Research article

REDUCTION IN GLOMERULAR AND RENAL TUBULAR DAMAGE IN KIDNEYS OF DIABETIC RATS SUPPLEMENTED WITH ALPHA-LIPOIC ACID

Tamyris Pontes FRAGA[1], Lanna Beatriz Neves Silva CORRÊA[1], Julia Galian Ribeiro TABOAS[1], Vivian Alves Pereira da SILVA[1], Gilson Teles BOAVENTURA[2], Mauricio Alves CHAGAS[1]*

[1]Federal Fluminense University, Biomedical Institute, Department of Morphology, Laboratory of Cellular and Extracellular Biomorphology, Niterói, RJ, Brazil; [2]Federal Fluminense University, Laboratory of Experimental Nutrition, Department of Nutrition and Dietetics, Niterói, RJ, Brazil

Chronic hyperglycemia caused by diabetes results in systemic damage to the body, and the kidney ends up being the target of tissue damage. The use of antioxidants such as alpha-lipoic acid (ALA) has been shown to improve clinical and biochemical parameters in diabetic patients as they help in the prevention of oxidative stress. The present study aimed to undertake a comparative evaluation of the renal morphology of normal and diabetes-induced Wistar rats supplemented with ALA. The experiment had 4 experimental groups (n=7): a control group and a diabetic group that were fed commercial food; an alpha-lipoic group and an alpha-lipoic diabetic group that received the ALA supplement. Diabetes was induced with a single intraperitoneal injection of alloxane monohydrate administered after fasting. After 60 days of the experiment, the kidneys were excised to study the histomorphometric and stereological parameters of the glomeruli and renal tubules and the immunohistochemical parameters with a podocyte count. In the evaluation, the ALA promoted improvement in all of the relevant parameters of the glomerular areas and attenuation of tubular lesions of diabetic animals (p<0.001). Supplementation with ALA also showed beneficial effects in diabetic animals, such as reducing hyperglycemia and the damage promoted by oxidative stress alongside promoting a protective effect on the renal parenchyma. The reduction of glomerular and tubular damage delayed the advance of diabetic nephropathy and its complications, preventing renal failure in diabetic groups.

Keywords: Alloxan, antioxidant, hyperglycemia, nephropathy, thiocytic acid, alpha-lipoic acid

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*Corresponding author: e-mail: chagas.m@gmail.com

INTRODUCTION

Diabetes is one of the most critical endocrinopathies in small animal clinics. It is characterized by insufficient secretion of insulin or its deficient action [1,2]. Chronic hyperglycemia damages the body, causing clinical and metabolic complications [3]. In such cases, the kidney is one of those organs that are the most affected [4,5].

One of the main changes experienced by a diabetic patient is developing a kidney disease related to diabetes known as diabetic nephropathy (DN). Diabetic patients have a high incidence of DN; it occurs in about 30–40% of the cases [6] and it is the main cause of end-stage kidney disease. Further, the mortality rate among diabetic patients with concomitant nephropathy is 30 times higher [7]. Incidentally, it must be noted that once nephropathy is established, it is possible to delay its progression to a terminal illness [8].

The mechanisms that lead to the development of DN after hyperglycemia are yet to be understood entirely [9]. However, it is known that several physiological processes co-occur with the activation of innate immunity leading to the development of a low-grade chronic response. This response increases the production of reactive oxygen species (ROS), a recognized factor in the pathogenesis of DN [10]. Once DN is established, the main changes found in a patient are the following: enlargement of the glomerular basement membrane, thickening of the tubular basement membrane [11,12], segmental and focal glomerulosclerosis, fibrosis [4], hyperfiltration and proteinuria [13].

The use of antioxidants has been described in the prevention of oxidative stress and as efficient compounds in treating the changes caused by diabetes. Alpha-lipoic acid (ALA) is an important antioxidant produced by the mitochondria. It can also be obtained through food [14]. ALA is especially promising in diabetic conditions due to its antioxidant action [15]. It acts as a regulator of glucose metabolism, increasing sensitivity to peripheral insulin and aiding glycemic control [16,17]. Unlike other antioxidants such as Vitamin C and Vitamin E, ALA has an amphipathic behavior [18], making it more efficient. Its use has been shown to yield promising results in chronic pathologies associated with oxidative stress, such as aging [19], cancer [20], neurodegenerative diseases [21], and diabetes and its complications [22-25].

According to the scientific literature, the supplementation with ALA promotes beneficial effects in the physiological parameters of several organs and systems, including the kidneys [2]. However, few studies have evaluated the action of ALA on renal morphology, especially in cases of long-term diabetic hyperglycemia.

This study aimed to evaluate the action of ALA supplementation on the renal morphology of diabetic rats. For this, the Wistar rat was used as an experimental model. The induction of diabetes was carried out using alloxan, which is frequently used in studies related to diabetes [26].

MATERIAL AND METHODS

Twenty-eight male *Rattus norvegicus* rats of the Albinus variety and Wistar lineage that were 90 days old and weighed 350 g were kept apart in individual cages. The animals were housed at a temperature of 21–23°C, relative humidity of 60%, with light-dark cycle control (12h/12h) and *ad libitum* water supply. The use of these animals for the experiment was approved by the Ethics Committee for the Use of Animals at the Federal Fluminense University (CEUA-UFF protocol number 799/16).

The selected animals were randomly divided into 4 experimental groups (n=7/ group), as follows: a control group (CG): fed with a standard diet based on Nuvilab® commercial feed; a diabetic group (DG): experimentally induced rats submitted to Nuvilab® commercial diet; an alpha-lipoic acid (non-diabetic) group (ALAG): fed with commercial feed with the addition of alpha-lipoic acid (Sigma 62320) at a dose of 300mg/kg/BW; and an alpha-lipoic acid diabetic group (ALAD): experimentally induced animals fed with Nuvilab® commercial food with the addition of ALA (Sigma 62320) at a dose of 300mg/kg/BW.

