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A High-salt diet induces renal dysfunction and alters cardiometabolic homeostasis through elevation of circulating PCSK9 levels in Wistar rats

J.C.K. Ligan[1], T.C.M. Medehouenou[1], A.G.J. Segbo[1], E.M.S. Fiogbe[1], F.E.E. Kougnimon[1], A.A.E. Anago[1], D.D.J. Mensah[1], K.M. Obossa[1], S.E.R. Tcheoubi[1], C. Agbangla[2], D.C. Akpovi[1*]

[1]*Unité de Recherche sur les Maladies Non Transmissibles et le Cancer (UR-MNTC), Laboratoire de Recherche en Biologie Appliquée, Ecole Polytechnique d'Abomey-Calavi, University of Abomey-Calavi. 01BP 2009 Cotonou, Benin*

[2]*Laboratoire de Génétique Moléculaire et d'Analyses des Génomes (LGMAG), Faculté des Sciences et Techniques (FAST), University of Abomey-Calavi, 01 BP 4521 Cotonou, Benin*

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Abstract

There is increasing evidence in support of a decisive role played by the diet in the development of non-communicable diseases. The present study aimed to assess the effect of salt overfeeding on renal function and on the cardiovascular system in Wistar rats. Four groups of rats were exposed to diets with various salt levels, 0.8% for control, and 2%, 4% and 8% for overfed rats during 12 weeks. Blood Glucose, Triglycerides, Total cholesterol, LDL cholesterol (LDL-c) and HDL cholesterol (HDL-c) levels were determined by enzymatic method and serum PCSK9 by ELISA. Kidneys' histology sections were treated with hematoxylin-eosin staining. Serum creatinine (17.54±2.35 mg/L vs. 11.06±0.95 mg/L; $p<0.05$) and urea (1.48±0.37 g/L vs. 0.48±0.04 g/L; $p<0.05$) were significantly increased in overfed rats compared to control. Structural alteration of glomeruli and extensive tubular lesions were observed in rats fed with 4% and 8% salt. High salt intake caused a significant increase in Na^+ (171.9±2.1 mEq/L vs. 148.4±2.6 mEq/L; $p<0.01$), K^+ (8.3±0.4 mEq/L vs. 7.2±0.1 mEq/L; $p<0.05$), and Cl^- (109.9±5.1 mEq/L vs. 96.0±1.6 mEq/L; $p<0.01$) levels at week 12 compared to control. Triglycerides (1.49±0.31 g/L vs. 0.89±0.05 g/L; $p<0.05$), LDL-c (0.59±0.09 g/L vs. 0.31±0.03 g/L; $p<0,05$) and serum PCSK9 (10.28±3.21 ng/ml vs. 0.98±0.11 g/L; $p<0.001$) were significantly increased in salt overfed rats compared to control. Our results suggest that high salt intake impairs renal morphology and function and increases the risks of cardiovascular disease through circulating PCSK9 level increasing.

* **Corresponding Author:** D.C. Akpovi ✉ casimir.akpovi@gmail.com

Introduction

Non-communicable diseases (NCDs) are responsible for 70% of deaths worldwide, making them the leading cause of death globally (OMS, 2017). Cardiovascular diseases (CVD), diabetes, and renal failure are all NCDs that are prevalent worldwide (Levenson *et al.*, 2017; Vlad *et al.*, 2019). Chronic kidney disease (CKD) affects hundreds of millions of people worldwide (Mills *et al.*, 2015). It was the 12th leading cause of death in 2015 (Wang *et al.*, 2016). The global prevalence of CKD is around 10% and it usually progresses to end-stage renal disease (ESRD) thus increasing the risk of mortality for those affected (Jha *et al.*, 2013; Radhakrishnan *et al.*, 2014). The management of ESRD represents a heavy economic burden on patients and health systems due to the high costs of dialysis and renal transplantation (Ayodele and Alebiosu, 2010). Low- and middle-income countries pay the highest price (OMS, 2014) and more than 75% of people with CKD live in developing countries (Mills *et al.*, 2015).

CKD is a risk factor for cardiovascular diseases (James *et al.*, 2010). Management of CKD requires control of blood pressure and reduction of proteinuria (Garofalo *et al.*, 2018). High blood pressure (HBP) and proteinuria are indeed the major components of CVD and renal failure, which can be prevented or controlled by appropriate dietary interventions (Palmer *et al.*, 2017). It has been reported that in patients with CKD, blood pressure is generally sensitive to sodium (de Borst and Navis, 2016). Reducing salt intake has been shown to be effective in lowering blood pressure and proteinuria in CKD patients (Garofalo *et al.*, 2018; Koh, 2018). Excessive consumption of salt has been associated with the occurrence of kidney malfunction in rats and CKD in humans (du Cailar *et al.*, 2002; Ohta *et al.*, 2013; Susic *et al.*, 2011; Washino *et al.*, 2018). In the past, population source of salt was the one naturally contained in food and the average daily salt consumption was below 0.5 grams (MacGregor and de Wardener, 1999). Nowadays, the discovery of the preservative properties of salt has contributed to increased its daily consumption to nearly 10 grams in many communities (Powles *et al.*, 2013; Thout *et al.*, 2019). Populations in Sub-Saharan Africa have a daily consumption of salt that is far higher than the 5 grams/day recommended by the World Health Organization (WHO) (Oyebode *et al.*, 2016; WHO, 2016).

Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) is a major regulator of lipid metabolism (Cariou *et al.*, 2011). It is implicated in the occurrence of hypertension through various mechanisms, one of which involves its ability to reduce renal expression of epithelial sodium channels (ENaC) (Sharotri *et al.*, 2012). Studies have reported that hypercholesterolemia observed in ESRD patients is associated with elevated PCSK9 blood levels (Haas *et al.*, 2016; Kwakernaak *et al.*, 2013; Sucajtys-Szulc *et al.*, 2016). Similarly, elevated blood PCSK9 levels have been associated with the occurrence of CKD and its cardiovascular complications (Abujrad *et al.*, 2014; Jin *et al.*, 2014; Konarzewski *et al.*, 2014). PCSK9 could serve as a predictive biomarker for the occurrence of renal damage associated with lipid metabolism disorders. However, the literature reports contradiction regarding the involvement of PCSK9 in renal function disturbances (Morena *et al.*, 2017; Rogacev *et al.*, 2016). The etiology of renal disorders would be the major cause of such diversities observed in the literature data. We think it is important to clarify salt influence in blood PCSK9 level variations and its involvement in renal impairment. Moreover, the involvement of PCSK9 in the occurrence of CKD and cardiovascular damage associated with salt overeating has not been demonstrated. The results of the present study will make it possible considering PCSK9 as a predictive biomarker of CKD and cardiovascular homeostasis disturbances due to high-salt intake.

Materials and methods

Animals

We carried out an experimental study to assess the effect of salt overfeeding on renal function and on the cardiovascular system in the *Rattus norvegicus* (Wistar rats). We used "resource equations" method

(Charan and Kantharia, 2013; Festing and Altman, 2002) to determine the number of rats needed for the study. This method is based on the determination of the degree of freedom (E) of the analysis of variance (ANOVA) test and is adequate for our study design (Charan and Kantharia, 2013). The degree of freedom is calculated by the following formula:

E = N – n, With N: total number of animals to be used; and n: number of groups to be formed A total of 24 rats including 12 males and 12 females were used. They were all 10 to 12 weeks old and weighed between 150 and 200 g at the beginning of the study.

Experimental protocol

The selected rats were randomly divided into 4 groups. Each group was consisted of 3 males and 3 females. Before starting experiments, the rats were given 2 weeks of acclimatization to physiologically adapt to their new living environment. They were put on a normal diet consisting of granules and water *ad libitum* during the acclimatization phase. Rats were weighed and then subjected to a retro-orbital blood sample just before the salt diet exposures. From the first day of experiments, rats in the control group (not exposed) were fed on a normal diet with 0.8% salt. The other three (exposed) groups were overfed with 2%, 4%, and 8% salt, respectively. At the week 4, 8, and 12 of the study, rat weighing, and blood sampling were performed again.

Laboratory measurements

Two blood sample tubes were collected from each rat after an overnight fast. Samples were stored at 4°C until centrifugation was performed (5 minutes, 3000 r/min). Plasma from fluoride tube was used for glucose test and serum was used for the other parameters. Blood glucose, triglycerides, total cholesterol, LDL-cholesterol (LDL-c) and HDL-cholesterol (HDL-c) levels were measured using an enzymatic end point method (Trinder). We used urease kinetic method and Jaffé kinetic method to determine respectively blood urea and creatinine. Total proteins were determined by biuret method. Potassium and sodium ions were determined by the precipitation method with sodium tetraphenylborate and uracil acetate, respectively. Chloride ion was determined by the mercury thiocyanate method. The calcemia was determined with arsenazo III, a complexometric dye method. Blood magnesium levels were performed using the calmagit complexometric method. All kits were purchased from Elitech Group (Puteaux, France) with the except for PCSK9 which was assayed by ELISA kit from MyBioSource Inc. (San Diego, USA).

Histology

Rats were sacrificed and dissected immediately to remove their kidneys. Kidneys were fixed in a 10% formalin solution. After washing with water, kidneys were dehydrated by passing through an ascending ethanol series (70°, 95° and 100°), and embedded in paraffin wax. The paraffin containing the kidneys was then poured into cassettes, taking care to properly orient the organs. Tissue sections were made by sectioning 4-micron thick paraffin blocks. Histological sections obtained were treated with hematoxylin-eosin (HE) staining.

