UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

EFEITO DA N-ACETILCISTEÍNA SOBRE O METABOLISMO DOS HORMÔNIOS TIREOIDIANOS, FUNÇÃO CARDÍACA E ESTRESSE OXIDATIVO EM MODELO ANIMAL DE SÍNDROME DO T3 BAIXO.

TATIANA EDERICH LEHNEN

Porto Alegre, outubro de 2017.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

EFEITO DA N-ACETILCISTEÍNA SOBRE O METABOLISMO DOS HORMÔNIOS TIREOIDIANOS, FUNÇÃO CARDÍACA E ESTRESSE OXIDATIVO EM MODELO ANIMAL DE SÍNDROME DO T3 BAIXO.

TESE DE DOUTORADO

TATIANA EDERICH LEHNEN

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Doutor em endocrinologia.

Orientadora: Profª. Dra. Simone Magagnin Wajner

Co-orientadora: Profª. Dra. Ana Luiza Maia

Porto Alegre, outubro de 2017.

CIP - Catalogação na Publicação

Lehnen, Tatiana Ederich
EFEITO DA N-ACETILCISTEÍNA SOBRE O METABOLISMO DOS HORMÔNIOS TIREOIDIANOS, FUNÇÃO CARDÍACA E ESTRESSE OXIDATIVO EM MODELO ANIMAL DE SÍNDROME DO T3 BAIXO / Tatiana Ederich Lehnen. -- 2017.
55 f.
Orientadora: Simone Magagnin Wajner.

Coorientadora: Ana Luiza Maia.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Porto Alegre, BR-RS, 2017.

1. Hormônios tireoidianos. 2. Síndrome do T3 baixo. 3. Desiodases. 4. Função cardíaca. 5. Estresse oxidativo. I. Wajner, Simone Magagnin, orient. II. Maia, Ana Luiza, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

À Profª Dra **Simone Magagnin Wajner** que me orientou em todo doutorado, estando sempre disponível em todos os momentos que precisei.

À Profª **Dra Ana Luiza Maia** que me deu a oportunidade de iniciar o meu projeto de doutorado.

Aos colegas de laboratório do grupo da Tireoide que pude contar sempre que precisei para tirar dúvidas e que confiei plenamente para me ajudar em análises bioquímicas.

Aos amigos do Instituto do Cardiologia (Max, Rafael e Melissa) que me ajudaram em muitos momentos que precisei.

Aos meus pais, **Álvaro** e **Bete**, que me ensinaram a batalhar por aquilo que eu quero sem jamais desistir. Aos meus irmãos, que sempre acreditaram em mim. E aos meus sogros, que estiveram presentes como pais nesta minha jornada, principalmente me dando no período que estive fora fazendo o doutorado sanduíche.

Ao meu marido, **Alexandre Machado Lehnen**, pela dedicação, carinho e compreensão, me apoiando em todos os momentos e consagrando, mais do que nunca, a difícil arte de amar. É uma pessoa que admiro demais e tenho muito orgulho.

Ao meu filho **Felipe**, uma criança espetacular, que sempre me entendeu e conheceu muito cedo a palavra saudade, ficando longe de mim quase 5 meses quando fiz o doutorado sanduíche e, mesmo assim, continuou sendo uma criança dócil e carinhosa. Ver o sorriso dele é o que me traz muita alegria e paz no coração, me deixando calma e tranquila.

Esta tese de doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de 3 manuscritos sobre o tema da Tese:

- Artigo de revisão (1): Effects of Non Thyroidal Illness Syndrome in Cardiovascular Disease.

- Artigo original (2): N-acetylcysteine prevents low T3 syndrome and attenuates cardiac dysfunction in a male rat model of myocardial infarction (publicado na revista Endocrinology em 2017 - Fator de Impacto 4.286).

- Artigo original (3): Oxidative stress induces type 3 deiodinase in multiple tissues after myocardial infarction: implications to Low T3 Syndrome pathophysiology.

LISTA DE ABREVIATURAS E SIGLAS

ATP	Adenosine triphosphate
CHF	Chronic heart failure
D1	Type 1 iodothyronine deiodinase
D2	Type 2 iodothyronine deiodinase
D3	Type 3 iodothyronine deiodinase
EF	Ejection fraction
FT4	Free Thyroxine
GSH	Glutathione
GSSG	Oxidized glutathione
LV	Left ventricle
LVDd	Left ventricular diastolic
LVDs	Left ventricular systolic
MCT-10	Monocarboxylate transporters 10
MCT-8	Monocarboxylate transporters 8
MI	Myocardial infarction
mRNA	Messenger RNA
NAC	N-acetylcysteine
NTIS	Nonthyroidal illness syndrome
ROS	Reative oxygen species
rT3	Reverse triiodothyronine
SERCA 2	Sarcoplasmic reticulum calcium adenosine triphosphatase
T2	Diiodothyronine
T3	Triiodothyronine
T4	Thyroxine
TH	Thyroid hormones
TR	Thyroid hormone receptors
Trx	Thioredoxin
TSH	Thyrotropin

SUMÁRIO

INTRODUÇÃO

A síndrome do T3 baixo se caracteriza por alterações no metabolismo dos hormônios tireoidianos. Independente da condição clínica existente, à medida que ocorre a piora da doença, ocorre mudanças na conversão periférica do T4 em T3, diminuindo os níveis séricos de T3. Esta síndrome ocorre em cerca de 75% dos pacientes em unidades de terapia intensiva, sendo também normalmente observada em pacientes com outras patologias como infarto agudo do miocárdio. Em todas as situações os baixos níveis séricos de T3 estão inversamente correlacionados com a mortalidade.

A conversão periférica dos hormônios tireoidianos é feita pelas enzimas iodotironinas desiodases tipo 1, 2 e 3 (D1, D2 e D3, respectivamente. As iodotironinas deiodinases são oxiredutases que catalisam a remoção de iodo do anel externo (D1 e D2) ou interno (D3) dos hormônios tireoidianos, ativando ou inativando esses hormônios.

A alteração na função das desiodases tem sido observada em várias doenças e está relacionada à presença do stress oxidativo. Trabalhos prévios do nosso grupo desenvolveram estudos em modelo celular demonstrando que o aumento da IL-6, observado em todas as situações de doença, estimula NADPH oxidase, que forma radical superóxido, levando a um aumento das espécies reativas de oxigênio, que por sua vez consome as reservas antioxidantes da célula, levando à disfunção das desiodases e síndrome do T3 baixo.

Foi observado que esse desequilíbrio redox resulta na alteração da atividade das desiodases, inibindo D1 e D2, diminuindo a conversão de T4 em T3, e aumentando D3, que inativa o T3. Recentemente, foram feitos estudos com antioxidantes na tentativa de corrigir a Síndrome do T3 baixo. E um dos antioxidantes usados tem sido a N-acetilcisteína (NAC), potente antioxidante que restaura os níveis de glutationa evitando o desequilíbrio redox. Um estudo clínico randomizado realizado em pacientes com infarto

do miocárdio que receberam tratamento NAC e placebo mostrou que o tratamento com NAC reverte a Síndrome do T3 baixo, corrigindo o metabolismo dos hormônios tireodianos e a função cardíaca.

O infarto do miocárdio leva ao estresse oxidativo e altera a função das desiodases, resultando na síndrome do T3 baixo. Demonstramos que a NAC corrige o metabolismo dos hormônios tireodianos. Considerando todos esses pontos, e devido à limitações de estudos que avaliem o efeito do stress oxidativo gerado pela doença em nível tecidual em humanos, o objetivo da nossa pesquisa foi avaliar o efeito da NAC sobre a função cardíaca, as enzimas que metabolizam os hormônios tireoidianos e o estresse oxidativo no sangue e em múltiplos tecidos na Síndrome do T3 baixo em ratos submetidos ao infarto do miocárdio.

ARTIGO I

Effects of Nonthyroidal Illness Syndrome in Cardiovascular Disease

Tatiana Ederich Lehnen, Ana Luiza Maia, Simone Magagnin Wajner

Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.

Word count: 3.354

Corresponding author and person to who reprints should be addressed:
Dr. Simone Magagnin Wajner
Serviço de Endocrinologia
Hospital de Clínicas de Porto Alegre
Rua Ramiro Barcelos, 2350
CEP 90035-003 – Porto Alegre, RS, Brasil.
Phone/Fax: 55-51-33310207
E-mail: simone.wajner@ufrgs.br

ABSTRACT

Non-thyroidal illness syndrome (NTIS) is characterized by changes in the metabolism of thyroid hormones (TH), regulated by enzymes called iodothyronine deiodinases (type 1, 2 and 3). Alterations on the function of deiodinases has been observed in several diseases, mainly in the cardiovascular diseases, being generally observed in patients with myocardial infarction (MI). These alterations leads to an inverse correlation of low T3 levels with poor prognosis and mortality. However, the mechanisms of this syndrome are still poorly understood. Thus, given the importance of TH metabolism in cardiovascular diseases, mechanisms involving the pathogenesis of NTIS are studied *in vivo* and *in vitro* models, and due to several lacks in the understanding of the pathophysiological mechanisms of NTIS, there is no currently approved treatment for these set of TH derangements.

Key-words: thyroid hormone, non-thyroidal illness syndrome, cardiovascular diseases.

1. INTRODUCTION

Thyroid hormones (TH) are essential for energy metabolism, cell growth and differentiation. Although Thyroxine (T4) is the main product of the thyroid gland, Thyroxine (T3) is the biologically active hormone. Most of the T3 production (80-90%) occurs in the peripheral tissues through the action of iodothyronine deiodinases types 1, 2 and 3 (D1, D2 and D3, respectively), which controls TH activation and inactivation.

The nonthyroidal illness syndrome (NTIS), also known as low T3 syndrome reflects alterations in TH levels that occur in almost every form of acute and chronic illness (Larsen and Baxter 2008). The acute phase of critical illness is marked by low T3 and free T3 (FT3) and high rT3 levels. T4 levels may be normal or reduced without increased thyroid-stimulating hormone (TSH) levels (Warner and Beckett 2010). While decreased T4 levels are associated with the severity of disease, low FT3 levels have been recognized as a marker of poor prognosis (Iervasi, Pingitore et al. 2003, Wang, Pan et al. 2012, Ozcan, Osmonov et al. 2014, Jabbar, Pingitore et al. 2017). The exact mechanisms leading to TH alterations in the acute phase of disease are unknown but changes in peripheral hormone metabolism are implicated. NTIS is commonly observed in patients with several pathologies, among them the majority of cardiovascular disease (Kimura, Kanda et al.

2000, Pavlou, Kliridis et al. 2002), such as myocardial infarction (MI) and chronic heart failure (CHF) (Li, Guo et al. 2011, Ozcan, Osmonov et al. 2014).

TH have a direct effect on the cardiac muscle and the impact of T3 levels on heart disease can be explained by the physiological actions of these hormones regulating cardiac function, having a large participation in myocardial contractility, systolic and diastolic function (Bengel, Nekolla et al. 2000). TH metabolism is altered in myocardial infarction (MI), cardiac hypertrophy and heart failure (Ojamaa, Kenessey et al. 2000, Henderson, Danzi et al. 2009, Pol, Muller et al. 2010).

TH also have a direct positive inotropic effect on the heart through genomic and nongenomic effects on components of the adrenergic receptor complex (Klein and Ojamaa 2001) and the sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA2) channels has a genomic action, regulating contractile function and heart rhythm. Low levels of T3 stimulate phospholamban, inhibiting SERCA. This reduces calcium removal in the cytosol by decreasing systolic heart function (Carr and Kranias 2002). T3 enters the cardiomyocyte through membrane transporters and is also activated intracellularly by the conversion of T4. At the nucleus, T3 binds to thyroid hormone receptors (TR), regulating transcription. Elevations of TH levels increase oxidative capacity and fatty acid utilization in skeletal muscle, which is the main target of thyroid hormone signaling (Simonides, Mulcahey et al. 2008).

The changes in TH availability modulate the contractile and metabolic properties of skeletal muscle, increasing SERCA and indicating that these hormones are in dynamic equilibrium. This balance helps explain the muscle changes that characterize insufficiency and excess in TH. It is also suggested that there may be a temporally restricted expression of DIO2 and DIO3 in satellite cells during differentiation and repair of muscle cells (Salvatore, Simonides et al. 2014).

Although the severity of the disease is the main determinant of NTIS, the production and function of THs seems to be affected by the increased levels of oxidative stress and cytokines. Interleucin-6 (IL-6) has a negative correlation with serum T3 levels in hospitalized patients, especially after MI (Papanas, Papatheodorou et al. 2008). Augmented IL-6 increases oxidative stress, decreasing T3 and increasing rT3 levels (Pingitore, Iervasi et al. 2006, Wajner, Goemann et al. 2011, Vidart, Wajner et al. 2014). Given the importance of TH metabolism, mechanisms involving the pathogenesis of NTIS

are studied *in vivo* and *in vitro* models. Here we review the alterations on NTIS related to cardiovascular diseases.

2. NONTHYROIDAL ILLNESS SYNDROME

The pathophysiology of NTIS is complex and poorly understood. The relationship between changes in TH parameters and the severity of the disease is a *continuum* that seems to depend not on the type of disease but on the severity and prognosis of the underlying disease (Iervasi, Pingitore et al. 2003). NTIS is associated with an increase cardiovascular mortality and morbidity in patients with acute coronary syndrome (Adawiyah, Norasyikin et al. 2010). With the progression of the disease, serum T3 remains falling to extremely low levels, with concomitant increase in rT3. Changes in T4 are more complex. In mild forms, T4 tends to be normal, and the fall is observed only in very severe cases (Docter, Krenning et al. 1993). The decrease in T3 levels can be observed in up to 75% of patients admitted to the intensive care unit, while the T4 decrease occurs in up to 50% of cases (Ray, Macduff et al. 2002). Until now, it is unclear whether the observed changes correspond to an adaptive mechanism intended to reduce energy consumption, or it is a form of secondary hypothyroidism that could benefit with treatment (Mebis and van den Berghe 2009).

Inflammatory cytokines has an important pathophysiological role in NTIS, since the serum IL-6 levels are usually high and have an inverse relationship with T3 levels (Boelen, Platvoet-Ter Schiphorst et al. 1993). In the acute phase of the disease some mechanisms are implicated in the peripheral alterations of TH. Particularly, one of them is the direct effect of augmented systemic IL-6 on T3 production, decreasing the peripheral conversion of T4 to T3 and elevating rT3 (Peeters, Wouters et al. 2003). Interestingly, cardiovascular diseases, as myocardial infarction (MI), increases the production of pro-inflammatory cytokines, decreasing T3 levels in cardiomyocytes in the left ventricle (LV) and causing an intracellular hypothyroid state (Pol, Muller et al. 2011). Other mechanism is the monocarboxylate transporters 8 e 10 (MCT8 and MCT10), adenosine 5-triphosphate (ATP) dependent transporters (Visser, van Mullem et al. 2011). MCT8 carries T4 and T3 and is expressed in the liver, kidney, brain, heart and thyroid gland; and MCT10 transports T3 instead of T4 and is expressed in the liver, kidney, skeletal muscle and placenta (Heuer, Maier et al. 2005, Nishimura and Naito 2008, Visser, van Mullem et al. 2011). These transporters (MCT8 and MCT10) depend on adenosine 5-triphosphate (ATP) to

uptake TH by the cell membrane. It is known that the transport of TH in tissues may be reduced in NTIS (Boelen, van der Spek et al. 2017) but the association with decreased expression of transporters is controversial (Friesema, Jansen et al. 2008).

2.1 Role of peripheral thyroid hormone metabolism alterations on NTIS

The activation and inactivation process of thyroid hormones is dependent on the action of three enzymes known as iodothyrononine deiodases types 1, 2 and 3 (D1, D2 and D3, respectively) (Baqui, Botero et al. 2003). The iodothyronine deiodinases (D1, D2 and D3) constitute a family of oxidoreductases, which removes a molecule of iodine from the outer (tyrosyl) and/or the inner (phenolic) ring of TH. D1 and D2 are hormone-activating enzymes, converting T4 to T3, although D1 also act as inactivating enzyme. On the other hand, D3 exclusively inactivates T3 and T4, leading to the formation of T2 and rT3, which are inactive compounds (Maia, Goemann et al. 2011, Wajner, Goemann et al. 2011). The majority of T3 production (80-90%) occurs in the peripheral tissues through the action of iodothyronine deiodinases (Maia, Kim et al. 2005) and T3 levels increase as it increases D2 activity in the heart, improving cardiac contractile function (Trivieri, Oudit et al. 2006).

While D1 is found in the plasma membrane, D2 is located in the endoplasmic reticulum. Both have the active site in the cytosol, thus allowing access to the yet unknown cofactor(s) (Bianco, Salvatore et al. 2002, Gereben, Zavacki et al. 2008). D3 is also found in the plasma membrane but the location of its active site is not still identified (Wajner, Goemann et al. 2011). D1 is located in the liver, kidney, thyroid and pituitary, while D2 is located in the thyroid, heart and skeletal muscle in humans and in animals is also located in the pituitary, brain and brown adipose tissue. D3 is located in the brain, skin, uterus, placenta and fetus (St Germain, Galton et al. 2009).

Besides converting T4 into T3 in peripheral tissues, these enzymes are highly expressed in the thyroid gland (Toyoda, Nishikawa et al. 1992, Salvatore, Tu et al. 1996). D2 and D3 are widely expressed during development (Galton 2005) and in the fetal period D3 regulates the degree of exposure of the fetus to TH (Cooper, Gibbens et al. 1983, Mortimer, Galligan et al. 1996) since the deficiency of these hormones can lead to neurological disturbances (Zoeller and Rovet 2004, Williams 2008).

The interaction between D2 activation and D3 inactivation in tissues expressing these enzymes regulates intracellular T3 concentration, providing flexibility for the tissue regeneration and development process (Larsen and Zavacki 2012). The function of deiodinases influences the levels and action of TH. Several diseases and fasting alter deiodinases function, decreasing T3 and T4 without a compensatory increase of serum TSH levels (Kmiec, Kotlarz et al. 1996, De Groot 2006, Boelen, Wiersinga et al. 2008).

