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N-acetyl-L-cysteine exacerbates kidney dysfunction caused by a chronic high-sodium diet in renal ischemia and reperfusion rats

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ABSTRACT

Aims: To investigate the effect of long-term *N*-acetyl-L-cysteine (NAC) treatment in Wistar rats subjected to renal ischemia and reperfusion (IR) and a chronic high-sodium diet (HSD).

Main methods: Adult male Wistar rats received an HSD (8.0% NaCl) or a normal-sodium diet (NSD; 1.3% NaCl) and NAC (600 mg/L) or normal drinking water starting at 8 weeks of age. At 11 weeks of age, the rats from both diet and NAC or water treatment groups underwent renal IR or Sham surgery and were followed for 10 weeks. The study consisted of six animal groups: NSD + Sham + water; NSD + IR + water; NSD + IR + NAC; HSD + Sham + water; HSD + IR + water; and HSD + IR + NAC.

Key findings: Tail blood pressure (tBP) increased with IR and NAC treatment in the NSD group but not in the HSD group. The serum creatinine level was higher after NAC treatment in both diet groups, and creatinine clearance was decreased in only the HSD + IR + NAC group. Albuminuria increased in the HSD + IR + water group and decreased in the HSD + IR + NAC group. Kidney mass was increased in the HSD + IR group and decreased with NAC treatment. Renal fibrosis was prevented with NAC treatment and cardiac fibrosis was decreased with NAC treatment in the HSD + IR group.

Significance: NAC treatment promoted structural improvements, such as decreased albuminuria and fibrosis, in the kidney and heart. However, NAC could not recover kidney function or blood pressure from the effects of IR associated with an HSD. Therefore, in general, long-term NAC treatment is not effective and is deleterious to recovery of function after kidney injury.

1. Introduction

Acute kidney injury (AKI) has an incidence of 5–7% in hospitalized patients [1], and a leading cause of AKI is ischemia and reperfusion (IR), which contribute to 80–90% of other renal etiologies [2]. The AKI mortality rate is high, approximately 45–90%, and an AKI diagnosis increases the risk of mortality by 5.5- to 6.5-fold compared with a similarly ill patient without AKI. In general, patients who survive an episode of AKI requiring dialytic therapy and recover function are at increased risk of chronic kidney disease (CKD), which is estimated to account for 3% of the overall yearly incidence of CKD.

Developments in the food industry have contributed to high sodium intake in the population; this trend is a major concern because high sodium intake at a young age is a risk factor for increased blood pressure [3,4], hypertension and renal tissue remodeling with proinflammatory and profibrotic effects [5]. In recent years, the prevalence of kidney disease has increased worldwide. Dietary intervention is one strategy for preventing and delaying CKD progression. Recent guidelines have proposed a daily dietary sodium intake of < 2.0 g (5.0 g of NaCl) for patients with CKD [6]. In populations that have or are at an increased risk of CKD, including African American individuals, seniors (> 55 years), and patients with diabetes or hypertension, the recommended dietary sodium intake is < 1.5 g/d (3.8 g of NaCl) [7]. An experimental analysis of the effects of high sodium intake on IR insult and recovery time would further elucidate how sodium intake influences CKD progression after IR, because evidence indicates that high sodium intake increases NADPH oxidase activity in the renal cortex, suggesting redox regulation of renal salt and water handling [8,9].

N-acetyl-L-cysteine (NAC), a thiol-containing antioxidant, is metabolized in the kidney, resulting in increased renal levels of the intracellular antioxidant glutathione and yielding sulfhydryl groups that directly scavenge reactive oxygen species (ROS), such as superoxide

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(O_2^-), hydrogen peroxide (H_2O_2), hypochlorus acid (HOCl) and hydroxyl radicals (OH) [10]. The protective effect of NAC is mediated by its antioxidant activity and by induction of vasodilation in the renal medulla via stabilization of nitric oxide and inhibition of angiotensin-converting enzyme (ACE) [10]. ROS are important mediators of tissue damage in renal IR injury and in chronic renal failure [10,11]. An imbalance in ROS production causes oxidative stress, which could be responsible for incomplete recovery from AKI, leading to CKD [12,13]. NAC treatment has been shown to have a renoprotective function against radiocontrast-induced renal failure [14–16]. In patients with end-stage renal disease, a 40% reduction in the primary endpoint of cardiovascular disease events has been shown; however, NAC treatment for more than one year has not shown any effect on mortality [17]. In L-NAME hypertensive rats, NAC treatment increased nitric oxide synthase levels in the heart and kidney, decreased ROS levels in the abdominal aorta and prevented an increase in blood pressure [18]. In contrast, NAC treatment did not improve kidney function during the first 80 min after renal IR [10].

We proposed using an IR model in combination with a high-sodium diet (HSD) to study the long-term repercussions of AKI because in many clinical cases, AKI is identified at a late stage or remains undiagnosed, and the underlying causes have not been examined. Experimentally, many studies have investigated the acute phase of IR, but few studies have investigated IR in the long term. Recovery is known to be incomplete after AKI; studies performed by Basile et al. [19] and Spurgeon-Pechman et al. [20] suggested that permanent alterations in renal structure and function occur after IR injury in rats, and such changes are associated with the development of features indicative of chronic renal disease. The authors observed compromised sodium homeostasis and a predisposition to hypertension when the rats were exposed to an HSD.

We hypothesized that rats subjected to IR in combination with a chronic HSD would advance to renal impairment and that NAC treatment would prevent the progression of this disorder.

2. Materials and methods

The experimental protocol was submitted to and approved by the Research Ethics Committee of the School of Medicine of São Paulo University (protocol number 037/14).

