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L-Arginine supplementation blunts resistance exercise improvement in rats with chronic kidney disease

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ABSTRACT

Chronic kidney disease (CKD) patients present L-arginine (L-arg) deficiency and L-arg supplementation has been used as a treatment. In addition, sarcopenia is another common problem in CKD population, resistance training (RT) is one of the conservative strategies developed to prevent CKD progression, and however there are no evidences of a combination of these two strategies to treat CKD outcomes. The aim of this study was to evaluate the effects of oral L-arg supplementation combined with RT in an experimental model of CKD. Twenty-five Munich-Wistar male rats, 8-week-old were divided in 5 groups: Sham (sedentary control), Nx (CKD sedentary), Nx L-arg (CKD sedentary supplemented with 2% of L-arg), Nx RT (CKD exercised) Nx RT + L-arg (CKD exercised and supplemented with 2% of L-arg). CKD model was obtained by a subtotal 5/6 nephrectomy. RT was performed on a ladder climbing, three weekly sessions on non-consecutive days, with an intensity of 70% maximum carrying capacity. They were submitted to RT and/or L-arg supplementation for 10 weeks. There was a significant improvement in muscle strength, renal function, anti-inflammatory cytokines, arginase metabolism and renal fibrosis after RT. However, the combination of RT and L-arg impaired all the improvements promoted by RT alone. The L-arg supplementation alone did not impair renal fibrosis and renal function. In conclusion, RT improved inflammatory balance, muscle strength, renal function and consequently decreased renal fibrosis. Nevertheless, the association with L-arg supplementation prevented all these effects promoted by RT.

1. Introduction

Chronic kidney disease (CKD) is increasing worldwide and became a public health problem [1]. Patients with CKD are a very high-risk group for cardiovascular disease and, consequently, mortality [2]. CKD could progress to an end-stage renal disease (ESRD), which is the worst complication in renal function since it requires treatment with dialysis and transplant. Both of these treatments are very expensive, not completely effective and lead to a reduction in the quality of life of the patients [3]. It has been suggested that conservative treatment could be used as an alternative strategy to prevent ESRD [3,4]. In this regard, new treatments strategies to the benefit of CKD and delay expensive treatments such as dialysis are required.

The exercise training has been used as an important treatment to prevent and atenuate CKD disabilities, such as low physical capacity [5], high oxidative stress [6], muscle atrophy [7], and low glomerular filtration rate [8]. A recent review reported that the loss of muscle mass and muscle strength in CKD were strongly related to morbidity, disability and mortality [9]. Moreover, the same authors assumed resistance training (RT) as the main strategy to prevent and treat

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sarcopenia in this population [9]. Nevertheless, the effects of RT on inflammatory profile and renal function in rats submitted to oral supplementation of L-arg are unknown.

Furthermore, oral supplementation of ergogenic aids has also been used as a strategy to counteract CKD disabilities [10,11]. Mainly, L-arginine (L-arg) which is a semi-essential amino-acid synthesized mostly in the kidneys has been used to treat CKD disorders [12,13]. In fact, patients with CKD showed a reduced production of L-arg by kidneys [14], and this reduction seems to be correlated with the progression of CKD and mortality [10,15]. Thus, L-arg supplementation has been used as a treatment in CKD patients based on improvements observed in clinical and experimental studies [12,13,16,17].

The exclusive use of L-arg supplementation in an animal model of CKD, reduced the expression of L-arg transporter, decreasing the availability of substrate for nitric oxide (NO) production, an important component involved in inflammatory system [18]. Furthermore, there are evidences that combined exercise with L-arg supplementation could be an alternative treatment for CKD outcomes, some previous studies demonstrated an improvement in endothelial function by an increase of NO biodisponibility after a program aerobic exercise in CKD rats [19,20].

In this regard, we hypothesized that the combination of both treatments could provide an additive effect on renal function in a CKD model. For this reason, the purpose of this research was to determine the effects of RT and/or oral L-arg supplementation on inflammation, renal function and muscle strength in an experimental CKD model.

2. Methods

2.1. Animals

Twenty-five male Munich-Wistar rats, 8-week-old, from the breeding colony of the Faculty of Medicine, University of São Paulo (FMUSP São Paulo, Brazil), with an initial weight of approximately 240 ± 20 g, were used. The animals were kept in collective cages (5 rats per cage), with water and chow *ad libitum*, they were kept at a constant temperature (23 ± 2 °C), in a 12 h light/dark cycle, with light from 06:00 a.m. to 06:00 p.m. The rodents were fed with a standard rat chow diet (Purina, Descalvado, São Paulo, Brazil). This research was approved by the Committee of Experimental Animals of the Federal University of São Paulo (protocol No. 0243/12). All the procedures with the animals were conducted in conformity with the Guide for care and use of laboratory animals.

2.2. Renal ablation

Renal ablation was obtained by 5/6 nephrectomy (Nx). The procedure initiated with an intramuscular anesthesia composed by 50 mg/kg of ketamine (Syntec®, São Paulo, Brazil) and 10 mg/kg of xylazine (Syntec®, São Paulo, Brazil). After that, 2/3 of the left kidney was infarcted and the right kidney was extracted. To simulate the same procedure in Sham group, these rats were anesthetized and had their kidneys manipulated, without removal. After surgery, the rodents received 5 mg/kg enrofloxacin (Schering Plough®, São Paulo, Brazil). After recovery, free access to water and rodent chow were given.

