

Dietary salt restriction in hyperthyroid rats. Differential influence on left and right ventricular mass

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Abstract

This study assessed the impact of salt restriction on cardiac morphology and biochemistry and its effects on hemodynamic and renal variables in experimental hyperthyroidism. Four groups of male Wistar rats were used: control, hyperthyroid, and the same groups under low salt intake. Body weight, blood pressure (BP), and heart rate (HR) were recorded weekly for 4 weeks. Morphologic, metabolic, plasma, cardiac, and renal variables were also measured. Low salt intake decreased BP in T₄-treated rats but not in controls. Low salt intake reduced relative left ventricular mass but increased absolute right ventricular weight and right ventricular weight/BW ratio in both control and hyperthyroid groups. Low salt intake increased Na⁺/H⁺ exchanger-1 (NHE-1) protein abundance in both ventricles in normal rats but not in hyperthyroid rats, independently of its effect on ventricular mass. Mammalian target of rapamycin (mTOR) protein abundance was not related to left or right ventricular mass in hyperthyroid or controls rats under normal or low salt conditions. Proteinuria was increased in hyperthyroid rats and attenuated by low salt intake. In this study, low salt intake produced an increase in right ventricular mass in normal and hyperthyroid rats. Changes in the left or right ventricular mass of control and hyperthyroid rats under low salt intake were not explained by the NHE-1 or mTOR protein abundance values observed. In hyperthyroid rats, low salt intake also slightly reduced BP and decreased HR, proteinuria, and water and sodium balances.

Keywords: Low salt intake, hyperthyroidism, ventricular hypertrophy, Na⁺/H⁺ exchanger-1, mammalian target of rapamycin

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Introduction

Hyperthyroidism is associated with increased blood pressure (BP), cardiac hypertrophy, and proteinuria,¹ which are exacerbated by an increased saline intake.² Cardiac mass can be modulated by sodium concentrations *in vitro* or dietary sodium intake *in vivo*. In cultured neonatal rat myocardial myoblasts, cellular protein content and cell size increased with higher sodium concentration in the medium.³ In humans, sodium intake (assessed by urinary sodium excretion) and left ventricular mass index were positively correlated in hypertensive and normotensive subjects.⁴ In animals, a high sodium intake increased cardiac mass in normotensive rats⁵ and exacerbated cardiac hypertrophy in hypertensive rats.⁶ Our group recently observed that saline loading increased the ventricular weight (VW) and ventricular-to-body weight (VW/BW) ratio in control, hyperthyroid, and hypothyroid rats and increased the susceptibility of hyperthyroid rats to saline-induced cardiac hypertrophy.² The same study

found that proteinuria levels were twofold higher in hyperthyroid rats than in controls and that a high salt intake did not change these levels in normal and hypothyroid rats but markedly increased them in hyperthyroid rats.

Conversely, salt restriction has been reported to have beneficial effects on cardiac hypertrophy, proteinuria, and BP in normotensive and hypertensive rats. Thus, low-sodium intake prevented cardiac hypertrophy associated with two-kidney, one-clip Goldblatt hypertension⁷ and angiotensin II (ANG II) hypertension,⁸ independently of the effect on BP. Moreover, the ANG II-induced rise in albuminuria was blunted in sodium-restricted rats.⁹ However, the beneficial effect of dietary sodium restriction on BP is controversial, and a low salt intake has been associated with reductions,^{10,11} slight decreases,⁹ or no change^{7,8,12} in various models of hypertension. In contrast with these experimental reports, epidemiological studies have recently associated lower sodium excretion with higher

cardiovascular disease mortality,¹³ and that extreme salt restriction increases the risk of all-cause mortality.¹⁴

Activity or expression of the aminopeptidase A (APA) responsible for the downstream cleavage of angiotensin II^{15,16} has been implicated in tumor growth and tumor angiogenesis.¹⁷ This APA is changed in several tissues in rats with thyroid dysfunction,^{18,19} but the potential modulatory role of thyroid hormone on APA activity in cardiac hypertrophy is unknown.