Induction of diabetes

Before receiving the experimental diets, 14 of the DG and ALAD rats started a 30-hour feeding restriction. During this period, these animals did not receive food; they only had *ad libitum* access to water. After the fasting period, these animals were manually restrained. Thereafter, they received a single intraperitoneal injection of alloxane monohydrate + 98% (Cayman Chemical, code 9002196) which was previously diluted in a solution of 0.9% sodium chloride at a ratio of 600 mg of alloxane in 40 ml of saline solution at a dose of 150 mg/kg [26]. The CG and ALAG groups were also submitted to food restriction and the same intraperitoneal injection protocol, with the alloxane replaced by a sterile saline solution.

After 72 hours of alloxane administration and 6-hour fasting, a drop of blood was punctured from the tail vein. With the Accu-Check Performa glucometer (Glucometer, Roche), the glycemia of the animals was analyzed following the protocol used by Aguirre-Arias and collaborators [27]. The rats that presented blood glucose levels equal to or greater than 270 mg/dL (range 270–700) were considered induced to diabetes and included in the experiment [26,28]. The same methodology was used weekly until the end of the experiment to control the glycemia of the animals.

Diet

The experiment lasted a total of 8 weeks. The CG and DG rats consumed commercial feed (Nuvilab®, Nuvital, Paraná, Brazil) mash prepared in pellets. The ALAG and ALAD rats were supplemented with 4g/kg of ALA R-isomer purified Sigma Aldrich 62320 (Table 1). This experimental diet was prepared in the Experimental Nutrition Laboratory (LabNE) of Federal Fluminense University. The ingredients were weighed

and homogenized in a Hobart® industrial mixer (São Paulo, SP, Brazil) with warm water (60°C). The obtained mass was transformed into pellets and dried in a ventilated oven (Fabbe-Primar® no 171, São Paulo, SP, Brazil) at 60°C for 12 hours and, after identification, stored under refrigeration until use.

Table 1. Food composition of experimental groups

Humidity (max)	125 g/kg
Crude protein (min)	220 g/kg
Ethereal extract (min)	40 g/kg
Mineral matter (max)	90 g/kg
Gross Fiber (max)	70 g/kg
Calcium (min-max)	10-14 g/kg
Phosphorus (min)	8 g/kg
Alpha lipoic acid *	4-6 g/kg
Vitamin A (min)	13.000 IU
Sodium (min)	2.7 g/kg
Iron (min)	0.05 g/kg
Manganese (min)	0.06 g/kg
Zinc (min)	0.06 g/kg
Copper (min)	0.01 g/kg
Iodine (min)	0.002 g/kg
Selenium (min)	5×10^{-5} g/kg
Cobalt (min)	0.0015 g/kg
Fluorine (max)	0.08 g/kg
Lysine (min)	12 g/kg
Methionine (min)	4 g/kg

Composition for each kg of food (minimum and / or maximum quantities).
The groups consumed pelleted commercial food (Nuvilab®, Nuvital, Paraná, Brazil)
*Addition in Alpha Lipoic acid group and Diabetic Alpha Lipoic acid group.

Renal assessment

At the end of the experimental period, the animals were euthanized. They were anesthetized with 75 mg/kg of ketamine + 10 mg/kg of xylazine, and the previously calculated dose was intraperitoneally administered. Once the anesthesia condition was achieved, as assessed by the absence of the foot reflex, the animals were subjected to bleeding by an intracardiac puncture, from which 10 mL of blood were obtained. After bleeding, an additional dose of anesthesia was given, which led to the animal's death.

After euthanasia, the kidneys were collected and the adipose tissue together with the renal capsule removed. For renal evaluation, the volume of the left kidney was determined using the method of Scherle [29] and weighed on a semi-analytical

digital balance (Bel® brand, 0.01 g). The right kidneys were destined for digital histomorphometric analysis.

Corticomedullary relationship

For the corticomedullary evaluation, the Cavalieri method was used, involving the calculation of the proportional area of the cortical and medullary region of the kidney. The left kidney was transversely sectioned at an interval of two-millimeter thickness, resulting in an average of 7 sections per kidney. The transverse surface of each fragment was photographed under a microscope with the Axiocam 506 color camera (Carl Zeiss Microscopy, LLC, Jena, Germany) coupled to the Stereo Discovery V8 stereomicroscope (Carl Zeiss) on 4x objective lens, together with a millimeter ruler for further calibration in the Image J program. The measurement of images to determine the proportional area of the renal cortex and medullary was performed after the calibration of the Image J software program, FIJI extension v 1.50G. The total area and area of the medullary region of each renal section were measured, and the subtraction of the total areas determined the area of the cortical region. The volume obtained by the Scherle method made it possible to calculate the volume of the cortical and medullary regions [30].

Histomorphometry

The rats' right kidneys were fixed in the Bouin for 5 hours and then in a 10% formalin solution. To measure their areas, uniform sagittal sections were made and dehydrated kidneys embedded in paraffin with the longest axis face down. Afterward, 5 µm-thick sections were made and stained with hematoxylin and eosin or prepared for immunohistochemistry [31].

Glomerular histomorphometry

The sections stained with hematoxylin and eosin were examined using an Olympus BX-51 optical microscope coupled to an Olympus DP-72 digital camera. Microphotographs of the kidneys were used to evaluate the renal corpuscle area, capsular space area, and glomerular area. Glomeruli stained in hematoxylin and eosin were captured at 400x magnification, digitized in "tiff" files, and measured using the FIJI v 1.50a software. After calibrating the images with the "freehand" tool, the area of the renal and glomerular corpuscle was measured with reference to the equatorial poles of the glomerulus, the subtraction of the aforementioned areas determined the area of the capsular space [32].

