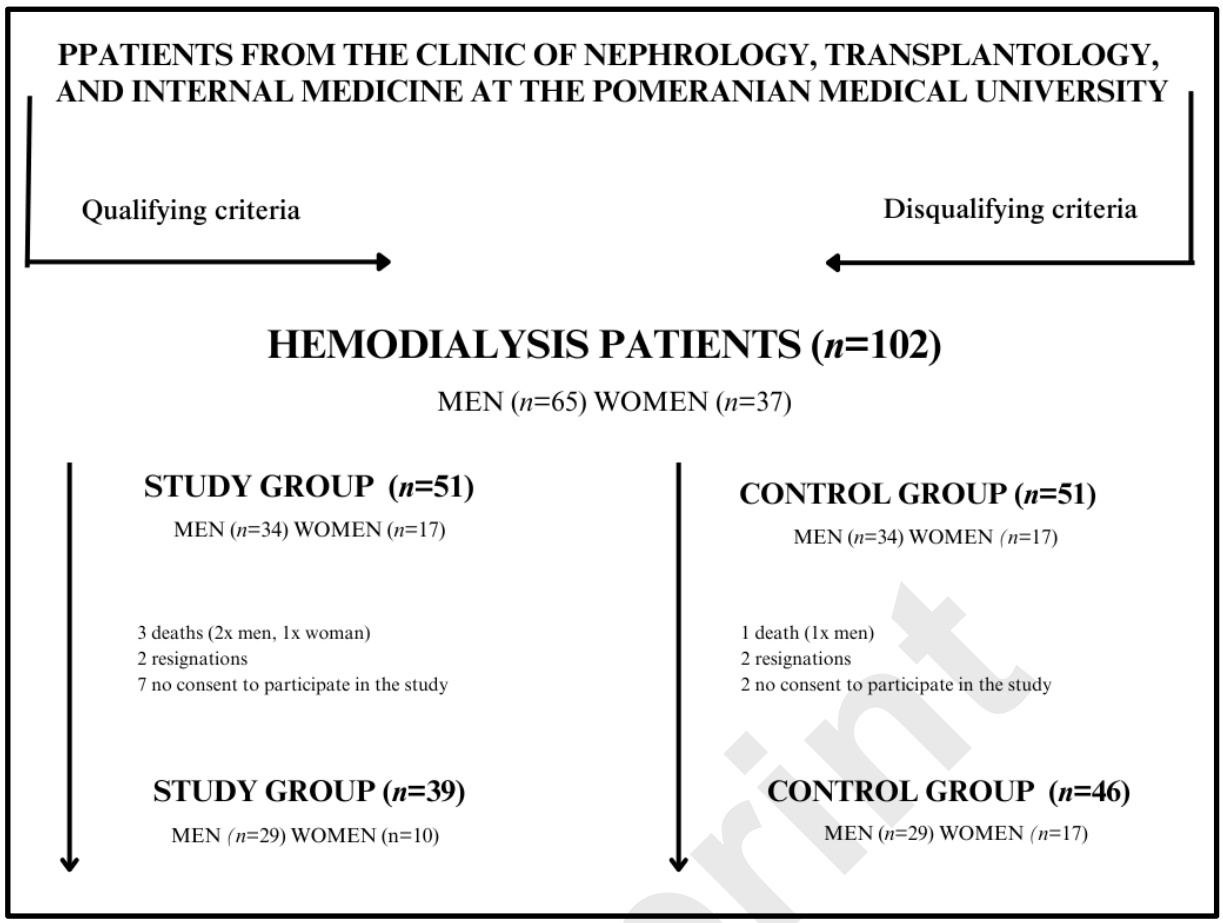


Figure 1. Qualification framework for the research.

The Table 2 presents a comparison of the values of four laboratory parameters (FOXO-1, FOXO-2, FOXO-3, and myostatin between the study and control groups. For each parameter, the mean (M), standard deviation (SD), median (Me), lower quartile (Q1), and upper quartile (Q3) are presented for both groups. In measurement 2, a statistically significant difference between the groups was noted ($p = 0.041$), where the study group showed lower FOXO-1 values compared to the control group. In the remaining parameters (FOXO-2, FOXO-3, and myostatin), no statistically significant differences were found between the groups in any of the measurements ($p > 0.05$).

Table 2. The examination of variations in the laboratory parameters assessed between the experimental and control groups.