Na⁺/H⁺ exchanger isoform-1 (NHE-1) and mammalian target of rapamycin (mTOR) are essential physiological pathways involved in ventricular hypertrophy and are activated by the main hypertrophic factors.^{20–24} Thus, it has been reported that thyroid hormone increases the expression of the NHE-1 protein^{25,26} and that hypertrophy of hyperthyroid hearts is prevented by NHE-1 inhibition.²⁷ Thyroid hormone is also an endogenous activator of mTOR in cardiomyocytes,^{28–30} which has been proposed as a key factor in hyperthyroid cardiac hypertrophy.^{29,31} Thus, mTOR inhibition with rapamycin prevented cardiac hypertrophy induced by thyroid hormone both *in vitro* and *in vivo*.^{30–32}

With this background, the objective of this study was to evaluate the effects of low salt intake in controls and in hyperthyroid rats, which have manifested an increased salt sensitivity,² analyzing the response of BP, heart rate (HR), morphological variables, and renal function. In order to identify the cause of changes in ventricular mass, we investigated APA activity and the abundance of the NHE-1 and of mTOR proteins in both cardiac ventricles.

Methods

Animals

Male Wistar rats born and raised in the experimental animal service of the University of Granada were used. Experiments were performed according to European Union guidelines for the ethical care of animals and were approved by the ethical committee of the University of Granada. Rats initially weighing 280–300 g were maintained on standard chow and tap water *ad libitum* except where stated. The animals were divided into two groups: control and hyperthyroid rats allocated to a diet of normal chow with 0.4% NaCl or a diet of low salt chow with 0.02% NaCl (n=8 each group), which commenced simultaneously with hyperthyroidism induction.

Hyperthyroidism was induced by injecting s.c. thyroxine (75 µg/rat/day), as previously reported.^{1,33} This treatment was administered for 4 weeks. Tail systolic BP (SBP) and HR were recorded by tail-cuff plethysmography in unanesthetized rats (LE 5001-Pressure Meter, Letica SA, Barcelona, Spain).

Experimental protocol

When the experimental period was completed, all rats were housed in metabolic cages (Panlab, Barcelona, Spain) with free access to food and their respective drinking fluid for a 4-day period (2 days for adaptation + 2 experimental days), during which food and fluid intakes were measured and urine samples collected. We measured 24-h urine volume

and total urinary sodium, potassium, proteinuria, and creatinine. Mean values of all intake and urinary variables obtained during the 2 experimental days were compared among groups. Water and sodium balances were calculated with respect to renal losses, without taking extra-renal losses into account. After completion of the metabolic study, rats were anesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 units of heparin in isotonic sterile NaCl solution was inserted into the femoral artery to measure intra-arterial BP, HR, and pulse pressure in conscious rats and to draw blood samples. The catheter was tunneled subcutaneously and brought out through the skin at the dorsal side of the neck. Intra-arterial BP was measured at 24 h after femoral catheter implantation. Direct BP and HR were recorded continuously for 60 min with a sampling frequency of 400/s (McLab, AD Instruments, Hastings, UK). BP and HR values obtained during the last 30 min were averaged for inter-group comparisons. Subsequently, blood samples taken with the femoral catheter were used to determine the following plasma variables: urea, creatinine, total proteins, electrolytes (sodium and potassium), and thyroid hormones (FT₃ and FT₄). Finally, the rats were killed by the injection of sodium pentobarbital and lidocaine, and the kidneys and ventricles were removed and weighed. Ventricles were divided into right ventricle and left ventricle plus septum. The auricles were cut and discarded. The following ratios were measured: VW/BW, as index of cardiac hypertrophy; left ventricular weight (LVW)/BW and right ventricular weight (RVW)/BW, as indices of left and right ventricular hypertrophy, respectively; and LVW/VW, RVW/VW, and LVW/RVW as indices of absolute left and right ventricular hypertrophy.

Renal and plasma variables

Proteinuria was measured by the method of Bradford.³⁴ Plasma and urinary electrolytes and creatinine were measured in an autoanalyzer (Hitachi-912, Roche, Spain). Plasma thyroid hormone levels (free circulating T₃ and T₄) were determined with rat radioimmunoassay kits according to the manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA, USA).

Cardiac biochemical variables

NHE-1, mTOR, and aminopeptidase A (ApA) abundance were quantified by indirect ELISA using an enzyme linked immunosorbent assay (ELISA) kit purchased from Bethyl Laboratories Inc. (Montgomery, TX, USA). Tissues were homogenized in 50 mM Tris-HCl, pH 7.5 with 1% Triton and Sigmafast protease inhibitor cocktail tablets (Sigma, St Louis, MO, USA). Homogenates containing 2 mg/mL of total protein were fixed in a 96-well plate overnight at 4°C. After blocking, the plate was probed with 1 µg/mL of rabbit anti-NHE-1 or anti-mTOR antibody (Sigma-Aldrich, St Louis, MO, USA) as primary antibody and with 0.2 µg/mL of mouse anti-rabbit horseradish peroxidase (HRP)-linked IgG antibody (KPL Inc., Gaithersburg, MD, USA) as secondary antibody. Goat anti-ApA antibody (1 µg/mL) and rabbit anti-goat HRP-linked IgG antibodies were purchased from Everest Biotech (Upper Heyford, UK).