Stereology of glomeruli and renal tubules

A total of 50 histological areas were evaluated from 5 sections, which were obtained from serial sections with a spacing of 25 micrometers between them in order to avoid

overlapping structures. From each section, 10 histological areas were analyzed. The M42 point system was used for counting. This system works by superimposing the analyzed images with a quadrangular grid delimited by lines containing 21 pairs of points (from a total of 42 points) joined by a long line.

The images were analyzed with 20x and 10x objectives, respectively; the evaluation was carried out by counting points on the structures determined by volume density (Vv) by the ratio (Pp/Pt)x100 between the number of countable points on the structures (Pp) and the total number of points of the grid (Pt).

Immunohistochemical processing

Obtained histological slides were dewaxed and rehydrated in a decreasing alcohol solution. For antigen retrieval, the sections were immersed in a high pH citrate buffer solution and placed in a Cuisinart pressure cooker (Inopat) at 98°C for 1 hour. After washing in phosphate buffer solution (PBS), the sections were incubated for 1 hour with a pre-diluted primary antibody Wilm's tumor 1 protein (Willm's, 1:100, clone 6F-H2-Dako, US). They were then incubated with an amplifier (EnVision + Dual Link System-HRP K-4063, Dako) for 20 minutes. The sections were then washed, revealed with diaminobenzidine chromogen (DAB), and counterstained with hematoxylin to visualize the markings. Wilm's tumor protein 1 (WT1) immunostaining is commonly used as a podocyte nuclei marker [33-35].

Podocyte number

The number of podocytes was counted from 50 glomeruli randomly obtained through the FIJI v 1.50a software, using a cell counter plugin, for the presentation of the average of podocytes.

Statistical analysis

The data gathered from the experiment were presented as mean and standard deviation. The normal distribution of the values found was evaluated using the Shapiro-Wilk test. For the present study, the one-way ANOVA test and the Tukey-Kramer multiple comparisons test were used. Statistical analyses were performed using the GraphPad Prism statistical package version 3.01 program (San Diego, CA, USA). The graphs shown were performed using the GraphPad Prism version 5. The significance in all tests was established at $p < 0.05$.

RESULTS

Blood glucose

After 72 hours, type 1 diabetes was established with a blood glucose level above 270 mg/dl. After 60 days, the DG and ALAD rats showed a significant increase

(p<0.001) in hyperglycemia. In the ALAG rats, the increase in blood glucose was not statistically significant when compared to the CG rats. At the end of the experiment, the hyperglycemia in the diabetic groups was significantly greater than in the control groups. Considering the weekly average, the glycemia of the ALAD rats was lower than that of the DG rats, but the difference was not significant. The results related to kidney weight did not significantly differ between the experienced groups (p>0.05), as observed in Table 2.

Table 2. Mean and standard deviation of blood glucose, weight, corticomedullary ratio, glomerular areas, volume density of glomeruli and tubular lesions, and the podocyte count of the experienced groups.

Parameters	Groups				P
	CG	ALAG	DG	ALAD	
Blood glucose (mg/dl)	122,53±8,24a	134,18±14,33a	506,45± 32,60b	490,53±28,53b	<0,0001
Kidney wheight (g)	1,46±0,14	1,71±0,19	1,65± 0,18	1,67±0,14	0,05
Corticomedullary ratio	0.72± 0.10a	0.73±0.05a	0.36± 0.02b	0.59±0.16c	0,001
Renal corpuscle area (μm²)	10179.38± 591.45a	9846.75± 659.16a	7051.11± 579.04b	8435.12± 970.76ab	0,001
Glomerular area (μm²)	7496.54± 730.47a	7193.53± 812.41a	5227.19± 737.38b	6809.27± 884.77ab	0,001
Capsular space area (μm²)	3243.59± 349.02a	3308.88± 407.81a	1656.68± 240.37b	2292.52± 354.42ab	0,001
Glomerular volume density (Vv-%)	5.62±0.77a	5.42±1.24a	3.21±0.59b	4.12±0.51ab	<0.0001
Tubular lesions volume density (Vv-%)	0.01±0.00a	0.1±0.00a	10.73±3.49b	5.05±1.30c	<0.0001
Podocyte count	12.07±1.95	9.93±0.75	9.325±0.05	10.19±0.95	0,05

Letters a, b and c indicates significant differences between groups; CG - control group; ALAG - alpha-lipoic acid group; DG - diabetic groups; ALAD - alpha-lipoic acid diabetic group.

Feed consumption

After 8 weeks, the DG and ALAD animals showed increased feed consumption compared to the CG and ALAG rats. There were no significant changes in feed intake of the ALAG rats when compared to the CG rats (p>0.05). Among the diabetic animals, those that received alpha-lipoic acid supplementation (ALAD group) showed a constant reduction in their feed intake, with a significant reduction seen in the seventh to eighth week (p<0.05) when compared with DG (Figure 1).

Body mass

From the second week of the experiment, it was observed that the DG and ALAD presented a significant reduction in their body mass compared to the CG and ALAG

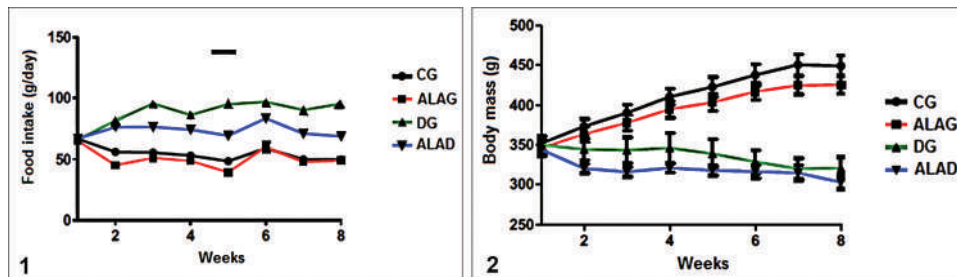(p<0.0001). There was no significant change between the DG and ALAD (p>0.05), as shown in Figure 2.

