HEALTH SCIENCES

Physical exercise associated with vitamin D chronic supplementation reduces kidney injury induced by monosodium glutamate

KÉSIA ZANUZO, ZOÉ M. GUARESCHI, ANNA CAROLINY DETOGNI, LUIZ PIERRE HUNING, PATRICK F. RODRIGUES, ELAINE M. PORTO, SABRINA GRASSIOLLI & JOÃO PAULO A. AMORIM

Abstract: The aim was to evaluate the effects of chronic vitamin D (VD) supplementation associated with regular swimming over renal histomorphometric aspects in obese rats. Thirty Wistar male rats (5 days old) were used. Twenty four rats were given subcutaneous injections of monosodium glutamate (MSG; 4 g/kg), and six control rats were given an equimolar saline solution. At 21-days-old, the MSG-treated rats were randomly distributed among sedentary animals (S) and exercised (E, swimming; 3x/week). These groups were subdivided into groups orally supplemented with VD (12 µg/kg; 3x/week) or not supplemented (NS), totaling Five experimental groups (n = 6 rats/group): MSG, MSG-SVD, MSG-ENS, MSG-EVD and control groups. In MSG-obese rats, there was such as a decrease in the diameter of the, glomerular tuft, Bowman's capsule, Bowman's space areas, and renal cortical thickness, compared to the control group. In MSG-SVD, MSG-ENS, and MSG-EVD animals, there was an increase in the cortical thickness in relation to the MSG group. In MSG-ENS and MSG-EVD animals, there was a reduction of tubular degeneration in relation to the MSG group. We conclude that physical exercise associated with Vitamin D supplementation can prevent of renal injury, increasing the thickness of the renal cortex and decrease the tubular degeneration.

Key words: Monosodium glutamate, hypothalamic obesity, physical exercise, renal tubular degeneration, cholecalciferol.

INTRODUCTION

Acute kidney injury (AKI) is a frequent complication of hospitalization, and the most common causes are sepsis, hypovolemia and exposure to nephrotoxic drugs, and is associated with an increased risk of chronic kidney disease (CKD), end-stage kidney disease (ESRD) and mortality (Vikrant et al. 2018). Although AKI is a known risk factor for short-term adverse outcomes, more recent data suggest that the risk of mortality and renal dysfunction extends well beyond hospital discharge. The magnitude of this risk seems highly dependent on the presence of comorbidities, including cardiovascular disease, hypertension (AH), diabetes mellitus (DM), pre-existing CKD and renal recovery (Fortrie et al. 2019). DM and AH frequently co-exist with CKD, and can modify kidney disease outcomes. Importantly, both of these conditions have been associated with heightened risks of AKI in several clinical settings (James et al. 2015).

The CKD, resulting manly from AH, leads to the final stage of decreased kidneys, with the cortical region and the parenchyma thinned (indicative of atrophy), and hyper-congenics, which is indicative of sclerosis and fibrosis

(small, dense and echogenic kidney), indicating irreversible alterations associated with a worse prognosis (Orozco et al. 2004, Kariyanna et al. 2010, Totou et al. 2018). As long as the fibrosis evolves, the damaged tubular epitheliums will lose their regenerative capacity and suffer apoptosis, leading to tubular atrophy and forming non-functional glomerulus (Webster et al. 2017). Diabetic kidney disease develops in approximately 40% of patients who are diabetic and is the leading cause of CKD worldwide. The natural history of diabetic kidney disease includes glomerular hyperfiltration, progressive albuminuria, declining Glomerular Filtration Rate (GFR), and ultimately, ESRD. Metabolic changes associated with diabetes lead to glomerular hypertrophy, glomerulosclerosis, and tubulointerstitial inflammation and fibrosis (Alicic et al. 2017).

Obesity also causes many structural, hemodynamic, and metabolic alterations in the kidney, being that the obesity-related glomerulopathy (ORG) is the most known of such a state of diseases (Tsuboi et al. 2017). ORG which is pathologically defined as the occurrence of glomerulomegaly in the presence or absence of focal and segmental glomerulosclerosis (FSGS). Renal hemodynamic changes, renin-angiotensin-aldosterone system (RAAS), insulin resistance (IR), mitochondrial dysfunction, inflammation, and abnormal lipid metabolism can all contribute to ORG progression (Chagnac et al. 2000, 2003, Xu et al. 2017).

In order to study the renal disease induced by obesity similar to human physiotherapy, it is important to set out animal models of obesity, together with glucose deregulation, hyperlipidemia, and hypertriglyceridemia (Glastras et al. 2016). Experimentally, obesity can be induced in rodents by the neonatal administration of monosodium glutamate – the nominated MSG model. This model

is characterized by hypercortisolism, hyperinsulinemia, dyslipidemia, and an increase in the activation of the autonomous renal nervous system (Hermanussen et al. 2006, Collison et al. 2011, Gaspar et al. 2016, Martin et al. 2016). Besides that, it presents renal histopathologic alterations, such as glomerulus with different stages of degeneration and tubulointerstitial injury (Contini et al. 2017).

In this context, as a form of prevention or treatment of obesity, as well as for the serious pathologies that alter renal function, the studies related to vitamin D (VD) and regular physical exercise (RPE), show that they can be used as alternative strategies to other therapeutic options, such as pharmaceuticals and surgery (Scomparin et al. 2011, Mccracken et al. 2018, Guareschi et al. 2019).

