BBA - General Subjects 1866 (2022) 130239

Contents lists available at ScienceDirect

BBA - General Subjects

journal homepage: www.elsevier.com/locate/bbagen

Role of thyroid hormones-induced oxidative stress on cardiovascular physiology

María Laura Barreiro Arcos *

School of Engineering and Agrarian Sciences, Pontifical Catholic University of Argentina (UCA), Institute of Biomedical Research (BIOMED-UCA-CONICET), Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Hyperthyroidism
Cardiac dysfunction
Oxidative stress
Antioxidants

ABSTRACT

Thyroid hormones (THs) play an essential role in the maintenance of cardiovascular homeostasis and are involved in the modulation of cardiac contractility, heart rate, diastolic function, systemic vascular resistance, and vasodilation. THs have actions on cardiovascular physiology through the activation or repression of target genes or the activation of intracellular signals through non-genomic mechanisms. Hyperthyroidism alters certain intracellular pathways involved in the preservation of the structure and functionality of the heart, causing relevant cardiovascular disorders. Reactive oxygen species (ROS) play an important role in the cardiovascular system, but the exacerbated increase in ROS caused by chronic hyperthyroidism together with regulation on the antioxidant system have been associated with the development of cardiovascular dysfunction. In this review, we analyze the role of THs-induced oxidative stress in the cellular and molecular changes that lead to cardiac dysfunction, as well as the effectiveness of antioxidant treatments in attenuating cardiac abnormalities developed during hyperthyroidism.

1. Introduction

Hyperthyroidism is the most frequent pathology among endocrine diseases, affecting approximately 0.8–1.3% of the population of adult women and a lower percentage of men. In countries with deficient iodine intake, the prevalence of hyperthyroidism is significantly higher [1]. About 6–15% of patients with hyperthyroidism can develop cardiovascular complications. THs has both direct and indirect actions on the cardiovascular system [2]. Patients with thyroid disease, especially those with hyperthyroidism, often have symptoms and signs indicating hemodynamic changes and contractile dysfunction of the heart. In the cardiovascular system, THs produce hypertension and cause several effects ranging from physiological cardiac hypertrophy with enhanced function to cardiac dilation and heart failure [2]. Several reports have associated hyperthyroidism with cardiac dysfunction in both experimental animals and human patients, indicating that excess THs could be a potential risk factor for heart failure [3,4]. Cardiac complications are the main cause of death in hyperthyroid patients. Return of hyperthyroid patients to euthyroidism through treatments that include antithyroid drugs, radioactive iodine therapy, or surgery, improves cardiac function and decreases the mortality [5].

THs are key regulators of cellular metabolism. THs increase the levels of many enzymes involved in the catabolism of glucose, fats, and proteins, as well as mitochondrial enzymes that participate in oxidative phosphorylation. Several studies have shown evidence that THs increase the production of ROS in most tissues, including heart tissue [6,7].

The effects of THs on the cardiovascular system are the result of their genomic actions that alter the transcription of cardiac genes and their non-genomic actions that regulate the function of structural proteins and ion channels, among others [8,9]. ROS exert essential functions in cell physiology, acting as redox signaling molecules and activating or repressing the activity of several kinases and transcription factors that participate in signaling pathways that lead to the transcription of cardiac target genes [10]. ROS regulate several physiological processes;

Abbreviations: THs, thyroid hormones; ROS, reactive oxygen species; T3, 3,3′,5′-triiodo-L-thyronine; T4, L-thyroxine; TSH, thyroid-stimulating hormone; MHC, myosin heavy chain; cTn I, cardiac troponin I; PKA, protein kinase A; AC, adenylyl ciclase; cAMP, cyclic adenosine monophosphate; SR Ca^{2+}/ATPase, sarcoplasmic reticulum calcium-dependent ATPase; SR, sarcoplasmic reticulum; G protein, guanine nucleotide-binding protein; β-AR, β-adrenergic receptor; PL, phospholamban; NOS, nitric oxide synthase; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; XO, xanthine oxidase; XDH, xanthine dehydrogenase; GSH, glutathione; GSSG, glutathione disulfide; MMPs, extracellular matrix metalloproteinases; CoQ10, coenzyme Q10; NAC, *N*-acetylcysteine.

* Corresponding author at: BIOMED-CONICET-UCA, Av. Alicia Moreau de Justo 1600 (C1107AAZ), Buenos Aires, Argentina.
E-mail address: mlbarreiro@yahoo.com.ar.

https://doi.org/10.1016/j.bbagen.2022.130239
Received 30 September 2021; Received in revised form 21 December 2021; Accepted 9 August 2022
Available online 3 September 2022

however, their excess production can cause oxidative damage to proteins, lipids and DNA, leading to cellular dysfunction. THs-induced oxidative stress differs widely between tissues, with greater effects of oxidative damage on cell types more metabolically sensitive to THs, such as the liver, muscle tissue, lymphoid tissue, and the heart [11]. A set of enzymatic and non-enzymatic antioxidants are responsible for the removal of ROS within cells, avoiding oxidative effects on macromolecules and cell damage, but when oxidative stress is high as in hyperthyroid conditions, the antioxidant system is unable to scavenge excess intracellular ROS [11].

THs-mediated transduction pathways are well documented and have been discussed by several authors [8,12–14].

The aim of this review is to summarize the effects of hyperthyroidism on cardiovascular physiology, analyzing the role of oxidative stress induced by THs on cellular and molecular changes in cardiac tissue, as well as the signaling pathways involved in these events. This review also examines the effectiveness of antioxidant treatments to attenuate abnormalities in hemodynamic parameters and cardiac function during hyperthyroidism.

This review focuses on the analysis of hyperthyroid cardiac pathology from two points of view: (1) analyzing the direct action of THs and (2) analyzing the role of ROS in cardiac dysfunction, and points out the need for adjuvant therapeutic treatments that attenuate the effects of both, HTs and ROS.

2. Hyperthyroidism and the cardiovascular system

Hyperthyroidism is an endocrine disorder characterized by an excessive activity of the thyroid gland that manifests with the overproduction and secretion of 3,3′,5′-triiodo-L-thyronine (T3) and L-thyroxine (T4). High serum levels of THs produce a negative feedback on the pituitary, causing the inhibition of thyroid-stimulating hormone (TSH) secretion [15]. The prevalence of overt hyperthyroidism is 0.2–1.3% worldwide with an incidence of 20 to 50 new cases per 100,000 inhabitants per year. In general, the incidence of hyperthyroidism is correlated with the intake of iodine in the population's diet, with higher rates of hyperthyroidism in countries with iodine deficiency, mainly due to excess thyroid nodular disease in elderly patients [16,17].

Several pathophysiological causes can lead to hyperthyroidism. The most common cause of hyperthyroidism is Graves' disease, an endocrine autoimmune disorder caused by the presence in serum of TSH receptor-stimulating autoantibodies that induce the overproduction of THs by the thyroid gland [15]. Other important causes of hyperthyroidism include toxic adenoma, toxic multinodular goiter, or thyroiditis. Toxic adenoma and toxic multinodular goiter are hyperfunctional nodules of the thyroid gland that autonomously secrete a large amount of THs, whereas thyroiditis is an inflammation of the thyroid gland caused by pregnancy, autoimmunity or viral infections that generates an excess in the storage of THs which are then released into the blood. In patients with thyroid disease, antithyroid treatments have been shown to improve both endocrine disorder and heart function. Treatment options for thyroid disease include antithyroid drugs (such as propylthiouracil, methiamazole, or carbimazole), radioactive iodine ablation, and surgery. These three options are effective for the treatment of patients with Graves' disease, however patients with toxic multinodular goiter or toxic adenoma rarely go into remission after antithyroid drug therapy and, should therefore receive radioactive iodine therapy or surgery [17–19].

The clinical manifestations of hyperthyroidism depend on the patient's age, severity, and duration of hyperthyroidism prior to treatment. The most common symptoms and signs of hyperthyroidism include nervousness, anxiety and irritability, increased sweating and heat intolerance, mild hand tremors, menstrual dysfunction, diarrhea, fatigue, muscle weakness and weight loss, thin skin and fine hair, enlarged thyroid gland (goiter), and heart problems (palpitations, arrhythmia and tachycardia with a frequency of more than 100 beats per minute). Patients with Graves' disease also usually have diffuse goiter, pretibial myxedema, ocular signs such as exophthalmos, edema of the eye conjunctiva, and eyelid retraction and, rarely, abnormalities of the fingertips and nails [15,20].

The cause of Graves' disease is multifactorial, involving genetic and environmental factors. Several studies have shown that certain polymorphisms in immunoregulatory genes such as HLA, CD40, CTLA4, PTPN22 and FCRL3 could be involved in the susceptibility of individuals to develop the disease [21]. Non-genetic risk factors such as stress, pregnancy, certain infections, smoking and female sex could induce epigenetic alterations in immunoregulatory genes, altering their expression and thus contributing to the development of Graves' disease [21]. Due to the higher prevalence of the disease in women, it is suspected that sex hormones and chromosomal factors, such as inactivation of the X chromosome, could be triggers for the disease [18].

THs are involved in the regulation of metabolism, development, tissue differentiation, survival, and cell proliferation. THs increase the basal metabolism in most tissues, regulating the biosynthesis of proteins, fats, carbohydrates and vitamins and, exerting control of body temperature. They have effects on numerous tissues including bone, muscle, and nervous and cardiovascular tissue [22].

The most adverse effects of hyperthyroidism are due to the action of THs on the cardiovascular system [23]. Hyperthyroidism can lead to various cardiac complications including increased heart rate, abnormal heart rhythm (atrial fibrillation), and congestive heart failure. The main cause of death associated with hyperthyroidism is cardiac dysfunction [24]. Several authors have studied cardiac functions and the alteration of hemodynamic parameters in patients with hyperthyroidism compared to euthyroid subjects. T3 increases the resting heart rate and the strength of myocardial contractions (positive chronotropic and inotropic effects, respectively), increases stroke volume and cardiac output, and decreases the time to induce myocardial relaxation (positive lusitropic effect).

Patients with hyperthyroidism have an accelerated heart rate (tachycardia), with a frequency greater than 90 beats per minute in resting conditions due to the action of the sympathetic nervous system that increases the slope of the action potential. During physical exercise, these patients show an increase in heart rate greater than expected in euthyroid subjects [12,25–27]. It has been shown that hyperthyroid patients have an increased sensitivity to catecholamines due to a positive regulation on the genomic expression of β-adrenergic receptors (β-AR) in the cell membrane of cardiomyocytes [28]. Administration of β-AR antagonists such as propranolol or atenolol to patients with hyperthyroidism slows the heart rate to values similar to those in euthyroid subjects [29].

Hyperthyroid patients have increased left ventricular systolic and diastolic contractility as a result of increased expression of several proteins that regulate myocardial tissue contractility and participate in the regulation of intracellular Ca^{2+} transport [12,30]. In addition, T3 reduces systemic vascular resistance by dilation of blood vessels and arterioles of the peripheral circulation through direct action on vascular smooth muscle that involves the functional alteration of Na^{+} and K^{+} channels, which leads to a decrease in smooth muscle contractility and vascular tone. A lower systemic vascular resistance produces a decrease in effective arterial volume causing an increase in renin release and activation of the angiotensin-aldosterone axis. This, in turn, induces an increase in Na^{+} reabsorption in the renal tubules causing an increase in total blood volume. By another way, T3 stimulates the secretion of erythropoietin in the kidney, which induces the production of red blood cells in the bone marrow, contributing to the increase in blood volume. Higher blood volume increases cardiac preload prior to myocardial contraction or systole, further causing an increase in cardiac output [12]. Lower systemic vascular resistance and higher stroke volume increase intraventricular pressure during systole, left ventricular blood ejection fraction, and blood flow rate to the aorta artery, allowing greater flow of oxygenated blood to tissues. This is essential in hyperthyroid conditions since the tissues have a higher oxygen requirement

due to the increase in cellular metabolism induced by THs [11]. During diastole, there is a decrease in intraventricular pressure (isovolumic relaxation) and the filling of the left ventricle through blood flow from the atrium [12].

In patients with hyperthyroidism, cardiac output (defined as the blood volume ejected by the heart per minute) is significantly high, reaching values up to 3 fold higher than in euthyroid subjects. The higher cardiac output found in hyperthyroid patients is the result of the THs-induced alteration in hemodynamic parameters, which include a decrease in systemic vascular resistance, an increase in total blood volume, an increase in heart rate, an increase in myocardial contractility and an increase in left ventricular blood ejection fraction [12].

