Current Trends in Endocrinology
Vol. 8, 2016

Original Communication

Salt intake and renal inflammatory factors: Modulatory role of thyroid hormone

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ABSTRACT

This study assessed the effects of changes in saline intake on renal cortex inflammatory cytokines (interleukin 6 (IL6), tumour necrosis factor alpha (TNF-α) and vascular endothelial growth factor (VEGF)) and hemodynamic, morphologic, and plasma variables in experimental thyroid disorders. In the present study, eight groups of male Wistar rats were used: euthyroid, hypothyroid, and hyperthyroid groups on a diet of normal salt (0.4% NaCl); the same groups on a high salt diet (8% *via* food intake); and euthyroid and hyperthyroid groups on a low salt (0.02%) diet. Blood pressure (BP) and heart rate were directly recorded, and the morphologic, plasma and renal variables were measured. BP increased and decreased in hyper- and hypothyroid rats, respectively. BP increased with high salt treatment and decreased with low salt intake in hyperthyroid rats but did not change in euthyroid or hypothyroid rats. The levels of all renal cortex inflammatory cytokines significantly decreased in hypothyroid rats but did not change in hyperthyroid rats. High salt diet reduced the levels of all renal inflammatory cytokines in euthyroid and hyperthyroid rats, and the reduction was significant. Low salt diet increased the inflammatory cytokine levels in renal cortex of euthyroid rats. In summary, this study demonstrates that: (i) salt intake is inversely related to renal inflammatory variables; (ii) the salt-sensitive model of hypertension induced by hyperthyroidism is not accompanied by an increase in renal inflammatory cytokines and (iii) hypothyroidism is associated with a reduction in inflammatory cytokines in renal tissues, which might contribute to its protective effects against renal injury.

KEYWORDS: salt intake, hyperthyroidism, hypothyroidism, inflammation, cytokines

INTRODUCTION

Thyroid disorders are associated with important changes in renal sodium handling [1, 2]. Thyroxine-treated rats have shown a reduced ability to excrete sodium after hypertonic saline loading [3] and a blunted pressure-diuresis-natriuresis response [4]. However, increased natriuresis has been reported in hypothyroid rats after salt- or water-loading [5, 6]. Thyroid disorders are also accompanied by major changes in renal sodium transporters [7], which may contribute to the abnormalities in BP and renal sodium handling observed in these diseases [2]. In addition, our group observed an enhanced salt sensitivity in hyperthyroid rats, which evidenced increased BP, oxidative stress and renal injury signs after a high salt intake, whereas hypothyroid rats were resistant to salt-induced BP elevation and renal injury signs [8].

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Several studies have provided convincing evidence that an increased saline intake causes greater oxidative stress [8, 9], and others have reported that oxidative stress activates NF-κB [10], which plays an essential role in the induction of multiple genes involved in the inflammatory response [11]. Thus, an increase in saline intake was found to increase NF-κB levels in deoxicorticosterone-salt rats [12]. High salt intake also increased proinflammatory/profibrotic cytokines, renal interstitial T cells, and macrophage infiltration in the renal cortex of salt-sensitive and salt-resistant male Dahl rats [13]. In contrast, a high salt diet was recently found [14] to attenuate vascular inflammation and atherogenesis in apolipoprotein E knockout mice and to reduce the gene expression of proinflammatory cytokines and adhesion molecules in their aorta in comparison to the mice receiving normal chow. In the same study, a low salt diet resulted in a four-fold increase in plaque accumulation with the increased vascular expression of adhesion molecules and inflammatory cytokines throughout the aorta [14]. In agreement with these observations, a population-based cohort study associated lower sodium excretion with higher cardiovascular disease mortality [15]. These data are consistent with reports showing that cardiovascular and total mortality are inversely associated with salt intake [16-18] and that extreme salt restriction augments the risk of all-cause mortality [19]. More recently, these observations have been supported by the 2013 Institute of Medicine report [20] and several additional papers [21, 22], including the Graudal *et al.* meta-analysis summarizing the findings from 274,683 individuals from 25 studies [23].

With this background, the objective of this study was to examine the influence of changes in salt intake on renal inflammatory variables and the possible modulatory effects of thyroid hormones on the response of these variables to these changes. A further objective was to analyze the possible role of these inflammatory factors in the salt sensitivity of rats with thyroid hormone excess or deficiency.