All samples were determined in duplicate and results were expressed as a percentage of the mean absorbance in the control group.

Statistical analyses

The time course of BW, SBP, and HR was compared by using a nested design, with groups and days as fixed factors and rat as random factor. When the overall difference was significant, Bonferroni's method with an appropriate error was employed. Two-way ANOVA was used for intergroup comparisons of each variable at the end of the experiments. When the overall ANOVA was significant, pairwise comparisons were performed using Bonferroni's method. Regression analyses and ANOVAs were performed, using Statgraphics software, to establish the correlation between variables. $P < 0.05$ and $|r| > 0.5$ were considered a strong correlation.

Results

Time course of BW, BP, and HR

Data obtained are depicted in Figure 1. The time course of BW was severely attenuated in the T_4 group with respect to controls; the BW was increased by the low salt diet in hyperthyroid rats, but this increase did not reach significance in

normal rats. At the end of the 4-week study period, the BW gain was significantly lower in the T_4 group ($\Delta BW = 17 \pm 1.1$; $p < 0.01$) than in controls ($\Delta BW = 59 \pm 3.8$). Low salt intake produced a tendency to an increased BW in controls ($\Delta BW = 76 \pm 3.7$; NS) and a significantly increased BW in T_4 -treated rats ($\Delta BW = 30 \pm 2.1$; $p < 0.05$). SBP values were higher in the T_4 group than in control group. Low salt intake did not change the BP in control group but produced a slight but significant decrease in the SBP in T_4 -treated rats at the end of the study. The HR was increased in T_4 -treated rats and was reduced by the low salt diet in both groups, more markedly in controls. The SBP and HR data were confirmed by the direct recording giving similar results to the last tail measurement (data not shown).

Morphological variables

Data obtained are exhibited in Table 1 and Figure 2. Absolute kidney weight (KW) was similar among all groups. The KW/BW ratio was significantly increased in the T_4 group and was nonsignificantly decreased by low salt intake in both groups.

The VW, LVW, RVW, and VW/BW, LVW/BW, and RVW/BW ratios were significantly increased in the T_4

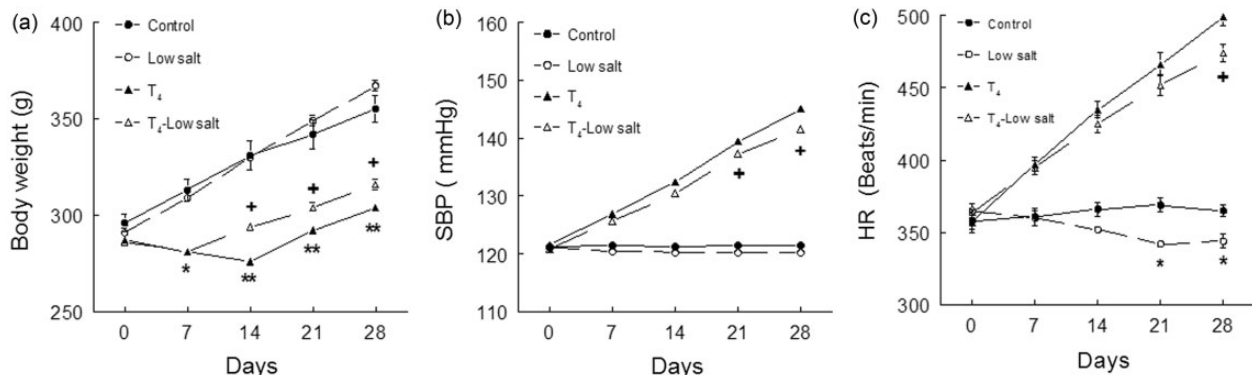


Figure 1 Time course of body weight (BW), tail systolic blood pressure (SBP), and heart rate (HR) in the experimental groups, measured by plethysmography. Data are means \pm SEM. * $p < 0.01$, ** $p < 0.001$ versus control group. + $p < 0.05$ versus T_4 group