2.1. Animals

Adult male Wistar rats were acquired from the Animal Care Center of the School of Medicine of São Paulo University and kept in a 22 °C environment with a 12-hour light/dark cycle and ad libitum access to food and water. We decided to study male Wistar rats because our previous studies showed that males are more susceptible to increased blood pressure after high sodium loading and that males have higher tissue and circulatory renin-angiotensin-aldosterone system responsiveness to sodium variations than females [21,22].

2.2. Diets

The animals received an HSD (8.0% NaCl and 25.0% protein) or a normal-sodium diet (NSD; 1.3% NaCl and 25.0% protein) starting at 8 weeks of age. All components of the diets were the same except the sodium content, and the diets were purchased from Harlan (Harlan Laboratories, Madison, WI 53744-4220, USA). The HSD was given at 8 weeks of age because this period corresponds to the young adult stage in humans. The diet protocol was implemented until the rats were 20 weeks old.

2.3. Renal IR

IR was induced at 11 weeks of age in both diet groups. The animals were anesthetized with a mixture of 20 mg/mL xylazine and 50 mg/mL ketamine (2:5 vol/vol) at a dose of 0.15 mL/100 g body weight. After an abdominal incision was made, the renal arteries of both kidneys were isolated and clamped for 45 min to induce ischemia. Then, the clamps were removed, and reperfusion was visualized by the color change in the renal tissue. Next, the abdominal incision was sutured, and the animals received the antibiotic penicillin G benzathine in a single dose and analgesic treatment with dipyrone for 3 days [23]. Additionally, the study included Sham animals that underwent mock surgery in which all procedures for IR were performed except clamping of the renal artery.

2.4. Treatment

The animals from both diet groups that were subjected to IR or Sham procedures were divided into two groups and received one of the following treatments from 8 to 20 weeks of age:

1. NAC (A7250, Sigma-Aldrich): 600 mg/L given in the drinking water.
2. Water (control): filtered water.

An NAC concentration of 600 mg/L was chosen for this protocol based on previous experimental studies that showed beneficial effects for kidney disorders or hemodynamic effects [16,24].

2.5. Experimental protocol

The following treatment groups were established:

NSD + Sham + water: the rats were fed an NSD, subjected to Sham surgery and provided normal water — (number of animals: 8);

NSD + IR + water: the rats were fed an NSD, subjected to renal IR and provided normal water — (number of animals: 16);

NSD + IR + NAC: the rats were fed an NSD, subjected to renal IR and provided a 600 mg/L NAC solution — (number of animals: 12);

HSD + Sham + water: the rats were fed an HSD, subjected to Sham surgery and provided normal water — (number of animals: 8);

HSD + IR + water: the rats were fed an HSD, subjected to renal IR and provided normal water — (number of animals: 16); and

HSD + IR + NAC: the rats were fed an HSD, subjected to renal IR and provided a 600 mg/L NAC solution — (number of animals: 12).

Animal body weight was evaluated every week using a balance specific for animals (model AS5500C, Marte Balanças, São Paulo, SP, Brazil); however, only the body weight at 10 weeks after IR was included in the analysis. Food and liquid (water or NAC) intake and the urine excretion volume were measured 10 weeks after IR in a metabolic cage for 24 h [Tecniplast S.P.A. Buguggiate (VA), Italy].

2.6. Tail blood pressure (tBP)

tBP measurements were performed via blood flux occlusion with a cuff and a pulse sensor, both of which were placed gently on the tail (Kent Scientific Corporation, USA, model RTBP 2045). The measurement principle is based on detection of arterial vessel distension caused by the blood pulse flowing through the tail, which is monitored by a highly sensitive piezoelectric sensor. The tBP measurement was performed at 10 weeks after IR.

2.7. Renal function

We measured urinary protein concentrations using the Denis-Ayer method [25], serum and urine creatinine concentrations using the modified Heinegard-Tiderstrom method (Creatinine kit K-ref: 96; Labtest Diagnostica S.A., Lagoa Santa-MG), serum urea concentrations (Ureia UV Liquiform kit, ref.: 104; Labtest Diagnostica S.A.) using a Cobas Mira analyzer, and serum and urine sodium and potassium concentrations using a flame spectrophotometer (model FC 280, CELM®, São Paulo, Brazil).

2.8. Biochemical parameters

The serum total antioxidant status (TAS) (Randox Antioxidant Products, County Antrim, United Kingdom), serum uric acid level (Acido Urico Liquiform kit, ref.: 140-1/100; Labtest Diagnostica S.A., Lagoa Santa-MG) and serum malondialdehyde level (MDA; nmol/L) were measured via the thiobarbituric acid reactive substances (TBARS) method. Measurements were performed at 10 weeks after IR.

2.8.1. MDA

A spectrophotometric TBARS assay was conducted. In this assay, MDA in serum samples reacts with thiobarbituric acid and trichloroacetic acid at 100 °C to form a TBARS-MDA adduct, yielding a pink color. The serum samples were read at 540 nm with a spectrophotometer.

Creatinine clearance and the fractional excretion of sodium (FENa) and potassium (FEK) were calculated using serum and urine analysis data and the 24-hour urine volume.

2.9. Kidney and heart masses and histology

The kidney and heart mass was evaluated using a balance, and the femur length was measured using a pachymeter. The results are presented as g/cm femur length.

For histological analysis, renal and cardiac tissues were fixed in formalin buffer, embedded in paraffin and sectioned to a thickness of 4 μm with a microtome (2065 Jung Supercut, Leica, Germany). Periodic acid-Schiff and Masson's trichrome staining and Panoramic Digital Slide Scanners (3DHISTECH, Budapest, Hungary) were used to assess renal cortical lesions and renal and cardiac fibrosis in a blinded manner with the Panoramic Viewer program. To evaluate renal cortical lesions, we assessed the tubulointerstitium and observed the presence of infiltrating cells, necrosis, vacuolar degeneration, tubular epithelial swelling and desquamation. Renal damage was graded as 0, < 5%; I, 5–25%; II, 26–50%; III, 51–75%; or IV, > 75% [26,27].

