



Original Article

Effect of acute intradialytic strength physical exercise on oxidative stress and inflammatory responses in hemodialysis patients



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Background: Oxidative stress and inflammation are common findings in chronic kidney disease (CKD) patients, and they are directly related to the increased risk of developing cardiovascular disease, which is the major cause of death in these patients, particularly for those undergoing hemodialysis (HD). Strength physical exercise is a new therapeutic approach to reduce these complications in CKD patients. Following this, the purpose of this study was to assess the effect of acute intradialytic strength physical exercise on oxidative stress and inflammatory responses in HD patients.

Methods: Sixteen HD patients were studied (11 women; 44.4 ± 14.6 years; body mass index 23.3 ± 4.9 kg/m²; 61.6 ± 43.1 months of dialysis) and served as their own controls. Acute (single session) intradialytic physical exercise were performed at 60% of the one-repetition maximum test for three sets of 10 repetitions for four exercise categories in both lower limbs during 30 minutes. Blood samples were collected on two different days at exactly the same time (30 minutes and 60 minutes after initiating the dialysis—with and without exercise). Antioxidant enzymes activity [superoxide dismutase (SOD), catalase, and glutathione peroxidase], lipid peroxidation marker levels (malondialdehyde), and inflammatory marker levels (high-sensitivity C-reactive protein) were determined.

Results: SOD plasma levels were significantly reduced after acute physical exercise from 244.8 ± 40.7 U/mL to 222.4 ± 28.9 U/mL ($P=0.03$) and, by contrast, increased on the day without exercise (218.2 ± 26.5 U/mL to 239.4 ± 38.6 U/mL, $P=0.02$). There was no alteration in plasma catalase, glutathione peroxidase, malondialdehyde, or high-sensitivity C-reactive protein levels in on either day (with or without exercise). Additionally, there was no association between these markers and clinical, anthropometric, or biochemical parameters.

Conclusion: These data suggest that acute intradialytic strength physical exercise was unable to reduce oxidative stress and inflammation, and in addition, it seems to reduce plasma SOD levels, which could exacerbate the oxidative stress in HD patients.

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Introduction

Chronic kidney disease (CKD) patients have a constant state of oxidative stress, which results from an imbalance between reactive oxygen species (ROS) production and insufficient endogenous antioxidant defense mechanisms [1]. Therefore, oxidative stress promotes the activation of factors that induce the inflammatory processes, establishing a vicious cycle between oxidative stress and inflammation in these patients [2,3]. This mechanism is directly related to the increased risk of developing cardiovascular disease (CVD), which is the major cause of death in these patients, particularly for those on dialysis [3,5].

Mechanisms involved in the inactivation of the oxidative stress and inflammation have been highlighted for being considered promising approaches to minimize cardiovascular complications. Recent studies showed that physical exercises can reduce oxidative stress markers as well as improve the antioxidant defense system in hemodialysis (HD) patients [6,7]; furthermore, they may also reduce the inflammatory process in these patients [8,9].

A notable quantity of research has been conducted on physical exercise in patients with CKD, with chronic aerobic physical exercise being the most studied [7,10,11]. However, few studies have been conducted with acute aerobic physical exercise [12–14]. Previous results from our research group showed that chronic resistance exercise in HD patients had beneficial anti-inflammatory effects [8]; however, no study has evaluated the effects of acute strength physical exercise on inflammation or oxidative stress in these patients. Following this, the purpose of this study was to assess the effect of a single session (acute) of intradialytic strength physical exercise on inflammatory and oxidative stress markers in CKD patients undergoing HD.

Patients and methods

Patients

This study enrolled 16 CKD patients undergoing HD at Renal Vida Clinic in Rio de Janeiro, Brazil. The study included patients with the following features: men and women aged 18 years or over; maintenance dialysis for at least 6 months before the study, using biocompatible membranes (low-flux polysulfone); no residual renal function; arteriovenous fistula for vascular access in the upper limb; absence of motor skill disorders; and ability to perform strength physical exercise. Patients were excluded for the following conditions: acute dialysis; autoimmune, infectious, and inflammatory diseases; uncontrolled hypertension; malignant arrhythmias; unstable angina; smoking; lower limb amputee; history of stroke (either ischemic or hemorrhagic), neurological or cardiovascular disease (moderate-intensity physical activity is not routinely recommended); chronic lung disease; pregnant; cancer; AIDS; and hospitalization within the 3 months prior to the study.

The studied patients were evaluated by a multidisciplinary team and classified as fit for physical therapy during the dialysis session, and each patient served as their own control on a nonphysical-exercise day (the nonexercise group). The dialysis process duration was 3–4.5 h/session, three times/wk with a blood flow >250 mL/min and a dialysate flow of

500 mL/min. The study protocol was approved by the Ethics Committee of the School of Medicine at the Federal Fluminense University (project number 301/11). All of the selected patients gave their informed consent.

