Cardiac ischemia/reperfusion injury is inversely affected by thyroid hormones excess or deficiency in male Wistar rats

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Abstract

Aim
Thyroid dysfunctions can increase the risk of myocardial ischemia and infarction. However, the repercussions on cardiac ischemia/reperfusion (IR) injury remain unclear so far. We report here the effects of hypothyroidism and thyrotoxicosis in the susceptibility to IR injury in isolated rat hearts compared to euthyroid condition and the potential role of antioxidant enzymes.

Methods
Hypothyroidism and thyrotoxicosis were induced by administration of methimazole (MMZ, 300 mg/L) and thyroxine (T4, 12 mg/L), respectively in drinking water for 35 days. Isolated hearts were submitted to IR and evaluated for mechanical dysfunctions and infarct size. Superoxide dismutase types 1 and 2 (SOD1 and SOD2), glutathione peroxidase types 1 and 3 (GPX1 and GPX3) and catalase mRNA levels were assessed by quantitative RT-PCR to investigate the potential role of antioxidant enzymes.

Results
Thyrotoxicosis elicited cardiac hypertrophy and increased baseline mechanical performance, including increased left ventricle (LV) systolic pressure, LV developed pressure and derivatives of pressure (dP/dt), whereas in hypothyroid hearts exhibited decreased dP/dt. Post-ischemic recovery of LV end-diastolic pressure (LVEDP), LVDP and dP/dt was impaired in thyrotoxic rat hearts, whereas hypothyroid hearts exhibited improved LVEDP and decreased infarct size. Catalase expression was decreased by thyrotoxicosis.
Conclusion
Thyrotoxicosis was correlated, at least in part, to cardiac remodeling and increased susceptibility to IR injury possibly due to down-regulation of antioxidant enzymes, whereas hypothyroid hearts were less vulnerable to IR injury.

Introduction
Thyroid hormones (TH) are known to play crucial roles in the regulation of cardiovascular system homeostasis. By binding to nuclear receptors, TH regulates the expression of several contractile and calcium-handling proteins, ion channels and sympathetic tonus [1]. As a result, cardiac inotropic, chronotropic, lusitropic and dromotropic properties, as well as vascular resistance, are directly affected by TH levels.

It has been widely recognized from clinical data that thyroid dysfunction is correlated to increased cardiovascular morbidity and mortality [2]. Overall, tachycardia and tachyarrhythmic events, cardiac hypertrophy and heart failure have been frequently reported in conditions of TH excess, such as Graves’ disease, thyroid or pituitary adenoma, and toxic multinodular goitre [3,4]. On the other hand, bradyarrhythmias, mild hypertension, impaired systolic and diastolic functions have been associated to TH deficiency [4,5].

Importantly, clinical reports have demonstrated that hyperthyroid and hypothyroid patients are more predisposed to myocardial ischemia and acute myocardial infarction (AMI), the leading cause of death among cardiovascular diseases, when compared to euthyroid patients [6,7]. In case of TH excess, coronary artery spasm and increased prothrombotic state can elicit coronary artery occlusion and myocardial ischemia [8–10]. Myocardial ischemia can be further worsened by increased cardiac workload and oxygen demand elicited by TH excess [11]. In hypothyroid patients, dyslipidaemia and increased circulating cholesterol levels are the main contributors to the formation of atherosclerotic plaque and development of coronary artery disease [3,10]. Nonetheless, decreased blood vessel density and increased arterial wall stiffness account for the declined coronary blood flow and the higher risk of myocardial ischemia, despite the decreased cardiac workload induced by TH deficiency [11–13].

Reperfusion remains the most effective therapeutic maneuver to rescue ischemic myocardium and has been widely recommended by both American Heart Association and European Society of Cardiology [14,15]. Even so, reperfusion per se recruits pro-apoptotic pathways that culminate in cardiomyocyte death and further infarct expansion, a condition referred to as reperfusion injury [16]. Among several pathophysiological mechanisms, Redox imbalance has been demonstrated to play a pivotal role in the progression of ischemia/reperfusion (IR) injury by promoting oxidative damage and cell apoptosis [17].

