Hypertrophic response of the Association of Thyroid Hormone and Exercise in the Heart of Rats

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Abstract

Background: **Cardiac hypertrophy is a component of cardiac remodeling occurring in response to an increase of the activity or functional overload of the heart.**

Objective: **Assess hypertrophic response of the association of thyroid hormone and exercise in the rat heart.**

Methods: **We used 37 Wistar rats, male, adults were randomly divided into four groups: control, hormone (TH), exercise (E), thyroid hormone and exercise (H + E); the group received daily hormone levothyroxine sodium by gavage at a dose of 20 μg thyroid hormone/100g body weight, the exercise group took swimming five times a week, with additional weight corresponding to 20% of body weight for six weeks; in group H + E were applied simultaneously TH treatment groups and E. The statistics used was analysis of variance, where appropriate, by Tukey test and Pearson correlation test.**

Results: **The T4 was greater in groups TH and H + E. The total weight of the heart was greater in patients who received thyroid hormone and left ventricular weight was greater in the TH group. The transverse diameter of cardiomyocytes increased in groups TH, E and H + E. The percentage of collagen was greater in groups E and H + E Correlation analysis between variables showed distinct responses.**

Conclusion: **The association of thyroid hormone with high-intensity exercise produced cardiac hypertrophy, and generated a standard hypertrophy not directly correlated to the degree of fibrosis. (Arq Bras Cardiol. 2014; 102(2):187-191)**

Keywords: **Thyroid Hormones; Exercise; Rats; Heart; Hyperthyroidism.**

Introduction

The thyroid hormone and physical exercise produce general elevation of basal metabolism and as a consequence, there occurs a greater oxygen consumption by the tissues. This increased demand is supplied, in part, by elevation of cardiac frequency, blood pressure, both systolic and diastolic, and cardiac output[1].

Clinical and experimental trials suggest that cardiac adaptations are the result of direct effects of the hormone on the heart and blood vessels, and indirectly result from serum elevation of cathecolamines[2].

Cardiac hypertrophy is a component of cardiac remodeling involving changes in the geometric structure, the biochemical composition, the generation and electric conduction, the volume of muscle cells in the organization of collagen matrix and blood vessels. It results from changes of genetic, humoral and molecular nature that can arise spontaneously or be induced by stressing mechanisms of different types and various forms of action[3].

Physical training, within reasonable limits, induces cardiac hypertrophy of the physiological type, producing adaptations that improve the performance of the cardiovascular system allowing the heart to withstand increases in demands during exercise. Cardiac hypertrophy is accompanied by increase in ventricular filling time and ejection fraction of the left ventricle, with consequent reduction of the heart rate at rest[4,5].

The main differences that determine the pattern of cardiac hypertrophy are the type and duration of stimulation that the heart receives. In situations in which athletes receive pressure overloads only during physical activity, cardiac hypertrophy is usually physiological. In contrast, in pathological situations the heart is continuously exposed to functional overload that occurs for a long time, determining pathological hypertrophy[6].

The excess thyroid hormone can promote these two patterns of hypertrophy described above (mixed cardiac hypertrophy). This response appears to be due, in part, the volume of overload due to increased venous return produced by the TH and also the direct effects of hormônio[7,8].

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Objective

Evaluate the hypertrophic response of the association of the thyroid hormone and exercise in the heart of rats.

Method

The procedures were performed after approval of the study protocol by the Ethics Committee on animal use of the institution under the numbers 077/10. The study conducted was of the experimental type.

We used 37 adult Wistar rats, male, weighing approximately 250 grams, derived from the laboratory of animal experiments (CEBEA-UFU). The environmental conditions for all groups were similar with regard to temperature, relative humidity of the air, level of noise and brightness in accordance with the circadian rhythm. The rats were fed with ration and water *"ad libitum"*.

The rats were weighed and randomly divided into four groups identified as: control (C) 8 animals, hormone (TH) 10 animals, exercise (E) 9 animals, hormone and exercise (H+E) 10 animals; after distributed, the animals underwent an adjustment period of 15 days in the laboratory before the experiment started.

The type of exercise used was of an anaerobic character. For training, we used a glass with 250 mm in diameter[9]. The water column height in the glass corresponded to 150% of the rat's body length. The water temperature was maintained between 30°C and 32°C, which is considered thermally neutral in relation to body temperature of the animal[10]. The load used in training was that corresponding to 20% of body weight of the rate because this is considered to be an overload that increases the concentration of lactate[11]. The referred work load consisted of lead blocks fixed to a vest in the region before the rats' trunk. The load was adjusted weekly according to the variation in the weight of the animals.

