Short-Term Thyroid Hormone Excess Affects the Heart but Does not Affect Adrenal Activity in Rats

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Abstract

Background: Hyperthyroidism (Hy) exerts a broad range of influences on a variety of physiological parameters. Its disruptive effect on cardiovascular system is one of its most remarkable impacts. Moreover, Hy has been clinically associated with stress – induced hyperactivation of the hypothalamic-pituitary-adrenal axis.

Objective: Evaluate the impact of short-term Hy on cardiac performance and adrenal activity of rats.

Methods: Induction of Hy in Wistar rats through injections of T3 (150 μg/kg) for 10 days (hyperthyroid group - HG) or vehicle (control group). The cardiovascular performance was evaluated by: echocardiography (ECHO); heart weight/body weight (mg/gr) ratio; contractility of isolated papillary muscles (IPM) and direct measurement of blood pressures. Adrenal activity was evaluated by adrenal weight/body weight (mg/gr) ratio and 24-hour fecal corticosterone (FC) levels on the, 5th and 10th days of T3 treatment.

Results: In HG, the ECHO showed reduction of the End Systolic and End Diastolic Volumes, Ejection, Total Diastolic and Isovolumic Relaxation Times, Diastolic and Systolic Areas and E/A ratio. Heart Rate, Ejection Fraction and Cardiac Output increased. The heart weight/body weight ratio was higher. Similarly, in IPM, the maximum rate of force decay during relaxation was higher in all extracellular calcium concentrations. Systolic blood pressure (SBP) levels were higher. ($p \leq 0.05$). On the other hand, there was no difference in the adrenal weight/body weight ratio or in the 24-hour FC levels.

Conclusions: Hy induces positive inotropic, chronotropic and lusitropic effects on the heart by direct effects of T3 and increases SBP. Those alterations are not correlated with changes in the adrenal activity. (Arq Bras Cardiol. 2014; 102(3):270-278)

Keywords: Thyroid Hormones; Hyperthyroidism / complications; Adrenal Glands; Rats; Cardiovascular Diseases.

Introduction

Thyroid hormones (TH) induce marked changes in the functioning of many physiological systems, including the cardiovascular and stress systems. Indeed, most studies have focused on the abnormalities of cardiac and blood vessel function in the presence of hypo and hyperthyroidism (Hy). In general, TH increase basal metabolism and oxygen consumption acting directly on the heart and blood vessels[1-4]. Clinical and experimental evaluation are often based on the demonstration of an increase in myocardial contractility and heart rate (HR) on the one side and reduced peripheral vascular total resistance (PVTR) followed by a decrease in cardiac efficiency in the long run on the other side. Molecular bases have revealed this phenomenon, showing regulation of genes related to excitation-contraction coupling (ECC) of the heart, known to be associated with increased positive inotropic, chronotropic and lusitropic effects. Furthermore, TH excess has also been clinically associated with increased activity of the hypothalamic-pituitary-adrenal axis (HPAA). The reason is that hyperthyroid patients need to receive cortisol, indicating a diminished adrenal reserve[5]. Although TH seems to interact with the HPAA, only few studies have addressed whether the association in humans and animals is true[5-9]. Moreover, there is lack of knowledge on the role of TH and the effects of Hy on corticosterone secretion. The effects of Hy *in vivo* (Echocardiography - ECHO) and *in vitro* (Isolated Papillary Muscles – IPM) on the systolic and diastolic functions and the adrenocortical function have never been confirmed in any study. Additionally, we measured corticosterone in feces (concentration of Fecal Corticosterone - FC), allowing the monitoring of corticosterone concentrations by a feedback-free technique.

Using an animal model of Hy, in which cardiac alterations could be confirmed and the stress status was unaltered by manipulations or invasive techniques, we aimed to make a comprehensive analysis of the cardiac performance, both *"in vivo"* and *"in vitro"*, as well as to verify the impact of short-term Hy on adrenocortical function.

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Methods

Animals

Two-month-old male Wistar rats, weighing about 250 grams were randomly separated into two groups: control (CG, n = 12) and hyperthyroid (HG, n= 12).

Rats from the HG were injected (*i.p.*) with T3 (150 μg/kg) (Sigma Aldrich Chemicals, St. Louis, MO)[10] daily for 10 days. The CG received daily *i.p.* injections of vehicle. The rats were kept in individual cages and under a 12-hour dark/light cycle. The Animal Experimentation Ethics Committee of the Biological Sciences Section at Federal University of Paraná approved all experimental protocols used in this study.

Basal hormonal levels

We measured the concentration levels of T4 and T3 in both groups of animals using a solid-phase, two-site chemiluminescent immunometric assay performed by the Immulite 2000 analyzer.