2.3. Experimental groups

Four weeks after renal ablation, the rats submitted to Nx surgery were randomly distributed into 4 experimental groups (5 animals in each group): Nx (CKD sedentary), Nx L-arg (CKD sedentary supplemented with L-arg), Nx RT (CKD submitted to RT), Nx RT + L-arg (CKD supplemented with L-arg and submitted to RT). An additional Sham group (n = 5) was used for control. The RT or L-arg supplementation protocol started immediately after the group allocation.

2.4. L-Arginine supplementation

The Nx L-arg and Nx RT + L-arg groups received 2% of L-arg solution (Sigma Aldrich®, São Paulo, Brazil) dissolved in 2% of sucrose (Synth® Brazil Ltda, São Paulo, Brazil) in drinking water. Sham, Nx, and Nx RT received 2% of sucrose (Synth® Brazil Ltda) in drinking water (placebo). The sucrose solution was used as a vehicle in order to improve L-arg ingestion. The solution was prepared in a 500 mL glass bottle, covered with a brown paper to protect from the light. The supplementation was offered without restrictions of quantity or period. The bottles were completed to 500 mL every week. The animals were supplemented for 10 weeks. The L-arg dose was based on that used in a previous study [21].

2.5. Resistance exercise training

After experimental groups distribution, all the animals were adapted to the resistance training (RT) protocol, that consisted to climb a vertical ladder (110 × 18 cm, 2 cm grid, 85 degrees of inclination) The length of the implement allowed rats to perform between 8 and 12 dynamic movements per climb [22]. The familiarization consisted of placing the animals at the bottom of the ladder and let them climb it. When the animals reached the top of the ladder, they entered in a housing chamber (20 × 20 × 20 cm), where they rested for 60 s. Rodents were stimulated until they voluntarily repeat this procedure for 3 consecutive times without stimuli; the familiarization was performed 4 weeks after renal ablation, for 5 consecutive days, with 5 climbs without load.

To determine the first maximal carrying capacity (MCC), 4–8 climbs on the implement with progressively heavier loads was carried out, it was performed 3 days after the familiarization trials. The initial climb load was set at 75% of the animal's body mass with weights attached to their tails by wrapping the proximal portion of the tail with a self-adhesive foam strip, an incremental load of 30 g in each climb was added until the animal failure to complete the climb. Failure was considered when the animal could not progress the ladder up after 3 successive stimuli (gently prodding). The highest load successfully carried along the entire length of the ladder was considered as the rat's MCC, which was used for the training intensity determination. MCC was performed in the middle (10^{th} week) and at the end (15^{th} week) of the experimental protocol to readjust the training intensity; each new MCC test began with 30%, 50%, 75% and 100% of the last MCC, then an additional load of 30 g was added, until a new MCC was determined.

Training sessions was performed with Nx RT and Nx RT + L-arg group for 10 weeks, three times per week, on non-consecutive days, it was consisted of 8 ladder climbs, with twice 30%, 50%, 60% and 70% of their MCC determined in the previous session, 60 s of rest between ladder climbs. The RT protocol was adapted from [22], according to the needs of our research. Fig. 1 describes all the procedures.

2.6. Tissue collection and biochemistry analysis

Forty-eighty hours after the end of the protocol, the animals were placed in a 24 h metabolic cage, where urine samples were collected for the measurements of 24 h proteinuria (UProt) and 24 h albuminuria (UAlb). UProt and UAlb were assessed using a commercially available enzymatic kit (Labtest Diagnostica, São Paulo, Brazil) and a radial immunodiffusion assay [23], respectively. 24h followed these procedures, rats were anesthetized with 10 mg/kg xylazine and 50 mg/kg ketamine intramuscular. After that, blood samples were collected from the abdominal aorta for serum urea analyses using a commercially available enzymatic kit (Labtest Diagnostica, São Paulo, Brazil). The kidneys were also collected, two midcoronal renal slices were made, one of them was post fixed in buffered 4% formaldehyde for posterior histomorphometric analysis, and the other one in -80 degrees for gene expression analysis.

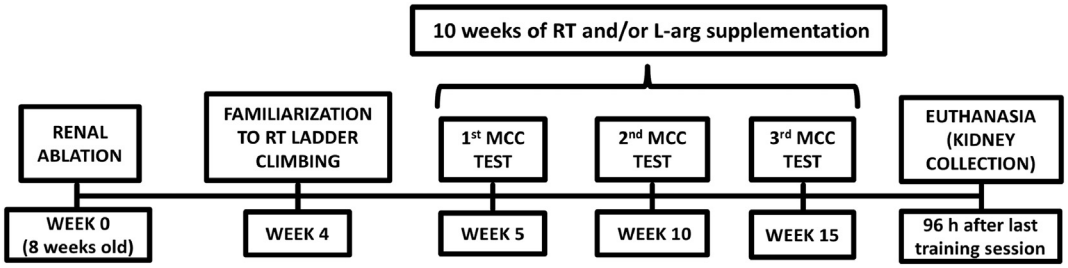

Fig 1. Experimental design.
RT = resistance training, MCC = maximal carrying capacity, Nx = 5/6 nephrectomy.

