UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS - ENDOCRINOLOGIA

Influence of thyroid hormone mechanisms on the progression of metabolic dysfunction associated with fatty liver disease (MAFLD)

TESE DE DOUTORADO

RAFAEL AGUIAR MARSCHNER

Porto Alegre

2022

Rafael Aguiar Marschner

INFLUENCE OF THYROID HORMONE MECHANISMS ON THE PROGRESSION OF METABOLIC DYSFUNCTION ASSOCIATED WITH FATTY LIVER DISEASE (MAFLD)

Tese apresentada como requisito parcial à obtenção do título de doutor em Endocrinologia pelo Programa de Pós-graduação em Ciências Médicas: Endocrinologia da/do Faculdade de Medicina da Universidade Federal do Rio Grande do Sul.

Orientador: Profa. Dra. Simone Magagnin Wajner

Porto Alegre

2022

Esta tese segue o formato proposto pelo Programa de Pós- Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul. Ela será constituída de: 1) Introdução; 2) Artigo original: Influence of altered thyroid hormone mechanisms in the progression of metabolic dysfunction associated with fatty liver disease (MAFLD): A systematic review; 3) Artigo original Role of Type 3 Deiodinase in the progression of non-alcoholic liver disease: Interconnections between Oxidative Stress, Respiratory Changes and Macrophage Activation; 4) Perspectivas; 5) Produção cientifica

Para minha avó, Eneida da Rosa Teixeira de Aguiar.

"As montanhas da vida não existem apenas para que você chegue ao topo, mas para que você aprenda o valor da escalada."

Autor Desconhecido

AGRADECIMENTOS

À Profª Dra Simone Magagnin Wajner, pelo exemplo, estímulo, amizade e confiança em todos os momentos que convivemos neste período.

A minha namorada, Paula da Rosa Bettim, que incansavelmente me apoiou e me estimulou a desenvolver todos os trabalhos com os quais me comprometi.

Aos meus pais e irmão, Oscar Roberto Marschner, Susana Aguiar Marschner e Bruno Aguiar Marschner, que sempre me motivaram a continuar na vida academia aperfeiçoando meus conhecimentos.

Aos colaboradores Prof. Dr. Mario Reis, Profª Dra. Larisse Longo, Profª Dra Ana Cristina Roginski, Me. Rafael Ribeiro por toda a ajuda no desenvolvimento do presente trabalho.

Aos meus colegas e amigos Profª Dra. Daysi Crispim, Profª Dra Natália Emerim, Me. Cristine Dieter e o Secretario Pedro Serafim por toda colaboração e indispensável contribuição no meu desenvolvimento pessoal e acadêmico.

RESUMO

Nas últimas décadas, com o aumento do consumo de alimentos industrializados, (ricos em gordura) somados com a falta da pratica de exercício físico obtivemos um aumento exponencial da prevalência de obesidade mundial. Relacionado a isso, doenças secundarias a obesidade vem ganhando destaque, esse é o caso da doença hepática gordurosa associada à disfunção metabólica (MAFLD), uma das formas mais comuns de doenças hepáticas crônicas. Nos últimos anos houve um incremento da sua prevalência, estima-se que em torno de 20-30% na população ocidental e 5-18% no Oriente tenham algum estagio da MAFLD, na América do Sul ela é estimada em 30%. A etiologia da doença inicia-se com a obesidade, resistência insulínica, diabetes mellitus do tipo 2 (DM-2) e dislipidemia sendo essas as causas primárias para o seu desenvolvimento. A história natural da MAFLD começa com a deposição de gordura no fígado, seguido dos primeiros estagio da esteatose hepática, ocasionando inflamação crônica e reparação compensatória do tecido podendo progredir a esteato-hepatite não-alcoólica (EHNA). Posteriormente ocorre o acúmulo de colágeno levando a cirrose e a longo prazo carcinoma hepatocelular (CHC). Embora já exista um grande conhecimento sobre a fisiopatologia da doença e seus fatores predisponentes, ainda existem mecanismos envolvidos no desenvolvimento e na progressão da MAFLD que foram pouco explorados. Neste sentido, o metabolismo dos hormônios tireoidianos (HTs) podem ter papel importante associado à doença. Os HTs são essenciais para o crescimento, desenvolvimento e metabolismo em praticamente todos os sistemas do corpo. Apesar da Tiroxina (T4) ser o principal produto, o triiodotironina (T3) é o biologicamente ativo. Sendo regulados pela atividade da família das iodotironinas desiodases. As do tipo 1 (Dio1) e 2 (Dio2) convertem T4 intracelular em T3, enquanto a do tipo 3 (Dio3) converte T3 em rT3 (T3 inativo). No fígado o papel dos HTs influenciam diretamente o metabolismo de lipídios e carboidratos, via lipogênese hepática, oxidação lipídica, homeostase do colesterol. Em situações normais os hepatócitos apresentam uma grande expressão de Dio1 e baixa expressão de Dio3, mantendo a conversão dos HTs adequada e sua atividade hepática preservada. Recentemente, alteração na disponibilidade dos T3 vem sendo observadas, apresentando uma possível associação com a progressão MAFLD. Contudo, pouco se sabe sobre os mecanismos envolvidos no metabolismo dos HTs na fisiopatologia da MAFLD.