Table 1 Morphologic variables in the experimental groups

Groups	Control	Low salt	T_4	T_4 low salt
KW (mg)	1032 \pm 18	1087 \pm 22	1079 \pm 33	1066 \pm 26
VW (mg)	891.5 \pm 6.0	917.7 \pm 31.9	1132 \pm 30*	1120 \pm 19*
LVW (mg)	718 \pm 9	683 \pm 31	913 \pm 19*	876 \pm 11*
RVW (mg)	173.2 \pm 9.2	234.2 \pm 8.9*	218 \pm 17.2*	285.5 \pm 13.9*,+
KW/BW (mg/g)	2.82 \pm 0.07	2.74 \pm 0.06	3.40 \pm 0.07*	3.26 \pm 0.08*
VW/BW (mg/g)	2.43 \pm 0.06	2.35 \pm 0.09	3.57 \pm 0.09*	3.42 \pm 0.06*
LVW/VW	0.81 \pm 0.01	0.75 \pm 0.01*	0.81 \pm 0.01	0.75 \pm 0.01*,+
RVW/VW	0.19 \pm 0.01	0.26 \pm 0.01*	0.19 \pm 0.01	0.25 \pm 0.01*,+
LVW/RVW	4.25 \pm 0.29	2.94 \pm 0.14*	4.44 \pm 0.28	3.01 \pm 0.17*,+

Data expressed as means \pm SEM.

BW: body weight; HW: Heart weight; KW: kidney weight; LVW: left ventricular weight; RVW: right ventricular weight.

* $P < 0.05$ versus control group; + $P < 0.05$ versus T_4 group.

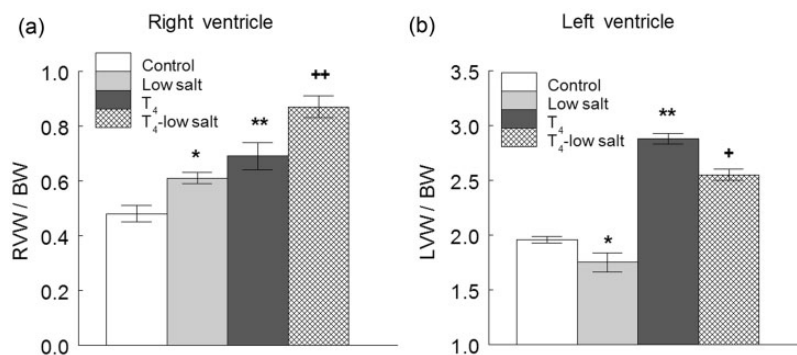


Figure 2 Right ventricular weight/body weight (RVW/BW) and left ventricular weight/body weight (LVW/BW) ratios. Data are means \pm SEM. * $p < 0.01$, ** $p < 0.001$ versus controls. + $p < 0.01$, ++ $p < 0.001$ versus T₄ group

Table 2 Plasma variables in the experimental groups

Groups	Control	Low salt	T ₄	T ₄ low salt
Na (mEq/L)	142.0 \pm 0.26	139.8 \pm 0.89*	143.0 \pm 0.90	140.4 \pm 0.14+
K (mEq/L)	3.97 \pm 0.18	3.84 \pm 0.15	4.24 \pm 0.12	4.23 \pm 0.11
Ca (mg/dL)	9.1 \pm 0.19	9.35 \pm 0.18	9.31 \pm 0.11	9.67 \pm 0.16
Urea (mg/dL)	32.4 \pm 3	30 \pm 0.53	31.3 \pm 1.71	31.9 \pm 1.75
Creatinine (mg/dL)	0.33 \pm 0.01	0.30 \pm 0.02	0.32 \pm 0.02	0.26 \pm 0.01*,+
Total Proteins (g/dL)	5.12 \pm 0.08	5.14 \pm 0.15	4.71 \pm 0.07*	5.24 \pm 0.09
FT ₃ (pg/mL)	3.81 \pm 0.18	4.06 \pm 0.20	7.1 \pm 0.74**	7.72 \pm 0.47**
FT ₄ (ng/dL)	2.73 \pm 0.14	3.06 \pm 0.24	5.99 \pm 0.30**	6.24 \pm 0.30**

Data expressed as means \pm SEM.

FT₃: free triiodothyronine; FT₄: free thyroxine.

* $P < 0.05$, ** $P < 0.01$ versus control group; + $P < 0.05$ versus T₄ group.