Nevertheless, studies approaching the role of deiodinases in disease produce controversital results. An animal study using three NTIS models (acute systemic inflammation by intraperitoneal injection of bacterial, chronic local inflammation by a turpentine injection in the hind limb and severe pneumonia and sepsis by intranasal inoculation with *Streptococcus pneumonia*) to study TH metabolism showed that peripheral TH metabolism is differentially regulated during disease, depending on the organ and the severity of the disease. This study showed that NITS differentially affects the expression and activity of D1 and D3 in the liver, resulting in a decrease in D1 and D3 activity in acute inflammation. In muscle, the decrease in TH concentrations is less severe than the observed decrease in liver (Boelen, van der Spek et al. 2017). In addition, other studies have shown that fasted rats increased *in vitro* D2 activity in the rat hypothalamus, resulting in an increase in T3 levels in this tissue (Coppola, Hughes et al. 2005, Fekete, Sarkar et al. 2005). Nevertheless, D3 induction is observed *in vivo* in response to tissue injury and may influence healing or regenerative processes in tissue injury and inflammation under conditions of hypoxia and / or oxidative stress (Lamirand, Pallud-Mothre et al. 2008, Simonides, Mulcahey et al. 2008). Therefore, an increase in D3 has been observed in animal models at sites of inflammation and in the heart with MI (Olivares, Marassi et al. 2007). In contrast, a recent animal study showed that in response to stress, such as caloric restriction or disease, in the liver and kidneys there was a decrease in TH signaling, increasing D3 activity. However, in the brain, muscle and heart, the TH signaling was preserved (Visser, Heuer et al. 2016). Interestingly, the induction of post-MI D3 cardiomyocytes is associated with a 50% reduction in T3 levels in the tissue, leading to a local hypothyroid condition and affecting the remodeling of the LV in hearts of animals that were severely hypothyroid when compared to euthyroid controls (Pol, Muller et al. 2010).

NTIS is correlated with decreased D1 in liver and D3 in liver and skeletal muscle in critically ill patients (Peeters, van der Geyten et al. 2005, Peeters, Wouters et al. 2005). Studies in humans have shown a decrease in D1 in the liver and an increase in D3 in skeletal muscle and liver in states of fasting and/or illness (Chopra 1997, De Groot 2006).

3. ROLE OF OXIDATIVE STRESS ON NTIS PATHOPHYSIOLOGY IN CARDIOVASCULAR DISEASE

Oxidative stress is characterized by unbalance between the production of pro-oxidant substances and antioxidant defenses and one of the most important oxidants is the ROS (Breitkreuz and Hamdani 2015). Recent study showed that oxidative stress induces functional damage in tissues and plays an important role in the pathophysiology of NTIS and the synthesis, activity and metabolism of TH. One of the possible mechanisms related to oxidative stress is the induction of cytokines, since all of three deiodinases require a still undefined cofactor, probably a thiol, which acts as a reducing agent releasing iodine from the selenocysteine residue and regenerating the active enzyme (Wajner, Goemann et al. 2011).

The alteration of deiodinases in cardiovascular diseases is directly linked to the changes in TH and one of the mechanisms involved in the relationship between TH imbalance to MI is the oxidative stress (Friberg, Werner et al. 2002, Lamirand, Pallud-Mothre et al. 2008), being associated with a low ejection fraction, which may interfere with in the improvement of the patient (Rybin and Steinberg 1996, Jankauskiene, Orda et al. 2015, Wang, Guan et al. 2015). The redox balance of the heart is an important factor, since ROS concentration can be related with the promotion of cardiac damage (Schenkel, Tavares et al. 2010), which can lead to changes inactivation of T3 and T4. In addition, the increased ROS production can produce cell damage, contributing to the pathophysiology of myocardial infarction.

Stress state, such as MI, leads to an adrenergic overload by increasing intracellular calcium in cardiomyocytes, reducing SERCA and altering the efficiency of cardiomyocytes (Weber 2016). Deiodinases also seem to alter this equilibrium in disease. Moreover, TH exert profound effects on the heart and cardiovascular hemodynamics, maintaining the cardiac mass and stress of the wall while preserving the LV ellipsoidal geometry (Pol, Muller et al. 2010). This effects on cardiovascular hemodynamics includes reduction of cardiac output, increase in vascular resistance and decrease in diastolic filling rates (Klein and Ojamaa 2001).

Several clinical and experimental studies also report an important role of D3 in NTIS (Peeters, Wouters et al. 2003, Peeters, van der Geyten et al. 2005, Rodriguez-Perez, Palos-Paz et al. 2008). D3 activity is nearly undetectable in the healthy heart and is

induced in various models of heart failure and MI (Hamilton, Stevenson et al. 1990, Kinugawa, Minobe et al. 2001). It is known that cardiovascular diseases induce IL-6, increasing oxidative stress and decreasing GSH and other antioxidants (Wajner et al. 2012). However, the increase in IL-6 has an inverse correlation with T3 levels in patients with NTIS, reducing the activity of deiodases. Recent studies have shown that the inhibitory action of IL-6 is corrected by the administration of N-acetylcysteine (NAC), indicating that IL-6 inhibits the function of D1 and D2 by increasing cellular ROS. Differently, IL-6 stimulates endogenous D3-mediated inactivation of T3, which is probably secondary to the plasma membrane location of D3 that allows this enzyme to have ready access to extracellular GSH and other antioxidants (Wajner and Maia 2012, Vidart, Wajner et al. 2014) (Figure 1).

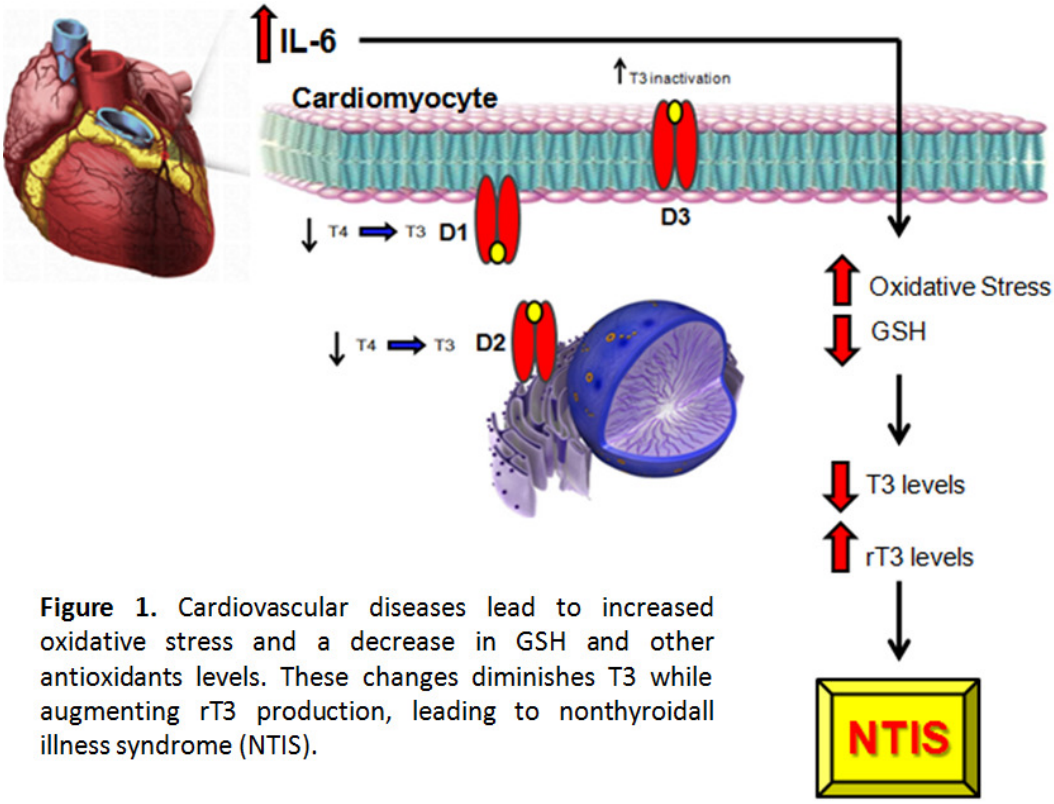

Figure 1. Cardiovascular diseases lead to increased oxidative stress and a decrease in GSH and other antioxidants levels. These changes diminishes T3 while augmenting rT3 production, leading to nonthyroidall illness syndrome (NTIS).

Changes in redox balance are known to disrupt deiodinases function and studies have made use of *N*-acetylcysteine (NAC) as a treatment in order to prevent these changes (Wajner, Goemann et al. 2011). *N*-acetylcysteine (NAC) is an antioxidant that increases intracellular cysteine levels and increase GSH levels. Thus, it can restore the deidinases activity and restoring redox equilibrium, preventing the inhibitory effectt of IL-6 on D1 and D2 mediated T4 to T3 conversion. NAC is a glutathione precursor that can correct the *upregulation* of D3 induced by ROS. The mechanism implicated in this phenomena is still not fully understood, but it is possible that D3 is protected by

extracellular GSH during the process of D3 endosomal internalization or that its active center is facing outward. After all, as previously described, GSH does not cross the plasma membrane. Thus explaining why its activity in intact cells parallels the D3 activity in sonicates of the same cells (Wajner, Goemann et al. 2011).

Lehnen et al (2017), demonstrated in animals submitted to AMI that NAC treatment prevents decreases in T3 levels after MI and preserves cardiac function in rats with NTIS, improving the diastolic and systolic diameters of the right and left ventricles, with a strong positive correlation between the ejection fraction and serum T3 levels. Moreover, NAC corrects the activity of D3 in cardiomyocytes after AMI through the improvement of the altered redox state (Lehnen, Santos et al. 2017). NAC also increases the antioxidant capacity, prevents apoptosis (Galang, Sasaki et al. 2000) and restores cardiomyocytes contractility (Kubin, Skoumal et al. 2011, Andre, Fauconnier et al. 2013). A recent study showed that NAC administration TH disorders that normally occurs in patients during the acute phase of MI (Vidart, Wajner et al. 2014). NAC rapidly reverses the acute thyroid disorders produced by oxidative stress to normal levels in patients with MI. In addition, the improvement of redox imbalance related to MI by NAC prevents abnormalities in TH metabolism and improves cardiac performance (Vidart, Wajner et al. 2014).

Due to its importance on heart metabolism, thyroid hormone administration was studied in order to improve cardiac function in cardiovascular disease. Animal studies showed that hormonal treatment with T3 and T4 promotes an improvement in cardiac function (de Castro, Tavares et al. 2014, Corssac, de Castro et al. 2016). The TH may have a protective effect after MI (Pantos, Mourouzis et al. 2007, Pantos, Mourouzis et al. 2008, Forini, Lionetti et al. 2011) and in CHF (Townsend, Nichols et al. 2015), since the concentrations of intracellular and circulating TH (mainly T3) decreases. Accordingly, low T3 administration (~ 6 μg / kg / day) following MI improves cardiac performance and reverses adverse changes in the gene expression without negative effects (Rajagopalan, Zhang et al. 2016).

Although long-term thyroid hormone administration has been shown to improve chronic cardiac function in post-MI heart failure in rats, experience with hormone replacement in humans is limited. The lack of benefit in the long-term outcomes and concerns about adverse effects, such as increased myocardial demand, arrhythmias and suppression of the hypothalamic-pituitary-thyroid axis, have reduced enthusiasm for T3 administration in acute settings. In humans, a study with post-ischemic patients who received T3 by continuous infusion showed that a short-term T3 administration reduced the activation of

the neuroendocrine system and improved systolic volume in LV in patients with ventricular dysfunction and NTIS (Pingitore, Galli et al. 2008). According to some authors, the therapy of TH replacement should be used only in patients with NTIS or subclinical hypothyroidism (Jabbar, Pingitore et al. 2017). And although T3 treatment improves cardiac function in patients with CHF, the true functional significance of low T3 and the effect of chronic T3 therapy remain obscure (Wassner, Jugo et al. 2017). Moreover, as in NTIS one of the major alterations is in the peripheral conversion of T4 to T3 and not the availability of T4 itself, the oral administration of T4 might not be of benefit as a treatment option.

5. FINAL REMARKS

The mechanisms behind NTIS are complex and encompass all stages of hormonal production and metabolism. So far, clinical studies have shown that the hormonal changes as seen in NTIS are common to different illness and associated with worse prognosis. In the acute phase of critical disease, TH changes were once thought as adaptive, as it could reduce energy expenditure and protein intake. However, the increase in morbidity and mortality associated with NTIS and the persistence of TH alterations in the chronic phase of disease, in which catabolism is deleterious, has stimulated further studies and development of treatment. Whether these changes are a mere marker of poor health or a part of the pathophysiological circle that sustains systemic derangement, thus leading to an increased morbidity and mortality is still a matter of debate. Given the available evidence, and probably due to several lacks in the understanding of the pathophysiological mechanisms of NTIS, there is no currently approved treatment for this set of TH derangements.

References

Adawiyah, J., A. W. Norasyikin, N. H. Mat, A. S. Shamsul and K. N. Azmi (2010). "The non-thyroidal illness syndrome in acute coronary syndrome is associated with increased cardiac morbidity and mortality." Heart Asia **2**(1): 11-14.

Andre, L., J. Fauconnier, C. Reboul, C. Feillet-Coudray, P. Meschin, C. Farah, G. Fouret, S. Richard, A. Lacampagne and O. Cazorla (2013). "Subendocardial increase in reactive oxygen species production affects regional contractile function in ischemic heart failure." Antioxid Redox Signal **18**(9): 1009-1020.

Baqui, M., D. Botero, B. Gereben, C. Curcio, J. W. Harney, D. Salvatore, K. Sorimachi, P. R. Larsen and A. C. Bianco (2003). "Human type 3 iodothyronine selenodeiodinase is located in the plasma membrane and undergoes rapid internalization to endosomes." J Biol Chem **278**(2): 1206-1211.

Bengel, F. M., S. G. Nekolla, T. Ibrahim, C. Weniger, S. I. Ziegler and M. Schwaiger (2000). "Effect of thyroid hormones on cardiac function, geometry, and oxidative metabolism assessed noninvasively by positron emission tomography and magnetic resonance imaging." J Clin Endocrinol Metab **85**(5): 1822-1827.

Bianco, A. C., D. Salvatore, B. Gereben, M. J. Berry and P. R. Larsen (2002). "Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases." Endocr Rev **23**(1): 38-89.

Boelen, A., M. C. Platvoet-Ter Schiphorst and W. M. Wiersinga (1993). "Association between serum interleukin-6 and serum 3,5,3'-triiodothyronine in nonthyroidal illness." J Clin Endocrinol Metab **77**(6): 1695-1699.

Boelen, A., A. H. van der Spek, F. Bloise, E. M. de Vries, O. V. Surovtseva, M. van Beeren, M. T. Ackermans, J. Kwakkel and E. Fliers (2017). "Tissue thyroid hormone metabolism is differentially regulated during illness in mice." J Endocrinol **233**(1): 25-36.

Boelen, A., W. M. Wiersinga and E. Fliers (2008). "Fasting-induced changes in the hypothalamus-pituitary-thyroid axis." Thyroid **18**(2): 123-129.

Breitkreuz, M. and N. Hamdani (2015). "A change of heart: oxidative stress in governing muscle function?" Biophys Rev **7**(3): 321-341.

Carr, A. N. and E. G. Kranias (2002). "Thyroid hormone regulation of calcium cycling proteins." Thyroid **12**(6): 453-457.

Chopra, I. J. (1997). "Clinical review 86: Euthyroid sick syndrome: is it a misnomer?" J Clin Endocrinol Metab **82**(2): 329-334.

Cooper, E., M. Gibbens, C. R. Thomas, C. Lowy and C. W. Burke (1983). "Conversion of thyroxine to 3,3',5'-triiodothyronine in the guinea pig placenta: in vivo studies." Endocrinology **112**(5): 1808-1815.

Coppola, A., J. Hughes, E. Esposito, L. Schiavo, R. Meli and S. Diano (2005). "Suppression of hypothalamic deiodinase type II activity blunts TRH mRNA decline during fasting." FEBS Lett **579**(21): 4654-4658.

Corssac, G. B., A. L. de Castro, A. V. Tavares, C. Campos, R. O. Fernandes, V. D. Ortiz, R. Siqueira, T. R. Fernandes, A. Bello-Klein and A. S. Araujo (2016). "Thyroid hormones effects on oxidative stress and cardiac remodeling in the right ventricle of infarcted rats." Life Sci **146**: 109-116.

de Castro, A. L., A. V. Tavares, C. Campos, R. O. Fernandes, R. Siqueira, A. Conzatti, A. M. Bicca, T. R. Fernandes, C. L. Sartorio, P. C. Schenkel, A. Bello-Klein and A. S. da Rosa Araujo (2014). "Cardioprotective effects of thyroid hormones in a rat model of myocardial infarction are associated with oxidative stress reduction." Mol Cell Endocrinol **391**(1-2): 22-29.

De Groot, L. J. (2006). "Non-thyroidal illness syndrome is a manifestation of hypothalamic-pituitary dysfunction, and in view of current evidence, should be treated with appropriate replacement therapies." Crit Care Clin **22**(1): 57-86, vi.

Docter, R., E. P. Krenning, M. de Jong and G. Hennemann (1993). "The sick euthyroid syndrome: changes in thyroid hormone serum parameters and hormone metabolism." Clin Endocrinol (Oxf) **39**(5): 499-518.

Fekete, C., S. Sarkar, M. A. Christoffolete, C. H. Emerson, A. C. Bianco and R. M. Lechan (2005). "Bacterial lipopolysaccharide (LPS)-induced type 2 iodothyronine deiodinase (D2) activation in the mediobasal hypothalamus (MBH) is independent of the LPS-induced fall in serum thyroid hormone levels." Brain Res **1056**(1): 97-99.

Forini, F., V. Lionetti, H. Ardehali, A. Pucci, F. Cecchetti, M. Ghanefar, G. Nicolini, Y. Ichikawa, M. Nannipieri, F. A. Recchia and G. Iervasi (2011). "Early long-term L-T3 replacement rescues mitochondria and prevents ischemic cardiac remodelling in rats." J Cell Mol Med **15**(3): 514-524.

Friberg, L., S. Werner, G. Eggertsen and S. Ahnve (2002). "Rapid down-regulation of thyroid hormones in acute myocardial infarction: is it cardioprotective in patients with angina?" Arch Intern Med **162**(12): 1388-1394.

Friesema, E. C., J. Jansen, J. W. Jachtenberg, W. E. Visser, M. H. Kester and T. J. Visser (2008). "Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10." Mol Endocrinol **22**(6): 1357-1369.