ABSTRACT

In the last decades, with the increase in the consumption of industrialized foods, (rich in fat) added to the lack of physical exercise, we obtained an exponential increase in the prevalence of obesity worldwide. Related to this, diseases secondary to obesity have been gaining prominence, this is the case of metabolic dysfunction associated with fatty liver disease (MAFLD), one of the most common forms of chronic liver disease. In recent years there has been an increase in its prevalence, it is estimated that around 20-30% in the western population and 5-18% in the East have some stage of MAFLD, in South America it is estimated at 30%. The etiology of the disease begins with obesity, insulin resistance, type 2 diabetes mellitus (DM-2) and dyslipidemia, which are the primary causes for its development. The natural history of MAFLD begins with the deposition of fat in the liver, followed by the first stages of hepatic steatosis, causing chronic inflammation and compensatory tissue repair that can progress to nonalcoholic steatohepatitis (NASH). Subsequently, collagen accumulation occurs leading to cirrhosis and long-term hepatocellular carcinoma (HCC). Although there is already a great knowledge about the pathophysiology of the disease and its predisposing factors, there are still mechanisms involved in the development and progression of MAFLD that have been little explored. In this sense, the metabolism of thyroid hormones (THs) may play an important role associated with the disease. THs are essential for growth, development and metabolism in virtually every system in the body. Although thyroxine (T4) is the main product, triiodothyronine (T3) is the biologically active one. Being regulated by the activity of the iodothyronine deiodases family. Types 1 (Dio1) and 2 (Dio2) convert intracellular T4 to T3, while type 3 (Dio3) converts T3 to rT3 (inactive T3). In the liver, the role of THs directly influence the metabolism of lipids and carbohydrates, via hepatic lipogenesis, lipid oxidation, cholesterol homeostasis. In normal situations, hepatocytes show a high expression of Dio1 and low expression of Dio3, maintaining the adequate conversion of THs and their hepatic activity preserved. Recently, changes in the availability of T3 have been observed, showing a possible association with MAFLD progression. However, little is known about the mechanisms involved in the metabolism of THs in the pathophysiology of MAFLD.

LISTA DE ABREVIATURAS E SIGLAS

ALT: alanina aminotransferase

AST: aspartato aminotransferase

CHC: carcinoma hepatocelular

DDC: dieta deficiente em colina

DDMC: dieta deficiente em metionina e colina

DCV: doença cardiovascular

DHDC: dieta hiperlipídica deficiente em colina

MAFLD: doença hepática gordurosa associada à disfunção metabólica

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

EHNA: esteato-hepatite não-alcoólica

DM-2: diabetes mellitus tipo-2

SM: síndrome metabólica

ELSA: Estudo Longitudinal de Saúde do Adulto

GLP-1: glucagon-1

GSH: glutationa reduzida

GR: glutationa redutase

GPx: glutationa peroxidase

GST: glutationa S-transferase

SOD: superóxido dismutase

MDA: malondialdeído

SUIT: substrate-uncoupler inhibitor titration protocol

GDH: glutamato desidrogenase

α-KGDH: alfa-cetoglutarato desidrogenase

MAPK: proteína quinase ativada por mitógeno

ERK1: extracellular signal related kinase 1

p-ERK1: phosphorylated-extracellular signal related kinase 1

p38: cell differentiation and apoptosis

p-p38: phosphorylated-cell differentiation and apoptosis

UCP-2: proteína desacopladora mitocondrial 2

NAC: N-acetilciesteina

THR-β: receptor do hormônio tireoidiano beta

Dio1: deiodinase 1

Dio2: deiodinase 2

Dio3: deiodinase 3

T3: tri-iodotironina

T4: tiroxina

HTs: hormônios tireoidianos

HCPA: Hospital de Clínicas de Porto Alegre

HDL: lipoproteína de alta densidade

H&E: hematoxilina e eosina

IL: interleucina

IMC: índice de massa corporal

LDL: lipoproteína de baixa densidade

LOLA: L-ornitina L aspartato

TCA: ácido tricloroacético

TNF-α: fator de necrose tumoral-α

UAMP: Unidade de Análises Moleculares e de Proteínas

UEA: Unidade de Experimentação Animal

VLDL: lipoproteínas de muito baixa densidade

G1: Group 1

G2: Group2

G3: Group 3

G4: Group 4

G5: Group 5

FFA: free fatty acid

HTLG: Hepatic triacyiglycerol lipase

PGC-1α: Peroxisome proliferator-activated receptor-gamma coactivator-alpha

CPT-1α: Carnitine palmitoyltransferase-1alpha

CAT: catalase

CYP7A1: cholesterol 7-alpha-monooxygenase

G6PC: glucose-6-phosphatase

PCK1: Phosphoenolpyruvate Carboxykinase 1

LXR-α: Liver X receptor-alpha

PPAR-α: Peroxisome proliferator-activated receptor-alpha

FGF21: Fibroblast growth factor 21

ROS: reactive oxygen species

GSSG: Oxidized glutathione

TGF- β: transforming growth factor-beta

Akt: Protein kinase B

THRβ: Thyroid Hormone Receptor-Beta

Me1: Malic Enzyme 1

NO: nitric oxide

↑: increases

↓: decreases

Sumário

1.   REVISÃO BIBLIOGRÁFICA

1.1 Doença Hepática Gordurosa Associada à Disfunção Metabólica

Durante muito tempo o abuso de álcool e as hepatites virais crônicas foram as principais causas de morbimortalidade por doenças hepáticas em todo o mundo, entretanto, nas últimas décadas, em decorrência do aumento da prevalência de obesidade, a doença hepática gordurosa associada à disfunção metabólica (MAFLD) tem sido reconhecida como uma das formas mais comuns de doenças hepáticas crônicas, particularmente nos países industrializados [1, 2]. A MAFLD abrange um amplo espectro de doenças hepáticas em indivíduos que não consomem álcool em quantidades consideradas danosas ao fígado, ou seja, inferior a 20g de etanol/dia para mulheres e 30g/dia para os homens [1, 2].