In patients with hyperthyroidism, sinus tachycardia is the most common heart rhythm disorder. However, more than 10% of patients have atrial fibrillation, a heart rate disturbance in which the atrial chambers beat uncoordinated from the ventricular chambers. The prevalence of atrial fibrillation is higher in patients 60 years of age or older and is usually treated with anticoagulant drugs. In younger patients, atrial fibrillation caused by hyperthyroidism is rare. Atrial fibrillation increases the risk of systemic embolization and can also lead to chest pain, heart attack, or heart failure [31,32].

Several reports indicate that THs induce cardiomyocyte hypertrophy, which contributes to increased myocardial contractility and could be a compensatory mechanism to increase oxygen delivery to tissues [33]. Cardiac hypertrophy is associated with increased heart function. However, patients with cardiac hypertrophy and chronic hyperthyroidism may have reduced cardiac output, poor cardiac contractility, and symptoms and signs of heart failure. It is more frequent in hyperthyroid patients with persistent sinus tachycardia or atrial fibrillation [11]. In animals models and in human patients it has also been described that chronic hyperthyroidism can cause dilated cardiomyopathy due to the prolonged action of THs on the cardiovascular system [34].

The alteration of hemodynamic parameters during hyperthyroidism are the result of signal transduction mediated by THs that alter gene transcription pathways or modulate the activity of ion channels in the cell membranes of cardiomyocytes [12]. THs increase the cellular metabolism of most tissues, leading to increased oxygen consumption and increased ROS production [7]. ROS are highly reactive species that can react quickly and thus act as second messengers, introducing reversible oxidative changes in a lot of cellular components and thus modulating their biological activity or altering intracellular signaling pathways. ROS can also produce deleterious effects on cardiac cell physiology through oxidative changes on macromolecules [35]. The joint activation of the signaling pathways triggered by THs causes changes in the physiology of the cardiovascular system, altering its anatomy and functionality, which could lead to cardiovascular complications that are the main cause of death in hyperthyroid patients.

3. Thyroid hormone-mediated transduction signals in the cardiovascular system

The thyroid gland produces and releases mainly T4 and to a lesser extent T3 into the blood. In the blood circulation, THs are bound to transporter proteins, with only 0.05% remaining free. Only free or unbound hormone has metabolic activity [36].

T3 is the biologically active TH in cardiomyocytes and cells of other tissues. Most of T3 in the blood is produced by deiodinase-mediated monodeiodination of T4 in the liver and kidney [37]. There is no expression and activity of deiodinases in cardiomyocytes, therefore myocardial tissue acts in response to minimal fluctuations in serum T3 levels [38].

The action of THs on the cardiovascular system is mediated by transduction signals that are triggered through its binding to nuclear receptors that lead to the modulation of gene transcription (genomic effects) or through non-genomic actions [39].

THs are soluble in lipids, however, they have a low rate of diffusion through cell membranes due to their size and not being charged molecules. It has been reported that T3 is transported into myocardial cells through carriers that are dependent on ATP and the Na^+ ion gradient [38]. In addition, the monocarboxylate transporter MCT-8, whose expression is prominent in cardiac tissue, has been shown to be the specific transporter for THs in cardiomyocytes [40].

Within the nucleus, T3 binds to nuclear receptors that recognize specific sequences in DNA and activates the transcription of target genes [41]. Numerous genes have been identified that are targets of the transcriptional activation of T3, among which are structural proteins of myocardial tissue and proteins that regulate cardiac functionality. These genes include the α-myosin heavy chain (α-MHC), the sarcoplasmic reticulum calcium-dependent ATPase (SR Ca^{2+}/ATPase), the Na^+/K^+ ATPase, the β1-adrenergic receptor (β1-AR), the guanine nucleotide-binding protein (G protein), voltage-gated potassium channels and cardiac troponin I [12,42]. Using cDNA microarray, other authors have identified more than 37 genes whose expression is increased in hyperthyroid heart tissue, which include various types of proteins related to metabolism, matrix and cytoskeletal structures, transcription factors, growth factors, transporters and Ca^{2+} channels [43]. In contrast, under hyperthyroid conditions the transcriptional repression of several cardiac genes has been described, such as β-myosin heavy chain (β-MHC), phospholamban and the Na^+/Ca^{2+} exchanger [44,45]. MHC (α-and β) are contractile myofibrillar proteins of cardiac myocytes, thus the regulation of their genetic expression by THs alters the contractile properties of the myocardium. More recently it has been reported that the regulation of MHC gene expression by THs involves epigenetic modifications in chromatin histones associated with MHC genes [46]. Cardiac troponin I is also increased under hyperthyroid conditions. Its phosphorylation mediated by Protein Kinase A (PKA) contributes to an increase in the strength of cardiac contractility and the rate of myocardial relaxation [47].

The release of Ca^{2+} and its reuptake in the sarcoplasmic reticulum (SR) are processes necessary for systolic contractility and diastolic relaxation of the heart. These processes are mediated by proteins whose gene expression is regulated by THs. Active Ca^{2+} transport to the lumen of the SR is mediated by SR Ca^{2+}/ATPase whose activity is negatively regulated by non-phosphorylated phospholamban located in the reticulum membrane. The phosphorylation of phospholamban by kinases causes an increase in Ca^{2+} uptake by SR Ca^{2+}/ATPase, increasing the Ca^{2+} pool in the SR and promoting its release through Ca^{2+} channels (ryanodine receptor), a process that increases cardiac contractility [12,14]. THs regulate phospholamban activity by decreasing its genomic expression and increasing the expression of PKA, which causes its phosphorylation. In addition, an increase in the Ca^{2+} pool of the SR lumen and its release has been described in transgenic mice that do not express phospholamban, demonstrating the importance of the negative regulation of its gene expression on the ionotropic effects of the heart [48].

THs also modulate the gene expression of ion transporters in the plasma membrane of the cardiomyocyte, such as Na^+/K^+ ATPase, the Na^+/Ca^{2+} exchanger and voltage-gated potassium channels, altering the electrochemical and mechanical properties of the tissue cardiac. Regulation of gene expression of both structural proteins and ion transporters increases myocardial contractility during systole and decreases myocardial relaxation time during diastole [49].

The β-adrenergic system participates in the increase of myocardial contractility and heart rate during the hyperthyroidism. In myocardial tissue, the β1-AR is the predominant subtype, representing 70–80% of the total population of β-AR [50]. The β1-AR is coupled to a subunit of the G protein known as Gs, which activates adenylyl cyclase causing an increase in the intracellular concentration of cyclic adenosine monophosphate (cAMP) in cardiomyocytes [51,52]. Increased levels of cAMP cause the activation of PKA, which phosphorylates key Ca^{2+} transport proteins, such as the sarcolemmal L-type Ca^{2+} channel, phospholamban and the Ca^{2+} release channel of the SR. Phosphorylation of sarcolemmal

L-type Ca^{2+} channels increases intracellular Ca^{2+} concentration, while phosphorylation of both phospholamban and Ca^{2+} release channels of the SR increases Ca^{2+} turnover in the SR, causing a positive inotropic effect [52]. Genomic transduction signals induced by THs in cardiac myocytes are shown in Fig. 1.

Serum catecholamine concentrations in hyperthyroid patients are normal or low; however, a higher susceptibility to catecholamines has been described during the hyperthyroidism. This could be explained by the increase in THs-mediated genomic expression of both the β1-AR and the receptor-coupled G protein [53]. The role of the β-adrenergic system in the ionotropic and chronotropic effects of the heart mediated by THs is controversial and has been discussed by several authors. Thus, Bachman et al. have reported that transgenic mice lacking all β-adrenergic receptor subtypes showed metabolic and cardiovascular responses similar to those of wild-type mice under conditions of excess THs [54]. Ojamaa et al. have described an increase in the gene expression of the β1-AR/G protein complex, but a decrease in the gene expression of adenylyl cyclase types V and VI that could result in normal adrenergic stimulation in hyperthyroidism [51].

THs also activate other metabolic pathways that lead to increased genomic expression of nitric oxide synthase (NOS) in vascular endothelium. NOS catalyzes the synthesis of nitric oxide ($NO^{\bullet}$), responsible for vascular smooth muscle relaxation, vasodilation, and decreased peripheral vascular resistance, which contributes to increased cardiac output [55].

THs modulate the transcription of several genes involved in cardiac functionality, but they also exert non-genomic actions through the activation of multiple intracellular signaling pathways [56]. The non-genomic mechanisms triggered by THs cause short-term effects because they do not require gene transcription. THs modulate the activity of several ion channels, altering the electrophysiological properties in cardiac myocytes and increasing the ionotropic and chronotropic effects on the heart. In addition, ROS could act as mediator molecules for several of the non-genomic actions of THs through oxidation-reduction reactions on proteins involved in the intracellular signaling pathways that regulate the functionality of the cardiovascular system.

ROS are small molecules, characterized by their high reactivity and biological activity. These include superoxide anion ($O_2\bullet^-$), hydroxyl

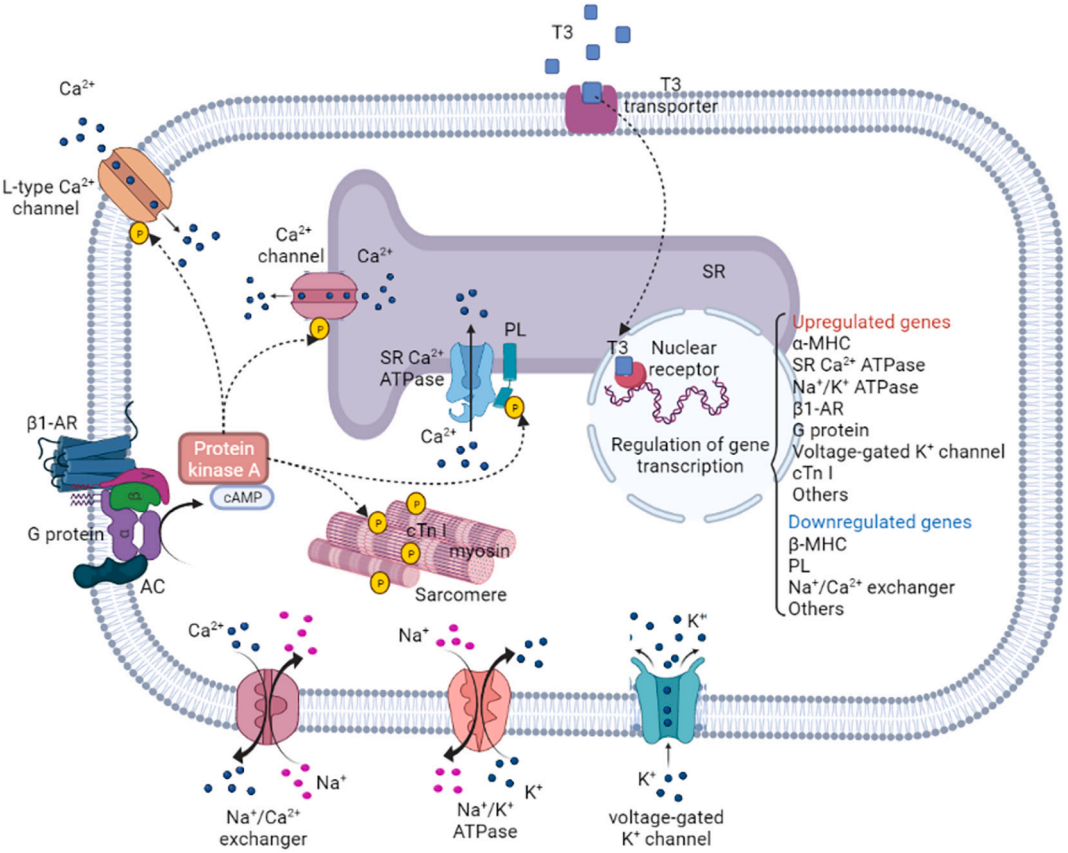

Fig. 1. Genomic signals triggered by thyroid hormones involved in the contraction-relaxation of cardiac myocytes.
T3 enters cardiac myocytes through specific transporters and binds to their nuclear receptors. Within the nucleus, the T3-nuclear receptor complex upregulates the transcription of several target genes such as α-myosin heavy chain (α-MHC) and cardiac troponin I (cTn I) in sarcomeres, Ca^{2+} ATPase of the sarcoplasmic reticulum (SR Ca^{2+} ATPase), β1-adrenergic receptor (β1-AR), G protein, Na^+/K^+ ATPase, and voltage-gated K^+ channel. In contrast, T3 downregulates the gene transcription of β-myosin heavy chain (β-MHC), phospholamban (PL) and the Na^+/Ca^{2+} exchanger, among others.
Intracellular signals that induce myocardial contraction are initiated with the entry of Ca^{2+} through the L-type Ca^{2+} channels of the sarcolemma, which induces the release of Ca^{2+} from the sarcoplasmic reticulum (SR) through the Ca^{2+} channels. Ca^{2+} released to the cytoplasm binds to cardiac troponin I (cTn I) of sarcomeres and causes contraction of cardiac myofibrils in myocytes. During relaxation, part of the cytoplasmic Ca^{2+} is extruded from the cell by the Na^+/Ca^{2+} exchanger, but most of it is recaptured by the SR through SR Ca^{2+}ATPase. SR Ca^{2+}ATPase activity is negatively regulated by non-phosphorylated PL located in the SR. T3 decreases the gene transcription of PL and induces its phosphorylation mediated by Protein Kinase A (PKA), which increases the activity of SR Ca^{2+}ATPase. The G protein coupled to the β1-AR activates adenylyl cyclase (AC) causing an increase in the intracellular concentration of cAMP that induces the activation of PKA. PKA phosphorylates the L-type Ca^{2+} channel, PL and the Ca^{2+} release channel of the SR. Phosphorylation of L-type Ca^{2+} channel increases intracellular Ca^{2+} concentration, while phosphorylation of both PL and Ca^{2+} channel increases Ca^{2+} turnover in the SR.
T3 also modulate the gene expression of several ion transporters in the plasma membrane, altering the electrochemical and mechanical properties of the tissue cardiac.

radical (•OH), hydrogen peroxide (H_2O_2), and peroxynitrite (ONOO-). ROS regulate several physiological processes including proliferation, differentiation, migration, angiogenesis, and metabolism, and play an important role in cardiovascular physiology in both physiological and pathological conditions [57].