Physical exercise program

The exercise was performed during 30 minutes in the second half-hour of the dialysis session on 1 day, and consisted of four different exercises in both lower limbs. The patients remained seated while performing the exercises with ankle-cuffs and elastic bands (Theraband, Akron, OH, USA). The exercise intensity was 60% of the one-repetition maximum test [15] and was administered by a physical therapist following an adopted protocol [16]. (1) The first exercise was knee extension from 90° to 0°. The patient remained in the 0° position for 5 seconds (an isometric contraction) and then returned to the starting position (90°). (2) The second exercise consisted of a triple flexion followed by extension of the lower limbs. The patient flexed the thigh, knee, and ankle (elastic band placed at the level of the metacarpals) followed by a double extension of the thigh and knee. (3) In a coisometric contraction, the patient performed a leg extension against the ankle-cuff resistance (located under the distal third of the leg at the level of the malleolus) for 10 seconds. (4) The patient performed a unilateral hip joint flexion of the lower limb with the knee extended by rising to their functional limit (the leg that did not perform the movement remained at rest on a bench with the knee and thigh flexed).

The patient rested for 3 minutes between the four exercise categories and the three sets of each exercise. The patient rested for 1 minute between each set of 10 repetitions.

Nutritional assessment

The following anthropometric parameters were measured: dry body weight (kg), height (m), waist circumference (cm), arm circumference (cm), and skinfold measurement (mm) at four standard sites (biceps, triceps, subscapular, and suprailiac) using a Lange Skinfold Caliper (Cambridge Scientific Products, Cambridge, MA, USA). All measurements were performed after the dialysis session by a trained staff member.

The body mass index (BMI) was calculated as the dry body weight (kg) divided by the squared height (m) and was used to assess the patient's nutritional status according to the World Health Organization [17]. The arm muscle area was calculated according to the Heymsfield equation [18]. Body fat was calculated from the skinfold measurement according to Durnin and Womersley's equation [19], and the percentage of body fat was calculated using Siri's equation [20]. The fat-free mass was calculated by subtracting the fat mass from the dry body weight.

Analytical procedures and biochemical analyses

Blood samples were drawn in the morning on different days of the week (1 day with 1 session of intradialytic physical exercise and 1 day without exercise) at exactly the same time (30 minutes and 60 minutes after initiating the dialysis) to avoid variations in the assay conditions. The blood was collected into Vacutainer tubes containing ethylenediaminetetraacetic acid (1.0 mg/mL) as an anticoagulant and was centrifuged (15 minutes, 3,000 × g, 4 °C) and stored in tubes at

–80°C until analysis. Routine biochemical parameters, including albumin, calcium, phosphorus, potassium, creatinine, hemoglobin and hematocrit. Urea pre- and postdialysis were measured for all patients according to standard methods at the routine clinical laboratory. The kinetic index of dialysis adequacy (Kt/V) was calculated according to Daugirdas [21].

Plasma antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity were assessed using an enzyme-linked immunosorbent assay from a commercial kit according to the manufacturer's instructions (No. 706002, 707002, and 703102; Cayman, Ann Arbor, MI, USA).

Malondialdehyde (MDA) plasma levels were measured by reaction with thiobarbituric acid. Samples were diluted with thiobarbituric acid (0.6% m/v), brought to heating up to 95°C for 60 minutes, and the supernatant was separated, after centrifugation, and read at 532 nm.

The inflammatory marker high-sensitivity C-reactive protein (hs-CRP) activity was detected by the immunoturbidimetric method using the autoanalyzer equipment Selectra (BS-120; Bioclin, Shenzhen, China) from a commercial kit according to the manufacturer's instructions (No: K079; Bioclin, Belo Horizonte, Minas Gerais, Brazil).

Table 1. Clinical, anthropometric and biochemical profile of the hemodialysis patients

Parameters	Total (n=16)	Women (n=11)	Men (n=5)
Age (y)	44.4 ± 14.6	43.6 ± 16.7	46.2 ± 10.1
BMI (kg/m ²)	23.3 ± 4.9	23.7 ± 5.8	22.6 ± 2.5
Waist circumference (cm)	79.4 ± 11.7	79.3 ± 13.3	79.6 ± 8.7
AMA (cm ²)	32.0 ± 12.5	31.2 ± 12.4	34.1 ± 13.9
Fat mass (%)	31.8 ± 8.0	34.8 ± 6.0	25.3 ± 8.5 *
Fat-free mass (%)	41.0 ± 8.1	38.0 ± 7.6	47.4 ± 5.2 *
Hemoglobin (g/dL)	10.4 ± 1.8	10.4 ± 1.6	10.3 ± 2.4
Hematocrit (%)	31.4 ± 5.4	31.6 ± 4.7	31.1 ± 7.4
Potassium (mg/dL)	4.7 ± 0.6	4.5 ± 0.6	5.1 ± 0.6
Phosphorus (mg/dL)	6.8 ± 1.8	6.7 ± 2.1	7.0 ± 1.0
Calcium (mg/dL)	8.8 ± 0.6	8.8 ± 0.6	9.0 ± 0.7
Albumin (g/dL)	4.1 ± 0.2	4.1 ± 0.3	4.0 ± 0.2
Creatinine (mg/dL)	11.0 ± 2.7	9.8 ± 1.1	13.6 ± 3.4 *
Urea predialysis (mg/dL)	171.0 ± 27.0	173.0 ± 23.4	167.0 ± 36.6
Urea postdialysis (mg/dL)	45.4 ± 13.5	41.4 ± 10.2	54.4 ± 16.6