2.7. Picrosirius red analysis for renal fibrosis

In order to evaluate fibrosis, kidneys were paraffin-embedded and picrosirius red stained sections were performed in the left renal tissue. An objective of 20× was used under polarized light to calculate fibrotic tissue. An AxioPhot I microscope combined with an ICc3 AxioCam camera were used to examine the slides. To obtain the images, the AxioVision software v 4.7.2.0 capture system (Carl Zeiss, Goettingen, Germany) was utilized. To quantify the bright of sirius red areas, the ImageJ 1.43a 64-bit software were used. Structures such as sub-capsular cortex, medulla and vessels were avoided when acquiring the images. The percentage of positively stained area was determined in 10 consecutive, nonoverlapping, 200× fields, of 0.09 mm^2, per animal. All the analyses were expressed by area (μm^2) [24,25].

2.8. Analysis of gene expression

Gene expressions were analyzed by quantitative polymerase chain reaction (qPCR). RNA of renal tissue was isolated with TRIzol Reagent (Life Technologies, USA) following the manufacturer's protocol, and a Nano Drop (Thermo Scientific, USA) was used to determine the RNA concentrations. After that, the cDNA synthesis was executed with a Super Script III RT (Life Technologies). The TaqMan PCR assay (Applied Biosystems, UK) was used to perform qPCR, each sample was measured in triplicate, the samples were analyzed with an ABI Prism 7300 sequence detection system (Life Technologies). Gene expression of interleukin (IL)-1β, IL-4, IL-6, IL-10, endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS) and arginase were obtained and each molecule was normalized to HPRT [26].

2.9. Enzymatic activity of arginase

To analyze the enzymatic activity of arginase, renal tissue was weighted and homogenized in 5 volumes of 50 mM Tris-HCl (pH 7.4) containing 0.5 M of PMSF and a cocktail of protease inhibitors. The samples were centrifuged at 4 °C for 5 min, at 1000 G. 120 μL of supernatant were separated into tubes of 1.5 mL and 120 μL of 10 mM $MnCl_2$ were added. The samples were heated to 55 °C for 10 min. After that, the tubes were placed under ice and 240 μL of L-arg (0.5 M) were added. The samples were mixed and divided into 4 tubes (100 μL in each tube). Two tubes were placed in the stove at 37 °C for 1 h, the others 2 tubes were left on ice (white reaction). Then, all the samples were left on room temperature and 500 μL of the acid mix were added. Next, 25 μL of ISPF 9% were added. The tubes were sealed with teflon tape and then crepe tape avoiding the light and boiled at 100 °C for 45 min in a water bath. The samples of the standard urea curve were processed from the addition of the acid mix. 200 μL of the samples were pipetted on an ELISA plate read in a spectrophotometer at 540 nm. The results were expressed in nmol of protein (urea/min/mg) [27].

2.10. Statistical analysis

Differences among five groups were assessed using one-way analysis of variance (ANOVA), followed by pairwise Tukey's post hoc test. MCC differences were assessed using two-way ANOVA for repeated-measures, followed by pairwise Bonferroni post-hoc tests. To assume that the group variances were statistically equal in ANOVA test, Brown-Forsythe test was used to validate the values in *F*-test. A p values < 0.05 were considered significant. Results are presented as mean ± standard deviation (SD). Data analyses were performed using Prism® 5.0 (GraphPad® Software, USA).

3. Results

3.1. Muscle strength

MCC data is showed in Fig. 2. After 10 weeks of RT, Nx RT group significantly increased the MCC at the tenth week (533 ± 57 g) when compared with the first week (533 ± 57 vs. 248 ± 44 g, $p < 0.0001$). We also observed higher values of MCC at the tenth week in the Nx RT group when compared to MCC baseline values of the Sham (533 ± 57 vs. 298 ± 117 g, $p < 0.01$), Nx (533 ± 57 vs. 272 ± 60 g, $p < 0.01$), Nx L-arg (533 ± 57 vs. 252 ± 58 g, $p < 0.01$), and Nx RT + L-arg groups (533 ± 57 vs. 315 ± 51 g, $p < 0.01$).

3.2. Renal function

Serum urea levels (Fig. 3A) were higher in the Nx, Nx L-arg groups when compared with the Sham group ($p < 0.01$). No difference was showed in the Nx RT group when compared with the Sham group ($p > 0.05$). Nx RT + L-arg group showed higher levels of serum urea when compared with the Sham group ($p < 0.01$).

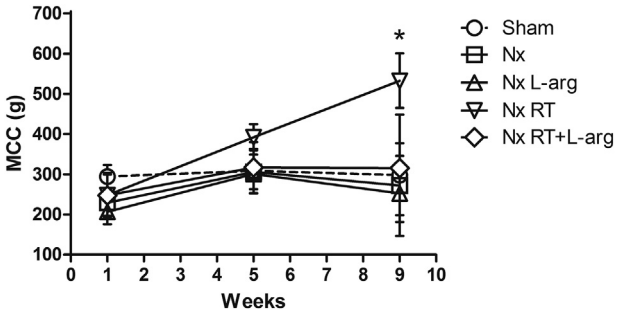

Fig 2. Maximal carrying capacity (MCC).
Expressed in grams (g). Main effects: interaction – group x time $p < 0.0001$; group effect $p < 0.0001$; time effect $p < 0.0001$. No statistical difference ($p > 0.05$) between Sham, Nx, Nx L-arg, Nx RT + L-arg groups during all times. Statistical difference ($p < 0.01$) at the tenth week of the Nx RT vs. all groups. Tenth week vs. first week in Nx RT group statistical different ($p < 0.01$).