Vitamin D has played an important role in the modulation of renal inflammation, because it can supress the factor nuclear kappa B via (NFkB) a key factor of transition that is thought to mediate acute and chronic inflammation and fibrogenesis by regulation of the gene expression of cytokines, chemokines, and adhesion molecules, including interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), and tumoral necrosis factor-α (TNF-α) (Guijarro & Egido 2001, Kim & Kim 2014).

Experimental studies in animal models have also described the benefits of RPE for renal diseases associated with AH being that exercises based on the ground and in the water induce different outcomes in renal function. Swimming exercises show different results in renal function when compared to running, in which only rats trained to swim showed a better profile of proteinuria and glomerulosclerosis (Totou et al. 2018).

Thus, the aim of this study was to evaluate the effects of chronic supplementation with VD

associated with regular swimming on the renal structure of MSG-obese rodents.

MATERIALS AND METHODS

Animals

A sample of 30 rats (n = 6) was calculated considering the variables obesity, exercise and vitamin D, with α of 5% and test power of 80% (GPower 3.1 software). Twenty-four Wistar male rats were given subcutaneous injections of monosodium glutamate (MSG 4 g/kg/day of corporal weight) during the first five days of life (Olney 1969). At the same time, six rats were given subcutaneous injections of an equimolar sodium chloride solution (NaCl) in the dose 1.25 g/kg of corporal weight for the control group (CON). On the 21st day of life, the MSG-treated and CON animals were weaned and maintained at the Sectorial Bioterium of the Physiology Laboratory (Center for Biological and Health Sciences, State University of Western Paraná, UNIOESTE, Cascavel – PR), in controlled conditions of temperature (22 $\pm$ 3°C), relative humidity (about 55%), and luminosity-photoperiod (7:00~19:00h). All the animals were given water and rations (BIOBASE®, SC, Brazil) with 1.1% calcium, 0.8% phosphorus, and 4 UI/g of VD freely, according to the recommendations of the American Institute of Nutrition (Reeves et al. 1993). The experimental procedures were taken in accordance with the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA), and they were approved by the Ethics Committee of Animal Use (CEUA) at UNIOESTE. The study is based on the ARRIVE Guidelines 2.0: updated guidelines for reporting animal research (Percie du Sert et al. 2020).

Experimental design

After the weaning, MSG-treated animals were randomly distributed in subgroups according to protocols of exercise (E) or supplementation with vitamin D. Five experimental groups were organized (n= 6 rats/group) as follows: Control sedentary non-supplemented (CON); MSG-Sedentary non-supplemented (MSG); MSG-Sedentary + Vitamin D (MSG-S$_{VD}$), MSD-Exercised non-supplemented (MSG-E$_{NS}$); MSG-Exercised + Vitamin D (MSG-E$_{VD}$).

Supplementation with VD

The rodents submitted to supplementation with VD (Supra D Kley Hertz®, 1 drop = 200 Ul of cholecalciferol) were given 12 µg/kg/day of VD dissolved in corn oil by gavage (Al-Rasheed et al. 2015). The supplementation with VD was given from the 21st to 90th days of life, taking place 3x/week between 8:00-10:00. The non-supplemented groups (NS) received only corn oil no VD with the same frequency and time as the other groups.

Physical training

For all the exercised groups (E), the physical swimming training started on the 21st day of life and continued until the 90th day. The swimming protocol was similar to the one established by Leite et al. (2013). The swimming was carried out in swimming pools for rats, with warm water (32 $\pm$ 2°C) and a duration of 30 minutes 3x/week). In order to avoid adaptation, a load equivalent to 5% of the corporal weight was attached to the animal's tail. All the sessions started at 5:00 pm. At the end of each section, the animals were dried and sent back to the Bioterium. Sedentary animals (S) were not submitted to any kind of physical training during the experiment.

Euthanasia, weight of organs, and adiposity

On the 90th day of life, the animals were weighed (g), their nasal-anal length was taken (cm), and afterward, they were euthanized by decapitation in a guillotine after a brief desensitization with carbon gas (CO_2). The obesity was evaluated indirectly by the Lee Index calculus, formula: (cubic root of body weight (g)/nasal-anal length (cm)) (Bernardis & Patterson 1968). In addition, after euthanasia, the animals were submitted to abdominopelvic laparotomy in order to remove the deposits of visceral fat (retroperitoneal) to determine the percentage of body fat (Von Diemen & Trindade 2010), in the case the Lee Index not show difference between group. The kidney was removed, weighed, and transferred to the fastening solution before being submitted to histological technics, as described as follows. All the deposits of retroperitoneal fat, as well as the kidney, were described in g/g of corporal weight.

Morphological and morphometric analysis of the kidneys

The kidneys were fixed in ALFAC (alcohol, formaldehyde, and acetic acid) for 24 hours, washed in running water, and stocked in 70% alcohol. Afterward, they were processed with a suitable methodology for light microscopy, with the inclusion in Paraplast Plus® (Sigama-Aldrich). For the morphological analysis, semi-serial cuts of 5µm thickness were prepared using a manual rotary microtome (Olympus 4060) equipped with a disposable steel razor. The cuts were deparaffinized with xylol, hydrated with distilled water, and submitted to hematoxylin-eosin (HE) staining technic for analysis.

For the morphometric and renal medullary tubular degeneration analysis, always in the same plane, a renal histological section was carried out, and three others were ignored throughout the organ, totalizing an average of 10 sections/animal. Fifty glomeruli per kidney were selected, and the following parameters were measured: diameter of glomerular tuft, area of glomerular tuft, area of Bowman's capsule, and space. In order to find out the area and space of Bowman, a calculus of the capsule's area subtracting the glomerular tuft area was carried out. To measure the cortical renal thickness, all kidneys were sectioned always in the same plane, five measurements per section were carried out (Danilewicz & Wagrowska-Danilewicz 1998, Dixit et al. 2014). For the analysis of the renal medullary tubular degeneration, three random microscopic examinations/section, ten sections/animal were analyzed.The tubular degeneration was measured to percentage of the affected area, as described by Moqbel et al. (2017).