2.10. Statistical analysis

Results are presented as the mean ± standard error (SE). We used GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA) for all statistical analyses. First, we evaluated the normality of the data using a D'Agostino test and then performed the appropriate data analysis. The parameters were analyzed via two-way ANOVA in which diet and NAC treatment were considered factors; after a Bonferroni post-test correction, a value of $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Baseline characteristics

Because previous studies have shown that chronic high sodium intake influences body weight, food and water intake and blood pressure [28], we believed investigating these parameters associated with IR and NAC treatment was important. We found that the HSD induced lower body weight; however, the NAC treatment did not influence body weight (Fig. 1A). Significantly higher blood pressure was observed in the NSD + IR + water and NSD + IR + NAC groups than in the NSD + Sham + water group, and the NSD + IR + NAC group presented higher blood pressure values than the NSD + IR + water group. In addition, higher blood pressure was observed in the HSD + IR + water and HSD + IR + NAC groups than in the HSD + Sham + water group. The HSD increased blood pressure in the HSD + Sham and HSD + IR + water groups compared with the NSD groups (Fig. 1B). Food intake and liquid intake were higher in the HSD group than in the NSD group (Fig. 1C and D, respectively).

3.2. Kidney parameters

NAC treatment has shown positive or indifferent results related to kidney function depending on the exposure period. For example, 3 days of NAC treatment did not alter kidney function [29]; however, with two weeks of NAC treatment after a single paracetamol dose [30], the kidney function did not improve. To evaluate how an extended NAC treatment time (10 weeks) interferes with kidney function recovery associated with chronic high sodium intake, creatinine, urea and urine volume excretion were determined. NAC treatment significantly increased serum creatinine independent of diet at 10 weeks after IR (Fig. 2A). In addition, NAC treatment decreased creatinine clearance in both diet groups, but clearance was lower in the HSD group with NAC treatment than in the HSD + Sham + water group (Fig. 2B). The blood nitrogen urea level was lower in the HSD group than in the NSD group (Fig. 2C). The HSD significantly increased the urine excretion volume compared with the NSD independent of IR or NAC treatment (Fig. 2D).

The FENa increases during renal reperfusion after ischemia, and reports have shown that previous NAC treatments do not completely prevent an increase in FENa [31] or even worsen FENa [10]. To investigate how NAC modulates sodium and potassium excretion associated with chronic high sodium intake, we evaluated the FENa, FEK, urine protein and albuminuria. The FENa presented a significant interaction and was higher in the HSD + Sham + water, HSD + IR + water and HSD + IR + NAC groups than in the corresponding NSD groups and was higher in the HSD + IR + NAC group than in the HSD + Sham + water and HSD + IR + water groups (Fig. 3A). The FEK increased significantly in the IR + NAC groups that received the NSD and HSD compared with the Sham + water and IR + water groups that received the same diets (Fig. 3B). The urine protein concentration was higher in the HSD group than in the NSD group and in the HSD + IR + NAC group compared with the NSD + IR + NAC group (Fig. 3C). In addition, the HSD significantly influenced the albuminuria level, which was higher in the HSD + IR + water group than in the HSD + Sham + water and NSD + IR + water groups, and NAC treatment decreased this parameter in both diet groups, with a lower level in the HSD + IR + NAC group than in the HSD + Sham + water group (Fig. 3D).

Studies have shown that kidney and heart mass increase with chronic high sodium intake [32,33]. To investigate whether NAC can prevent this increase, we evaluated kidney and heart mass. The kidney mass was significantly influenced by diet and NAC treatment. Kidney mass values were found to be higher in the HSD + IR + water group than in the HSD + Sham + water and NSD + IR + water groups and lower due to NAC treatment in the HSD + IR + NAC group than in the HSD + IR + water group (Fig. 4A). The HSD significantly increased the heart mass in the HSD + IR + water group compared with the HSD + IR + water and NSD + IR + water groups. A significant effect on heart mass was observed, with NAC treatment decreasing the heart mass in the HSD + IR + NAC group compared with the HSD + IR + water group, whereas no difference was observed between the NSD groups (Fig. 4B).

3.3. Histological parameters

In a previous study in our laboratory with rats given a chronic HSD and NAC, we found that NAC decreased interstitial fibrosis without improvement in blood pressure [32]. To test whether NAC could also be effective when associated with IR and an HSD, we evaluated renal and cardiac fibrosis and the renal cortical score. Renal fibrosis was higher in the NSD + IR + water group than in the NSD + Sham + water group and in the HSD + IR + water group than in the HSD + Sham + water group. NAC treatment significantly decreased renal fibrosis

