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Exercise Changes Oxidative Profile and Purinergic Enzymes Activity in Kidney Disease

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Abstract: Chronic Kidney Disease (CKD) patients are inactive and have reduced physical performance. The CKD lead to abnormalities in various systems including the hemostatic and oxidative systems. The platelet activation occurs with the participation of adenine nucleotides such as ATP and ADP. This nucleotides are part of a system calls purinergic signaling, that is a cell-cell communication pathway, present in several physiological mechanisms such as immune responses, pain, inflammation, cell proliferation, oxidative stress and platelet aggregation. In this work we evaluate the physical mobility, functional capacity and changes in oxidative profile and purinergic enzymes activity in patients with CKD during hemodialysis treatment before and after the protocol of resistance exercise (RE) development. Patients during hemodialysis section were recruited (n = 34). All patients underwent a RE three times a week for eight weeks. The data were analyzed in two moments: before the exercises (BE) and after the exercises (AE). Physical training significantly reduced the markers of oxidative stress after RE by increasing enzymatic and non-enzymatic antioxidant defenses. In addition, the activity of the enzymes of the purinergic system was significantly lower by ATP and AMP hydrolysis after RE. We showed, for the first time, that RE decreased significantly the oxidative stress markers after exercise when compared to previous exercise through increased enzymatic and non-enzymatic antioxidant defenses in CDK patients. These results reinforce the main role of RE in patients with chronic disease and future uses to increase the quality of life of CKD patients.

Keywords: Chronic Kidney Disease (CKD), Purinergic Signaling, Oxidative Stress, Platelet Aggregation, Resistance Exercise

1. Introduction

Chronic Kidney Disease (CKD) is defined by international guidelines as a decrease of kidney function, shown by glomerular filtration rate less than 60 mL/min per 1·73 m^2, markers of kidney damage, or both, of at least during 3 months, regardless of underlying cause [1]. It is recognized as a public health priority worldwide [1]. Patients with CKD display a wide range of abnormalities in the hemostatic pathway that may result in their increased risk for both thrombotic events and bleeding [2]. The hemostatic changes resulting mainly from blood contact with the hemodialysis fistula and extracorporeal circuit. The swirling in this circuit results in platelet and monocytes activation, with expression of tissue factor, contributing to the formation of clots and thrombotic events [2].

The platelets are one of the most important components of blood, they are critical for the body's response to vascular injury, participating in the maintenance of vascular integrity via adhesion, aggregation and subsequent thrombus formation. To maintain homeostasis, they rely on a signal transduction machinery, which is optimized to sense and respond to minor changes in the environment, besides, synthesizes enzymes, ATP, ADP, proteins, prostaglandins and growth factor [3].

In normal conditions, platelets circulated in the blood in a non-active form, due to the negative charge on their surface and vascular endothelial cells, which are electrostatically repulsed [3]. When a blood vessel injury occurs, platelet adhesion to the subendothelial matrix and platelet activation occurs [4], with the participation of adenine nucleotides - ATP and ADP, released by damaged cells and secreted by the dense granules of platelets. This mechanisms contributing to platelet activation and stabilization of platelet aggregates already formed [5].

Purinergic signaling is a cell-cell communication pathway, present in several physiological mechanisms such as immune responses, pain, inflammation, cell proliferation and platelet aggregation [6]. The main components of the purinergic system are extracellular nucleotides – ATP, ADP and AMP and their nucleoside adenosine. Their receptors and ectoenzymes regulate the levels of these molecules, being the adenine nucleotides important in platelet activation and aggregation [7].

Thus, patients with CKD are inactive and have reduced physical performance, aerobic training interventions have been used to increase maximal oxygen uptake, which can improve blood pressure control, lipid profile and mental health of these individuals. In addition, there are notes that resistance training appears to increase muscle flexibility, strength and function. Above all, there is evidence that exercise is safe and beneficial for CKD. Recently, interventions during hemodialysis sessions have become more popular and shown to be the safest. The training and physical exercise programs are safe and effective as non-pharmacological proposals for this population, mainly for the improvement of the quality of life [8].