The training period was started after 7 days of adaptation to the water mean. Training was conducted in 6 weeks and consisted of five weekly sessions of swimming limited by exhaustion.

The hormone was administered by means of oro-gastric probing performed once a day for 6 weeks. The dose of thyroid hormone was 20 μg/100 g of body weight of a suspension of T4 at 0.1% which was obtained from 10 mg tablets of 100 μg of T4 diluted in 10 ml of distilled water[12].

After the 6 weeks of the experiment, the rats were sacrificed under anesthesia, proceeding to open the chest for blood collection by direct cardiac puncture and removal of the heart. Confirmation of exposure to high levels of thyroid hormone was performed by serum dosage of T3 and T4 by the ELISA method.

The heart was weighed and preserved in formaldehyde; this process lasted 24 hours. After the use of formaldehyde, the atria were removed and separated from the ventricles for weighing; the material was then forwarded for histological processing.

To analyze the transverse diameters of cardiomyocytes, slides were stained with eosin/ hematoxylin and to quantify collagen, the slides were stained with picrosirius. We obtained five sections of each ventricle and measured the smallest diameters of five cells (with visible core) in five different fields. For measures, we made analyses on digital images captured randomly by an Olympus BX40 binocular microscope with a 40× objective. To measure the values of the diameters of cardiomyocytes, we used software HL Image (Western Vision).

The quantification of collagen was made by suppression technique of pixels, where three sections were obtained from each ventricle and selected five different fields of each histological section. It was suggested that the area located in the middle portion between the visceral endocardium and epicardium. The measurement was performed by a single observer who was unaware of which group belonged to the slide examined.

To compare the level of hormone, total weight of the heart, left ventricular weight, cardiomyocyte transversal diameter and percent of collagen between the groups after treatment, was carried out using complemented variance analysis (ANOVA) where necessary by Tukey's test. To analyze the existence or not of correlation between total weight of the heart, left ventricular weight, cardiomyocyte transversal diameter and percentage of collagen, we used Pearson's correlation test.

Results

The serum T4 was greater in groups TH and H+E, compared to groups C and E. The values of T3 did not differ among the groups. The results found are shown in Table 1.

The results relative to the total weight of the heart (Pcor), left ventricular weight (LVW), cardiomyocyte transversal diameter (CTD) and the percentage of collagen are shown in Table 2.

PCor was greater in groups TH and HE, compared to groups C and E. LVW was greater in group TH in comparison with E. CDT of the left ventricle was greater in groups TH, E and H+E in comparison to C, being observed the highest values in group TH.

Correlation analysis between variables PCor, LVW, CDT and percentage of collagen of the groups showed different responses. The variables in group C showed positive correlation between LVW and PCor ($r = 0.74$; $p < 0.05$). The other comparisons did not prove significant.

Applying the same analyses to group TH, we found a positive correlation only between CDT and PCor ($r = 0.70$; $p < 0.05$).

In group E there was a positive correlation between LVW and PCor ($r = 0.67$, $p < 0.05$) and between the percentage of collagen and LVW ($r = 0.79$, $p < 0.05$). There was also a negative correlation between CDT and LVW ($r = 0.62$; $p < 0.05$) and between the percentage of collagen and CDT ($r = 0.67$; $p < 0.05$).

In the H+E group was a positive correlation between LVW and PCor ($r = 0.80$, $p < 0.05$) and between CDT and LVW ($r = 0.71$, $p < 0.05$).

Table 1 - Mean and standard deviation of serum concentration of T3 and T4 after six weeks of intervention

Hormone Level	Groups			
	CONTROL	HORMONE	EXERCISE	H + E
T3(µg/ml)	2.67 ± 1.31	2.91 ± 0.99	2.45 ± 0.9	2.89 ± 1.89
T4(µg/dl)	9.08 ± 0.58	13.76 ± 0.56*	9,81 ± 0.36	12.47 ± 0.29*

**p < 0,05 (ANOVA-Tukey)*

Table 2 - Mean and standard deviation of the heart weight, left ventricular weight and transversal diameter of cardiomyocites after six weeks of intervention

VARIABLE ANALYZED	Groups			
	CONTROL	HORMONE	EXERCISE	H+E
Heart weight(g)	1.64 ± 0.18	1.80 ± 0.1*	1.56 ± 0.11	1.88 ± 0.17*
LV weight(g)	0.71 ± 0.07	0.79 ± 0.06*	0.70 ± 0.06	0.77 ± 0.07
CDT (µm)	11.6 ± 0.63	13.5 ± 0.77*†	12.7 ± 0.54*	12.2 ± 0.63*
Collagen (%)	1.38 ± 0.28	1.16 ± 0.24	1.51 ± 0.33*	2.12 ± 0.43*

**p < 0,05 (ANOVA-Tukey)*

Discussion

Several experimental models have been used in the study involving cardiac remodeling. This experiment was conducted in order to check aspects obtained in the interaction between stimulation produced by the thyroid hormone and by the anaerobic exercise in cardiac remodeling.