A história natural da MAFLD envolve desde a simples deposição de gordura no fígado, a esteatose hepática, podendo progredir para esteato-hepatite não-alcoólica (EHNA), cirrose e carcinoma hepatocelular (CHC) [2-4]. Os estádios iniciais da doença podem ocasionar inflamação crônica e reparação compensatória do tecido, posteriormente ocorre o acúmulo de colágeno e cicatriz (fibrose e/ou cirrose) [5-7]. A cirrose está associada à perda progressiva da função orgânica e constitui a base para o desenvolvimento do CHC, embora o tumor possa ocorrer em fígado não-cirrótico [5, 8, 9]. A MAFLD é assintomática até o surgimento de complicações em seu estádio final, podendo as alterações tornarem-se irreversíveis, quando o transplante hepático passa a ser a única opção terapêutica [3, 5, 10, 11]. Assim, a identificação dos fatores de risco, diagnóstico precoce e intervenções são fundamentais para o manejo clínico da doença [2, 11].

A MAFLD tem distribuição mundial com prevalência variada, dependendo da população estudada e do método diagnóstico empregado [1, 6]. Ela ocorre em indivíduos de todas as idades, entretanto, é mais comum nos adultos e tende a aumentar com a idade [1, 12]. Estima-se que a prevalência da MAFLD seja algo em torno de 20-30% na população ocidental e 5-18% no Oriente [13]. Sua prevalência na América do Sul tem sido estimada em 30% [14]. Ademais, Goulart *et al.* relataram uma

frequência de 34,4% de MAFLD em 195 sujeitos de pesquisa avaliados por ultrassonografia no Estudo Longitudinal de Saúde do Adulto (ELSA)-Brasil [15]. Atualmente, a MAFLD é a segunda causa de transplante hepático nos Estados Unidos [16], e é notável que seja ainda a causa que mais cresce nos últimos anos, naquele país [17, 18]. Estimativas recentes dão conta que nos próximos anos a MAFLD vai se tornar mais frequente e mais grave, não só nos Estados Unidos, mas em todo o mundo [19, 20]. De fato, até 2030 estima-se um incremento no número de casos de cirrose descompensada em 168%, de CHC em 137% e de 178% nos casos de morte hepática relacionados à MAFLD [19].

Em indivíduos com peso normal, a prevalência de MAFLD é de aproximadamente 16%, aumentando para 43 a 60% em pacientes com diabetes *mellitus* tipo-2 (DM-2) e 91% em pacientes obesos submetidos à cirurgia bariátrica [1]. Devido à alta prevalência na população obesa, a MAFLD tem sido considerada uma manifestação hepática da síndrome metabólica (SM), além de ser considerada um fator de risco independente para o desenvolvimento de doença cardiovascular (DCV) [3, 21-23]. Do ponto de vista etiológico, obesidade, resistência insulínica, DM-2 e dislipidemia são as causas primárias para o desenvolvimento dessa doença [1, 7, 13]. Já o aparecimento secundário, inclui a exposição à xenobióticos, nutrição parenteral prolongada e algumas intervenções cirúrgicas, como transplante hepático e derivação jejunoileal [12, 13, 22].

Neste sentido, algumas hipóteses têm sido levantadas na fisiopatologia da MAFLD e em sua evolução para EHNA, destacando a teoria dos múltiplos golpes propostos por Day & James [24, 25]. A resistência à insulina seria a condição inicial para o acúmulo de ácidos graxos nos hepatócitos, uma vez que favorece a lipogênese e inibe a lipólise, aumentando significativamente o aporte de ácidos graxos a esse órgão (primeiro golpe), enquanto que o acúmulo progressivo de gordura atua como um sinalizador para o estresse oxidativo, disfunção mitocondrial, processo inflamatório e endotoxemia crônica (segundo golpe), mecanismos fundamentais para a progressão da doença hepática [24, 25]. Contudo, embora se conheçam os fatores

predisponentes, ainda existem mecanismos que foram pouco explorados e que podem contribuir para a progressão da doença [7, 21, 26]. Neste aspecto, damos destaque para a função dos hormônios tireoidianos no fígado, que ainda não possui uma associação com o desenvolvimento e a progressão da MAFLD.

1.2 Metabolismo Tireoidiano

Como descrito, a MAFLD é uma doença complexa, por tratar-se de uma doença multifatorial outros fatores genéticos, epigenéticos e ambientais podem estar envolvidos na sua patogênese. Neste sentido, existem condições metabólicas associadas à MAFLD que foram pouco exploradas até o momento, dentre elas destacamos o metabolismo dos hormônios tireoidianos (HTs) que podem ter papel fundamental na progressão para outros estágios da doença.

Os HTs são essenciais para o crescimento, desenvolvimento e metabolismo em praticamente todos os sistemas do corpo. Apesar da Tiroxina (T4) ser o principal produto da tireoide, o hormônio biologicamente ativo é o triiodotironina (T3). Ambos HTs entram nas células através de um transportador especifico, posteriormente são regulados pela atividade da família das iodotironinas desiodases tipo 1, 2 e 3. As desiodases tipos 1 (Dio1) e 2 (Dio2) tem função de converter o T4 intracelular em T3 [27], enquanto a do tipo 3 (Dio3) converte o T3 em rT3 (T3 inativo) [28].

A função desempenhada pelos HTs no fígado é de suma importância. Seu papel influencia diretamente o metabolismo de lipídios e carboidratos, pelos processos de lipogênese hepática, oxidação lipídica, homeostase do colesterol e gliconeogênese [29, 30]. Grande parte destas vias metabólicas envolvem genes que são regulados pelos HTs via receptor do hormônio da tireóide-β (THR-β), sendo essa a principal isoforma expressa no fígado [31]. Além disso os HTs também demonstram ter papel na estimulação da lipofagia hepática, mobilizando os triglicerídeos hepáticos e liberando ácidos graxos livres para a β-oxidação mitocondrial. Essa funcionalidade dos HTs no fígado parece gerar de forma aguda um mecanismo de hidrolise de triglicerídeos, enquanto em situações crônicas aumenta genes ligados a lipase hepática [32].