Table 3 Metabolic variables in the experimental groups

Groups	Control	Low salt	T ₄	T ₄ low salt
Food intake (g)	16.43 \pm 1.31	20.71 \pm 1.02*	20.42 \pm 1.13*	26.67 \pm 0.96*,+
Water intake (mL)	38.14 \pm 3.60	27.71 \pm 1.23*	49.42 \pm 5.01*	33.67 \pm 2.91+
Water balance (mL)	21.73 \pm 0.67	19.67 \pm 0.77	25.36 \pm 0.84*	18.50 \pm 0.94+
Sodium balance (mmol)	0.56 \pm 0.06	0.13 \pm 0.01*	0.78 \pm 0.10*	0.12 \pm 0.01*,+

Data expressed as means \pm SEM. All data are referred to 24 h.

* $P < 0.05$ versus the control group; + $P < 0.05$ versus T₄ group.

group, as expected. Low salt intake produced a decrease in LVW/BW, LVW/VW, and LVW/RVW ratios and an increase in absolute RVW values and RVW/BW and RVW/VW ratios; these changes were of a similar magnitude in both controls and T₄-treated rats except for the reduction in LVW/BW, which was greater in the low-salt T₄ group. These data demonstrate that salt restriction produced right ventricular hypertrophy and reduced left ventricular hypertrophy in control and T₄-treated rats (Figure 2).

Plasma variables and thyroid hormone levels

Data obtained are summarized in Table 2. Plasma sodium was slightly decreased in both low-salt groups, while plasma potassium and calcium levels were similar among

all groups. Plasma urea was not significantly modified in any group, while plasma creatinine was decreased in the T₄-low salt group. Total plasma protein levels were reduced in the T₄ group. FT₃ and FT₄ values were significantly increased in both T₄-treated groups.

Metabolic and urinary variables

Data obtained are presented in Tables 3 and 4. Food intake, water intake, water balance, and sodium balance were increased in T₄-treated rats. Low salt intake increased the food intake and reduced the water intake in both control and T₄-treated rats. The water and sodium balance was higher in the T₄ group, and the low salt diet decreased the sodium balance in both control and T₄-treated rats. The decrease in water balance in the two low-salt groups did

Table 4 Urinary and renal variables in the experimental groups

Groups	Control	Low salt	T ₄	T ₄ low salt
Diuresis (mL/100 g)	4.49 ± 1.23	2.09 ± 0.28*	7.87 ± 1.41*	4.60 ± 0.66+
U _{Na} ⁺ V (μmol/100 g)	87.6 ± 13.08	16.2 ± 1.63*	141.6 ± 19.48*	39.0 ± 2.40*,+
U _K ⁺ V (μmol/100 g)	594.1 ± 65.7	385.8 ± 51.7*	743.1 ± 44.8*	984.1 ± 87.5*,+
Proteinuria (mg/mg crea)	14.2 ± 1.83	16.4 ± 1.22	36.5 ± 2.18*	25.8 ± 2.14*,+
U _{Cr} V (mg/100 g)	2.58 ± 0.39	2.60 ± 0.20	2.42 ± 0.15	2.44 ± 0.23
CrC (mL/min/g kidney)	1.40 ± 0.07	1.42 ± 0.10	1.62 ± 0.27	1.48 ± 0.31

Data expressed as means ± SEM.

CrC: creatinine clearance; U_{Cr}V: total creatinine excretion.

All data are referred to 24 h. *P < 0.05 versus the control group; +P < 0.05 versus T₄ group.