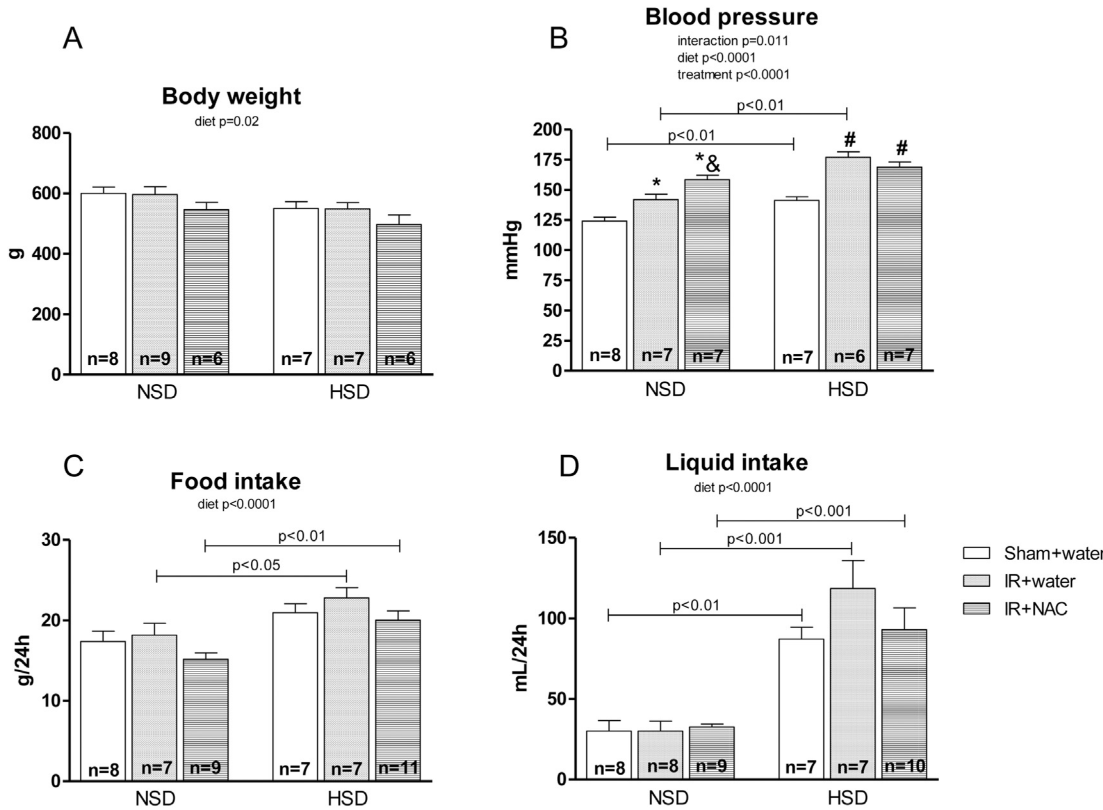

Fig. 1. Body weight (A), tail blood pressure (B), food intake (C) and liquid intake (D) in the normal-sodium diet (NSD) and high-sodium diet (HSD) groups subjected to Sham surgery (Sham + water) or ischemia and reperfusion (IR) and *N*-acetyl-L-cysteine (NAC) treatment (IR + NAC) or water (IR + water) in the 10th week after IR. Two-way ANOVA with a Bonferroni post-test. *p < 0.05 vs the NSD + Sham + water group; &p < 0.05 vs the NSD + IR + water group; and #p < 0.05 vs the HSD + Sham + water group.

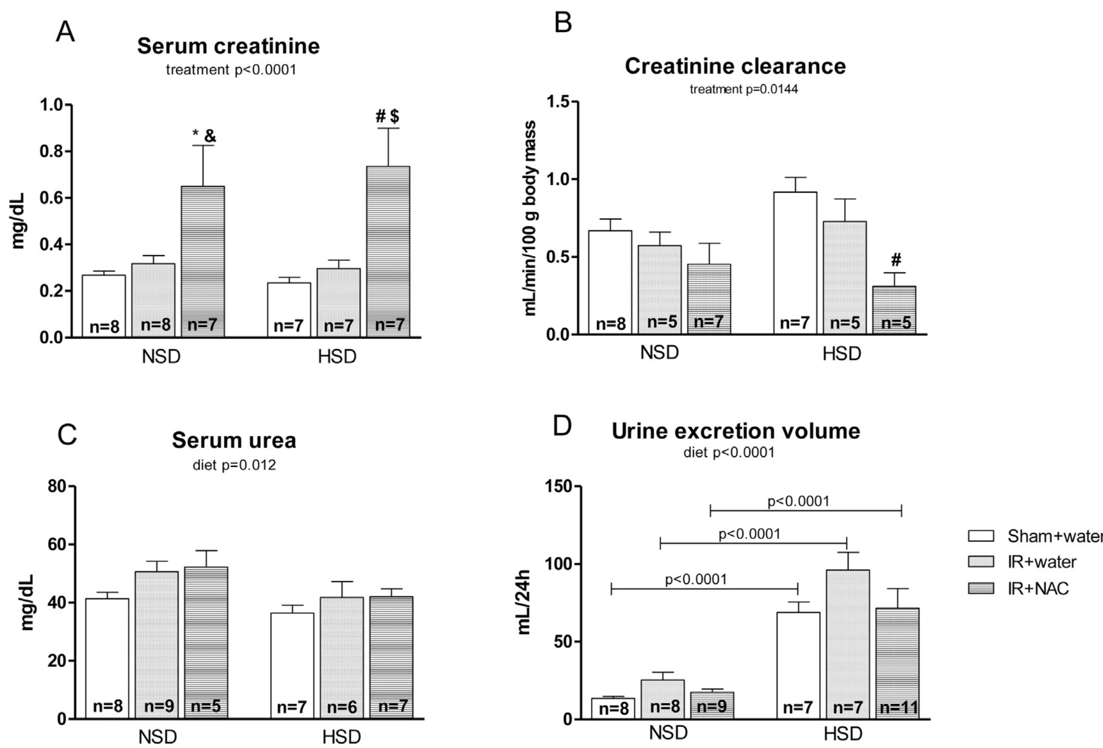

Fig. 2. Serum creatinine (A), creatinine clearance (B), serum urea (C) and urine excretion volume (D) in the normal-sodium diet (NSD) and high-sodium diet (HSD) groups subjected to Sham surgery (Sham + water) or ischemia and reperfusion (IR) and *N*-acetyl-L-cysteine (NAC) treatment (IR + NAC) or water (IR + water) in the 10th week after IR. Two-way ANOVA with a Bonferroni post-test. *p < 0.05 vs the NSD + Sham + water group; #p < 0.05 vs the HSD + Sham + water group; and $p < 0.05 vs the HSD + IR + water group.