A importância dos HTs no fígado já está bem estabelecida, sendo de conhecimento geral que em situações normais (¨fígado saudável¨) os hepatócitos apresentam uma grande expressão de Dio1 e baixa expressão de Dio3, mantendo a conversão dos HTs adequada e sua atividade hepática preservada. Mais recentemente, evidencias tem demonstrando alteração na disponibilidade dos T3, tendo uma possível associação com a progressão MAFLD [33-35]. Além disso, estudos em modelos animais tem demostrado o papel do T3 na organização da rede microtubular hepática, renovação mitocondrial e autofagia, que são comprometidas na MAFLD [36, 37].

Outro aspecto importante a ser falado são os recentes dados demonstrando uma associação macrocitaria com a atividade das desiodases. Como descrito, a MAFLD induz inflamação o que gera uma sinalização e mobilização do sistema imune ao local de lesão. Recentemente, estudos tem mostrado uma co-localização da Dio2 e Dio3 em macrófagos após situações de lesão [38-40]. Esses dados demonstram uma visão inédita do crosstalk na disponibilidade de T3, dando uma nova perspectiva de tratamento para doenças agudas e crônicas, incluindo a MAFLD.

Neste contexto, compreender melhor os mecanismos envolvidos no metabolismo dos HTs na doença podem trazer uma ¨luz¨ na compreensão da fisiopatologia da MAFLD, abrindo a possibilidade para potencias terapêuticos relacionados a atividade dos HTs no fígado.

CAPÍTULO 1

Influence of altered thyroid hormone mechanisms in the progression of metabolic dysfunction associated with fatty liver disease (MAFLD): A systematic review.

Influence of Altered Thyroid Hormone Mechanisms in the Progression of Metabolic Dysfunction Associated with Fatty Liver Disease (MAFLD): A Systematic Review

Rafael Aguiar Marschner [1], Fernanda Arenhardt [1], Rafael Teixeira Ribeiro [2] and Simone Magagnin Wajner [1,3,*]

1 Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre,
   Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre 90035-003, RS, Brazil;
   rafamarschner@gmail.com (R.A.M.); farenhardt@gmail.com (F.A.)
2 Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul (UFRGS),
   Porto Alegre 90035-003, RS, Brazil; rafaelrated@hotmail.com
3 Department of Internal Medicine, Universidade Federal do Rio Grande do Sul (UFRGS),
   Porto Alegre 90035-003, RS, Brazil
* Correspondence: simone.wajner@ufrgs.br

Abstract: We performed a systematic review of the mechanisms of thyroid hormones (THs) associated with metabolic dysfunction associated with fatty liver disease (MAFLD). This systematic review was registered under PROSPERO (CRD42022323766). We searched the MEDLINE (via PubMed) and Embase databases from their inception to March 2022. We included studies that assessed thyroid function by measuring the serum level of THs and those involved in MAFLD. We excluded reviews, case reports, editorials, letters, duplicate studies and designed controls. Forty-three studies included MAFLD, eleven analyzed THs, and thirty-two evaluated the mechanisms of THs in MAFLD. Thyroid hormones are essential for healthy growth, development and tissue maintenance. In the liver, THs directly influence the regulation of lipid and carbohydrate metabolism, restoring the homeostatic state of the body. The selected studies showed an association of reduced levels of THs with the development and progression of MAFLD. In parallel, reduced levels of T3 have a negative impact on the activation of co-regulators in the liver, reducing the transcription of genes important in hepatic metabolism. Overall, this is the first review that systematically synthesizes studies focused on the mechanism of THs in the development and progression of MAFLD. The data generated in this systematic review strengthen knowledge of the impact of TH changes on the liver and direct new studies focusing on therapies that use these mechanisms.

Keywords: thyroid hormones; liver; MAFLD

1. Introduction

Thyroid hormones (THs) are crucial for of all systems through the human body to work properly. Although thyroxine (T_4) is the main product of the thyroid, the biologically active hormone is triiodothyronine (T_3). Both THs enter cells through specific transporters and are subsequently regulated by the activity of the type 1, 2 and 3 iodothyronine deiodinases family [1], which activates (D1 and D2) and inactivates (D3) both T3 and T4 [2]. THs play a fundamental role in liver metabolism through a well-known metabolic network [3]. They directly influence lipid and carbohydrate metabolism in hepatocytes, maintaining plasma levels of triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol [4], leading to hepatic lipogenesis, lipid oxidation and gluconeogenesis [5,6]. The circulating levels of THs are directly associated with liver metabolism [7]. Evidence has shown that lower availability of T_3 or dysfunction of thyroid hormone receptors (THRs) leads to a reduction in free fatty acid (FFA) uptake by hepatocytes, lower mitochondrial β-oxidation and changes the lipogenesis processes.

In recent decades, MAFLD was recognized as the most common form of chronic liver disease, particularly in industrialized countries due to increased obesity in this population [8,9]. It is estimated that the prevalence of MAFLD is around 20–30% in the Western countries and 5–18% in the East [10]. MAFLD is a complex and multifactorial disease, with genetic, epigenetic and environmental factors involved in its pathogenesis. The alterations begin with the deposit of fat in the liver (secondary to a high fat diet (HFD)). The excess of fat leads to lipotoxicity, generating chronic inflammation. MAFLD then progress to more advanced stages such as non-alcoholic steatohepatitis (NASH), characterized by high levels of oxidative stress and increased damage to liver tissue. Next, the hepatic tissue deposits collagen in the injured cells in an attempt to reduce the damage generated, leading to the fibrosis phase. If there is no change, the next stage is cirrhosis and sometimes hepatocellular carcinoma (HCC) [9,11,12].