MATERIALS AND 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 the European Union guidelines for the ethical care of animals and were approved by the ethical committee of the University of Granada. Rats initially weighing 275-300 g were maintained on standard chow and tap water *ad libitum* except where stated. The animals were divided into eight groups (n = 8 in each): euthyroid, hyperthyroid and hypothyroid groups on a diet of normal salt (0.4% NaCl); the same groups on a high salt diet (8% NaCl); and euthyroid and hyperthyroid groups on a low salt diet (0.02% NaCl). In the euthyroid group, the high salt diet was prepared by mixing 76 g of NaCl with 924 g of chow. In the hyper- and hypothyroid groups, the NaCl concentration in the chow was adjusted in accordance with weekly body weight (BW) measurements and daily food intake records in order to deliver the same dosage as in euthyroid rats. Hyperthyroidism was induced by the subcutaneous (s.c.) injection of thyroxine (75 μg/rat/day), while hypothyroidism was induced by continuous administration of 0.03% methimazole *via* drinking water, as previously reported [3, 4, 7]. These treatments were administered for six weeks. Hypothyroid group was not studied under low salt diet, as we previously observed that methimazole-treated rats showed a reduced salt sensitivity [8].

Experimental protocol

When the experimental period was completed, the rats were anesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 units of heparin in isotonic sterile NaCl solution was then inserted into the femoral artery to measure intra-arterial BP 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 24 h after femoral catheter implantation. Direct BP was recorded continuously for 60 min with a sampling frequency of 400/s (McLab, AD Instruments, Hastings, UK). BP 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 plasma urea, creatinine and thyroid hormone (FT_3 and FT_4) levels. Finally, the rats were killed by exsanguination and the kidneys were removed and weighed. The kidneys were then dissected to separate cortex and medulla.

Analytical procedures

Plasma urea and creatinine were measured in an autoanalyzer (Hitachi-912, Roche, Spain). Plasma thyroid hormone levels (free circulating T_3 and T_4) were determined using rat radioimmunoassay kits according to the manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA, USA).

Inflammatory factors

Three recognized pro-inflammatory factors were measured in the renal cortex, which was homogenized in 50 mM Tris-HCl (pH 7.4) containing 1% Triton X-100 and centrifuged for 15 min at 1000 g. The abundance of renal cortex cytokines IL6, TNF-α and VEGF were analyzed by Luminex x-MAP technology using a kit purchased from Millipore (Billerica, MA, USA). Tissue protein was determined using the DC protein assay kit (Bio-Rad, Madrid, Spain).

Statistical analyses

One-way analysis of variance (ANOVA) was used to compare the variables at the end of the experiments. When the overall ANOVA was significant, pairwise comparisons were performed using Bonferroni's method. SPSS-Windows 15.0 (IBM, Chicago, IL) was used for the analyses. $P < 0.05$ was considered significant in all tests.

Linear regression and analysis of variance were used to study the correlation of total urinary sodium excretion ($U_{Na+}V$) with TNF-α, IL-6 and VEGF in the renal cortex at the end of the experiment. $P < 0.05$ and $|r| > 0.5$ was considered as a strong correlation. Statgraphics software was used for these analyses.

RESULTS

Biological variables

Tables 1 and 2 exhibit the values obtained for the variables known to be affected by thyroid excess or deficiency and show the effects of the increased or reduced saline intake. At the end of the six-week study period, BW was significantly lower in hyper- and hypothyroid groups than in euthyroid animals. High salt intake produced an additional decrease in BW in hyper- and hypothyroid rats. Low salt intake produced a tendency to increase BW in euthyroid rats, and a significant increase in hyperthyroid rats. Kidney weight increased in hyperthyroid rats and decreased in hypothyroid rats. High salt intake produced an additional increase in kidney weight in hyperthyroid rats. Low salt diet did not alter kidney-to-BW ratio in either euthyroid or hyperthyroid animals.

In comparison to the euthyroid rats, mean arterial pressure (MAP) values were higher in hyperthyroid rats and lower in hypothyroid rats on a normal salt diet. MAP values did not change with high salt intake in euthyroid and hypothyroid rats but increased in hyperthyroid rats. Low salt intake did not change the BP in euthyroid rats but produced a slight but significant decrease in MAP in the hyperthyroid group. The heart rate increased in hyperthyroid rats and decreased in hypothyroid rats on a normal salt diet. It was not affected by high salt intake in any group but was reduced by low salt intake in euthyroid and hyperthyroid rats.

FT_3 and FT_4 values increased in hyperthyroid rats and decreased in hypothyroid rats on a normal salt diet and reduced with high salt intake in hyperthyroid rats. These values were not affected by low salt intake in euthyroid or hypothyroid rats. Plasma urea levels were similar in all groups except for a significant increase in the hypothyroid group. Plasma creatinine levels reduced in hyperthyroid rats and augmented in hypothyroid rats, but they were not significantly modified by high or low salt intake in any group. All these data confirm the efficacy of the treatments administered.