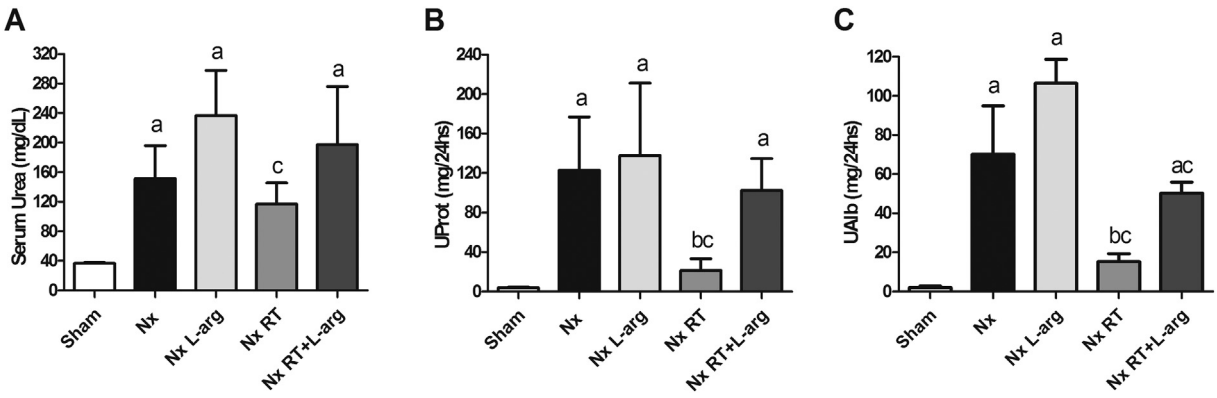

Fig 3. Renal function.
Serum Urea (panel A) expressed in mg/dL, UProt (panel B) expressed in mg/24 h, UAlb (panel C) expressed in mg/24 h. a = vs. Sham $p < 0.01$; b = vs. Nx $p < 0.01$; c = vs. Nx L-arg $p < 0.01$.

UProt showed the same pattern of results (Fig. 3B), with higher levels in the Nx, Nx L-arg groups when compared with the Sham group ($p < 0.01$). No difference was showed in the Nx RT group when compared with the Sham group ($p > 0.05$). Nx RT group presented lower levels of UProt when compared with the Nx and Nx L-arg groups ($p < 0.01$). Nx RT + L-arg group showed higher levels of UProt when compared with the Sham group ($p < 0.01$).

UAlb levels (Fig. 3C) were higher the Nx, Nx L-arg groups when compared with the Sham group ($p < 0.01$). Nx RT group compared with the Sham group ($p > 0.05$). Nx RT group showed lower levels of UAlb when compared with Nx and Nx RT + L-arg groups ($p < 0.01$). Nx RT + L-arg group showed higher levels of UAlb when compared with the Sham group ($p < 0.01$).

3.3. Renal fibrosis

Renal fibrosis analysis (Fig. 4) revealed increased collagen deposition in the Nx, Nx L-arg and Nx RT + L-arg groups when compared with the Sham group ($p < 0.01$). Collagen deposition was reduced in Nx RT group when compared with the Nx, Nx L-arg and Nx RT + L-arg groups ($p < 0.01$).

3.4. Cytokines expression in renal tissue

The expression of pro-inflammatory cytokine IL-1β (Fig. 5A) was only increased in NxRT + L-arg group when compared with Sham group ($p < 0.05$). IL-6 (Fig. 5B) was elevated in Nx and Nx RT + L-arg group compared to Sham animals ($p < 0.05$). A decrease of IL-6 was observed in Nx L-arg and Nx RT when compared to Nx group ($p < 0.05$). There was an increase of anti-inflammatory cytokine IL-4 (Fig. 5C) in Nx L-arg, Nx RT and Nx RT + L-arg group compared to Sham group ($p < 0.05$). Interleukin-10 (Fig. 5D), also an anti-inflammatory cytokine, was only increased in Nx RT group compared with Sham group ($p < 0.05$). The IL-6/IL-10 ratio (Fig. 5E) demonstrated an increase in Nx group when compared to Sham group ($p < 0.05$); the Nx L-arg, Nx RT and Nx RT + L-arg groups decreased this ratio when compared to Nx group ($p < 0.05$).

3.5. Nitric oxide synthase enzymes gene expression in renal tissue

The expression of renal eNOS (Fig. 6A) in Nx L-arg group was increased in comparison to Shan and Nx groups ($p < 0.01$); the same pattern was observed when Nx RT + L-arg group was compared to Sham and Nx animals ($p < 0.05$). Nx RT group had a reduction of eNOS when compared to Nx L-arg ($p < 0.01$). There was an increase of iNOS enzyme (Fig. 6B) expression in Nx L-arg group when compared to Sham animals ($p < 0.05$), however in Nx RT + L-arg group iNOS reduced when compared to Nx-L-arg ($p < 0.05$). The eNOS/iNOS ratio (Fig. 6C) demonstrated an increase in Nx RT + L-arg group compared to Sham ($p < 0.05$), Nx ($p < 0.01$), Nx L-arg ($p < 0.01$) and Nx RT ($p < 0.01$) groups.

3.6. Arginase gene expression and enzymatic activity of arginase in renal tissue

It was observed a decrease of renal arginase gene expression (Fig. 7A) in Nx and Nx L-arg groups compared to Sham group ($p < 0.01$). In enzymatic activity of arginase (Fig. 7B), Nx group decreased when compared to Sham group ($p < 0.05$), on the other hand, Nx RT and Nx RT + L-arg had an increase compared to Nx animals ($p < 0.05$).