The clinical relevance of changes on TH level in the progression of IR injury and the repercussion on AMI outcomes are unclear, whereas experimental data remain conflicting so far. Administration of 3,5,3’-triiodothyronine (T3), the main cellular active thyroid hormone, has been reported to potentiate post-ischemic recovery of myocardial mechanical properties in experimental models of IR injury [18,19]. Conversely, IR-induced myocardial damage and ventricular arrhythmias have been shown to be attenuated in hypothyroid rat hearts in comparison to euthyroid and thyrotoxic rats [20,21]. Therefore, this study aimed to assess both cardiac mechanical properties, as well as the extent of myocardial damage induced by IR in rats exposed to long-term TH deficiency and excess in comparison to euthyroid rats. The potential role of antioxidant enzyme imbalance was also investigated.
Materials and methods

Animals

This study followed the standards and ethical guidelines of the Ethics Committee for Research of the Federal Rural University of Rio de Janeiro and of the Federal University of Rio de Janeiro. It was approved by the Ethics Committee for Research of the Federal Rural University of Rio de Janeiro and of the Federal University of Rio de Janeiro under the number IBCCF194-07/16. Furthermore, all the standards proposed by the Guide for the Care and Use of Laboratory Animals (U.S. National Institutes of Health (NIH) Publication No. 85–23, revised 1996) were observed.

Experimental protocol

Male Wistar rats (8 weeks-old, 170–200 g) obtained at the Central Vivarium (Carlos Chagas Filho Institute of Biophysics, UFRJ, Brazil) were housed in cages under controlled temperature (21 ± 2°C), daily exposed to 12-hour light–dark cycle (lights off at 7:00 pm) and water and standard chow ad libitum. The animals were divided into three groups: euthyroid (CTL, N = 18), hypothyroid (MMZ, N = 14) and thyrotoxic (T4, thyroxine group, N = 17). Hypothyroidism and thyrotoxicosis were induced by methimazole (300 mg/L) and L-thyroxine (12 mg/L), respectively, administered in drinking water for 35 days [22]. No animal exhibited adverse symptoms during all period of vehicle, T4 and MMZ exposure. After treatments, all animals were euthanized under anesthesia with isoflurane by exsanguination, and blood samples were collected for serum T4 and T3 measurements by radioimmunoassay. Hearts were excised and weighed, and the tibia length was measured for pathological analysis. The susceptibility to IR injury was evaluated in isolated hearts using a Langedorff apparatus. Cardiac samples were collected and stored at -80°C for posterior molecular analyses.

Ex vivo IR experiments

Methods for isolated rat heart experiments were similar to those previously described [23]. Excised hearts were weighted and placed in modified Krebs-Henseleit solution (KHS) (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 10 mM glucose, 1.8 mM CaCl2, saturated with 95% O2 and 5% CO2). Aortas were hung on the cannula of a modified Langendorff apparatus and hearts were artificially perfused with modified KHS adjusted for pH 7.4 and 37°C at a constant flow (10 mL/min). A latex balloon was inserted into the left ventricular (LV) chamber through an atrial incision to assess mechanical performance. Hearts were kept immersed in perfusion solution and baseline LV end-diastolic pressure (LVEDP) was set at 10 mmHg. After 20 min of baseline period, wherein heart rate, diastolic and systolic pressures were stable, the peristaltic pump was stopped and hearts were submitted to 30 min of global ischemia and subsequent 60 min of reperfusion. LV developed pressure (LVDP), LV systolic pressure (LVSP), LV end-diastolic pressure, LV maximal derivative of pressure (max. dP/dt) and LV minimal derivative of pressure (min. dP/dt) waveforms were recorded with a pressure transducer (PT 300, Grass Technologies). The transducer was connected to an amplifier (ML 110 ADInstruments), which was connected to an analogical/digital converter (PowerLab 400, ADInstruments). All recordings were digitized and stored on a computer for later analysis using the program LabChart 5.0 (ADInstruments®).