The half-life of ROS in tissues is very brief, however due to their high reactivity with other molecules they can act as second messengers modulating intracellular signaling pathways as well as intercellular communication. The radical $O_2•^-$ can dismute to H_2O_2 spontaneously or by action of the enzyme superoxide dismutase (SOD). Hydrogen peroxide can in turn react with transition metals to form •OH with high oxidation capacity, through the Fenton reaction. The radical $O_2•^-$ can also react with nitric oxide (NO•) to form ONOO- or can react with H_2O_2 to form •OH through the Haber-Weiss reaction [58]. Reactions underlying the generation and degradation of ROS are shown in Fig. 2. Both, H_2O_2 and ONOO- can cross cell membranes and act as intracellular or intercellular messengers. The $O_2•^-$ radical could only act as a signaling molecule at the formation sites due to its brief half-life, although some authors have demonstrated the ability of $O_2•^-$ to cross the outer membrane of the mitochondria. Due to its short half-life, it is thought that the •OH radical could not act as an intracellular signaling molecule [59].

Although different ROS and their sources of production have been identified in cardiac tissue, their exact contribution to the development of heart pathologies cannot be clarified due to the high speed (of the order of milliseconds) with which oxygen radicals are interconverted in other reactive species (Fig. 2) and to their high reactivity with biological macromolecules. Thus, the damage that each oxidative species can cause in cardiac tissue will depend on its half-life, its reactivity and its ability to diffuse through cell membranes, which causes oxidative damage at distant sites of its formation. In addition, its harmful effects on tissues will depend to a great extent on the activity of the antioxidant system. Thus, the reactivity of $O_2•^-$ is weak, but it can cross biological membranes and cause damage to specific targets, it is also the initiator of chain reactions that give rise to other free radicals with greater reactivity such as •OH, H_2O_2 and ONOO-. H_2O_2, due to its greater stability and intracellular molar concentration, is the ROS with the highest oxidative activity and, although it is not a free radical, it can diffuse to other cell locations, giving rise to •OH and spreading oxidative damage. •OH has the ability to react with almost all biological molecules, mainly with polyunsaturated fatty acids in cell membranes, a process called lipid peroxidation [58,59].

There are several sources of ROS production in cardiac tissue, such as

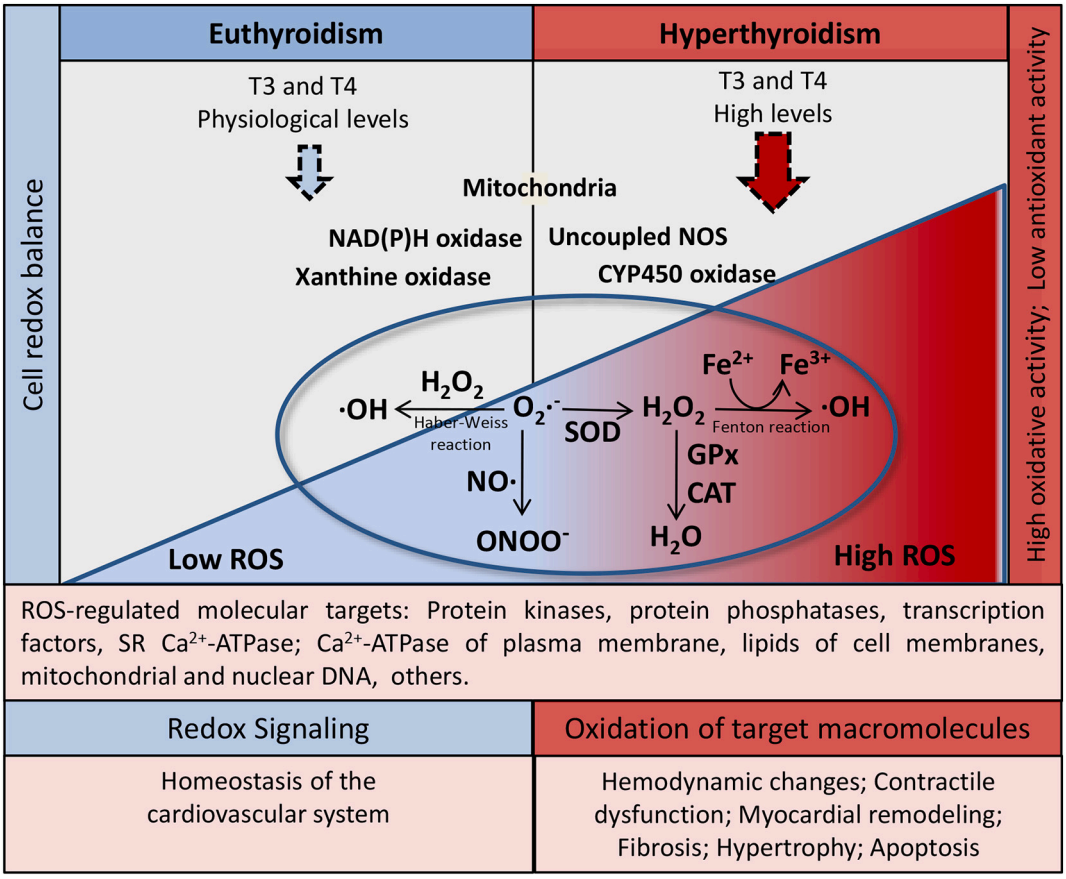

Fig. 2. ROS generation mediated by thyroid hormones and their role in the oxidative regulation of signaling molecules involved in cardiac function.
In euthyroid conditions, ROS is produced at low levels and participates in cell redox signaling, exerting an essential role in cardiac tissue homeostasis. In hyperthyroid conditions, the excess of THs increases the basal metabolism of the cells causing a higher consumption of oxygen and production of ROS. Hyperthyroidism induces oxidative stress as a result of increased oxidative enzyme activity and decreased antioxidant levels. Oxygen free radicals include the superoxide anion ($O_2•^-$), hydroxyl radical (·OH), and peroxynitrite ($ONOO^-$), which together with hydrogen peroxide (H_2O_2) are responsible for the oxidation of many biological macromolecules. The enzyme superoxide dismutase (SOD) catalyzes the conversion of $O_2•^-$ to H_2O_2, which in turn is degraded by glutathione peroxidase (GPx) or catalase (CAT). Single electron reduction of H_2O_2 can lead to the formation of ·OH, either by the Fenton reaction in the presence of iron or by the Haber-Weiss reaction when reacting with $O_2•^-$. In addition, $O_2•^-$ reacts with nitric oxide (NO·) forming the $ONOO^-$, a highly reactive molecule with biological macromolecules. Several proteins involved in cardiac functionality are susceptible to oxidation by ROS, including protein kinases, phosphatases, transcription factors, and ion transport system proteins. ROS also oxidize membrane lipids, altering their biological properties, and oxidize mitochondrial and nuclear DNA, modulating apoptotic processes.
In pathological conditions such as hyperthyroidism, ROS produces alterations in hemodynamic parameters as well as structural and functional changes in cardiac tissue, leading to dysfunction of the cardiovascular system.

mitochondria, NADPH-oxidases, NOS, xanthine oxidase, and cytochrome P450 [60]. Cardiac tissue has higher oxygen requirements than other tissues due to the high number of active mitochondria that produce chemical energy to maintain cardiac contractile function [61]. On the other hand, it has been reported that THs increase cellular metabolism in most tissues, including heart tissue, which leads to higher oxygen consumption and increased oxidative stress.

Mitochondria are both the main sites of production and the main target of ROS, which can lead to mitochondrial dysfunction and cell damage [62]. The increase in cellular metabolism induced by THs accelerates the oxidation-reduction reactions carried out in the mitochondrial respiratory chain and produces large amounts of $O_2\bullet^-$, which in turn can lead to the generation of other ROS [58]. It has been estimated that up to 5% of the oxygen consumed by the mitochondria can be used by different enzyme complexes of the respiratory chain for the production of $O_2\bullet^-$. The major source of $O_2\bullet^-$ is complex I NADH-dehydrogenase, but the semiquinone-Q radical and complex III of the respiratory chain can also produce $O_2\bullet^-$ [63].

Mitochondrial DNA encodes 13 polypeptides of the mitochondrial respiratory chain that include seven subunits of complex I, one subunit of complex III, three subunits of complex IV and two subunits of complex V [64]. However, the vast majority of mitochondrial proteins are encoded by the nuclear genome and are imported into the mitochondria through translocation systems localized in the mitochondrial membranes [65]. THs induce genomic expression of mitochondrial proteins encoded by both nuclear and mitochondrial DNA [66]. Additionally, several reports indicate the presence of T3 receptors in mitochondria suggesting a possible direct action of THs on mitochondrial functionality [67].

A decrease in the number of mitochondria per cell has been observed in hyperthyroid heart tissue [68], however, the increase in the amount and activity of the proteins of the mitochondrial respiratory chain cause a higher mitochondrial activity that leads to an increase in the production of ROS [66,69].

NAD(*P*)H-oxidase was first identified in phagocytic cells where it plays an essential role in the elimination of pathogens, but numerous reports indicate that NADPH-oxidase is expressed in the plasma membrane of numerous cell types including cardiac myocytes. Each member of the NAD(*P*)H-oxidase family contains a catalytic unit called Nox involved in the transfer of electrons from NAD(*P*)H to molecular oxygen resulting in the formation of superoxide anion. There are five isoforms of Nox that are expressed in different tissues (Nox1–5). The Nox2 and Nox4 isoforms are expressed in cardiac myocytes, endothelial cells, and fibroblasts while the Nox1 isoform is highly expressed in vascular smooth muscle cells [70]. The NAD(*P*)H oxidase proteins Nox2 and Nox4 are major enzymatic sources of ROS involved in cell signaling in cardiomyocytes [71]. Under physiological conditions, NAD(*P*)H-oxidase regulate key vascular functions through redox-sensitive signaling pathways, but under pathological conditions, NAD(*P*)H-oxidase can increase its expression and activity leading to the overproduction of $O_2\bullet^-$ [72]. Ago et.al have shown that Nox4, located mainly in the mitochondria of cardiac myocytes, is involved in enhanced ROS production and cardiac remodeling due to pressure overload [73], pointing out its role in hyperthyroidism-mediated cardiac dysfunction. In this same sense, other authors have reported the participation of NAD(*P*)H-oxidase in the induction of oxidative stress in the hyperthyroid status and its association with cardiovascular dysfunction [72].

Xanthine oxidoreductases are synthesized as NAD^+-dependent xanthine dehydrogenase (XDH), which catalyzes the synthesis of NADH and urate from xanthine, but this enzyme can be converted to O_2-dependent Xanthine Oxidase (XO), which produces $O_2\bullet^-$, H_2O_2, and urate. Both forms of the enzyme can reduce O_2 but the binding of NAD^+ to the active site of XDH inhibits the binding of O_2 to the enzyme. XDH can be converted to XO by oxidation of its sulfhydryl residues or by proteolysis. Both mechanisms induce a conformational change that blocks the access of the NAD^+ substrate to the flavin adenine dinucleotide (FAD) cofactor and changes the electrostatic environment of the active site, however these changes do not affect the binding of O_2 to the FAD, which causes a switch of substrate specificity [74]. In a context of oxidative stress induced by T3, the most probable mechanism of conversion of XDH to XO could be due to the oxidation of sulfhydryl groups and the formation of intra-subunit disulfide bonds that induce conformational changes that allow enzymatic conversion.