Statistical analysis

Data were recorded using Microsoft Excel 2016. Statistical analysis as well as graphs were performed with SigmaPlot version 14 software. Results are presented as mean ± standard error of the mean (SEM). We used the classic ANOVA test (parametric test) and the non-parametric alternative Kruskal-Wallis ANOVA test. Multiple comparison test (post-hoc test) and Dunnett's test were also used. The exposed groups (B, C and D) were compared to group A (control). A p-value under 5% was considered as significant.

Results

Impact of salt overfeeding on weight

The rats' weight did not change significantly from the beginning of the study (W0) to the week 4 (W4) and the week 8 (W8) (Fig. 1). At week 12, however, the weight of rats under 2% salt diet (group B) was significantly reduced compared to the control rats (group A).

Table 1. Variation of renal biomarkers and electrolytes.

Parameters were determined at baseline at week 0 (W0), week 4 (W4), week 8 (W8) and week 12 (W12). The comparisons were made at each of these four times, between rats of groups B, C and D which were fed respectively with a diet of 2%, 4% and 8% salt versus rats of group A (control).

Parameters	Weeks	A (0.8%)	B (2%)	C (4%)	D (8%)
Water consumption (ml/24h)	W0	42.00 ± 0.62	41.86 ± 0.67	42.14 ± 0.91	41.57 ± 0.65
	W4	50.43 ± 1.02	50.29 ± 0.52	56.57 ± 1.21	65.71 ± 0.81*
	W8	54.00 ± 0.54	53.71 ± 1.06	134.57 ± 1.13***	134.43 ± 1.07***
	W12	55.00 ± 1.29	56.43 ± 1.02	134.29 ± 1.30***	134.00 ± 1.40***
Urea (g/l)	W0	0.49 ± 0.06	0.50 ± 0.06	0.45 ± 0.02	0.55 ± 0.06
	W4	0.48 ± 0.04	0.38 ± 0.03	1.06 ± 0.23	1.48 ± 0.37*
	W8	0.93 ± 0.05	1.67 ± 0.25	1.32 ± 0.16	1.19 ± 0.34
	W12	0.40 ± 0.02	1.05 ± 0.50	1.34 ± 0.03	2.00 ± 0.28**
Creatinine (mg/l)	W0	9.62 ± 0.83	10.40 ± 0.42	9.77 ± 0.72	10.27 ± 0.44
	W4	10.30 ± 0.42	9.47 ± 0.32	12.61 ± 1.53	12.54 ± 1.05
	W8	11.06 ± 0.95	10.30 ± 0.81	14.95 ± 2.35	17.54 ± 2.35*
	W12	11.45 ± 1.07	11.63 ± 1.86	17.83 ± 0.95*	16.57 ± 1.94*
Total protein (g/l)	W0	64.25 ± 5.14	61.58 ± 1.42	57.85 ± 4.28	67.74 ± 1.56
	W4	71.64 ± 2.23	67.13 ± 2.82	46.35 ± 8.28	66.03 ± 11.74
	W8	55.06 ± 6.41	31.22 ± 2.79**	37.88 ± 5.14	39.62 ± 1.32
	W12	71.70 ± 1.24	28.47 ± 0.77***	38.97 ± 3.46***	52.22 ± 9.01*
Na^+ (meq/l)	W0	149.13 ± 2.86	152.98 ± 2.69	151.15 ± 2.54	148.77 ± 2.58
	W4	158.74 ± 2.38	150.23 ± 7.76	154.08 ± 3.33	154.73 ± 1.32
	W8	156.54 ± 3.08	158.85 ± 4.56	168.20 ± 4.08	169.30 ± 2.04
	W12	148.40 ± 2.12	155.37 ± 3.40	171.87 ± 4.32**	171.50 ± 8.11**
K^+ (meq/l)	W0	7.23 ± 0.13	7.41 ± 0.11	7.33 ± 0.14	7.22 ± 0.10
	W4	7.67 ± 0.12	7.28 ± 0.35	7.46 ± 0.21	7.49 ± 0.16
	W8	7.57 ± 0.20	7.68 ± 0.19	8.10 ± 0.15	8.15 ± 0.12
	W12	7.20 ± 0.12	7.52 ± 0.06	8.27 ± 0.09**	8.25 ± 0.37**
Cl^- (meq/l)	W0	96.95 ± 1.70	98.78 ± 1.44	97.65 ± 1.87	96.13 ± 1.33
	W4	102.26 ± 1.62	97.10 ± 4.71	99.32 ± 2.71	99.85 ± 2.07
	W8	100.94 ± 2.60	102.33 ± 2.48	108.00 ± 1.85	115.17 ± 6.65*
	W12	96.03 ± 1.56	100.23 ± 0.79	110.07 ± 1.23**	109.87 ± 5.08**
Ca^{2+} (mg/l)	W0	88.67 ± 4.37	87.17 ± 5.44	85.67 ± 5.92	86.38 ± 5.51
	W4	86.60 ± 1.21	67.33 ± 3.48	77.69 ± 9.31	78.33 ± 8.75
	W8	86.40 ± 12.65	80.33 ± 3.88	74.50 ± 0.96	89.80 ± 3.10
	W12	91.75 ± 1.80	75.67 ± 9.33	78.33 ± 2.73	88.27 ± 5.74
Mg^{2+} (mg/l)	W0	19.58 ± 0.93	18.72 ± 0.60	20.83 ± 2.97	20.59 ± 0.67
	W4	22.54 ± 1.90	21.57 ± 1.03	32.82 ± 4.15	32.40 ± 3.03
	W8	28.06 ± 5.94	25.57 ± 0.64	26.15 ± 0.79	27.99 ± 2.78
	W12	22.78 ± 1.04	23.33 ± 1.16	25.77 ± 0.79	26.47 ± 2.18

