ABSTRACT

Background: The role of the chloride anion on the deleterious effects of excessive salt (NaCl) intake is unknown and whether its effects are independent of the presence of sodium.

Objective: The aim of this study was to demonstrate that both chloride and sodium overload in the diet produce independent deleterious effects on systolic blood pressure (SBP), renal function and kidney markers of oxidative stress.

Methods: Male Wistar rats were divided into four groups (n=8/group) and fed different diets for three weeks: C: control (standard diet), NaCl: high sodium-high chloride diet; Na: high sodium without chloride diet and Cl: high chloride without sodium diet. Systolic blood pressure (SBP) and renal function were measured, and thiobarbituric acid reactive species (TBARS) production, and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymatic activity and expression were evaluated in the renal cortical tissue.

Results: After three weeks, SBP increased (*) in the two groups fed with chloride. Fractional excretion of sodium and chloride increased (*) in the NaCl and Na groups. Diuresis and TBARS increased (*) in the renal cortex with the three diets, with no changes in SOD and CAT activity and expression. GPx activity increased (*) in the two groups that received chloride (* p <0.05 vs. C).

Conclusions: Both sodium and chloride overload are associated with a higher oxidative state characterized by increased lipid peroxidation in the renal cortex. However, only chloride overload is associated with higher GPx activity and hypertension without changes in urinary chloride excretion, suggesting a higher renal pro-oxidant state in this experimental group with respect to the Na group.

Key words: Chloride - Hypertension - Kidney - Lipid Peroxidation - Glutathione

RESUMEN:

Introducción: Se desconoce el papel del anión cloruro en los efectos deletéreos del consumo excesivo de sal (NaCl) y si sus efectos son independientes de la presencia del sodio.

Objetivo: Demostrar que tanto una sobrecarga de cloruro como una sobrecarga de sodio en la dieta producen efectos deletéreos, en forma independiente, sobre la presión arterial sistólica (PAS), la función renal y los marcadores de estrés oxidativo en el riñón.

Materiales y métodos: Ratas Wistar macho fueron divididas en cuatro grupos (n = 8/grupo) y fueron alimentadas con diferentes dietas durante tres semanas: C: control (dieta estándar), NaCl: hipersódica-hiperclórica, Na: hipersódica sin cloruro, Cl: hiperclórica sin sodio. Se determinaron la presión arterial sistólica (PAS) y la función renal y en la corteza renal se evaluó la producción de especies reactivas del ácido tiobarbitúrico (en inglés: TBARS) y la actividad y la expresión de las enzimas superóxido dismutasa (SOD), catalasa (CAT) y glutatión peroxidasa (GPx).

Resultados: Al cabo de tres semanas, la PAS aumentó (*) en los dos grupos alimentados con cloruro. La excreción fraccional de sodio y de cloruro aumentó (*) en los grupos NaCl y Na. La diuresis y los TBARS en la corteza renal aumentaron (*) con las tres dietas, sin cambios en la actividad y en la expresión de SOD y CAT. La actividad de la GPx aumentó (*) en los dos grupos que recibieron cloruro; (* p < 0,05 vs C).

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Sources of funding: This work was supported by grants from the National Agency for Scientific and Technological Promotion [Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT)], the University of Buenos Aires, the Argentine Society of Arterial Hypertension and the Héctor A. Barceló Foundation

This work received the 2020 Braun Menéndez Award

Received: 01/09/2021 – Accepted: 02/19/2021

REV ARGENT CARDIOL 2021;89:96-104. http://dx.doi.org/10.7775/rac.v89.i2.20034

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INTRODUCTION
Excessive salt intake in the diet is a risk factor for the development of high blood pressure. Saline overload in the kidney induces oxidative stress and inflammation, regardless of the blood pressure value. Clinical studies suggest that blood pressure is not increased by a high sodium diet (Na+) in the absence of chloride (Cl-). (1-3) Since sodium bicarbonate does not have the same pressor effect as sodium chloride. (NaCl) in hypertensive people. (4, 2) Recent evidence suggests that chloride may have a more specific role in "salt sensitive" hypertension, independent of the hypertensogenic effect of sodium. (5-9)

Our working group has demonstrated the presence of acute and chronic pro-inflammatory and profibrotic effects that NaCl overload causes in the kidney. (10-13) A high NaCl diet induces activation of the angiotensinogen gene, increased synthesis of renal angiotensin II, and increased oxidative stress leading to the development of hypertension. (14-16) However, to date, the possible harmful effects of a chloride overload in the kidney, and whether its effects are independent of the presence of sodium have not been described or clarified.