The images of the renal glomerulus were observed with magnification of 400X, renal cortex with magnification of 40X and tubular degeneration with magnification of 100X. The sections were analyzed using a BX60 Olympus microscope, and the images were captured using DP71 Olympus digital camera and DP Controller 3.2.1.276 software, and analyzed by the Pro-Plus 4.1 program.

Statistical analysis

The data are presented as the mean $\pm$ standard error mean (SEM). A Student's t-test was used to analyze the ability of the neonatal MSG treatment to induce obesity in animals between the control group and the MSG group. The effect of VD supplementation, associated or not with swimming training, was evaluated in CON lean rats and MSG-treated rats, separately, using two-way ANOVA. When F values were significantly different, the Tukey post-test was perfomed. All the statistical analyses were carried out using *GraphPad Prism 6* (GraphPad Software, San

Diego, CA). The differences were considered statistically significant when ($P < 0.05$).

RESULTS

On the 90th day of life, the MSG-obese animals had lower corporal weight (25.5%) and smaller nasal-anal length when compared to the animals from the CON group ($P < 0.05$), with no statistical difference on the Lee Index. However, the MSG-obese rats had higher adiposity, observed by a great increase in the weight of the deposit of retroperitoneal fat (100%) in relation to the CON group ($P < 0.05$; Table I).

The animals of the CON group had the renal structure preserved, in which the cortical region is darker and granular, while the medulla had striated and pale regions (Figure 1c). In the cortex, it is possible to observe red granules similar to dots, which are the renal corpuscles. In the renal corpuscles, there are the glomerulus, which are tufts of glomerular capillaries (Figure 1c). In the renal corpuscle of the control animals, we observed that the glomerulus is invaginated in the Bowman's capsule, the vascular pole, and the urinary pole, which is the continuation region

between the renal corpuscle and the proximal tubule, which drains the Bowman's space. At this junction, the simple squamous epithelium of the parietal layer from the Bowman's capsule joins together with the cubic epithelium of the tubule. The kidneys of control rats showed the renal corpuscles surrounded by renal tubules (Figure 1a).

In this study, treatment with MSG modified renal histology. We observed that in the kidney of MSG-obese animals, there is smaller diameter of the glomerular tuft (8%), area of glomerular tuft (21%), area of Bowman's capsule (28%), area of Bowman's space (37%), and renal cortical thickness (16%) when compared to the same renal parameters of the CON group ($P < 0.05$; Figure 1f-j). Also, the weight of the kidney was lower (19.5%) in the MSG-obese animals in comparison to the CON group ($P < 0.05$; Figure 1e). The MSG-obese group presented tubular degeneration, too (Figure 3a).

The supplementation with VD and regular swimming promoted changes in the biometric parameters evaluated in the MSG-obese animals. There was a statistical difference for the physical exercise practice taken in isolation (F = 10.67; P = 0.0021) on the deposit of retroperitoneal fat;

**Table I.** Adiposity and Biometric Parameters in MSG-treated rats.

	CON	MSG	*P*-value
Body Weight (g)	335.80 ± 6.27	250.20 ± 7.57*	<0.0001
Naso-anal Length (cm)	22.53 ± 0.20	20.27 ± 0.45*	0.0002
Lee Index	0.30 ± 0.003	0.31 ± 0.007	0.6726
Retroperitoneal Fat (g/g BW)	0.004 ± 0.0004	0.008 ± 0.0003*	<0.0001

Data are mean±SEM; n= 6 rats/group. BW: Body weight; CON: control and MSG: rats treated with monosodium glutamate, sedentary and non-supplemented.*P < 0.05 in Student´s t test.

thus, it was observed that the MSG-E$_{NS}$ group had an inferior average of 37.5% in the deposit of retroperitoneal fat in comparison to the MSG group ($P < 0.05$). When the interaction between the physical exercise and the supplementation with VD was evaluated, significant differences were observed concerning the isolated action of supplementation with VD (F = 4.336; $P = 0.0428$) on corporal weight, so that the MSG-E$_{VD}$ group presented an average 9% inferior of corporal weight in comparison to the MSG group, but with no significant difference when the post-test was carried out. Also, significant differences were observed when the interaction between VD and physical exercise (F = 11.41; $P = 0.0015$) was evaluated, being that the MSG-E$_{VD}$ group had a reduction (12.5%) in the deposit of

retroperitoneal fat when compared to the MSG group, but with no statistical difference when the post-test was carried out (Table II).