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Impact of salt overfeeding on kidneys and blood electrolytes

Table 1 depicts the variation of renal biomarkers and electrolytes in the three experiment groups in comparison to the control one. At week 4 of the study, water consumption significantly increased in rats under 8% salt diet compared to control ($p < 0.05$). At weeks 8 and 12, water consumption in 4% ($p < 0.001$) and 8% ($p < 0.001$) salt-supplemented rats was almost 3 times higher than in the control group.

Table 2. Variation of blood glucose and lipid parameters.

Parameters were determined at baseline at week 0 (W0), week 4 (W4), week 8 (W8) and week 12 (W12). The comparisons were made at each of these four times, between rats of groups B, C and D which were fed respectively with a diet of 2%, 4% and 8% salt versus rats of group A (control).

Parameters	Weeks	A (0.8%)	B (2%)	C (4%)	D (8%)
Glucose (g/l)	W0	0.97 ± 0.13	0.67 ± 0.05	0.94 ± 0.12	0.74 ± 0.05
	W4	1.34 ± 0.11	0.55 ± 0.04*	1.15 ± 0.14	1.29 ± 0.24
	W8	0.98 ± 0.15	0.92 ± 0.08	0.77 ± 0.07	0.70 ± 0.04
	W12	0.57 ± 0.08	0.61 ± 0.02	0.62 ± 0.05	0.92 ± 0.16
Triglycerides (g/l)	W0	0.90 ± 0.08	1.08 ± 0.15	1.07 ± 0.14	1.15 ± 0.10
	W4	0.85 ± 0.08	0.93 ± 0.04	1.75 ± 0.22*	1.91 ± 0.36*
	W8	1.04 ± 0.18	1.58 ± 0.34	0.90 ± 0.06	1.33 ± 0.28
	W12	0.89 ± 0.05	0.87 ± 0.05	0.91 ± 0.04	1.49 ± 0.31*
Total cholesterol (g/l)	W0	0.94 ± 0.06	0.80 ± 0.11	0.82 ± 0.06	0.79 ± 0.05
	W4	1.14 ± 0.11	1.09 ± 0.15	0.80 ± 0.08	0.82 ± 0.06
	W8	0.93 ± 0.17	1.01 ± 0.13	0.48 ± 0.09	0.98 ± 0.13
	W12	1.09 ± 0.03	0.94 ± 0.25	0.89 ± 0.06	1.18 ± 0.07
HDL-c (g/l)	W0	0.42 ± 0.03	0.34 ± 0.04	0.41 ± 0.05	0.34 ± 0.02
	W4	0.47 ± 0.04	0.39 ± 0.08	0.41 ± 0.04	0.58 ± 0.09
	W8	0.39 ± 0.06	0.47 ± 0.06	0.40 ± 0.05	0.42 ± 0.07
	W12	0.40 ± 0.02	0.45 ± 0.17	0.44 ± 0.07	0.43 ± 0.07
LDL-c (g/l)	W0	0.28 ± 0.03	0.22 ± 0.06	0.25 ± 0.04	0.22 ± 0.05
	W4	0.30 ± 0.04	0.32 ± 0.06	0.21 ± 0.05	0.23 ± 0.08
	W8	0.24 ± 0.04	0.23 ± 0.14	0.35 ± 0.08	0.40 ± 0.05
	W12	0.31 ± 0.03	0.36 ± 0.08	0.39 ± 0.04	0.59 ± 0.09*

* $p < 0.05$.