Renal cortex inflammatory factors

Figure 1 (top) shows that renal cortex TNF-α, IL-6 and VEGF levels decreased in hypothyroid group but were similar in the hyperthyroid and euthyroid groups on a normal sodium diet.

The renal cortex cytokine levels were similar in all the groups on a high salt diet, indicating that thyroid hormone does not modulate inflammatory cytokines when rats receive a high salt intake. Renal cortex TNF-α, IL-6 and VEGF levels are shown in figure 1 (bottom).

Figure 2 shows that TNF-α, IL-6 and VEGF levels increased in euthyroid rats on low salt diet and decreased in those on high salt diet, showing negative correlations between inflammatory variables and total urinary sodium excretion, which is the

Table 1. Morphological and hemodynamic variables in the experimental groups under high, normal and low salt intake.

Groups	FBW (g)	KW (mg)	MAP (mmHg)	HR (Beats/min)
Euthyroid-High salt	363 ± 7.4	1038 ± 35	102 ± 48	352 ± 3.1
Euthyroid-Normal salt	388 ± 11	1028 ± 17	100 ± 0.36	348 ± 2.1
Euthyroid-Low salt	401 ± 2.8	1090 ± 23	99 ± 0.17	327 ± 5*
Hyperthyroid-High salt	283 ± 5.8***,$^{+}$	1265 ± 24***,$^{++}$	135 ± 0.28***,$^{++}$	470 ± 10.0***
Hyperthyroid-Normal salt	302 ± 7.0***	1149 ± 27***	120 ± 0.31***	466 ± 9.0***
Hyperthyroid-Low salt	316 ± 2.7***,$^{+}$	1135 ± 26***	117 ± 0.20***,$^{+}$	442 ± 6***,$^{+}$
Hypothyroid-High salt	253 ± 3.2***,$^{+}$	621 ± 21***	95 ± 42**	302 ± 2.0**
Hypothyroid-Normal salt	265 ± 5.7***	606 ± 17***	96 ± 0.23**	303 ± 1.2**

Data are expressed as mean ± s.e.m; FBW, final body weight; KW, kidney weight; MAP, Mean arterial pressure; HR, heart rate. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ *vs* the control group; +: $p < 0.05$, ++: $p < 0.01$ *vs* their respective saline untreated group.

Table 2. Plasma variables and thyroid hormone levels in the experimental groups under high, normal and low salt intake.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	FT_3 (pg/ml)	FT_4 (ng/dl)
Euthyroid-High salt	42.6 ± 1.4	0.40 ± 0.01	3.49 ± 0.09	2.98 ± 0.11
Euthyroid-Normal salt	44.7 ± 2.2	0.41 ± 0.02	3.21 ± 0.05	2.68 ± 0.13
Euthyroid-Low salt	40 ± 0.53	0.40 ± 0.02	3.66 ± 0.20	3.06 ± 0.24
Hyperthyroid-High salt	40.1 ± 3.1	0.22 ± 0.01***	5.03 ± 0.37**,$^{++}$	4.58 ± 0.58***,$^{++}$
Hyperthroid-Normal salt	41.3 ± 1.5	0.26 ± 0.04**	8.10 ± 0.30***	7.56 ± 0.23***
Hyperthyroid-Low salt	41.9 ± 1.7	0.26 ± 0.01***	7.72 ± 0.47***	6.24 ± 0.30***,$^{++}$
Hypothyroid-High salt	46.7 ± 2.5	0.49 ± 0.02*,$^{+}$	1.47 ± 0.07***	0.081 ± 0.005***
Hypothyroid-Normal salt	52.2 ± 2.1*	0.58 ± 0.01***	1.65 ± 0.11***	0.073 ± 0.003***

Data are expressed as mean ± s.e.m.; FT3, free triiodothyronine; FT4, free thyroxine. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ *vs* the control group; +: $p < 0.05$, ++: $p < 0.01$ *vs* their respective saline untreated group.

most reliable index of salt intake (Figure 3). High salt diet reduced the levels of all renal cortex inflammatory cytokines in hyperthyroid rats. Figure 4 depicts TNF-α, IL-6 and VEGF values.

DISCUSSION

The main findings of the present study were that renal cortex inflammatory cytokines are inversely related to salt intake in euthyroid rats. These variables decreased in hypothyroid rats but did not change in hyperthyroid rats, despite hyperthyroid rats are hypertensive. Moreover, an increase in dietary sodium reduced renal cortex inflammatory cytokine levels in hyperthyroid rats, despite their exacerbated hypertension.