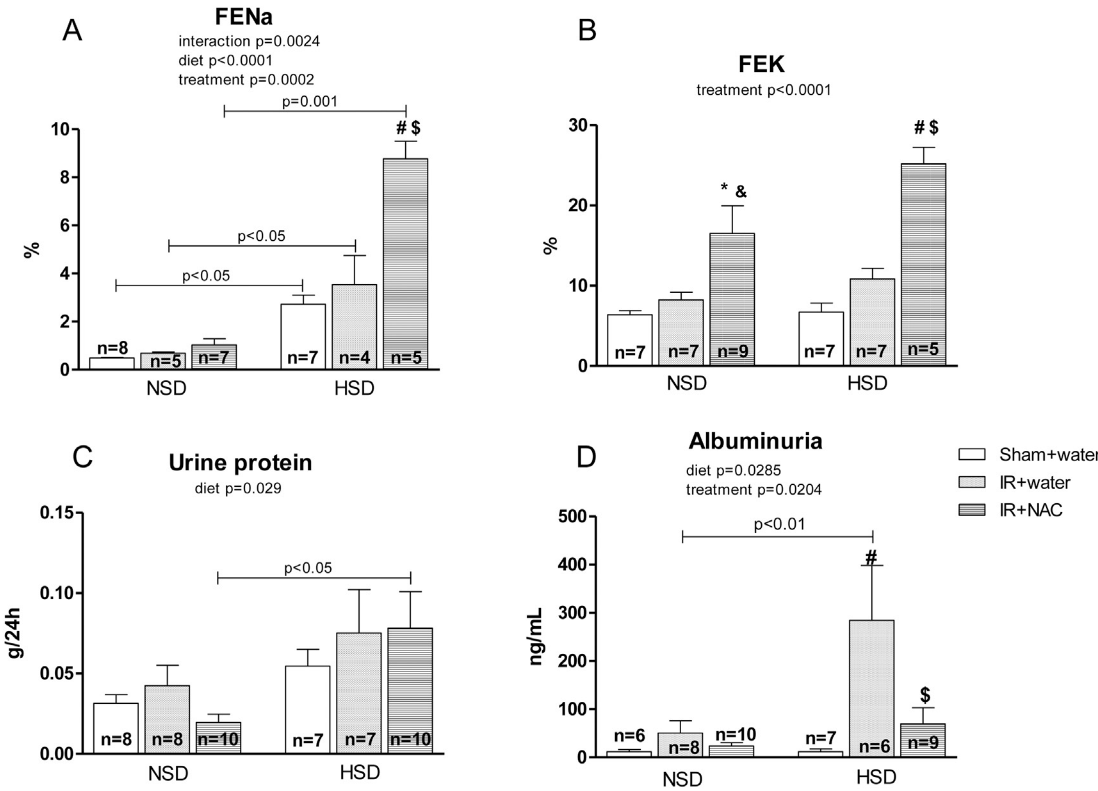

Fig. 3. Fractional excretion of sodium (FENa) (A), fractional excretion of potassium (FEK) (B), urine protein (C) and albuminuria (D) in the normal-sodium diet (NSD) and high-sodium diet (HSD) groups subjected to Sham surgery (Sham + water) or ischemia and reperfusion (IR) and *N*-acetyl-L-cysteine (NAC) treatment (IR + NAC) or water (IR + water) in the 10th week after IR. Two-way ANOVA with a Bonferroni post-test. *p < 0.05 vs the NSD + Sham + water group; & p < 0.05 vs the NSD + IR + water group; #p < 0.05 vs the HSD + Sham + water group; and $p < 0.05 vs the HSD + IR + water group.

independently of diet, shown by the observation that the NSD + IR + NAC and HSD + IR + NAC groups were not different from their respective Sham groups (Fig. 5A).

Cardiac fibrosis was significantly influenced by diet and NAC treatment. The degree of fibrosis was higher in the HSD + IR + water group than in the HSD + Sham + water and NSD + IR + water groups and lower in the HSD + IR + NAC group than in the HSD + IR + water group (Fig. 5B). NAC treatment significantly increased the renal cortical lesion score in the NSD + IR + NAC and HSD + IR + NAC groups compared with that in the NSD + IR + water and HSD + Sham + water groups, respectively (Fig. 5C).

3.4. Oxidative stress parameters

An imbalance in ROS production causes oxidative stress, which could be responsible for incomplete recovery from AKI, leading to CKD [12,13]. As an antioxidant, NAC might be able to reestablish the balance between antioxidants and oxidants. To verify the status of this balance, we examined the serum MDA level, TAS and uric acid level. Then, oxidative stress was evaluated via TBARS assays, and the results showed that the serum MDA levels were higher in the NSD + IR + water group than in the NSD + Sham + water group, while NAC treatment significantly decreased the serum MDA levels in both diet groups compared with their corresponding Sham and IR + water groups (Fig. 6A). The level of the antioxidant parameter