Hypothesis
The chloride anion (Cl-), independently of the sodium cation (Na+), would also be involved in the oxidative stress of the kidney and blood pressure elevation. These alterations would be attenuated if Cl- were replaced by another anion (for example, citrate) or if Na+ were replaced by other cations.

Objectives
The aim of this study was to determine the independent effects of dietary chloride and sodium overload on the following parameters:

- systolic blood pressure (SBP)
- renal function
- kidney markers of oxidative stress.

METHODS
Animals used
Thirty-two 7-week-old male Wistar rats, weighing 155-165 g at the beginning of the diet, were used.

Diets
The animals were divided into a control group and three experimental groups (n=8/group). They received drinking tap water ad libitum and were fed the following diets (17) for 3 weeks:

1. Control: standard diet (0.4% W/W NaCl in food)
2. NaCl: high sodium-high chloride diet (8%)
3. Na: high sodium diet without chloride (Na2C6H5O7 11.8%) (equimolar in Na+ with group 2)
4. Cl: high chloride diet without sodium (CaCl2 3.80%; KCl 3.98% and MgCl2 1.30%) (equimolar in Cl- with group 2).

Assessment of systolic blood pressure
Baseline SBP was measured at 1, 2 and 3 weeks in the rat tail using a sphygmomanometer (Hatteras Instruments, Cary, NC, US), between 9 and 11 a.m., after training the animals for 3 consecutive days.

Assessment of food, calorie and water intake
During the third week, food (g) and water (mL) intake was assessed in three consecutive days. Calorie intake (kcal) was estimated as: 3.3 kcal/g*food intake (g).

Assessment of urinary and plasma parameters and evaluation of excretory renal function
After 3 weeks of diet, the animals were housed in metabolic cages for two days: one for acclimatization and the other for 24-hour urine collection to measure diuresis, and urinary concentrations of Na+, Cl−, creatinine (mg/dL). Before euthanizing the animals, the final body weight (BW) was obtained, and blood was drawn from the retrocaval sinus under anesthesia with ketamine (60 mg/kg) and xylazine (2 mg/kg). Plasma concentrations of Na+, Cl−, creatinine, glucose and urea were assessed by means of an autoanalyzer. Plasma osmolarity (mOsm/kg) was estimated as:

\[ 2 \times \text{plasma sodium (mEq/L)} + 1/18 \times \text{blood glucose (mg/dL)} + 1/6 \times \text{plasma urea (mg/dL)} \]

Creatinine clearance was calculated as:

\[ \text{CrCl} = \text{(urinary creatinine/plasma creatinine)} \times \text{diuresis/time/body weight (BW)} \]

Tubular function was assessed by means of filtered load (FL), urinary excretion (UE), fractional excretion (FE), tubular reabsorption (TR) and fractional reabsorption (FR) of the different ions, using the following standard formulas:

\[
\begin{align*}
\text{FLNa} &= \text{CrCl} \times \text{plasma sodium} \\
\text{UENa} &= \text{diuresis}/\text{urea} \\
\text{TRNa} &= \text{FlNa} \times \text{UENa} \\
\text{RENa} &= \text{URENa} \times \text{FRNa} \times 100 \\
\text{TRCl} &= \text{FlCl} \times \text{URECl} \\
\text{FECI} &= \text{URECl} \times \text{FRCl} \times 100 \\
\text{Diuresis, CrCl and FlL, TR and UE} &= \text{normaliz} \text{ed by the BW of each rat and were expressed in mL/day/kg, mL/min/kg or mEq/day/kg, while FE and FR are expressed as percentage (%).}
\end{align*}
\]

Euthanasia, kidney removal and sample processing
Under anesthesia, both kidneys were removed by abdominal laparotomy. The renal cortex was dissected, homogenized in saline phosphate buffer (7.6 mM KH2PO4, 42.4 mM K2HPO4, 150 mM NaCl, pH: 7.4) and centrifuged at 600 g for 20 minutes at 4°C. TRARS, and the antioxidant enzyme activity: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined in the supernatants. The protein expression of these enzymes was assessed by Western Blot in renal cortex homogenates, and protein content was measured by the Lowry method. (18) The animals were euthanized by decapitation.