The inverse relationship between renal inflammatory cytokines and salt intake agrees with a previous report [14] that a six-week low salt diet (0.03% NaCl) increases the gene expression of proinflammatory cytokines (IL-6 and TNF-α) and adhesion molecules in the aorta and is associated with a four-fold increase in plaque accumulation in apolipoprotein E knockout mice in comparison to mice receiving normal chow (0.3% NaCl). In contrast, a high salt diet (3% NaCl) was found to attenuate vascular inflammation and atherogenesis. The potential mechanisms underlying the effects of dietary salt on renal cortex inflammatory cytokines have not been elucidated but may involve the activation and inhibition of the renin-angiotensin

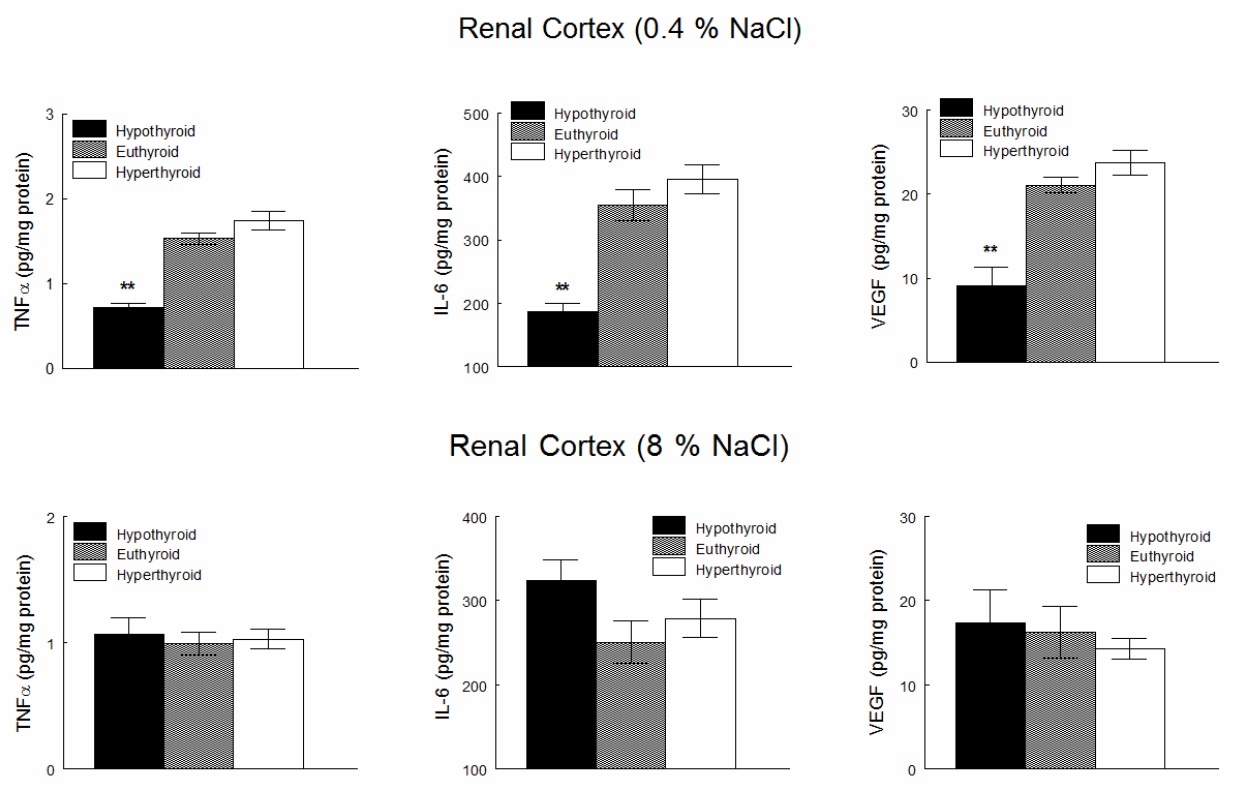

Figure 1. Inflammatory variables in the renal cortex of euthyroid, hyperthyroid and hypothyroid rats after six weeks on a normal salt (0.4% NaCl, top panels) and a high salt (8% NaCl, bottom panels) diet. TNF-α, tumor necrosis factor-α; IL6, interleukin 6; and VEGF, vascular endothelial growth factor. Data are expressed as mean ± s.e.m. **: $p < 0.01$ *versus* euthyroid rats.