Figure 1. Food intake (g/day) throughout the experiment
Figure 2. Evolution of animal body mass (g) throughout the experiment
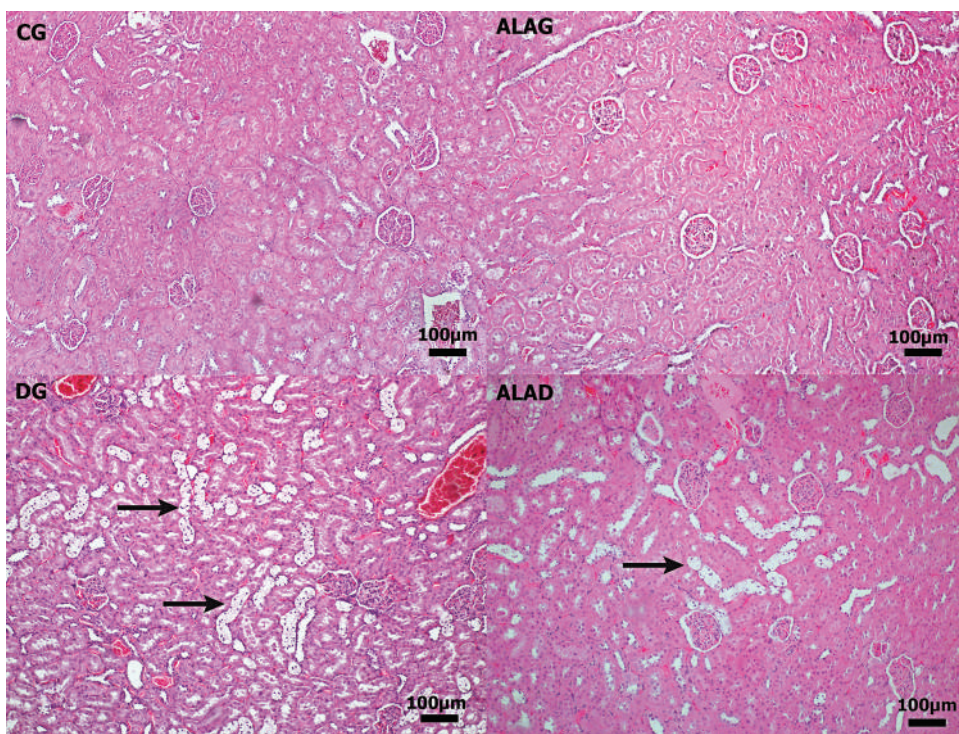

Figure 3. Histological images of rat renal glomerulus, evidencing tubular lesions
Legend: Representative photomicrographs of kidney of: control group (CG); alpha-lipoic acid group (ALAG); diabetic group (DG); - alpha-lipoic acid diabetic group (ALAD): (haematoxylin and eosin, bar: 100 μm)

Corticomedullary relationship

Based on the corticomedullary relationship (Table 2), the proportional areas between the cortical and medullary regions of the DG rats' kidneys were inferior to those of the CG, ALAG and ALAD (p<0.001). There was no significant reduction in the ALAD compared to what was seen in the CG and ALAG (p>0.05).

Histomorphometry and stereology

By histomorphometry of glomerular areas (renal corpuscle area, glomerular area, capsular space area), the diabetic groups had lower means compared to the CG (p<0.001). However, in all of the parameters, the ALAD group showed greater preservation of their glomerular areas with means higher than the DG (p<0.001), as seen in Figure 3.
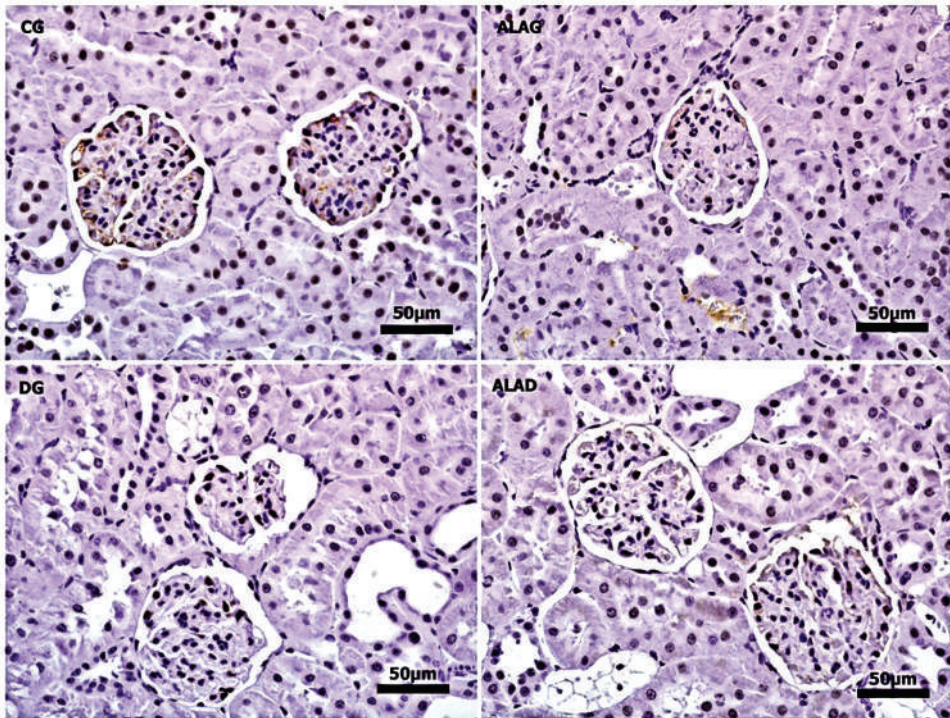

Figure 4. Histological images of rat renal glomerulus
Legend: Immunohistochemical (IHC) detection of Wilms' tumor 1 protein (WT1) as a marker for podocytes in renal glomeruli in the control group (CG); alpha-lipoic acid group (ALAG); diabetic group (DG); - alpha-lipoic acid diabetic group (ALAD): (DAB, counterstain haematoxylin, bar: 50 μm).