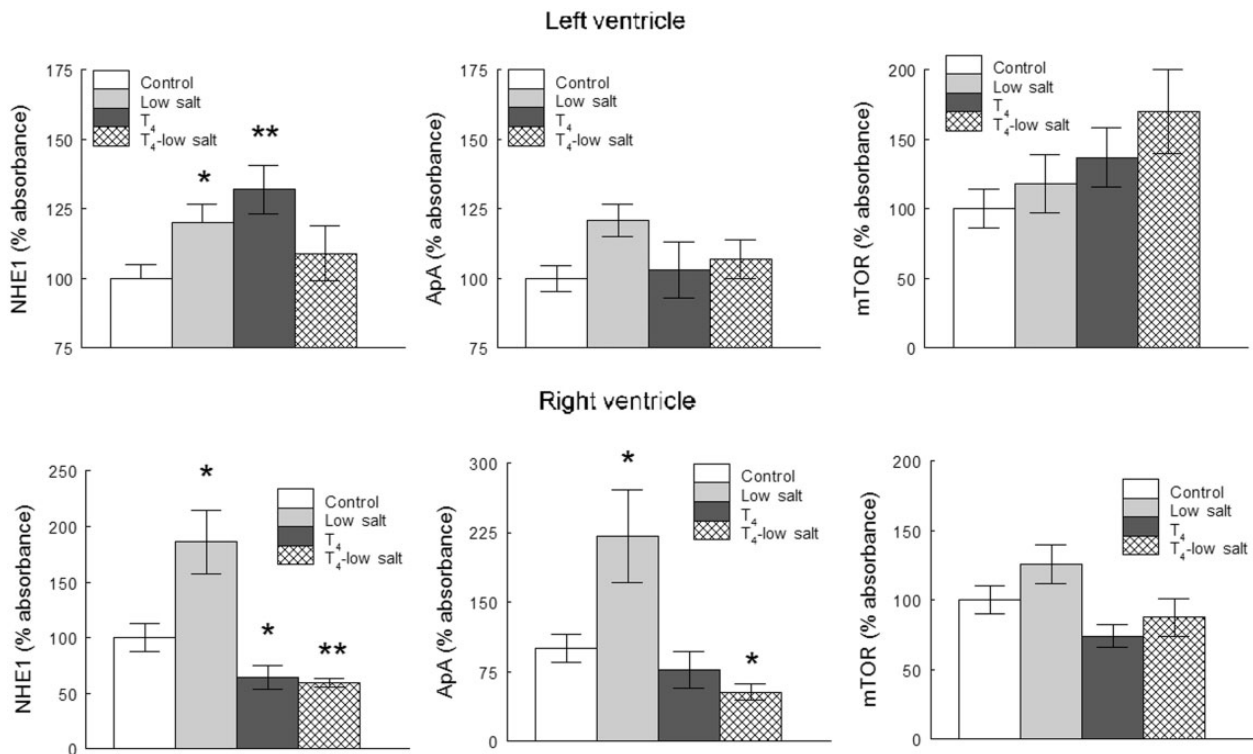


Figure 3 Variables measured in left and right ventricular tissue. Protein abundances in left and right cardiac ventricles of: NHE-1, Na⁺/H⁺ exchanger 1; ApA, aminopeptidase A; and mTOR, mammalian target of rapamycin protein. Data are means ± SEM. *p < 0.01, **p < 0.001 versus control group

not reach significance in normal rats (Table 3). In consonance with these findings, daily urine volume and total sodium excretion were higher in T₄-treated rats and were reduced by the low salt intake in control and T₄-treated rats (Table 4).

Total potassium excretion was higher in the T₄ group than in the controls and was reduced by low salt intake in controls but increased by this diet in the T₄-treated rats. Creatinine excretion was similar between controls and the T₄ group, and it was not changed by the low salt intake. Proteinuria levels were twofold higher in the T₄ group than in controls, and they were not changed by low salt intake in the controls but were reduced by this diet in T₄-treated rats. Creatinine clearance values were similar among all groups (Table 4).

Biochemical variables in heart ventricles

Data obtained are depicted in Figure 3. In the left ventricle, ApA expression was increased in the low-salt group but unchanged in the T₄ and low-salt T₄ groups. The NHE-1 protein abundance was higher in the T₄ group than in the controls and was increased by low salt intake in normal rats but reduced by this diet in T₄-treated rats. The mTOR protein abundance in the left ventricle was not significantly changed by any treatment.

In the right ventricle, ApA and NHE-1 expression showed a similar pattern in the different groups, with both being increased in the low-salt group, decreased in the T₄ group, and additionally reduced in the low-salt T₄ group. The mTOR protein abundance in the right ventricle was not significantly changed by treatments.

Correlation studies

A strong positive relationship was found between the LVW/BW ratio and the final SBP ($r=0.91$; $p<0.001$; 4th week) and between the final SBP \times HR, an index of cardiac work, and the VW/BW ratio ($r=0.908$; $p<0.001$, Figure 4) when all groups were pooled in a common regression line. LVW/BW and RVW/BW showed a positive and negative correlation, respectively, with total sodium excretion (U_{Na+V}) in the control and hyperthyroid groups. LVW/BW- U_{Na+V} values were $r=0.84$, $p<0.001$ in controls and $r=0.64$, $p<0.01$ in hyperthyroid animals; RVW/BW- U_{Na+V} values were $r=-0.58$, $p<0.05$ in controls and $r=-0.69$, $p<0.01$ in hyperthyroid animals. When the data of all groups were pooled, ApA and NHE-1 protein abundances showed positive correlations in the right ($r=0.90$; $p<0.0001$) and left ventricles ($r=0.68$; $p<0.0001$). Positive correlations were found between NHE-1 and mTOR ($r=0.60$; $p=0.0001$) and between ApA and mTOR ($r=0.52$; $p=0.0008$) in the right ventricle but not in the left ventricle.