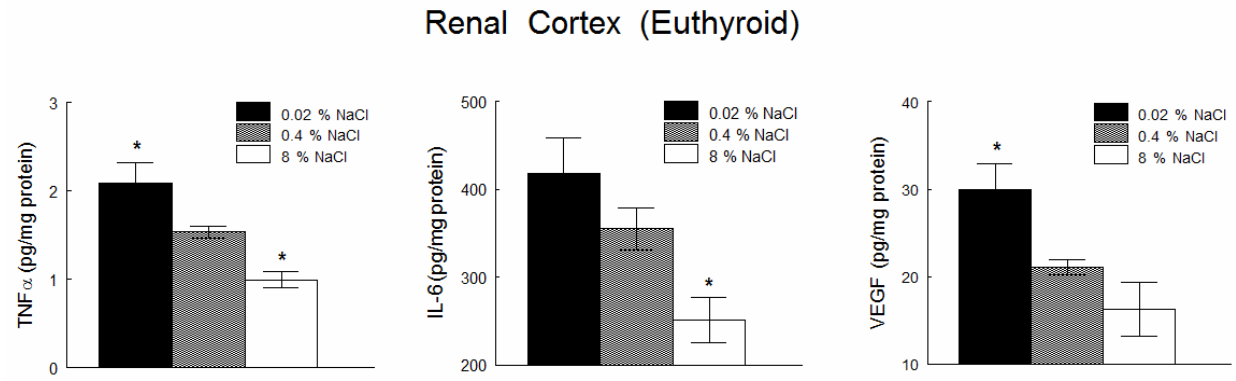

Figure 2. Inflammatory variables in the renal cortex of euthyroid rats after six weeks on a high (8% NaCl), normal (0.4% NaCl) and low (0.02% NaCl) salt diet. TNF-α, tumor necrosis factor-α; IL6, interleukin 6; and VEGF, vascular endothelial growth factor. Data are expressed as mean ± s.e.m. *: $p < 0.05$ *versus* groups on a normal salt diet (0.4% NaCl).

system (RAS) induced by low and high salt intake, respectively. Thus, activation of the AT1 receptor stimulates the production of proinflammatory cytokines and the expression of adhesion molecules, which promote inflammation and tissue damage [13]. In consonance with these data, Tikellis *et al.* [14] reported that the reduced gene expression of proinflammatory cytokines in the aorta is associated

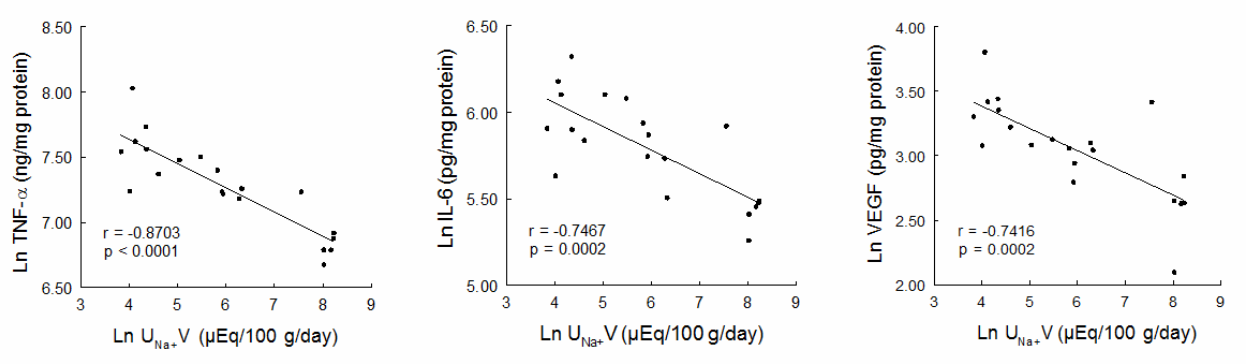

Figure 3. Relationship between inflammatory variables in the renal cortex and total urinary sodium excretion ($U_{Na+}V$) when all data from euthyroid rats on the different sodium diets were pooled in a common regression line.