Several studies have shown that XO is an important source of $O_2\bullet^-$, which leads to the formation of other oxidant molecules in hyperthyroid heart tissue [75]. In this sense, some authors have shown that allopurinol, an inhibitor of XO activity, attenuates oxidative stress in the hyperthyroid status [76].

XO is expressed in the heart of many mammals, however it has been found that this enzyme has low activity in the human myocardium [77].

NAD(*P*)H cytochrome P450 reductase (CYP450) is an important source of ROS production. It is expressed mainly in the liver where it plays an essential role in drug detoxification, but its expression has been reported in the kidney, lung, adrenal and heart [78]. Several studies have shown an increased expression and activity of CYP450 in hyperthyroid hearts [43]. Reports have indicated that CYP450 participates in the progression of cardiovascular diseases, including cardiac hypertrophy and heart failure in experimental animal models, as well as in human patients. In addition, different CYP isoforms expressed in cardiac tissue can be involved in drug metabolism within the heart and influence pharmacologic efficacy [79].

Nitric oxide ($NO^\bullet$) is produced during the conversion of L-arginine to L-citrulline mediated by NOS. There are three isoforms of NOS expressed in cardiomyocytes, called endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). NOS isoforms are expressed in subcellular compartments and synthesize $NO^\bullet$ in proximity to their cellular signaling pathway [80]. $NO^\bullet$ is essential in normal cardiac physiology, regulating several processes such as coronary vasodilation, inhibition of neutrophil and platelet adhesion, and modulation of myocardial contractile function [81]. In the heart, $NO^\bullet$ inhibits L-type Ca^{2+} channels and stimulates sarcoplasmic reticulum Ca^{2+} release, modulating cardiac contractility [80]. $NO^\bullet$ has a protective role in heart disease by modulating various signaling pathways, however, it can exert toxic effects on heart tissue when it is produced in excess. Several reports have indicated an increased expression and activity of all NOS isoforms with overproduction of $NO^\bullet$ in cardiac tissue of hyperthyroid animals. In addition, increased $NO^\bullet$ production has been associated with cardiac hypertrophy and cardiac dysfunction in hyperthyroid animal models [82–84]. In the absence of L-arginine or the cofactor tetrahydrobiopterin, the NOS enzyme can uncouple and produce $O_2\bullet^-$ instead of NO• [85,86]. The $O_2\bullet^-$ can react with NO• to form the highly reactive oxidant ONOO-. Thus, the cytotoxic effects of the $O_2\bullet^-$ may be due to its direct action as an oxidant molecule or through the inactivation of the cytoprotector NO• [87].

A wide range of oxidation-reduction modifications in numerous proteins are involved in redox signaling and regulate the functionality of cardiac tissue [88]. ROS induces post-translational modifications in proteins through the oxidation of their amino acid residues cysteine. Cysteine oxidation can also occur by reacting with NO• or ONOO-. ROS-mediated oxidation of thiol groups can modulate the activity of numerous proteins in which cysteine groups are critical for substrate binding [89]. Furthermore, the oxidation of cysteine residues can cause the formation of intra- or intermolecular disulfide bonds, altering the conformational structure of proteins and their biological activity [90,91]. ROS-mediated protein oxidation is reversible and is regulated by intracellular antioxidants such as glutathione that can remove disulfide bonds in oxidized proteins and restore their activity [10].

ROS regulates the activity of several proteins such as kinases, phosphatases transcription factors and proteins of the ion transport system. Protein kinases whose activity is modulated by ROS include Ca^{2+}/Calmodulin-dependent Kinase II (CaMKII), Protein Kinase G (PKG), PKA, Protein Kinase C (PKC), Extracellular signal-Regulated Kinases

(ERK), Phospholipid-dependent kinase-1 (PDK), tyrosine kinase Src, Phosphatidylinositol-3 kinase (PI3K), Akt kinase, p38 MAP kinase, and c-Jun N-terminal kinase (JNK). It has not been demonstrated whether ROS exerts a direct effect on kinase activation or an indirect effect through inhibition of phosphatase activity [59,71,92,93]. Some authors have described the ROS-mediated regulation of several phosphatases such as Protein Phosphatase 1 (PP1) and Phosphatase and Tensin homolog (PTEN), which could participate in intracellular signaling pathways triggered by oxidative stress in cardiac tissue. ROS also modulates the activity of several transcription factors, including Nuclear Factor NF-κB, Nuclear factor erythroid-derived 2-like (Nrf-2), Hypoxia-Inducible Factor 1 (HIF-1) and Signal Transducer and Activator of Transcription (STAT3/STAT5). Protein kinases, phosphatases, and transcription factors regulated by redox signaling participate in the transcription of several genes involved in cardiac physiology, and that contribute to hypertrophy, metabolic changes, cytoprotection, and angiogenesis [59,71,92,93].

In addition, ROS are involved in the regulation of ion transport through their effects on ion channels and pumps. ROS have a biphasic effect on Ca^{2+} transport in the cardiac myocyte. Thus, ROS activate the SR Ca^{2+}/ATPase, which causes the reduction of Ca^{2+} levels in the cytoplasm, but ROS also inactive the Ca^{2+}-ATPase of the plasma membrane and open the Transient Receptor Potential-Melastatin-2 channels (TRPM2), which induce the loading of Ca^{2+} in the cell [59,94].

Physiological levels of ROS control cell proliferation and angiogenesis [95]. Redox signaling is also involved in protection against ischemia and hypoxia, however, ROS levels that exceed physiological values can induce apoptosis or autophagy, and remodeling of cardiac tissue with the appearance of arrhythmias and contractile dysfunction of the heart [96].

4. Role of the antioxidant system in the regulation of oxidative stress mediated by thyroid hormones

Redox signaling is involved in the maintenance of the physiological homeostasis of the heart by regulating several key proteins necessary for the normal function of cardiac tissue, however the excessive production of ROS mediated by THs causes harmful effects on the functionality of the cardiomyocytes. Cells have efficient enzymatic and non-enzymatic defense systems to counteract the action of ROS and, thereby, protect cells from oxidative damage.

The cellular redox state is defined as the balance between the production of ROS and its removal by the antioxidant system. Several antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), as well as other non-enzymatic antioxidants (vitamin E, ascorbic acid, β-carotene, ubiquinone and glutathione) are involved in the scavenger or degradation of ROS to non-toxic molecules [58,97]. Oxidative stress occurs when ROS production is increased with respect to the content of enzymes and molecules with antioxidant capacity.

SOD enzymes catalyze the conversion of $O_2\bullet^-$ to H_2O_2 and O_2. The three isoforms of SOD (extracellular membrane bound-EcSOD, MnSOD, and CuZnSOD) are expressed in heart tissue [98]. While EcSOD is the major extracellular $O_2\bullet^-$ scavenger, the other SOD isoenzymes are heterogeneously expressed in cardiac tissue and scavenge $O_2\bullet^-$ within cells [99].

GPx is a key enzyme that catalyzes the oxidation of glutathione (GSH) to glutathione disulfide (GSSG) using H_2O_2. GPx plays an essential role in antioxidant defense against oxidative stress induced by THs in cardiac tissue. GPx not only removes H_2O_2 and hydroperoxides, but also prevents the formation of other highly reactive molecules such as the $^{\bullet}OH$. CAT enzyme catalyzes the conversion of H_2O_2 to O_2 and H_2O. GPx has a higher affinity and capacity to reduce H_2O_2 than CAT and is expressed in relatively high amounts in cardiac tissue, mainly in the cytosol and mitochondria [100,101].

Although all the evidence concludes that hyperthyroidism induces oxidative stress in tissues, both the expression and activity of antioxidant enzymes and the content of endogenous antioxidant molecules in hyperthyroid heart tissue is controversial [7,102–104]. Some authors have reported an increased activity of MnSOD and CuZnSOD in hyperthyroid heart tissue, while other authors have reported no changes or a decreased activity of these enzymes [11,103,105]. CAT activity has been reported to be unchanged or decreased in hyperthyroid hearts [11,105]. These discrepancies have been attributed to differences in the treatment period, the iodothyronine used (T3 or T4) or the age of the animals at the beginning of treatment [7]. Regarding this last point, it is known that oxidative stress in tissues increases with age, therefore a modulation of antioxidant activity with aging is to be expected [106].

Several authors have reported that oxidative stress can induce the expression of antioxidant enzymes in several cell types, including cardiac tissue, as a compensatory mechanism to counteract the increase in ROS. Thus, they have described the participation of the transcription factor Nrf-2 in the regulation of gene expression of a variety of redox-sensitive proteins, which are essential for antioxidant defense, as well as for cell survival. ROS oxidize the sulfhydryl groups of Keap-1, a cytoplasmic inhibitor of Nrf-2, causing the translocation of Nrf-2 to the cell nucleus, where it induces the gene transcription of antioxidant enzymes [97,107].

Glutathione, a low molecular weight thiol, is the main antioxidant molecule involved in the scavenger of ROS. Intracellular GSH and GSH/GSSG ratio were decreased in cardiac tissue from hyperthyroid animals, which is evidence of tissue oxidative stress [82,105]. Several studies have indicated alterations in the plasma levels of ascorbic acid, vitamin E and coenzyme Q10 in patients with hyperthyroidism, however the results are not conclusive. It is hypothesized that the disparity in the results could be due to the incorporation of these antioxidants in the diet and not due to the regulation of oxidative metabolism in hyperthyroidism [108]. A decreased activity of the enzymatic antioxidant system or reduced concentrations of endogenous antioxidants may further contribute to the increase in ROS mediated by hyperthyroidism [58]. Thus, when the antioxidant system is not sufficient to eliminate excess ROS induced by hyperthyroidism, oxidative stress exerts harmful effects on the functional and structural integrity of cardiac tissue. ROS can react with biological macromolecules such as lipids, proteins, and nucleic acids, causing irreversible cell damage and apoptosis, which has been implicated in a wide range of cardiovascular diseases including hyperthyroidism [7,109].

The mitochondria is one of the main sources of ROS production in the myocardium. Excessive ROS production in mitochondria causes significant damage to mitochondrial DNA and functional loss of the organelle, leading to increased ROS generation and heart tissue dysfunction [58,110,111]. On the contrary, oxidative damage has not been observed in genomic DNA, which can be attributed to the elimination of ROS by the cytosolic antioxidant system before its arrival in the nucleus, to the DNA structure in nucleosomes that protect from ROS attack or to the DNA repair system of the cell nucleus [112–114]. Polyunsaturated fatty acids are especially susceptible to attack by ROS. Several evidences indicate an increased lipid peroxidation in hyperthyroid heart tissue [104,105,115,116]. Some authors point out that this increase is higher in older animals than in young animals, which could be explained by the regulation of the antioxidant system mediated by aging [117]. Other authors have reported increased protein carbonylation, a type of protein oxidation, in the hearts of hyperthyroid animals [105].

ROS oxidize membrane phospholipids and ion channels, causing electrophysiological alterations in the heart [118]. ROS also oxidize the proteins involved in excitation-contraction coupling, altering their activity and causing contractile dysfunction in the myocardium of hyperthyroid hearts. Oxidative stress plays an important role in the regulation of cardiac myocyte growth and survival. In addition, several reports suggest that the increase in ROS induced by THs is associated with myocardial hypertrophy, myocardial infarction and heart failure [119].

ROS activate a broad variety of protein kinases and transcription

factors that induce proliferation of cardiomyocytes and fibroblasts and activate extracellular matrix metalloproteinases (MMPs), leading to hypertrophy and remodeling of cardiac tissue. Thus, Tsutsui et al. have reported that the oxidative stress induced by THs increases the expression of the IGF-1 receptor in the cardiomyocyte membrane. Furthermore, they have reported that the binding of the IGF-1 receptor with its ligand stimulates cardiomyocyte proliferation via Akt and ERK signaling [58]. Other authors have also reported the involvement of Akt-1 in THs-induced cardiac hypertrophy and have suggested the redox activation of the Akt-1 and JUN/FOS signaling pathways with H_2O_2 acting as a possible intracellular messenger in the adaptive response to experimental hyperthyroidism [82]. On the other hand, the modulation of ROS-mediated signaling pathways and their effects on cell physiology could be dependent on the intracellular levels of ROS. Thus, low levels of H_2O_2 would be associated with physiological processes such as protein synthesis, while high levels of ROS would induce apoptosis and remodeling of cardiac tissue. These biological effects would respond to the differential activation of protein kinases and signaling pathways [120].