Discussion

One of the main findings of this study is that a low salt intake produced an increase in right ventricular mass in normal and hyperthyroid rats. It was also found that low dietary sodium slightly attenuated hypertension and decreased the HR, left ventricular hypertrophy, albuminuria, and water and sodium balance associated with T_4 -induced hyperthyroidism in rats.

Our group previously reported that a high salt intake increased the BP in hyperthyroid rats.² Conversely, in the present study, a low salt intake reduced the SBP in hyperthyroid rats. These BP changes in response to dietary sodium are the basis of the well-documented phenomenon of salt sensitivity. A decreased sensitivity to the pressor effect of angiotensin II may participate in the mechanism(s) underlying the reduced SBP in the present study.⁹

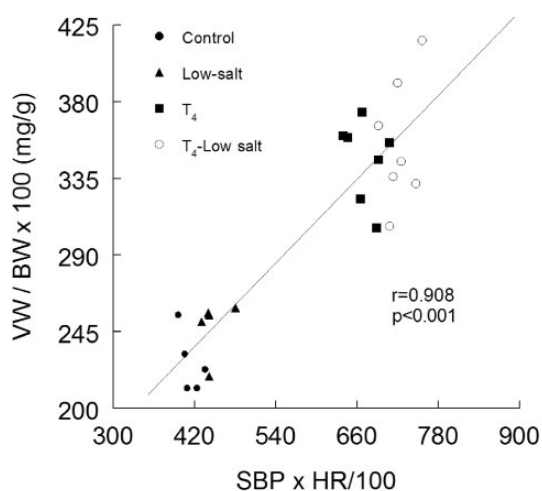


Figure 4 Graphs showing the relationship between SBP \times HR, an index of cardiac work, and VW/BW ratio, index of cardiac hypertrophy, when all data were pooled in a common regression line

In addition, a decrease in sympathetic activity secondary to decreased neural oxidative stress may be involved in the reduced HR produced by a low salt intake in both control and hyperthyroid rats.³⁵

The morphological study showed significantly increased absolute and relative cardiac mass values in the T_4 -treated group. However, LV/VW ratio, an index of left ventricular cardiac hypertrophy, was not significantly modified in the T_4 -treated group, indicating that hyperthyroidism affected left and right ventricular mass equally, as previously reported.^{1,2} It is known that dietary sodium intake can modulate cardiac mass⁹ and that a high-sodium intake increases cardiac mass in normal rats^{2,5} and exacerbates cardiac hypertrophy in hyperthyroid rats.² Conversely, a low-sodium intake prevents cardiac hypertrophy associated with two-kidney, one-clip Goldblatt hypertension⁷ and ANG II hypertension,⁸ independent of any BP reduction. In the present study, low salt intake decreased LVW/BW and LVW/VW ratios in control and hyperthyroid groups. Moreover, positive correlations were found between LVW/BW ratio and total sodium excretion in control and hyperthyroid groups, indicating the influence of sodium intake on the left ventricle mass. However, low salt intake unexpectedly increased the RVW in control and hyperthyroid rats, both in absolute values and relative to BW or VW. RVW/BW ratio was negatively correlated with total sodium excretion in control and hyperthyroid groups, showing a clear association between low salt intake and right ventricular hypertrophy.

KW and KW/BW ratio were significantly increased in hyperthyroid rats, as previously reported.² Control and hyperthyroid rats on a low salt diet showed a tendency to a decreased KW/BW that did not reach significance.

The mechanisms by which TH produces cardiac hypertrophy are poorly understood. Some studies demonstrated that T_3 can induce hypertrophy in cultured cardiomyocytes, indicating a direct hypertrophic effect,³⁶ but hypertrophy was not developed by heterotopically transplanted hearts exposed to TH in the absence of loading conditions.³⁷ Our results indicate that the loading conditions make a major contribution to the development of heart ventricular hypertrophy, because a strong and positive relationship was observed between VW/BW ratio and the final SBP or estimated heart work. However, these observations do not rule out the role of a combination of direct and indirect factors in cardiac hypertrophy in hyperthyroidism, as previously suggested.³⁰

Given this evidence of an association between low salt diet and left ventricular atrophy and, unexpectedly, right ventricular hypertrophy in control and hyperthyroid rats, we explored the potential cause of these cardiac changes by analyzing the status of NHE-1 and of mTOR and ApA protein abundance in both ventricles.