After the 90th day of life, supplementation with VD and regular swimming resulted in changes in the renal histomorphometric parameters evaluated on the MSG-obese animals. There was a statistical difference when the supplementation with VD in isolation was evaluated (F = 4.241; $P = 0.0455$) and also in the interaction between physical exercise and supplementation with VD (F = 5.156, $P = 0.0282$) on the kidney's weight; thus, it was observed that the MSG-E$_{VD}$ group had inferior an average of 25.55% less in kidney weight in comparison with the MSG-E$_{NS}$ group ($P < 0.05$; Figure 2e). Significant differences were observed in the isolated action
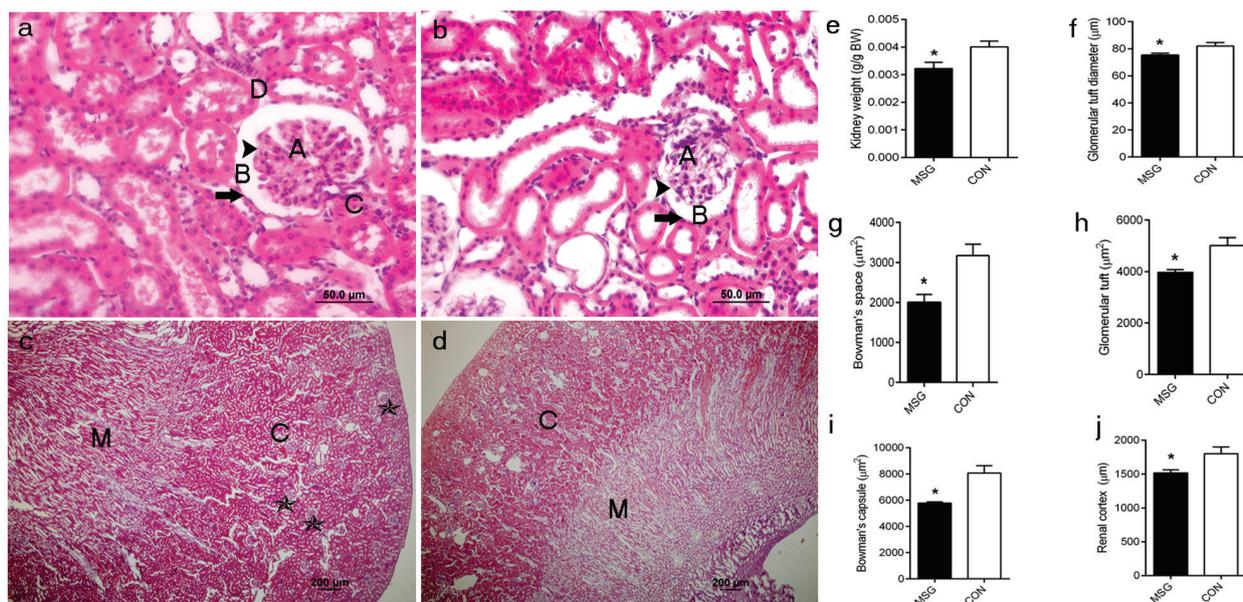
Figure 1. Comparative photomicrography of the renal structure of the animals from group CON and MSG. **a.** Glomerulus with normal aspect in the animals from group CON (A – glomerular tuft, B= Bowman's space, arrow= Bowman's Capsule, arrow head= area of the glomerular tuft, C= vascular pole, D= urinary pole. **b.** Group MSG with alteration (reduction) in all the evaluated renal parameters compared to group CON and renal tubules display dilated lumen. **c.** Renal cortex (C), marrow (M) and renal corpuscle (star) with normal aspect in group CON. **d - j.** Renal cortex (C) with smaller thickness in the group MSG and more presence of renal corpuscle with no presence of glomerular tuft (total atrophy). **e.** Kidney's weight (g/g;BW). **f.** Diameter of the glomerular tuft (μm). **g.** Area of Bowman's space (μm²). **h.** Area of glomerular tuft (μm²). **i.** Area of Bowman's Capsule (μm²). Graphs present the mean±SEM. The symbol "*" above the bars represent statistical difference in Student's T test ($P<0.05$). Staining = Harris Hematoxylin and Eosin.

of supplementation with VD (F = 6.351; P = 0.0156) and physical exercise (F = 45.99; P < 0.001) in the percentage of tubular degeneration, so that the MSG-E_{NS} group and MSG-E_{VD} group had lower percentages of 30.33% and 47.10%, respectively, of renal tubular degeneration in comparison with the MSG group. The MSG-E_{VD} group also presented a reduction (43.25%) in renal tubular degeneration when compared with the MSG-S_{VD} group (P < 0.05; Figure 3e). There was a statistical difference in the thickness of the renal cortex, both in supplementation with VD and physical exercise in isolation (F = 5.459; P = 0.0306 and F = 12.57; P = 0.0022, respectively) and in the interaction between both (F = 5.459; P = 0.0360), thus, it was observed that the MSG-S_{VD} group, MSG-E_{NS} and the MSG-E_{VD} group had higher averages of 18.73%; 21.50% and 27.11%, respectively, in the comparison with the MSG group (P < 0.05; Fig. 2j).

Although significant differences between the averages of the other evaluated parameters (diameter of the glomerular tuft, area of the tuft, Bowman's capsule, and Bowman's space)

were not observed, Figures. 2a-d shows that the MSG-E_{VD} group seems to have suffered less renal atrophy, presenting higher averages, when compared to MSG-S_{VD} and MSG-E_{NS} groups.

DISCUSSION

In our study, the animals from the MSG groups on the 90th day of life had lower corporal weight, nasal-anal length and an increase of adiposity in comparison to the CON group. This is typical because these rats do not have hyperphagia and maintain their weight in a normal range despite the accumulation of visceral fat (Hirata et al. 1997). Besides that, the lower corporal weight and smaller nasal-anal length of the MSG rodents are derived from the deficiency in releasing growth hormone, due to the relative loss of the liberator factor of the growth hormone by the MSG action on the arcuate nucleus (Hermanussen et al. 2006, Gaspar et al. 2016).

Table II. Adiposity and biometric parameters in MSG-treated rats submitted to VD supplementation and exercise at long of life.