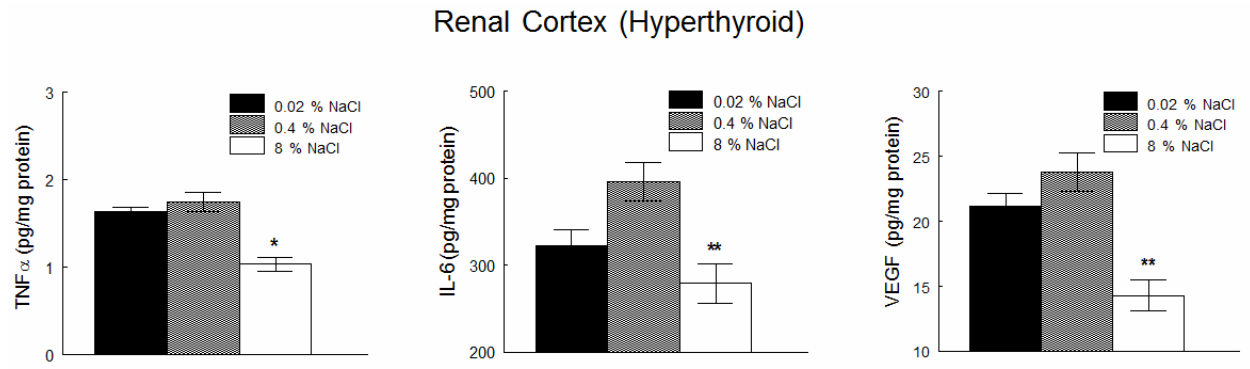

Figure 4. Inflammatory variables in the renal cortex of hyperthyroid rats after six weeks on a high (8% NaCl), normal (0.4% NaCl) and low (0.02% NaCl) salt diet. TNF-α, tumor necrosis factor-α; IL6, interleukin 6; and VEGF, vascular endothelial growth factor. Data are expressed as mean ± s.e.m. *: $p < 0.05$, **: $p < 0.01$ *versus* groups on a normal salt diet (0.4% NaCl).

with RAS suppression, that a low salt diet increases vascular inflammation in this model, which is associated with higher RAS activity, and that this increase can be attenuated by pharmacological RAS blockade.

The reduction in renal cortex proinflammatory cytokines in hypothyroid rats may be related to the beneficial effects of the hypothyroid state on renal diseases [24, 25] and salt sensitivity [8]. Thus, a slower deterioration of renal function was observed in thyroidectomized rats with induced renal insufficiency or ischemia reperfusion [24, 25]. The mechanism underlying this protection is poorly understood but may be caused by alterations in proximal tubular protein reabsorption, prevention of oxidative stress, or a reduction in RAS activity. In this regard, lower levels of immunohistochemically-determined oxidative stress markers were reported in the renal tissue of hypothyroid rats [25]. However, in an earlier study our group observed that urinary isoprostane and H_2O_2 levels were similar in control and hypothyroid rats under baseline conditions. These variables were augmented by high salt intake, reaching similar levels, in both groups [8]. Hence, the reduced levels of inflammatory factors in the hypothyroid rats cannot be explained by a reduction in oxidative stress. Finally, the hypothyroid state reduced the activity of the RAS [26], an important promoter of inflammation [13].

Some researchers have suggested that thyroid hormones can promote the inflammatory process, showing that T_3 at nanomolar concentrations selectively increases IL-6 and IL-8 production by osteoblasts and bone marrow stromal cells [27] and IL-10 production by dendritic cells [28]. However, the present data reveal that none of the cytokines evaluated in the renal cortex are significantly modified in hyperthyroid rats.

Salt sensitivity is a condition in which high salt intake produces a greater BP elevation. The mechanisms underlying salt sensitivity are complex and not fully elucidated, but it has been proposed that they depend on the frequent association of inflammation and oxidative stress in tubulointerstitial areas of the kidney [29]. Thus, the beneficial effects of immunosuppression on salt sensitivity may in part be related to a direct inhibition of the inflammatory response in the kidneys of salt-sensitive models [30, 31]. Salt sensitivity is associated with upregulation of the intrarenal angiotensin system. A high salt diet produces angiotensin-II-positive cells [13] and initiates AT_1 receptor activation, resulting in increased ROS production and oxidative stress *via* NAD(P)H oxidase in the kidney and cardiovascular tissues [32, 33]. Consistent with these reports, our group recently defined hyperthyroid hypertension in rats as a salt-sensitive model [8] that manifests increased BP, cardiac hypertrophy, oxidative stress (augmented urinary isoprostanes and H_2O_2) and renal injury signs in response to high salt intake. However, the fact that the high salt diet decreased rather than increased the renal abundance of inflammatory variables in salt-sensitive hyperthyroid rats contrast with the above-reported findings. This discrepancy is difficult to explain, but the decrease in inflammatory cytokines observed in the present study may be mediated by a high salt intake-induced inhibition of the proinflammatory RAS, consistent with the aforementioned findings by Tikellis *et al.* [14] in the aortic tissue.