The oxidation of macromolecules such as proteins, lipids and DNA can alter their biological function, causing cell damage and triggering signaling pathways that lead to apoptosis [121]. Some authors have reported an increase in pro-apoptotic proteins and caspase activity in hypertrophied cardiac tissue with impaired cardiac function, but apoptosis has not been reported when cardiac tissue function is still preserved [122]. In addition, ROS cause oxidation and breakage of DNA strands, which activates the nuclear enzyme poly (ADP-ribose) polymerase-1 (PARP-1) involved in the expression of inflammatory mediators, which facilitate the progression of cardiac remodeling [123]. ROS can also induce the expression of MMPs through the activation of transcription factors NF-kappaB and Activator Protein-1 (AP-1). MMPs are a family of proteolytic enzymes involved in tissue remodeling processes, such as proliferation, cell migration, invasion, and apoptosis [124].

The increase in ROS mediated by THs induces relevant functional and structural changes in cardiac tissue, which leads to alteration of myocardial contractile function, hypertrophy, interstitial fibrosis and apoptosis of cardiomyocytes, and can cause heart failure [125].

5. Effects of antioxidant treatment on cardiac dysfunction developed in hyperthyroidism

Clinical trials and studies in experimental hyperthyroidism models have described the effect of treatment with antioxidant molecules or inhibitors of several ROS-producing sources on cardiac dysfunction.

Vitamin E (α-tocopherol) is a lipophilic molecule that interacts with the lipids of cell membranes, where it exerts an antioxidant action, eliminating ROS and thus inhibiting both lipid peroxidation and protein oxidation. The protective effect of vitamin E on lipid peroxidation in hyperthyroid hearts has been described [126]. Some authors have shown that vitamin E plays an essential role in the elimination of ROS from the mitochondria. Thus, these authors have reported that vitamin E reacts with peroxyl radicals faster than the molecules of polyunsaturated fatty acids, protecting mitochondrial membranes from oxidative damage. In addition, they found that supplementation with vitamin E reduces the levels of ROS induced by THs in the mitochondria of hyperthyroid animals [62]. Some authors have suggested that vitamin E or curcumin could interact with Keap-1 (Nrf-2 inhibitor), which would lead to the activation of the NRf-2 signaling pathway and the transcription of antioxidant enzymes, reducing oxidative stress in heart tissue [127]. Vitamin E scavenges ROS, preventing the oxidation of ionic channels and pumps, proteins involved in cardiac excitation-contraction and lipids of cell membranes. Several authors have demonstrated in experimental models that the administration of vitamin E partially attenuates the THs-mediated alteration of electrical activity in cardiac tissue [128]. Thus, they have described a decrease in heart rate and a shortening of the duration of the action potential in hyperthyroid animals treated with vitamin E [129]. Although there is some evidence of the effect of vitamin E on cardiac activity, its action on the improvement of myocardial hypertrophy is not conclusive. While some authors have reported that vitamin E does not induce effects on myocardial hypertrophy [130], others have pointed out a decrease in heart weight gain induced by hyperthyroidism and have proposed that vitamin E would be useful to prevent cardiac remodeling during stress oxidative [84,130]. Studies on the therapeutic use of vitamin E are controversial and some authors have questioned its effectiveness in improving heart disease. Short-term dietary supplementation with vitamin E reduced the levels of oxidative stress biomarkers and showed improvements in cardiac function. However, when vitamin E is administered in high doses or for long-term, it worsens cardiovascular function and induces heart failure, which may be associated to the formation of the pro-oxidative vitamin E radical [57,130].

Coenzyme Q10 (CoQ10) or ubiquinone is part of enzyme complexes in the mitochondrial electron transport chain and is a powerful antioxidant. Several reports have indicated that CoQ10 treatment significantly inhibits structural disorganization and mitochondrial damage in hyperthyroid heart tissue. Furthermore, the administration of CoQ10 decreased the THs-mediated increase in iNOS and eNOS expression levels and oxidative stress in cardiomyocytes [131]. Clinical studies in patients showed that CoQ10 supplementation reduces oxidative stress, improves endothelial dysfunction, and decreases mortality from cardiovascular causes. In addition, CoQ10 supplementation for prolonged periods is safe and well-tolerated [132].

N-acetylcysteine (NAC) is a compound that contains a thiol group, that can act both as precursor to reduced glutathione synthesis or as direct scavenger of ROS. Glutathione is the main thiol compound involved in the intracellular ROS scavenger, but under some conditions where there is a significant decrease in glutathione, NAC can act as a direct scavenger of oxidizing species [133]. NAC reduces the peroxidation in lipids and proteins due to its high capacity to react with free radicals and because it causes the breaking of disulfide bonds in oxidized macromolecules. Several authors have indicated that treatment with NAC in hyperthyroid animals partially reduces the chronotropic effects induced by THs on cardiac tissue, but has no effect on the reversal of cardiac hypertrophy [129].

Another antioxidant is Vitamin C, also known as L-ascorbic acid. Humans are unable to synthesize vitamin C, so they must incorporate it through the consumption of citrus fruits and vegetables [134]. Several authors have reported that vitamin C can decrease lipid peroxidation reducing oxidative damage in hyperthyroid heart tissue and can improve some altered hemodynamic parameters during hyperthyroidism [57,135,136]. Vitamin C also participates in the regeneration of other antioxidants such as vitamin E [137].

Despite the crucial role of ROS in the pathogenesis of cardiovascular disease, dietary supplementation with antioxidants has not shown the expected therapeutic effects.

Several authors have analyzed the effect of specific inhibitors of different ROS-producing sources on the alteration of hemodynamic parameters and cardiac hypertrophy induced by THs. Thus, the effect of allopurinol (XO inhibitor), apocinin or pravastatin (NADPH-oxidase inhibitors), L-NIO (NOS inhibitor) or Mito-TEMPO (mitochondria-targeted antioxidant) on cardiac pathophysiology has been described. These inhibitors showed a partial reversal of lipid and protein oxidation and an improvement in the cardiac function of hyperthyroid hearts, although they were ineffective in reducing the structural alterations of the cardiac tissue induced by hyperthyroidism. Only pravastatin decreased the size of cardiomyocytes although it was unable to reverse the THs-induced increase in heart weight [11,84,138–141].

6. Concluding remarks and perspectives

THs induce alterations in hemodynamic parameters and in the

functionality of the cardiovascular system through genomic and non-genomic actions. The genomic actions of T3 involve the transcriptional induction or repression of target genes that encode structural and functional proteins essential for the normal function of cardiac tissue. However, T3 can act through non-genomic mechanisms, exerting direct effects on the activity of ion channels and pumps or, increasing cellular metabolism and ROS production in cardiac tissue. Physiological levels of ROS are necessary for homeostasis of cardiovascular tissue; however, the THs-mediated increase in ROS causes oxidative tissue damage and cardiovascular dysfunction. Oxidative stress has been reported in hyperthyroid heart tissue due to the imbalance between the production of oxidative species and the activity of the antioxidant system. ROS causes the oxidation of lipids and proteins involved in the transport of ions or in processes of excitation-contraction of the cardiac muscle, altering the electrophysiological mechanisms in the heart. In addition, ROS can also modulate the activity of protein kinases and transcription factors that participate in signaling pathways that lead to apoptosis, tissue remodeling, or hypertrophy of cardiac tissue.

Due to the multiple sites of action and the complexity of the intracellular signals triggered by THs, I believe that therapeutic strategies that focus on the joint inhibition of the aberrant genomic and non-genomic actions of THs will be more effective in attenuating damage to cardiovascular tissue caused by hyperthyroidism.

In patients with hyperthyroidism, antithyroid treatments have been reported to improve both endocrine disorder and cardiac function. Thionamide antithyroid drugs such as propylthiouracil, methiamazole, or carbimazole are effective in reverting the plasma levels of THs to euthyroid values, and they also exert antioxidant effects [142]. However, antithyroid treatment must be accompanied by adjuvant therapies that help reduce oxidative stress, attenuating oxidative damage in cardiovascular tissue. In this sense, the efficacy of therapeutic strategies that reduce oxidative stress has been analyzed, ranging from the use of inhibitors of ROS production sources to dietary supplementation with antioxidants.

Since the physiological levels of ROS are essential in the maintenance of cardiac homeostasis, the activity of oxidative enzymes cannot be completely blocked, therefore inhibitors that partially block the enzyme activity have been considered. In this context, the efficacy of inhibitors of p47phox, a subunit that is required for the complete activation of the NOX1 and NOX2 isoforms of NADPH oxidase, has been demonstrated. Pharmacological inhibition of p47phox decreases enzyme activity, but does not completely inhibit ROS production [143]. NADPH oxidase is an attractive target for partial pharmacological inhibition given its relevance as a source of ROS production in cardiac tissue. However, improvements in efficacy, pharmacokinetics and specificity are required prior to clinical trials. Pharmacological inhibitors of other ROS-producing sources in cardiac tissue are under study.

Despite the crucial role that ROS play in the pathogenesis of cardiovascular disease in hyperthyroid conditions, dietary supplementation with antioxidants has not shown the expected beneficial effects.

Vitamins E and C showed limited effects on the reversal of THs-mediated oxidative damage in cardiac tissue. A explanation is that these antioxidant compounds are not specifically targeted at ROS formation sites, such as the mitochondria, mainly involved in the pathogenesis of hyperthyroid cardiovascular disease. Furthermore, vitamins could react much more slowly with ROS than these free radicals could react with biological macromolecules to induce oxidative damage [143]. In recent years, significant advances have been made in the delivery of antioxidants to the mitochondria. New strategies focus on the conjugation of antioxidants to lipophilic cationic molecules (such as triphenylphosphonium or MitoQ) that, due to their positive charge and the negative potential gradient of the inner mitochondrial membrane, are concentrated within the mitochondria, decreasing the production of ROS and mitochondrial damage without interfering with the function of the respiratory chain. The delivery of antioxidants to specific ROS production sites in animal models of cardiovascular disease has shown promising results, however more studies must be performed to demonstrate the efficacy of these treatments.

Credit author statement

María Laura Barreiro Arcos: Conceptualization, funding acquisition and writing-original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the National Agency for Scientific and Technical Promotion (Grant PICT-2016-0696).

References

[1] P.N. Taylor, D. Albrecht, A. Scholz, G. Gutierrez-Buey, J.H. Lazarus, C.M. Dayan, O.E. Okosieme, Global epidemiology of hyperthyroidism and hypothyroidism, Nat. Rev. Endocrinol. 14 (2018) 301–316, https://doi.org/10.1038/nrendo.2018.18.

[2] H. Vargas-Uricoechea, A. Bonelo-Perdomo, C.H. Sierra-Torres, Effects of thyroid hormones on the heart, Clin. Investig. Arterioscler. 26 (2014) 296–309, https://doi.org/10.1016/j.arteri.2014.07.003.

[3] J.A. Kuzman, T.A. Thomas, K.A. Vogelsang, S. Said, B.E. Anderson, A.M. Gerdes, Effects of induced hyperthyroidism in normal and cardiomyopathic hamsters, J. Appl. Physiol. 99 (2005) 1428–1433.

[4] S.Y. Sohn, E. Lee, M.K. Lee, J.H. Lee, The association of overt and subclinical hyperthyroidism with the risk of cardiovascular events and cardiovascular mortality: meta-analysis and systematic review of cohort studies, Endocrinol. Metab. 35 (2020) 786–800, https://doi.org/10.3803/enm.2020.728.

[5] F. Osman, M.D. Gammage, J.A. Franklyn, Hyperthyroidism and cardiovascular morbidity and mortality, Thyroid 12 (2002) 483–487, https://doi.org/10.1089/105072502760143854.

[6] M. Messarah, M. Saoudi, A. Boumendjel, M.S. Boulakoud, A.E. Feki, Oxidative stress induced by thyroid dysfunction in rat erythrocytes and heart, Environ. Toxicol. Pharmacol. 31 (2011) 33–41, https://doi.org/10.1016/j.etap.2010.09.003.

[7] P. Venditti, S. Di Meo, Thyroid hormone-induced oxidative stress, Cell. Mol. Life Sci. 63 (2006) 414–434, https://doi.org/10.1007/s00018-005-5457-9.

[8] M. Giammanco, C.M. Di Liegro, G. Schiera, I. Di Liegro, Genomic and non-genomic mechanisms of action of thyroid hormones and their catabolite 3,5-diiodo-L-thyronine in mammals, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21114140.

[9] A. Iordanidou, M. Hadzopoulou-Cladaras, A. Lazou, Non-genomic effects of thyroid hormone in adult cardiac myocytes: relevance to gene expression and cell growth, Mol. Cell. Biochem. 340 (2010) 291–300, https://doi.org/10.1007/s11010-010-0430-9.

[10] K.M. Holmström, T. Finkel, Cellular mechanisms and physiological consequences of redox-dependent signalling, Nat. Rev. Mol. Cell Biol. 15 (2014) 411–421, https://doi.org/10.1038/nrm3801.