4. Discussion

Previous studies demonstrated that both RT [28,29] and L-arg supplementation [13,17] had been successful as conservative strategies alone to prevent CKD progression, however no previous study evaluated the combined effect of these two treatments in an experimental model of CKD. It was expected that the combination of RT and L-arg would promote an additive effect on CKD improvement. Nevertheless, when both treatments were combined, all the improvements seem with the exercise treatment exclusively were prevented after association with L-arg.

The Nx RT + L-arg group showed a decrease in renal function (albuminuria, proteinuria and urea) and an increase of renal fibrosis after the treatment. There are some explanations for these results, the prevalence expression of pro-inflammatory cytokines in the kidney (increase of IL-1β and IL-6); over the anti-inflammatory ones, (increase of IL-4 but not IL-10), created a pro-inflammatory environment. In addition, the increase of renal endothelial nitric oxide synthase (eNOS) expression in this pro-inflammatory environment, may have led to an elevation of some oxidative molecules like peroxynitrite ($ONOO^-$), increasing the oxidative stress in the kidney.

It is well know the relationship between inflammation and oxidative stress in CKD [30,31], in a pro-inflammatory environment, eNOS can increase the redox balance by NO production and its reaction with superoxide (O^{2-}), forming peroxynitrite; furthermore, the uncoupling of eNOS leads to a direct production of superoxide, and the reduced levels of hydrogen peroxide would also be an indicator of reactive oxygen species (ROS) increase in renal tissue [32,33]. It is reasonable to speculate that this excess of nitrogen donned by L-arg decreased the renal function and led it to fibrosis, specially by the larger amounts NOS and reactive oxygen species, that probably increased the nitrogen reactive species in the organism and increased the inflammatory process.

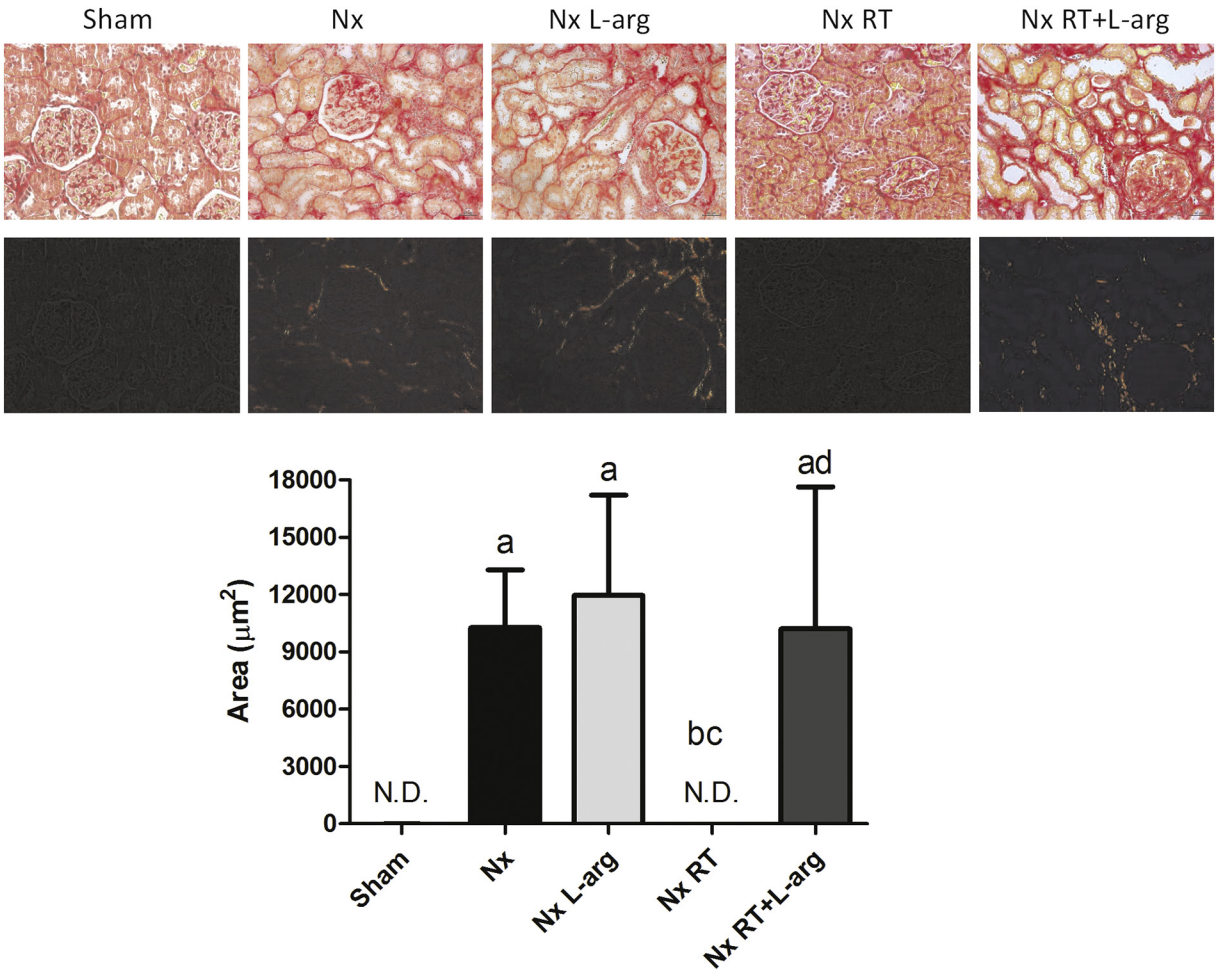

Fig 4. Renal fibrosis.
Expressed in μm^2. Lower images obtained with polarized light. a = vs. Sham $p < 0.01$; b = vs. Nx $p < 0.01$; c = vs. Nx L-arg $p < 0.01$; d = vs. Nx RT $p < 0.01$ $p < 0.01$. N.D. = non detected collagen deposition. Magnification 50×.