* $P < 0.05$ between women and men.

Data are presented as means ± standard deviation.
AMA, arm muscle area; BMI, body mass index.

Table 2. Plasma levels of oxidative stress and inflammatory markers in hemodialysis (HD) patients

Parameters	Nonexercise group (n=16)		Exercise group (n=16)	
	Before (30 min in HD session)	After (60 min in HD session)	Before (30 min in HD session)	After (60 min in HD session)
SOD (U/mL)	218.2 ± 26.5	239.4 ± 38.6 *	244.8 ± 40.7 †	222.4 ± 28.9 ‡
CAT (U/mL)	30.5 ± 19.2	30.0 ± 12.2	32.6 ± 18.9	40.7 ± 30.3
GPx (μM/min)	52.0 ± 10.5	51.1 ± 8.3	40.3 ± 6.6 †	38.9 ± 9.7
MDA (mM)	10.9 ± 8.2	8.9 ± 7.1	7.8 ± 5.3	11.7 ± 9.1
hs-CRP (mg/L)	9.5 ± 9.7	9.3 ± 9.5	11.6 ± 7.6	10.3 ± 6.6

* $P < 0.02$ in the same group at the baseline.

† $P < 0.05$ between exercise and nonexercise group at the baseline.

‡ $P < 0.03$ in the same group before exercise (baseline).

Data are presented as means ± standard deviation.

CAT, catalase; GPx, glutathione peroxidase; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; SOD, superoxide dismutase.

Statistical analysis

The distribution of the variables was analyzed using the Shapiro–Wilk test. The results are expressed as the mean ± standard deviation, median (interquartile range), or percentage, as applicable. The differences of the variables were analyzed using nonparametric tests (paired tests, Wilcoxon W, Mann–Whitney U, McNemar) or parametric tests (independent samples *t* test or paired samples *t* test). The correlation between variables was analyzed using Spearman or Pearson coefficients depending on the distribution of the variable. The values were compared after exercise with their own controls. The statistical analyses were performed using SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA).

Results

The clinical, anthropometric, and biochemical data are summarized in Table 1. The time on dialysis was 61.6 ± 43.1 months and Kt/V was 1.57 ± 0.4 . The main causes of CKD were hypertension ($n=11$), chronic glomerulonephritis ($n=2$), diabetes ($n=1$), and unknown causes ($n=2$). According to the BMI, the majority (68.8%) of patients were normal weight, three patients (18.8%) were overweight/obese, and two patients (12.5%) presented a BMI value < 18.5 kg/m². The waist circumference was within normal values in 75% of patients, the arm muscle area in 93.8%, and 62.5% of patients presented a high % of body fat. In addition, the majority of patients presented normal values of potassium (68.8%) and higher levels of phosphorus (87.5%).

After 30 minutes of intradialytic physical exercise, plasma SOD levels were reduced. By contrast, on the day without exercise plasma, SOD levels increased significantly after a 1-hour dialysis session (Table 2). There was no significant difference in plasma CAT, GPx, MDA, and hs-CRP levels in both moments (day with or without exercise). However, SOD and GPx levels were significantly different between the nonexercise and exercise day before the exercise session (baseline). Additionally, there was no correlation between the oxidative stress and inflammatory markers activities and analyzed parameters. Moreover, we also could see that these markers did not show any difference according to sex.

Discussion

Our study investigated the response of acute intradialytic strength physical exercise on oxidative stress damage and

inflammation in HD patients. The physical exercise was unable to increase plasma antioxidant enzymes and decrease MDA or hs-CRP levels in these patients. In addition, plasma SOD levels were reduced after acute physical exercise, which could exacerbate the oxidative stress in HD patients.