Galang, N., H. Sasaki and N. Maulik (2000). "Apoptotic cell death during ischemia/reperfusion and its attenuation by antioxidant therapy." Toxicology **148**(2-3): 111-118.

Galton, V. A. (2005). "The roles of the iodothyronine deiodinases in mammalian development." Thyroid **15**(8): 823-834.

Gereben, B., A. M. Zavacki, S. Ribich, B. W. Kim, S. A. Huang, W. S. Simonides, A. Zeold and A. C. Bianco (2008). "Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling." Endocr Rev **29**(7): 898-938.

Hamilton, M. A., L. W. Stevenson, M. Luu and J. A. Walden (1990). "Altered thyroid hormone metabolism in advanced heart failure." J Am Coll Cardiol **16**(1): 91-95.

Henderson, K. K., S. Danzi, J. T. Paul, G. Leya, I. Klein and A. M. Samarel (2009). "Physiological replacement of T3 improves left ventricular function in an animal model of myocardial infarction-induced congestive heart failure." Circ Heart Fail **2**(3): 243-252.

Heuer, H., M. K. Maier, S. Iden, J. Mittag, E. C. Friesema, T. J. Visser and K. Bauer (2005). "The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations." Endocrinology **146**(4): 1701-1706.

Iervasi, G., A. Pingitore, P. Landi, M. Raciti, A. Ripoli, M. Scarlattini, A. L'Abbate and L. Donato (2003). "Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease." Circulation **107**(5): 708-713.

Jabbar, A., A. Pingitore, S. H. Pearce, A. Zaman, G. Iervasi and S. Razvi (2017). "Thyroid hormones and cardiovascular disease." Nat Rev Cardiol **14**(1): 39-55.

Jankauskiene, E., P. Orda, E. Rumbinaite, D. Zaliaduonyte-Peksiene, R. Steponaviciute, A. Krasauskiene, J. J. Vaskelyte and R. Bunevicius (2015). "Left ventricular function by speckle-tracking echocardiography in patients with low-T3 syndrome and acute myocardial infarction." Medicina (Kaunas) **51**(4): 209-216.

Kimura, T., T. Kanda, N. Kotajima, A. Kuwabara, Y. Fukumura and I. Kobayashi (2000). "Involvement of circulating interleukin-6 and its receptor in the development of euthyroid sick syndrome in patients with acute myocardial infarction." Eur J Endocrinol **143**(2): 179-184.

Kinugawa, K., W. A. Minobe, W. M. Wood, E. C. Ridgway, J. D. Baxter, R. C. Ribeiro, M. F. Tawadrous, B. A. Lowes, C. S. Long and M. R. Bristow (2001). "Signaling pathways responsible for fetal gene induction in the failing human heart: evidence for altered thyroid hormone receptor gene expression." Circulation **103**(8): 1089-1094.

Klein, I. and K. Ojamaa (2001). "Thyroid hormone and the cardiovascular system." N Engl J Med **344**(7): 501-509.

Kmiec, Z., G. Kotlarz, B. Smiechowska and A. Mysliwski (1996). "Thyroid hormones homeostasis in rats refed after short-term and prolonged fasting." J Endocrinol Invest **19**(5): 304-311.

Kubin, A. M., R. Skoumal, P. Tavi, A. Konyi, A. Perjes, H. Leskinen, H. Ruskoaho and I. Szokodi (2011). "Role of reactive oxygen species in the regulation of cardiac contractility." J Mol Cell Cardiol **50**(5): 884-893.

Lamirand, A., S. Pallud-Mothre, M. Ramauge, M. Pierre and F. Courtin (2008). "Oxidative stress regulates type 3 deiodinase and type 2 deiodinase in cultured rat astrocytes." Endocrinology **149**(7): 3713-3721.

Larsen, P. R. and J. D. Baxter (2008). "Cardiovascular and metabolic issues in patients with thyroid dysfunction will be highlighted at the Fifth Anniversary of ATA Spring Meetings in March 2008." Thyroid **18**(2): 101.

Larsen, P. R. and A. M. Zavacki (2012). "The role of the iodothyronine deiodinases in the physiology and pathophysiology of thyroid hormone action." Eur Thyroid J **1**(4): 232-242.

Lehnen, T. E., M. V. Santos, A. Lima, A. L. Maia and S. M. Wajner (2017). "Preventing low T3 syndrome attenuates cardiac dysfunction in a male rat model of myocardial infarction." Endocrinology.

Li, L., C. Y. Guo, J. Yang, E. Z. Jia, T. B. Zhu, L. S. Wang, K. J. Cao, W. Z. Ma and Z. J. Yang (2011). "Negative association between free triiodothyronine level and international normalized ratio in euthyroid subjects with acute myocardial infarction." Acta Pharmacol Sin **32**(11): 1351-1356.

Maia, A. L., I. M. Goemann, E. L. Meyer and S. M. Wajner (2011). "Deiodinases: the balance of thyroid hormone: type 1 iodothyronine deiodinase in human physiology and disease." J Endocrinol **209**(3): 283-297.

Maia, A. L., B. W. Kim, S. A. Huang, J. W. Harney and P. R. Larsen (2005). "Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans." J Clin Invest **115**(9): 2524-2533.

Mebis, L. and G. van den Berghe (2009). "The hypothalamus-pituitary-thyroid axis in critical illness." Neth J Med **67**(10): 332-340.

Mortimer, R. H., J. P. Galligan, G. R. Cannell, R. S. Addison and M. S. Roberts (1996). "Maternal to fetal thyroxine transmission in the human term placenta is limited by inner ring deiodination." J Clin Endocrinol Metab **81**(6): 2247-2249.

Nishimura, M. and S. Naito (2008). "Tissue-specific mRNA expression profiles of human solute carrier transporter superfamilies." Drug Metab Pharmacokinet **23**(1): 22-44.

Ojamaa, K., A. Kenessey, R. Shenoy and I. Klein (2000). "Thyroid hormone metabolism and cardiac gene expression after acute myocardial infarction in the rat." Am J Physiol Endocrinol Metab **279**(6): E1319-1324.

Olivares, E. L., M. P. Marassi, R. S. Fortunato, A. C. da Silva, R. H. Costa-e-Sousa, I. G. Araujo, E. C. Mattos, M. O. Masuda, M. A. Mulcahey, S. A. Huang, A. C. Bianco and D. P. Carvalho (2007). "Thyroid function disturbance and type 3 iodothyronine deiodinase induction after myocardial infarction in rats a time course study." Endocrinology **148**(10): 4786-4792.

Ozcan, K. S., D. Osmonov, E. Toprak, B. Gungor, A. Tatlisu, A. Ekmekci, A. Kaya, G. Tayyareci and I. Erdinler (2014). "Sick euthyroid syndrome is associated with poor prognosis in patients with ST

segment elevation myocardial infarction undergoing primary percutaneous intervention." Cardiol J **21**(3): 238-244.

Pantos, C., I. Mourouzis, K. Markakis, A. Dimopoulos, C. Xinaris, A. D. Kokkinos, M. Panagiotou and D. V. Cokkinos (2007). "Thyroid hormone attenuates cardiac remodeling and improves hemodynamics early after acute myocardial infarction in rats." Eur J Cardiothorac Surg **32**(2): 333-339.

Pantos, C., I. Mourouzis, K. Markakis, N. Tsagoulis, M. Panagiotou and D. V. Cokkinos (2008). "Long-term thyroid hormone administration reshapes left ventricular chamber and improves cardiac function after myocardial infarction in rats." Basic Res Cardiol **103**(4): 308-318.

Papanas, N., K. Papatheodorou, D. Papazoglou, T. Gioka, C. Antonoglou, S. Kotsiou and E. Maltezos (2008). "Post-thyroidectomy thyroxine replacement dose in patients with or without compensated heart failure: the role of cytokines." Cytokine **41**(2): 121-126.

Pavlou, H. N., P. A. Kliridis, A. A. Panagiotopoulos, C. P. Goritsas and P. J. Vassilakos (2002). "Euthyroid sick syndrome in acute ischemic syndromes." Angiology **53**(6): 699-707.

Peeters, R. P., S. van der Geyten, P. J. Wouters, V. M. Darras, H. van Toor, E. Kaptein, T. J. Visser and G. Van den Berghe (2005). "Tissue thyroid hormone levels in critical illness." J Clin Endocrinol Metab **90**(12): 6498-6507.

Peeters, R. P., P. J. Wouters, E. Kaptein, H. van Toor, T. J. Visser and G. Van den Berghe (2003). "Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients." J Clin Endocrinol Metab **88**(7): 3202-3211.

Peeters, R. P., P. J. Wouters, H. van Toor, E. Kaptein, T. J. Visser and G. Van den Berghe (2005). "Serum 3,3',5'-triiodothyronine (rT3) and 3,5,3'-triiodothyronine/rT3 are prognostic markers in critically ill patients and are associated with postmortem tissue deiodinase activities." J Clin Endocrinol Metab **90**(8): 4559-4565.

Pingitore, A., E. Galli, A. Barison, A. Iervasi, M. Scarlattini, D. Nucci, A. L'Abbate, R. Mariotti and G. Iervasi (2008). "Acute effects of triiodothyronine (T3) replacement therapy in patients with chronic heart failure and low-T3 syndrome: a randomized, placebo-controlled study." J Clin Endocrinol Metab **93**(4): 1351-1358.

Pingitore, A., G. Iervasi, A. Barison, C. Prontera, L. Pratali, M. Emdin, D. Giannessi and D. Neglia (2006). "Early activation of an altered thyroid hormone profile in asymptomatic or mildly symptomatic idiopathic left ventricular dysfunction." J Card Fail **12**(7): 520-526.

Pol, C. J., A. Muller and W. S. Simonides (2010). "Cardiomyocyte-specific inactivation of thyroid hormone in pathologic ventricular hypertrophy: an adaptative response or part of the problem?" Heart Fail Rev **15**(2): 133-142.

Pol, C. J., A. Muller, M. J. Zuidwijk, E. D. van Deel, E. Kaptein, A. Saba, M. Marchini, R. Zucchi, T. J. Visser, W. J. Paulus, D. J. Duncker and W. S. Simonides (2011). "Left-ventricular remodeling after myocardial infarction is associated with a cardiomyocyte-specific hypothyroid condition." Endocrinology **152**(2): 669-679.

Rajagopalan, V., Y. Zhang, K. Ojamaa, Y. F. Chen, A. Pingitore, C. J. Pol, D. Saunders, K. Balasubramanian, R. A. Towner and A. M. Gerdes (2016). "Safe Oral Triiodo-L-Thyronine Therapy Protects from Post-Infarct Cardiac Dysfunction and Arrhythmias without Cardiovascular Adverse Effects." PLoS One **11**(3): e0151413.

Ray, D. C., A. Macduff, G. B. Drummond, E. Wilkinson, B. Adams and G. J. Beckett (2002). "Endocrine measurements in survivors and non-survivors from critical illness." Intensive Care Med **28**(9): 1301-1308.

Rodriguez-Perez, A., F. Palos-Paz, E. Kaptein, T. J. Visser, L. Dominguez-Gerpe, J. Alvarez-Escudero and J. Lado-Abeal (2008). "Identification of molecular mechanisms related to nonthyroidal illness syndrome in skeletal muscle and adipose tissue from patients with septic shock." Clin Endocrinol (Oxf) **68**(5): 821-827.

Rybin, V. and S. F. Steinberg (1996). "Thyroid hormone represses protein kinase C isoform expression and activity in rat cardiac myocytes." Circ Res **79**(3): 388-398.

Salvatore, D., W. S. Simonides, M. Dentice, A. M. Zavacki and P. R. Larsen (2014). "Thyroid hormones and skeletal muscle--new insights and potential implications." Nat Rev Endocrinol **10**(4): 206-214.

Salvatore, D., H. Tu, J. W. Harney and P. R. Larsen (1996). "Type 2 iodothyronine deiodinase is highly expressed in human thyroid." J Clin Invest **98**(4): 962-968.

Schenkel, P. C., A. M. Tavares, R. O. Fernandes, G. P. Diniz, M. Bertagnolli, A. S. da Rosa Araujo, M. L. Barreto-Chaves, M. F. Ribeiro, N. Clausell and A. Bello-Klein (2010). "Redox-sensitive prosurvival and proapoptotic protein expression in the myocardial remodeling post-infarction in rats." Mol Cell Biochem **341**(1-2): 1-8.

Simonides, W. S., M. A. Mulcahey, E. M. Redout, A. Muller, M. J. Zuidwijk, T. J. Visser, F. W. Wassen, A. Crescenzi, W. S. da-Silva, J. Harney, F. B. Engel, M. J. Obregon, P. R. Larsen, A. C. Bianco and S. A. Huang (2008). "Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats." J Clin Invest **118**(3): 975-983.

St Germain, D. L., V. A. Galton and A. Hernandez (2009). "Minireview: Defining the roles of the iodothyronine deiodinases: current concepts and challenges." Endocrinology **150**(3): 1097-1107.

Townsend, N., M. Nichols, P. Scarborough and M. Rayner (2015). "Cardiovascular disease in Europe 2015: epidemiological update." Eur Heart J **36**(40): 2673-2674.

Toyoda, N., M. Nishikawa, Y. Mori, M. Yoshimura, H. Masaki, A. Gondou, T. Yonemoto and M. Inada (1992). "Identification of a 27-kilodalton protein with the properties of type I iodothyronine 5'-deiodinase in human thyroid gland." J Clin Endocrinol Metab **74**(3): 533-538.

Trivieri, M. G., G. Y. Oudit, R. Sah, B. G. Kerfant, H. Sun, A. O. Gramolini, Y. Pan, A. D. Wickenden, W. Croteau, G. Morreale de Escobar, R. Pekhletski, D. St Germain, D. H. Maclennan and P. H. Backx (2006). "Cardiac-specific elevations in thyroid hormone enhance contractility and prevent pressure overload-induced cardiac dysfunction." Proc Natl Acad Sci U S A **103**(15): 6043-6048.

Vidart, J., S. M. Wajner, R. S. Leite, A. Manica, B. D. Schaan, P. R. Larsen and A. L. Maia (2014). "N-acetylcysteine administration prevents nonthyroidal illness syndrome in patients with acute myocardial infarction: a randomized clinical trial." J Clin Endocrinol Metab **99**(12): 4537-4545.

Visser, W. E., H. Heuer and T. J. Visser (2016). "Triiodothyroacetic Acid Treatment in MCT8 Deficiency: A Word of Nuance." Thyroid **26**(5): 615-617.

Visser, W. E., A. A. van Mullem, J. Jansen and T. J. Visser (2011). "The thyroid hormone transporters MCT8 and MCT10 transport the affinity-label N-bromoacetyl-[(125)I]T3 but are not modified by it." Mol Cell Endocrinol **337**(1-2): 96-100.

Wajner, S. M., I. M. Goemann, A. L. Bueno, P. R. Larsen and A. L. Maia (2011). "IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells." J Clin Invest **121**(5): 1834-1845.

Wajner, S. M. and A. L. Maia (2012). "New Insights toward the Acute Non-Thyroidal Illness Syndrome." Front Endocrinol (Lausanne) **3**: 8.

Wajner, S. M., H. C. Rohenkohl, T. Serrano and A. L. Maia (2015). "Sodium selenite supplementation does not fully restore oxidative stress-induced deiodinase dysfunction: Implications for the nonthyroidal illness syndrome." Redox Biol **6**: 436-445.

Wang, F., W. Pan, H. Wang, S. Wang, S. Pan and J. Ge (2012). "Relationship between thyroid function and ICU mortality: a prospective observation study." Crit Care **16**(1): R11.

Wang, W., H. Guan, A. M. Gerdes, G. Iervasi, Y. Yang and Y. D. Tang (2015). "Thyroid Status, Cardiac Function, and Mortality in Patients With Idiopathic Dilated Cardiomyopathy." J Clin Endocrinol Metab **100**(8): 3210-3218.

Warner, M. H. and G. J. Beckett (2010). "Mechanisms behind the non-thyroidal illness syndrome: an update." J Endocrinol **205**(1): 1-13.

Wassner, A. J., R. H. Jugo, D. M. Dorfman, R. F. Padera, M. A. Maynard, A. M. Zavacki, P. Y. Jay and S. A. Huang (2017). "Myocardial Induction of Type 3 Deiodinase in Dilated Cardiomyopathy." Thyroid **27**(5): 732-737.

Weber, K. T. (2016). "Depressed Myocardial Contractility: Can It Be Rescued?" Am J Med Sci **352**(4): 428-432.

Williams, G. R. (2008). "Neurodevelopmental and neurophysiological actions of thyroid hormone." J Neuroendocrinol **20**(6): 784-794.

Wu, X. Y., A. Y. Luo, Y. R. Zhou and J. H. Ren (2014). "N-acetylcysteine reduces oxidative stress, nuclear factorkappaB activity and cardiomyocyte apoptosis in heart failure." Mol Med Rep **10**(2): 615-624.

Zoeller, R. T. and J. Rovet (2004). "Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings." J Neuroendocrinol **16**(10): 809-818.

ARTIGO II

N-Acetylcysteine Prevents Low T3 Syndrome and Attenuates Cardiac Dysfunction in a Male Rat Model of Myocardial Infarction

Tatiana Ederich Lehnen,[1] Marcus Vinicius Santos,[1] Adrio Lima,[1] Ana Luiza Maia,[1] and Simone Magagnin Wajner[1]

[1]Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre 90035-003, Rio Grande do Sul, Brazil

Nonthyroidal illness syndrome (NTIS) affects patients with myocardial infarction (MI). Oxidative stress has been implicated as a causative factor of NTIS, and reversed via *N*-acetylcysteine (NAC). Male Wistar rats submitted to left anterior coronary artery occlusion received NAC or placebo. Decreases in triiodothyronine (T3) levels were noted in MI-placebo at 10 and 28 days post-MI, but not in MI-NAC. Groups exhibited similar infarct areas whereas MI-NAC exhibited higher ejection fraction than did MI-placebo. Left ventricular systolic and diastolic diameters were also preserved in MI-NAC, but not in MI-placebo. Ejection fraction was positively correlated with T3 levels. Oxidative balance was deranged only in MI-placebo animals. Increased type 3 iodothyronine deiodinase expression was detected in the cardiomyocytes of MI-placebo compared with normal heart tissue. NAC was shown to diminish type 3 iodothyronine deiodinase expression and activity in MI-NAC. These results show that restoring redox balance by NAC treatment prevents NTIS- related thyroid hormone derangement and preserves heart function in rats subjected to MI. (***Endocrinology*** **158: 1502–1510, 2017)**

Thyroid hormones are essential for growth, development, and metabolism in virtually every body system. Thyroid hormone homeostasis is critically regulated by the synchronized activities of the iodothyronine deiodinases. Type 1 (D1) and type 2 iodothyronine deiodinases catalyze the conversion of the prohormone thyroxine (T4) into the biologically active triiodothyronine (T3) via outer ring deiodination. In contrast, type 3 iodothyronine deiodinase (D3) catalyzes the inactivation of T4 and T3 via inner-ring deiodination (1).