Echocardiography

ECHO was performed in anesthetized animals (50 mg/kg of ketamine – Fort Dodge; Iowa, USA and 10 mg/kg of xylazine – Bayer; Germany, intramuscularly[11]). The functional analysis was carried out by a two-dimensional device for transthoracic echocardiography (5500 Sonos, Hewlet Packard) with S12 (5 – 12 MHz) sectors conductor and 15L6 (7–15 MHz), which allowed the analysis of up to 160 MHz. The transducer was placed on the left anterior-lateral region of the thorax. The hearts were visualized in two-dimensions with an axial view of the left ventricle, including the mitral and aortic valves and the apex in the same image. The digital conversion image was performed by the delimitation of the ventricular septum and the posterior wall of the left ventricle. The measures of left ventricular-end diastolic volume (EDV), left ventricular-end systolic volume (ESV), ejection time (ET), isovolumic relaxation (IRT) and total diastolic time (TDT) were registered in five consecutive cardiac cycles. EDV, ESV and left ventricular ejection fraction (EF) were measured using the Simpson method. Systolic output (SO) was estimated by subtracting the ESV of the EDV. The TDT was measured from the onset of aortic valve closure to closure of the mitral valve. The ET was measured from the opening to closure of the aortic valve. The IRT was measured from the interval between the aortic valve closure and the start of mitral flow. The isovolumic contraction time (ICT) was obtained by measuring the time interval of the onset of mitral closure to its reopening (MCMR) and subtracting it from the measured sum of IRT and ET. The left ventricular filling pattern was assessed by the E/A ratio, which was calculated by dividing the peak early diastolic filling velocity (E) by the peak atrial systolic flow velocity (A). The HR was calculated as the inverse of the period between two QRS complex peaks in the electrocardiogram (ECG). The Tei index, which indicates global ventricular systolic and diastolic function[12], was calculated by the sum of isovolumic contraction time and isovolumic relaxation time divided by ejection time as previously described[13]. The A variable (AV) was calculated by the sum of systolic time and IRT. The EDV/body weight ratio was calculated as an index of the ventricular intrinsic performance according to the animal weight. The systolic work (SW) evaluates the ventricular performance by multiplying the SO and the mean arterial blood pressure (MABP). The observer variability was registered for two measurements in a random sample of rats. The correlation coefficient and standardization of the estimated error was calculated in accordance to Bland and Altman method.

Blood pressure, Heart weight/body weight ratio, Adrenal weight/body weight ratio and Isolated papillary twitch measurements

After 24 hours, the animals were anesthetized as described above. The blood pressure was measured by placing a cannula connected to a pressure transducer (WPI-World Precision Instrument, model BLPR) inside the right carotid artery fed into a chart recorder and an analog/digital converter (Data Translation 2821) connected to a PC for data acquisition and analysis using a Lab Tech Pro software. After 15 min of stabilization, we measured the systolic (SBP), the diastolic (DBP) and the MABP. The total peripheral vascular resistance (TPVR) was calculated by dividing the MABP by the CO. Using the same cannula, blood samples were separated into serum aliquots, centrifuged and frozen at – 20^0 C for posterior hormonal dosage.

The heart and right adrenal were then removed and their wet weight measured and expressed as mg/gr of body weight. Following, the heart was perfused with Tyrode's solution continuously bubbled with 95 % O_2 and 5 % CO_2, through a cannula inserted into the aorta. The papillary muscles from the right ventricle were removed and their ends were clamped using O-shaped clamps. The muscles were transferred to a 10 mL muscle chamber where one end was fixed to a movable arm and the other to a force transducer (WPI, F10). They were then superfused with Tyrode's solution. The force transducer´s output was fed into the strip chart recorder (Lafayette Instruments Company) and the digital data acquisition system. The papillary muscles were electrically stimulated at 0.5 Hz with supramaximal square pulses via parallel platinum plate electrodes using an isolated pulse generator. All muscles were stretched to the length at which we obtained their maximal twitch force. The length and diameters of the preparations were measured under a microscope. After the experiments, we determined blotted wet muscle weight (verificar com o autor). Assuming a uniform cross-sectional area and muscle density of 1.06 g/cm^3, the cross-sectional area (CSA in mm^2) was calculated by dividing the wet weight by the product of the muscle length and density: CSA= mass (mg)/(length(mm) x density (1.06 g/cm^3). In all cases, the papillary muscles were stimulated for a 45 min equilibration period. Then, Tyrode's solution calcium concentration was consecutively changed to 1.0; 2.0; 3.0; 5.0 and 10.0 mM. The maximum rate of force development during contraction (dF/dt_{max}) and the maximum rate of force decay during relaxation (dF/dt_{min}) were measured in all calcium concentrations. In order to evaluate the time dependence of the rest in the contractile force generated by the papillary muscles, post-rest potentiation (PRP) was measured by interrupting the electrical stimulation for 1, 3, 5, 10 and 20 seconds[14]. To measure the amount of potentiation, the force produced by the first contraction after the rest period was considered as PRP and normalized to previous steady state contraction.