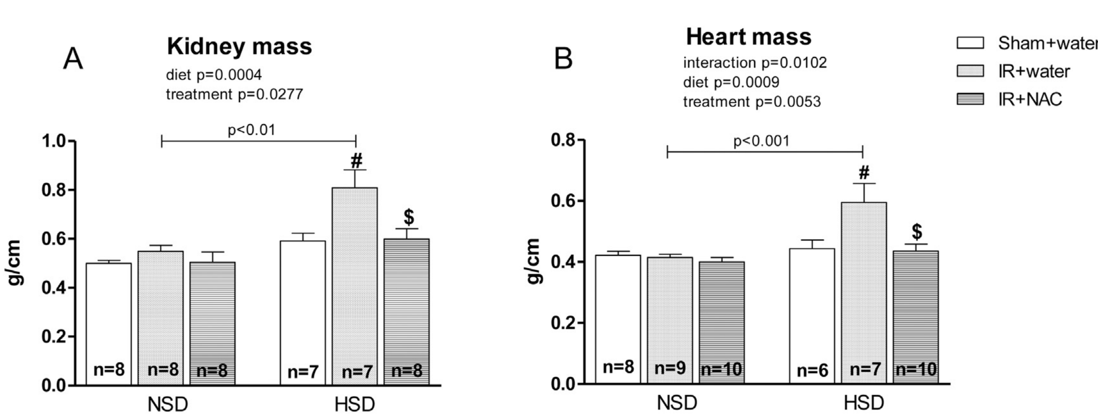

Fig. 4. Kidney mass (A) and heart mass (B) in the normal-sodium diet (NSD) and high-sodium diet (HSD) groups subjected to Sham surgery (Sham + water) or ischemia and reperfusion (IR) and *N*-acetyl-L-cysteine (NAC) treatment (IR + NAC) or water (IR + water) in the 10th week after IR. Two-way ANOVA with a Bonferroni post-test. #p < 0.05 vs the HSD + Sham + water group and $p < 0.05 vs the HSD + IR + water group.

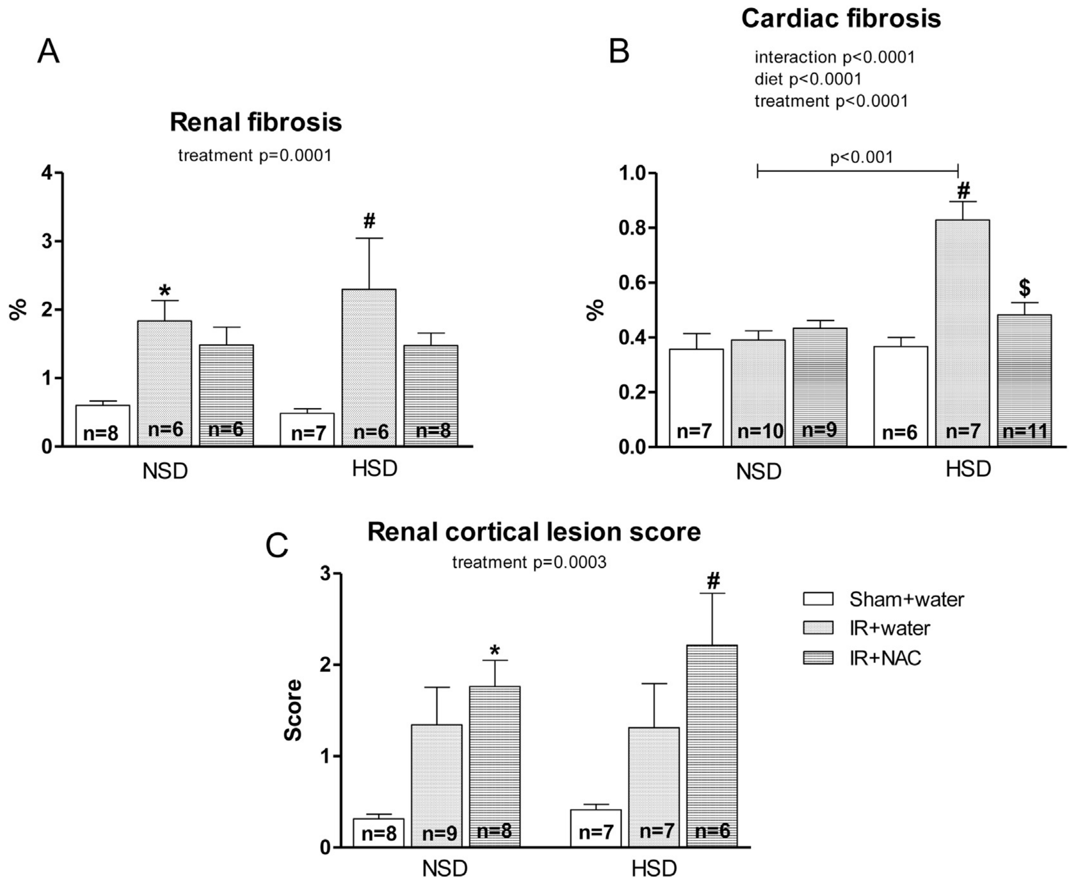

Fig. 5. Renal fibrosis (A), cardiac fibrosis (B), and renal cortical lesion scores (C) in the normal-sodium diet (NSD) and high-sodium diet (HSD) groups subjected to Sham surgery (Sham + water) or ischemia and reperfusion (IR) and *N*-acetyl-L-cysteine (NAC) treatment (IR + NAC) or water (IR + water) in the 10th week after IR. Two-way ANOVA with a Bonferroni post-test. *p < 0.05 vs NSD + Sham + water; #p < 0.05 vs the HSD + Sham + water group and $p < 0.05 vs the HSD + IR + water group.

serum TAS was lower in the NSD + IR + NAC group than in the NSD + Sham + water group, and the serum uric acid level was higher in the HSD group than in the NSD group (Fig. 6B and C).

4. Discussion

From our hypothesis, we demonstrated that rats subjected to IR in combination with a chronic HSD advanced to renal impairment, evidenced by high blood pressure, albuminuria, and increased kidney and heart mass and fibrosis. On the other hand, NAC treatment did not completely prevent the progression of this disorder, because while some kidney parameters improved, others worsened. The main improvements were decreased albuminuria, kidney and heart mass and fibrosis, but in contrast, NAC treatment worsened kidney function by inducing an increase in serum creatinine, a decrease in creatinine clearance and an increase in FENa and FEK and renal cortical lesions. In general, NAC did not improve kidney function in the IR model with a chronic HSD.