[11] M.T. Elnakish, A.A.E. Ahmed, P.J. Mohler, P.M.L. Janssen, Role of oxidative stress in thyroid hormone-induced cardiomyocyte hypertrophy and associated cardiac dysfunction: an undisclosed story, Oxidative Med. Cell. Longev. 2015 (2015) 854265, https://doi.org/10.1155/2015/854265.

[12] I. Klein, K.N. Ojamaa, Thyroid hormone and the cardiovascular system, N. Engl. J. Med. 344 (2001) 501–509, https://doi.org/10.1056/nejm200102153440707.

[13] J.H. Bassett, C.B. Harvey, G.R. Williams, Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions, Mol. Cell. Endocrinol. 213 (2003) 1–11, https://doi.org/10.1016/j.mce.2003.10.033.

[14] A. Jabbar, A. Pingitore, S.H. Pearce, A. Zaman, G. Iervasi, S. Razvi, Thyroid hormones and cardiovascular disease, Nat. Rev. Cardiol. 14 (2017) 39–55, https://doi.org/10.1038/nrcardio.2016.174.

[15] T.J. Smith, L. Hegedüs, Graves' disease, N. Engl. J. Med. 375 (2016) 1552–1565, https://doi.org/10.1056/nejmra1510030.

[16] P.N. Taylor, D. Albrecht, A. Scholz, G. Gutierrez-Buey, J.H. Lazarus, C.M. Dayan, O.E. Okosieme, Global epidemiology of hyperthyroidism and hypothyroidism, Nat. Rev. Endocrinol. 14 (2018) 301–316, https://doi.org/10.1038/nrendo.2018.18.

[17] D.S. McLeod, D.S. Cooper, The incidence and prevalence of thyroid autoimmunity, Endocrine 42 (2012) 252–265.

[18] S. De Leo, S.Y. Lee, L.E. Braverman, Hyperthyroidism, Lancet 388 (2016) 906–918, https://doi.org/10.1016/S0140-6736(16)00278-6.

[19] I. Singh, J.M. Hershman, Pathogenesis of hyperthyroidism, Compr. Physiol. 7 (2016) 67–79, https://doi.org/10.1002/cphy.c160001.

[20] J.L. Johnson, J.V. Felicetta, Hyperthyroidism: a comprehensive review, J. Am. Acad. Nurse Pract. 4 (1992) 8–14, https://doi.org/10.1111/j.1745-7599.1992.tb01105.x.
[21] M. Marinò, F. Latrofa, F. Menconi, L. Chiovato, P. Vitti, Role of genetic and non-genetic factors in the etiology of Graves' disease, J. Endocrinol. Investig. 38 (2015) 283–294.
[22] R. Mullur, Y.Y. Liu, G.A. Brent, Thyroid hormone regulation of metabolism, Physiol. Rev. 94 (2014) 355–382, https://doi.org/10.1152/physrev.00030.2013.
[23] L. Sabatino, A. Colantuoni, G. Iervasi, Is the vascular system a main target for thyroid hormones? From molecular and biochemical findings to clinical perspectives, Curr. Vasc. Pharmacol. 3 (2005) 133–145, https://doi.org/10.2174/1570161053586921.
[24] N.A. Samuel, J.J. Cuthbert, O.I. Brown, S. Kazmi, J.G.F. Cleland, A.S. Rigby, A. L. Clark, Relation between thyroid function and mortality in patients with chronic heart failure, Am. J. Cardiol. 139 (2021) 57–63, https://doi.org/10.1016/j.amjcard.2020.10.034.
[25] T. Feldman, K.M. Borow, D.H. Sarne, A. Neumann, R.M. Lang, Myocardial mechanics in hyperthyroidism: importance of left ventricular loading conditions, heart rate and contractile state, J. Am. Coll. Cardiol. 7 (1986) 967–974, https://doi.org/10.1016/s0735-1097(86)80213-3.
[26] S. Danzi, I. Klein, Thyroid hormone and the cardiovascular system, Med. Clin. N. Am. 96 (2012) 257–268, https://doi.org/10.1016/j.mcna.2012.01.006.
[27] M. Zhou, J. Tan, J. Liu, L.X. Yin, S.J. Wang, L. Xie, Z.Y. Guo, W.J. Zhang, Changes in left ventricular function and contractile homogeneity in young adults with newly diagnosed hyperthyroidism due to Graves' disease, J. Clin. Ultrasound 48 (2020) 216–221, https://doi.org/10.1002/jcu.22802.
[28] A.W. Fox, E.N. Juberg, J.M. May, R.D. Johnson, P.W. Abel, K.P. Minneman, Thyroid status and adrenergic receptor subtypes in the rat: comparison of receptor density and responsiveness, J. Pharmacol. Exp. Ther. 235 (1985) 715–723.
[29] G. Amos, D. Kerr, C. Sernia, L. Brown, Beta-adrenoceptor antagonism and the hyperthyroid rat heart, J. Cardiovasc. Pharmacol. 24 (1994) 336–343, https://doi.org/10.1097/00005344-199424020-00021.
[30] S. Vinzio, O. Morel, J.L. Schlienger, B. Goichot, Cellular mechanisms of thyroid hormone action, Presse Med. 34 (2005) 1147–1152, https://doi.org/10.1016/s0755-4982(05)84141-7.
[31] T. Shimizu, S. Koide, J.Y. Noh, K. Sugino, K. Ito, H. Nakazawa, Hyperthyroidism and the management of atrial fibrillation, Thyroid 12 (2002) 489–493, https://doi.org/10.1089/105072502760143863.
[32] P.H. Chan, J. Hai, C.Y. Yeung, G.Y. Lip, K.S. Lam, H.F. Tse, C.W. Siu, Benefit of anticoagulation therapy in hyperthyroidism-related atrial fibrillation, Clin. Cardiol. 38 (2015) 476–482, https://doi.org/10.1002/clc.22427.
[33] M.J. Legato, L.A. Mulieri, N.R. Alpert, The ultrastructure of myocardial hypertrophy: why does the compensated heart fail? Eur. Heart J. 5 (1984) 251–269, https://doi.org/10.1093/eurheartj/5.suppl_f.251.
[34] L.E. Wildemberg, L.L. Sousa, L.P. Fonseca, M.V. Souza, Reversible dilated cardiomyopathy related to hyperthyroidism, Arq. Bras. Endocrinol. Metabol. 51 (2007) 1533–1538, https://doi.org/10.1590/s0004-27302007000900018.
[35] M.L. Circu, T.Y. Aw, Reactive oxygen species, cellular redox systems, and apoptosis, Free Radic. Biol. Med. 48 (2010) 749–762, https://doi.org/10.1016/j.freeradbiomed.2009.12.022.
[36] T.M. Ortiga-Carvalho, M.I. Chiamolera, C.C. Pazos-Moura, F.E. Wondisford, Hypothalamus-pituitary-thyroid Axis, Compr. Physiol. 6 (2016) 1387–1428, https://doi.org/10.1002/cphy.c150027.
[37] S. Mondal, K. Raja, U. Schweizer, G. Mugesh, Chemistry and biology in the biosynthesis and action of thyroid hormones, Angew. Chem. Int. Ed. Eng. 55 (2016) 7606–7630, https://doi.org/10.1002/anie.201601116.
[38] M.E. Everts, F.A. Verhoeven, K. Bezstarosti, E.P. Moerings, G. Hennemann, T. J. Visser, J.M. Lamers, Uptake of thyroid hormones in neonatal rat cardiac myocytes, Endocrinology 137 (1996) 4235–4242, https://doi.org/10.1210/endo.137.10.8828482.
[39] A. Iordanidou, M. Hadzopoulou-Cladaras, A. Lazou, Non-genomic effects of thyroid hormone in adult cardiac myocytes: relevance to gene expression and cell growth, Mol. Cell. Biochem. 340 (2010) 291–300, https://doi.org/10.1007/s11010-010-0430-9.
[40] E.C.H. Friesema, S. Ganguly, A. Abdalla, J.E. Manning Fox, A.P. Halestrap, T. J. Visser, Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter, J. Biol. Chem. 278 (2003) 40128–40135, https://doi.org/10.1074/jbc.m300909200.
[41] P.M. Yen, S. Ando, X. Feng, Y. Liu, P. Maruvada, X. Xia, Thyroid hormone action at the cellular, genomic and target gene levels, Mol. Cell. Endocrinol. 246 (2006) 121–127, https://doi.org/10.1016/j.mce.2005.11.030.
[42] W.H. Dillmann, Biochemical basis of thyroid hormone action in the heart, Am. J. Med. 88 (1990) 626–630, https://doi.org/10.1016/0002-9343(90)90530-q.
[43] K. De, G. Ghosh, M. Datta, A. Konar, J. Bandyopadhyay, D. Bandyopadhyay, S. Bhattacharya, A. Bandyopadhyay, Analysis of differentially expressed genes in hyperthyroid-induced hypertrophied heart by cDNA microarray, J. Endocrinol. 182 (2004) 303–314, https://doi.org/10.1677/joe.0.1820303.
[44] K. Ojamaa, J.D. Klemperer, S.S. MacGilvray, I. Klein, A. Samarel, Thyroid hormone and hemodynamic regulation of beta-myosin heavy chain promoter in the heart, Endocrinology 137 (1996) 802–808, https://doi.org/10.1210/endo.137.3.8603588.
[45] Y. Kimura, K. Otsu, K. Nishida, T. Kuzuya, M. Tada, Thyroid hormone enhances Ca^{2+} pumping activity of the cardiac sarcoplasmic reticulum by increasing Ca^{2+} ATPase and decreasing phospholamban expression, J. Mol. Cell. Cardiol. 26 (1994) 1145–1154, https://doi.org/10.1006/jmcc.1994.1133.
[46] F. Haddad, W. Jiang, P.W. Bodell, A.X. Qin, K.M. Baldwin, Cardiac myosin heavy chain gene regulation by thyroid hormone involves altered histone modifications, Am. J. Physiol. Heart Circ. Physiol. 299 (2010) H1968–H1980, https://doi.org/10.1152/ajpheart.00644.2010.
[47] J. Layland, R.J. Solaro, A.M. Shah, Regulation of cardiac contractile function by troponin I phosphorylation, Cardiovasc. Res. 66 (2005) 12–21, https://doi.org/10.1016/j.cardiores.2004.12.022.
[48] E. Kiss, A.G. Brittsan, I. Edes, I.L. Grupp, G. Grupp, E.G. Kranias, Thyroid hormone-induced alterations in phospholamban-deficient mouse hearts, Circ. Res. 83 (1998) 608–613, https://doi.org/10.1161/01.res.83.6.608.
[49] W.H. Dillmann, Cellular action of thyroid hormone on the heart, Thyroid 12 (2002) 447–452, https://doi.org/10.1089/105072502760143809.
[50] S.W. Bahouth, Thyroid hormones transcriptionally regulate the beta 1-adrenergic receptor gene in cultured ventricular myocytes, J. Biol. Chem. 266 (1991) 15863–15869.
[51] K. Ojamaa, I. Klein, A. Sabet, S.F. Steinberg, Changes in adenylyl cyclase isoforms as a mechanism for thyroid hormone modulation of cardiac beta-adrenergic receptor responsiveness, Metabolism 49 (2000) 275–279, https://doi.org/10.1016/s0026-0495(00)92262-5.
[52] A. Perjés, A.M. Kubin, A. Kónyi, S. Szabados, A. Cziráki, R. Skoumal, H. Ruskoaho, I. Szokodi, Physiological regulation of cardiac contractility by endogenous reactive oxygen species, Acta Physiol. 205 (2012) 26–40, https://doi.org/10.1111/j.1748-1716.2011.02391.x.
[53] G.S. Levey, I. Klein, Catecholamine-thyroid hormone interactions and the cardiovascular manifestations of hyperthyroidism, Am. J. Med. 88 (1990) 642–646, https://doi.org/10.1016/0002-9343(90)90533-j.
[54] E.S. Bachman, T.G. Hampton, H. Dhillon, I. Amende, J. Wang, J.P. Morgan, A. N. Hollenberg, The metabolic and cardiovascular effects of hyperthyroidism are largely independent of beta-adrenergic stimulation, Endocrinology 145 (2004) 2767–2774, https://doi.org/10.1210/en.2003-1670.
[55] M.A. Carrillo-Sepúlveda, G.S. Ceravolo, Z.B. Fortes, M.H. Carvalho, R.C. Tostes, F. R. Laurindo, R.C. Webb, M.L. Barreto-Chaves, Thyroid hormone stimulates NO production via activation of the PI3K/Akt pathway in vascular myocytes, Cardiovasc. Res. 85 (2010) 560–570, https://doi.org/10.1093/cvr/cvp304.
[56] P.J. Davis, F.B. Davis, Nongenomic actions of thyroid hormone on the heart, Thyroid 12 (2002) 459–466, https://doi.org/10.1089/105072502760143827.
[57] Y.J.H.J. Taverne, A.J.J.C. Bogers, D.J. Duncker, D. Merkus, Reactive oxygen species and the cardiovascular system, Oxidative Med. Cell. Longev. 2013 (2013) 862423, https://doi.org/10.1155/2013/862423.
[58] H. Tsutsui, S. Kinugawa, S. Matsushima, Oxidative stress and heart, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H2181–H2190, https://doi.org/10.1152/ajpheart.00554.2011.
[59] A.V. Krylatov, L.N. Maslov, N.S. Voronkov, A.A. Boshchenko, S.V. Popov, L. Gomez, H. Wang, A.S. Jaggi, J.M. Downey, Reactive oxygen species as intracellular signaling molecules in the cardiovascular system, Curr. Cardiol. Rev. 14 (2018) 290–300, https://doi.org/10.2174/1573403X14666180702152436.
[60] Y. Zhang, C.G. Tocchetti, T. Krieg, A.L. Moens, Oxidative and nitrosative stress in the maintenance of myocardial function, Free Radic. Biol. Med. 53 (2012) 1531–1540, https://doi.org/10.1016/j.freeradbiomed.2012.07.010.
[61] J. Marín-García, Thyroid hormone and myocardial mitochondrial biogénesis, Vasc. Pharmacol. 52 (2010) 120–130, https://doi.org/10.1016/j.vph.2009.10.008.
[62] G. Napolitano, G. Fasciolo, S. Di Meo, P. Venditti, Vitamin E supplementation and mitochondria in experimental and functional hyperthyroidism: a mini-review, Nutrients 11 (2019) 2900, https://doi.org/10.3390/nu11122900.
[63] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem. J. 417 (2009) 1–13, https://doi.org/10.1042/bj20081386.
[64] P. Sharma, H. Sampath, Mitochondrial DNA integrity: role in health and disease, Cells 8 (2019) 100, https://doi.org/10.3390/cells8020100.
[65] N. Wiedemann, N. Pfanner, Mitochondrial machineries for protein import and assembly, Annu. Rev. Biochem. 86 (2017) 685–714, https://doi.org/10.1146/annurev-biochem-060815-014352.
[66] M.J. Goldenthal, H.R. Weiss, J. Marín-García, Bioenergetic remodeling of heart mitochondria by thyroid hormone, Mol. Cell. Biochem. 265 (2004) 97–106, https://doi.org/10.1023/b:mcbi.0000044321.17680.a2.
[67] C. Wrutniak-Cabello, F. Casas, G. Cabello, Mitochondrial T3 receptor and targets, Mol. Cell. Endocrinol. 458 (2017) 112–120, https://doi.org/10.1016/j.mce.2017.01.054.
[68] P.D.M. Rosaroll, V. Di Maio, M. Valente, S. Di Meo, T. de Leo, Thyroid hormone and the mitochondrial population in the rat heart, J. Endocrinol. Investig. 11 (1988) 559–565, https://doi.org/10.1007/bf03350180.
[69] E. Fernández-Vizarra, J.A. Enríquez, A. Pérez-Martos, J. Montoya, P. Fernández-Silva, Tissue-specific differences in mitochondrial activity and biogénesis, Mitochondrion 11 (2011) 207–213, https://doi.org/10.1016/j.mito.2010.09.011.
[70] J.D. Lambeth, NOX enzymes and the biology of reactive oxygen, Nat. Rev. Immunol. 4 (2004) 181–189, https://doi.org/10.1038/nri1312.
[71] C.X.C. Santos, S. Raza, A.M. Shah, Redox signaling in the cardiomyocyte: from physiology to failure, Int. J. Biochem. Cell Biol. 74 (2016) 145–151, https://doi.org/10.1016/j.biocel.2016.03.002.
[72] Y. Maejima, J. Kuroda, S. Matsushima, T. Ago, J. Sadoshima, Regulation of myocardial growth and death by NADPH oxidase, J. Mol. Cell. Cardiol. 50 (2011) 408–416, https://doi.org/10.1016/j.yjmcc.2010.12.018.
[73] T. Ago, J. Kuroda, J. Pain, C. Fu, H. Li, J. Sadoshima, Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in