Measurement of infarct size

Ventricular sections were sliced into approximately 1.5 mm from apex to base and incubated in 1% (w/v) TTC in phosphate buffer (pH 7.4) for 5 min at 37°C. All slices were placed in a
10% (v/v) formaldehyde solution for 24 h to improve contrast between stained (viable) and unstained (necrotic) tissues. Ventricle slices were placed between two glass slides and their images were digitally acquired in a scanner. Infarct size was determined using ImageJ software (NIH Image): National Institute of Health, USA, version 1.22). Values were expressed as % of total ventricular area [24].

Radioimmunoassay for total serum T4 and T3

Blood samples were collected at the end of treatment after euthanasia (35th day). Samples were centrifuged at 1931.9 g for 20 min, and sera were separated and stored at -20°C. Serum T3 and T4 were determined by specific Coated-Tube Radioimmunoassay kits (MP Biomedicals, LLC, USA). All the procedures were carried out following the kit recommendation.

Quantitative polymerase-chain reaction (qPCR)

To estimate mRNA expression levels of superoxide dismutase type 1 (SOD1), SOD2, catalase, glutathione peroxidase type 1 (GPX1) and GPX3 (Table 1), total RNA was extracted from LV tissue samples using RNeasy® Fibrous Tissue Mini Kit (QIAGEN) and cDNA was prepared from 1 μg of total RNA using High-Capacity Reverse Transcription kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. mRNA levels of target genes (Table 1) were evaluated by qRT-PCR. Amplification reactions containing 1ng of cDNA were performed at 60°C during the annealing and extension cycles. The expression of chosen genes was normalized to GAPDH as an internal control. The quantification of selected mRNA was determined by $2^{-\Delta\Delta CT}$ method in a Via7 Software v1.2.4 and expressed as fold change of MMZ and T4 group compared to the control group.

Statistical analysis

Data are presented as mean ± standard error of mean (S.E.M.). Normal statistical distribution of all data was determined by Shapiro-Wilks test (Statext v2.7, http://www.statext.com/index.php). One-way ANOVA followed by Bonferroni post-hoc test were used to compare MMZ and T4 groups to CTL group (Prism®, GraphPad). Statistical differences were considered significant when P < 0.05.

Results and discussion

The present study provides evidence that the pathophysiological progression of myocardial IR injury can be distinctly affected by TH excess or deficiency. While thyrotoxic rat-hearts were more vulnerable to post-ischemic myocardial stunning than euthyroid rat hearts, post-ischemic recovery of mechanical properties was improved and infarct size was decreased in hypo-thyroid rat hearts. Increased susceptibility to redox imbalance and oxidative damage might contribute to these deleterious effects, given that thyrotoxic rat-hearts exhibited decreased mRNA expression level of antioxidant enzymes SOD2 and catalase.

TH deficiency is the most commonly diagnosed thyroid dysfunction and can result from several different environmental and physiological disturbances, such as iodine deficiency, primary atrophic hypothyroidism and Hashimoto’s thyroiditis [25]. The most frequent causes of TH excess are Graves’ disease, toxic multinodular goitre and thyroid adenoma [25]. Despite the limitations to mimic all features of human thyroid dysfunctions, delivery of MMZ and T4 in drinking water have been frequently used as non-invasive experimental models of hypothyroidism and thyrotoxicosis, respectively [22]. MMZ inhibits thyroidal enzyme thyroperoxidase and incorporation of iodine into thyroglobulin, resulting in drop of T3 and T4 production, as
evidenced in the MMZ group (Table 2, \( P < 0.05 \) vs. CTL group). Unsurprisingly, long-term T4 exposure increased serum level of T4 (Table 2, \( P < 0.01 \) vs. CTL group). In addition, serum T3 levels were also increased in the T4 group (Table 2, \( P < 0.01 \) vs. CTL group), given that T4 can be converted into T3 by deiodinases (DIO) type 1 and 2 in target tissues. Taken together, these findings confirm that MMZ and T4 groups developed hypothyroidism and thyrotoxicosis, respectively as expected. TH-deficient rats exhibited decreased mean body weight (Table 2, \( P < 0.001 \) vs. CTL group), in agreement with the essential role of TH in the process of musculoskeletal growth by regulating pituitary and hypothalamic growth hormone synthesis [26]. Furthermore, TH deficiency has been correlated to decreased food intake and decline on body weight gain [26]. Conversely, body weight gain was not significantly affected by long-term T4 exposure (Table 2, \( P > 0.05 \) vs. CTL group). Although previous data have demonstrated that food intake can be increased by TH excess, it has been widely recognized that metabolic rate can also be increased in such a condition, which might elicit balanced body weight gain [27].