Urea levels were 3-fold higher ($p < 0.05$) in rats overfed with 4% salt (group C) and 5-fold higher ($p < 0.01$) in rats with 8% salt (group D) compared with control rats (group A) at week 12 of treatment. Creatinine levels were significantly higher in rats with 4% salt diet ($p < 0.05$) and 8% salt diet ($p < 0.05$) compared to control rats after 12 weeks of treatment. Compared with control rats, serum total protein decreased significantly at week 8 in rats with 2% salt ($p < 0.01$) and at week 12 of treatment in rats with 2% ($p < 0.001$), 4% ($p < 0.001$) and 8% ($p < 0.05$) salt. The natraemia and kalaemia did not significantly vary during the first 8 weeks of treatment. But at week 12, natraemia significantly increased in rats under 4% salt ($p < 0.01$) and 8% salt ($p < 0.01$) diets compared to control ones. Kalemia significantly increased at week 12 in rats with 4% salt diet ($p < 0.01$) and 8% salt diet ($p < 0.01$) compared to controls. Chloremia significantly increased ($p < 0.05$) at weeks 8 of treatment in rats with 8% salt diet compared to controls. After 12 weeks of treatment, chloride was significantly increased in rats with 4% salt diet ($p < 0.01$) and 8% salt diet ($p < 0.01$) compared to controls. Calcium and magnesium levels did not vary significantly in any group throughout the study (Table 1).The histological study of the kidney sections showed normal renal cortex and a fairly compact glomeruli (G) in control rats. Renal tubules (Black arrow) also showed normal morphology (Fig. 2A). In rats with 2% salt diet (Fig. 2B), a beginning of

structural alteration of some glomeruli was observed (Yellow arrow). Rats under 4% salt (Fig. 2C) and rats under 8% salt (Fig. 2D) showed altered glomeruli, with detachment of some podocytes and enlargement of Bowman's space (Red arrow).

There was also detachment of tubular epithelial cells from the basal lamina, with necrosis of some of them (Blue arrowhead). Rats with 8% salt (Fig. 2D) showed, in addition, lesions of the basal lamina of the tubular epithelium (Green arrowhead) and tubules swelling, accompanied by intrusion of the epithelial cells into the tubular lumen (Star).

Impact of salt overfeeding on cardiometabolic parameters

Blood glucose levels did not change significantly during the study, excepted for week 4 when levels decreased significantly ($p < 0.05$) in rats with 2% salt compared to control rats (Table 2). Triglycerides and LDL-c levels did not vary significantly during the first 8 weeks of the study. At week 12, rats fed with 8% salt diet showed significantly higher triglycerides ($p < 0.05$) and LDL-c ($p < 0.05$) levels compared to control rats.

Total cholesterol and HDL-c levels did not vary significantly during the study (Table 2). Circulating PCSK9 levels were significantly increased at week 8 ($p < 0.05$) and week 12 ($p < 0.05$) in rats with 4% salt compared to control ones. The increase was higher in rats with 8% salt at week 8 ($p < 0.05$) and week 12 ($p < 0.001$) compared to controls (Fig. 3).

Discussion

This study showed the impact of salt overfeeding on renal biomarkers and cardiometabolic risk biomarkers.

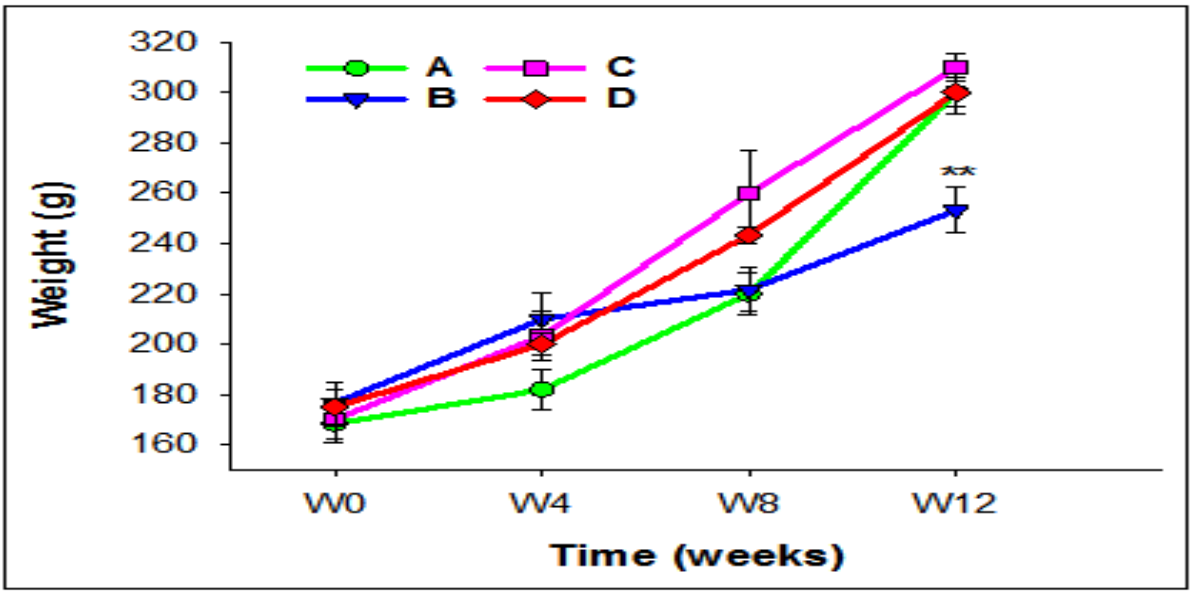

Fig. 1. Variation in rat weight.