Similar to our data, Vaziri, Bai et al. [34] demonstrated that up-regulation of inflammatory system increasing oxidative stress and consequently the progression of renal disease in rats submitted to Nx model of CKD. In addition, rats with hyperoxaluric kidneys also presented an increase of oxidative stress after 42 days of 0.6% of L-arg supplementation in drinking water [35].

Besides that, L-arg supplementation impaired the gain of muscle strength in Nx RT + L-arg group; there is no experimental studies demonstrating an interference of L-arg supplementation on muscle strength of rodents submitted to RT, furthermore, studies with RT in humans had failed to show muscle strength additional improvements after oral L-arg supplementation [36–39], but none of them demonstrated impairments of RT effects after L-arg supplementation.

Although the combination of L-arg and resistance training brought interesting results in experimental studies, it is possible that the excess of nitrogen generated by the renal injury itself, and these metabolic alterations were able to make the supplementation deleterious. It is possible that a good kidney function would be necessary to convert L-arg in NO and CKD harm its metabolization [40,41].

Contrary to our expectation, L-arg supplementation did not improve renal function and renal fibrosis, it was expected that L-arg would improve renal function as previous studies had already demonstrated [12,13], these two studies supplemented with 1% of L-arg in drinking water for 14–16 weeks, observed an improvements in renal function of diabetic and CKD rats, respectively. Nevertheless, in our study, the group supplemented with 2% L-arg had a decrease in all biochemical renal function parameters and an increase of fibrosis in the kidney with only 10 weeks, demonstrating that this treatment was not successful in our experimental model of CKD, and the progression with this protocol would clearly decrease the renal function of our animals.

In consonance with these findings, Liu, Haylor et al. [42] evaluated the effects of L-arg supplementation (1% in drinking water) in Nx rats for 4 and 16 weeks. This study observed a compensatory renal growth and tubule-intestinal damage, which was associated with cellular growth factors and intraglomerular capillary pressure. The possible mechanisms involve an excessive glomerular NO production and the shunt of L-arg to urea cycle pathway. Moreover, these data suggest that even in low doses and for short periods, oral L-arg supplementation seems to impair the kidney function and morphology.

In our findings however, we could not support that urea cycle metabolism was involved in renal fibrosis, because arginase expression was decreased after renal ablation and L-arg supplementation did not alter this parameter; arginase activity presented a small reduction in Nx L-arg compared to Sham group, however this decrease was not sufficiently low to show statistical difference. Moreover, this is the first study to evaluate the effects of L-arg supplementation on NO synthase enzymes expression in the kidney of Nx rats, our data demonstrated that eNOS had an improvement in Nx L-arg group, this is probably a mechanism trying to regulate NO biodiponibility by endothelial cells in renal tissue [14]. The inducible nitric oxide synthase (iNOS) isoform was also elevated in renal tissue of Nx L-arg group, this enzyme is known as an arginase regulator, as they compete for the same substrate,