Since there are few studies in the literature that report the effectiveness of physical exercise in the purinergic system and in oxidative stress in CKD, this study aimed to evaluate the physical mobility and functional capacity of patients with CKD in hemodialysis treatment in a clinic in the west of the state of Santa Catarina, before and after the performance of resistance exercise (RE). For the first time, we showed that RE decreased significantly the oxidative stress markers after exercise when compared to previous exercise through increased enzymatic and non-enzymatic antioxidant defenses in CDK patients.

2. Method

CKD hemodialysis patients were recruited (n=34) in a reference clinic in the west of Santa Catarina, Brazil. The project received ethical approval by the Federal University of South Frontier committee under protocol number 56468716.0.0000.5564 and all patients signed the consent form.

All patients underwent a RE three times a week for eight weeks. The data analyzed were collected in two moments: before the exercises (BE) and after the exercises (AE). The inclusion criteria were they did not perform physical exercise and were on hemodialysis. The data was obtained by medical records and by interview with the participants.

2.1. Study Design & Experimental Procedures

The participants attended three separate sessions distributed over a seven-day period during eighth typical week. The first week was adaptation and was not computed. Series of RE using loads the 1 to 2 kilograms was used, which encompassed the main muscle groups, and should be performed alternately, three sets for each exercise [9]. The sequence of the protocol of RE and each of these exercises was performed as follows:

a. Biceps Flexion with dumbbell: sitting with dumbbell in each hand, held in semi-pronation, inhale and flex the forearms, either simultaneously or alternatively, expire at the end of the movement. 3 sets - 12 to 15 repetitions, with progressive loading.
b. Triceps: Sitting, arms along the body, elbows flexed, hands holding the dumbbell, in the inspiration extends the forearm, taking care not to move the elbows away from the body, expire at the end of the movement.
c. Seated development: Shoulder-level dumbbells supported by hands in pronation, inspire and develop until you extend your arms vertically. Expires at the end of the move.
d. Lateral lifting of the arms with dumbbells: sitting, with the back straight, the arms along the body, a dumbbell in each hand, raise the arms horizontally, with elbows slightly flexed, return to the starting position.
e. Elevation Frontal of arms with dumbbells: Sitting with the arms along the body, with the hand holding the dumbbell in pronation, to inspire and to raise the arm forward until the level of the eyes, to exhale at the end

of the movement.

f. Posterior of the shoulders with theraband: sitting, with the trunk supported, the arms extended forward, hands holding the dumbbells, inhale, move the arms away and approach the scapulas at the end of the movement and exhale.
g. Abduction and Adduction with the Ball: Sitting with your hands holding the ball, arms extended vertically or slightly flexed to relieve the elbow articulation, inhale, and then open the arms to the horizontal, and exhale at the end of the movement when returning the arm to rest.
h. Flexion and Hand Extension with the Ball: Sitting with your hands holding the ball, arms resting on the chair, inhaling and extending your hands, flexing your hands by squeezing the ball, exhale at the end of the movement.
i. Extension of knees with ankles: Sitting, with your hands supported, to keep the trunk immobile, knees flexed, ankles with the anklets, to inspire and to extend an extension of the legs until the horizontal, to exhale at the end of the movement.
j. Flexion and leg extension with ankles: Sitting with your hands supported to stabilize the trunk, extended knees, ankles with ankles, inhale and perform a combined hip and knee flexion, exhale at the end of the movement, at the extension.
k. Hip adductor with ball: sitting with your hands supported to stabilize the trunk, knees flexed, with ball between the legs, inhale and perform an adduction by compressing the ball, contracting one leg over the other, expires at the end of the movement.
l. Calf: Sitting, with feet supported, perform an extension of the feet, ankles in passive flexion, inspires in dorsi-flexion, expires in the planti-flexion.

Blood samples were collected before and after the protocol of RE. Platelet-rich plasma was prepared by the method of Pilla and col. [10] modified by Lunkes and col. [11]. Protein was measured by the method of Bradford [12] using bovine serum albumin as standard.

2.2. Sitting-Rising Test (SRT)

The SRT assesses components of musculoskeletal fitness through evaluation of the subject's ability to sit and rise from the floor, assigning a partial score for each of the two required actions [13]. These test was performed in the beginning and the end of the 8-week practice of resistance exercises.