Previous studies showed that serum levels of THs are associated with the start and progression of metabolic dysfunction associated with fatty liver disease (MAFLD) [11–13]. The main association was between hypothyroidism and MAFLD, leading to increased risk of hepatic fibrosis in the long term. However, the exact mechanisms that orchestrate thyroid hormone regulation of hepatic metabolism are still unclear when related to the progression to more severe cases of the disease. Thus, this systematic review aimed to better understand the newly recognized mechanisms involved in the dysfunctional metabolism of THs in MAFLD progression.

2. Results

2.1. Identified Records

Our search initially identified 480 titles and abstracts of potentially eligible studies through database searching. After duplicate removal, 411 records were screened, and m texts were assessed for eligibility. Of these, 133 did not provide information about the outcome of interest. Forty-three studies were included in the analysis, eleven related to changes in serum levels of THs and the liver, and thirty-two evaluating alterations in the metabolism of THs in relation to the progression of MAFLD (Figure 1).

2.2. Altered Circulating Levels of Thyroid Hormone and the Liver

The studies selected in Table 1 verified the effect of serum levels of thyroid hormones on the liver, mainly related to the progression of MAFLD. Eleven articles evaluated the serum levels of THs in the context of MAFLD. Of these, one developed an experimental model [14] and the other ten were clinical studies. Among the clinical studies, most had a cross-sectional or retrospective design; only one study was of a prospective cohort [15].

The selected clinical articles used two types of MAFLD assessment technique. Seven were based on imaging tests, while three studies performed biopsies, considered the gold standard for the diagnosis of the disease. The TH groups were separated into euthyroid, hypothyroid or hyperthyroid. With respect to the liver profile, groups were divided into with or without MAFLD, fibrosis or steatosis. One study also verified the action of type 2 diabetes (DM2) on MAFLD and levels THs [16]. All studies demonstrated an association between hypothyroidism with the development of MAFLD.

One study verified the hepatic effects of hyperthyroidism [14]. Interestingly, the authors suggest that increasing the availability of T3 protects, to some extent, the liver from disease progression. However, as it is an experimental study it is not possible to draw definitive conclusions, and further studies are necessary to assess this hypothesis. Another study evaluated gene mutation of the TH receptor, THR-β [17]. A decrease in the availability of the receptor in the liver tissue also increased the risk of developing MAFLD.

The data in Table 1 is a compilation of these results and demonstrates an overall direct association between hypothyroidism and MAFLD. Fat deposition in the liver increases in situations of low serum T3 levels. A long-term, increased risk for MAFLD progression to more severe stages, such as fibrosis, cirrhosis, and hepatocellular carcinoma can be considered when the hormone is not supplemented to euthyroid levels.
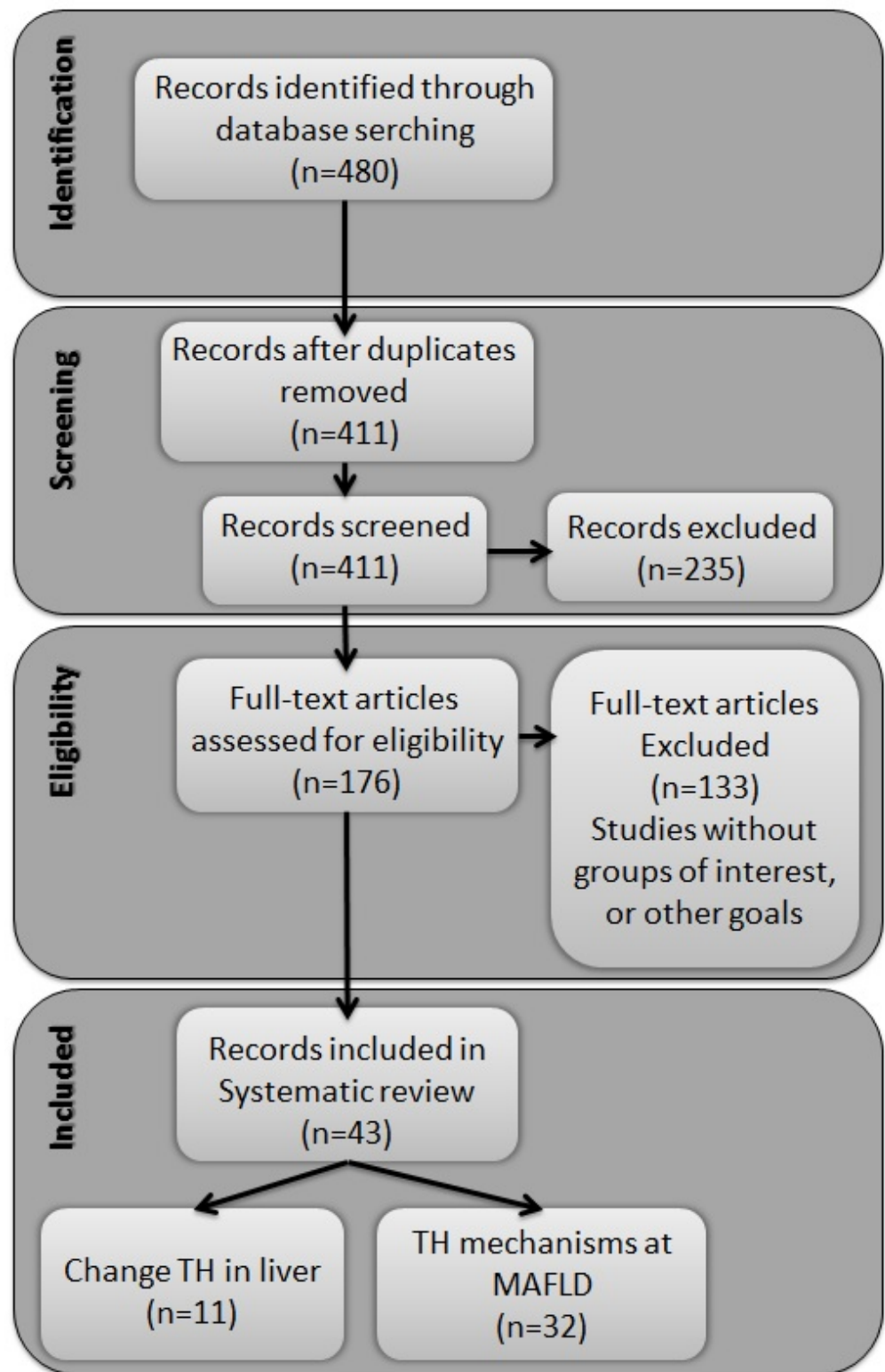
Figure 1. Flowchart of study selection.