Palabras clave: Cloruro – Hipertensión – Riñón - Peroxidación de lípido - Glutatión peroxidasa

Conclusion: Tanto la sobrecarga de sodio como la de cloruro se asocian a mayor estado oxidativo caracterizado por un incremento en la peroxidación lipídica en la corteza renal. Sin embargo, solo el exceso de cloruro se asocia a mayor actividad de la GPx y de la hipertensión, sin cambios en la excreción urinaria de cloruro, sugiriendo un mayor estado prooxidante renal en comparación con el grupo Na.
**Results**

Body weight, food, calorie and water intake

The three groups fed with experimental diets showed a lower final-initial BW difference than the control group, accompanied by a higher intake of water after three weeks of dietary treatment (Table 1).

**Evolution of systolic blood pressure**

Control rats remained normotensive during the 3 weeks of diet. In the three groups fed with experimental diets, SBP increased since the second week, and the differences were significant with respect to baseline values and the control group for NaCl and Cl diets.

The highest SBP values were reached at 2 and 3 weeks in the NaCl group, while the SBP rises in the Cl and Na groups were lower than those reached in the NaCl group. As can be seen in Figure 1, SBP in the Na group showed a lower increase than in the other two experimental groups, but without reaching significant differences with respect to the control group.

Plasma and urinary parameters

Plasma creatinine, sodium, chloride and osmolality (estimated from plasma sodium, glucose and urea) did not change in any of the groups. Urinary creatinine decreased in the three groups with respect to the control group, and urinary sodium increased in the groups with a high sodium diet (NaCl and Na) and decreased in the Cl group. The urinary Na⁺/Cl⁻ index, which assesses urinary equimolarity between the two ions, increased significantly in the Na group, and reached values very close to equimolarity in the Cl group (Table 2).

Excretory renal function parameters

Diuresis increased in the three groups with respect to control, while CrCl decreased in the NaCl and Na groups.

In the NaCl and Na groups, FLNa, TRNa, FRNa, FLCl, TRCl and FRCI decreased and UENa, FENa, UECl and FECI increased with respect to control. Compared with the NaCl group, in the Na group we observed an increase in UENa and a decrease in UECl. Moreover, in the Na group, FECI was lower than FENa, while FRCI was higher than FRNa.

The CI group did not show significant changes with respect to the control group, but evidenced differences when compared with the other two groups: it had higher FLNa, TRNa, FRNa, FLCl, TRCl and FRCI with respect to the NaCl group, changes that were accompanied by less urinary and FE of both ions.

**Table 1. Body weight, food, calorie and water intake**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaCl</th>
<th>Na</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (n)</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>152 ± 4</td>
<td>151 ± 6</td>
<td>156 ± 10</td>
<td>175 ± 6</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>293 ± 22</td>
<td>265 ± 21</td>
<td>247 ± 15</td>
<td>290 ± 13</td>
</tr>
<tr>
<td>Body weight difference (g)</td>
<td>141 ± 7</td>
<td>114 ± 9*</td>
<td>91 ± 15*</td>
<td>115 ± 3*</td>
</tr>
<tr>
<td>Estimated food intake (g)</td>
<td>27 ± 2</td>
<td>29 ± 3</td>
<td>35 ± 8</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>Estimated calorie intake (kcal)</td>
<td>91 ± 7</td>
<td>95 ± 9</td>
<td>116 ± 26</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>Estimated water intake (mL)</td>
<td>21 ± 1</td>
<td>50 ± 4*</td>
<td>61 ± 9*</td>
<td>31 ± 1*</td>
</tr>
</tbody>
</table>

NaCl: high sodium-high chloride diet; Na: high sodium diet without chloride; Cl: high chloride diet without sodium. * p <0.05 vs. Control.
ROLE OF CHLORIDE ANION IN HYPERTENSION / Nicolás M. Kouyoumdzian et al.

by an increase in lipid peroxidation in the renal cortex, demonstrated by an increase in the production of TBARS. However, compared with the Na group, only excess of chlorides is associated with greater GPx activity and the development of hypertension with greater urinary retention of both ions, suggesting a higher pro-oxidant and oxidative stress state in the kidney in the presence of chloride overload.

DISCUSSION

Body weight, food, calorie and water intake
The intake of Na⁺, Cl⁻ or both ions was associated with lower BW gain, with respect to the control group, during the three weeks of diet. These results are consistent with that reported in the literature, where a hypopersaline diet was associated with a decrease in total

With respect to the Na group, the Cl group had lower urinary and FE, and greater FR of both ions (Table 3).