2.3. Oxidative Profile

The lipid peroxidation was assessed by thiobarbituric acid reactive substances, measured according to Olas [14] with some modifications. The SOD assay was carried out according to Misra and Fridovich [15]. The measurement of protein carbonyls following their covalent reaction with DNPH was pioneered by Levine et al. [16] and has become the most widely utilized measure of protein oxidation in several human diseases. The plasma activity of pro-inflammatory enzyme myeloperoxidase (MPO) was measured spectrophotometrically [17]. The reduced glutathione was determined by the method of Beutler, Duron & Kelly [18]. The determination of vitamin C serum status was performed by direct method, by the photometric analysis [19].

2.4. Purinergic Enzymes Activity

E-NTPDase activity was determined by the method of Lunkes and col. [11]. The E-5'-nucleotidase was determined by the method of Heymann and col. [20]. ADA activity was determined according to Giusti and Galanti [21].

2.5. Statistical Analysis

Statistical analyses were performed with GraphPad Prism 6. Normality was tested by the Shapiro-Wilk test. The differences between the groups, in relation to the variables of the study, were evaluated through analysis paired T test. The results were presented as mean and standard deviation. The differences in the probability of rejection of the null hypothesis as being less than 5% ($p < 0.05$) were considered statistically significant.

3. Results

The clinical characteristics and biochemical parameters were evaluated (Table 1). The parameters evaluated were: weight, blood pressure, albumin, creatinine, urea, low density lipoproteins, high density lipoproteins, hematocrit and hemoglobin. For these parameters, no statistical difference was observed. The mean age of the group was 50.95 years (± 18.4), being 16 women and 18 men.

3.1. Sitting-Rising Test (SRT)

There was a reduction of the execution time of the test in 75% of the participants. This would explain the improvement in the times after the execution of the protocol of this work (Figure 1).

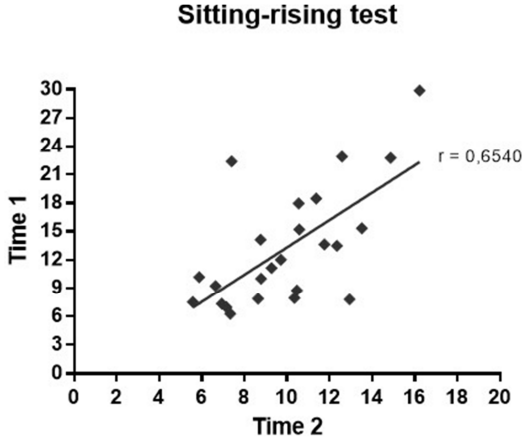

Figure 1. *The sitting-rising test (SRT) evaluated the ability of sitting and rising from the floor of people with Chronic Kidney Disease. The time 1 was realized before and the time 2 was realized after exercise protocol.*

3.2. Oxidative Damage

The damage caused by oxidative stress has dangerous effects in patients with CKD. Based on this, we evaluated the protein carbonylation and lipid peroxidation (TBARs) in our study group and observed that the exercise training was decreased significantly after exercise when compared to before exercise in both analyses (Figure 2).

3.3. Enzymatic and Non-enzymatic Antioxidants Defense

To understand the mechanism by the exercise was able to decrease protein carbonylation and lipid peroxidation, we evaluated enzymatic and non-enzymatic antioxidant defenses and found very impressive data. Only the glutathione reductase activity was significant decreased after exercise (Figure 3). The enzymes myeloperoxidase and superoxide dismutase are significantly increased after the exercise when compared to before exercise training (Figure 3). The ascorbic acid, a non-enzymatic antioxidant, was significant increased after exercise when compared to before exercise (Figure 3).