Evaluation of the influence of Hy in the FC

To monitor the influence of Hy in the adrenocortical activity, we performed the extraction and concentration measurement of FC, according to fecal weight, as described[15,16]. The 24-hours feces samples of the two groups of animals were collected on the first, the fifth and the tenth days of treatment. They were dried, weighted and stored at -20 C. The sample gathering was made at 1 p.m and on days when the cages were not cleaned[15,17]. Moreover, the adrenal weight/body weight ratio was used to combine the data analysis, since this parameter is altered only in conditions of enhanced pituitary stimulation of the adrenal cortex[7].

Statistical analyses

Two samples were compared using Students t-test. A one-way analysis of variance was applied for multiple comparisons followed by Tukey, using SigmaPlot 9.0 and Sigma Stat. 2.0 software. To ensure better control of type I error, for repeated samples we applied the Bonferroni post-test, as it is more accurate than Tukey's post-test when there is a small number of groups, as the significance level is divided by the total number of groups used (α/n). Data was expressed as the means ± SE of at least 8 observations. Statistical significance was assumed for $p \leq 0.05$.

Results

Basal hormonal levels

T4 levels were slightly lower in the T3 treated group: 2.54 ± 0.4 μg/dL and 2.1 ± 0.47 μg/dL, respectively ($p > 0.05$). Serum T3 levels were significantly higher in the T3 treated group: 35 ± 11.3 ng/dL and 103 ± 16.4 ng/dL, respectively ($p \leq 0.05$).

Echocardiography

ECHO data is shown in Tables 1 and 2. HG showed reduction of ESV, EDV, TDT, ET, IRT, Diast. area, Syst. area, AV and E/A ratio; HR, EF and CO increased. In both groups, there was no alteration between groups regarding the ICT, SO, Tei index and DT. The EDV/body weight ratio was not different between groups (CG: 0.002 mL/gr ± 0.0001 and HG: 0.0018 mL/gr ± 0.0001) and SW was higher in the HG (CG: 33.792 mL/mmHg ± 1.11 and HG: 38.11 mL/mmHg ± 1.36).

Blood pressure, Heart weight/body weight ratio and Papillary twitch measurements

SBP levels were higher in the HG. SBP, DBP and MABP (± SE, in mmHg) obtained in the control group were 112 ± 14; 97.6 ± 11 and 102.4 ±12 respectively while in the hyperthyroid rats they were 135.4 ± 15.0;116.7 ± 19 and 122.93 ± 16.5 respectively.

There was no difference in the TPVR (mmHg/mL/min) between the groups. The calculated values in the CG and HG were: 1.15 ± 0.1 and 1.04 ± 0.09, respectively.

The body weight (g) was 309 ± 19 in the CG and 296 ± 25 in the HG. The heart weight/body weight ratio (mg/gr) was higher in the HG. The values (± SE) were 3.3 ± 0.16 in the CG and 5.1 ± 0.36 in the HG ($p \leq 0.05$).

The papillary CSA from control and hyperthyroid groups were 0.42 ± 0.16 and 1.05 ± 0.6 mm^2 respectively. As shown in Figure 1, the groups did not show statistically differences in the maximum twitch force (mN/mm^2) (CG: 62 ± 28.7; HG: 56.3 ± 22.1) and in the PRP (1, 3, 5, 10 and 20 seconds) in any of the extracellular calcium concentrations used as shown in Figure 1 for the calcium concentration of 2 mM. An original record of 3 sec PRP of IPM being electrically stimulated at 0.5 Hz from the HG is shown in Figure 2. Nevertheless, the concentration of external calcium necessary to produce 50 % of the maximal force was statistically different in the groups. This concentration (mM) was 0.85 ± 0.25 in the CG and 0.66 ± 0.2 in the HG.

In the HG, the dF/dt_{max} which indicates the speed of contraction of IPM was higher only at 2 mM of Ca^{2+} (Figure 3). However, the dF/dt_{min} which indicates the speed of relaxation of IPM was higher at 1, 2, 3 and 5 mM of Ca^{2+} (Figure 4).

Table 1 – Cardiac performance data from control (CG) and hyperthyroid (HG) animals obtained by Echocardiography

	ESV	EDV	IRT	ICT	TDT	HR	ET	EF
CG	0.19 ± 0.01	0.52 ± 0.02	27 ± 0.006	16 ± 0.002	131 ± 0.01	270 ± 14	84 ± 0.001	63 ± 0.5
HG	0.12* ± 0.01	0.43* ± 0.02	18* ± 0.002	15 ± 0.001	100* ± 0.004	380* ± 9.2	59* ± 0.001	72* ± 1.2

*Values are means ± SE of eight animals. * p < 0.05 compared to control animals. Time - in msec. Volume - in mL. ESV: end systolic volume; EDV: end diastolic volume; IRT: isovolumic relaxation time; ICT: isovolumic contraction time; TDT: total diastolic time; HR: heart rate; EF: ejection fraction. ET: Ejection time.*

Table 2 – Cardiac Performance Data from Control (CG) and Hyperthyroid Animals (HG) obtained by Echocardiography