Rats were weighed at the start of the study (W0), week 4 (W4), week 8 (W8) and week 12 (W12). The comparisons were made at each of these four times, between rats of groups B, C and D which were fed respectively with a diet of 2%, 4% and 8% salt versus rats of group A (control). ** $p < 0,01$.

Adult Wistar rats were randomized into four groups and fed with different salt levels in diet. An experimental study had suggested that the early effects of salt overfeeding vary by sex in salt-sensitive Dahl rats (Jackson *et al.*, 2018). In order to avoid any variability due to the sex of the rats, we made sure that all the groups contained an equal number of rats of both sexes. There was no significant difference between the mean values of the parameters withing the four groups of rats at the beginning of the study. This shows the homogeneity of the rat groups in the study.

High salt intake and Kidney

At 4th week of study, the water consumption significantly increased in rats under 8% salt diet and almost tripled that of controls at 8th week. These results are consistent with report from other experimental studies performed in both rats and mice (Fonseca-Alaniz *et al.*, 2007; Kitada *et al.*, 2017; Ramachandran *et al.*, 2019). It was shown that water consumption is multiplied by 3.1 in mice under 4% NaCl compared to 0.1% (Kitada *et al.*, 2017). There was no major change in weight between the groups, excepted rats on 2% salt diet that exhibited a significant reduced weight at week 12 compared to the control group. However, the fact that control rats and those under 8% salt did not show significant variation in weight suggests that weight reduction observed in rats under 2% salt is not related to the salt load level. High salt intake has been associated with increased fat tissue mass in rats with no impact on overall body mass (Fonseca-Alaniz *et al.*, 2007; Kitada *et al.*, 2017). Creatinine and uremia were significantly increased with salt supplementation compared to control. They are used as key biomarkers of renal function (Earley *et al.*, 2012; Levey *et al.*, 2014; Shannon, 1935). In addition, renal histology showed structural alteration of the glomeruli, with damaged podocytes in rats overfed with salt. Significant tubular damage was also observed in rats overfed with salt.