Variable	Study group		Control group		p (statistical power of the test)
	M (SD)	Me (Q1-Q3)	M (SD)	Me (Q1-Q3)	
Measurement 1					
FOXO-1	23.66 (65.07)	7.49 (3.90-13.18)	13.36 (11.12)	8.53 (6.88-18.39)	0.176 (0,25)
FOXO-2	4.71 (4.91)	2.92 (1.63 -5.93)	4.67 (4.47)	2.37 (1.80-7.11)	0.917 (0.05)
FOXO-3	4.88 (4.92)	2.66 (1.64-6.44)	4.74 (6.05)	2.31 (1.78-5.62)	0.966 (0.05)
Myostatin	681.40 (590.68)	565.755 (140.7250-1272.15)	517.21 (422.23)	412.13 (250.7- 657.1)	0.483 (0.31)
Measurement 2					
FOXO-1	10.14 (14.06)	4.88 (3.47-9.92)	13.87 (12.34)	9.03 (6.46-19.43)	0.041* (0,23)
FOXO-2	3.48 (3.68)	2.23 (1.34-3.92)	5.10 (4.95)	2.84 (1.82-5.89)	0.169 (0.50)
FOXO-3	4.85 (6.23)	3.06 (1.45-5.90)	5.26 (4.97)	3.18 (2.07-6.89)	0.151 (0.061)
Myostatin	669.18 (659.03)	416.34 (194.79-1087.70)	618.51 (602.94)	404.29 (123.54-1061.42)	0.522 (0.065)

Legend: Measurement 1 - measurement on the day of the study (E0); Measurement 2 - measurement after 3 months (E3); M - mean; SD - standard deviation; Me - median; Q1 - lower quartile; Q3 - upper quartile; p - statistical significance; * $p<0.05$ (Mann-Whitney U test)

The Table 3 presents a comparison of the correlations between two measurements (measurement 1 and measurement 2) of four laboratory parameters (FOXO-1, FOXO-2, FOXO-3, and myostatin) in the study and control groups. In the study group, a statistically significant correlation between measurements 1 and 2 in the FOXO1 measurement was observed ($p = 0.045$). In the control group, no statistically significant correlation was found ($p = 0.434$). In both groups, no statistically significant correlations between measurements 1 and 2 were found for the remaining parameters ($p > 0.05$).

Table 3. The examination of the relationship between the initial and subsequent laboratory test measurements within both the study and control groups.

Variable	Study Group	Control Group
	p (statistical power of the test)	p (statistical power of the test)

FOXO1	Measurement 1	0.045* (0.94)	0.434 (0.35)
	Measurement 2		
FOXO2	Measurement 1	0.075 (0.15)	0.777 (0.09)
	Measurement 2		
FOXO3	Measurement 1	0.658 (0.08)	0.502 (0.21)
	Measurement 2		
Myostatin	Measurement 1	0.829 (0.89)	0.875 (0.95)
	Measurement 2		

Legend: Measurement 1 - measurement on the day of the study (E0); Measurement 2 - measurement after 3 months (E3); p - statistical significance; * $p<0.05$ (Wilcoxon test)

4. Discussion

In the conducted study assessing the impact of virtual reality exercises performed over a three-month period on FoXO proteins and myostatin concentrations, a statistically significant difference was observed in FOXO-1 protein levels between the experimental and control groups after three months. Additionally, a reduction in FOXO-1 protein concentration was noted in the experimental group following the three-month exercise intervention. No significant statistical differences were found for the other analyzed parameters. The observed differences between groups may be attributed to the implementation of the exercise intervention using VR technology, which—by increasing patient engagement and diverting

attention from the discomfort of the dialysis environment—may have contributed to more consistent and effective participation in physical activity. This, in turn, could have influenced favorable changes in biomarkers such as myostatin and FOXO protein levels. However, we cannot exclude the influence of additional factors, such as individual motivation, fatigue level, or undetected differences in the clinical status of participants.

An increasing number of research is concentrating on the encouragement of physical activity among patients undergoing hemodialysis, highlighting its advantages for both mental and physical well-being, which encompass enhanced laboratory outcomes and improved physical performance. However, motivating these patients to exercise is a challenge due to their poor condition and lack of hope for full recovery. The use of virtual reality (VR) technology in dialysis settings may reduce patients' subjective perception of treatment duration, improve mood, increase engagement in intradialytic exercise, and support adherence to therapeutic recommendations [38-44].

In the study, after a three-month intervention using virtual reality (VR) during physical activity, a statistically significant difference in FOXO-1 protein concentrations was found between the study group and the control group. Patients from the study group who participated in the VR program showed lower FOXO-1 concentrations compared to the control group. A notable decrease in FOXO-1 levels was observed following a three-month intervention with the NefroVR system in the experimental group. The changes observed in FOXO-1 are specific to this protein and may be the result of the body's specific response to VR-related stimuli. The findings suggest that physical activity, particularly engaging and motivating forms such as those involving VR, may induce changes regulated by FOXO-1. No comparable studies exist in the literature that have evaluated these parameters within a group of patients undergoing hemodialysis. Therefore, the study can be considered pioneering in this field.

Few studies on FOXO expression were found in the literature. For example, in an experimental study performed by Reed et al. animal model showed that FOXO expression in skeletal muscles increased significantly during the period of cachexia (3.1-fold in the soleus). In response to inflammation, FOXO1 activity increases (1.8-fold in the soleus), and aerobic exercise may slightly reduce its acetylation [45].