	SO	CO	AV	DTEW	Diast. area	Syst. area	Tei index	E/A
CG	0.33 ± 0.01	89 ± 7.98	127 ± 0.002	45 ± 0.002	0.99 ± 0.014	0.54 ± 0.01	0.5 ± 0.03	1.9 ± 0.24
HG	0.31 ± 0.02	118* ± 6.82	92* ± 0.002	43 ± 0.002	0.9* ± 0.02	0.42* ± 0.01	0.54 ± 0.02	1.57* ± 0.35

*Values are means ± SE of eight animals. * p < 0.05 compared to control animals. Time - in msec. Volume - in mL. Area – in cm^2. SO: systolic output; CO: cardiac output; Diast. area: Diastolic area; Syst. area: Systolic area; AV: A variable; DTEW: deceleration time of E wave; E/A: ratio of the maximal early to atrial mitral flow velocities.*

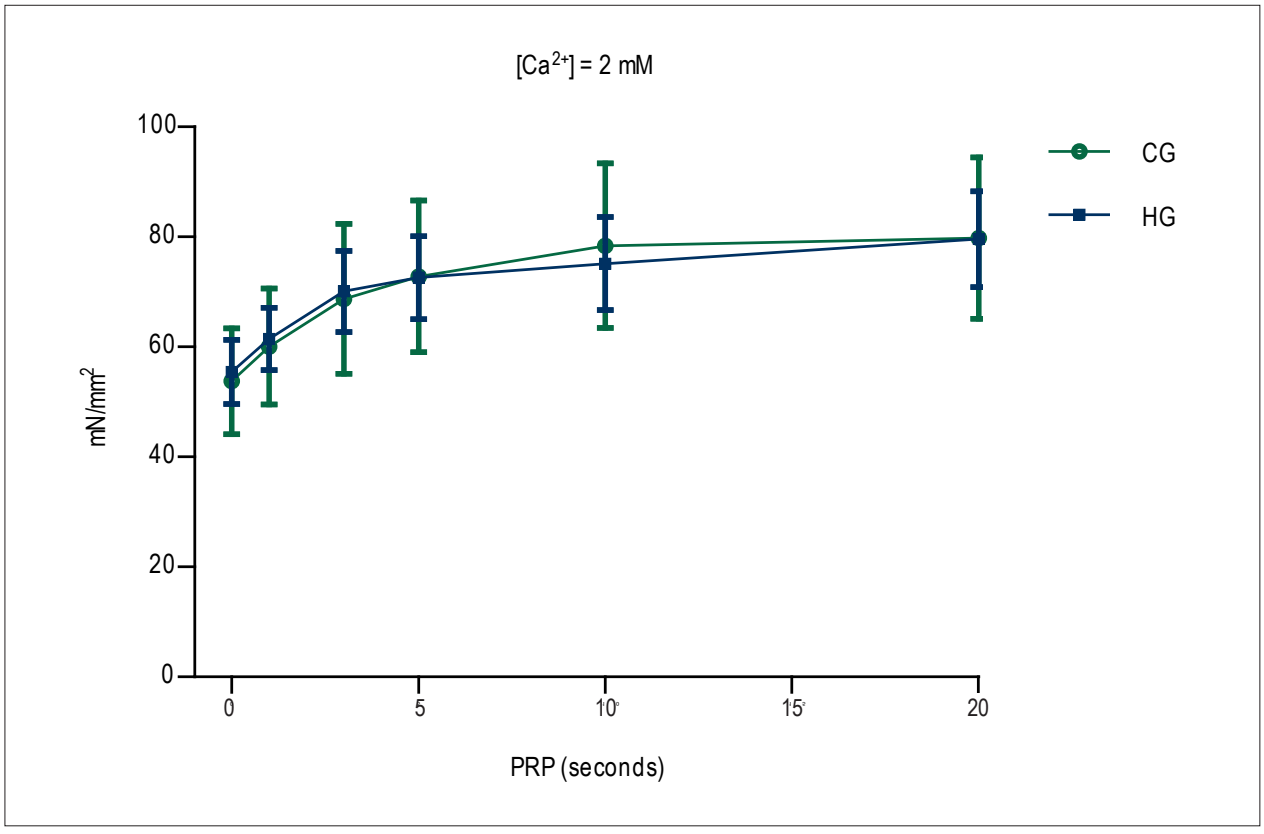

Figure 1 – *PRP of IPM from CG and HG at [Ca²⁺] of 2 mM. Values are means ± SE of eight animals.*