Table 1. Changes in thyroid hormones and their effects on the liver.

Manuscript	Sample	Study Design	MAFLD Assessment Technique	Groups	Serum Levels of THs	Effect on Liver
Klieverik et al. [14] (2009)	Rats	—	—	G1: Euthyroid G2: Hypothyroidism G3: Thyrotoxic	G2: ↓ THs G3: ↑ THs	Hypothyroidism ↓ Absorption of FFAs in oxidative tissues ↑ FFAs WAT absorption Thyrotoxic ↑ Absorption of oxidative tissue FFAs
Liangpunsakul et al. [18] (2003)	Human	Retrospective study	Biopsies and imaging	G1: Control G2: NASH	G2: ↓ T3	Increased risk to NASH development
Chung et al. [19] (2012)	Human	Cross sectional	Imaging	G1: Euthyroidism with NAFLD G2: Subclinical hypothyroidism with NAFLD	G2: ↓ T3	↑ NAFLD prevalence
Bano et al. [15] (2016)	Human	Prospective cohort	Imaging	G1: NAFLD Euthyroidism G2: NAFLD Hypothyroidism G3: NAFLD Hyperthyroidism	G2: ↓ THs	↑ Fibrosis ↑ Risk for NAFLD progression
Kim et al. [20] (2018)	Human	Cross sectional	Biopsies	G1: NAFLD strict-normal thyroid function G2: NAFLD low thyroid function	G2: ↓ THs	↑ Fibrosis ↑ Risk of progression to NASH
Manka et al. [21] (2019)	Human	Retrospective study	Imaging	G1: NAFLD grade 1 G2: NAFLD grade 2 G3: NAFLD grade 3 G4: NAFLD grade 4	G1: ↓ T3 G2: ↓↓ T3 G3: ↓↓ T3 G4: ↓↓↓ T3	↑ Risk of Fibrosis

Table 1. *Cont.*

Manuscript	Sample	Study Design	MAFLD Assessment Technique	Groups	Serum Levels of THs	Effect on Liver
Kim et al. [22] (2020)	Human	Retrospective study	Imaging	G1: NAFLD strict-normal thyroid function G2: NAFLD low thyroid function	G2: ↓ THs	↑ Fibrosis ↑ Risk for all-cause and cardiovascular mortality
D'Ambrosio et al. [23] (2021)	Human	Retrospective Single-Center study	Biopsies	G1: NASH G2: Fibrosis G3: Steatosis	G1: ↓ T3 G2: ↓↓ T3 G3: ↓↓↓ T3	↑ Risk of NAFLD progression
Du et al. [16] (2021)	Human	Retrospective study	Imaging	G1: DM2 with NAFLD without Fibrosis G2: DM2 + NAFLD + Fibrosis	G2: ↓ T3	↑ Fibrosis
Chaves et al. [17] (2021)	Human	Cross sectional	Imaging	G1: R243Q mutation of the THR-β gene G2: Their wild-type first-degree relatives	G1: ↓ THR-β	↑ Risk for NAFLD progression
Wang et al. [24] (2021)	Human	Cross sectional	Imaging	G1: Hypothyroidism G2: Hypothyroidism + NAFLD	G2: ↓ T3	↑ Liver FFAs

G1 (Group 1); G2 (Group2); G3 (Group 3); G4 (Group 4); FFA (free fatty acid); NAFLD (non-alcoholic fatty liver disease); NASH (non-alcoholic steatohepatitis); ↑ increases; ↓ decreases; ↓↓ greater decreases; ↓↓↓ severe decreases.

2.3. Metabolic Action of Thyroid Hormone in Liver with MAFLD

The publications that aimed to understand the mechanisms involved in thyroid hormones implicated in the progression of MAFLD were selected and organized into two tables: Table 2 (clinical studies) and Table 3 (experimental studies).

Table 2 presents the selected clinical studies that verified the altered mechanisms of THs metabolism and their effects on the liver and on the progression of MAFLD. Designs included a cross-sectional study [25], a cohort [26], a randomized controlled trial [27] and an extension study [28]. The selected works used two MAFLD evaluation techniques. One study used imaging tests [25], while the other three performed biopsies. Regarding the study groups, we observe that all were composed of patients with or without MAFLD. Of the selected works, two studies carried out the use of MGL-3196, an analogue of THR-β [27,28] as proposed treatment. Serum THs levels were measured by biochemical tests. Interestingly, the studies demonstrated an alteration in the REDOX state and a reduction in the expression of genes positively stimulated by T3 and linked to metabolic functions in the liver [25,26]. When the THR-β analog treatment was used, the mitochondrial capacity improved, reducing liver fat and decreasing the risk of progression to other stages of MAFLD [27,28].