Exposure of animals to increased concentrations of thyroid hormone was obtained by detecting levels of T4 significantly greater at the end of the trial period in the groups receiving levothyroxine sodium (51% group HT and 37% group H+E) compared to group C. The values of T3 did not differ from the post-treatment groups.

The behavior of the total weight of the heart, left ventricular weight, transverse diameter and percentage of collagen in groups C, HT and E corroborate the results found in the literature[13-15].

Detailed analysis of the results obtained of the hypertrophy obtained in the group that associated physical exercise and thyroid hormone, draws the attention on two issues. The first of them concerns the model of hypertrophy and the second refers to the absence of added hypertrophic effect. As already mentioned, physiological hypertrophy maintains existing relations between the muscle fibers, the collagen structure and myocardial vascularization. This opinion seems to be the pattern mostly found in cardiac hypertrophy derived from exercises and excess thyroid hormone[16].

A few pathways, which, activated, produce physiological cardiac hypertrophy, are involved in the cardiovascular actions of exercise and thyroid hormone. One of the is that which involves the growth factor similar to insulin (IGF-1) which binds to its receptor of tyrosine kinase (IGF-1R) activating and autophosphorylating tyrosine residues that promote recruitment and activation of a lipid kinase PI3K (phosphatidylinositol-3-kinase). These processes start the activation of intracellular pathways that produce increased protein synthesis and consequent cardiac hypertrophy[17,18].

To explain the lack of added hypertrophic effect in the H+E group, it can be suggested that both physical exercise as thyroid hormone have induced hypertrophy using the same inducing pathway, that is, that of PI3K-AKT-mTOR a pathway which is closely related to physiological cardiac hypertrophy[19]. The experimental model used in this study induced hyperthyroidism by an approximate period of 4.4% of the animal's expected life (34 months) and as the activation of the pathway of PI3K-AKT-mTOR takes place in a short period of time, this hypothesis becomes quite plausible. An alternative to this explanation could be that the sum of the stimuli to cardiac hypertrophy has produced a depletion of hypertrophic mechanisms and thus possibly even acting through different pathways, they had reached a plateau of response preventing further growth in the cardiac muscle in the H+E group.

Analyzing the correlations between the studied variables, it was found that exposure to thyroid hormone produced an increase in heart weight in a direct proportion to the increase in the transverse diameter of cardiomyocytes. This correlation found is positive and strong. This fact seems to be directly linked to the failure to detect accumulation of collagen in the hearts of the HT group indicating that the increase in heart weight is dependent on the increase in the transverse diameter of cardiomyocytes. In the literature consulted, we did not find any reference to this pattern of hypertrophy.

The same analysis applied to group E, identified that there was strong and positive correlation between heart weight and left ventricular weight and between collagen and

left ventricular weight. There was also a negative correlation between the transverse diameter of cardiomyocytes and left ventricular weight and between collagen and transverse diameter of cardiomyocytes.

The detected correlation between the weight of the heart and left ventricular weight restores the pattern obtained in group C and indicates that the increased collagen may be directly implicated in increased left ventricular weight. At the same time, the negative correlation between the transverse diameter of cardiomyocytes and left ventricular weight and the percentage of collagen and the transverse diameter of cardiomyocytes reinforce this hypothesis and seem to suggest that the participation of cardiomyocytes, although showing a transverse diameter of cardiomyocytes greater than that of group C, has a less important role on cardiac hypertrophy.

The association of exercise to the hormone maintained the positive correlation between left ventricular weight and heart weight and between the transverse diameter of cardiomyocytes and left ventricular weight, although no correlation has been found involving collagen. This result is interesting because the sum of the effects appears to reduce the accumulation of collagen, a fact which may have clinical relevance and needs to be further studied in future experimental models.

Conclusion

The association of thyroid hormone with high-intensity exercise produced cardiac hypertrophy characterized by increased heart weight and transverse diameter of cardiomyocytes without concomitant increase of the left ventricle.

This association led to a hypertrophic pattern not directly correlated to the degree of fibrosis.

Author contributions

Conception and design of the research and Critical revision of the manuscript for intellectual content: Souza FR, Resende ES; Acquisition of data, Statistical analysis and Obtaining funding: Souza FR; Analysis and interpretation of the data: Souza FR, Lopes L, Gonçalves A; Writing of the manuscript: Souza FR, Chagas R, Fidale T, Rodrigues P.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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