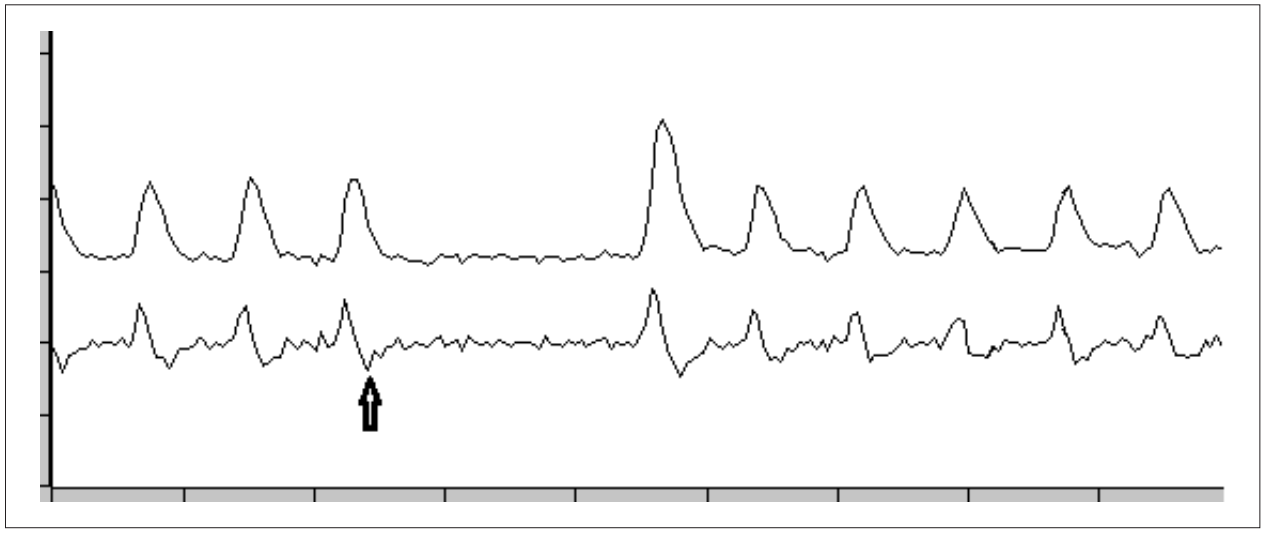

Figure 2 – *Original record of 3 sec PRP of IPM electrically stimulated at 0.5 Hz from the HG. [Ca²⁺] = 2 mM. Note the pause period between contractions of 3 sec after the arrow.*

Influence of short-term Hy in the adrenal weight/body weight ratio and FC

There was no difference in the adrenal weight/body weight ratio (mg/gr). The values (± SE) were 0.091 ± 0.01 in the CG and 0.1 ± 0.013 in the HG. In contrast, there was a difference in the FC on the fifth day of treatment. However, there was no difference on day 10 when the experiments were carried out (Table 3).

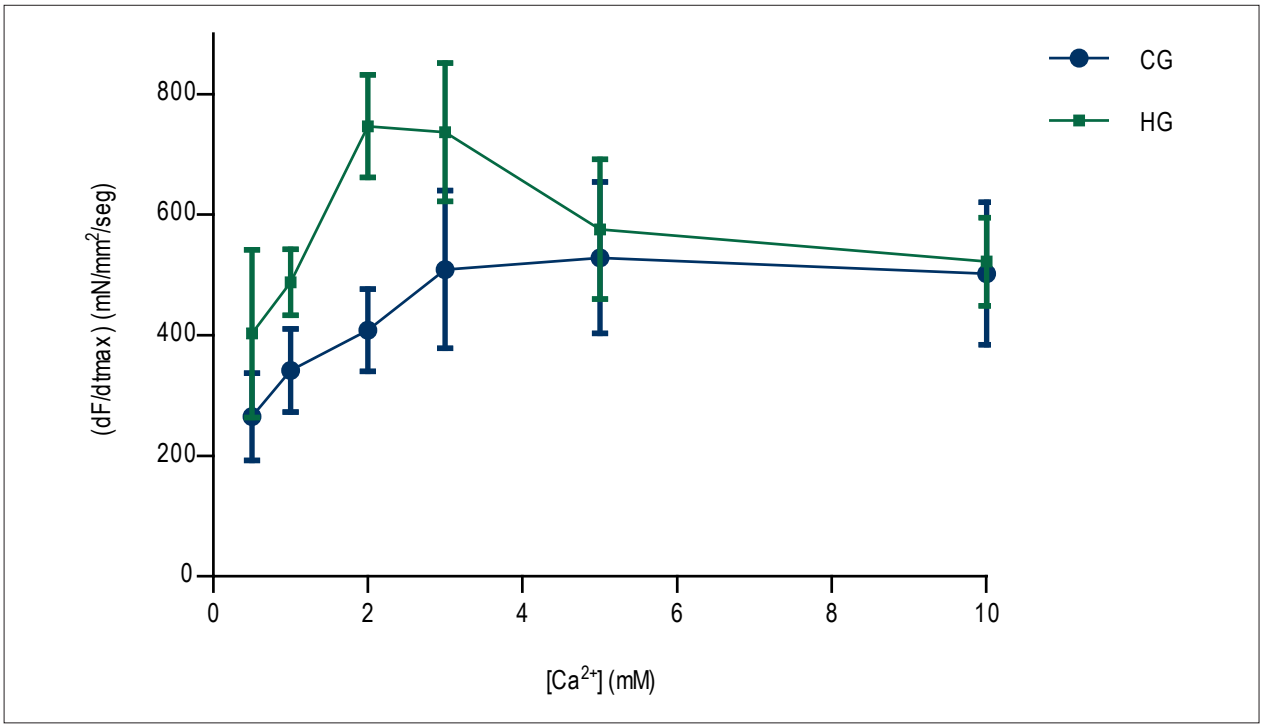

Figure 3 – *Maximum rate of force development during contraction (dF/dt_{max}) of IPM from the CG and HG.*