Clinical and pre-clinical data have demonstrated that TH excess can induce substantial cardiac morphological changes [28,29]. Indeed, thyrotoxic rats exhibited increased HW (Table 2, \( P < 0.01 \) vs. CTL group), HW/BW (Table 2, \( P < 0.001 \) vs. CTL group) and HW/tibia length (Table 2, \( P < 0.01 \) vs. CTL group) in comparison to euthyroid rats, suggesting development of cardiac hypertrophy. At initial phases, cardiac growth in response to TH involves proportional rates of cardiomyocyte enlargement and proliferation of other cell types, such as fibroblasts and vascular cells [30–32]. At the transcriptional level, contractile and calcium-handling proteins are up-regulated, resulting in a condition frequently referred as physiological cardiac

Table 1. Primer sequences.

| Targets     | Forward               | Reverse               | Amplicon length |
|-------------|-----------------------|-----------------------|-----------------|
| SOD1        | TGTGTCCATTGAAAGATCSTGG | CTGCCAGATTCCAGCTTTT   | 138 bp          |
| SOD2        | GACAAACCTGAGCCCTAGA   | CAAAGCCCCAAGTCAGCC    | 81 bp           |
| GPX1        | AATCACGGTTTGACATGGAG  | GAGGTCAAGAGGGGTGAG    | 150 bp          |
| GPX3        | CAGCTACTGAGGCTGACAG   | ACTAGGGGAGATTCGGAGC   | 145 bp          |
| Catalase    | CAAGCTGGTTATGCGGATGG  | TTGAAAGATCCTGGAGGCC   | 141 bp          |
| GAPDH       | CCATCACCACCCCTTCATT   | GACCCGCTCCCCATTCAG    | 110 bp          |

SOD1 and SOD2 = Superoxide dismutase types 1 and 2; GPX1 and GPX3 = glutathione peroxidase types 1 and 3; GAPDH = Glyceraldehyde 3-phosphate dehydrogenase.

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Table 2. Thyroid hormone levels and biometric parameters.

| Parameters               | CTL                   | MMZ                   | T4                     |
|--------------------------|-----------------------|-----------------------|------------------------|
| Total T4 (µg/dL)         | 4.10 ± 0.25           | 2.06 ± 0.13*          | 12.19 ± 1.35**         |
| Total T3 (ng/dL)         | 27.86 ± 5.54          | 8.74 ± 0.92*          | 347.1 ± 36.82**        |
| Initial BW (g)           | 160.3 ± 2.431         | 162.0 ± 3.209         | 159.6 ± 3.156          |
| Final BW (g)             | 262.0 ± 3.804         | 194.4 ± 4.925***      | 279.4 ± 10.35          |
| HW (g)                   | 1.217 ± 0.04112       | 0.8520 ± 0.04352**    | 1.582 ± 0.07506**      |
| HW/BW (mg/g)             | 4.287 ± 0.1180        | 4.382 ± 0.1818        | 9.913 ± 0.4274***      |
| HW/Tibia length (g/cm)   | 0.4953 ± 0.02398      | 0.4338 ± 0.03431      | 0.6833 ± 0.03318**     |

T4 = thyroxine; T3 = 3,5,3’ triiodothyronine; BW = body weight; HW = heart weight. Data are mean ± S.E.M. N = 14–18.