The antioxidant enzymes SOD, CAT, and GPx form the main line of defense against ROS. They are produced by the cycle between oxidative stress and inflammation in HD patients [2,3]. The data regarding the plasma CAT and GPx levels after the dialysis session are controversial. Some studies have reported an increase [4,5], whereas others have reported no alteration [4,22–24] or even a decrease after a HD session [25–27]. In this study, we observed no significant differences in these enzymes 1 hour after initiating the dialysis session. Similar findings were found by Bianchi et al [22] and Toborek et al [23]. Both studies demonstrated no significant difference in CAT activity at the end of the dialysis session, which supports the results of this study. Ramakrishna et al [4] and Wu et al [24] also observed that GPx activity did not change after the dialysis session. By contrast, researchers have shown a reduction in SOD activity after a session of dialysis [25,26]. Interestingly, our results showed an increase in plasma SOD levels 1 hour after initiating the dialysis session. Varan et al [5] and Stepniewska et al [28] found the same results at the end of the dialysis session. This increase may be explained by a compensatory mechanism that mobilizes the antioxidant defense system and prevents the free radicals produced during the dialysis session.

Additionally, in this study we observed an intriguing situation; plasma SOD and GPx levels were significantly different between the nonexercise and exercise day in the same patients before the exercise session (baseline). These results may be explained by the variation of patient's values on different days of HD. Nevertheless, there is no confirmed study that has tried to analyze the possible variation of these enzymes values on different days of the HD session.

According to exercise effects, there are no proven studies about the effects of strength physical exercise (acute or chronic) on oxidative stress and inflammation markers in HD patients, nevertheless there are few studies about aerobic (acute and chronic) physical exercise. According to Fatouros et al [14], one session of aerobic physical exercise seems to exacerbate the oxidative stress damage in HD patients; nonetheless, the chronic effect of this exercise is still controversial. In a recent pilot study, Wilund et al [7] observed that thiobarbituric acid reactive substances, a marker of oxidative stress, were reduced by 38% in HD patients after 4 months of intradialytic exercise training (cycling).

The present study did not show significant differences in plasma CAT and GPx levels after acute strength physical exercise in HD patients. Although chronic strength physical exercise can increase plasma SOD levels in HD patients [6], acute aerobic physical exercise seems to decrease them [29]. We also verified that plasma SOD levels decreased after acute strength physical exercise. We hypothesize, that in this case, the compensatory system previously reported that increased plasma SOD levels to combat free radicals produced after 1 hour of dialysis, failed to compensate excessive production of free radicals formed from the HD process [5,30], CKD *per se* [1,31], and physical exercise [13,14] all at the same time, leading to this reduction.

Studies in healthy individuals also show contradictions. The effect of acute aerobic physical exercise on antioxidants

enzymes levels has been studied in healthy individuals, and authors have observed an increase in plasma CAT and GPx levels after exercise [32,33]. Interestingly, Gwozdziński et al [34] observed no change in the antioxidant capacity of plasma immediately after exercise, but 1 hour after the exercise an increase was detected in young untrained men.

MDA is a lipid peroxidation marker and its levels are commonly increased in CKD patients [5,26]. Although chronic strength physical exercise can decrease plasma MDA levels in HD patients [6], acute aerobic physical exercise seems to increase them [13,29]. In the present study, the plasma MDA levels were not significantly increased after acute intradialytic strength physical exercise. This difference may be explained by the type, time, and intensity of exercise.

The measurement of inflammatory markers such as hs-CRP may provide a useful method for detecting the risk of CVD [35]. Plasma hs-CRP, an inflammation marker, is also normally elevated in HD patients [31]. The HD procedure *per se* induces the production of ROS by inflammatory cells, and the ultimate result is an increase in inflammatory reactions characterized by the synthesis and release of inflammatory mediators [5]. According to Wilund et al [7] and Gołębiowski et al [36], chronic aerobic physical exercise during the dialysis session did not influence the inflammation markers. However, previous results from our group showed that chronic resistance exercise in HD patients had beneficial anti-inflammatory effects [8]. In this study, the plasma hs-CRP levels were not decreased after acute intradialytic strength physical exercise.

In this study, we decided to collect data 30 minutes after initiating the dialysis session based on other studies that collected data using similar timing frames, and also in order to allow patients to adapt to the dialysis process. Because the exercise duration was 30 minutes, we collected the blood samples 60 minutes after initiating the dialysis session, i.e., immediately after the exercise session.

Some limitations should be taken into consideration when interpreting our results, such as a relatively small sample size and inpatient variability. However, this was a very well-controlled protocol, which allowed us to conclude that the results are considerably relevant.

In conclusion, these data suggest that acute intradialytic strength physical exercise was unable to reduce oxidative stress damage and inflammation in CKD patients undergoing HD. Additionally, this exercise seems to reduce plasma SOD levels, which could exacerbate the oxidative stress in these patients. Thus, considering the important benefits of physical exercise and the scarcity of information on this subject, further studies with the same or an alternative exercise modality are needed to evaluate the regulation of oxidative stress and inflammatory effects in HD patients.

Conflict of interest

All contributing authors declare no conflicts of interest.

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