The results of our study indicate no significant differences in FOXO-2 and FOXO-3 protein concentrations in patients with stage 5 chronic kidney disease treated with hemodialysis who participated in a regular physical activity program using VR technology. Although previous studies have suggested a significant role of FOXO proteins, especially FOXO3, in the regulation of cellular mechanisms related to the response to oxidative stress, autophagy and in the transition from acute to chronic kidney disease, our results did not confirm clear changes in the concentration of these proteins as a result of physical intervention.

It is important to emphasize that the absence of notable variations in the concentrations of FOXO-2 and FOXO-3 does not diminish the possible health advantages linked to consistent physical activity among individuals with CKD. As other studies show, exercise can improve inflammation, cardiovascular function and overall quality of life in patients, even if it does not directly affect the expression of specific transcription proteins.

It is worth noting that in our study, FOXO protein concentrations were determined using the ELISA method, while most previous studies focused on the analysis of FOXO gene expression at the mRNA level. Differences in methodological approach may partially explain the lack of significant differences in our results. Determining proteins directly from plasma samples allows for the assessment of their actual concentration in the body, but this does not always reflect changes in their gene expression that may occur at earlier stages of the transcription process [46, 47].

Myostatin, known as a myokine that negatively regulates muscle growth, is a well-studied factor whose levels may change with physical activity. Previous studies have shown that regular physical exercise can reduce the concentration of myostatin, which helps increase muscle mass and improve muscle strength [38, 44, 48]. However, in the context of our study, in which patients underwent three months of virtual reality exercises, no significant changes in myostatin levels were observed. The lack of significant change may be due to several factors. First, the intensity and type of physical activity may not be sufficient to induce changes in myostatin concentrations. While virtual reality can increase motivation to exercise, it can also reduce the intensity of exercise, which is key to inducing significant changes in myostatin levels. Second, a three-month period could be too short to see noticeable changes, especially in patients with chronic kidney disease, whose metabolism and body responses may be slowed. Additionally, individual differences in response to exercise may also influence results.

The lack of clear changes in the concentration of the analyzed proteins may be due to several factors. Possible reasons include the relatively short duration of the intervention, the moderate intensity of the exercises, as well as the large individual variability in the physiological response. Additionally, the results could have been influenced by confounding factors such as nutritional status, chronic inflammation level, or vitamin D level, which were not controlled in this study.

Our study did not include a group of healthy individuals as a reference point, so we cannot directly compare myostatin and FOXO protein values to the healthy population. Studies suggest that circulating FOXO1 levels in healthy adults are in the tens of ng/ml range. For example, in healthy pregnant women, the mean serum FOXO1 concentration was approximately 29.1 ± 3.2 ng/ml [49]. There are no reference data for FOXO2 protein in the literature. It is assumed that circulating FOXO2 levels in healthy individuals are similar to those reported for FOXO3. For example, in a group of healthy adults, the median FOXO3

concentration was approximately 30 ng/ml (range ~5–56 ng/ml) [50]. The reference range for myostatin in healthy adults is approximately 7–32 ng/ml, and its concentration decreases slightly with age [51]. The above values constitute reference points (reference levels) for the healthy population, which enables comparison with the results of patients in the interventional study.

Further research should focus on more intense exercise programs and longer monitoring periods to better understand the effects of VR on FOXO protein expression and myostatin levels. Such studies can provide important information on the long-term effects of VR interventions on the metabolic health and overall health of dialysis patients, which is crucial for the development of effective rehabilitation strategies and motivation for physical activity. Furthermore, to determine whether the observed effect on FOXO-1 concentration results specifically from the use of VR or from the physical activity itself, further studies are needed to compare groups performing identical training with and without VR.

Limitations

The study conducted had many limitations. First of all, the Covid-19 pandemic limited the study to one dialysis center, which affected the representativeness and sample size. Additionally, the deteriorating health status of patients with CKD and the presence of comorbidities could have significantly influenced the study results. Vitamin D status was not assessed in our study, which is a limitation. Since myostatin levels may be dependent on vitamin D, it is necessary to include this measurement in future studies. The degree of fatigue of the patients may have influenced the results, especially in the context of physical activity performed during dialysis. Although fatigue was not assessed quantitatively in our study, the observations of the physiotherapist and the subjective feelings of the patients suggested good

exercise tolerance. Nevertheless, this could have been a potential confounding factor. Motivating hemodialysis patients to regularly participate in an exercise program was also a challenge. The study group experienced a reduction in population throughout the project, attributed to various independent factors, including kidney transplantation and participant mortality. The incorporation of VR as a motivational instrument may have enhanced patients' willingness to engage in the prescribed intervention. Future research should consider expanding both the study and control groups to facilitate a more accurate evaluation of the intervention's effects and to support a more detailed development of research findings within the cohort of patients suffering from CKD.

5. Conclusions

The findings of the study suggest that that training performed 3 times a week for 3 months through virtual reality (VR) could reduce FOXO-1 protein levels. The study is pioneering as no previous work has been found on these parameters in the context of CKD patients participating in VR programs.