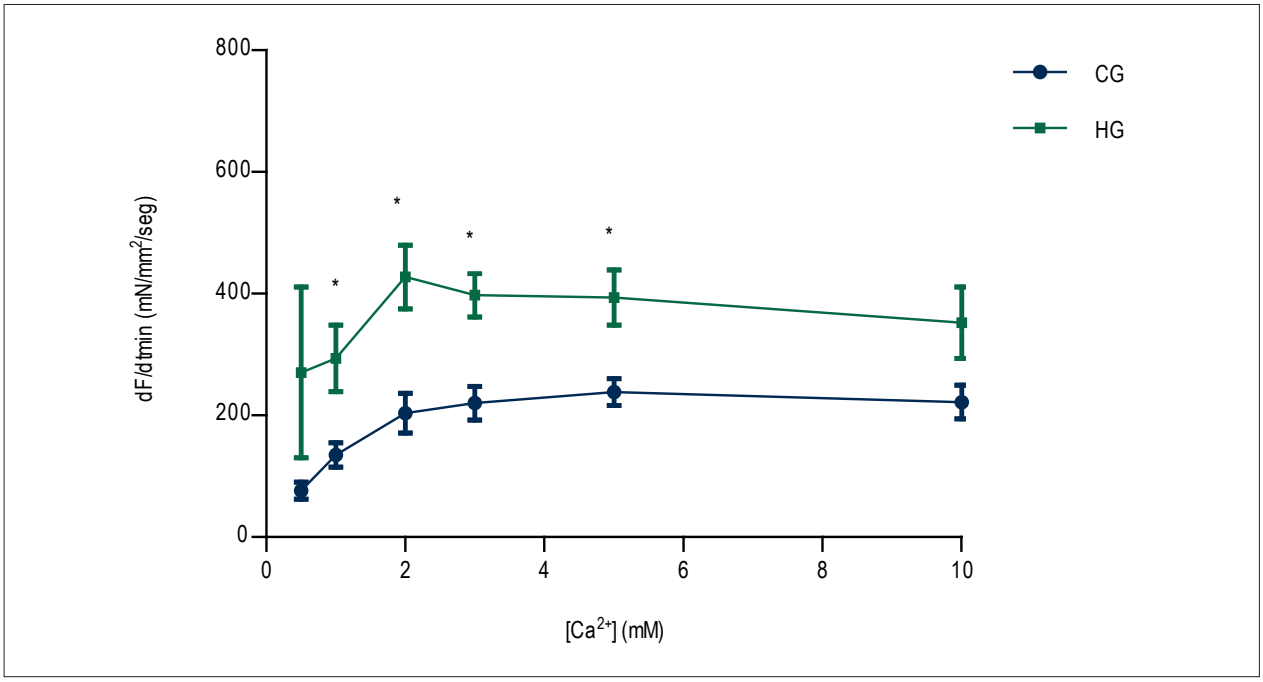

Figure 4 – *Maximum rate of force decay during relaxation (dF/dt_{min}) of IPM from the CG and HG*

Discussion

Many studies have shown the interactions between TH and the cardiovascular system[1-3,6,18-20].

Although only a few studies have addressed the impact of TH excess on the HPAA of humans and animals[5-7,9], the fact that there is an impact has been clinically accepted.

Table 3 – Fecal Corticosterone from Control (CG) and Hyperthyroid (HG) Animals

CG 1	HG 1	CG 5	HG 5*	CG 10	HG 10
273,19 ± 62,73	317,32 ± 48,96	272,94 ± 30,83	613,54 ± 104,74	305,82 ± 65,79	361,27 ± 65,99

*Values are means ± SE of eight animals. * $p < 0.05$ compared to control animals. Concentration, in ng/g of feces. 1: 1st day of injection; 5: 5th day of injection; 10: 10th day of injection.*

Nevertheless, to the best of our knowledge, this is the first study to demonstrate the consequences of short-term Hy on the function of the adrenal cortex in the presence of physiologically-analyzed cardiac performance alterations both "*in vivo*" and "*in vitro*". We used an animal model and, through different approaches, we showed the direct (myocardium hypothesis) and the hemodynamic (vascular hypothesis – Frank Starling Law) effects of TH on CO. We also demonstrated that the influences of short-term Hy on the cardiovascular system do not result in alterations of the adrenal cortex function, *i.e.,* the alterations seen in the short-term hyperthyroid rats are independent from the TH effects on the adrenal cortex.