*P<0.05

**P<0.01 and

***P<0.001 vs. CTL.

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hypertrophy [33,34]. As demonstrated elsewhere, TH can potentiate calcium-induced calcium release from sarcoplasmic reticulum, as well as cytosolic calcium concentration during systolic period by up-regulation of ryanodine receptor expression level [33–34]. In addition, up-regulation of sarcoplasmic Ca\[^{2+}\] ATPase, as well as down-regulation of phospholambam expression levels can potentiate calcium uptake by sarcoplasmic reticulum, which has been correlated to increased relaxation rate during diastole, and further calcium release in the following excitation-contraction cycle [33–34]. Consequently, hyperthyroid patients can present increased fractional shortening and ejection fraction compared to euthyroid patients [35]. In keeping with these evidences, baseline contractility and relaxation properties were potentiated by TH excess, as evidenced by increased LVSP (Table 3, P < 0.05 vs. CTL group), LVDP (Table 3, P < 0.05 vs. CTL group) max. dP/dt (Table 3, P < 0.001 vs. CTL group) and min. dP/dt (Table 3, P < 0.05 vs. CTL group).

On the other hand, there is no consensus regarding cardiac structural changes elicited by TH deficiency. In general, clinical studies have demonstrated no significant changes on LV structure of hypothyroid patients [36–38]. Even so, cardiac atrophy has been reported in few experimental clinical studies, whereas cardiac hypertrophy and dilation can be observed during the progression towards heart failure [39]. Corroborating these evidences, TH-deficient rats did not show significant changes in relative HW/BW and HW/tibia length (Table 3, P > 0.05 vs. CTL group) in comparison to euthyroid rats, although absolute HW was decreased (Table 2, P < 0.01 vs. CTL group). Furthermore, contractility (Table 3, P < 0.001 vs. CTL group) and relaxation (Table 3, P < 0.05 vs. CTL group) rates were significantly decreased by hypothyroidism, in agreement with the neuralgic role played by TH in the regulation of contractile and calcium-related proteins. Indeed, decreased fractional shortening, ejection fraction, stroke volume and cardiac output have been strictly correlated to TH-deficiency-related diseases [4,5].

After the onset of ischemia, LVEDP progressively rose in all experimental groups (Fig 1). Ischemic contracture, also known as stone heart, has been attributed to reduced ATP bioavailability. The shift towards glycolysis and the unbalanced metabolic demand result in increased lactate production and cytosolic acidification, an effect counterbalanced by the extrusion of H\(^+\) by the Na\(^+\)/H\(^+\) exchanger (NHX) [40]. As a result of increased NHX-induced sodium influx, the activity of Na\(^+/\)Ca\(^{2+}\) exchanger (NCX) also increases, as does intracellular calcium concentration and LVEDP [41–43]. Interestingly, ischemic contracture was delayed (Fig 1F), whereas post-ischemic LVEDP reached the lowest levels among hypothyroid rat hearts (Table 4, P < 0.05 vs. CTL group). In agreement with our findings, Mourouzis et al. observed decreased post-ischemic LVEDP in hypothyroid rat hearts, which resulted in increased LVDP

### Table 3. Baseline hemodynamic parameters.

| Mean baseline parameters | CTL     | MMZ     | T4       |
|--------------------------|---------|---------|----------|
| LVSP (mmHg)              | 80.34 ± 5.086 | 87.28 ± 3.898 | 98.33 ± 3.345* |
| LVEDP (mmHg)             | 11.06 ± 0.8729 | 10.50 ± 0.8639 | 9.110 ± 0.7380 |
| LVDP (mmHg)              | 70.69 ± 5.100 | 80.31 ± 4.717 | 89.22 ± 3.226* |
| Max. dP/dt (mmHg/s)      | 2709 ± 130.3 | 1825 ± 99.36*** | 3517 ± 94.76*** |
| Min. dP/dt (mmHg/s)      | -1860 ± 78.35 | -1462 ± 57.27* | -2205 ± 119.9* |

LVSP = left ventricle systolic pressure; LVEDP = left ventricle end-diastolic pressure; LVDP = left ventricle developed pressure; max. dP/dt = maximal derivative of pressure; min. dP/dt = minimal derivative of pressure. Data are mean ± S.E.M. N = 4–6 per group.