	MSG	MSG-S_{VD}	MSG-E_{NS}	MSG-E_{VD}	*P*-value VD	*P*-value exercise	*P*-value interaction
Body Weight (g)	250.20 ± 7.57	246.90 ± 7.06	260.8 ±13.58	227.50 ± 7.89	0.0428	0.6177	0.0906
Naso-anal Length (cm)	20.27 ± 0.45	19.96 ± 0.25	20.69 ± 0.40	19.55 ± 0.28	0.0512	0.9919	0.2546
Lee Index	0.31 ± 0.007	0.31 ± 0.003	0.30 ± 0.003	0.31 ± 0.002	0.4027	0.4094	0.7003
Retroperitoneal Fat (g/g BW)	0.008 ± 0.0003[c]	0.007 ± 0.0003[c]	0.005 ± 0.0005[a,b,d]	0.007 ± 0.0004[c]	0.2289	0.0021	0.0015

Data are mean±SEM; n=6 rats/group. MSG: rats treated with monosodium glutamate, sedentary and non-supplemented; MSG-S_{VD}: rats treated with monosodium glutamate, sedentary and VD supplemented; MSG-E_{NS}: rats treated with monosodium glutamate, exercise and non-supplemented; MSG-E_{VD}: rats treated with monosodium glutamate, exercise and VD supplemented. BW: Body weight. Letters above numbers show statistical differences between groups by two-way Anova with Tukey post-test (P < 0.05). [a]MSG; [b]MSG-S_{VD}; [c]MSG-E_{NS} and [d]MSG-E_{VD}.

The Lee Index, which can be used for indicating obesity level, did not show statistical differences between the MSG and CON groups in our study. It is known that the Lee Index is not accurate for determining the percentage corporal fat of rodents. It is the equivalent, for rats, of the Body Mass Index for humans, which takes into consideration the distribution of mass throughout the body surface. This way, the animals with low weight and reduced nasal-anal length may be obese or not, which can be determined by the percentage of body fat (Von Diemen & Trindade 2010).

In this study, the practice of physical exercise in isolation decreased the deposit of retroperitoneal fat in comparison to the MSG group. Previous studies showed similar

protection of RPE in the reduction of adiposity in rats treated with MSG (Ribeiro et al. 2014, Scomparin et al. 2011). The reduced adiposity in the groups that practiced swimming is probably an improved activity of the sympathetic nervous system (SNS). RPE activates circuits of the SNS that provide activation of the sympathetic-adrenal axis, as well as the hypothalamic-hypophyseal axis. Besides that, RPE increases the activity of the lipase sensitive to hormones in the adipose tissue via reinforced action of the SNS (Nonogaki 2000).

The supplementation with VD in sedentary MSG-obese did not show any reduction in the deposits of fat and corporal weight, which was also observed in the exercise and supplemented groups. In our study, it was observed a probable
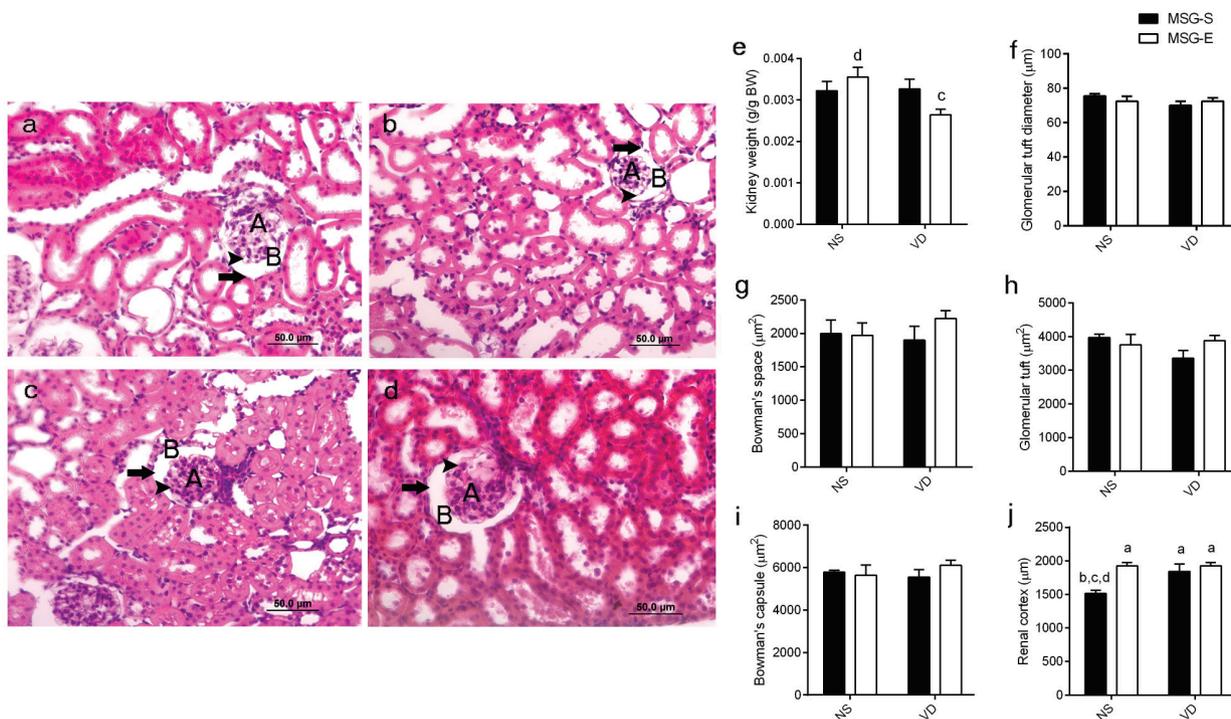