The selected experimental studies are shown in Table 3; these studies explore the mechanisms of TH metabolism and its relationship with MAFLD. The selection features different experimental designs. Four studies used a cell model [29–32], while the others used an animal model, demonstrating different ways of inducing MAFLD. The cell studies used the administration of FFAs [29] or palmitic and oleic acid [30]. The animal models show heterogeneity regarding the induction of the model, but the vast majority used ae high fat diet (HFD) [33–40]; one study used a pork-derived diet [41], while other models did not induce MAFLD but induced situations of hypo and hyperthyroidism [42–49], subsequently evaluating its effects on the liver. Most of the selected articles included some type of hormone supplementation. Some studies used T2, T3 and T4 hormones [29–32,34,35,38,39,42,45,46,50,51], while others used THR analogs [35,38,39,52]. One study used soy oil and fish oil supplements [48] and one study used an antioxidant compound [40], whereas other studies worked with gene mutations [52–54].

We observe that the data described in these studies relates to three main mechanisms. The first is the inflammatory process, which is due to a high-fat diet and low levels of physical exercise. An excess of fat in hepatocytes generates a signaling cascade of pro-inflammatory factors such as IL-6 and TNF-α. At the same time, an imbalance between antioxidant defenses and reactive oxygen species generates an altered (pro-oxidative) REDOX state, characterizing the second process. These disorders negatively affect the activity of enzymes capable of activating THs, decreasing the activity of type 1 deiodinase (Dio1), responsible for converting T4 to T3, and increasing the activity of type 3 deiodinase (Dio3), responsible for inactivating T3. This results in all mechanisms dependent on T3 levels in the liver being suppressed. This reduced signaling process decreases the activation of genes responsible for the metabolism of lipids and carbohydrates in the liver and causes systemic changes such as increased serum levels of cholesterol and triglycerides, and hepatic changes such as reduced lipid catabolism, reduced β- oxidation and increased lipid synthesis. The set of metabolic imbalances in the liver, secondary to these altered mechanisms, significantly increases the risk of developing MAFLD, leading to fibrosis, cirrhosis and, in more severe cases, hepatocarcinoma.

Table 2. Metabolic action modulated by thyroid hormone signaling in clinical studies.

Manuscript	Sample	Study Design	NAFLD Assessment Technique	Groups	Treatment	Dose	THs	TH Target	Effect on Liver
Mustafa et al. [25] (2009)	Human	Cross sectional	Imaging	G1: Control G2: NASH	—	—	G2: ↓ T3	↑ serum MDA ↑ serum NO ↓ serum GSH ↓ serum GPx	↑ Risk NASH progression
Krause et al. [26] (2018)	Human	Cohort Study	Biopsies	G1: NAFLD	—	—	G1: ↓ T3	↓ Dio1 mRNA ↓ THR-β mRNA	↑ Hyperlipidemia ↑ Risk NASH progression
Harrison et al. [27] (2019)	Human	Randomized Controlled Trial	Biopsies	G1: NASH + Placebo G2: NASH + MGL-3196	MGL-3196	80 mg	G2: ↑ THR-β	THR-β	↓ Hepatic fat Restoration of Mitochondrial function
Harrison et al. [28] (2021)	Human	Extension Study	Biopsies	G1: NASH	MGL-3196	80 or 100 mg	G1: ↑ T3 and ↓ rT3	THR-β	↓ Risk NASH progression

G1 (Group 1); G2 (Group2); G3 (Group 3); G4 (Group 4); G5 (Group 5); FFA (free fatty acid); NAFLD (non-alcoholic fatty liver disease); NASH (non-alcoholic steatohepatitis); Dio1 (iodothyronine deiodinase 1); GSH (reduced glutathione); THRβ (thyroid hormone receptor-beta); MDA (malondialdehyde); GPx (glutathione peroxidase); NO (nitric oxide); ↑ increases; ↓ decreases.

Table 3. Metabolic actions modulated by thyroid hormone signaling in experimental studies.

Manuscript	Sample	Groups	Treatment	Dose	THs	TH Target	Effect on Liver
Nozaki et al. [31] (1992)	Cells	HepG2 cells	T3	0.1/μg/mL 0.2/μg/mL 0.3/μg/mL	↑ T3	↑ HTGL mRNA ↑ Hepatic lipid hydrolysis	↑ Lipogenesis
Zhang et al. [32] (2004)	Cells	HepG2 cells with Luciferase Vectors-CPT-1	T3	100 nM	↑ T3	↑ PGC-1α mRNA ↑ CPT-1α mRNA	↑ Fatty acid β-oxidation
Grasselli et al. [29] (2011)	Cells	G1: Control G2: FFAs G3: FFAs + T2 G4: FFAs + T3	T2 T3	10^{-7} to 10^{-5} M 10^{-7} to 10^{-5} M	G3: ↑ T2 G4: ↑ T3	↓ PPAR-δ and -γ mRNA ↓ SOD ↓ CAT	↓ Excess fat ↓ TAG
Grasselli et al. [30] (2014)	Cells	G1: Control cells G2: Hepatoma cell + oleate/palmitate	T2 T3	10^{-6} M to 10^{-5} M	G2: ↑ T3	↑ UCP2 mRNA ↑ CPT-1 mRNA ↑ UCP2 protein ↑ CPT1 protein ↑ ROS ↓ CAT ↓ GSH	↓ Extracellular TAG ↑ Fatty-acid oxidation ↓ NAFLD progression