References

1. Webster, A.C.; Nagler, E.V.; Morton, R.L.; Masson, P. Chronic kidney disease. *Lancet* 2017, *389*, 1238–1252.
2. Kovesdy, C.P. Epidemiology of chronic kidney disease: An update 2022. *Kidney Int. Suppl.* 2022, *12*, 7–11.
3. Campbell, D.; Weir, M.R. Defining, Treating, and Understanding Chronic Kidney Disease—A Complex Disorder. *J. Clin. Hypertens.* 2015, *17*, 514–527.
4. Kalantar-Zadeh, K.; Jafar, T.H.; Nitsch, D.; Neuen, B.L.; Perkovic, V. Chronic kidney disease. *Lancet* 2021, *398*, 786–802.

5. Zheng CM, Hou YC, Liao MT, Tsai KW, Hu WC, Yeh CC, Lu KC. Potential role of molecular hydrogen therapy on oxidative stress and redox signaling in chronic kidney disease. Biomed Pharmacother. 2024 Jul;176:116802. doi: 10.1016/j.biopha.2024.116802.
6. Su Z, Klein JD, Du J, Franch HA, Zhang L, Hassounah F, Hudson MB, Wang XH. Chronic kidney disease induces autophagy leading to dysfunction of mitochondria in skeletal muscle. Am J Physiol Renal Physiol. 2017 Jun 1;312(6):F1128-F1140. doi: 10.1152/ajprenal.00600.2016.
7. Luyckx VA, Cherney DZI, Bello AK. Preventing CKD in Developed Countries. Kidney Int Rep. 2019 Dec 18;5(3):263-277. doi: 10.1016/j.ekir.2019.12.003.
8. Zhou Y, Hellberg M, Hellmark T, Höglund P, Clyne N. Muscle mass and plasma myostatin after exercise training: a substudy of Renal Exercise (RENEXC)-a randomized controlled trial. Nephrol Dial Transplant. 2021 Jan 1;36(1):95-103. doi: 10.1093/ndt/gfz210.
9. Stanislas Bataille, Philippe Chauveau, Denis Fouque, Michel Aparicio, Laetitia Koppe, Myostatin and muscle atrophy during chronic kidney disease, *Nephrology Dialysis Transplantation*, 2021, 36:(11), 1986–1993.
10. Zhang L, Rajan V, Lin E, Hu Z, Han HQ, Zhou X, Song Y, Min H, Wang X, Du J, Mitch WE. Pharmacological inhibition of myostatin suppresses systemic inflammation and muscle atrophy in mice with chronic kidney disease. FASEB J. 2011 May;25(5):1653-63. doi: 10.1096/fj.10-176917.
11. Wang XH, Mitch WE.. Mechanisms of muscle wasting in chronic kidney disease. *Nat Rev Nephrol* 2014; 10: 504–516.
12. Yano S, Nagai A, Isomura M, Yamasaki M, Kijima T, Takeda M, Hamano T, Nabika T. Relationship between Blood Myostatin Levels and Kidney Function:Shimane

CoHRE Study. PLoS One. 2015, 26;10(10):e0141035. doi: 10.1371/journal.pone.0141035.

13. Baczek, J.; Silkiewicz, M.; Wojszel, Z.B. Myostatin as a Biomarker of Muscle Wasting and other Pathologies-State of the Art and Knowledge Gaps. *Nutrients* 2020, *12*, 2401. https://doi.org/10.3390/nu12082401
14. Bataille S, Chauveau P, Fouque D, Aparicio M, Koppe L. Myostatin and muscle atrophy during chronic kidney disease. Nephrol Dial Transplant. 2021 Nov 9;36(11):1986-1993. doi: 10.1093/ndt/gfaa129.
15. Marchelek-Mysliwiec, M.; Nalewajska, M.; Turoń-Skrzypińska, A.; Kotrych, K.; Dziedziejko, V.; Sulikowski, T.; Pawlik, A. The Role of Forkhead Box O in Pathogenesis and Therapy of Diabetes Mellitus. *Int. J. Mol. Sci.* 2022, *23*, 11611.
16. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell 2004; 117(4): 421– 426. doi: 10.1016/s0092-8674(04)00452-0.2.
17. Lam EW, Brosens JJ, Gomes AR, Koo CY. Forkhead box proteins: tuning forks for transcriptional harmony. Nat Rev Cancer. 2013; 13(7): 482–495. doi: 10.1038/nrc3539.
18. Kaestner KH, Knochel W, Martinez DE. Unified nomenclature for the winged helix/Forkhead transcription factors. Genes Dev. 2000; 14(2): 142–146.
19. Du, S., Zheng, H. Role of FoxO transcription factors in aging and age-related metabolic and neurodegenerative diseases. *Cell Biosci*, 2021, 11, 188.
20. Xing YQ, Li A, Yang Y, Li XX, Zhang LN, Guo HC. The regulation of FOXO1 and its role in disease progression. Life Sci. 2018 Jan 15;193:124-131. doi: 10.1016/j.lfs.2017.11.030.
21. Calissi, G., Lam, E.WF. Link, W. Therapeutic strategies targeting FOXO transcription factors. *Nat Rev Drug Discov* 2021, 20, 21–38.