*P < 0.05, and
***P < 0.001 vs. CTL group.
In thyrotoxic rat hearts, though, LVDP recovery was significantly impaired (Table 4, $P < 0.05$ vs. CTL group) due to persistently increased LVEDP levels throughout the reperfusion period (Table 4, $P < 0.05$ vs. CTL group). Furthermore, contractility and recovery [20].

Table 4. Post-ischemic hemodynamic parameters.

| Reperfusion (60’) | CTL          | MMZ          | T4           |
|------------------|--------------|--------------|--------------|
| LVSP (mmHg)      | 95.44 ± 6.839 | 91.67 ± 5.672 | 88.91 ± 5.588 |
| LVEDP (mmHg)     | 54.21 ± 4.676  | 36.40 ± 5.631* | 77.54 ± 6.823* |
| LVDP (mmHg)      | 41.74 ± 7.203  | 55.27 ± 9.185* | 11.37 ± 1.574* |
| Max. dP/dt (mmHg/s) | 1700 ± 135.1  | 1497 ± 213.4  | 1032 ± 103.9*  |
| Min. dP/dt (mmHg/s) | -1286 ± 74.02 | -1111 ± 101.8 | -913.8 ± 79.95* |

LVSP = left ventricle systolic pressure; LVEDP = left ventricle end-diastolic pressure; LVDP = left ventricle developed pressure; max. dP/dt = maximal derivative of pressure; min. dP/dt = minimal derivative of pressure. Data are mean ± S.E.M. N = 4–6 per group.

$*P < 0.05$ vs. CTL group.
relaxation velocities reached the lowest levels among thyrotoxic rat hearts (Table 4, \( P < 0.05 \) vs. CTL group). These findings suggest that TH excess not only worsened IR-induced myocardial contracture, but also impaired the recovery of contractility properties, a condition known as myocardial stunning.

In contrast, acute and short-term exposure to T3 have been reported to improve IR-induced myocardial stunning in isolated rat hearts [18,19]. The increased glucose uptake induced by TH has been shown to elicit cardioprotection against doxorubicin toxicity, and this change might also elicit cardioprotection against IR injury, as evidenced by previous data [44]. At low doses or short-term exposure, TH might induce compensated cardiac hypertrophy and moderate increases on myocardial mechanical properties, consistent with TH positive inotropic and lusitropic effects [18]. However, long-term exposure to supraphysiological concentration of TH can elicit remarkable increases in ATP and oxygen consumption, which might turn thyrotoxic hearts more vulnerable to IR damage [45–47]. Conversely, hypothyroid hearts exhibited decreased metabolic rate which might be convenient in a condition marked by low bioavailability of oxygen and metabolic fuels such as ischemia [11,48]. Indeed, it has been postulated that the decreased myocardial conversion of T4 into T3 after AMI might be an adaptation to the new metabolic demand [49]. In keeping with this hypothesis, infarct size was decreased among hypothyroid rat hearts at approximately 45% compared to euthyroid rat hearts (Fig 2, \( P < 0.05 \) vs. CTL group). Furthermore, previous experimental data demonstrated reduced myocardial creatine kinase release in response to IR, which can be associated to decreased myocardial damage [20,21].