Out of the nine NHE isoforms identified to date, the NHE-1 isoform is present in cardiac cells.²⁰⁻²² Various pro-hypertrophic factors, including endothelin-1, angiotensin II, α_1 -adrenergic agonists, isoproterenol, aldosterone, and growth factors can stimulate NHE-1 activity.²⁰⁻²² NHE inhibitors block hypertrophic responses to these stimuli, and NHE-1 activation has been proposed as a

common response to mechanical stretch and as a key player in the hypertrophic process.^{20–22} Cardiac NHE-1 activity is increased in many different models of myocardial hypertrophy,^{38–40} which can be prevented by inhibition of this activity.^{38,41,42} In the right ventricle, an NHE-1 inhibitor (cariporide) was found to abrogate right ventricular hypertrophy and the associated increased NHE-1 mRNA in rats.⁴³ Our results show that NHE-1 protein abundance is increased in the hypertensive T₄ group. These results agree with reports showing that thyroid hormone, by the interaction of its receptor with the NHE-1 promoter, increases the expression of the NHE-1 protein,²⁵ and that hypertrophy of hyperthyroid hearts was prevented by NHE-1 inhibition.²⁶ In consonance, with these observations low salt intake reduces LVW ratios and LV NHE-1 protein abundance in hyperthyroid rats. However, low salt intake in normal rats increases NHE-1 protein abundance in both ventricles regardless of its effect on ventricular mass; in hyperthyroid rats, under low salt intake, NHE-1 protein abundance in right ventricle is also unrelated to the changes in ventricular mass. Therefore, when all results are taken together do not support a mandatory role for NHE-1 determining left and right ventricular mass, observations that contrast with the general assumption^{20–22} that NHE-1 abundance is relative to ventricular hypertrophy.

In the right ventricle, ApA values of the four experimental groups mirrored those of NHE-1 and showed a strong correlation between both variables. This correlation also was present, although less close, in the left ventricle. These data suggest that angiotensin II, which stimulates sodium–hydrogen exchange,²⁷ might modulate NHE-1 expression.

Inhibition of mTOR was reported to suppress myocardial hypertrophy induced by mechanical stresses in animal models,^{24,44} suggesting a role for this protein in ventricular hypertrophy.

It has been reported that mTOR activation plays a role in the cardiac hypertrophy induced by thyroid hormone excess^{29,31} that can be prevented by the mTOR inhibitor rapamycin.^{30–32} In the present study, however, mTOR protein abundance was not related to left or right ventricular mass in the hyperthyroid or control rats under normal or low salt conditions, suggesting that the abundance of this factor does not play a role in the ventricular mass changes observed in our experimental setting.

Proteinuria levels were twofold higher in the hyperthyroid rats than in the controls. Proteinuria is often present in hyperthyroid humans⁴⁵ and rats.^{46,47} Proteinuria was attenuated by a low sodium intake in the T₄-treated rats. Conversely, a higher sodium intake was reported to increase proteinuria in hyperthyroid rats.² All of these data indicate that salt intake modulates proteinuria in hyperthyroidism.

Finally, this study contributes original data demonstrating that the right ventricular mass is increased by a low sodium intake. It has not been addressed to date whether this type of hypertrophy is attributable to an increased size of the cardiomyocytes or to fibrosis or whether a low salt diet can induce pulmonary hypertension. In this context, the pulmonary circulation is the main site of angiotensin

II production, which is augmented under low salt intake and may therefore result in vasoconstriction of the pulmonary vasculature, and hence pulmonary hypertension. Furthermore, the molecular markers and inducers of cardiac hypertrophy measured in this study did not shed light on the mechanisms responsible for the changes in ventricular mass. Hence, this study opens up new perspectives in this research field, and further studies are warranted to establish the mechanisms underlying the onset of right ventricular hypertrophy under low salt intake and heart hypertrophy in the hyperthyroid state.

In summary, this study demonstrated that a low salt intake increases the right ventricular mass in normal and hyperthyroid rats and that NHE-1 and mTOR protein abundances do not explain the changes in left or right ventricular mass in these rats under low salt conditions. Low-salt diet also slightly attenuated hypertension and decreased heart rate, left ventricular hypertrophy, proteinuria, and water and sodium balance associated with T₄-induced hyperthyroidism in rats.

Author contributions: Conceived and designed the experiments: FV and AO. Performed the experiments: RW. Analyzed the data: IRG, RPA, AQ, and SMM. Wrote the paper: FV and RW.

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