22. Orea-Soufi A, Paik J, Bragança J, Donlon TA, Willcox BJ, Link W. FOXO transcription factors as therapeutic targets in human diseases. Trends Pharmacol Sci. 2022 Dec;43(12):1070-1084. doi: 10.1016/j.tips.2022.09.010.
23. Calissi G, Lam EW, Link W. Therapeutic strategies targeting FOXO transcription factors. Nat Rev Drug Discov. 2021 Jan;20(1):21-38. doi: 10.1038/s41573-020-0088-2.
24. Lin F. Molecular regulation and function of FoxO3 in chronic kidney disease. Curr Opin Nephrol Hypertens. 2020 Jul;29(4):439-445. doi: 10.1097/MNH.0000000000000616.
25. Lin F. Molecular regulation and function of FoxO3 in chronic kidney disease. Curr Opin Nephrol Hypertens. 2020 Jul;29(4):439-445. doi: 10.1097/MNH.0000000000000616.
26. Madhyastha H, Madhyastha R, Nakajima Y, Maruyama M. Deciphering the molecular events during arsenic induced transcription signal cascade activation in cellular milieu. Biometals. 2018;31(1):7-15. doi: 10.1007/s10534-017-0065-3.
27. Chen J, Yan S, Yin H, et al. Virtual reality technology improves the gait and balance function of the elderly: a meta-analysis of randomized controlled trials. Archives of Medical Science. 2024;20(6):1918-1929.
28. Shousha T, Abo-zaid N, Hamada HA, Abdelhamid Abdelsamee M, Behiry M. Virtual reality versus Biodex training in adolescents with chronic ankle instability: a randomized controlled trial. Archives of Medical Science. 2023;19(4):1059-1068.
29. Turoń-Skrzypińska A, Rotter I, Przybyciński J, Szylińska A, Mińko A, Ciechanowski K, Dutkiewicz G. Does Exercising with the Use of Virtual Reality during Haemodialysis Have an Impact on Plasma Levels of Interleukin 1β, Interleukin 6, and Interleukin 8? J Clin Med. 2023 Aug 17;12(16):5358. doi: 10.3390/jcm12165358.

30. Turoń-Skrzypińska A, Tomska N, Mosiejczuk H, Rył A, Szylińska A, Marchelek-Myśliwiec M, Ciechanowski K, Nagay R, Rotter I. Impact of virtual reality exercises on anxiety and depression in hemodialysis. Sci Rep. 2023 Aug 1;13(1):12435. doi: 10.1038/s41598-023-39709-y.
31. Lorenz E.C., Kennedy C.C., Rule A.D., LeBrasseur N.K., Kirkland J.L., Hickson L.J., Kidney International Reports, (9), 2021, s. 2270-2280 Dis 2010; 55 (1): 88-99.
32. Rutkowski S., Rutkowska A., Jastrzębski D., Racheniuk H., Pawełczyk W., Szczegielniak J., Effect of Virtual Reality-Based Rehabilitation on Physical Fitness inPatients with Chronic Obstructive Pulmonary Disease, J Hum Kinet, 2019, 69, s. 149-157. doi: 10.2478 / hukin-2019-0022.
33. Segura-Ortí E., Pérez-Domínguez B., Ortega-Pérez de Villar L., Meléndez-Oliva E., Martínez-Gramage J., García-Maset R., Gil-Gómez J.A., *Virtual reality exercise intradialysis to improve physical function: A feasibility randomized trial*. Scand J Med Sci Sports, 2019, 29(1), s.89-94. doi: 10.1111/sms.13304.
34. Cho H., Sohng K.Y., *The effect of a virtual reality exercise program on physical fitness, body composition, and fatigue in hemodialysis patients*. J Phys Ther Sci, 2014, 26(10), s. 1661-5. doi: 10.1589/jpts.26.1661.
35. Slopack D, Roudier E, Liu ST, Nwadozi E, Birot O, Haas TL. Forkhead BoxO transcription factors restrain exercise-induced angiogenesis. J Physiol. 2014 Sep 15;592(18):4069-82. doi: 10.1113/jphysiol.2014.275867.
36. Kamani CH, Gencer B, Montecucco F, Courvoisier D, Vuilleumier N, Meyer P, et al. Stairs instead of elevators at the workplace decreases PCSK9 levels in a healthy population. *Eur J Clin Invest.* 2015;45:1017–1024. doi: 10.1111/eci.12480.
37. Meléndez Oliva, E.; Villafañe, J.H.; Alonso Pérez, J.L.; Alonso Sal, A.; Molinero Carlier, G.; Quevedo García, A.; Turroni, S.; Martínez-Pozas, O.; Valcárcel Izquierdo,

N.; Sánchez Romero, E.A. Effect of Exercise on Inflammation in Hemodialysis Patients: A Systematic Review. *J. Pers. Med.* 2022, *12*, 1188.

