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# Role of Thyroid Hormones and mir-208 in Myocardial Remodeling in 5/6 Nephrectomized Rats

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*Background and Aims.* Thyroid hormones exert important effects on heart remodeling through mir-208. The process may have a role in myocardial changes in chronic kidney disease where thyroid abnormalities are common. In this study the effect of $T_4$ supplementation on left ventricle (LV) remodeling in 5/6 nephrectomized rats (5/6Nx) was analyzed.

*Methods.* 5/6Nx rats and 5/6Nx under $T_4$ supplementation (5/6Nx + $T_4$) were compared with control (C) and thyroidectomized (Tx) rats. After 8 weeks of follow-up, LV was analyzed for α-MHC, β-MHC, TGF-β, and mir-208 expression, hydroxyproline content, and myocardial fibrosis. Serum collagenase activity was also analyzed.

*Results.* Heart weight increased in 5/6Nx rats compared to C, which was prevented with $T_4$ supplementation (C, 1.5 ± 0.04; 5/6Nx, 1.8 ± 0.09; 5/6Nx + $T_4$, 1.6 ± 0.07 g, $p < 0.05$). The same pattern was seen for LV wall thickness, hydroxyproline content, LV fibrosis, and mRNA TGF-β expression (C, 0.47 ± 0.17; 5/6Nx, 10.55 ± 3.4; 5/6Nx + $T_4$, 3.01 ± 0.52, $p < 0.01$). Tx rats had reduction in heart weight, increased LV wall thickness, and fibrosis. Collagenase activity did not change in any group. mRNA expression of α-, β-MHC, and TGF-β increased in 5/6Nx in comparison to C and 5/6Nx + $T_4$. Expression of mir-208 decreased in 5/6Nx groups, and levels were restored with $T_4$ supplementation (4.21 ± 0.28, 3.39 ± 0.29, and 4.26 ± 0.37 RU, respectively, $p < 0.01$).

*Conclusions.* Decreased plasma level of thyroid hormones or sensitivity at tissue level observed in chronic kidney disease induced by 5/6Nx has an important effect in heart remodeling processes, some of it related or mediated by mir-208 and TGF-β expression in the heart. 

*Key Words:* Chronic kidney disease, Myocardial remodeling, mir-208, Heart failure, Heart fibrosis, Thyroid hormone.

## Introduction

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in patients with chronic kidney disease (CKD), particularly in patients in chronic dialysis (1–4). Heart failure is one of the most frequent forms of heart disease in this population; fluid and pressure overload are among the mechanisms underlying this phenomenon. Functional changes are associated with abnormal remodeling with heart enlargement and chamber dilatation, particularly of the left ventricle (LV) where cardiomyocyte hypertrophy and apoptosis, as well as interstitial fibrosis, occur.

Decreased expression of α-myosin heavy chain (α-MHC), overexpression of β-myosin heavy chain (β-MHC), and

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other proteins mainly expressed during fetal life are biochemical manifestations of myocardial remodeling. Myocardial fibrosis is of clinical interest because it contributes to diastolic dysfunction, one of the early alterations found in CKD patients (5). Myocardial fibrosis results from the imbalance between the synthesis and degradation of collagen molecules (6−8). Genetic factors, cytokines, and hormones can modify hypertrophy and fibrosis. Among these, one not-well-understood factor is the reduction in thyroid hormones, which seems to be part of this complex mechanism (9,10).

Low or low-normal plasma levels of triiodothyronine ($T_3$) and thyroxine ($T_4$) with normal thyroid stimulating hormone (TSH) is the hormonal pattern commonly seen in CKD patients (9,11). In some studies it has been reported that low levels of $T_3$ are inversely associated with mortality rates, both in hemodialysis and peritoneal dialysis patients, but the nature of the association is unclear (12−15); heart abnormalities are a possible explanation. Thyroid hormones are linked with the process of hypertrophy as well as fibrosis in the heart in several ways (10). Experimental and clinical studies have shown that thyroid hormones regulate expression of proteins associated with hypertrophy such as α-, and β-MHC and also prevent collagen deposit and/or increase collagen removal (16−20).

In the past few years, a growing number of reports have emerged concerning the post-transcriptional regulation of different proteins in various biological processes. MicroRNAs have a central role in this regulation. One of them, microRNA-208 (mir-208), is selectively expressed in myocardial tissue and is involved in the control of heart remodeling because it regulates the expression of β-MHC and myocardial fibrosis in response to various stimuli (21,22). Thyroid hormones are at the same time one of the most important factors regulating the expression of mir-208 at the pre-transcriptional level. In spite of their potential as regulators of myocardial remodeling, thyroid abnormalities have not been sufficiently studied in terms of myocardial changes in CKD patients or experimental models of uremia.

The aim of the present study was to analyze the effect of thyroxin supplementation on expression of mir-208 as well as of hypertrophy-related proteins and mechanisms of fibrosis in the myocardium of rats with induced CKD.

## Materials and Methods

### *Animals*

Male Sprague Dawley rats weighing 250−300 g were studied. Rats were allowed free access to standard chow (5008 Purina chow, Purina SA, Mexico) and tap water and were housed under controlled humidity and temperature with a 12-h light-dark cycle.

### *Experimental Design and Procedures*

Four groups of animals with at least eight rats each were formed. Group C, sham-operated rats, served as controls: Group 5/6Nx, rats with chronic kidney disease induced by 5/6 nephrectomy; Group 5/6Nx + $T_4$, 5/6Nx rats supplemented with L-thyroxine; Group Tx, thyroidectomized rats.

5/6Nx was performed as previously reported (23). In group 5/6Nx + $T_4$, thyroxin ($T_4$) (8 μg/kg/day) (Sigma Chemical Co., St. Louis, MO) was administered intraperitoneally. Hypothyroidism was surgically induced in animals of Tx group. Rats were anesthetized with xylazine-ketamine and the thyroid gland was dissected and excised. Parathyroid glands were dissected and implanted into the sternocleidomastoid muscles.

Rats were followed for 8 weeks after the last surgery. Blood pressure was measured weekly by a non-invasive method in the tail (CODA 2 system model; Kent Scientific Corporation, Torrington, CT). At the end of follow-up, rats were weighed and sacrificed using pentobarbital. Blood samples were taken, plasma was separated and kept frozen at −20°C until biochemical analysis, and the heart was removed and weighed. Left ventricle (LV) samples were prepared and stored in 10% formaldehyde and in physiological solution until assayed.

### *Methods of Analysis*

Serum samples were assayed for creatinine by standard methods in a clinical chemistry analyzer (Syncron CX5, Beckman, Fullerton, CA), and plasma assayed for $T_3$ and $T_4$ by ELISA with commercial kits (Milliplex Cat RTHY-30K, Billerica, MA).

### *Histology*

LV fragments fixed in 10% formaldehyde were embedded in paraffin, cut in 4-μm-thick slices and stained using Masson's trichromic method (24). Histological analysis was done using an Olympus BX51 microscope (Olympus American, Melville, NY) at different enlargement degrees and images digitalized and recorded with a VR Evolution half cybernetic digital camera (Madison, WI). Image analysis was done by using a color imaging Image-Pro Plus software v.5.1. Results are expressed as average of pixels for areas of fibrosis (stained blue with Masson trichrome) with the selected color in useful areas that were digitized at 10X recorded in 50 fields.

### *Immunohistochemistry*

Expression of transforming growth factor beta (TGF-β) in LV samples was carried out by conventional technique immunoperoxidase assay (ABC) using an anti-TGF-β antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Immunostained intensity for TGF-β was measured using color analysis capability of imaging software, positivity