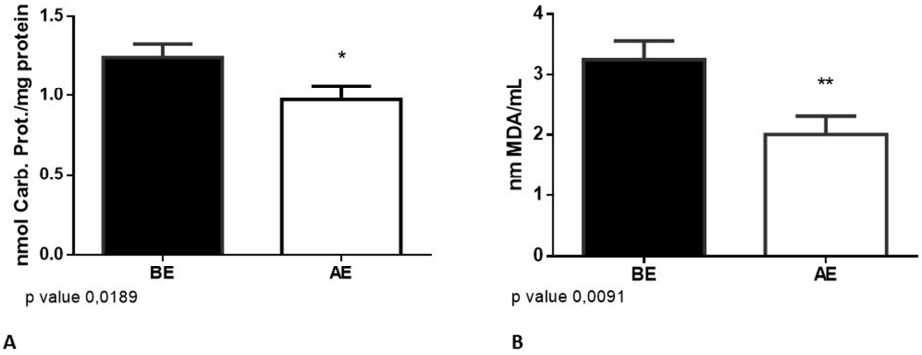

Figure 2. *Protein Carbonylation in serum and TBARs production in platelets of Chronic Kidney Disease patients before (BE) and after (AE) exercise protocol The Protein Carbonilation test is a highly sensitive assays for detection of protein carbonyls and involve derivatisation of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH), which leads to the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone product. The lipid peroxidation was assessed by thiobarbituric acid reactive substances (TBARS), and results were expressed as nanomoles of MDA/mg protein. Data are presented as means ± SEM.*Indicates a significant difference from the control group (Student's t test, p<0.05). **Indicates a significant difference from the control group (Student's t test, p<0.001).*

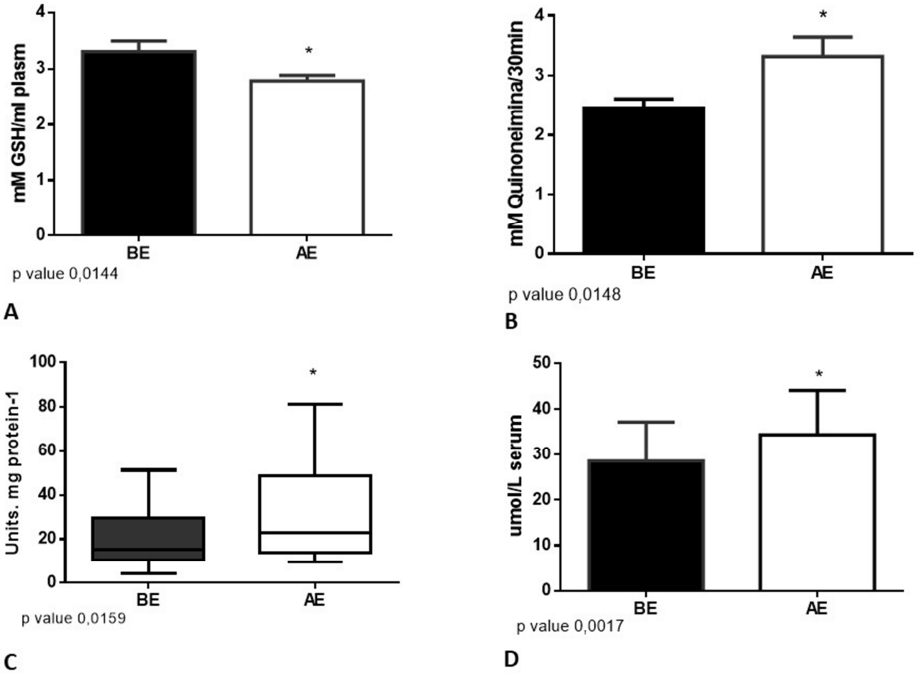

Figure 3. *Glutatione reductase activity, Myeloperoxidase activity in serum, SOD in total blood and Ascorbic Acid in serum of Chronic Kidney Disease patients before (BE) and after (AE) exercise protocol. The reduced glutathione was determined by addition of 2-nitrobenzoic acid (DTNB) in medium containing phosphate buffer, pH 8.0, allowed to obtain the maximum formation of the anion thiolate (TNB), of yellow color, measurable at 412nm. The plasma activity of pro-inflammatory enzyme myeloperoxidase (MPO) was measured spectrophotometrically by a modified peroxidase-coupled assay system involving phenol, 4-aminoantipyrine and H_2O_2. The SOD assay was based by epinephrine auto-oxidation at pH 10.2 to produce adrenochrome, a colored product that was detected at 480 nm. The addition of samples containing SOD inhibits the auto-oxidation of epinephrine. Ascorbic acid was oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2,4-dinitrophenylhydrazine to form the derivative bis-2,4-dinitrophenylhydrazone. Data are presented as means ± SEM. *Indicates a significant difference from the control group (Student's t test, p<0.05).*