The percentages of glomerular volume density (GVv) of the DG and ALAD were significantly lower compared to the CG and ALAG (p<0.05). The ALAD animals presented GVv that was superior to that of the DG. However, the results were not

significant. Regarding the Tubular lesion (vacuolization and hydropic degeneration) Vv (TLVv), the ALAD group presented a percentage reduction of 50% compared to the DG (p<0.001) (Table 2).

Podocytes

The DG and ALAD groups had a significantly smaller amount of podocytes compared to the CG (p<0.001), as observed in Figure 4. The ALAD rats presented a mean number of podocytes higher than DG, but the difference was not significant (p>0.05) (Table 2).

DISCUSSION

Chronic hyperglycemia caused by diabetes is a significant risk factor for vascular diseases [3], and it contributes to oxidative stress [36]. Diabetic nephropathy (DN) occurs mainly in patients with difficult glycemia control [36,37].

Diabetes mellitus was successfully established and maintained for 60 days after alloxane induction in a single dose of 150 mg/kg [26]. A similar finding was found by Singh and collaborators [38], who established hyperglycemia with an intraperitoneal injection of alloxane at a dose of 130mg/kg, but the experiment was short-lived (14 days).

Several relevant studies demonstrate that the use of antioxidants reduced the harmful oxidative effects of chronic hyperglycemia and preserved organs and tissues [17,39]. At the end of 8 weeks, a reduction in glycemia (not statistically significant) was observed in the diabetic rats supplemented with ALA compared to the non-supplemented diabetic group. This finding is in agreement with that of Mohasseb and collaborators [28], who observed a decrease in hyperglycemia with the associated use of ALA, tocopherol, and ascorbic acid. Kamenova [15] also demonstrated increased peripheral insulin sensitivity in human diabetic patients after 4 weeks of ALA supplementation.

The main symptoms associated with diabetes mellitus are polyuria, polydipsia, polyphagia, and weight loss [40]. Polyphagia occurs when hypoinsulinemia makes it impossible for glucose to adequately penetrate the satiety center located in the hypothalamus [41]. Furthermore, the deficient action of insulin causes protein catabolism and weight loss [42]. ALA is absorbed from the gastrointestinal tract and transported by tissues [43]. In the present study, an effect on polyphagia was only observed at the end of the experiment and did not influence the animals' body weight. This differs from the finding by Seo and collaborators [44] where there was a reduction in the body weight of rats that were on a fat-rich diet and supplemented with ALA. Although diabetic rats supplemented with 300g of ALA did not show weight gain when compared to the DG rats, it is possible that the regular consumption of ALA continues to reduce polyphagia through the mechanism of decreasing hyperglycemia.

Several physiological processes are involved in the development of DN [10]. However, glycemic imbalance is the main trigger [36,37]. The process of DN is established

when there are kidney lesions with concomitant proteinuria in the setting of chronic hyperglycemia, which can lead to end-stage renal failure [6]. When diabetes is established, severe alterations in the renal parenchyma are found, resulting from microvascular complications that cause lesions in glomeruli, renal tubules, and podocytes [3,11,12], with oxidative stress aiding the progression of DN [36].

ALA supplementation in diabetic animals demonstrated preservation in the area proportional to the cortex of approximately 39% compared to the diabetic group, demonstrating a protective effect on the renal parenchyma, delaying the progression of DN. A significant increase in the capsular space area was also observed in the ALAD group, suggesting a delay in the glomerulosclerosis process and improvement in the glomerular filtration process. This result corroborates the finding by Kustepe and collaborators [45], who compared antioxidants and found that intraperitoneal use of ALA for 15 days preserved renal morphology with reduced tissue apoptosis. In the study by Feng and collaborators [46], there was also the preservation of kidney functionality through the reduction of oxidative effects. Regardless, ALA was used intraperitoneally in both these studies, and a comprehensive histomorphometry was not performed in the renal parenchyma.

The characteristic structural changes resulting from DN are the enlargement of the basement membrane and thickening of the tubular membrane [11,12], segmental and focal glomerulosclerosis and fibrosis [4]. As for glomerular histomorphometry, diabetic animals supplemented with ALA showed greater preservation in all of the parameters of glomerular areas when compared with the non-supplemented diabetic group. These results may be related to ALA's antioxidant action [47,48] and attenuation of chronic hyperglycemia [15,16], ensuring a protective effect on nephropathy and delaying glomerular lesions.

There was a significant reduction in the glomerular volume in diabetic animals due to chronic hyperglycemia [49], causing vascular damage and consequent injury to the glomeruli [6]. Winiarska and collaborators [39] demonstrated that ALA supplementation in rabbits promoted attenuation of glomerular lesions. However, in the present study, despite the percentage increase in the glomerular volume in the ALAD, this was not statistically significant.

Tubular changes parallel glomerular changes [50,51], which are a predictor of DN [52]. According to Gilbert and Cooper [53], tubular changes may precede or accompany tubular changes in DN frames. In this study, after 60 days, the diabetic animals that were supplemented had 50% fewer tubular lesions, demonstrating that ALA was able to delay the progression of the observed lesions significantly. The use of ALA offered an important protection to the renal parenchyma, which suggests that diabetic patients can delay the progression of renal damage with its use as an auxiliary treatment. ALA contributes to the treatment of chronic diseases; in DN it improves clinical and biochemical parameters [19] by decreasing oxidative stress [39] by inhibiting renal apoptosis [45].