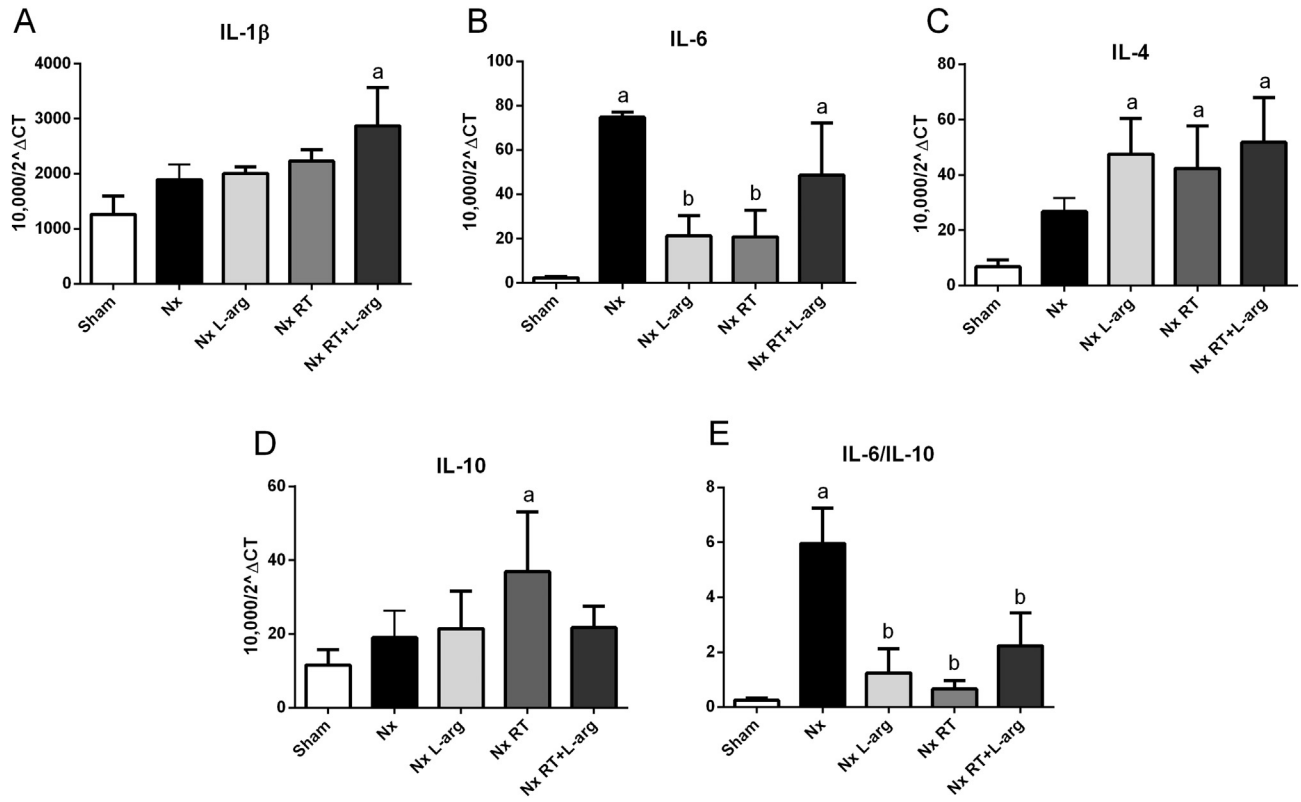

Fig 5. Cytokines mRNA expression in renal tissue.
Expressed in $10{,}000/2^{\Delta CT}$. Interleukin 1β (IL-1β; panel A). Interleukin 6 (IL-6; panel B). Interleukin 4 (IL-4; panel C). Interleukin 10 (IL-10; panel D). IL-6/IL-10 Ratio; panel E. a = vs. Sham $p < 0.05$; b = vs. Nx $p < 0.05$.

which explains the down regulation of arginase expression in renal tissue. The increase of iNOS was also observed by Peters, Border et al. [43] in an experimental model of glomerulonephritis after L-arg supplementation (1% in drinking water), this study showed an increase of proteinuria, mesangial cell injury and renal fibrosis after 6 days of supplementation. In our study, the increase of iNOS expression in renal tissue may have led to oxidative stress up-regulation with NO cytotoxic effects, collaborating to renal fibrosis and decrease of renal function in Nx L-arg group. In addition, a prospective study of Bahadoran, Mirmiran et al. [44] demonstrated that dietary animal-derived L-arg increases the risk of CKD in adults.

The iNOS increase in Nx L-arg group may have had some impact on pro-inflammatory signaling, but this was not clearly seen, except for IL-4. It is reported that IL-4 is an endogenous stimulator of arginase, on the other hand, NO production and iNOS increase suppresses arginase activity [45]. In our study, IL-4 had a mild effect on arginase, maintaining its enzymatic activity but not its gene expression in renal tissue of Nx L-arg group, probably by the high levels of iNOS.

On the other hand, RT prevented CKD progression, with an improvement of renal function and fibrosis. There was a different inflammatory balance in Nx RT group compared to the other groups; it was observed an increase of both IL-4 and IL-10, without an increase of IL-1β and IL-6, this anti-inflammatory environment collaborated to the improvement in renal fibrosis after 10 weeks of RT.

Similar to our data, Luiz Rda, Silva et al. [28] demonstrated a reduction in proteinuria and glomerular sclerosis after 5 weeks of swimming training. In complementation, besides the impairment of CKD progression and renal fibrosis, Peng, Chen et al. [29] demonstrated that

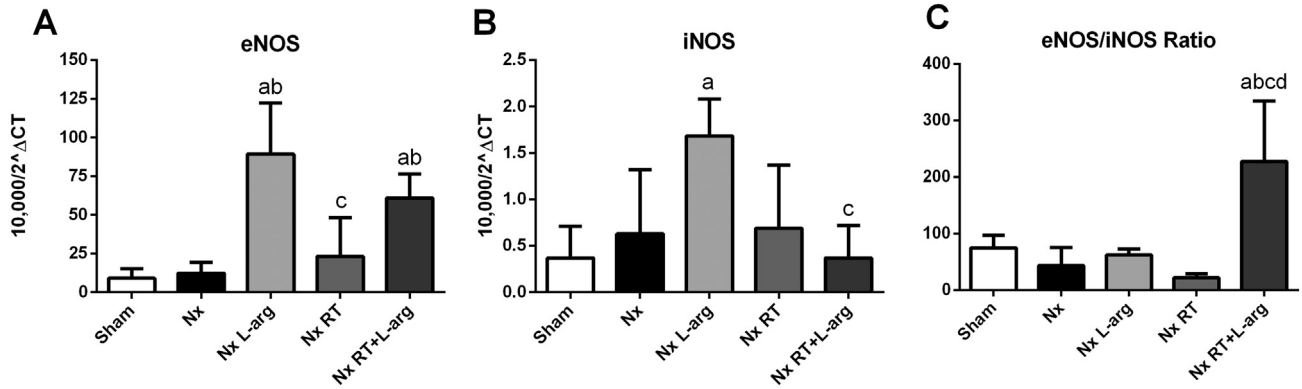

Fig 6. Nitric oxide synthase enzymes mRNA expression in renal tissue.
Expressed in $10{,}000/2^{\Delta CT}$. Endothelial nitric oxide synthase (eNOS; panel A). Inducible nitric oxide synthase (iNOS; panel B). eNOS/iNOS Ratio; panel C). a = vs. Sham $p < 0.05$; b = vs. Nx $p < 0.05$. c = vs. Nx L-arg $p < 0.05$; d = vs. Nx RT $p < 0.05$.