3.4. Purinergic Enzymes Activity

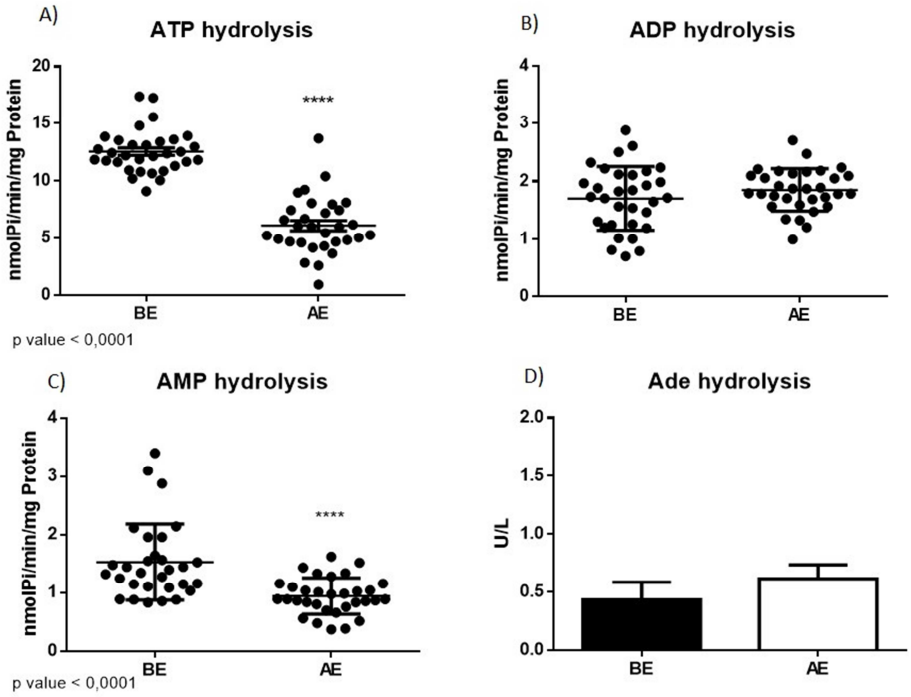

Figure 4. *ATP, ADP, AMP and Ade hydrolysis in platelets of Chronic Kidney Disease patients before (BE) and after (AE) exercise protocol. E-NTPDase (hydrolyzing ATP); E-NTPDase (hydrolyzing ADP), E-5'-Nucleotidase (hydrolyzing AMP) activity, ADA (Adenosine desaminase – hydrolyzing adenosine) in platelets. The E-NTPDase and E-5'-nucleotidase assays was followed as described in materials. Data are presented as means ± SEM. ***Indicates a significant difference from the control group (Student's t test, p<0.0001).*

Table 1. *Clinical characteristics of studied patients.*

Parameters	Before exercise (BE)	After exercise (AE)
Average weight (g)	65,576	65,667
Blood pressure (mmHg) (before hemodialysis)	139/82 (± 23/13)	138/80 (±19/8)
Albumin (g/dL)	3.80 (± 0.40)	3.69 (± 0.42)
Creatinine (mg/dL)	7.70 (± 2.40)	9.08 (± 2.99)
Urea (mg/dL)	133.40 (± 34.82)	141.93 (± 32.03)
Low density lipoproteins (mg/dL)	77.40 (± 28.27)	69.91 (± 23.52)
High density lipoproteins (mg/dL)	39.10 (± 12.74)	38.23 (± 11.40)
Hematocrit (%)	33.00 (± 4.23)	33.04 (± 4.18)
Hemoglobin (g/dL)	10.60 (± 1.43)	10.59 (± 1.40)

The current study assessed whether platelets from patients with CKD presented changes in the activity of E-NTPDase, E-5'Nucleotidase and ADA enzymes. The Figure 3 shows the results obtained of ATP and ADP hydrolysis by E-NTPDase (Figure 4) and revealed a significant decreased in ATP but no difference in ADP hydrolysis. The exercise training decrease ATP hydrolysis in platelets. The same occurred in E-5'Nucleotidase (Figure 4), which after exercise training the CKD patients showed a significant decrease in AMP hydrolysis. Similar to happened to ADP hydrolysis, the adenosine hydrolysis (Figure 4) had no difference between before and after exercise training. These results showed that physical training was successful in altering the activity of purinergic enzymes in platelets.