The metabolic signature of the nonthyroidal illness syndrome (NTIS), also known as low T3 syndrome, is the thyroid hormone level alterations that occur in almost every form of acute or chronic illness (2). The pathophysiology of NTIS is complex. It was recently shown in a cell culture system that changes in intracellular redox balance alter T3/T4 activation/inactivation during the acute phase of the disease (3). Increased reactive oxygen species (ROS) generation reduces D1 and type 2 iodothyronine deiodinase function while increasing D3 function, resulting in decreased serum T3 levels and increased reverse T3 (rT3) concentrations. These alterations can be prevented by *N*-acetylcysteine (NAC), an antioxidant that raises intracellular cysteine levels and corrects glutathione (GSH) levels, restoring redox equilibrium. Neuroendocrine abnormalities seem to predominate in the setting of prolonged disease, and a reduced thyrotropin (TSH) level seems to be the most frequently observed abnormality in this setting (4, 5). Interestingly, studies utilizing postmortem samples have shown that D3 expression is induced in several tissues in disease state, further implicating this enzyme in illness-related thyroid hormone derangements (6–9).

NTIS is commonly observed in patients with myocardial infarction (MI) (10, 11). Low T3 levels are an independent marker of myocardial damage and are associated with a poor prognosis (12–14). The impact of T3 levels on heart disease can be explained by the

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in USA

Received 15 August 2016. Accepted 13 February 2017.
First Published Online 16 February 2017

Abbreviations: D1, type 1 iodothyronine deiodinase; D3, type 3 iodothyronine deiodinase; EF, ejection fraction; GSH, glutathione; GSSG, oxidized glutathione; LV, left ventricle; LVDd, left ventricular diastolic diameter; LVSd, left ventricular systolic diameter; MI, myocardial infarction; NAC, *N*-acetylcysteine; NTIS, nonthyroidal illness syndrome; ROS, reactive oxygen species; rT3, reverse triiodothyronine; T2, 3,5-diiodothyronine; T3, triiodothyronine; T4, thyroxine; TSH, thyrotropin; v/v, volume-to-volume ratio.

physiological actions of the hormone (15–17). Thyroid hormone activity in the cardiomyocyte regulates myocardial contractility, diastolic and systolic function. T3 activates and regulates cardiac genes encoding proteins such as the myosin heavy chain isoforms and the sarcoplasmic reticulum calcium-activated adenosine triphosphatase pump. Thus, thyroid hormones exert profound effects on the heart and on cardiovascular hemodynamics, maintaining cardiac mass and wall stress while preserving ellipsoid left ventricular geometry (16). Thyroid hormones also have a direct inotropic effect on the heart through both genomic and nongenomic effects on components of the adrenergic receptor complex and on sodium, potassium, and calcium channels. Thyroid hormone metabolism is reportedly abnormal in acute heart disease, as well as in the setting of cardiac hypertrophy and heart failure (15–17).

We recently showed that NAC administration prevents the thyroid hormone derangements that commonly occur in patients during the acute phase of MI (18). In this study, we aimed to determine whether attenuating decreases in serum T3 levels impacts MI-related cardiac dysfunction in an animal model of NTIS (15). Additionally, we explored the physiopathological role of cardiomyocyte D3 expression in heart function alterations.

Materials and Methods

Reagents

Reagents were obtained from Invitrogen (Life Technologies, Grand Island, NY), Calbiochem-Novabiochem (St. Louis, MO), or Sigma-Aldrich (St. Louis, MO).

Animals and procedures

Male Wistar rats (weighing ± 250 g) were used in the experiments. All procedures and experiments involving animals were performed in accordance with the recommendations of the Brazilian College of Animal Experimentation. Our study complied with the ethical principles of the *Guide for the Care and Use of Laboratory Animals* (19) and the international standards for animal research of the Guidelines for Reporting Animal Research (20). This study was approved by the Research Ethics Committee of our institution (protocol 130501). All animals received standard food and water *ad libitum* and were kept in cages under 12-hour light/dark cycles.

On the day of MI induction, the animals underwent basal echocardiography and blood sample collection (retro-orbital plexus) before being subjected to anterior descending artery ligation, as previously described (21, 22), resulting in infarction of the left ventricle (LV) free wall. Briefly, the rats were anesthetized intraperitoneally (1 mL/kg ketamine and 0.5 mL/kg xylazine), intubated, and connected to a mechanical ventilator with ambient air at a volume of 2.5 mL and ventilatory rate of 65 breaths per minute using a pressure fan for rodents (Harvard Apparatus, Holliston, MA, model 683). Anesthesia was administered through a vaporizer (Surgivet, St. Louis, MN) containing isoflurane (1 mL/mL; Isoforine; Cristália, Itapira, SP, Brazil). After MI or sham procedure, tramadol (20 mg/kg, every 6 hours for 48 hours, Teuto, Anápolis, GO, Brazil) was administered and animals were kept under medical oxygen at temperatures of 25 to 27°C for 6 hours. Twelve hours after surgery, blood was collected for troponin measurements to confirm successful MI induction. The animals were then divided into two groups (10 or 28 days of follow-up) and randomly assigned to receive either intraperitoneal NAC (10 mg/kg, 12-hour light/dark cycle for 48 hours, n = 20) or the same volume of vehicle (saline, n = 20; placebo group). Serial cardiac functional assessments and measurements of serum TSH, T4, T3, and rT3 levels were performed on the day of the procedure and at 10 or 28 days after surgery. Previous publications showed that neither T3 levels nor echocardiographic parameters changed at 28 days post-MI (23, 24).

At the conclusion of each experimental period (10 or 28 days), the animals were euthanized [anesthetized with a vaporizer (Surgivet) containing isoflurane and decapitated with a guillotine] and tissues were rapidly removed and frozen in liquid nitrogen before being stored for subsequent analyses. Hearts were separated into LV infarcted tissue, LV noninfarcted tissue, and non–LV noninfarcted heart tissue. For each sample, before dissection, one portion of the heart was fixed in 4% paraformaldehyde and processed for immunohistochemistry. The noninfarcted areas used for experiments were selected from remote nonaffected LV tissue.

Echocardiography

Echocardiograms were performed before MI and at 48 hours and 10 or 28 days post-MI. Animals were anesthetized with 2.5% isoflurane (in 0.5 L/min O_2) via inhalation and placed in the left lateral decubitus position (45°) so that we could obtain parasternal and apical cardiac images. An EnVisor HD system by Philips Medical (Andover, MA) and a 12-3 MHz transducer, 2 cm deep, were used, as was fundamental harmonic imaging (25). All echocardiographic analyses were performed by the same blinded echocardiographer. Anterior and posterior end-diastolic and end-systolic wall thickness and relative wall thickness were measured. Systolic function was expressed as the ejection fraction (EF), which was calculated using Simpson's method.

Plasma thyroid hormone, troponin, and carbonyl measurements

Measurements of T3, T4, rT3, TSH, and troponin levels were performed via electrochemiluminescence immunoassay (Siemens ADVIA Centaur XP). The interassay coefficients of variation were as follows: T3, 7.1%; T4, 10%; FT4, 7%; and rT3, <10%. The intra-assay coefficients of variation were as follows: T3, 6.2% to 7%; T4, 3% to 8%; FT4, 3% to 6%; and rT3, 6% to 8%. For carbonyl measurements, duplicate aliquots of plasma (containing ~0.3 mg of protein) from each sample were incubated with 500 mL of 10 mM 2,4-dinitrophenylhydrazine or 1.0 mL of 2 M HCl (blank tube). After 30 minutes, 250 mL of 50% trichloroacetic acid was added to the aliquots. The samples were subsequently centrifuged at 8000*g* for 30 minutes to obtain the protein pellets, which were immediately washed with ethanol/ethyl acetate at a 1:1 volume-to-volume ratio (v/v). The final protein pellets were diluted in 500 μL of 8 M urea buffer and incubated at 50°C for 90 minutes. The difference between the 2,4-dinitrophenylhydrazine treated and

HCl-treated samples (blank) was used to calculate the carbonyl content determined at 370 nm. The carbonyl content was calculated using the millimolar absorption coefficient of hydrazine (e370 nm = 2.1×10^4 M^{-1} cm^{-1}), and the results were expressed as nanomoles carbonyl per milligram protein and evaluated by the Bradford method.

Determination of tissue protein carbonyl content

Duplicate aliquots of 0.3 mg of protein homogenate of each tissue type were incubated with 500 μL of 10 mM 2,4-dinitrophenylhydrazine or 1.0 mL of 2 M HCl (blank tube). After 30 minutes, 250 μL 50% trichloroacetic acid was added to the aliquots. The samples were subsequently centrifuged at 8000*g* for 30 minutes to obtain the protein pellets, which were immediately washed with ethanol/ethyl acetate at 1:1 (v/v). The final protein pellets were resuspended in 500 μL of 8 M urea buffer and incubated at 50°C for 90 minutes. The difference between the 2,4-dinitrophenylhydrazine–treated and HCl-treated samples (blank) was used to calculate the carbonyl content determined at 370 nm. Carbonyl content was calculated using the millimolar absorption coefficient of hydrazine (e370 nm = 2.1×10^4 M^{-1} cm^{-1}), and the results were expressed in nanomoles carbonyl per milligram of protein (26).

Nonenzymatic antioxidant defenses

Reduced GSH levels were measured according to a standard method. Briefly, tissues were homogenized in the presence of 300 μL of 20 mM sodium phosphate and 140 mM KCl buffer (pH 7.4). Proteins were precipitated by adding sodium metaphosphoric acid for a final ratio of 1:1. Samples were centrifuged for 10 minutes at 7000*g*. Fifteen microliters of tissue preparation was incubated with an equal volume of *o*-phthaldialdehyde (1 mg/mL methanol) at room temperature for 15 minutes in the presence of 20 volumes (1:20 v/v) of 100 mM sodium phosphate buffer (pH 8.0) containing 5 mM EDTA. Fluorescence was measured using excitation and emission wavelengths of 350 nm and 420 nm, respectively. A calibration curve was generated using standard GSH (0.001 to 0.1 mM), and GSH concentrations were calculated as nanomoles per milligram protein. Total GSH and oxidized GSH (GSSG) levels were determined using the enzymatic recycling method described by Teare *et al.* (27), with some modifications. Briefly, tissues were homogenized in 4 volumes of a sulfosalicylic acid solution (11%) and Triton X-100 (0.11%) (1:1 weight-to-volume ratio). After incubating for 5 minutes at 4°C with continuous shaking, the samples were centrifuged at 10,000*g* for 10 minutes (4°C), and the supernatant was collected for analyses of glutathione levels. For GSSG measurement, 10 μL of the supernatant was added to 110 μL of a GSH masking buffer [100 mM phosphate buffer, 1 mM EDTA, 1.1% 2-vinylpyridine (pH 7.4)] and incubated for 1 hour at room temperature. The samples prepared for total GSH and GSSG measurement were subjected to enzymatic analysis in a recycling buffer system containing 300 μM reduced form of NAD phosphate, 225 μM 5′,5′-dithio-bis(2-nitrobenzoic acid), 1.6 U/mL glutathione reductase, and 1.0 mM EDTA in 100 mM phosphate buffer (pH 7.4). The linear increase in absorbance at 405 nm over time was monitored using a microplate reader (SpectraMax M5; Molecular Devices, Sunnyvale, CA). A standard curve was generated using known amounts of GSH (100 μM) and GSSG (3.47, 6.95, and 13.89 μM).

D3 activity assay

D3 activity in tissue samples was determined using paper chromatography as previously described (3, 28). Tissues were

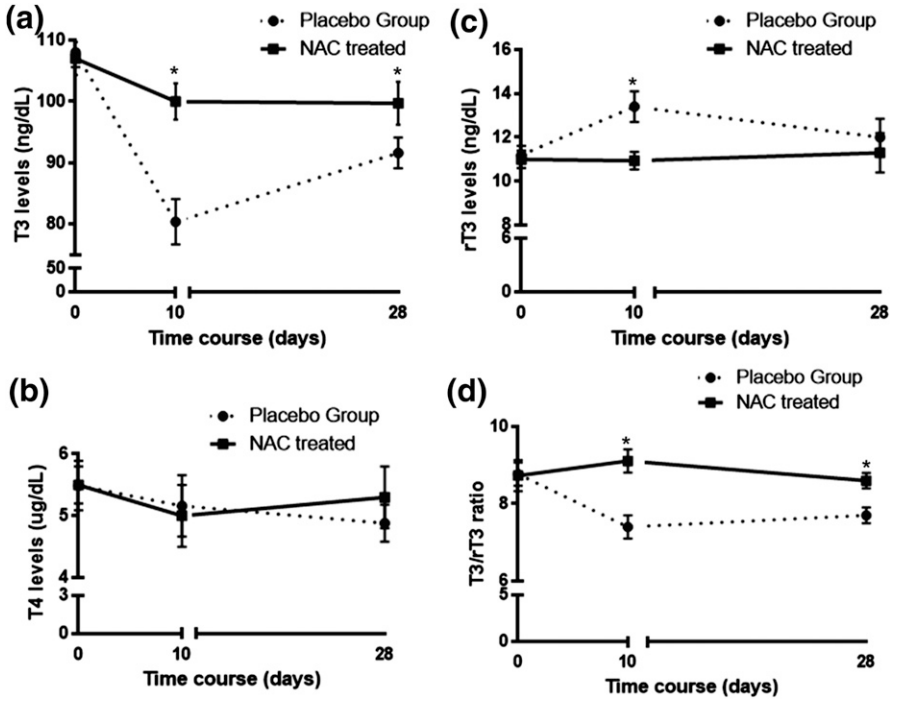

Figure 1. Changes in thyroid hormone levels in animals subjected to MI that received NAC or placebo. (a) Serum T3 levels decreased at 10 and 28 days post-MI in placebo animals, but not in NAC-treated animals. (b) Neither group exhibited significant changes in serum FT4 levels. (c) Serum rT3 levels were augmented in the placebo group at 10 days but stable in NAC-treated rats. The T3/rT3 ratio is progressively augmented in NAC-treated animals whereas it diminishes in the placebo group until day 10 (c). $^*P < 0.001$, n = 20 in each group.

individually homogenized and sonicated with 10 mM Tris-HCl, 0.25 sucrose buffer (pH 7.5), and 10 mM dithiothreitol. Protein concentration was quantified by Bradford assay using bovine serum albumin as a standard. The homogenates were incubated for 1 hour with 200,000 counts per minute ^{125}I-labeled T3, 2 nM T3, 20 mM dithiothreitol, and 1 mM propylthiouracil to inhibit any D1 activity. The addition of 200 nM of T3 completely abolished D3 activity in all samples. The reaction was stopped by adding 200 μL of ethanol (95%), 50 μL of NaOH (0.04 N), and 5 mg propylthiouracil. Deiodination was determined based on the amount of ^{125}I-3,5-diiodothyronine (T2) produced after separation of reaction products by paper chromatography. Results were expressed as the fraction of T2 counts minus the nonspecific deiodination (always <1.5%), obtained with the saturating concentration of T3 (200 nM). D3 activity was expressed as fentomoles T3 per minute per milligram of protein. The quantity of protein assayed was adjusted to ensure that <30% of the substrate was consumed. All experiments were performed in triplicate in at least two independent experiments.

Immunohistochemistry

D3 expression was evaluated via immunohistochemistry in matched normal and peri-infarcted tissue samples from placebo and NAC-treated rats. Heart tissue samples from animals subjected to surgery without artery ligation were used as controls (sham group, n = 20). D3 expression analysis was performed using 6-μm sections of formalin-fixed and paraffin-embedded tissues. The immunohistochemistry technique included tissue deparaffinization and rehydration, antigenic recovery, endogenous peroxidase inactivation, and unspecific reaction blockage. The samples were incubated with D3 anti-rabbit polyclonal antibodies (Novus Biologicals, Littleton, CO) overnight at 4°C at a dilution of 1:300 (the same antibody concentration was used for the positive-control samples), followed by incubation with biotinylated streptavidin/horseradish peroxidase–conjugated secondary antibodies (LSAB kit; Dako, Carpinteria, CA) and diaminobenzidine tetrahydrochloride (DAB kit; Dako). Placental tissue samples served as positive controls. Normal lung tissue was used as negative-control samples. Slides were examined using an Olympus BX51 microscope with an Olympus QColor 5 camera, and QCapturePro software was used to capture the images.

Statistical analysis

Unless otherwise specified, the results are presented as the mean ± standard deviation. Data were analyzed using a two-tailed Student *t* tests or one-way analysis of variance followed by *post hoc* Duncan multiple range tests when *F* was significant. Linear regression analysis was also used to verify concentration-dependent effects. Prism 6.0 software was used for statistical analyses, and $P < 0.05$ was considered significant.