38. Bennett PN, Thompson S, Wilund KR. An introduction to Exercise and Physical Activity in Dialysis Patients: Preventing the unacceptable journey to physical dysfunction. Semin Dial. 2019 Jul;32(4):281-282. doi: 10.1111/sdi.12816. PMID: 31264290.
39. Turoń-Skrzypińska A, Rotter I, Przybyciński J, Szylińska A, Mińko A, Ciechanowski K, Dutkiewicz G. Does Exercising with the Use of Virtual Reality during Haemodialysis Have an Impact on Plasma Levels of Interleukin 1β, Interleukin 6, and Interleukin 8? J Clin Med. 2023 Aug 17;12(16):5358. doi: 10.3390/jcm12165358.
40. Turoń-Skrzypińska A, Dutkiewicz G, Marchelek-Myśliwiec M, Rył A, Dziedziejko V, Safranow K, Ciechanowski K, Rotter I. Physical Activity versus Sclerostin and Interleukin 6 Concentration in Patients Receiving Renal Replacement Therapy by Hemodialysis. Risk Manag Healthc Policy. 2020 Sep 7;13:1467-1475.
41. Burrows, B.T.; Morgan, A.M.; King, A.C.; Hernandez, R.; Wilund, K.R. Virtual Reality Mindfulness and Personalized Exercise for Patients on Hemodialysis with Depressive Symptoms: A Feasibility Study. Kidney Dial. 2023, 3, 297-310.
42. Clarke, A.L.; Jhamb, M.; Bennett, P.N. Barriers and facilitators for engagement and implementation of exercise in end-stage kidney disease: Future theory-based interventions using the Behavior Change Wheel. *Semin. Dial.* 2019, *32*, 308–319.
43. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. 2019 Jun 15;11(3):45-63. PMID: 31333808; PMCID: PMC6628012.
44. Mansoor G, Tahir M, Maqbool T, Abbasi SQ, Hadi F, Shakoori TA, Akhtar S, Rafiq M, Ashraf M, Ullah I. Increased Expression of Circulating Stress Markers,

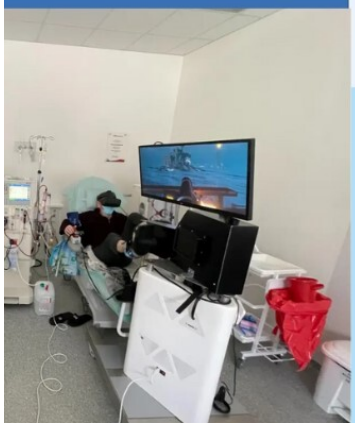
Inflammatory Cytokines and Decreased Antioxidant Level in Diabetic Nephropathy. Medicina (Kaunas). 2022 Nov 7;58(11):1604. doi: 10.3390/medicina58111604.

45. Reed SA, Sandesara PB, Senf SM, Judge AR. Inhibition of FoxO transcriptional activity prevents muscle fiber atrophy during cachexia and induces hypertrophy. FASEB J. 2012 Mar;26(3):987-1000. doi: 10.1096/fj.11-189977.
46. Du, S., Zheng, H. Role of FoxO transcription factors in aging and age-related metabolic and neurodegenerative diseases. Cell Biosci, 2021, 11, 188.
47. Frederik Seiler, Jan Hellberg, Philipp M. Lepper, Andreas Kamyschnikow, Christian Herr, Markus Bischoff, Frank Langer, Hans-Joachim Schäfers, Frank Lammert, Michael D. Menger, Robert Bals, Christoph Beisswenger; FOXO Transcription Factors Regulate Innate Immune Mechanisms in Respiratory Epithelial Cells. J Immunol 15 February 2013; 190 (4): 1603–1613.
48. Gonzalez-Gil AM, Elizondo-Montemayor L. The Role of Exercise in the Interplay between Myokines, Hepatokines, Osteokines, Adipokines, and Modulation of Inflammation for Energy Substrate Redistribution and Fat Mass Loss: A Review. Nutrients. 2020 Jun 26;12(6):1899. doi: 10.3390/nu12061899.
49. Akkaya Fırat, A, Alıcı Davutoğlu, E, Özel, A. et al. Serum FoxO1 and SIRT2 concentrations in healthy pregnant women and complicated by preeclampsia. Ir J Med Sci 194, 181–188 (2025).
50. Hofny, Eman R.M.; Twisy, Howida O.,; Bamatraf, Maysaa S.; Hasan, Howayda I.; Mohamed, Nahed A.; Mahran, Ayman M.. Serum versus tissue levels of FoxO3a, zinc, and iron in patients with vitiligo: is oxidative stress a local process?. Journal of the Egyptian Women's Dermatologic Society 20(1):p 26-32, 2023.

51. Han DS, Huang CH, Chen SY, Yang WS. Serum reference value of two potential doping candidates-myostatin and insulin-like growth factor-I in the healthy young male. J Int Soc Sports Nutr. 2017 Jan 5;14:2. doi: 10.1186/s12970-016-0160-9.