Oxidative stress parameters in the renal cortex
The production of TBARS increased in the renal cortex in the NaCl, Na and Cl groups compared with the control group. The activity and protein expression of SOD and CAT mitochondrial and cytosolic isoforms were not modified. While the protein expression of GPx was not modified in any group, the activity of this enzyme increased in the NaCl and Cl groups compared with the control and Na groups (Figure 2).

Summary of Results
Both excess of a high-sodium and high-chloride diet are associated with a higher oxidative state evidenced the increase in lipid peroxidation in the renal cortex, demonstrated by an increase in the production of TBARS. However, compared with the Na group, only excess of chlorides is associated with greater GPx activity and the development of hypertension with greater urinary retention of both ions, suggesting a higher pro-oxidant and oxidative stress state in the kidney in the presence of chloride overload.

Table 2. Plasma and urine parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaCl</th>
<th>Na</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine (mg/dL)</td>
<td>0.56 ± 0.04</td>
<td>0.64 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>Plasma sodium (mEq/L)</td>
<td>151 ± 5</td>
<td>144 ± 2</td>
<td>147 ± 3</td>
<td>144 ± 2</td>
</tr>
<tr>
<td>Plasma chloride (mEq/L)</td>
<td>102 ± 2</td>
<td>100 ± 1</td>
<td>101 ± 3</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>Glycemia (mg/dL)</td>
<td>138 ± 11</td>
<td>152 ± 15</td>
<td>153 ± 13</td>
<td>151 ± 14</td>
</tr>
<tr>
<td>Plasma urea (mg/dL)</td>
<td>27 ± 1</td>
<td>38 ± 4 *</td>
<td>49 ± 4 * $</td>
<td>22 ± 2 * $ Δ</td>
</tr>
<tr>
<td>Estimated plasma osmolarity (mOsm/kg)</td>
<td>319 ± 9</td>
<td>311 ± 4</td>
<td>321 ± 7</td>
<td>306 ± 5</td>
</tr>
</tbody>
</table>

NaCl: high sodium-high chloride diet; Na: high sodium diet without chloride; Cl: high chloride diet without sodium. * p <0.05 vs. Control; $ p <0.05 vs. NaCl; Δ p <0.05 vs. Na.

Table 3. Oxidative stress parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaCl</th>
<th>Na</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx activity (U/mg protein)</td>
<td>0.56 ± 0.04</td>
<td>0.64 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>SOD protein expression (U/mg protein)</td>
<td>151 ± 5</td>
<td>144 ± 2</td>
<td>147 ± 3</td>
<td>144 ± 2</td>
</tr>
<tr>
<td>CAT mitochondrial protein expression (U/mg protein)</td>
<td>102 ± 2</td>
<td>100 ± 1</td>
<td>101 ± 3</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>CAT cytosolic protein expression (U/mg protein)</td>
<td>138 ± 11</td>
<td>152 ± 15</td>
<td>153 ± 13</td>
<td>151 ± 14</td>
</tr>
</tbody>
</table>

NaCl: high sodium-high chloride diet; Na: high sodium diet without chloride; Cl: high chloride diet without sodium. * p <0.05 vs. Control; $ p <0.05 vs. NaCl; Δ p <0.05 vs. Na.
fat mass in mice that presented upregulation of genes involved in lipolysis and downregulation of genes related to lipogenesis. (25) In our work, at the time of euthanasia, we observed a decrease in epididymal and perirenal fat in rats that consumed NaCl and Na with respect to the other two experimental groups (data not included). These findings occurred despite the fact that all the diets were isocaloric. Animals fed with salt-overloaded diets consumed more water than controls. This may be caused by an initial acute increase in plasma osmolarity, which stimulates the thirst center, in order to compensate for that increase. (26)

**Evolution of systolic blood pressure**

We have shown that male Sprague Dawley rats, subjected to a diet with NaCl overload (8% W/W) increase their SBP after three weeks of diet, with values that exceed those defined as systolic hypertension (140 mmHg). (27)