Results

Animal model of MI and NTIS

Compared with baseline, serum T3 levels were decreased in MI-placebo animals at 10 (108 to 88.4 ng/dL; $P < 0.01$) and 28 days (108.5 to 91.6 ng/dL; $P < 0.03$) post-MI. In contrast, no significant decreases in T3 levels were noted in MI-NAC animals at 10 or 28 days post-MI (107 to 97.3 ng/dL, $P = 0.8$; 107 to 100 ng/dL, $P = 0.8$, respectively) [Fig. 1(a)]. No significant differences in serum T4 levels were observed between the two groups [5.49 ± 0.3 vs 5.5 ± 0.22 μg/dL; $P = 0.77$; Fig. 1(b)]. The baseline rT3 level was 11.2 ± 0.4 ng/dL in the MI-placebo group and increased to 14 ± 0.6 ng/dL at

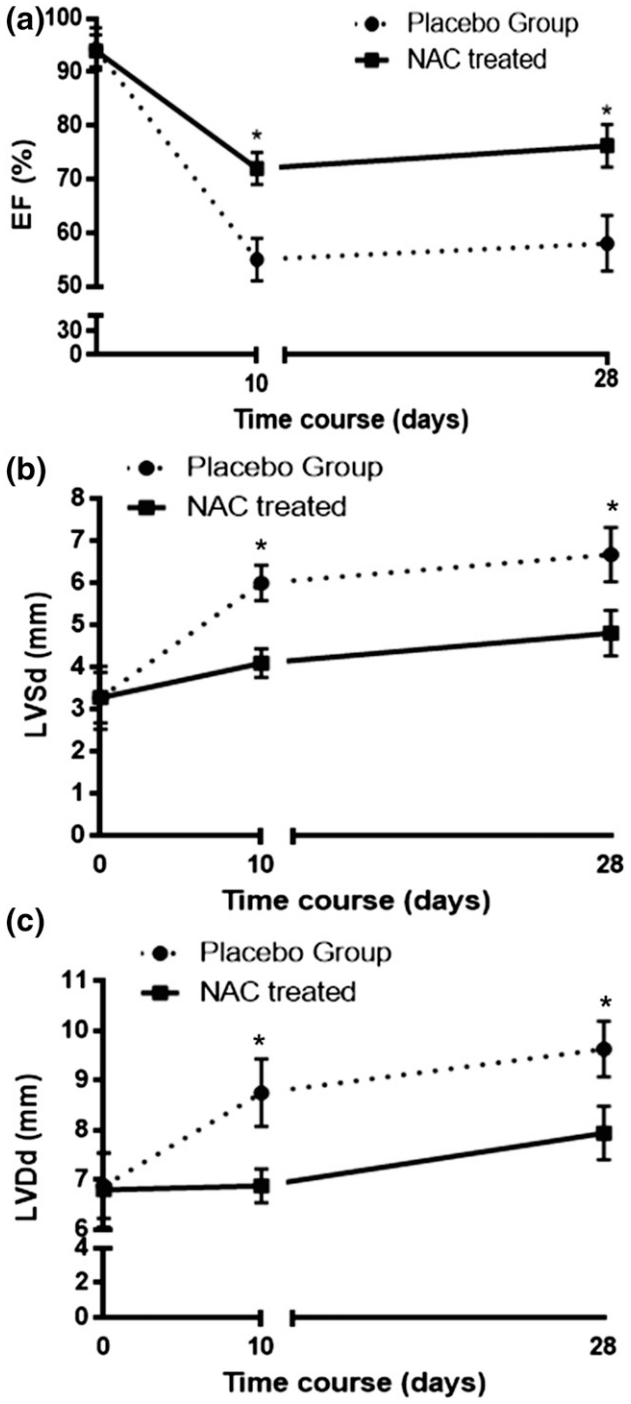

Figure 2. Effect of NAC administration on heart function. The NAC-treated group exhibited smaller decreases in EF (a), LVSd (b), and LVDd (c) compared with the placebo group. *$P < 0.001$, n = 20 in each group.

10 days post-MI [$P = 0.0001$, Fig. 1(c)]. No significant changes in rT3 levels were observed in the MI-NAC group (11.2 ± 0.4 vs 11 ± 0.4 vs 11.4 ± 0.9 ng/dL at baseline and 10 and 28 days post-MI, respectively; $P = 0.8$). Interestingly, whereas the T3/rT3 ratio remained low and stable in the placebo group [8.7 to 7.4 to 7.75 at baseline and 10 and 28 days post-MI, respectively; $P = 0.6$; Fig. 1(d)], it increased over time in the MI-NAC group [8.7 to 9.6 to 9.1 at baseline and 10 and 28 days post-MI, respectively; $P = 0.002$; Fig. 1(d)]. Serum TSH levels were virtually identical between the two groups (1.03 vs 1.12 ng/mL; $P = 0.8$).

Antioxidant treatment prevents thyroid hormone derangements and ameliorates MI-induced cardiac abnormalities

The placebo and NAC groups exhibited similar baseline echocardiographic measurements (Fig. 2). Twelve hours post-MI, both groups exhibited comparable infarcted area extensions as measured by echocardiography (~50%). Accordingly, troponin levels (46 vs 43 ng/L; $P = 0.7$) and heart rates were similar between the two groups ($P = 0.43$). The infarct size (expressed as a ratio of the percentage of infarcted area to total LV mass) at the time of euthanasia did not differ between the groups at 10 (40.3 ± 1.1 vs 39.6 ± 1.3, $P = 0.7$) and 28 (43.1 ± 1.5 vs 38.9 ± 2.5, $P = 0.2$) days. Heart weight was increased in the MI-placebo group (1.08 ± 0.06 vs 1.3 ± 0.3 vs 1.4 ± 0.05 g at baseline and 10 and 28 days post-MI, respectively; $P = 0.03$), in this case most likely due to hypertrophy and remodeling and was unchanged in the MI-NAC group (1.08 ± 0.06 vs 1.12 ± 0.06 vs 1.17 ± 0.1 g at baseline and 10 and 28 days post-MI, respectively; $P > 0.05$).

The EF was ~22% higher in MI-NAC animals than in MI-placebo animals at 10 (72 vs 57.3%; $P < 0.001$) and 28 days post-MI [72.3 vs 56%; $P < 0.001$; Fig. 2(a)]. Final left ventricular systolic (LVSd) and diastolic (LVDd) diameters were maintained in the MI-NAC group [3.28 to 4.1 mm and 7 to 7.5 mm, respectively; $P > 0.05$, Figs. 2(b) and 2(c)], but not in the MI-placebo group [3.2 to 6.4 mm and 6.9 to 8.7 mm, respectively; $P < 0.001$; Fig. 2 (b) and 2(c)], at 10 days post-MI. LVSd and LVDd were preserved to a greater extent in the MI-NAC group than in the MI-placebo group [4.8 vs 7 mm and 6.7 vs 10 mm, respectively; $P < 0.001$, Fig. 2(b) and 2(c)] at 28 days post-MI. The improvement of diastolic function was also evidenced by the lower early-to-late ratio of ventricular filling velocities in IM-NAC animals as compared with IAM-placebo (2 vs 2.6, $P = 0.005$ at 10 days; 2.1 vs 2.5, $P = 0.001$ at 28 days).

Next, we sought to evaluate whether serum T3 levels correlate with heart function parameters. Interestingly, the EF was positively correlated with T3 levels at 10 [$r = 0.85$, $P < 0.0001$, Fig. 3(a)] and 28 [$r = 0.7$, $P = 0.0001$, Fig. 3(b)] days post-MI.

NAC administration restores total carbonyl content and nonenzymatic antioxidant compounds

Total carbonyl content, a parameter of protein oxidation, was subsequently measured. Plasma carbonyl content increased during the initial hours post-MI (data not shown) and was higher in both the MI-placebo group and the MI-NAC group than in the sham group at 10 [$P < 0.002$, Fig. 4(a)] and 28 days post-MI [$P = 0.01$, Fig. 4(a)]. Peri-infarcted heart tissue samples exhibited increased carbonyl content in the MI-placebo group compared with the MI-NAC group at 10 [$P = 0.0001$, Fig. 4(b)] and 28 days post-MI [$P = 0.01$, Fig. 4(a)]. Noninfarcted heart tissue samples also exhibited increased carbonyl content in the MI-placebo group at 10 [$P = 0.004$, Fig. 4(c)] and 28 days post-MI [$P = 0.01$, Fig. 4(c)].

We then determined the concentrations of the nonenzymatic antioxidant GSH in all tissues. GSH content was diminished in the peri-infarcted tissue samples of MI-placebo animals, but not MI-NAC animals, at 10 [$P < 0.001$, Fig. 5(a)] and 28 days post-MI [$P = 0.01$, Fig. 5(a)]. GSH levels were diminished in the noninfarcted tissue samples of MI-placebo animals at 10 days post-MI ($P < 0.01$, Fig. 5B), but not at 28 days post-MI [$P > 0.05$, Fig. 5(b)]. The GSH/GSSG ratio was decreased in both tissue samples [$P < 0.0001$, Fig. 5(c) and 5(d))] at 10 days post-MI and in the peri-infarcted tissue samples at 28 days post-MI [$P < 0.001$, Fig. 5(c)].

D3 expression and activity in cardiomyocytes is affected by NAC

We subsequently determined the role of D3 expression in myocardial tissue damage. D3 protein expression levels

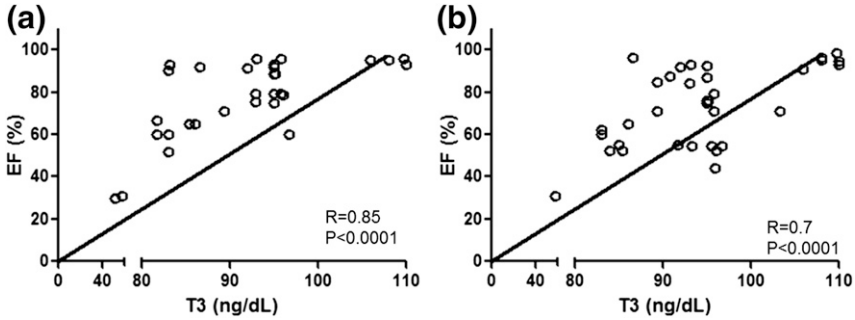

Figure 3. The EF was positively correlated with plasma T3 levels at both 10 (a) and 28 days post-MI (b). *$P < 0.001$, n = 20 in each group.

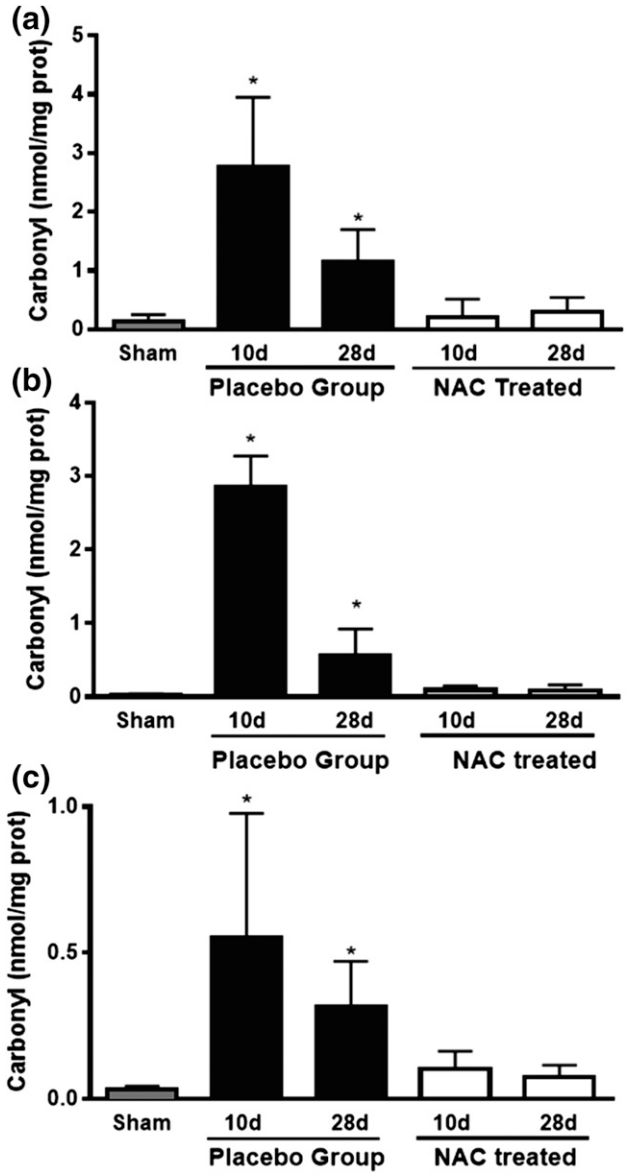

Figure 4. Oxidative stress biomarkers in rats subjected to MI that received NAC or placebo. (a) A significant augmentation in total plasma carbonyl content was observed in the placebo group, whereas total carbonyl content decreased significantly and reached the levels observed in sham in NAC-treated group. Total tissue carbonyl content was increased in the peri-infarcted (b) and noninfarcted heart tissue samples of the placebo group (c). *$P < 0.001$, n = 20 in each group.

were determined via immunohistochemistry and enzyme assay activity. We observed an increased D3 staining (fourfold) in peri-infarcted myocardial tissue of the MI-placebo group compared with sham tissue [$P < 0.001$, Fig. 6(a)]. However, D3 staining levels in the peri-infarcted samples of MI-NAC animals were not different from those in the tissue samples of sham animals [$P > 0.05$, Fig. 6(a)]. Accordingly, D3 activity levels were augmented at 10 and 28 days post-MI in the peri-infarcted tissue samples of the placebo group when compared with sham [7.4 ± 0.6 vs 5.22 ± 0.5 vs 2.18 ± 0.4 fmol/mg protein/min, respectively, $P < 0.0001$, Fig. 6(b)]. Interestingly, D3 activity in the peri-infarcted tissue of MI-NAC animals was not different from sham animals [4.6 ± 0.4 vs 3.7 ± 0.6 vs 2.18 ± 0.4 fmol/mg protein/min, respectively, $P > 0.05$, Fig. 6(b)].

Discussion

The present study was designed to investigate whether attenuating oxidative stress impacts NTIS and heart function in rats subjected to MI. We showed that NAC treatment prevents decreases in T3 levels and preserves heart function. Accordingly, the EF was shown to be strongly correlated with serum T3 levels. MI was associated with altered redox balance and increased D3 levels in infarcted heart tissue samples. Taken together, these findings indicate that a correlation exists among oxidative stress–induced thyroid hormone inactivation, low T3 levels, and altered heart function in MI-related NTIS.

NTIS is characterized by several changes in thyroid hormone physiology, the most striking of which are decreases in T3 levels and increases in rT3 levels, which are observed in a variety of illnesses. NTIS is commonly observed in patients suffering from MI, and low serum T3 levels are inversely correlated with mortality (12). Previous studies have noted improvements in cardiac systolic and diastolic function, as well as subsequent improvements in hemodynamics and myocardial perfusion, in patients treated with thyroid hormones (15, 29, 30). Accordingly, authors have attempted to restore serum T3 levels or T3-related gene expression in rat models of MI and low T3 syndrome by administering high doses of T3 or T4 (15, 29, 30). This approach has been shown to increase the EF by ~10% and to improve ventricular function but has not been shown to have any effect on left or right ventriclular mass or cardiac hypertrophy (15, 30). To date, all attempts to treat NTIS have involved administering thyroid hormone replacement therapy. In this study, confirming previous studies performed in cell culture systems and MI patients, we demonstrated that NAC administration attenuates MI-induced oxidative stress and prevent the characteristic changes in serum thyroid hormone levels as observed in NTIS. This indicates that thyroid hormone derangement occurs mainly as a result of changes in redox balance affecting peripheral thyroid hormone metabolism (3, 18). NAC-treated animals exhibited higher EF and better preserved LV systolic and diastolic diameters compared with placebo-treated group (Fig. 2). Consistently, we observed a positive correlation between plasma T3 levels and the EF (Fig. 3). Nevertheless, we cannot exclude an associated NAC-related beneficial effect on heart function. These results demonstrate that NAC attenuates

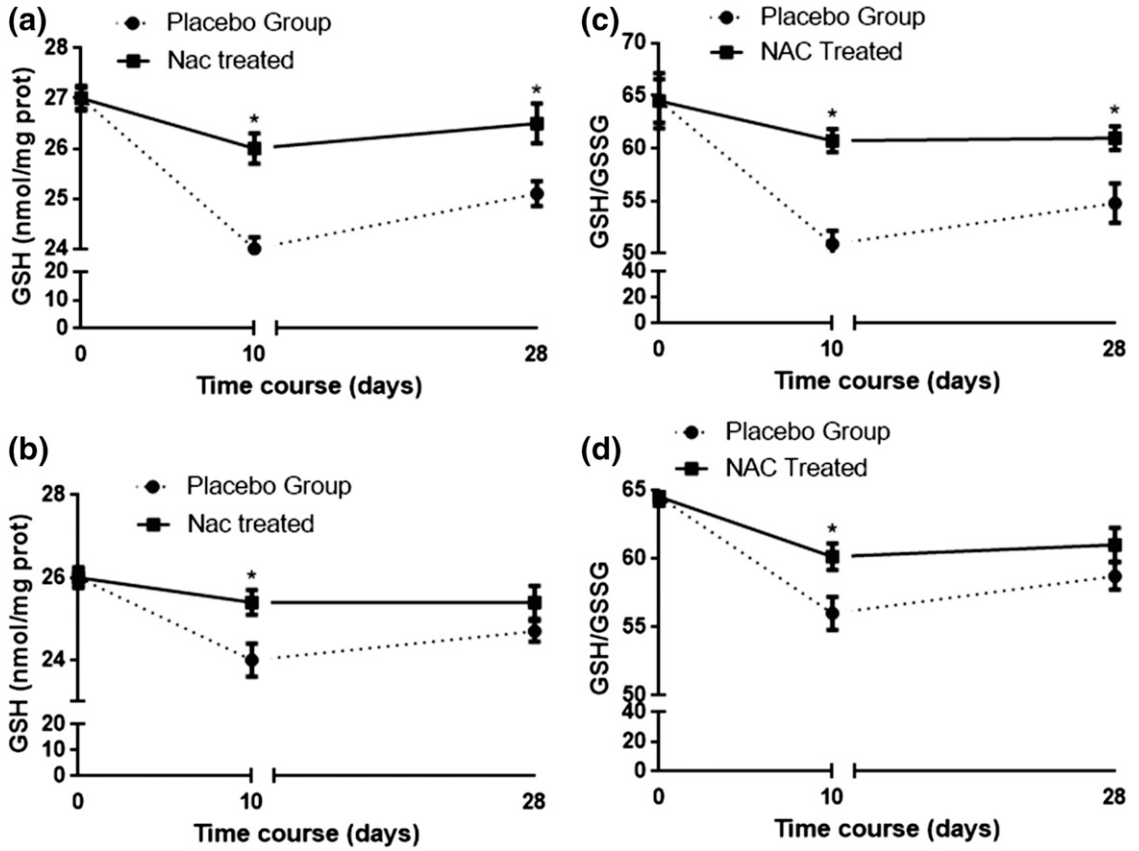

Figure 5. GSH levels were diminished in both the peri-infarcted (a) and in the noninfarcted (b) heart tissue of the placebo group, but not the NAC-treated animals. The GSH/GSSG ratio was shown to be reduced in the placebo group in both peri-infarcted (c) and noninfarcted (d) heart tissue. $*P < 0.001$, n = 20 in each group.