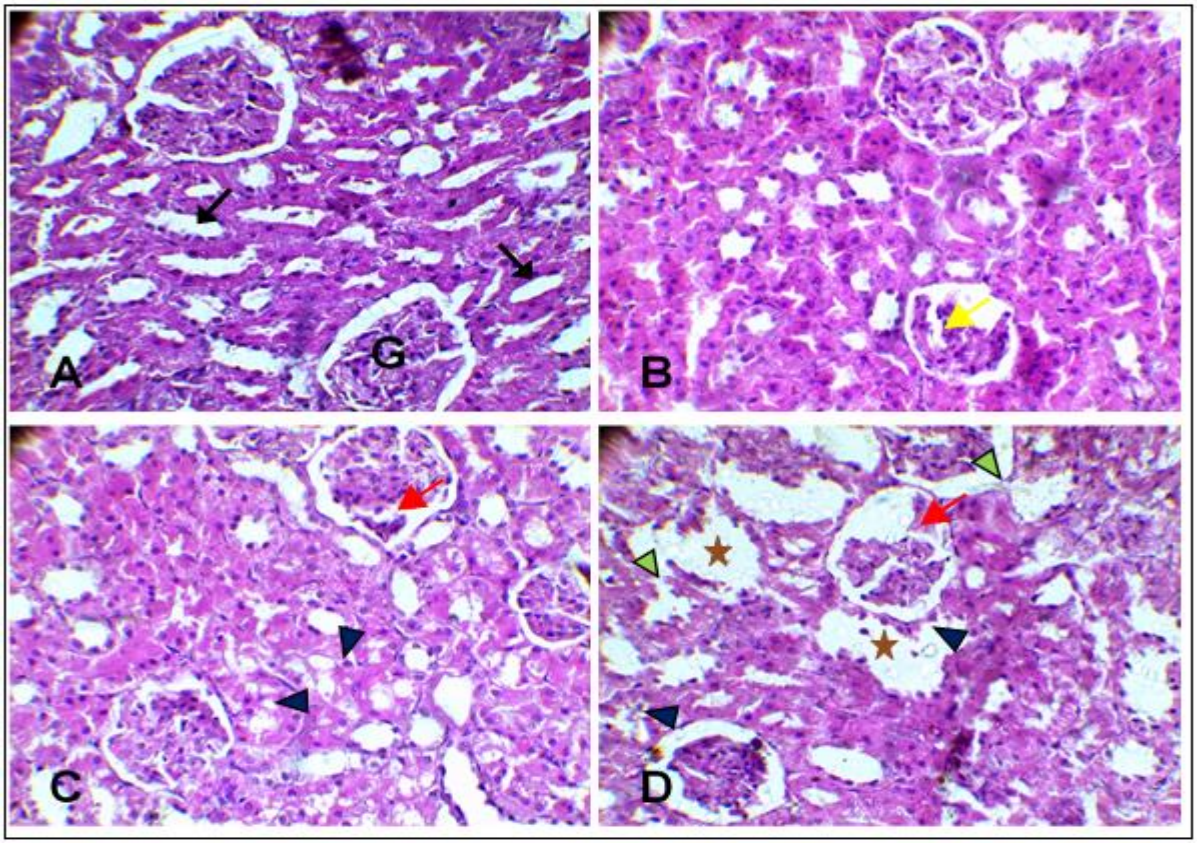

Fig. 2. Effect of salt overfeeding on renal morphology (HE, X 40).

Rats subjected to salt overfeeding were sacrificed at the end of the experiment at week 12, and histological sections of the kidneys were made. Sections were stained with eosin hematoxylin. Picture represent three independent experiments.

These structural abnormalities may be the cause of increased levels of biomarkers of renal function that appear after the structural damage. Our results suggest that salt overeating induces structural and functional impairment of the kidneys. Some studies performed both in animal models (Nakazawa *et al.*, 2019; Washino *et al.*, 2018) and in humans (MacGregor *et al.*, 1989; Ritz *et al.*, 2009) reported similar results. Renal dysfunction due to salt overfeeding has also been reported to extend tubular

damage to alterations in glomerular filtration function (Hosohata, 2017; Washino *et al.*, 2018). Our study showed a decrease in total protein levels in rats overfed with salt. Indeed, variations in proteinuria, a marker for kidney functional exploration, have also been associated with salt level in diet (Huang *et al.*, 2016; Maack *et al.*, 1979; McMahon *et al.*, 2013; Verhave *et al.*, 2004). Urinary levels of creatinine, proteins, and even electrolytes would have strengthened our conclusions, and represent some limitations of the present study.

Overfeeding with salt induced a significant elevation of serum sodium, potassium and chloride ions during this study. Similar results have been reported by studies performed on animal models (Kitada *et al.*, 2017; Nakazawa *et al.*, 2019). The increase in serum sodium must be associated with the increase in water consumption observed in rats overfed with salt. Excessive salt intake is known to inhibit the renin-angiotensin-aldosterone system (RAAS), thereby accelerating urinary sodium excretion and potassium retention, in order to maintain plasma sodium levels within normal ranges (MacGregor *et al.*, 1981).

In addition, the elevation of serum sodium results in an increase of the osmotic force in the intracellular environment, and a movement of fluids from the cells to the extracellular environment (He *et al.*, 2001). The increase in extracellular fluid volume to the detriment of cells allows a return of serum sodium levels to normal but also increases water requirement. The simultaneous elevation of natreamia and kaleamia in rats overfed with salt indicates a homeostatic imbalance. It showed in our study that the elevation of renal function markers such as urea and creatinine occurred at week 4 in rats overfed with 8% salt diet, much earlier than the elevation of sodium and potassium ions (week 12). These results suggest that the imbalance in sodium/potassium homeostasis is due to an earlier impairment of renal function (He *et al.*, 2020).