Figure 2. Renal structure photomicrography of the animals from MSG-obese groups. **a.** Glomerulus of animals from group MSG (A= glomerular tuft, B= Bowman's space= Bowman's Space, arrow= Bowman's Capsule, arrow head= area of glomerular tuft). **b.** Group MSG-S$_{VD}$. **c.** Grupo MSG-E$_{NS}$. **d.** Grupo MSG-E$_{VD}$. **e.** Kidney's weight (g/g/BW). f. Diameter of the glomerular tuft (µm). **g.** Area of Bowman's space (µm^2). **h.** Area of the glomerular tuft (µm^2). **i.** Area of Bowman's Capsule (µm^2). **j.** Renal cortical thickness (µm). Graphs present the mean±SEM. Letters above the bars show statistical differences between groups by two-way Anova with Tukey post-test ($P<0.05$). [a]MSG (MSG-S$_{NS}$); [b]MSG-S$_{VD}$; [c]MSG-E$_{NS}$ and [d]MSG-E$_{VD}$. Staining = Harris Hematoxylin and Eosin.

isolated action of the supplementation with VD on the corporal weight in comparison to the MSG group. In the study by Jin et al. (2018), 1,25(OH)2D3 injected (subcutaneous) at a dose of 1 µg/kg, 2x/week, for 16 weeks, significantly mitigated the induced obesity by MSG and the insulin resistance (IR). It is worth mentioning that in our study we offered the VD in a similar time, but orally.

However, there is experimental evidence that the concentration of VD in subcutaneous fat increases with supplementation of VD, and an *in vitro* study suggests that adipocytes of obese people with IR may have an impairment in the liberation of VD (Didriksen et al. 2015, Di Nisio et al. 2017). The 1.25(OH)2D hormonally active is produced in the proximal renal tubule by the CYP27BI enzyme after a second hydroxylation on the first position, and there are conditions in which

its activity can be abnormal, such as in diseases that have abnormalities in the concentration of VD, advanced hepatic insufficiency, obesity, poor intestinal absorption, nephrotic syndrome, or CKD (Schuster 2011, Quesada-Gomez & Bouillon 2018). The degenerative alterations in kidney of MSG rats have already been firmly stated in the proximal region of the contorted tubules, local of activation of VD, which can also have a negative effect on its activation (Al-Agha 2007).

In this study, treatment with MSG modified renal histology. We observed that in the kidneys of MSG-obese animals, there is an intense glomerular reduction and smaller renal area associated with intense tubular degeneration, suggesting severe renal atrophy. In the study by Elbassuoni et al. (2018), an oral dose of 35 mg/kg/day of MSG was administered, and a disorganized renal structure was also observed,
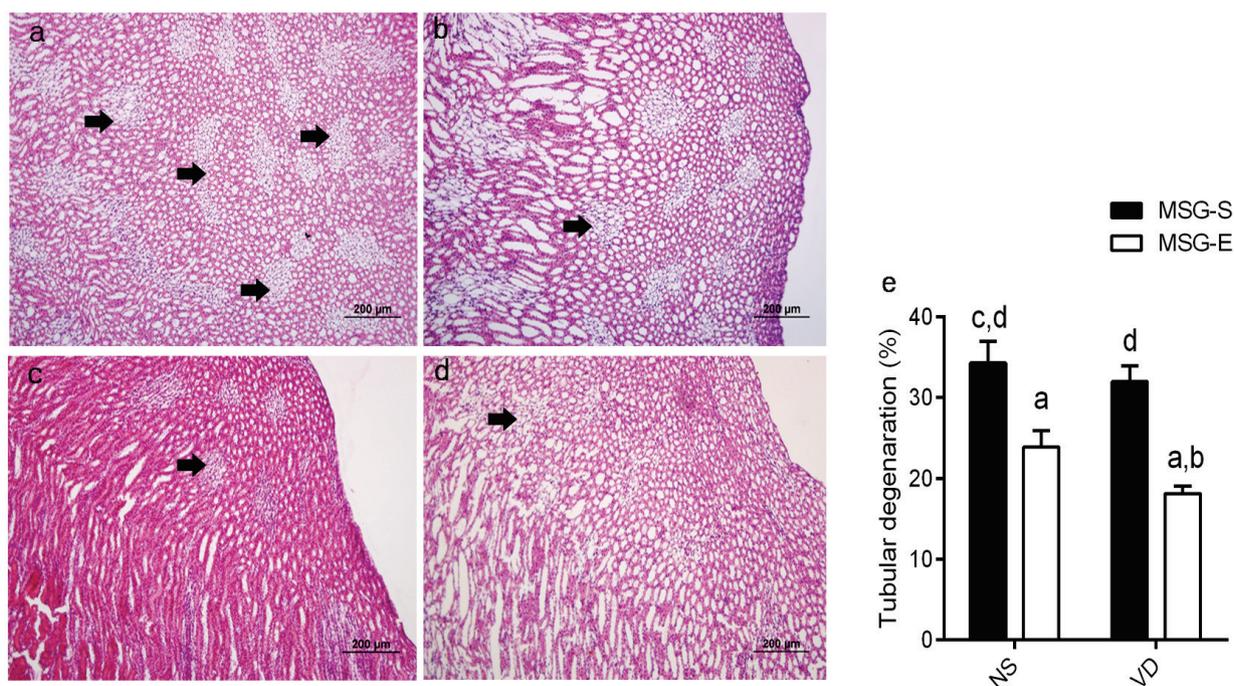
Figure 3. Renal medullary tubular degeneration photomicrography (black arrow) in MSG-obese groups. **a.** Group MSG. **b.** Group MSG-S$_{VD}$. In the group MSG-E$_{NS}$ (**c**) and group MSG-E$_{VD}$ (**d**) it is seen lower amounts of medullary tubular degeneration. **e.** Medullary tubular degeneration (%). Graphs present the mean±SEM. Letters above the bars show statistical differences between groups by two-way Anova with Tukey post-test (*P*<0.05). [a]MSG (MSG-S$_{NS}$), [b]MSG-S$_{VD}$; [c]MSG-E$_{NS}$ and [d]MSG-E$_{VD}$. Staining = Harris Hematoxylin and Eosin.