The results presented suggest that the increase in SBP is also related to chloride overload, since the Cl group reached pressure values greater than 140 mmHg, higher than those of the Na group. The Cl anion is a component of NaCl that could have a more specific role in salt-sensitivity and that could be even more decisive than Na⁺. (28) Other studies in “salt-sensitive” Dahl rats showed that over several weeks, hypertension developed in NaCl-consuming animals, in CrCl and FLNa and FLCl that we observed in the Cl group and the urinary Na⁺/Cl⁻ index was similar in both groups. In the Na group, it is possible that bicarbonate secretion and excretion increases, a result consistent with the increase in the urinary Na⁺/Cl⁻ index with respect to the other groups, suggesting that Cl⁻ is not the main counterion to excreted Na⁺. The objective of HCO₃⁻ secretion is to compensate for metabolic alkalosis in the animals that receive Na⁺ citrate and, as a consequence, the reabsorption of Cl⁻ would be increased and its excretion, decreased, since the chloride anion is accumulated in some compartment, such as the skin, and decrease the glomerular filtration rate as a consequence of this anion and can cause renal vasoconstriction and decrease the biological efficiency of physiological mechanisms to compensate for possible hypernatremia and/or hyperchloremia and to preserve plasma osmolarity.

As expected, urinary sodium and chloride increased in the NaCl group compared with the control group and the urinary Na⁺/Cl⁻ index was similar in both groups. In the Na group, it is possible that bicarbonate secretion and excretion increases, a result consistent with the increase in the urinary Na⁺/Cl⁻ index with respect to the other groups, suggesting that Cl⁻ is not the main counterion to excreted Na⁺. The objective of HCO₃⁻ secretion is to compensate for metabolic alkalosis in the animals that receive Na⁺ citrate and, as a consequence, the reabsorption of Cl⁻ would be increased and its excretion, decreased, since the Cl/HCO₃⁻ exchanger, independent of the Na⁺ cation, would present a higher expression in the apical cell membranes of the distal convoluted, cortical collector and connector tubules. (28) Regarding the Cl group, the low urinary Cl⁻ is striking with respect to control rats, suggesting that for its excretion, it is also necessary to eliminate Na⁺ as a counterion.

These results indicate that the chloride anion is accumulating in some compartment, such as the skin, since its levels are still normal in plasma. (32-34)

**Excretory renal function parameters**

The increase in diuresis in the three experimental groups, with respect to controls, agrees with the increase in water intake. Di Ciano et al. have reported increased diuresis in Wistar rats fed a saline diet. (35) These changes were accompanied by a lower filtration fraction, a finding that is consistent with the decrease in CrCl and FLNa and FLCI that we observed in the NaCl group.

Chloride overload leads to an increase in the supply of this anion and can cause renal vasoconstriction and decrease the glomerular filtration rate as a consequence of the tubule-glomerular feedback due to greater transport of Cl⁻ to the macula densa. This ef-

### Table 3. Kidney function parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaCl</th>
<th>Na</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine output (mL/day/kg)</td>
<td>10 ± 2</td>
<td>78 ± 14 *</td>
<td>92 ± 15 *</td>
<td>51 ± 21 * Δ</td>
</tr>
<tr>
<td>CrCl (mL/min/kg)</td>
<td>3.55 ± 0.55</td>
<td>2.21 ± 0.29 *</td>
<td>2.41 ± 0.19 *</td>
<td>3.01 ± 0.53</td>
</tr>
<tr>
<td>NaFL (mEq/day/kg)</td>
<td>790 ± 141</td>
<td>461 ± 61 *</td>
<td>511 ± 39 *</td>
<td>634 ± 105 $</td>
</tr>
<tr>
<td>NaUE (mEq/day/kg)</td>
<td>1.2 ± 0.3</td>
<td>22.9 ± 4.3 *</td>
<td>34.4 ± 6.2 *</td>
<td>1.1 ± 0.3 $ Δ</td>
</tr>
<tr>
<td>NaFE (%)</td>
<td>0.15 ± 0.04</td>
<td>5.24 ± 1.74 *</td>
<td>6.82 ± 0.97 *</td>
<td>0.15 ± 0.03 $ Δ</td>
</tr>
<tr>
<td>NaTR (mEq/day/kg)</td>
<td>789 ± 141</td>
<td>440 ± 64 *</td>
<td>477 ± 38 *</td>
<td>633 ± 105 $</td>
</tr>
<tr>
<td>NaFR (%)</td>
<td>99.85 ± 0.04</td>
<td>94.76 ± 1.74 *</td>
<td>93.18 ± 0.97 *</td>
<td>99.85 ± 0.03 $ Δ</td>
</tr>
<tr>
<td>CIFL (mEq/day/kg)</td>
<td>532 ± 92</td>
<td>319 ± 40 *</td>
<td>349 ± 26 *</td>
<td>435 ± 74 $</td>
</tr>
<tr>
<td>CIFE (%)</td>
<td>1.4 ± 0.3</td>
<td>26.5 ± 5.1 *</td>
<td>7.8 ± 1.5 * Δ</td>
<td>1.1 ± 0.3 $ Δ</td>
</tr>
<tr>
<td>CIFE (%)</td>
<td>0.27 ± 0.07</td>
<td>8.39 ± 2.70 *</td>
<td>2.23 ± 0.33 * $ @</td>
<td>0.24 ± 0.04 $ Δ</td>
</tr>
<tr>
<td>CITR (mEq/day/kg)</td>
<td>531 ± 92</td>
<td>295 ± 44 *</td>
<td>341 ± 26 *</td>
<td>434 ± 74 $</td>
</tr>
<tr>
<td>CIFR (%)</td>
<td>99.73 ± 0.07</td>
<td>91.61 ± 2.70 *</td>
<td>97.77 ± 0.33 * $ @</td>
<td>99.76 ± 0.04 $ Δ</td>
</tr>
</tbody>
</table>