It has been shown that Hy induces cardiac hypertrophy and exercise intolerance. Moreover, Hy results in cardiac failure in the long term[21-24]. It has also been suggested[25] that Hy is an independent risk factor for myocardial ischemia. Moreover, clinical findings have shown a strong correlation between thyroid function, cardiac mass and ventricular hypertrophy in subjects between 45 and 79 years[22]. Different from what could be expected from a so-called "physiological" hypertrophy, histological alterations were also found, *i.*e. disorganized intercalated discs, high HR at rest and decreased cardiac efficiency[22,23]. Indeed, tachycardia at rest, as well as an increased risk to develop atrial arrhythmias[24] are some of the electrophysiological effects commonly found in Hy. Supporting this hypothesis, our data on short–term Hy rats also showed high HR. In this respect, the HR may be changed by directly modifying the electrical properties or by changing the levels of neurohumoral factors that modulate the activity of the sinus node[26]. The molecular basis of positive chronotropic effect of T3 is not entirely clear. The alterations could be in the expression of the two genes responsible for the channel coding for I_f (hyperpolarizing current) and $I_{Ca,T}$ (calcium transitory current), although $I_{Ca,T}$ appears to be the main one and probably the most affected by Hy[27,28].

Although the impact of Hy on the cardiovascular system has been broadly studied, we made a strong contribution to a comprehensive analysis. By combining *"in vivo"* and *"in vitro"* experiments, we confirmed that the cardiac alterations seen in many studies exist in both situations. Furthermore, we used the same anesthetic regimen both "*in vivo*" and "*in vitro*" conditions to ensure an adequate correlation of results as previously[29] established.

In our study, the cardiac mass of HG increased as shown by the augmented heart weight/body weight ratio (mg/gr) of the HG. Furthermore, the increased hypertrophic morphology of the heart and its functional alterations were confirmed by the ECHO and IPM experiments. Our data showed an increase in HR, EF, CO (*in vivo)* and dF/dt_{min} (*in vitro)*. Likewise, our data showed a decrease in EDV, ESV, IRT, TDT, ET, AV and E/A ratio (*in vivo*), which altogether indicate positive inotropic, chronotropic and lusitropic effects. The decrease in ET and IRT, as well as the dF/dt_{min}, found in the HG may be explained by the increased rate of Ca^{2+} uptake to the sarcoplasmic reticulum (SR) and/or decreased levels of phospholamban, which characterizes a direct effect on the contractile mechanism of CO regulation. Our results can also explain the exercise intolerance[3] of hyperthyroid individuals due to an inability to further raise the parameters as required by exercise. This suggests that hyperthyroid individuals' hearts may be operating close to their maximum capacity, *i.e.*, the cardiac reserve is lowered, as it justifies the higher incidence of arrhythmias due to tachycardia and, in the long-term, ventricular dysfunction and heart failure.

In contrast, there was no difference in the *in vitro* measurements of maximum twitch force and the dF/dt_{max}. These findings suggest that a hyperthyroid heart is not capable of developing stronger or more rapid maximum contraction during systole than a normal heart is. On the other hand, the reason for no alteration on the maximum twitch force might be that the *in vivo* diminished ET, which resulted in less time to reach the maximum force. Such observation is in agreement with findings of no alteration *in vivo* SO and ICT. Those *in vitro* and *in vivo* correlated findings are also in agreement with studies on the cardiac function seen in Hy[2,3,6,18]. However, both groups' Tei index, which is used to detect both systolic and diastolic dysfunction[12,13], was not statistically different. This apparently contradictory result only occurred because in the HG, the ET decreased proportionally to the IRT.

Our findings of no alteration of PRP of 1, 3, 5, 10 or 20 seconds possibly indicates that the rate of Ca^{2+} uptake to the sarcoplasmic reticulum by SERCA2a may reflect, in both groups, along-enough rest for the calcium uptake to the sarcoplasmic reticulum. These results may be due to a decreased expression of the Na^+/Ca^{2+} exchanger in the HG. In fact, Reed et al., 2000, showed that the SERCA2a and Na^+/Ca^{2+} exchanger were increased and decreased, respectively, in Hy in mice[30]. In this manner, a smaller amount of Ca^{2+} leaves the cells, maintaining a sufficient cytosolic concentration to produce a PRP response similar to the one in the CG.

Regarding the impact of Hy on preload, it has been suggested that venous compliance is augmented in Hy. Therefore, the preload increases[26]. In fact, under physiological conditions (magnitude, radius, wall thickness and compliance of left ventricle) preload can be determined by EDV. Therefore, we could conclude from our ECHO data of lower EDV that preload is lowered in the HG. Nevertheless, due to the concentric hypertrophy, the high HR found in Hy should induce decrease in the EDV if preload

was not augmented. Thus, the EDV must be corrected for the body weight in order to achieve a more reliable preload index since the hyperthyroid animals lose weight[26]. In this manner, our results show no difference in EDV/body weight between the CG and HG, strongly indicating that preload is not augmented in hyperthyroid animals. Moreover, although SO is not different in the HG, which can be assumed by the lower EDV, EF rises and gives strong evidence of direct effect of TH on the heart, increasing contractility. In addition, the positive chronotropic effect of TH ensures a higher resting CO found in the HG in spite of the unchanged SO.