In the present study, the high blood pressure found in the IR group independent of diet could be attributed to reductions in peritubular capillary density, previously observed by Basile et al. [19], suggesting that IR induces a predisposition to hypersensitivity to pressure stimuli, such as infusion of angiotensin II or salt [34–36]. In support of this hypersensitivity to pressure stimuli notion, we observed that exposure to the HSD increased the blood pressure in rats subjected to IR. In addition, NAC treatment did not decrease the blood pressure; however, it was able to prevent a further increase in blood pressure. In a previous study, NAC was shown to prevent an increase in blood pressure in rats when administered concomitantly with N-nitro-L-arginine methyl ester (L-NAME) [18] or with an HSD [37]; on other hand, no effect on blood pressure levels was observed when NAC was administered as a therapeutic after 4 weeks of L-NAME administration [18]. However, the NAC concentrations used in these studies were different, with a much higher concentration (4.0 g/kg per day or 20 g/L) used in the previous study than in the present study (600 mg/L). Another difference is the experimental model used; an insult to the kidney was applied in our study, whereas the other studies only utilized drug administration. In a clinical study, no effect of NAC on blood pressure was observed in CKD patients whose blood pressure was controlled by anti-renin-angiotensin system drugs [38]. In the present study, although the renin-angiotensin system was suppressed by high sodium intake, which contributed to vasodilation [39] under NAC treatment, we also observed increased food and liquid intake in the HSD group that might contribute to maintenance of high blood pressure levels.

Therefore, NAC treatment was not able to decrease blood pressure in the HSD + IR group, likely due to hypersensitivity to pressure stimuli induced by salt through a reduction in peritubular capillary density in the kidney, however, NAC prevented an increase in blood pressure in animals subjected to IR and an HSD through synergism of the vasodilation mechanism of NAC and renin-angiotensin system suppression by the HSD.

The HSD induced an increase in albuminuria in animals subjected to IR, which is in accordance with Basile et al. [19] who showed that albuminuria began to increase after 16 weeks in animals subjected to IR. The concomitant increase in albuminuria and in kidney and heart mass associated with the HSD in the present study could be suggestive of manifestations of chronic renal disease [19]. However, NAC treatment was able to prevent the increase in albuminuria severity and kidney and heart mass in the animals fed an HSD. The present findings

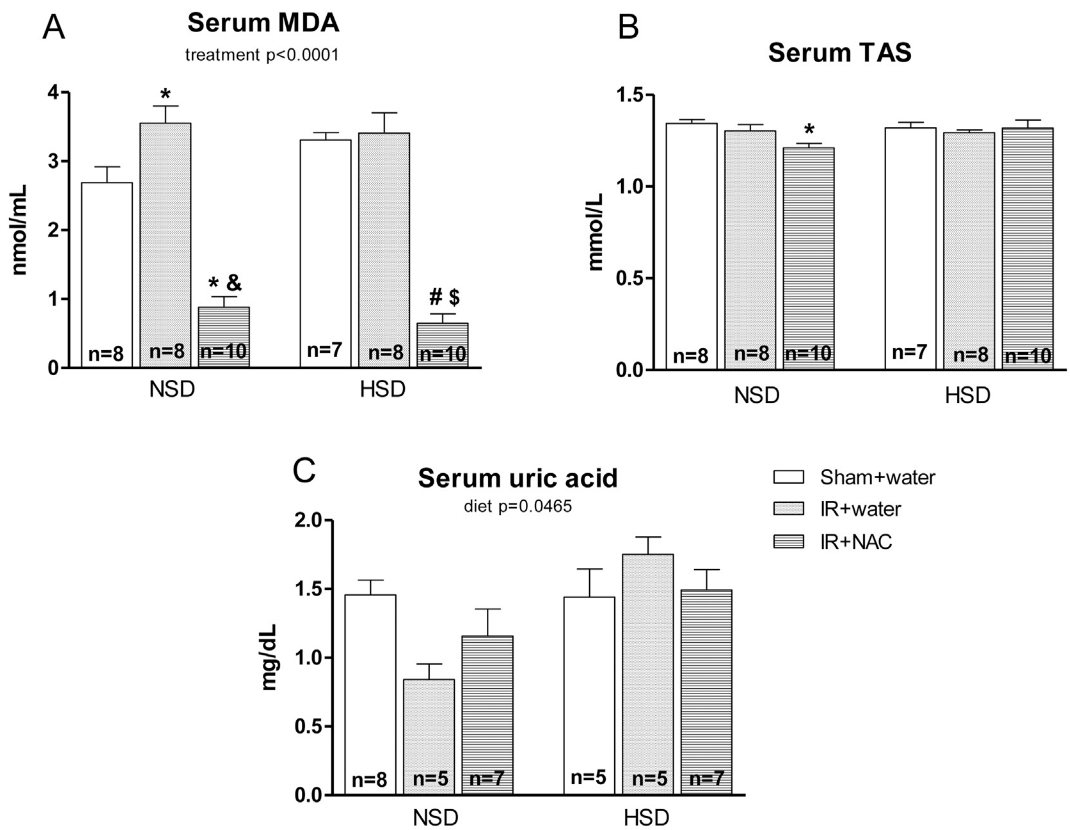

Fig. 6. Serum malondialdehyde (MDA) level (A), serum total antioxidant status (TAS) (B), and serum uric acid level (C) in the normal-sodium diet (NSD) and high-sodium diet (HSD) groups subjected to Sham surgery (Sham + water) or ischemia and reperfusion (IR) and *N*-acetyl-L-cysteine (NAC) treatment (IR + NAC) or water (IR + water) in the 10th week after IR. Two-way ANOVA with a Bonferroni post-test. *p < 0.05 vs the NSD + Sham + water group; & p < 0.05 vs the NSD + IR + water group; #p < 0.05 vs the HSD + Sham + water group; and $p < 0.05 vs the HSD + IR + water group.