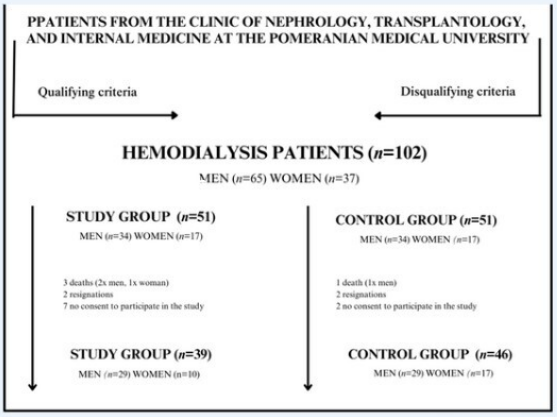
Intradialytic Exercise

NefroVR



Aim of the study

Assess the impact of regular physical activity using virtual reality on the concentration of plasma myostatin and FOXO proteins



Conclusion

The findings of the study suggest that consistent engagement in physical activity through virtual reality (VR) could influence the levels of FOXO-1 protein.

Table 1. Characteristics of the study and control groups.

		Study group (n=39)		**Control group** (n=46)		p
Age, mean±SD; Me		57.56±17.61; 63.0		62.63±15.47; 64.0		0.266
Sex, n (%)	males	29	74.36%	29	63.04%	0.264
	females	10	25.64%	17	36.96%	
BMI (kg/m2)		28.23±5.75; 28.28		27.89±5.79; 27.57		0.760
Professional activity, n (%)	no	26	72.22%	40	88.89%	0.103
	yes	10	27.78%	5	11.11%	
Professional activity before the start of haemodialysis, n (%)	no	10	27.78%	21	46.67%	0.082
	yes	26	72.22%	24	53.33%	
Type of job, n (%)	blue-collar	18	47.37%	18	54.55%	0.829
	white-collar	11	28.95%	7	21.21%	
	no job	9	23.68%	8	24.24%	
Currently smoking cigarettes, n (%)	no	30	76.92%	35	76.09%	0.868
	yes	9	23.08%	11	23.91%	
Number of cigarettes per day, mean±SD; Me		14.44±6.13; 15.0		14.09±7.41; 10.0		0.676
How many years ago quit smoking, mean±SD; Me		9.71±10.95; 5.0		16.67±16.17; 13.0		0.520
Number of HD per week, mean±SD; Me		2.95±0.23; 3.0		2.98±0.15; 3.0		0.805
Duration of dialysis [min], mean±SD; Me		223.85±20.47; 240.0		216.52±28.92; 210.0		0.110
Concomitant diseases						
Diabetes, n (%)		5	14.71%	13	28.89%	0.224
Arterial hypertension, n (%)		25	73.53%	32	71.11%	0.812
Epilepsy, n (%)		4	12%	3	7%	0.697
Ophthalmic, n (%)		8	24%	15	33%	0.484
Neurological, n (%)		2	6%	3	3	0.745
Treatment with another renal replacement therapy, n (%)		7	20.59%	8	17.78%	0.979

Legend: n - number of patients, SD - standard deviation, Me - median, HD - hemodialysis, p - level of statistical significance, * statistical significance

Table 2. Analysis of differences between the tested laboratory parameters in the study and control groups.