Additionally, we evaluated the effect of Hy on blood pressure, since thyroid dysfunction is associated with alterations in the systolic and/or diastolic pressures[4,18,31]. The measurements showed a higher SBP in the HG. This finding is in agreement with studies in humans[31] and gives rise to another evidence of increased heart contractility in the HG. This is supported by the higher EF (in face of decreased EDV and ESV), rather than TPVR alterations, since modifications in the TPVR in the absence of compliance disturbance, would lead to proportional increases in SBP and DBP. On the other hand, some studies have shown a reduced TPVR in the presence of Hy[32,33]. Considering that our study found no significant difference in the TPVR between groups, increased SBP could be a result of increased contractility due to direct action of TH on the heart, leading to increases force of contraction and HR.

Our findings suggest functional effects that are in agreement with biological studies concerning the direct actions of TH in terms of expression and activity of ion channels related to the cardiac ECC, *i.e.* augmented expression of dihydropyridine receptor, RyR, SERCA2a, α - MHC, actin, troponin I, Na^+/K^+ pump and voltage dependent K^+ channels and reduced expression of Phospholamban, β - MHC and Na^+/Ca^{2+} exchanger as previously described[2,27,30,34,35].

Finally, regarding the adrenal cortex, the activation of the rat HPAA has been associated with Hy[7,9]. The augmented activity in the HPAA is followed, within minutes, by increased levels of corticosterone (the main glucocorticosteroid in rats). However, the few studies that addressed the possibility of direct correlation between thyroid dysfunction and adrenal gland were conducted through measurements of basal plasma concentration of ACTH and corticosterone, both of which are elevated in thyrotoxicosis[6,36,37].

It has been shown that the glucocorticoid concentration in the blood is not an appropriate indicator of a stressful event, because it is influenced by the stressful sampling and it reflects a transitory situation[38,39]. In fact, the sampling of blood increases corticosterone levels if it is not terminated within two minutes and if the animal was being handled for the first time. For the rats, the capturing, the handling and the bleeding are stressful events. Therefore, they interfere with glucocorticoid concentrations[17]. In contrast, fecal sample collection is easily performed and allows monitoring of corticosterone concentrations without stressing the animal. It is a non–invasive technique to study the impact of different approaches on the adrenal activity.

In our study, we collected twenty-four hour fecal samples to monitor the influences of Hy on the HPAA. T3 injections followed and were given always at the same time, *i.e.* 1 p.m., since time interference can influence corticosterone levels[16]. Moreover, fecal collection was made only on days when the cages were not cleaned as defecation increases on days of cage replacement and this increase could interfere with the FC[17]. We measured FC on the 1st, the 5th and the 10th day of T3 treatment as 15% to 25% of corticosterone are excreted on feces. This way we eliminated the stress component that follows plasmatic measurement of corticosterone. The FC was higher in the HG on the fifth day. But there was no difference on the tenth day of treatment. We were able to exclude the possibility of a higher FC found in the HG due to initial stress response to the injections followed by adaptation to that stimulus, as we also applied vehicle injections to the CG. Instead, this finding shows that Hy initially alters the adrenal cortex metabolic rate. However, the alteration ceases as one maintains the hyperthyroid state and the glucocorticoid-ACTH negative feedback is intact[39,40]. This finding is in agreement with an unchanged adrenal weight/body weight ratio The adrenal growth is usually associated with enhanced pituitary stimulation of the adrenal cortex producing hypercortisolism[7,9]. This fact strongly suggests that Hy effects are independent from the HPAA activation by the TH. The last statement suggests that additional experimental and clinical human studies are necessary to establish the impact of Hy on the cardiovascular system, as well as its interaction with the adrenal gland and; therefore, the need for adrenal gland clinical intervention in thyrotoxicosis.

Conclusion

In conclusion, our study shows that short-term Hy increases the CO due to an increase in the HR. As well, it increases contractility - as shown by the reduction in the EDV, ESV, IRT, ET and TDT and EF. Finally, it accelerates relaxation, as shown by the higher dF/dt_{min} of IPM and IRT and TDT reductions (*i.e.* Hy induces positive inotropic, chronotropic and lusitropic effects on the heart as shown by both *in vivo* and *in vitro* experiments) and increases SBP. Such effects are not correlated with HPAA hyper-activation. Hy does not increase the weight of adrenal gland or FC in conditions where the stress status is not changed by manipulation or invasive techniques. Those results shall be taken into consideration for future clinical investigations.

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Author contributions

Conception and design of the research: Szkudlarek AC, Aldenucci B; Acquisition of data: Szkudlarek AC, Aldenucci B, Miyagui NI; Analysis and interpretation of the data: Szkudlarek AC, Moraes RN; Statistical analysis: Silva IK, Fogaça RTH; Obtaining funding: Szkudlarek AC, Silva IK; Writing of the manuscript: Szkudlarek AC; Critical revision of the manuscript for intellectual content: Szkudlarek AC, Fogaça RTH; Supervision: Ramos HE, Fogaça RTH.

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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