with atrophied glomerulus, dilated Bowman's space, and renal tubules that displayed dilated lumen. However, in the study by Dixit et al. (2014), adult albino Wistar rats were administered 4 mg/kg/day of MSG, by subcutaneous via, and the histomorphometry showed glomeruli with increased length and Bowman's capsule size, and with an increase in the Bowman's space. Ferreira et al. (2011) studied the glomerulosclerosis index (GSI) in a group of Wistar rats and a group of spontaneously hypertensive rats receiving daily subcutaneous injections of MSG (2 mg/kg/day and 1 mg/kg/day, respectively) in the neonatal period (up to the 11th day of life); and concluded that induction of neuroendocrine obesity in hypertensive rats significantly increased GSI. It should be noted that in our study, the route of administration of MSG was also via subcutaneous injection, but at a different dose and time of administration (4 g/kg/day and in the first five days of life).

This experimental model of obesity shows that metabolic alterations can lead to renal damage, such as excessive activation of SNS (Grassi 2006, Hoy et al. 2008, Da Silva et al. 2012). The sudden acquired renal injury constituting microcirculatory dysfunction, especially arteriolar glomerular afferent narrowing and afferent vasoconstriction, is key-mediators of this hypothesis (Ruiz-Hurtado & Ruilope 2018). Another hypothesis for the renal damage is atherosclerotic renal artery stenosis (ARAS), which is directly related to renal ischemia (reflecting a decrease in weight, volume, and thickness of the renal cortex) what can be decisive on the renal atrophy (Konopka et al. 2007). There is experimental evidence that renal cortical hypoxia can be caused by renal ischemia or by glomerular hyperfiltration, and that the fraction of filtration is a great determinant of the oxygen tension on the cortical tissue (PtO_2) and that the inefficient use of oxygen for absorbing

sodium causes renal hypoxia in pathological conditions, including DM (type 2), systemic arterial hypertension (SAH), and renovascular disease (RVD) (Lee et al. 2017). Besides that, it is known that an increase of renal artery pressure, characteristic of this experimental model, can cause tubular degeneration and interstitial fibrosis, while glomerular hypertension leads to glomerulosclerosis, glomerular atrophy, and proliferative glomerulitis. Together, these changes are associated with glomerular hyperfiltration and progression of tubular and glomerular damages, which results in the worsening of SAH and, eventually, renal insufficiency (Acierno & Labato 2004).

The deposition of visceral fat, also characteristic of this experimental model, could determine the compression of the renal capsule and to induce hydrosaline retention by activation of the renin-angiotensin-aldosterone system (RAAS) and an increase of the adrenergic activity. Both of these mechanisms could determine the alteration on the glomerular hemodynamic causing glomerular injury and the emergence of microalbuminuria (Hall et al. 2003).

According to Beland et al. (2010), an early sign of renal insufficiency is the progressive reduction of renal cortical thickness, and they suggest that the evaluation of cortical thickness would be a good evaluation sign for renal function because they established a statistically significant linear relationship between renal function and renal cortical thickness.

We also observed the presence of renal tubular degeneration in the animals of the MSG groups. Data similar to the one found in the study by Al-Agha (2007), in which two different oral doses of MSG, 2 mg/kg and 3 mg/kg for 21 and 45 days, respectively, were administered to adult albino male rats, the authors observed that the renal tubules had significant degeneration with considerable intertubular congestion and

inflammatory cellular infiltration. According to the authors, more serious tubular injuries occurred after elevated doses of MSG (3 mg/kg) probably due to the direct toxic effect of MSG on the renal cells. Vercoutere et al. (2004) observed that feed additives, such as MSG, caused alterations in the cellular lining of the contorted renal tubules, as well as on the Bowman's capsule, and they could also be related to variations in the threshold of tubular reabsorption, renal blood flow, and glomerular filtration rate (GFR). All these factors may contribute to the nephrotoxic effect of MSG, which leads to cellular and functional damages. Ortiz et al. (2006) observed that rats received who MSG at a dose of 4mg/kg intraperitoneally had tubular degeneration and renal necrosis at 15, 30, and 45 minutes after administration of MSG. It should be noted that MSG was administered by other routes (oral and intraperitoneal), whereas in our study, we administered MSG via subcutaneous injection.

Studies suggest that proper plasmatic levels of VD are necessary for the metabolic effects of RPE, such as the increase of anti-inflammatory adipokines (Hoseini et al. 2007). In this perspective, we combined RPE and supplementation with VD and evaluated the renal effect. Thus, it was observed that both RPE and VD increase renal cortical thickness in MSG-obese rats. Interestingly, the combination of RPE and VD did not maximize this effect, suggesting that RPE and VD act by distinct mechanisms to promote the increase in renal cortical thickness. The mechanisms involved in this response are not yet known. Nevertheless, it is well established that the increase in glomerular blood flow can contribute to the improvement of oxygenation, reduction of stress, and improve the effect of SNS and RAAS. These effects seem to be caused by RPE in patients with CKD (Cocks et al. 2013, Howden et al. 2017).