MI-related NTIS and results in myocardial protection. The data presented in this study have provided us with new insights regarding the pathophysiology of NTIS-associated thyroid hormone level changes and heart function.

Changes in redox balance are known to disrupt deiodinase function (31). Increases in ROS induce D3, an oxidoreductase that catalyzes iodine removal from the inner rings of thyroid hormones, inactivating them (1). The role of D3 has been implicated in NTIS by experimental and clinical studies (7–9, 32). Interestingly, it was recently shown that patients with low T3 syndrome secondary to postoperative atrial fibrillation presented with elevated T2 levels. Systemic D3 upregulation diminishes T3 levels but increases those of T2, further explaining why these patients do not benefit from exogenous thyroid hormone administration (33). ROS-induced D3 upregulation can be fully corrected by the antioxidant NAC, a thiol-containing radical scavenger and glutathione precursor (3, 34, 35). Although the effect of NAC on D3 expression and activity is still not fully understood, it is probably associated with the maintenance of intracellular GSH levels, as well as the correction of redox imbalance (Figs. 4 and 5). Interestingly, GSH has the unusual property that it becomes a more potent reductant and effective antioxidant as its concentration increases, as a consequence of the formation of disulfide bridges between two GSHs. Therefore, modest increases in GSH concentration result in exceptionally large effects on the antioxidant network. Thus, one can suppose that rather than increasing GSH levels, NAC administration immediately after the MI prevents a decrease in GSH levels, as observed in the placebo group. Elegant studies have shown that cardiomyocyte D3 induction post-MI is associated with a 50% reduction of tissue T3 levels, leading to a local hypothyroid condition and affecting LV remodeling (16). Thus, we decided to also investigate the effect of NAC treatment on heart D3 expression and activity. We show that cardiomyocyte D3 expression is increased in peri-infarcted tissue in the placebo-MI group but not in NAC-treated animals (Fig. 6), suggesting that the preservation of heart function is dependent on the maintenance of intracellular thyroid hormone levels by NAC administration. Interestingly, D3 immunostaining and activity showed similar results, suggesting that protein and activity levels correlate in heart tissue.

Taken together, our findings demonstrate that improvement of the MI-related redox imbalance by NAC prevents the abnormalities in thyroid hormone metabolism and improves cardiac performance. Moreover,

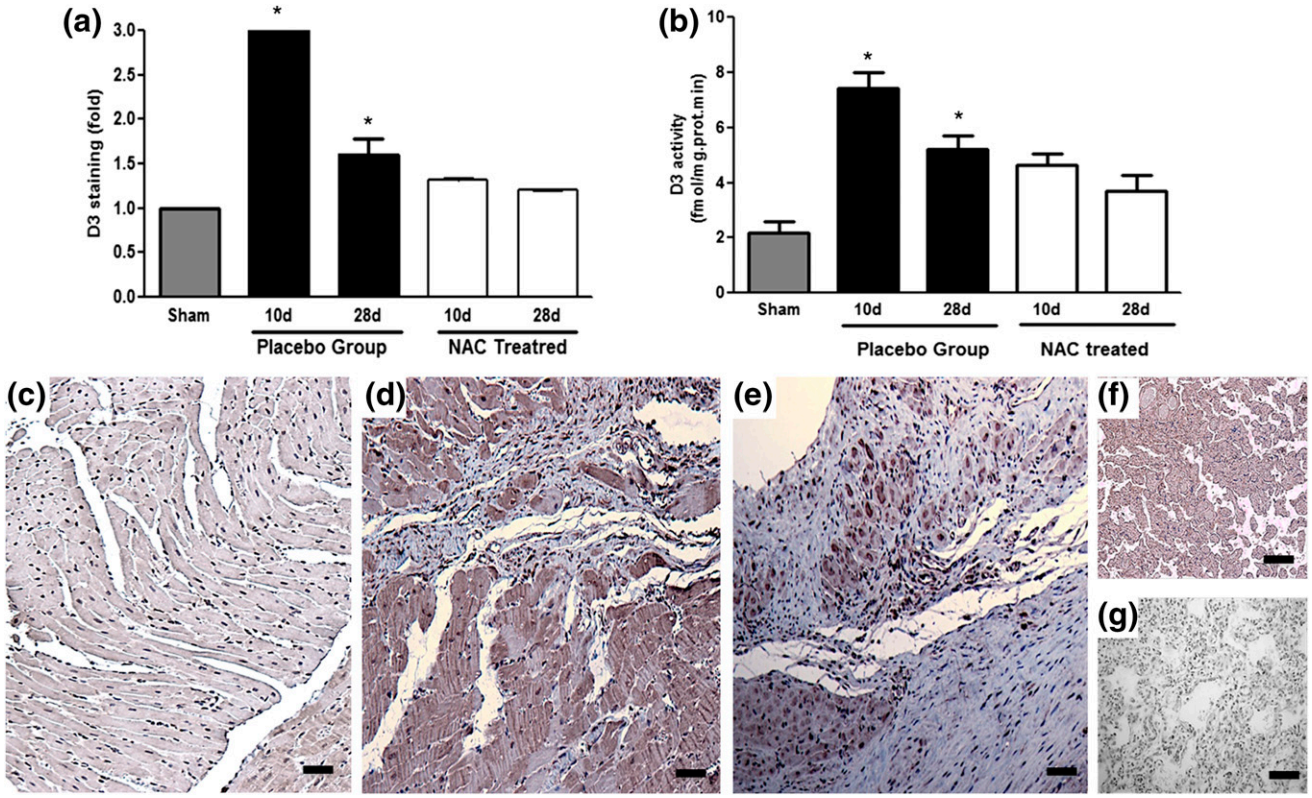

Figure 6. (a) Type 3 deiodinase staining is significantly augmented in the peri-infarcted tissue samples of the placebo group, but not in the NAC-treated group, at 10 and 28 days post-MI. (b) D3 activity is also augmented in the MI-placebo but not in MI-NAC group. $*P < 0.001$, n = 20 in each group. Representative micrographs of D3 brown–dark immunohistochemically staining heart tissue of sham (c), peri-infarcted heart of MI-placebo (d), and peri-infarcted heart of the NAC-treated group (e). Placenta was used as positive control for D3 staining (f), whereas normal lung was used as a negative control for D3 staining (g). Original magnification, ×200.

our results confirm that tissue D3 induction likely contributes to the cardiac injury associated with ischemic heart disease. Whether antioxidants such as NAC could be beneficial as an adjuvant therapy in MI patients, as well as its putative impact on patient survival, remains to be evaluated.

Acknowledgments

Address all correspondence and requests for reprints to: Ana Luiza Maia, MD, PhD, Serviço de Endocrinologia, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, CEP 90035-003–Porto Alegre, RS, Brazil. E-mail: almaia@ufrgs.br.

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio a Pesquisa do Rio Grande do Sul (FAPERGS), and Fundo de Incentivo a Pesquisa do Hospital de Clínicas de Porto Alegre (FIPE), Brazil.

Disclosure Summary: The authors have nothing to disclose.

References

1. Maia AL, Goemann IM, Meyer EL, Wajner SM. Deiodinases: the balance of thyroid hormone: type 1 iodothyronine deiodinase in human physiology and disease. *J Endocrinol*. 2011;**209**(3):283–297.
2. Larsen PR, Baxter JD. Cardiovascular and metabolic issues in patients with thyroid dysfunction will be highlighted at the fifth anniversary of ATA spring meetings in March 2008. *Thyroid*. 2008;**18**(2):101.
3. Wajner SM, Goemann IM, Bueno AL, Larsen PR, Maia AL. IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells. *J Clin Invest*. 2011;**121**(5):1834–1845.
4. de Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. *J Endocrinol*. 2015;**225**(3):R67–R81.
5. Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. *Thyroid*. 2014;**24**(10):1456–1465.
6. Peeters RP, van der Geyten S, Wouters PJ, Darras VM, van Toor H, Kaptein E, Visser TJ, Van den Berghe G. Tissue thyroid hormone levels in critical illness. *J Clin Endocrinol Metab*. 2005;**90**(12):6498–6507.
7. Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ, Van den Berghe G. Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. *J Clin Endocrinol Metab*. 2003;**88**(7):3202–3211.
8. Debaveye Y, Ellger B, Mebis L, Van Herck E, Coopmans W, Darras V, Van den Berghe G. Tissue deiodinase activity during prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone-releasing peptide-2. *Endocrinology*. 2005;**146**(12):5604–5611.
9. Rodriguez-Perez A, Palos-Paz F, Kaptein E, Visser TJ, Dominguez-Gerpe L, Alvarez-Escudero J, Lado-Abeal J. Identification of molecular mechanisms related to nonthyroidal illness syndrome in skeletal muscle and adipose tissue from patients with septic shock. *Clin Endocrinol (Oxf)*. 2008;**68**(5):821–827.

10. Kimura T, Kanda T, Kotajima N, Kuwabara A, Fukumura Y, Kobayashi I. Involvement of circulating interleukin-6 and its receptor in the development of euthyroid sick syndrome in patients with acute myocardial infarction. *Eur J Endocrinol*. 2000;**143**(2): 179–184.
11. Pavlou HN, Kliridis PA, Panagiotopoulos AA, Goritsas CP, Vassilakos PJ. Euthyroid sick syndrome in acute ischemic syndromes. *Angiology*. 2002;**53**(6):699–707.
12. Iervasi G, Pingitore A, Landi P, Raciti M, Ripoli A, Scarlattini M, L'Abbate A, Donato L. Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease. *Circulation*. 2003; **107**(5):708–713.
13. Adawiyah J, Norasyikin AW, Mat NH, Shamsul AS, Azmi KN. The non-thyroidal illness syndrome in acute coronary syndrome is associated with increased cardiac morbidity and mortality. *Heart Asia*. 2010;**2**(1):11–14.
14. Marenzi G, Assanelli E, Marana I, Lauri G, Campodonico J, Grazi M, De Metrio M, Galli S, Fabbiocchi F, Montorsi P, Veglia F, Bartorelli AL. *N*-acetylcysteine and contrast-induced nephropathy in primary angioplasty. *N Engl J Med*. 2006;**354**(26):2773–2782.
15. Ojamaa K, Kenessey A, Shenoy R, Klein I. Thyroid hormone metabolism and cardiac gene expression after acute myocardial infarction in the rat. *Am J Physiol Endocrinol Metab*. 2000;**279**(6): E1319–E1324.
16. Pol CJ, Muller A, Simonides WS. Cardiomyocyte-specific inactivation of thyroid hormone in pathologic ventricular hypertrophy: an adaptative response or part of the problem? *Heart Fail Rev*. 2010;**15**(2):133–142.
17. Henderson KK, Danzi S, Paul JT, Leya G, Klein I, Samarel AM. Physiological replacement of T3 improves left ventricular function in an animal model of myocardial infarction-induced congestive heart failure. *Circ Heart Fail*. 2009;**2**(3):243–252.
18. Vidart J, Wajner SM, Leite RS, Manica A, Schaan BD, Larsen PR, Maia AL. *N*-acetylcysteine administration prevents nonthyroidal illness syndrome in patients with acute myocardial infarction: a randomized clinical trial. *J Clin Endocrinol Metab*. 2014;**99**(12): 4537–4545.
19. National Institutes of Health. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academy Press; 1996.
20. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*. 2010;**8**(6):e1000412.
21. Lehnen TE, Lehnen AM, Tavares AM, Belló-Klein A, Markoski MM, Machado UF, Schaan B. Atorvastatin administered before myocardial infarction in rats improves contractility irrespective of metabolic changes. *Clin Exp Pharmacol Physiol*. 2014;**41**(12): 986–994.
22. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ Res*. 1979;**44**(4):503–512.
23. Schenkel PC, Tavares AM, Fernandes RO, Diniz GP, Ludke AR, Ribeiro MF, Araujo AS, Barreto-Chaves ML, Belló-Klein A. Time course of hydrogen peroxide–thioredoxin balance and its influence on the intracellular signalling in myocardial infarction. *Exp Physiol*. 2012;**97**(6):741–749.
24. Olivares EL, Marassi MP, Fortunato RS, da Silva AC, Costa-e-Sousa RH, Araújo IG, Mattos EC, Masuda MO, Mulcahey MA, Huang SA, Bianco AC, Carvalho DP. Thyroid function disturbance and type 3 iodothyronine deiodinase induction after myocardial infarction in rats a time course study. *Endocrinology*. 2007; **148**(10):4786–4792.
25. Cheitlin MD, Armstrong WF, Aurigemma GP, Beller GA, Bierman FZ, Davis JL, Douglas PS, Faxon DP, Gillam LD, Kimball TR, Kussmaul WG, Pearlman AS, Philbrick JT, Rakowski H, Thys DM, Antman EM, Smith SC, Jr, Alpert JS, Gregoratos G, Anderson JL, Hiratzka LF, Faxon DP, Hunt SA, Fuster V, Jacobs AK, Gibbons RJ, Russell RO; ACC; AHA; ASE. ACC/AHA/ASE 2003 Guideline Update for the Clinical Application of Echocardiography: summary article. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASE Committee to Update the 1997 Guidelines for the Clinical Application of Echocardiography). *J Am Soc Echocardiogr*. 2003; **16**(10):1091–1110.
26. Zanatta A, Viegas CM, Tonin AM, Busanello EN, Grings M, Moura AP, Leipnitz G, Wajner M. Disturbance of redox homeostasis by ornithine and homocitrulline in rat cerebellum: a possible mechanism of cerebellar dysfunction in HHH syndrome. *Life Sci*. 2013;**93**(4):161–168.
27. Teare JP, Punchard NA, Powell JJ, Lumb PJ, Mitchell WD, Thompson RP. Automated spectrophotometric method for determining oxidized and reduced glutathione in liver. *Clin Chem*. 1993;**39**(4):686–689.
28. Huang SA, Mulcahey MA, Crescenzi A, Chung M, Kim BW, Barnes C, Kuijt W, Turano H, Harney J, Larsen PR. Transforming growth factor-β promotes inactivation of extracellular thyroid hormones via transcriptional stimulation of type 3 iodothyronine deiodinase. *Mol Endocrinol*. 2005;**19**(12):3126–3136.
29. Katzeff HL, Powell SR, Ojamaa K. Alterations in cardiac contractility and gene expression during low-T3 syndrome: prevention with T3. *Am J Physiol*. 1997;**273**(5 Pt 1):E951–E956.
30. Chang KC, Figueredo VM, Schreur JH, Kariya K, Weiner MW, Simpson PC, Camacho SA. Thyroid hormone improves function and Ca^{2+} handling in pressure overload hypertrophy. Association with increased sarcoplasmic reticulum Ca^{2+}-ATPase and alpha-myosin heavy chain in rat hearts. *J Clin Invest*. 1997;**100**(7): 1742–1749.
31. Wajner SM, Maia AL. New insights toward the acute non-thyroidal illness syndrome. *Front Endocrinol (Lausanne)*. 2012;**3**:8.
32. Peeters RP, Wouters PJ, van Toor H, Kaptein E, Visser TJ, Van den Berghe G. Serum 3,3′,5′-triiodothyronine (rT3) and 3,5,3′-triiodothyronine/rT3 are prognostic markers in critically ill patients and are associated with postmortem tissue deiodinase activities. *J Clin Endocrinol Metab*. 2005;**90**(8):4559–4565.
33. Dietrich JW, Müller P, Schiedat F, Schlömicher M, Strauch J, Chatzitomaris A, Klein HH, Mügge A, Köhrle J, Rijntjes E, Lehmphul I. Nonthyroidal illness syndrome in cardiac illness involves elevated concentrations of 3,5-diiodothyronine and correlates with atrial remodeling. *Eur Thyroid J*. 2015;**4**(2):129–137.
34. Wajner SM, Rohenkohl HC, Serrano T, Maia AL. Sodium selenite supplementation does not fully restore oxidative stress-induced deiodinase dysfunction: implications for the nonthyroidal illness syndrome. *Redox Biol*. 2015;**6**:436–445.
35. Lamirand A, Pallud-Mothré S, Ramaugé M, Pierre M, Courtin F. Oxidative stress regulates type 3 deiodinase and type 2 deiodinase in cultured rat astrocytes. *Endocrinology*. 2008;**149**(7): 3713–3721.

ARTIGO III

Oxidative stress induces type 3 deiodinase in multiple tissues after myocardial infarction: implications to T3 Low Syndrome pathophysiology

Tatiana Ederich Lehnen, Rafael Marschner, Ana Luiza Maia, Simone Magagnin Wajner

Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.

Word count: 3.436

Corresponding author and person to who reprints should be addressed:
Dr. Simone Magagnin Wajner
Serviço de Endocrinologia
Hospital de Clínicas de Porto Alegre
Rua Ramiro Barcelos, 2350
CEP 90035-003 – Porto Alegre, RS, Brasil.
Phone/Fax: 55-51-33310207
E-mail: simone.wajner@ufrgs.br

Disclosure Statement: The authors have nothing to disclose.

ABSTRACT

Induction of type 3 deiodinase (D3) alters the metabolism of thyroid hormones in the low T3 syndrome. Imbalances in the redox status, existent in disease are involved in the change of deiodinases function. N-acetylcysteine (NAC), a potent antioxidant, corrects the oxidative damage and alterations seen in deiodinases in cell culture model. However, the effect of oxidative stress on D3 expression and activity in other tissues than the initially affected are unknown. Objective: Evaluate the oxidative stress and the D3 function and activity in liver, muscle and brain in an animal model of myocardial infarction (MI) and low T3 syndrome. Methods: Male Wistar rats submitted to left anterior coronary artery occlusion (MI) received NAC (10mg/kg, n=20) or placebo (NaCl; n=20). After 10 or 28 days post-MI the animals were sacrificed and tissues collected. The total carbonyl content and GSH were used as a parameter of intracellular redox balance. D3 expression and activity were measured. Results: As predicted, the formation of carbonyls, a marker of oxidative damage to proteins, was increased in heart ($P=0.0001$). Interestingly, liver, muscle and brain (all $P<0.001$) also showed increase in carbonyl levels in the placebo but not in NAC group. GSH levels were diminished and GSSG levels augmented in all tissues of the placebo group ($P<0.001$) while maintained in the NAC-treated animals. DIO3 expression and activity were increased in the placebo group in liver ($P=0.002$), muscle ($P=0.03$) and brain ($P=0.01$) as compared to the NAC group. Conclusion: Oxidative stress occurs after myocardial infarction and results in systemic induction of D3, which is related to the low T3 levels in disease. Antioxidant treatment prevents thyroid hormone derangement and corrects D3 activity to levels observed in controls.