Liu *et al.* [34] demonstrated that a high salt intake significantly increases plasma VEGF levels in salt-sensitive subjects and proposed this cytokine as a biomarker of salt sensitivity. In the present study, however, high salt intake did not significantly modify renal cortex VEGF levels in the euthyroid or hypothyroid groups and reduced these levels in the hyperthyroid group.

There is accumulating evidence of tubulointerstitial inflammation and a loss or rarefaction of peritubular capillaries in salt-sensitive models of hypertension [35, 36], and these changes have been proposed to contribute to the blunted pressure-natriuresis response [29. 31]. However, hyperthyroidism was not accompanied by increased levels of renal inflammatory mediators in the present study, and our group recently observed augmented capillarity rather than rarefaction in the renal cortex and medulla of salt-sensitive [8] hyperthyroid rats [37]. These findings rule out the implication of capillary loss and inflammation as factors implied in the attenuated pressure-natriuresis of these animals, in which the RAS [38] and an augmented oxidative stress [39] participate.

CONCLUSION

The following conclusions can be drawn from our findings: (i) there is an inverse relationship between renal cortical inflammatory cytokines and salt intake in euthyroid rats; (ii) the hyperthyroid state, which produces a salt-sensitive model of hypertension with increased oxidative stress, is not accompanied by an increase in the levels of renal inflammatory cytokines, which are even decreased when these rats are on a high salt diet; (iii) the modulatory role of thyroid hormone on renal inflammatory cytokines is lost when the rats are subjected to a high salt intake and (iv) the hypothyroid state is associated with a decrease in the levels of renal tissue inflammatory cytokines and this might contribute to the protective effects of hypothyroidism against renal injury.

ACKNOWLEDGEMENTS

The authors thank Richard Davies for help with the English version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. Therefore no competing financial interests exist.

FUNDING

This study was supported by a grant (CTS-6704) from Junta de Andalucía and from the Carlos III Health Institute of the Spanish Ministry of Health and Consumer Affairs (Red de Investigación Renal, REDinREN RD012/0021). “FEDER una manera de hacer Europa”.

REFERENCES

1. Bradley, S. E., Stephan, F., Coelho, J. B. and Reville, P. 1974, Kidney Int., 6, 346-365.
2. Vargas, F., Moreno, J. M., Rodríguez-Gómez, I., Wangensteen, R., Álvarez-Guerra, M., Osuna, A. and García-Estañ, J. 2006, Eur. J. Endocrinol., 154, 197-212 .