In the glomerulus, the capillary tufts are covered by podocytes, i.e., cells with intertwined extensions [54] and a low mitotic index that are responsible for preventing the passage of proteins [55]. Oxidative stress generated by chronic hyperglycemia leads to podocyte apoptosis [10]. This injury leads to loss of the filtration barrier integrity and dysregulation in glomerular permeability [56]. The count of podocytes in the studied groups revealed that the diabetic animals had a smaller number of cells when compared to the control group. ALA supplementation showed an increase in the podocyte count, but this was not statistically significant. These results are similar to the findings of Siu and collaborators [57], who tested ALA intraperitoneally and observed significant preservation of the number of podocytes. The difference observed in our results can be explained in terms of the route of administration, causing a drop in plasma levels due to intestinal absorption. Therefore, a higher dosage of ALA by the oral route may be necessary to produce more substantial effects on the glomerular podocytes.

Our study demonstrates that oral supplementation of alpha-lipoic acid (ALA) has beneficial effects in diabetic animals through the attenuation of hyperglycemia and tissue damage, promoting a protective effect on the renal parenchyma. The reduction of glomerular and tubular damage delayed the advance of DN and its complications, preventing renal failure in diabetic groups. Therefore, it can be concluded that ALA is a nutraceutical that could be included as standard supplemental therapy for diabetic animals, which can be added normally to the animal feed to maintain its therapeutic efficacy.

A limitation of this study is that the levels of oxidative stress markers were not evaluated, which is a gap that should be filled in future studies.

Authors' contributions

All authors named have participated in the work in a substantive way. TPF, LBNSC, VAPS and MAC the manuscript concept creators, have processed the experimental material, performed the stereological measurements, written majority of the manuscript, designed the figures, analyzed and interpreted results of the research group in broader context of literature. GTB was responsible for the formulation and preparation of the experimental feed, as well as for contributions and analyzes in the field of experimental nutrition. TPF, VAPS and JGR have performed Diabetes induction, histological stainings and light microscopy analysis/photography, as well as discussed some stereological aspects of the results. TPF, JGR and LBNSC have organized and conducted the experiment (work in the animal unit, daily care and treatment, sacrifice, kidney extraction, immunohistochemistry, etc.). MAC, VAPS and

LBNSC have carefully read and critically revised the manuscript for its scientific merit and intellectual content and have supplemented the discussion and literature survey. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Gilor C, Niessen S, Furrow E, DiBartola S: What's in a name? Classification of diabetes mellitus in veterinary medicine and why it matters. J Vet Intern Med 2016, 30(4):927-940.

2. Anthony RM, MacLeay JM, Jewell DE, Brejda JJ, Gross KL: Alpha-lipoic acid is an effective nutritive antioxidant for healthy adult dogs. Animals 2021, 11:274.

3. Sicree R: The global burden of diabetes. International Diabetes Federation, 2003, 43.

4. Ilatovskaya DV, Levchenko V, Lowing A, Shuyskiy LS, Palygin O, Staruschenko A: Podocyte injury in diabetic nephropathy: implications of angiotensin II–dependent activation of TRPC channels. Sci Rep 2015, 5(1):1-10.

5. Nelson RW, Couto CG: *Disorders of the endocrinal pancreas*. 6th ed. Small Animal Internal Medicine. 2019: Elsevier Health Sciences.

6. Vora J, Ibrahim H: Clinical manifestations and natural history of diabetic nephropathy, in *Comprehensive Clinical Nephrology* 2003, Elsevier Limited: Spain. 425-437.

7. Sangwan P, Khanna K: Consistency of Rat model for diabetic nephropathy. IJAR 2015, 1(9):157-159.

8. Group G: Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. Lancet 1997, 349(9069):1857-1863.

9. Luis-Rodríguez D, Martínez-Castelao A, Górriz JL,De-Álvaro F, Navarro-González JF: Pathophysiological role and therapeutic implications of inflammation in diabetic nephropathy. World J Diabetes 2012, 3(1):7.

10. Susztak K, Raff AC, Schiffer M, Böttinger EP: Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes 2006, 55(1):225-233.

11. Riella MC: Diabetes Renal Disease, in *Principles of nephrology and water-electrolyte imbalance*. 2018, 1558-1667.

12. Papadopoulou-Marketou N, Paschou SA, Marketos N, Adamidi S, Adamidis S, Kanaka-Gantenbein C: Diabetic nephropathy in type 1 diabetes. Minerva Med 2017, 109(3):218-228.

13. Slaughter TN, Paige A, Spires D, KojimaN, Kyle PB, Garrett MR, Roman RJ, Williams JM: Characterization of the development of renal injury in Type-1 diabetic Dahl salt-sensitive rats. Am J Physiol Regul Integr Comp Physiol 2013, 305(7):R727-R734.

14. Kouzi SA, Yang S, Nuzum DS, Dirks-Naylor AJ: Natural supplements for improving insulin sensitivity and glucose uptake in skeletal muscle. Front Biosci (Elite Ed), 2015, 7(1):94-106.

15. Kamenova P: Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid. Hormones (Athens) 2006, 5(4):251.

16. Moini H, Tirosh O, Park YC, Cho KJ, Packer L: R-α-lipoic acid action on cell redox status, the insulin receptor, and glucose uptake in 3T3-L1 adipocytes. Arch Biochem Biophys 2002, 397(2):384-391.

17. Corrêa LBNS, da Costa CAS, Ribas JAS, Boaventura GT, Chagas MA: Antioxidant action of alpha lipoic acid on the testis and epididymis of diabetic rats: morphological, sperm and immunohistochemical evaluation. Int Braz J Urol 2019, 45(4):815-824.

18. Bilska A, Wlodek L: Lipoic acid-the drug of the future. Pharmacol Rep 2005, 57(5):570-577.

19. Ansar H, Mazloom Z, Kazemi F, Hejazi N: Effect of alpha-lipoic acid on blood glucose, insulin resistance and glutathione peroxidase of type 2 diabetic patients. Saudi Med J 2011, 32(6):584-588.