4. Discussion

This work showed that the exercise training was decreased significantly the oxidative stress markers AE when compared to BE through of increased enzymatic and non-enzymatic antioxidant defenses. Besides that, the activity of the purinergic system enzymes was significantly decrease on ATP and AMP hydrolysis after exercise training, suggesting that the exercise protocol was powerful to modify biochemical parameters in CDK.

Several parameters were evaluated before and after the exercise protocol performed in patients (Table 1), and none of these parameters were affected by the exercise protocol applied, a fact of fundamental importance since they have a direct relationship with efficacy and survival in CKD. Studies have shown exacerbated oxidative stress in patients with CKD at different stages of the disease that can alter albumin, creatinine, urea, LDL and HDL cholesterol by high levels of lipid and protein peroxidation [22], but in this case, the exercise protocol was able to maintain stable such parameters.

The oxidative stress has been considered an important factor associated with inflammation, endothelial dysfunction, atherogenesis and cardiovascular disease in patients with CDK [22], so, we evaluated some oxidative parameters - the protein carbonylation and lipid peroxidation, before and after exercise protocol, and were demonstrated that after training exercise this parameters was decreased (Figure 1). Regarding lipid peroxidation damage, some studies measured increased MDA levels in plasma of CKD patients [23-25]. Moreover, another study [26], reported an increase in free radicals generation in CDK patients when compared to healthy subjects.

Even though there exacerbated oxidative stress and reduced antioxidant activity in patients with CKD, this exercise protocol increased the antioxidant defense, as shown in Figure 2. The antioxidant system are divided into enzymatic and non-enzymatic. Among the enzymatic defenses, exists the superoxide dismutase (SOD) and glutathione peroxidase, which remove free radicals and other reactive species. The non-enzymatic system consists of tocopherols (vitamin E), ascorbic acid and glutathione, among others, which protect against the toxic effect of polyunsaturated fatty acids. It has confirmed an expressive reduction of the antioxidant capacity in uremic patients, supporting the hypothesis that these individuals have less defense against the aggression of oxidative stress [22].

It is known that, the major complication of CKD is atherosclerosis, caused by proinflammatory substances, such cytokines, adhesion molecules, chemokines and reactive oxygen species, culminating in the formation of atherosclerotic plaque and leading to arterial occlusion [2]. Considering that CKD triggers high platelet reactivity, the effect of exercise on the purinergic system activity in platelets of individuals with CKD on hemodialysis was evaluated.

It was observed that the exercise was able to decrease significantly the ATP and AMP hydrolysis when compared to pre-exercise group. The hydrolysis of ADP, however, did not have a significant change between the groups. Regarding the hydrolysis of adenosine, a non-phosphated nucleoside, although visual differences were observed in the graph, no significant difference was observed between the groups. It seem that the exercise protocol was able to decrease the activity of the purinergic enzymes in the platelets of these patients with possible less adenosine being produced. Differently another study [27], they shown that in CKD patients was observed an increase in ATP and AMP hydrolysis in platelets and a decrease in ADP hydrolysis in both undergoing hemodialysis and not undergoing hemodialysis patients. Therefore, a possible positive correlation was being found between ATP, ADP and AMP hydrolysis and our exercise protocol.

Our results showed alterations in oxidative parameters and nucleotide hydrolysis in platelets of CKD patients after the exercise training protocol developed. It is possible that this finding can direct futures adjuvant therapies in treatment of CDK and could contribute to abnormalities in hemostasis found in CDK, besides to increase the quality of life of CKD patients.

5. Conclusion

In summary, despite humans study limitations, the whole of results suggest that RE protocol decreased significantly the oxidative stress markers after exercise when compared to before exercise through increased enzymatic and non-enzymatic antioxidant defenses in CDK patients. These results reinforce the main role of exercise training in patients with chronic disease.

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Conflict of Interest

All the authors do not have any possible conflicts of interest.

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