3. Vargas, F., Baz, M. J., Luna, J. D., Andrade, J., Jódar, E. and Haro, J. M. 1991, Clin. Sci., 81, 471-476.
4. Vargas, F., Atucha, N., Sabio, J. M., Quesada, T. and García-Estañ, J. 1994, Clin. Sci., 87, 323-328.
5. Holmes, E. W. and DiScala, V. 1970, J. Clin. Invest., 49, 1224-1236.
6. Michael, U. F., Barenberg, R. L., Chavez, R., Vaamonde, C. A. and Paper, S. 1972, J. Clin. Invest., 51, 1405-1412.
7. Moreno, J. M., Pérez-Abud, R., Wangensteen, R., Rodríguez Gómez, I., López Merino, I., Osuna, A. and Vargas, F. 2012, J. Endocrinol. Invest., 35, 735-741.
8. Pérez-Abud, R., Rodríguez-Gómez, I., Villarejo, A. B., Moreno, J. M., Wangensteen, R., Tassi, M., O´Valle, F., Osuna, A. and Vargas, F. 2011, Am. J. Physiol. Endocrinol. Metab., 301, E281-E287.
9. Cruz, A., Rodríguez-Gómez, I., Pérez-Abud, R., Vargas, M. A., Wangensteen, R., Quesada, A., Osuna, A. and Moreno, J. M. 2011, J. Biomed. Biotechnol., doi:10.1155/ 2011/469481.
10. Bowie, A. and O'Neill, L. A. J. 2000, Biochem. Pharmacol., 59, 13-23.
11. Barnes, P. J. and Karin, M. 1997, N. Engl. J. Med., 336, 1066-1071.
12. Beswick, R. A., Zhang, H., Marable, D., Catravas, J. D., Hill, W. D. and Webb, R. C. 2001, Hypertension, 37, 781-786.
13. Chandramohan, G., Bai, Y., Norris, K., Rodriguez-Iturbe, B. and Vaziri, N. D. 2008, Am. J. Nephrol., 28, 158-167.
14. Tikellis, C., Pickering, R. J., Tsorotes, D., Huet, O., Chin-Dusting, J., Cooper, M. E. and Thomas, M. C. 2012, Hypertension, 60, 98-105.
15. Stolarz-Skrzypek, K., Kuznetsova, T., Thijs, L., Tikhonoff, V., Seidlerová, J., Richard, T., Jin, Y., Olszanecka, A., Malyutina, S., Casiglia, E., Filipovský, J., Kawecka-Jaszcz, K., Nikitin, Y. and Staessen, J. A. 2011, JAMA., 305, 1777-1785.
16. Alderman, M. H., Cohen, H. and Madhavan, S. 1998, Lancet, 351, 781-785.
17. Cohen, H. W., Hailpern, S. M., Fang, J. and Alderman, M. H. 2006, Am. J. Med., 119, 275. e7-14.
18. Cohen, H. W., Hailpern, S. M. and Alderman, M. H. 2008, J. Gen. Intern. Med., 23, 1297-1302.
19. Taylor, R. S., Ashton, K. E., Moxham, F., Hooper, L. and Ebrahim, S. 2011, Am. J. Hypertens., 24, 843-853.
20. Graudal, N. 2015, Curr. Hypertens. Rep., 17, 9.
21. Katz, L. A. 2015, Am. J. Hypertens., 28, 952.
22. Alderman, M. H. 2016, Am. J. Hypertens., 29, 424-427.
23. Graudal, N., Jürgens, G., Baslund, B. and Alderman, M. H. 2014, Am. J. Hypertens., 27, 1129-1137.
24. Conger, J. D., Falk, S. A. and Gillum, D. M. 1989, Am. J. Kidney Dis., 13, 217-225.
25. Tenorio-Velázquez, V. M., Barrera, D., Franco, M., Tapia, E., Hernández-Pando, R., Medina-Campos, O. N. and Pedraza-Chaverri, J. 2005, BMC Nephrol., 6, 12.
26. Vargas, F., Rodríguez-Gómez, I., Vargas-Tendero, P., Jiménez, E. and Montiel, M. 2012, J. Endocrinol., 213, 25-36.
27. Siddiqi, A., Burrin, J. M., Wood, D. F. and Monson, J. P. 1998, J. Endocrinol., 157, 453-461.
28. Mascanfroni, I., del Mar Montesinos, M., Susperreguy, S., Cervi, L., Ilarregui, J. M., Ramseyer, V. D., Masini-Repiso, A. M., Targovnik, H. M., Rabinovich, G. A. and Pellizas, C. G. 2008, FASEB J., 22, 1032-1042.
29. Vaziri, N. D. and Rodríguez-Iturbe, B. 2006, Nat. Clin. Pract. Nephrol., 2, 582-593.
30. Rodríguez-Iturbe, B. and Johnson, R. J. 2010, Hypertens Res., 33, 975-980.
31. Rodríguez-Iturbe, B., Franco, M., Tapia, E., Quiroz, Y. and Johnson, R. J. 2012, Clin. Exp. Pharmacol. Physiol., 39, 96-103.
32. Landmesser, U., Cai, H., Dikalov, S., McCann, L., Hwang, J., Jo, H., Holland, S. M. and Harrison, D. G. 2002, Hypertension, 40, 511-515.
33. Rajagopalan, S., Kurx, S., Munzel, T., Freemann, B. A., Griendling, K. K. and Harrison, D. G. 1996, J. Clin. Invest., 95, 588-593.
34. Liu, F., Mu, J., Yuan, Z., Lian, Q., Zheng, S., Wu, G. and Liu, E. 2011, Med. Sci. Monit., 17, CR542-CR546.
35. Andoh, T. F., Johnson, R. J., Lam, T. and Bennet, W. M. 2001, Am. J. Transplant., 1, 222-227.

36. Ray, P. E., Suga, S., Liu, X. H., Huang, X. and Johnson, R. J. 2001, Kidney Int., 59, 1850-1858.
37. Rodríguez-Gómez, I., Banegas, I., Wangensteen, R., Quesada, A., Jiménez, R., Gómez-Morales, M., O'Valle, F., Duarte, J. and Vargas, F. 2012, J. Endocrinol., 216, 1-10.
38. García-Estañ, J., Atucha, N., Quesada, T. and Vargas, F. 1995, Am. J. Physiol., 268, E897-E901.
39. Moreno, J. M., Rodríguez-Gómez, I., Wangensteen, R., Álvarez-Guerra, M., Luna, J. D., García-Estañ, J. and Vargas, F. 2008, Am. J. Physiol. Reg. Integr. Comp. Physiol., 294, R867-R873.