NaCl: high sodium- high chloride diet; Na: high sodium diet without chloride; Cl: high chloride diet without sodium. CrCl: creatinine clearance, FL: filtered load, UE: Urinary excretion, FE: fractional excretion, TR: tubular reabsorption, FR: fractional reabsorption. * p <0.05 vs. Control; $ p <0.05 vs. NaCl; @ p <0.05 vs. FENa or FRNa; Δ p <0.05 vs. Na.
The decrease in CrCl in the NaCl group may explain lower FLNa and FLCI, compared with the control group. It was expected that both groups that received iso-osmolar Na⁺ overloads would present the same profile in terms of ionic excretion and retention parameters. But this profile was not observed in the Cl group, showing that the replacement of the Na⁺ ion by other cations causes dissimilar responses. In the Cl...
group, Na⁺ and Cl⁻ ions presented very similar excretion and reabsorption profiles, evidencing a clear urinary equimolarity, which reflects that the counterion that is eliminated with Cl⁻ is Na⁺.

**Oxidative stress parameters in the renal cortex**

Na⁺, Cl⁻ or both ions overload in the diet was associated with an increase in lipid peroxidation in the renal cortex, represented by the increase in the production of TBARS. The pro-oxidant state in these cells is characterized by an increase in the production of reactive oxygen species, a situation in which SOD produces the dismutation of the superoxide anion to H₂O₂ and molecular oxygen with a high reaction rate constant (2.3.10⁹ M⁻¹s⁻¹), while CAT converts H₂O₂ to molecular oxygen and water.

It was expected that increased TBARS production was accompanied by increased SOD and CAT activity and/or expression. But in our models these parameters were not affected. However, an increase in GPx activity was registered, which suggests a compensatory effect in the absence of SOD and CAT modifications. The regulation of its activity is related to post-translational modifications that take place in the active site of the enzyme and that occur regardless of whether or not its expression varies. (38)

**CONCLUSION**

These results suggest that the chloride anion is co-responsible, together with sodium, of triggering oxidative kidney damage and increased blood pressure. It is therefore important to take into account the reduction in the intake of both ions as a measure of non-pharmacological treatment of hypertension, considering that most commercial dietary products, substitutes for table salt, are based on potassium chloride.

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A) TBARS: Thiobarbituric acid reactive species. B) Mn-SOD: Manganese-superoxide dismutase (mitochondrial isoform of the enzyme). C) Cu/Zn-SOD: Copper/Zinc-superoxide dismutase (cytosolic isoform of the enzyme). D) CAT: Catalase. E) GPx: Glutathione peroxidase. NaCl: high sodium-high chloride diet; Na: high sodium diet without chloride; Cl: high chloride diet without sodium. * p < 0.05 vs Control; Δ p < 0.05 vs Na.


**Acknowledgements**

We thank Technician Cecilia Mambrin, from the Nutrition Chair of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires, for preparing the diets used in this project.

**Conflicts of interest**

None declared.

(See authors’ conflicts of interest forms on the website/Supplementary material)

**REFERENCES**