![Graph showing infarct size](https://doi.org/10.1371/journal.pone.0190355.g002)

**Fig 2. Measurement of infarct size.** Percentage of infarcted area in relation to total ventricular area of CTL (black box), MMZ (white box) and T4 (striped box). Representative images of TTC-stained heart slices are shown beneath each graph box. Data are expressed as Mean ± S.E.M. *\( P < 0.05 \) vs. CTL group. N = 5–6 per group.
The pathophysiological mechanisms involved in the opposite effects induced by TH excess and deficiency in the progression of IR injury remain unclear. However, pre-clinical studies have correlated the regulation of cellular metabolism and mitochondrial respiratory chain activity to reactive oxygen species (ROS) production and oxidative damage elicited by TH excess [45,50–54]. In a condition of redox imbalance, antioxidant enzyme provide defense against oxidative damage by promoting ROS clearance. Interestingly, catalase, SOD2 and GPX1 mRNA expression level (Fig 3) was down-regulated in thyrotoxic rat-hearts at 46.0% (P < 0.05 vs. CTL group), 37.6% (P > 0.05 vs. CTL group), and 51% (P > 0.05 vs. CTL group), respectively. On the other hand, SOD1 and GPX1 mRNA expression levels were up-regulated at 93.0% and 52.9% (Fig 3, P > 0.05 vs. CTL group), respectively, in hypothyroid rat-hearts. As previously demonstrated, SOD1, SOD2 and GPX knockout mice are more vulnerable to IR-induced damage and cell death [55–57]. On the other hand, overexpression of SOD and catalase have been correlated to post-ischemic mechanical improvements and decreased organ damage [58,59]. Previous data have also correlated decreased levels of GPX, coenzyme Q9 and Q10 to increased levels of lipid and proteins oxidative damage in thyrotoxic hearts [51–54]. Although myocardial oxygen level drops significantly after the onset of ischemia, residual
oxygen level remains at approximately 3–5 Torr, which enables the production of ROS at sub-lethal concentration [60–62]. Nevertheless, abrupt reestablishment of myocardial oxygen level at the onset of reperfusion further increases ROS production [60]. At high concentrations, ROS can induce oxidative damage and contribute to the opening of mitochondrial permeability transition pore and release of pro-apoptotic molecules, contributing to infarct expansion [17,63]. Conversely, experimental studies have demonstrated that mitochondrial ROS production and leak at complexes I and III are reduced in hypothyroid hearts, resulting in protection against oxidative damage [45,64,65]. Therefore, redox status might play a pivotal role in the switch between increased and decreased susceptibility to myocardial IR injury elicited by TH excess and deficiency, respectively.

It remains unclear, though, how cardiac antioxidant defense was down-regulated by thyrotoxicosis. Clinical studies have reported decreased antioxidant enzymes expression levels in hyperthyroid patients, which can be restored by antithyroid drug therapy [66,67]. However, several data have demonstrated that hyperthyroidism can be followed by increased expression levels of antioxidant enzymes, as a compensatory mechanism to increased ROS production [51–54]. Noteworthy, thyrotoxic rat-hearts were also exposed to IR in the present study, suggesting that thyrotoxicosis might be followed by decreased expression levels of antioxidant enzymes particularly in conditions of physiological challenge. In addition, these findings also suggest that secondary effects, instead direct transcriptional effect of TH receptors, might play important roles in thyrotoxicosis-induced redox imbalance. Indeed, it has been demonstrated that hyperthyroidism can elicit autonomic imbalance, severe insulin resistance and unbalanced adipokine bioavailability, including decreased circulating levels of vaspin, while visfatin and resistin levels can be increased, irrespective of changes on body weight [68–70]. Together, these metabolic abnormalities can elicit cardiomyocyte pro-inflammatory changes and mitochondrial dysfunctions, which can ultimately aggravate redox imbalance and IR damage [71,72].

Taken together, the present findings demonstrated that long-term exposure to TH excess or deficiency distinctly affected the progression of myocardial IR injury. Whereas post-ischemic recovery of mechanical properties was slightly improved and infarct size decreased by TH deficiency, myocardial stunning was worsened was increased in thyrotoxic rat-hearts. These findings were correlated to decreased expression of catalase in the condition of TH excess. However, additional studies will be necessary to investigate the role of redox imbalance and cardiac remodeling in the opposite effects induced by TH deficiency or excess in the susceptibility to IR injury.

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