cardiac myocytes, Circ. Res. 106 (2010) 1253–1264, https://doi.org/10.1161/circresaha.109.213116.
[74] C. Enroth, B.T. Eger, K. Okamoto, T. Nishino, T. Nishino, E.F. Pai, Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion, Proc. Natl. Acad. Sci. 97 (2000) 10723–10728, https://doi.org/10.1073/pnas.97.20.10723.
[75] D.A. Parks, D.N. Granger, Xanthine oxidase: biochemistry, distribution and physiology, Acta Physiol. Scand. Suppl. 548 (1986) 87–99.
[76] O. Makay, C. Yenisey, G. Icoz, N. Genc Simsek, G. Ozgen, M. Akyildiz, E. Yetkin, The role of allopurinol on oxidative stress in experimental hyperthyroidism, J. Endocrinol. Investig. 32 (2009) 641–646, https://doi.org/10.1007/bf03345734.
[77] S. Abadeh, P.C. Case, R. Harrison, Purification of xanthine oxidase from human heart, Biochem. Soc. Trans. 21 (1993) 99S, https://doi.org/10.1042/bst021099s.
[78] P.A. Ram, D.J. Waxman, Thyroid hormone stimulation of NADPH P450 reductase expression in liver and extrahepatic tissues. Regulation by multiple mechanisms, J. Biol. Chem. 267 (1992) 3294–3301.
[79] M. Sato, U. Yokoyama, T. Fujita, S. Okumura, Y. Ishikawa, The roles of cytochrome p450 in ischemic heart disease, Curr. Drug Metab. 12 (2011) 526–532, https://doi.org/10.2174/138920011795713715.
[80] L.A. Barouch, R.W. Harrison, M.W. Skaf, G.O. Rosas, T.P. Cappola, Z.A. Kobeissi, I.A. Hobai, C.A. Lemmon, A.L. Burnett, B. O'Rourke, E.R. Rodriguez, P.L. Huang, J.A.C. Lima, D.E. Berkowitz, J.M. Hare, Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms, Nature 416 (2002) 337–339, https://doi.org/10.1038/416337a.
[81] E. Takimoto, D.A. Kass, Role of oxidative stress in cardiac hypertrophy and remodeling, Hypertension 49 (2007) 241–248, https://doi.org/10.1161/01.hyp.0000254415.31362.a7.
[82] A.S. Araujo, P. Schenkel, A.T. Enzveiler, T.R. Fernandes, W.A. Partata, S. Llesuy, M.F. Ribeiro, N. Khaper, P.K. Singal, A. Belló-Klein, The role of redox signaling in cardiac hypertrophy induced by experimental hyperthyroidism, J. Mol. Endocrinol. 41 (2008) 423–430, https://doi.org/10.1677/jme-08-0024.
[83] A.S. Araujo, G.P. Diniz, F.E. Seibel, G. Branchini, M.F. Ribeiro, I.S. Brum, N. Khaper, M.L. Barreto-Chaves, A. Belló-Klein, Reactive oxygen and nitrogen species balance in the determination of thyroid hormones-induced cardiac hypertrophy mediated by renin-angiotensin system, Mol. Cell. Endocrinol. 333 (2011) 78–84, https://doi.org/10.1016/j.mce.2010.12.018.
[84] M.T. Elnakish, E.J. Schultz, R.L. Gearinger, N.S. Saad, N. Rastogi, A.A.E. Ahmed, P.J. Mohler, P.M.L. Janssen, Differential involvement of various sources of reactive oxygen species in thyroxin-induced hemodynamic changes and contractile dysfunction of the heart and diaphragm muscles, Free Radic. Biol. Med. 83 (2015) 252–261, https://doi.org/10.1016/j.freeradbiomed.2015.02.035.
[85] S. Luo, H. Lei, H. Qin, Y. Xia, Molecular mechanisms of endothelial NO synthase uncoupling, Curr. Pharm. Des. 20 (2014) 3548–3553, https://doi.org/10.2174/13816128113196660746.
[86] A.C. Montezano, R.M. Touyz, Reactive oxygen species and endothelial function–role of nitric oxide synthase uncoupling and Nox family nicotinamide adenine dinucleotide phosphate oxidases, Basic Clin. Pharmacol. Toxicol. 110 (2012) 87–94, https://doi.org/10.1111/j.1742-7843.2011.00785.x.
[87] P. Pacher, R. Schulz, L. Liaudet, C. Szabó, Nitrosative stress and pharmacological modulation of heart failure, Trends Pharmacol. Sci. 26 (2005) 302–310, https://doi.org/10.1016/j.tips.2005.04.003.
[88] V.J. Thannickal, B.L. Fanburg, Reactive oxygen species in cell signaling, Am. J. Phys. Lung Cell. Mol. Phys. 279 (2000) L1005–L1028, https://doi.org/10.1152/ajplung.2000.279.6.L1005.
[89] T. Finkel, Signal transduction by reactive oxygen species, J. Cell Biol. 194 (2011) 7–15, https://doi.org/10.1083/jcb.201102095.
[90] O. Rudyk, P. Eaton, Biochemical methods for monitoring protein thiol redox states in biological systems, Redox Biol. 2 (2014) 803–813, https://doi.org/10.1016/j.redox.2014.06.005.
[91] M.A. Wouters, S. Iismaa, S.W. Fan, N.L. Haworth, Thiol-based redox signalling: rust never sleeps, Int. J. Biochem. Cell Biol. 43 (2011) 1079–1085, https://doi.org/10.1016/j.biocel.2011.04.002.
[92] G.A. Knock, J.P. Ward, Redox regulation of protein kinases as a modulator of vascular function, Antioxid. Redox Signal. 15 (2011) 1531–1547, https://doi.org/10.1089/ars.2010.3614.
[93] P.H. Sugden, A. Clerk, Oxidative stress and growth-regulating intracellular signaling pathways in cardiac myocytes, Antioxid. Redox Signal. 8 (2006) 2111–2124, https://doi.org/10.1089/ars.2006.8.2111.
[94] A.V. Zima, L.A. Blatter, Redox regulation of cardiac calcium channels and transporters, Cardiovasc. Res. 71 (2006) 310–321, https://doi.org/10.1016/j.cardiores.2006.02.019.
[95] Y. Watanabe, R.A. Cohen, R. Matsui, Redox regulation of ischemic angiogenesis-another aspect of reactive oxygen species, Circ. J. 80 (2016) 1278–1284, https://doi.org/10.1253/circj.cj-16-0317.
[96] L. Zhang, K. Wang, Y. Lei, Q. Li, E.C. Nice, C. Huang, Redox signaling: potential arbitrator of autophagy and apoptosis in therapeutic response, Free Radic. Biol. Med. 89 (2015) 452–465, https://doi.org/10.1016/j.freeradbiomed.2015.08.030.
[97] M. Costilla, R. Macri Delbono, A. Klecha, G.A. Cremaschi, M.L. Barreiro Arcos, Oxidative stress produced by hyperthyroidism status induces the antioxidant enzyme transcription through the activation of the Nrf-2 factor in lymphoid tissues of Balb/c mice, Oxidative Med. Cell. Longev. 2019 (2019) 7471890, https://doi.org/10.1155/2019/7471890.
[98] M.V. Brahmajothi, D.L. Campbell, Heterogeneous basal expression of nitric oxide synthase and superoxide dismutase isoforms in mammalian heart: implications for mechanisms governing indirect and direct nitric oxide-related effects, Circ. Res. 85 (1999) 575–587, https://doi.org/10.1161/01.res.85.7.575.
[99] Z. Qin, K.J. Reszka, T. Fukai, N.L. Weintraub, Extracellular superoxide dismutase (ecSOD) in vascular biology: an update on exogenous gene transfer and endogenous regulators of ecSOD, Transl. Res. 151 (2008) 68–78, https://doi.org/10.1016/j.trsl.2007.10.003.
[100] F. Antunes, D. Han, E. Cadenas, Relative contributions of heart mitochondria glutathione peroxidase and catalase to H(2)O(2) detoxification in vivo conditions, Free Radic. Biol. Med. 33 (2002) 1260–1267, https://doi.org/10.1016/s0891-5849(02)01016-x.
[101] C.T. Le, L. Hollaar, E.J. van der Valk, A. van der Laarse, Buthionine sulfoximine reduces the protective capacity of myocytes to withstand peroxide-derived free radical attack, J. Mol. Cell. Cardiol. 25 (1993) 519–528, https://doi.org/10.1006/jmcc.1993.1062.
[102] M.S. Petrulea, I. Duncea, A. Muresan, Thyroid hormones in excess induce oxidative stress in rats, Acta Endocrinol. 5 (2009) 155–164, https://doi.org/10.4183/aeb.2009.155.
[103] S. Civelek, O. Seymen, A. Seven, G. Yigit, H. Hatemi, G. Burçak, Oxidative stress in heart tissue of hyperthyroid and iron supplemented rats, J. Toxicol. Environ. Health 64 (2001) 499–506, https://doi.org/10.1080/152873901753215957.
[104] P. Venditti, M. Balestrieri, S. di Meo, T. de Leo, Effect of thyroid state on lipid peroxidation, antioxidant defences, and susceptibility to oxidative stress in rat tissues, J. Endocrinol. 155 (1997) 151–157, https://doi.org/10.1677/joe.0.1550151.
[105] A.S.R. Araujo, M.F.M. Ribeiro, A. Enzveiler, P. Schenkel, T.R.G. Fernandes, W. A. Partata, M.C. Irigoyen, S. Llesuy, A. Belló-Klein, Myocardial antioxidant enzyme activities and concentration and glutathione metabolism in experimental hyperthyroidism, Mol. Cell. Endocrinol. 249 (2006) 133–139, https://doi.org/10.1016/j.mce.2006.02.005.
[106] H. Zhang, K.J.A. Davies, H.J. Forman, Oxidative stress response and Nrf2 signaling in aging, Free Radic. Biol. Med. 88 (2015) 314–336, https://doi.org/10.1016/j.freeradbiomed.2015.05.036.
[107] X. Zhang, Y. Yu, H. Lei, Y. Cai, J. Shen, P. Zhu, Q. He, M. Zhao, The Nrf-2/HO-1 signaling axis: a ray of hope in cardiovascular diseases, Cardiol. Res. Pract. 2020 (2020) 5695723, https://doi.org/10.1155/2020/5695723.
[108] U. Resch, G. Helsel, F. Tatzber, H. Sinzinger, Antioxidant status in thyroid dysfunction, Clin. Chem. Lab. Med. 40 (2002) 1132–1134, https://doi.org/10.1515/cclm.2002.198.
[109] R. Gredilla, G. Barja, M. Lopez-Torres, Thyroid hormone-induced oxidative damage on lipids, glutathione and DNA in the mouse heart, Free Radic. Res. 35 (2001) 417–425, https://doi.org/10.1080/10715760100300931.
[110] M. López-Torres, M. Romero, G. Barja, Effect of thyroid hormones on mitochondrial oxygen free radical production and DNA oxidative damage in the rat heart, Mol. Cell. Endocrinol. 168 (2000) 127–134, https://doi.org/10.1016/s0303-7207(00)00302-6.
[111] J.N. Peoples, A. Saraf, N. Ghazal, T.T. Pham, J.Q. Kwong, Mitochondrial dysfunction and oxidative stress in heart disease, Exp. Mol. Med. 51 (2019) 1–13, https://doi.org/10.1038/s12276-019-0355-7.
[112] H. Tsutsui, T. Ide, T. Shiomi, D. Kang, S. Hayashidani, N. Suematsu, J. Wen, H. Utsumi, N. Hamasaki, A. Takeshita, 8-oxo-dGTPase, which prevents oxidative stress-induced DNA damage, increases in the mitochondria from failing hearts, Circulation 104 (2001) 2883–2885, https://doi.org/10.1161/hc4901.101347.
[113] C. Richter, J.W. Park, B.N. Ames, Normal oxidative damage to mitochondrial and nuclear DNA is extensive, Proc. Natl. Acad. Sci. 85 (1988) 6465–6467, https://doi.org/10.1073/pnas.85.17.6465.
[114] R. Lu, H.M. Nash, G.L. Verdine, A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer, Curr. Biol. 7 (1997) 397–407, https://doi.org/10.1016/s0960-9822(06)00187-4.
[115] K. Asayama, K. Dobashi, H. Hayashibe, Y. Megata, K. Kato, Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism, Endocrinol. 121 (1987) 2112–2118, https://doi.org/10.1210/endo-121-6-2112.
[116] R. Mogulkoc, A.K. Baltaci, L. Aydin, E. Oztekin, A. Sivrikaya, The effect of thyroxine administration on lipid peroxidation in different tissues of rats with hypothyroidism, Acta Physiol. Hung. 92 (2005) 39–46, https://doi.org/10.1556/aphysiol.92.2005.1.6.
[117] R. Shinohara, T. Mano, A. Nagasaka, R. Hayashi, K. Uchimura, I. Nakano, F. Watanabe, T. Tsugawa, M. Makino, H. Kakizawa, M. Nagata, K. Iwase, Y. Ishizuki, M. Itoh, Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status, J. Endocrinol. 164 (2000) 97–102, https://doi.org/10.1677/joe.0.1640097.
[118] P. Venditti, T. de Leo, S. di Meo, Antioxidant-sensitive shortening of ventricular action potential in hyperthyroid rats is independent of lipid peroxidation, Mol. Cell. Endocrinol. 142 (1998) 15–23, https://doi.org/10.1016/s0303-7207(98)00123-3.
[119] A. Sabri, H.H. Hughie, P.A. Lucchesi, Regulation of hypertrophic and apoptotic signaling pathways by reactive oxygen species in cardiac myocytes, Antioxid. Redox Signal. 5 (2003) 731–740, https://doi.org/10.1089/152308603770380034.
[120] S.H. Kwon, D.R. Pimentel, A. Remondino, D.B. Sawyer, W.S. Colucci, H_2O_2 regulates cardiac myocyte phenotype via concentration-dependent activation of distinct kinase pathways, J. Mol. Cell. Cardiol. 35 (2003) 615–621, https://doi.org/10.1016/s0022-2828(03)00084-1.