20. Berkson BM, Rubin DM, Berkson AJ: Revisiting the ALA/N (α-Lipoic Acid/Low-Dose Naltrexone) protocol for people with metastatic and nonmetastatic pancreatic cancer: a report of 3 new cases. Integr Cancer Ther 2009, 8(4):416-422.

21. Hager K, Kenklies M, McAfoose J: Alpha-lipoic acid as a new treatment option for Alzheimer's disease--a 48 months follow-up analysis. Alter Med Rev 2008, 13(1):74-75.

22. Ramos CSCB, Silva VAPD, Corrêa LBNS, Abboud RS, Boaventura GT, Chagas MA: Long-Term Alpha-Lipoic Acid (ALA) Antioxidant Therapy Reduces Damage in the Cardiovascular System of Streptozotocin-Induced Diabetic Rats. J Diet Suppl 2021, 22:1-15.

23. Koya D, Hayashi K, Kitada M, Kashiwagi A, Kikkawa R, Haneda M: Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. J Am Soc Nephrol 2003, 14(suppl 3):S250-S253.

24. Tankova T, Koev D, Dakovska L: Alpha-lipoic acid in the treatment of autonomic diabetic neuropathy (controlled, randomized, open-label study). Rom J Inter Med 2004, 42(2):457-464.

25. Mittermayer F, Pleiner J, Francesconi M, Wolzt M: Treatment with α-lipoic acid reduces asymmetric dimethylarginine in patients with type 2 diabetes mellitus. Translational Res 2010, 155(1):6-9.

26. de Souza Abboud R, Chagas MA, de Amorim Ribeiro IC, Corrêa LBNS, Lange RM: A modified protocol of the alloxan technique for the induction of diabetes mellitus in Wistar rats. Med Veterinária (UFRPE) 2020, 14(4):315-318.

27. Aguirre-Arias MV, Velarde V, Moreno RD: Effects of ascorbic acid on spermatogenesis and sperm parameters in diabetic rats. Cell Tissue Res 2017, 370(2):305-317.

28. Mohasseb M, Ebied S, Yehia MA, Hussein N: Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. J Physiol Biochem 2011, 67(2):185-194.

29. Scherle W: A simple method for volumetry of organs in quantitative stereology. Mikroskopie 1970, 26:57-60.

30. Bagetti-Filho HJ, Sampaio FJ, Marques RG, Pereira-Sampaio MA: Different from renal artery only clamping, artery and vein clamping causes a significant reduction in number of rat glomeruli during warm ischemia. J Endourol 2012, 26(10):1335-1339.

31. Bancroft J, Layton C: The Hematoxylin and eosin In: Suvarna S. Theory Practice of histological techniques., 7th ed edn Philadelphia: Churchill Livingstone of El Sevier, Philadelphia: Churchill Livingstone of El Sevier, 2013.

32. de Oliveira Ramos C, Nardeli CR, Campos KKD, Pena KB, Machado DF, Bandeira ACB, de Paula Costa G, Talvani A, Bezerra FS: The exposure to formaldehyde causes renal dysfunction, inflammation and redox imbalance in rats. Exp Toxicol Pathol 2017, 69(6):367-372.

33. Chen J, Chen Y, Luo Y, Gui D, Huang J, He D: Astragaloside IV ameliorates diabetic nephropathy involving protection of podocytes in streptozotocin induced diabetic rats. Eur J Pharmacol 2014. 736:86-94.

34. Tunçdemir M, Öztürk M: The effects of angiotensin-II receptor blockers on podocyte damage and glomerular apoptosis in a rat model of experimental streptozotocin-induced diabetic nephropathy. Acta Histochem 2011, 113(8):826-832.

35. Su J, Li SJ, Chen ZH, Zeng CH, Zhou H, Li LS, Liu ZH: Evaluation of podocyte lesion in patients with diabetic nephropathy: Wilms' tumor-1 protein used as a podocyte marker. Diabetes Res Clin Pract 2010, 87(2):167-175.

36. Iseki K, Ikemiya Y, Kinjo K, Iseki C, Takishita S: Prevalence of high fasting plasma glucose and risk of developing end-stage renal disease in screened subjects in Okinawa, Japan. J Clin Exp Nephrol 2004, 8(3):250-256.

37. Henegar JR, Bigler SA, Henegar LK, Tyagi SC, Hall JE: Functional and structural changes in the kidney in the early stages of obesity. J Am Societ Nephrol 2001, 12(6):1211-1217.

38. Singh H, Singh R, Arora R, Mannan R, Buttar HS, Arora S, Singh B: Protective role of Phyllanthus fraternus in alloxan-induced diabetes in rats. J Ayurveda Integr Medicine 2020, 11(4):391-398.

39. Winiarska, K, Malinska D, Szymanski K, Dudziak M, Bryla J: Lipoic acid ameliorates oxidative stress and renal injury in alloxan diabetic rabbits. Biochimie 2008, 90(3):450-459.

40. Behrend E, Holford A, Lathan P, Rucinsky R, Schulman R: 2018 AAHA diabetes management guidelines for dogs and cats. J Am Animal Hospit Assoc 2018, 54(1):1-21.

41. Fracassi F, Ettinger S: Canine diabetes mellitus, in *Textbook of Veterinary Internal Medicine*. 2017, Elsevier: St. Louis, USA. p. 1767-81.

42. Davison LJ: Canine diabetes mellitus, in *BSAVA Manual of Canine and Feline Endocrinology*. 2012, British Small Animal Veterinary Association.

43. Golbidi S, Alireza Ebadi S, Laher I: Antioxidants in the treatment of diabetes. Curr Diabetes Rev 2011, 7(2):106-125.