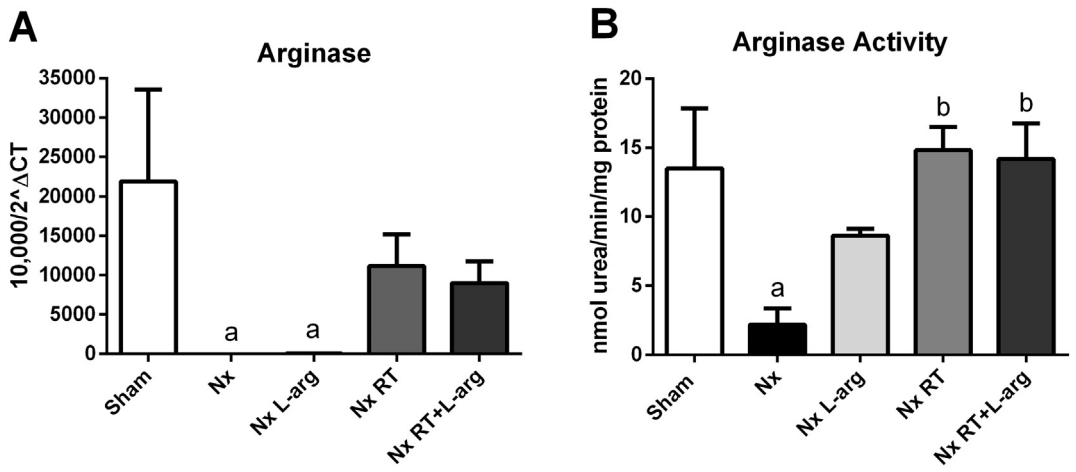

Fig 7. Arginase mRNA expression and enzymatic activity of arginase in renal tissue.
A Expressed in $10,000/2^{\Delta CT}$. B expressed in mol of protein (urea/min/mg). a = vs. Sham $p < 0.05$; b = vs. Nx $p < 0.05$.

this improvements occurred by the inhibition of mesangial cell activation and CD34 expression, down regulating IL-6, platelet-derived growth factor (PDGF) pathway, alpha-smooth muscle actin (α-SMA), and deregulating matrix metalloproteinases, suppressing myofibroblast transdifferentiation after 10 weeks of swimming training. Nevertheless, this type of exercise has one particular disadvantage, the rodents swim to avoid sinking and drowning, causing more stress than our voluntary ladder climbing exercise.

Although literature demonstrate that suppression of arginase pathway could improve CKD outcomes [19,20,46,47], a deficiency of this enzyme could also lead to some problems like hyperammonemia, cognitive and neurologic disorders [48,49]. This is the first time that arginase pathways down regulation was reported in an experimental model of CKD, furthermore, our data showed that RT restored arginase expression and activity. The normalization of arginase expression and activity was probably responsible for the maintenance of low levels of iNOS expression, reducing NO cytotoxic effects in renal tissue [45].

The improvements of muscle strength in Nx RT group confirm that RT is an important non-pharmacological strategy to overcome the CKD disabilities ([5,9]. The Nx RT group showed an increase of 215% in the muscle strength. As demonstrated by previous studies of our group [50,51], we had improvements in muscle function. In humans, Cheema, O'Sullivan et al. [52] observed that RT was safe and effective to increase muscle strength and quality of life of patients under hemodialysis treatment. Therefore, considering the association of muscle strength loss with a progression of CKD [53] and a higher risk of mortality [9], RT seems to be an important strategy for CKD treatment.

The main limitation of our study was the oxidative stress parameters that were not assessed to confirm that L-arg supplementation was causing damage in the kidneys. The strengths of our study were the analyses of some pro and anti-inflammatory cytokines expression, arginase activity and expression in renal tissue, which collaborated to understand the mechanisms behind renal fibrosis in CKD after L-arg supplementation and resistance exercise.

The practical implications of this study are the potential effect of RT on CKD population in order to prevent sarcopenia and improve strength gain, hence this population could improve their day life activities and reduce the risk of fall and fractures. Furthermore, L-arg supplementation may not be an interesting strategy for the treatment of patients with CKD associated with RT, and its effects should be extensively investigated to understand dose response and cause-effect in this population.

5. Conclusion

Our study demonstrated that 10 weeks of RT improve the muscle strength, inflammation, arginase metabolism and renal function, besides protecting against renal fibrosis in an experimental model of CKD. However, the supplementation of L-arg alone did not improve muscle strength, had no effect no inflammatory system and did not impair CKD progression. The combination of L-arg supplementation to RT prevents the benefits observed by the RT performed exclusively. Finally, further studies should be performed in order to unravel the mechanisms underlying the benefits of RT on CKD and to elucidate the safety of oral L-arg supplementation in CKD.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

MKS, MRM, TSR, CSP, RVPN, ASH: Performed the experiments, analyzed data, drafted manuscript, edited and revised manuscript, approved the final version of manuscript. MAC, SCA, CKF, SAT, MNM: Performed the experiments, edited and revised manuscript, approved the final version of manuscript. NOSC, APSF Edited and revised manuscript, approved the final version of manuscript.

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