[121] Y. Wang, B. Jiao, W. Guo, H. Che, Z. Yu, Excessive thyroxine enhances susceptibility to apoptosis and decreases contractility of cardiomyocytes, Mol. Cell. Endocrinol. 320 (2010) 67–75, https://doi.org/10.1016/j.mce.2010.01.031.
[122] R.O. Fernandes, G.J. Dreher, P.C. Schenkel, T.R.G. Fernandes, M.F.M. Ribeiro, A. S.R. Araujo, A. Belló-Klein, Redox status and pro-survival/pro-apoptotic protein expression in the early cardiac hypertrophy induced by experimental hyperthyroidism, Cell Biochem. Funct. 29 (2011) 617–623, https://doi.org/10.1002/cbf.1796.
[123] K. Ordog, O. Horvath, K. Eros, K. Bruszt, S. Toth, D. Kovacs, N. Kalman, B. Radnai, L. Deres, F. Gallyas, K. Toth, R. Halmosi, Mitochondrial protective effects of PARP-inhibition in hypertension-induced myocardial remodeling and in stressed cardiomyocytes, Life Sci. 268 (2021) 118936, https://doi.org/10.1016/j.lfs.2020.118936.
[124] D.A. Siwik, W.S. Colucci, Regulation of matrix metalloproteinases by cytokines and reactive oxygen/nitrogen species in the myocardium, Heart Fail. Rev. 9 (2004) 43–51, https://doi.org/10.1023/b:hrev.0000011393.40674.13.
[125] H. Tsutsui, T. Ide, S. Hayashidani, N. Suematsu, H. Utsumi, R. Nakamura, K. Egashira, A. Takeshita, Greater susceptibility of failing cardiac myocytes to oxygen free radical-mediated injury, Cardiovasc. Res. 49 (2001) 103–109, https://doi.org/10.1016/s0008-6363(00)00197-8.
[126] A. Saremi, R. Arora, Vitamin E and cardiovascular disease, Am. J. Ther. 17 (2010) e56–e65, https://doi.org/10.1097/MJT.0b013e31819cdc9a.
[127] P. Mishra, B. Paital, S. Jena, S.S. Swain, S. Kumar, M.K. Yadav, G.B.N. Chainy, L. Samanta, Possible activation of NRF2 by vitamin E/curcumin against altered thyroid hormone induced oxidative stress via NFκB/AKT/mTOR/KEAP1 signalling in rat heart, Sci. Rep. 9 (2019) 7408, https://doi.org/10.1038/s41598-019-43320-5.
[128] P. Venditti, T. De Leo, S. Di Meo, Vitamin E administration attenuates the triiodothyronine-induced modification of heart electrical activity in the rat, J. Exp. Biol. 200 (1997) 909–914.
[129] P. Venditti, T. de Leo, S. di Meo, Antioxidant-sensitive shortening of ventricular action potential in hyperthyroid rats is independent of lipid peroxidation, Mol. Cell. Endocrinol. 142 (1998) 15–23, https://doi.org/10.1016/s0303-7207(98)00123-3.
[130] K. Asayama, K. Dobashi, H. Hayashibe, K. Kato, Vitamin E protects against thyroxine-induced acceleration of lipid peroxidation in cardiac and skeletal muscles in rats, J. Nutr. Sci. Vitaminol. 35 (1989) 407–418, https://doi.org/10.3177/jnsv.35.407.
[131] F. Oztay, B. Ergin, S. Ustunova, H. Balci, A. Kapucu, M. Caner, C. Demirci, Effects of coenzyme Q10 on the heart ultrastructure and nitric oxide synthase during hyperthyroidism, Chin. J. Phys. 50 (2007) 217–224.
[132] Y. Rabanal-Ruiz, E. Llanos-González, F.J. Alcain, The use of coenzyme Q10 in cardiovascular diseases, Antioxidants 10 (2021) 755, https://doi.org/10.3390/antiox10050755.
[133] G. Aldini, A. Altomare, G. Baron, G. Vistoli, M. Carini, L. Borsani, F. Sergio, N-acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why, Free Radic. Res. 52 (2018) 751–762, https://doi.org/10.1080/10715762.2018.1468564.
[134] D. Giugliano, Dietary antioxidants for cardiovascular prevention, Nutr. Metab. Cardiovasc. Dis. 10 (2000) 38–44.
[135] H. Khodadadi, R. Pakdel, M. Khazaei, S. Niazmand, K. Bavarsad, M. Hadjzadeh, A comparison of the effects of *Portulaca oleracea* seeds hydro-alcoholic extract and vitamin C on biochemical, hemodynamic and functional parameters in cardiac tissue of rats with subclinical hyperthyroidism, Avicenna J. Phytomed. 8 (2018) 161–169.
[136] P.Y. Zhang, X. Xu, X.C. Li, Cardiovascular diseases: oxidative damage and antioxidant protection, Eur. Rev. Med. Pharmacol. Sci. 18 (2014) 3091–3096.
[137] R.A. Jacob, G. Sotoudeh, Vitamin C function and status in chronic disease, Nutr. Clin. Care 5 (2002) 66–74, https://doi.org/10.1046/j.1523-5408.2002.00005.x.
[138] M.T. Elnakish, L. Moldovan, M. Khan, H.H. Hassanain, P.M. Janssen, Myocardial Rac1 exhibits partial involvement in thyroxin-induced cardiomyocyte hypertrophy and its inhibition is not sufficient to improve cardiac dysfunction or contractile abnormalities in mouse papillary muscles, J. Cardiovasc. Pharmacol. 61 (2013) 536–544, https://doi.org/10.1097/fjc.0b013e31828d4b9d.
[139] F. Qin, M. Simeone, R. Patel, Inhibition of NADPH oxidase reduces myocardial oxidative stress and apoptosis and improves cardiac function in heart failure after myocardial infarction, Free Radic. Biol. Med. 43 (2007) 271–281, https://doi.org/10.1016/j.freeradbiomed.2007.04.021.
[140] H.E. Cingolani, J.A. Plastino, E.M. Escudero, B. Mangal, J. Brown, N.G. Perez, The effect of xanthine oxidase inhibition upon ejection fraction in heart failure patients, J. Card. Fail. 12 (2006) 491–498, https://doi.org/10.1016/j.cardfail.2006.05.005.
[141] W. Doehner, N. Schoene, M. Rauchhaus, F. Leyva-Leon, D.V. Pavitt, D. A. Reaveley, G. Schuler, A.J. Coats, S.D. Anker, R. Hambrecht, Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies, Circulation 105 (2002) 2619–2624, https://doi.org/10.1161/01.cir.0000017502.58595.ed.
[142] H. Kim, T. Lee, Y.S. Hwang, M.A. Bang, K.H. Kim, J.M. Suh, H.K. Chung, D.Y. Yu, K.K. Lee, O.Y. Kwon, H.K. Ro, M. Shong, Methimazole as an antioxidant and immunomodulator in thyroid cells: mechanisms involving interferon-gamma signaling and H_2O_2 scavenging, Mol. Pharmacol. 60 (2001) 972–980, https://doi.org/10.1124/mol.60.5.972.
[143] T. Münzel, G.G. Camici, C. Maack, N.R. Bonetti, V. Fuster, J.C. Kovacic, Impact of oxidative stress on the heart and vasculature: part 2 of a 3-part series, J. Am. Coll. Cardiol. 70 (2017) 212–229, https://doi.org/10.1016/j.jacc.2017.05.035.