On the other hand, the molecular mechanisms behind the protecting actions of VD in the kidney can be more related to the decrease of oxidative stress and a significant increase in the total antioxidant capacity of the renal tissue (Elbassuoni et al. 2018, Finch et al. 2012). The VD receptor (VDR) has potent anti-inflammatory activities, and low expression of VDR is associated with renal injury activity (Sun et al. 2019). Xu et al. (2015) said that renal activation of VDR results in lower production of pro-inflammatory renal cytokines induced by lipopolysaccharides (LPS). In the kidney, VDR is mainly expressed in proximal and distal tubular epithelial cells, podocytes, and collecting epithelial duct cells (Yang et al. 2018). According to Gembillo et al. (2020), proper level of VD has a protective role on the proximal renal tubule. Megalin-Cubilin-Amnionless and the FGF23-Klotho axis represent two VD-linked mechanisms that could modulate and ameliorate the damage response at the renal tubular level, balancing VD therapy with an effect potent enough to contrast the inflammatory cascades. Megalin-Cubilin-Amnionless interacts with 25(OH)D3 and DBP (Vitamin D Binding Protein) complex to modulate the uptake of 25(OH)D3 in the proximal tubule. FGF23, VD and Klotho have a central role in the homeostasis of tubular function, primarily regulating renal calcium and phosphate reabsorption. When the phosphate level is increased in the urine, this will contribute to tubular injury and interstitial fibrosis (Gembillo et al. 2020).

VD also may interact with other kidney hormones such as renin and erythropoietin. This interaction would be responsible for some of the systemic and renal effects associated with VDR activation (Santoro et al. 2015). VD inhibits RAAS, which is enlarged in MSG rat models, and it is already well described by several studies that this increase is an important risk factor responsible for the progression of renal diseases (Li et al. 2002).

The interaction of plasma renin and VD is tightly connected with the VDR status: in case of VD deficiency there is a reduced transcription of VDR and an enhanced degradation of unliganded VDR, with a decrease in both unliganded and liganded VDR. This deficiency of liganded VDR, as previously mentioned, would improve the transcription of renin whereas the lack of unliganded VDR would enhance the transcription of angiotensinogen and Angiotensin II Type I Receptors (AT1Rs) via modulation of p53 expression (Gembillo et al. 2019).

Nevertheless, it is important to mention that the effect of VD on CKD survival is controversial, and in spite of studies that have shown a decrease of proteinuria, it is common to observe a significant reduction of the renal function (decrease of GFR, hypercalcemia) in patients with CKD that received treatment with VD (Christiansen et al. 1978, Palmer et al. 2007, De Zeeuw et al. 2010, Agarwal et al. 2011). Interestingly, in this study, we observed for the first time that there is a stronger effect on renal tubular protection with the combination of RPE and VD. Thus, it is possible that the tubular protecting action of VD, associated with a better renal blood flow favored by RPE, works positively on the tubular protection and consequently favors the activation of VD.

CONCLUSION

We conclude that physical exercise associated with Vitamin D supplementation can play important roles in the prevention of renal injury, increasing the thickness of the renal cortex and decrease the tubular degeneration.

Acknowledgments

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How to cite

KÉSIA ZANUZO[1]
https://orcid.org/0000-0002-6397-2160

ZOÉ M. GUARESCHI[2]
https://orcid.org/0000-0002-3852-7073

ANNA CAROLINY DETOGNI[3]
https://orcid.org/0000-0003-1003-0097

LUIZ PIERRE HUNING[2]
https://orcid.org/0000-0001-9158-9583

PATRICK F. RODRIGUES[2]
https://orcid.org/0000-0002-7692-597X

ELAINE M. PORTO[3]
https://orcid.org/0000-0002-0538-2461

SABRINA GRASSIOLLI[1,2]
https://orcid.org/0000-0001-5647-7877

JOÃO PAULO A. AMORIM[1,3]
https://orcid.org/0000-0003-2245-548X

[1]Programa de Pós-Graduação em Ciências Aplicadas a Saúde, Universidade Estaudual do Oeste do Paraná/UNIOESTE, Rodovia Vitório Traiano, Km 02, Contorno Leste, Água Branca, 85601-970 Francisco Beltrão, PR, Brazil

[2]Universidade Estadual do Oeste do Paraná/UNIOESTE, Centro de Ciências Biológicas e da Saúde, Laboratório de Endocrinologia e Fisiologia Metabólica, Rua Universitária, 2069, Universitário, 85819-110 Cascavel, PR, Brazil

[3]Universidade Estadual do Oeste do Paraná/UNIOESTE, Centro de Ciências Biológicas e da Saúde, Laboratório de Biologia Tecidual e da Reprodução, Rua Universitária, 2069, Universitário, 85819-110 Cascavel, PR, Brazil

Correspondence to: **João Paulo de Arruda Amorim**
 E-mail: amorimjpa@yahoo.com.br

Author contributions

Késia Zanuzo (analysis, interpretation of data, writing of the text and critical revision) Anna Caroliny Detogni (data collection and analysis), Zoé Maria Guareschi (interpretation of data and writing of the text), Luiz Pierre Huning and Patrick Fonntes Rodrigues (data collection), Elaine Manoela Porto (analysis and interpretation of data), Sabrina Grassiolli (interpretation of data, writing of the text and critical revision) and João Paulo de Arruda Amorim (data collection, analysis, interpretation of data, writing of the text and critical revision). All the authors read and approved the final text.