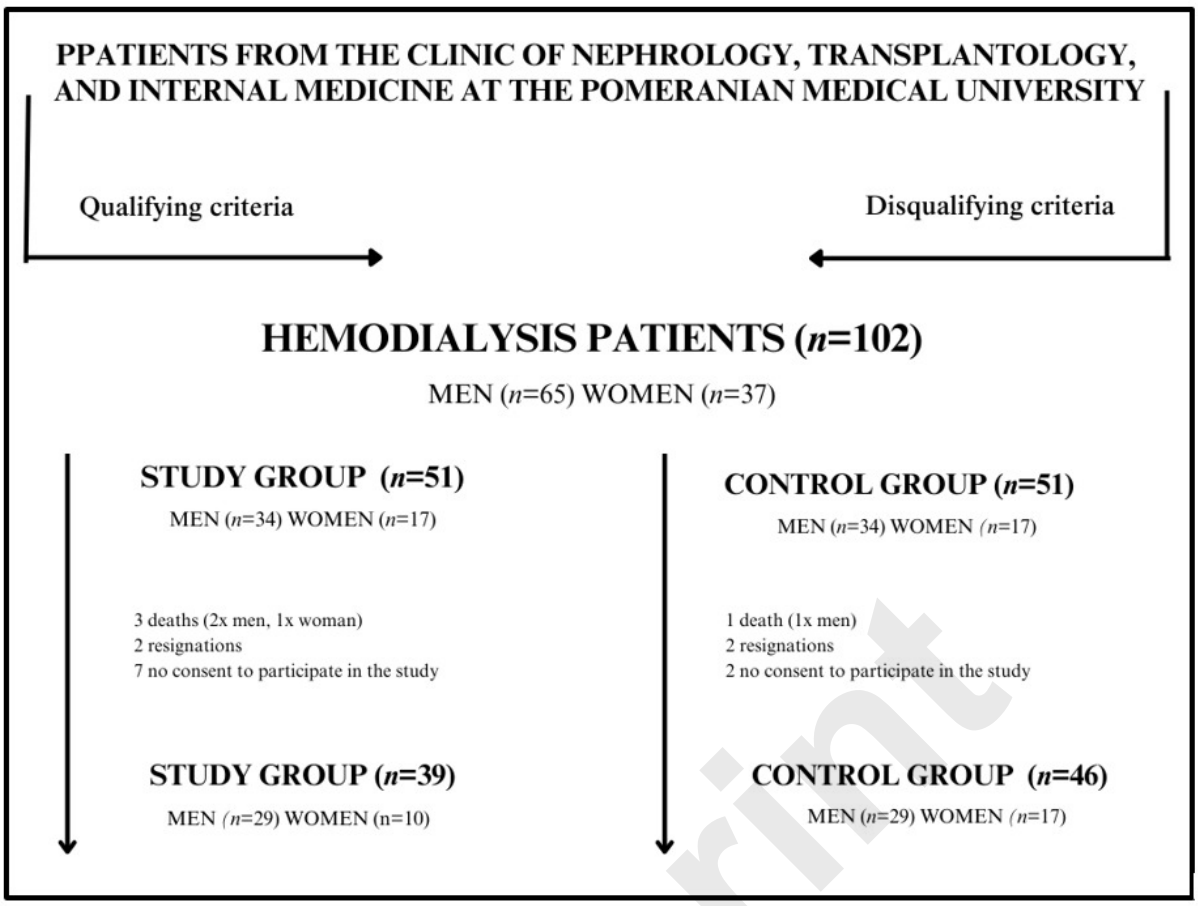
Variable	Study group	Control group	p
	M (SD)	M (SD)	
Measurement 1			
FOXO-1	23.66 (65.07)	13.36 (11.12)	0.176
FOXO-2	4.71 (4.91)	4.67 (4.47)	0.917
FOXO-3	4.88 (4.92)	4.74 (6.05)	0.966
Myostatin	681.40 (590.68)	517.21 (422.23)	0.483
Measurement 2			
FOXO-1	10.14 (14.06)	13.87 (12.34)	0.041
FOXO-2	3.48 (3.68)	5.10 (4.95)	0.169
FOXO-3	4.85 (6.23)	5.26 (4.97)	0.151
Myostatin	669.18 (659.03)	618.51 (602.94)	0.522

Legend: M - mean; SD - standard deviation; p - statistical significance, * p<0.05

Table 3. Analysis of the relationship between the first and second measurement of laboratory tests in the study and control groups.

Variable		Study group	p	Control group	p
		M (SD)		M (SD)	
FOXO1	Measurement 1	23.66 (65.07)	0.045	13.36 (11.12)	0.434
	Measurement 2	10.14 (14.06)		13.87 (12.34)	
FOXO2	Measurement 1	4.71 (4.91)	0.075	4.67 (4.47)	0.777
	Measurement 2	3.48 (3.68)		5.10 (4.95)	
FOXO3	Measurement 1	4.88 (4.92)	0.658	4.74 (6.05)	0.502
	Measurement 2	4.85 (6.23)		5.26 (4.97)	
Myostatin	Measurement 1	681.40 (590.68)	0.829	517.21 (422.23)	0.875
	Measurement 2	669.18 (659.03)		618.51 (602.94)	

Legend: M - mean; SD - standard deviation; p - statistical significance, * p<0.05


PPATIENTS FROM THE CLINIC OF NEPHROLOGY, TRANSPLANTOLOGY, AND INTERNAL MEDICINE AT THE POMERANIAN MEDICAL UNIVERSITY
Qualifying criteria
Disqualifying criteria
HEMODIALYSIS PATIENTS (n=102)
MEN (n=65) WOMEN (n=37)
STUDY GROUP (n=51)
MEN (n=34) WOMEN (n=17)
3 deaths (2x men, 1x woman)
2 resignations
7 no consent to participate in the study
STUDY GROUP (n=39)
MEN (n=29) WOMEN (n=10)
CONTROL GROUP (n=51)
MEN (n=34) WOMEN (n=17)
1 death (1x men)
2 resignations
2 no consent to participate in the study
CONTROL GROUP (n=46)
MEN (n=29) WOMEN (n=17)

Eligibility and disqualification criteria for participation in the study

Qualifying Criteria

- total lack of diuresis,
- undergoing haemodialysis treatment for at least 3 months (three times a week),
- age above 18 years.

Disqualifying Criteria

- musculoskeletal disorders preventing participation,
- severe cardiovascular diseases (heart failure in NYHA class III or IV),
- acute coronary syndrome within the last three months,
- uncontrolled hypertension,
- uncorrectable vision impairments,
- poorly controlled diabetes (HbA1c above 8% for 3 months),
- senile dementia,
- other neurological or psychiatric
- diseases preventing consent or understanding of the study,
- nature and participation conditions,
- malignant tumours,
- surgeries in the preceding month,
- lower limb amputation,
- preventing analysis,
- epilepsy.