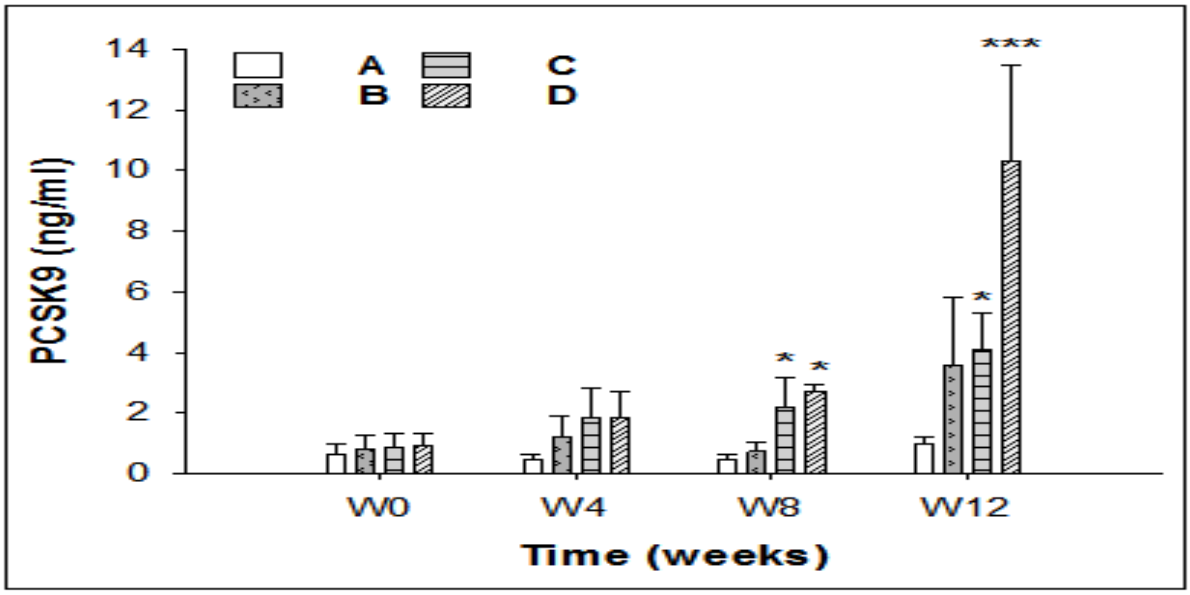

Fig. 3. Serum PCSK9 variation depending on salt intake.
Serum PCSK9 was determined at the beginning of the study (W0), week 4 (W4), week 8 (W8) and week 12 (W12). The results obtained in rats overfed with 2% (group B), 4% (group C) and 8% (group D) salt were compared to the control group (group A). *p <0.05; ***p <0.001.

High salt intake and cardiovascular system

During the 12-week study period, there was no major change in plasma glucose, total cholesterol and HDL-c levels. Harsha *et al.* reported that salt level in diet does not significantly impact lipid balance in humans after a short duration of exposure (Harsha *et al.*, 2004), while another study conducted in human suggest an association between reduced salt intake

and increased plasma lipid levels (Graudal *et al.*, 2017). We showed in this study that triglyceridemia (week 12), LDL-c (week 12) and serum PCSK9 level (from week 8) were significantly increased in rats with high-salt intake compared to controls. PCSK9 plays an important role in the regulation of circulating lipoprotein levels through its interaction with the LDL and VLDL receptors (Guo *et al.*, 2021; Park *et al.*, 2004; Wang *et al.*, 2012). The increase in serum PCSK9 reduces membrane expression of LDLR in hepatocytes, leading to an increase of circulating LDL-c (Park *et al.*, 2004; Wang *et al.*, 2012). This would explain the elevation of serum PCSK9 which occurred 4 weeks earlier than that of LDL-c in this study. Serum triglycerides accurately reflects the levels of triglyceride-rich lipoproteins (Chapman *et al.*, 2011).This report is corroborated by the simultaneous elevation of triglycerides and PCSK9 showed in our study. Increased serum PCSK9 levels as well as dyslipidemia have been associated with cardiovascular diseases (Vlad *et al.*, 2019; Zheng-Lin and Ortiz, 2018). Our results suggest that the cardiometabolic disorders reported in salt overeating would be associated with PCSK9 overexpression.

High salt diet-induced renal damage and serum PCSK9

Renal function impairment was observed as early as week 4, while increased serum PCSK9 and dyslipidemia occurred at weeks 8 and 12, respectively. These results suggest that the impact of salt overeating on the cardiovascular system is mediated by a prior impairment of renal function. Studies in both rats (Liu and Vaziri, 2014) and humans (Haas *et al.*, 2016; Jin *et al.*, 2014; Kwakernaak *et al.*, 2013) have associated renal disturbances with increased serum PCSK9 and LDL-c. One of the main mechanisms by which renal disturbances induce overexpression of PCSK9 involves inflammation. Damaged podocytes increase their secretion of TNF-α, a pro-inflammatory cytokine (Rosa *et al.*, 2012; Tipping, 2008). TNF-α induces an intracellular increase in c-Inhibitor of apoptosis (c-IAP) in liver. c-IAP enhances PCSK9 maturation on the one hand and leads to its ubiquitination on the other one. Thus, overexpressed PCSK9 prevents recycling of cell surface expressed LDLR while promoting intracellular destruction of newly synthesized LDLR (Xu *et al.*, 2012). Finding in this study showed that salt overeating alters renal morphology and function, disturb hydro-electrolytic balance, and cardiometabolic biomarkers.

Conclusion

The present study shows that exposure of Wistar rats to a high-salt diet leads to disturbances in renal function and increases the risk of cardiovascular diseases. In chronological order, our results suggest that salt overfeeding first leads to morphological alteration of the kidneys followed by disruption of electrolyte homeostasis. The elevation of blood PCSK9 levels, which occurs after the renal disturbance, contribute to the occurrence of dyslipidemia, sign of increased risk of cardiovascular disease.

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