agree with those of Shimizu et al. [40] in an experimental subtotal nephrectomy study that showed a significant protective effect of NAC on renal inflammation and reduced proteinuria. The underlying mechanism explaining the association between albumin and inflammation is that albumin can stimulate proinflammatory cytokine production in proximal tubule cells via nuclear factor kappa B (NF-κB) activation, which is an important transcription factor for a number of cytokine genes, thereby inducing endothelial cell death and generating a local inflammatory reaction characterized by the release of endothelial-derived cytokines [41,42]. However, NAC inhibits the activation of some protein kinases and of NF-κB [43]. Thus, the significant decrease in albuminuria and consequent decrease in kidney and heart mass were influenced by the anti-inflammatory effect of NAC treatment.

The unexpected decrease in kidney and heart mass without a decrease in blood pressure under NAC treatment in animals fed an HSD is interesting, and the mechanism underlying the decrease in the mass of these organs must be identified. Similar results were found by Shimizu et al. [40], who demonstrated no reduction in blood pressure and decreased heart mass with NAC treatment in experimental subtotal nephrectomy. In addition, similar results were obtained with administration of the antioxidant vitamin E in a rat model of chronic nitric oxide synthase inhibition [44] in which renal injury was reduced, but there was no concomitant improvement in hypertension. Previous studies with antihypertensive drugs have shown no apparent effect on blood pressure and demonstrated a significant reduction in atherosclerotic lesion size in Apo E-deficient mice [39] or a decrease in lipid peroxidation and NADPH oxidative activity in Dahl salt-sensitive rats [45]. In a previous study in our laboratory with rats administered a chronic HSD and NAC, we found that NAC decreased heart mass as well as cardiomyocyte diameter and interstitial fibrosis without improvement in blood pressure [32]. Basic studies have shown that NAC impacts cell cycle regulation, including inhibition of normal mammalian human cell proliferation, via inhibition of DNA synthesis induction and modulation of various target gene and/or protein levels [46]. In addition, NAC has been shown to inhibit the spread of local endothelial ACE activity in patients with chronic renal disease and experimental animals, improve endothelial dysfunction and prevent vascular and myocardial structural changes [47]. Other studies have shown that NAC decreases angiotensin II receptor binding in vascular smooth muscle cells [48]. High sodium intake inhibits the renin-angiotensin system, and NAC inhibits cellular proliferation; thus, both actions together could contribute to a decrease in renal and cardiac mass without affecting hemodynamic parameters.

Another structural improvement induced by NAC was the reduction in cardiac fibrosis in rats fed an HSD and subjected to IR. Additionally, in the kidney, we observed fibrosis reduction in both diet groups.

NAC treatment worsened kidney function, evidenced by the increased serum creatinine level and decreased creatinine clearance in the HSD- and NAC-treated groups. These data suggest that long-term NAC treatment could interfere with the creatinine level. A previous study found that administration of 150 mg/kg NAC for 2 weeks plus paracetamol in Wistar rats did not return serum creatinine levels back to normal [30], while three days of NAC treatment did not change the creatinine serum level in rats with unilateral hydronephrosis [29].

Epithelial cell damage typically results in reduced reabsorption of many ions, including Na and K [49], and the FENa increases during renal reperfusion after ischemia and in a gentamicin-induced nephrotoxicity model [10,31,49]. In the present study, we observed that NAC treatment also increased the FENa and FEK. These data suggest that NAC treatment induced a further reduction in Na and K reabsorption, contributing to worsening of kidney function. In the literature, NAC has been shown to improve kidney function by decreasing the FENa in a gentamicin-induced nephrotoxicity rat model [40]. Patients with advanced, stable chronic renal failure and in sodium balance exhibit a high sodium fraction due to chronic reduction in the glomerular filtration rate and a lack of simultaneous reduction in salt intake [50]. In the present study, the long-term NAC treatment after IR in rats likely contributed to high FENa and FEK.

We expected that long-term NAC treatment would have an overall renal protective effect; however, the opposite result was observed, including decreased renal function and the presence of renal cortical lesions. Most likely, the lower serum MDA, which suggests lower antioxidant levels caused by long-term NAC treatment, contributed to the deleterious activity of NAC. Deleterious activity of NAC has been demonstrated in vivo and in vitro, including autoxidation of thiols, an increase in OH generation and DNA damage. Clinically, in the absence of significant oxidative stress, it is suggested to avoid NAC administration [45]. In addition, one study showed that after 2 h of oral ^{35}S-NAC administration, the highest concentration of ^{35}S was found in the kidney and liver [51]. Therefore, the long-term NAC treatment proposed in the present study caused deleterious effects under stable conditions after IR and contributed to renal cortical lesion development.

We observed that TAS and uric acid levels did not change with NAC treatment, while MDA levels decreased significantly independent of diet. Maintenance of the equilibrium between antioxidants and oxidants is known to be important for avoiding oxidative stress. The decrease in MDA levels likely caused the lack of change in TAS and uric acid levels, thereby maintaining this equilibrium [52].

A limitation of this study is that we did not include inulin determination for kidney function evaluation or later time points to observe the chronic kidney course.

5. Conclusions

NAC treatment promoted structural improvements, such as decreased albuminuria and fibrosis in the kidney and heart. However, NAC could not recover kidney function or blood pressure from the effects of IR associated with an HSD. Therefore, in general, long-term NAC treatment is not effective and is deleterious to recovery of function after kidney injury.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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