44. Seo EY, Ha AW, Kim WK: α-Lipoic acid reduced weight gain and improved the lipid profile in rats fed with high fat diet. Nutr Res Pract 2012, 6(3):195-200.

45. Kayhan Kuştepe E, Bahar L, Zayman E, Sucu N, Gül S, Gül M: A light microscopic investigation of the renoprotective effects of α-lipoic acid and α-tocopherol in an experimental diabetic rat model. Biotech Histochem 2020, 95(4):305-316.

46. Feng B, Yan XF, Xue JL, Xu L, Wang H: The protective effects of α-lipoic acid on kidneys in type 2 diabetic Goto-Kakisaki rats via reducing oxidative stress. Int J Mol Sci 2013, 14(4):6746-6756.

47. Portari GV, Moraes RCM, Deminice R, Orsatti FL, Merino S: Effects of the supplementation with alpha-lipoic acid on muscular antioxidant biomarkers of trained mice. Med Exp 2017, 4.

48. Turkyilmaz IB, Bilgin Sokmen B, Yanardag R: Alpha lipoic acid prevents brain injury in rats administered with valproic acid. J Biochem Molecul Toxicol 2020, 34(11):e22580.

49. Schnackenberg CG, Wilcox CS: The SOD mimetic tempol restores vasodilation in afferent arterioles of experimental diabetes. Kidney Int 2001, 59(5):1859-1864.

50. Huang HC, Preisig PA: G1 kinases and transforming growth factor-β; signaling are associated with a growth pattern switch in diabetes-induced renal growth. Kidney Int 2000, 58(1):162-172.

51. Van Buren PN, Toto R: Hypertension in diabetic nephropathy: epidemiology, mechanisms, and management. Adv Chronic Kidney Dis 2011, 18(1):28-41.

52. Jheng HF, Hirotsuka M, Goto T, Shibata M, Matsumura Y, Kawada T: Dietary low-fat soy milk powder retards diabetic nephropathy progression via inhibition of renal fibrosis and renal inflammation. Mol Nutr Food Res 2017, 61(3):1600461.

53. Gilbert RE, Cooper ME: The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? Kidney Int 1999, 56(5):1627-1637.

54. Cunningham JG, Klein BG: *Veterinary physiology*. 2007: Saunders Elsevier Philadelphia.

55. Garovic VD, Wagner SJ, Turner ST, Rosenthal DW, Watson WJ, Brost BC, Rose CH, Gavrilova L, Craigo P, Bailey KR, Achenbach J, Schiffer M, Grande JP: Urinary podocyte excretion as a marker for preeclampsia. Am J Obstet Gynecol 2007, 196(4):320.e1-7.

56. Ojima A, Ishibashi Y, Matsui T, Maeda S, Nishino Y, Takeuchi M, Fukami K, Yamagishi S: Glucagon-like peptide-1 receptor agonist inhibits asymmetric dimethylarginine generation in the kidney of streptozotocin-induced diabetic rats by blocking advanced glycation end product–induced protein arginine methyltranferase-1 expression. Am J Pathol 2013, 182(1):132-141.

57. Siu B, Saha J, Smoyer WE, Sullivan KA, Brosius FC: Reduction in podocyte density as a pathologic feature in early diabetic nephropathy in rodents: prevention by lipoic acid treatment. BMC Nephrol 2006, 7(1):1-11.

SMANJENJE OŠTEĆENJA GLOMERULA I TUBULA U BUBREZIMA PACOVA SA DIJABETESOM SUPLEMENTIRANIH ALFA-LIPOIčNOM KISELINOM

Tamyris Pontes FRAGA, Lanna Beatriz Neves Silva CORRÊA, Julia Galian Ribeiro TABOAS, Vivian Alves Pereira da SILVA, Gilson Teles BOAVENTURA, Mauricio Alves CHAGAS

Hronična hiperglikemija, izazvana dijabetesom dovodi do oštećenja organa pri čemu se na kraju bubrezi nalaze kao ciljni organ tkivnog oštećenja. Upotreba antioksidanasa kao što je alfa-lipoična kislina (ALA) dovodi do poboljšanja kliničkih i biohemijskih parametara kod pacijenata sa dijabetesom, a obzirom da dolazi do prevencije oksida-tivnog stresa. Cilj studije je bio da obavi komparativnu evaluacija morfologije bubrega kod normalnih i kod Wistar pacova kod kojih je indukovan dijabetes, suplementiranih

sa ALA. U okviru eksperimenta, formirane su četiri grupe sa po 7 jedinki. Pacovi kontrolne kao i grupe pacova sa dijabetesom, dobijali su komercijalnu hranu. Životinje u alfa-lipoičnoj i alfa-lipoičnoj grupi pacova sa dijabetesom, dobijale su ALA suplement u hrani. Dijabetes je indukovan jednom intraperitonealnom injekcijom aloksan monohidrata, koja je aplikovana posle perioda gladovanja. Posle 60 dana trajanja eksperimenta, obavljena je ekscizija bubrega u cilju ispitivanja histomorfoloških parametara glomerula i renalnih tubula kao i imunohistohemijskih parametara sa brojem podocita. U okviru evaluacije, ustanovljeno je da ALA dovodi do poboljšanja svih relevantnih parametara regije glomerula pri čemu je ustanovljeno smanjenje intenziteta lezija tubula kod životnja sa dijabetesom (p<0.001). Prikazan je povoljan efekat ALA suplementacije kod životinja sa dijabetesom i to u odnosu na smanjenje hiperglikemije i oštećenja kao posledice oksidativnog stresa pri čemu je uočen i zaštitni efekat na parenhim bubrega. Smanjenje oštećenja glomerula i tubula je dovelo do odlaganja progresije nefrotpatije uslovljene dijabetesom kao i komplikacija u smislu sprečavanja otkazivanja ovog organa kod jedinki sa dijabetesom.