Key-words: thyroid hormone, non-thyroidal illness syndrome, N-acetylcysteine, oxidative stress.

INTRODUCTION

The low T3 syndrome, also known as non-thyroidal illness syndrome (NTIS), is a clinical condition characterized by altered levels of thyroid hormones in critically ill patients (Larsen and Baxter, 2008). With worsening of the clinical condition, we can first observe a decrease in serum triiodothyronine (T3) levels, accompanied by increased reverse T3 (rT3), and subsequently a decrease in plasma thyroxine (T4), without elevated levels of thyroid stimulating hormone (TSH) (Warner and Beckett 2010). It is not yet known if these changes represent an attempt to protect metabolism in a state of disease or if it is a consequence of the disease process. However, the alterations in thyroid hormone levels are directly related to the prognosis of the patients (Iervasi, Pingitore et al. 2003, Wang, Pan et al. 2012, Ozcan, Osmonov et al. 2014). The pathophysiology of NTIS is characterized by profound changes in the peripheral conversion of T4 to T3. This process occurs by the action of a group of enzymes, the deiodinases types 1, 2 and 3 (D1, D2 and D3, respectively). D1 and D2 convert T4 to T3, while D3 exclusively inactivates both T4 and T3. This enzymes regulate the homeostasis of thyroid hormones (Maia, Goemann et al. 2011). All three deiodinases require an as-yet undefined cofactor, probably a thiol, which acts as a reducing agent releasing iodine from the selenocysteine residue and regenerating the active enzyme.

The low T3 syndrome is commonly observed in patients with myocardial infarction (MI). Although the pathophysiological process is yet unknown, one of the mechanisms involves the increase in the production of reactive oxygen species (ROS). Changes in the intracellular redox state can decrease deiodinase function by independent mechanisms. The decrease in intracellular glutathione (GSH) levels leads to redox imbalance. Oxidative stress compromises deiodinases function, altering the metabolism of thyroid hormones (Wajner, Goemann et al. 2011, Wajner, Rohenkohl et al. 2015). Upon oxidation, the cysteine residues within proteins can be modified to different products, including reversible inter- or intra-molecular disulfide bonds and GSH-mixed disulfides. This might be particularly relevant for deiodinase function (Kuiper, Klootwijk et al. 2002, Lu and Holmgren 2009).

Indeed, a recent randomized clinical study performed in patients with MI, showed that oxidative stress alters the thyroid hormone metabolism and that N-acetylcysteine

(NAC), a potent antioxidant that increases intracellular cysteine and GSH levels avoids thyroid hormone derangement. It was demonstrated that NAC administration in this scenario prevented the derangement in thyroid hormone concentrations commonly occurring in the acute phase of MI, indicating that oxidative stress is involved in the NTIS pathophysiology in humans (Vidart, Wajner et al. 2014).

The effect of NAC in type 3 deiodinase in the heart tissue was then evaluated in an animal model of low T3 syndrome and MI (Lehnen, Santos et al. 2017). The redox imbalance after MI altered D3 function, resulting in diminished T3 levels and both T3 and D3 function were corrected with the administration of NAC. Moreover, significant improvement in cardiac performance was shown (Lehnen, Santos et al. 2017). These results demonstrated that restoring redox balance by NAC treatment prevents low T3 syndrome-related thyroid hormone derangement and preserves heart function in rats subjected to MI. However, one can easily question whether only the heart tissue alterations on D3 could result in a systemic cascade of thyroid hormone modifications. Thus, in this study, we aimed to evaluate the effect of oxidative stress and D3 function in the liver, skeletal muscle and brain in an animal model of MI and NTIS.

MATERIAL AND METHODS

Reagents

Reagents were obtained from Invitrogen (Life Technologies Inc., NY, USA), Calbiochem-Novabiochem (St. Louis, MO, USA), or Sigma-Aldrich (St. Louis, MO, USA).

Animals and procedures

Male Wistar rats (weighing ± 250g) were used in the experiments. All procedures and experiments involving animals were performed in accordance with the recommendations of the Brazilian College of Animal Experimentation (COBEA).Our study complied with the ethical principles of the Guide for the Care and Use of Laboratory Animals (NIH 1996) and the international standards for animal research of the Guidelines for Reporting Animal Research (Kilkenny, Browne et al. 2010). This study was

approved by the Research Ethics Committee of our institution (Protocol #130501). All animals received standard food and water *ad libitum* and were kept in cages under 12-hour light/dark cycles.

On the day of MI induction, the animals underwent basal echocardiography and blood sample collection (retro-orbital plexus) before being subjected to anterior descending artery ligation, as previously described(Pfeffer, Pfeffer et al. 1979, Lehnen, Lehnen et al. 2014), resulting in infarction of the left ventricle (LV) free wall. Briefly, the rats were anesthetized intraperitoneally (1 mL/kg of ketamine and 0.5 mL/kg of xylazine), intubated and connected to a mechanical ventilator with ambient air at a volume of 2.5 mL and ventilatory rate of 65 breaths per minute using a pressure fan for rodents (Harvard Apparatus, model 683, Massachusetts, USA). Anesthesia was administered through a vaporizer (Surgivet, Saint Louis, MN, USA) containing isoflurane (1 mL/mL, Isoforine, Cristália, Itapira, SP, Brazil). After MI or Sham procedure, it was administered Tramadol (20 mg/kg, every 6h for 48 h, Teuto, GO, Brazil) and animals were kept under medical oxygen at temperatures of 25-27°C for 6 hours. Twenty four hours after surgery, echocardiography was performed to measure cardiac parameters and to determine the MI extension. Blood was collected for troponin measurements to confirm successful MI induction. The animals were then divided into two groups (10 or 28 days of follow-up) and randomly assigned to receive either intraperitoneal NAC (10 mg/kg, 12/12 h for 48 h n=20) or the same volume of vehicle (saline, n=20; placebo group). Serial cardiac functional assessments and measurements of serum TSH, T4, T3 and rT3 levels were performed on the day of the procedure and at 10 or 28 days after surgery. Previous publications showed that neither T3 levels nor echocardiographic parameters changed after 28 days post-MI (Olivares, Marassi et al. 2007, Schenkel, Tavares et al. 2012).

At the conclusion of each experimental period (10 or 28 days), the animals were euthanized (anesthetized with vaporizer (Surgivet, Saint Louis, MN, USA) and tissues were rapidly removed and frozen in liquid nitrogen before being stored for subsequent analyses.

Carbonyl measurements

Duplicate aliquots of 0.3 mg of protein homogenate of each tissue were incubated with 500 µL of 10 mM 2.4-dinitrophenylhydrazine or 1.0 mL of 2 M HCl (blank tube). After

30 min, 250 µL of 50% trichloroacetic acid was added to the aliquots. The samples were subsequently centrifuged at 8000 *g* for 30 min to obtain the protein pellets, which were immediately washed with ethanol-ethyl acetate at a 1:1 (v/v) ratio. The final protein pellets were resuspended in 500µL of 8 M urea buffer and incubated at 50ºC for 90 min. The difference between the 2.4-dinitrophenylhydrazine-treated and HCl-treated samples (blank) was used to calculate the carbonyl content determined at 370 nm. Carbonyl content was calculated using the millimolar absorption coefficient of hydrazine (e370 nm=21,000000 M^{-1} cm^{-1}), and the results were expressed in nmol carbonyl/mg protein(Zanatta, Viegas et al. 2013).

Non-enzymatic antioxidant defenses

Reduced glutathione (GSH) levels were measured according to a standard method. Briefly, tissues were homogenized in the presence of 300µL of 20 mM sodium phosphate and 140 mMKCl buffer, pH 7.4. Proteins were precipitated by adding sodium metaphosphoric acid for a final ratio of 1:1. Samples were centrifuged for 10 min at 7,000 *g*. Fifteen microliters of tissue preparation was incubated with an equal volume of ophthaldialdehyde (1 mg/mL methanol) at room temperature for 15 min in the presence of 20 volumes (1:20, v/v) of 100 mM sodium phosphate buffer, pH 8.0, containing 5 mM EDTA. Fluorescence was measured using excitation and emission wavelengths of 350 nm and 420 nm, respectively. A calibration curve was generated using standard GSH (0.001–0.1 mM), and GSH concentrations were calculated as nmol/mg protein. Total glutathione (tGS) and oxidized glutathione (GSSG) levels were determined using the enzymatic recycling method described elsewhere (Teare, Punchard et al. 1993), with some modifications. Briefly, tissues were homogenized in 4 (w/v) volumes of a sulfosalicylic acid solution (11%) and Triton X-100 (0.11%) (1:1 ratio). After incubating for 5 min at 4°C with continuous shaking, the samples were centrifuged at 10,000 g for 10 min (4°C), and the supernatant was collected for analyses of glutathione levels. For GSSG measurement, 10 µL of the supernatant was added to 110 µL of a GSH masking buffer (100 mM phosphate buffer, 1 mM EDTA, 1.1% 2-vinylpyridine), pH 7.4, and incubated for 1 h at room temperature. The samples prepared for tGS and GSSG measurement were subjected to enzymatic analysis in a recycling buffer system containing 300 µM NADPH, 225 µM DTNB, 1.6 U/mL GR and 1.0 mM EDTA in 100 mM phosphate buffer (pH 7.4). The linear increase in absorbance at 405 nm over time was monitored using a microplate reader

(SpectraMax M5, Molecular Devices, California, US). A standard curve was generated using known amounts of GSH (100 µM) and GSSG (3.47, 6.95, 13.89 uM).

D3 activity assay

D3 activity in tissue samples was determined using paper chromatography as previously described (Huang, Mulcahey et al. 2005). Tissues were individually homogenized and sonicated with 10mMTris-HCl, 0.25 sucrose buffer (pH 7.5), and 10mM dithiothreitol (DTT). Protein concentration was quantified by Bradford assay using BSA as a standard. The homogenates were incubated for an hour with 200,000 cpm 125I-labeled T3, 2 nM T3, 20mM DTT, and 1mM propylthiouracil (PTU) in order to inhibit any D1 activity. The addition of 200nM of T3 completely abolished D3 activity in all samples. The reaction was stopped by adding 200 uL ethanol 95%, 50 uL NaOH (0.04 N), and 5mg PTU. Deiodination was determined based on the amount of 125I-3, 3'-T2 produced after separation of reaction products by paper chromatography. Results were expressed as the fraction of T2 counts minus the nonspecific deiodination (always < 1.5%), obtained with the saturating concentration of T3 (200 nM). D3 activity was expressed as fentomoles T3 per minute per milligram protein. The quantity of protein assayed was adjusted to ensure that < 30% of the substrate was consumed. All experiments were performed in triplicate in at least two independent experiments.

Real Time PCR

Total RNA was extracted from tissues with an RNeasy kit (Qiagen) and used to synthesize cDNA (SuperScript First-Strand Synthesis System for RT-PCR; Invitrogen). The generated cDNAs were used in a real-time PCR with a SYBR Green PCR Master Mix (Applied Biosystems) in ABI Prism 7500 Sequence Detection System (Applied Biosystems). Standard curves representing 5-point serial dilution of cDNA of the experimental and control groups were analyzed and used as calibrators of the relative quantification of product generated in the exponential phase of the amplification curve. The r2 was greater than 0.99, and the amplification efficiency varied between 80% and 100%. Samples were measured by relative quantification (change in expression in the experimental group versus control; untreated versus treated cells). The data generated by

ABI Prism 7500 system SDS software (Applied Bio systems) were then transferred to an Excel spreadsheet (Microsoft), and the experimental values were corrected to that of the cyclophilin A standard. The primers used for D3 were (5'-TCCAGAGCCAGCACATCCT-3' and 5'-ACGTCGCGCTGGTACTTAGTG-3'), with the internal controller cyclophilin A (5'-GTCAACCCCACCGTGTTCTTC-3' and 5'-ACTTGCCACCAGTGCCATTATG-3').

Western Blotting

Tissues were collected and prepared by homogenization and lysis at 4°C. After centrifugation at 13,000 ×g for 15 min at 4°C, the total protein concentration was determined using the BCA assay. Protein extracts (30–50 mg protein of each sample was fractionated by 8–12% SDS–PAGE and blotted onto an Immobilon PVDF membrane (Millipore, Billerica, MA, USA). Non-specific binding sites were blocked by incubation with 5% nonfat dry milk in Tris-buffered saline 0.1% Tween-20. The following primary antibodies were used: anti-D3 (1:400; Novus Biologicals), anti-a-tubulin B7 (1:500; Santa Cruz Biotechnology) and anti-b-actin (1:10 000; Sigma–Aldrich). The antigen–antibody complexes were visualized using HRP-conjugated secondary antibody and an enhanced chemiluminescence system (GE Healthcare, Pittsburgh, PA, USA). Expression was quantified using image densitometry with ImageJ analysis software.

Statistical analysis

Unless otherwise specified, the results are presented as the mean ± standard deviation. Data were analyzed using 2-tailed Student's t tests or one-way analysis of variance (ANOVA) followed by post hoc Duncan multiple range tests when F was significant. Prism 6.0 software was used for statistical analyses and $P<0.05$ was considered significant.

RESULTS

Plasma T3 levels and low T3 syndrome

Compared to baseline, serum T3 levels were decreased in MI-placebo animals at 10 (108 to 88.4 ng/dL, P<0.01) and 28 days (108.5 to 91.6 ng/dL, P<0.03) as previously shown (Lehnen, Santos et al. 2017). In contrast, no significant decreases in T3 levels were noted in MI-NAC animals at 10 or 28 days after MI.

NAC administration restores nonenzymatic antioxidant compounds and total carbonyl content in peripheral tissues

We next determined the concentrations of the non-enzymatic antioxidant GSH in all tissues. GSH content was diminished in the liver (37.2 to 32.3 nmol/mg prot, P<0.001, Figure 1A), muscle (30.8 to 25.4 nmol/mg prot, P<0.001, Figure 1B) and brain (30.8 to 25.8 nmol/mg prot, P<0.001, Figure 1C) tissue samples of MI-placebo animals at 10 days after MI. At 28 days no difference in GSH content was observed in all tissues (Figure 1A, 1B and 1C). The GSH/GSSG ratio was decreased in all tissue samples (P<0.001, Figure 1A, 1B and 1C) at 10 days in placebo group, but not in 28 days.

The tissue carbonyl content, a parameter of protein oxidation, was increased in the placebo group in 10 days in the liver (0.12 to 0.9 nmol/mg prot, P<0.001, Figure 2A), muscle (0.08 to 2.9 nmol/mg prot, P<0.001, Figure 2B) and brain (0.03 to 0.89 nmol/mg prot, P <0.001, Figure 2C). At 28 days, carbonyl content was still increased only in the liver (0.12 to 0.89 nmol/mg prot, P<0.001, Figure 2A) and in the brain (0.03 to 0.46 nmol/mg prot P<0.001, Figure 2C), but not in muscle (0.08 to 0.13 nmol/mg prot, P >0.05, Figure 2B) in the placebo group. In the NAC group, the carbonyl content was not different from controls in 10 or 28 days (Figure 2A, 2B and 2C).

D3 expression and activity in peripheral tissues is affected by NAC

D3 expression was increased in the Placebo group at 10 days post-MI in liver (~4.2, P<0.001, Figure 3A), muscle (~11 fold, P<0.001, Figure 3B) and brain (~3 fold, P<0.001, Figure 3C). At 28 days, D3 expression in the Placebo group was increased in liver (~2.5 fold, P<0.001, Figure 3A) and brain (~2 fold, P<0.001, Figure 3C), but not in the muscle (P>0.05, Figure 3C). We observed that NAC treatment prevented the increase of D3 expression at 10 and 28 days post MI in all tissues (Figure 3A, 3B and 3C). Accordingly, D3 activity levels were augmented at 10 days post-MI in all tissue samples of placebo

group when compared to sham (liver 1 to 4.7 fmol/ mg prot/min; muscle 1.8 to 8 fmol/ mg prot/min and brain 1.6 to 5.2 fmol/ mg prot/min, all $P < 0.001$, Figures 4A, 4B and 4C). At 28 days, D3 activity levels were not significantly increased ($P>0.05$, Figures 4A, 4B and 4C). NAC treatment prevented the increase of D3 activity at 10 and 28 days after MI in all tissues ($P >0.05$, Figures 4A, 4B and 4C). We next sought to determine the protein content of the samples at day 10 and observed that it was augmented in the placebo group in all tissues but not in the NAC treated animals (Figures 5A, 5B and 5C).

DISCUSSION

The present study aimed to investigate whether oxidative stress induced alterations in D3 in peripheral tissues in rats submitted to MI. The observed oxidative stress secondary to MI, here determined as augmented carbonyl and diminished GSH content decreased serum T3 levels and resulted in induced D3 function in all peripheral tissues. Treatment with NAC prevented redox imbalance, preserved T3 levels, and corrected D3 expression and activity. Taken together, these findings indicate that altered D3 function is observed systemically in oxidative stress- induced low T3 syndrome related to MI.

The pathophysiology of NTIS is characterized by several changes in the metabolism of thyroid hormones. Generally, NTIS is observed in patients after MI and the low serum T3 levels are inversely correlated with the mortality of these patients (Iervasi, Pingitore et al. 2003). Clinical and experimental studies showed increased inactivation of thyroid hormone by robust stimulation of D3 playing a significant role in the low T3 syndrome (Peeters, Wouters et al. 2003, Peeters, Wouters et al. 2005, Rodriguez-Perez, Palos-Paz et al. 2008). Previous studies also demonstrated that the correction of oxidative stress induced in disease prevented the low T3 syndrome, indicating that changes in redox balance, directly affect the peripheral metabolism of the thyroid hormones (Wajner, Goemann et al. 2011, Vidart, Wajner et al. 2014). NAC, a potent antioxidant that restores intracellular reduced gluthatione (GSH) levels, prevents the deleterious effects of altered intracellular redox state on thyroid hormone metabolism.

The function of deiodinases is affected by changes in the redox balance (Wajner and Maia 2012). Type 3 deiodinase is a selenocysteines oxireductases enzyme that catalyzes the removal of iodine from the inner rings of thyroid hormones, inactivating them (Maia, Goemann et al. 2011). Although the effect of NAC on oxidative damage and D3

expression and activity is not yet totally understood, it is probably associated with the maintenance of intracellular GSH levels and with the correction of redox imbalance. NAC is the major cysteine donator, not only to GSH but also to several thiol enzymes which might be critical to proteins dependent on thiols (Peeters, Wouters et al. 2003). Moreover, GSH is known to have its antioxidant effect potentiated since small increases in GSH concentration result in large antioxidant effects (Lamirand, Pallud-Mothre et al. 2008, Wajner, Goemann et al. 2011, Wajner, Rohenkohl et al. 2015). All these mechanisms can be of importance to D3 function maintenance.

Importantly, studies with ill patients were manly designed with septic patients or have analyzed only the primary affected organ. It was not demonstrated, until now that oxidative stress occurred in the course of MI was able to alter D3 function in tissues primarily unaffected by the disease, thus leading to low T3 syndrome. Additionally, the administration of NAC protects proteins from oxidative damage as well as corrects the impaired total body antioxidant capacity. These results further confirms that the T3 decrease is mainly due augmented thyroid hormone inactivation due to oxidative stress and that type 3 deiodinase might be the most important enzyme in the low T3 syndrome context.

In conclusion, our findings demonstrate that oxidative stress occurs after MI and results in systemic induction of D3. The improvement of MI-related redox imbalance by NAC prevents changes in thyroid hormone metabolism by preventing the increase in D3 expression and activity systemically. These results suggest that type 3 deiodinase might be the main altered enzyme that orchestrates the low T3 syndrome.

Funding

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio a Pesquisa do Rio Grande do Sul (FAPERGS), and Fundo de Incentivo a Pesquisa do Hospital de Clínicas de Porto Alegre (FIPE), Brazil.

REFERENCES

Huang, S. A., M. A. Mulcahey, A. Crescenzi, M. Chung, B. W. Kim, C. Barnes, W. Kuijt, H. Turano, J. Harney and P. R. Larsen (2005). "Transforming growth factor-beta promotes inactivation of extracellular thyroid hormones via transcriptional stimulation of type 3 iodothyronine deiodinase." Mol Endocrinol **19**(12): 3126-3136.

Iervasi, G., A. Pingitore, P. Landi, M. Raciti, A. Ripoli, M. Scarlattini, A. L'Abbate and L. Donato (2003). "Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease." Circulation **107**(5): 708-713.

Kilkenny, C., W. J. Browne, I. C. Cuthill, M. Emerson and D. G. Altman (2010). "Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research." PLoS Biol **8**(6): e1000412.

Kuiper, G. G., W. Klootwijk and T. J. Visser (2002). "Substitution of cysteine for a conserved alanine residue in the catalytic center of type II iodothyronine deiodinase alters interaction with reducing cofactor." Endocrinology **143**(4): 1190-1198.

Lamirand, A., S. Pallud-Mothre, M. Ramauge, M. Pierre and F. Courtin (2008). "Oxidative stress regulates type 3 deiodinase and type 2 deiodinase in cultured rat astrocytes." Endocrinology **149**(7): 3713-3721.

Lehnen, T. E., A. M. Lehnen, A. M. Tavares, A. Bello-Klein, M. M. Markoski, U. F. Machado and B. Schaan (2014). "Atorvastatin administered before myocardial infarction in rats improves contractility irrespective of metabolic changes." Clin Exp Pharmacol Physiol **41**(12): 986-994.

Lehnen, T. E., M. V. Santos, A. Lima, A. L. Maia and S. M. Wajner (2017). "Preventing low T3 syndrome attenuates cardiac dysfunction in a male rat model of myocardial infarction." Endocrinology.

Lu, J. and A. Holmgren (2009). "Selenoproteins." J Biol Chem **284**(2): 723-727.

Maia, A. L., I. M. Goemann, E. L. Meyer and S. M. Wajner (2011). "Deiodinases: the balance of thyroid hormone: type 1 iodothyronine deiodinase in human physiology and disease." J Endocrinol **209**(3): 283-297.

NIH (1996). Guide for the Care and Use of Laboratory Animals. Washingtion, DC, National Academy Press.

Olivares, E. L., M. P. Marassi, R. S. Fortunato, A. C. da Silva, R. H. Costa-e-Sousa, I. G. Araujo, E. C. Mattos, M. O. Masuda, M. A. Mulcahey, S. A. Huang, A. C. Bianco and D. P. Carvalho (2007). "Thyroid function disturbance and type 3 iodothyronine deiodinase induction after myocardial infarction in rats a time course study." Endocrinology **148**(10): 4786-4792.

Ozcan, K. S., D. Osmonov, E. Toprak, B. Gungor, A. Tatlisu, A. Ekmekci, A. Kaya, G. Tayyareci and I. Erdinler (2014). "Sick euthyroid syndrome is associated with poor prognosis in patients with ST segment elevation myocardial infarction undergoing primary percutaneous intervention." Cardiol J **21**(3): 238-244.

Peeters, R. P., P. J. Wouters, E. Kaptein, H. van Toor, T. J. Visser and G. Van den Berghe (2003). "Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients." J Clin Endocrinol Metab **88**(7): 3202-3211.

Peeters, R. P., P. J. Wouters, H. van Toor, E. Kaptein, T. J. Visser and G. Van den Berghe (2005). "Serum 3,3',5'-triiodothyronine (rT3) and 3,5,3'-triiodothyronine/rT3 are prognostic markers in critically ill patients and are associated with postmortem tissue deiodinase activities." J Clin Endocrinol Metab **90**(8): 4559-4565.

Pfeffer, M. A., J. M. Pfeffer, M. C. Fishbein, P. J. Fletcher, J. Spadaro, R. A. Kloner and E. Braunwald (1979). "Myocardial infarct size and ventricular function in rats." Circ Res **44**(4): 503-512.

Rodriguez-Perez, A., F. Palos-Paz, E. Kaptein, T. J. Visser, L. Dominguez-Gerpe, J. Alvarez-Escudero and J. Lado-Abeal (2008). "Identification of molecular mechanisms related to nonthyroidal illness syndrome in skeletal muscle and adipose tissue from patients with septic shock." Clin Endocrinol (Oxf) **68**(5): 821-827.

Schenkel, P. C., A. M. Tavares, R. O. Fernandes, G. P. Diniz, A. R. Ludke, M. F. Ribeiro, A. S. Araujo, M. L. Barreto-Chaves and A. Bello-Klein (2012). "Time course of hydrogen peroxide-thioredoxin balance and its influence on the intracellular signalling in myocardial infarction." Exp Physiol **97**(6): 741-749.

Teare, J. P., N. A. Punchard, J. J. Powell, P. J. Lumb, W. D. Mitchell and R. P. Thompson (1993). "Automated spectrophotometric method for determining oxidized and reduced glutathione in liver." Clin Chem **39**(4): 686-689.

Vidart, J., S. M. Wajner, R. S. Leite, A. Manica, B. D. Schaan, P. R. Larsen and A. L. Maia (2014). "N-acetylcysteine administration prevents nonthyroidal illness syndrome in patients with acute myocardial infarction: a randomized clinical trial." J Clin Endocrinol Metab **99**(12): 4537-4545.

Wajner, S. M., I. M. Goemann, A. L. Bueno, P. R. Larsen and A. L. Maia (2011). "IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells." J Clin Invest **121**(5): 1834-1845.

Wajner, S. M. and A. L. Maia (2012). "New Insights toward the Acute Non-Thyroidal Illness Syndrome." Front Endocrinol (Lausanne) **3**: 8.

Wajner, S. M., H. C. Rohenkohl, T. Serrano and A. L. Maia (2015). "Sodium selenite supplementation does not fully restore oxidative stress-induced deiodinase dysfunction: Implications for the nonthyroidal illness syndrome." Redox Biol **6**: 436-445.

Wang, F., W. Pan, H. Wang, S. Wang, S. Pan and J. Ge (2012). "Relationship between thyroid function and ICU mortality: a prospective observation study." Crit Care **16**(1): R11.

Warner, M. H. and G. J. Beckett (2010). "Mechanisms behind the non-thyroidal illness syndrome: an update." J Endocrinol **205**(1): 1-13.

Zanatta, A., C. M. Viegas, A. M. Tonin, E. N. Busanello, M. Grings, A. P. Moura, G. Leipnitz and M. Wajner (2013). "Disturbance of redox homeostasis by ornithine and homocitrulline in rat cerebellum: a possible mechanism of cerebellar dysfunction in HHH syndrome." Life Sci **93**(4): 161-168.

FIGURES

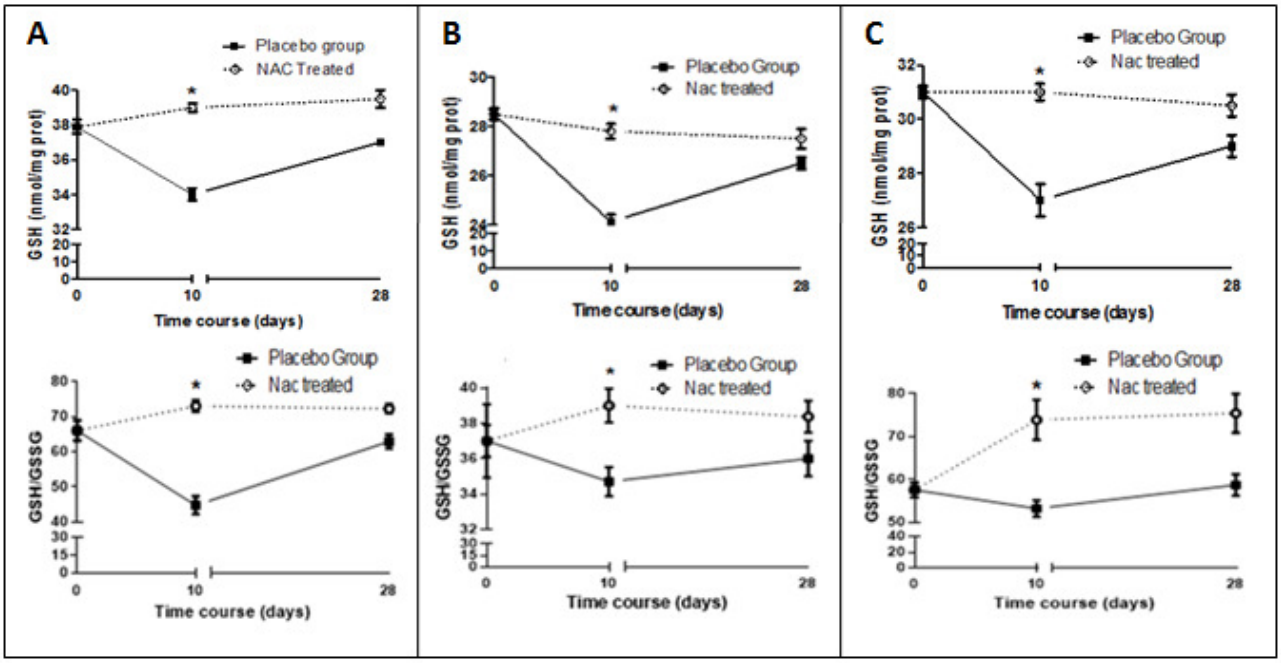

Figure 1. Changes in GSH levels and GSH/GSSG ratio in the liver (A), muscle (B) and brain (C) in rats subjected to myocardial infarction that received NAC or placebo. GSH content was diminished in the liver, muscle and brain tissue samples of MI-placebo animals, but not MI-NAC animals (Figures 1A, 1B and 1C) at 10 days after AMI. At 28 days we observed that the GSH content returned to initial levels. The GSH/GSSG ratio was decreased in both tissue samples (Figures 1A, 1B and 1C) at 10 days post-AMI, indicating augmented oxidation. All data with $P<0.001$. * $P < 0.001$, *vs* Placebo group, n = 20 in each group.

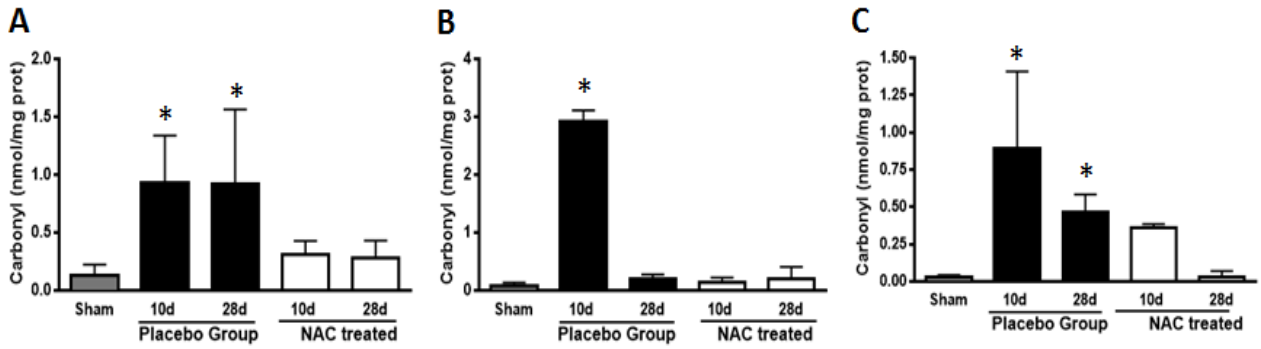

Figure 2. Oxidative stress biomarkers in rats subjected to myocardial infarction that received NAC or placebo. The carbonyl content was increased in the placebo group in 10 in the liver (Figure 2A), muscle (Figure 2B) and brain (Figure 2C). At 28 days, carbonyl content increased only in the liver (Figure 2A) and in the brain (Figure 2C) in the placebo group. In the NAC group, the carbonyl content was decreased by 10 and 28 days (Figures 2A, 2B and 2C). All data with P<0.001. * P < 0.001, *vs* Sham, n = 20 in each group.

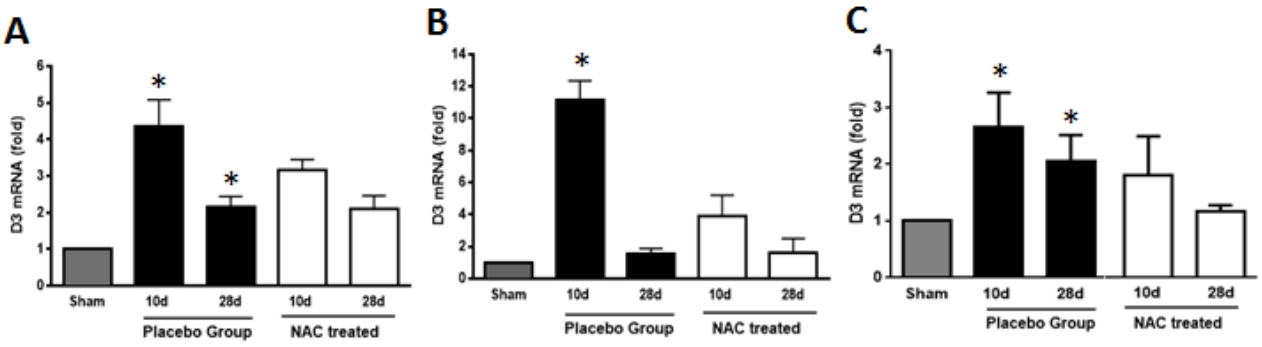

Figure 3. D3 mRNA in rats subjected to myocardial infarction that received NAC or placebo. D3 expression was increased in the Placebo group at 10 days post-MI in liver (Figure 3A), muscle (Figure 3B) and brain (Figure 3C). At 28 days, D3 expression in the Placebo group was increased just in liver (Figure 3A) and brain (Figure 3C). In muscle, this difference did not exist anymore at 28 days (Figure 3C). NAC treatment with NAC prevented the increase of D3 expression at 10 and 28 days post-MI in all tissues (Figures 3A, 3B and 3C). All data with P<0.001. * P < 0.001, *vs* Sham, n = 20 in each group.

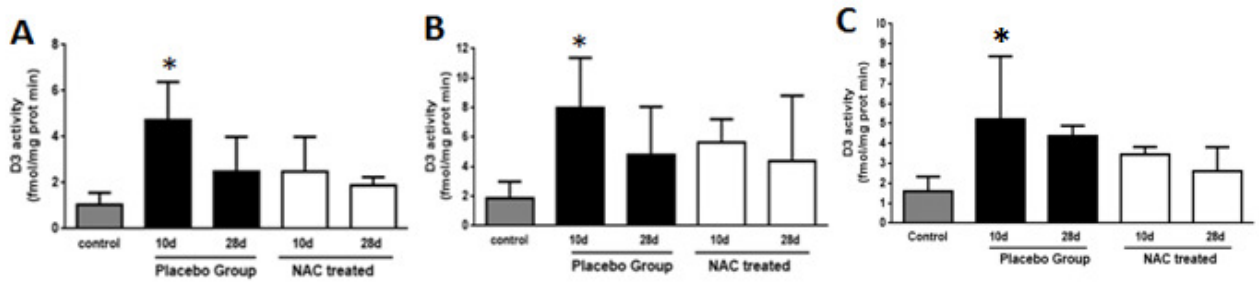

Figure 4. D3 activity and Western Blotting in rats subjected to myocardial infarction that received NAC or placebo. D3 activity levels was augmented at 10 days post-MI in all tissue in placebo group (Figures 4A, 4B and 4C). At 28 days, D3 activity levels were not significantly increased (Figures 4A, 4B and 4C). NAC treatment prevented the increase of D3 activity at 10 and 28 days after MI in all tissues (Figures 4A, 4B and 4C).

Figure 5. Illustrative western blot images demonstrating that the amount of protein was increased at 10 days in Placebo group in liver, muscle and brain (Figures 5A, 5B and 5C).

CONCLUSÃO GERAL

Concluímos que o estresse oxidativo induzido pelo infarto do miocárdio resulta em um aumento local e sistêmico da desiodase tipo D3, contribuindo para a diminuição dos níveis circulantes de T3. A NAC, pela sua natureza antioxidante, restaura o equilíbrio redox e preserva a função cardíaca, evitando a síndrome do T3 baixo. Ainda, o tratamento com NAC previne a disfunção observada da desiodase tipo D3 em vários tecidos (além do cardíaco), prevenindo a síndrome do T3 baixo. A correção do desequilíbrio redox pela NAC está associada à prevenção de anormalidades sistêmicas no metabolismo dos hormônios tireoidianos em ratos submetidos ao infarto do miocárdio.