Table 3. *Cont.*

Manuscript	Sample	Groups	Treatment	Dose	THs	TH Target	Effect on Liver
Ness et al. [46] (1990)	Rats	G1: Normal G2: Hypophysectomized	T3	10 µg/100 g	G2: ↑ T3	↑ CYP7A1 mRNA	↑ Cholesterol metabolism
Huang et al. [44] (1998)	Rats	G1: Hypothyroidism G2: Hyperthyroidism G3: Euthyroidism	—	—	G1: ↓ THs G2: ↑ THs	Hyperthyroid ↑ Acetyl-CoA carboxylase mRNA Hypothyroid ↓ Acetyl-CoA carboxylase mRNA	Hyperthyroid ↑ Hepatic lipogenesis Hypothyroidism ↓ Hepatic lipogenesis
Feng et al. [43] (2000)	Mice	G1: Control G2: Hyperthyroid	—	—	G2: ↑ T3 + THR	↑ G6PC ↑ PCK1	↑ Glycogenolysis ↑ Gluconeogenesis
Jackson-Hayes et al. [53] (2003)	Mice	Transgenics (CPT-1α-luciferase) with/without the 1st intron of the CPT-1α gene	—	—	↑ T3 + THR	↑ CPT-1α gene	↑ Fatty acid β-oxidation
Noguchi-Yachide et al. [49] (2007)	Mice	G1: Euthyroidism G2: Thyrotoxic G3: Hypothyroidism	—	—	G2: ↑ T3 + THR	↑ LXR-α mRNA ↑ CYP7A1 mRNA	Lipid homeostasis
Liu et al. [52] (2007)	Mice	G1: WT control G2: Mutation in THR	—	—	G2: ↓ T3 + THR	↓ PPARα protein	↓ Fatty acid β-oxidation
Lopez et al. [45] (2007)	Rats	G1: Normal G2: Hypophysectomized G3: Thyroidectomy	T3	10 µg/100 g	G3: ↑ T3	↑ LDL receptor mRNA	↑ FFA absorption
Cable et al. [55] (2009)	Rats	G1: NASH + Vehicle G2: NASH + T3 G3: NASH + MB07811	MB07811 T3	1–50 mg/kg/day 650 µg/kg/day	G2: ↑ T3 G3: ↑ T3 + THR	↑ CPT-1 mRNA ↑ PGC-1α mRNA	↑ Mitochondrial β-oxidation
Mollica et al. [38] (2009)	Rats	G1: Control G2: HFD G3: HFD + T2	T2	25 µg/100 g	G3: ↑ T2	↑ PPAR-α ↑ CPT-1	↑ mitochondrial respiration ↓ degree of steatosis
Adams et al. [50] (2010)	Mice	G1: C57BL/6 control G2: C57BL/6 with T3	PBS T3	500 µg/kg	G2: ↑ T3	↑ FGF21 mRNA	↑ lipolysis ↑ hepatic fatty acid oxidation
Sousa et al. [48] (2011)	Rats	G1: Euthyroidism G2: Hypothyroidism	Soybean oil Fish oil	0.5 mL	G2: ↓ T3	↑ PPARα protein ↓ D1 mRNA	↓ Serum triglycerides ↓ Hepatic TAG levels
Grasselli et al. [34] (2012)	Rats	G1: DP G2: HFD G3: HFD + T2 G4: DP + T2	T2	25 µg/100 g	G3: ↑ T2 G4: ↑ T2	↑ PPARγ mRNA ↑ acyl-CoA oxidase mRNA	↓ Inflammation ↓ Adipose triglyceride lipase ↑ FFA oxidation

Table 3. *Cont.*

Manuscript	Sample	Groups	Treatment	Dose	THs	TH Target	Effect on Liver
Santana-Farré et al. [47] (2012)	Rats	G1: Neonatal Hypothyroidism G2: Age-matched euthyroid G3: Euthyroid weight paired	—	—	G1: ↓ T3	↑ PPARα mRNA ↓ LXR mRNA ↓ CD36 mRNA ↓ genes uptake Ags	↓ Absorption of FFAs in the liver
Cavallo et al. [42] (2013)	Rats	G1: Euthyroid G2: Hypothyroid G3: Hypothyroid + T2	T2	150 µg/100 g	G3: ↑ T2	↑ CPT-1 protein ↑ OXPHOS	↑ Fatty acid β-oxidation ↓ Adiposity ↓ Dyslipidemia
Alonso-Merino et al. [51] (2016)	Cells Rats	G1: Euthyroid G2: Hyperthyroid	T4 T3	T4 7 ng/g T3 35 ng/g	G2: ↑ T3 + THR	↓ TGF-β mRNA	↓ Fibrosis progression
Iannucci et al. [35] (2017)	Rats	G1: Control G2: HFD G3: HFD + T2 G4: HFD + T3	T2 T3	25 µg/100 g 2.5 µg/100 g	G3: ↑ T3 + THR G4: ↑ T3 + THR	↑ CPT-1α protein ↑ UCP2 protein ↑ p-ERK protein ↑ p-Akt protein	↑ Lipolysis ↑ Autophagy ↑ Fatty acid β-oxidation
Senese et al. [39] (2017)	Rats	G1: Control G2: HFD G3: HFD + T2 G4: HFD + T3	T2 T3	25 µg/100 g^{-1} 2.5 µg/100 g^{-1}	G3: ↑ T2 G4: ↑ T3	↑ Dio1 mRNA ↑ THRβ mRNA	↓ TAG ↓ Lipogenesis ↑ Fatty acid oxidation
Bruinstroop et al. [56] (2018)	Rats	G1: Control G2: MCD diet	—	—	G2: ↓ T3	↓ T3 hepatic ↓ Dio1 mRNA	↑ NAFLD progression
Xia et al. [40] (2019)	Mice	G1: C57BL/6 control G2: C57BL/6 HFD G3: C57BL/6 HFD + Myr	Myricetin	100 mg/kg^{-1}	G3: ↑ T4 and ↑ T3	↑ Dio1 mRNA ↑ Dio1 protein ↑ Dio1 activity ↑ THRβ mRNA ↑ THRβ protein	↓ Hepatic steatosis ↑ Lipid metabolism
Luong et al. [37] (2020)	Rats	G1: Control G2: HFD G3: HFD + MGL-3169 (5.0 mg/kg) G4: HFD + MGL-3169 (1.5 mg/kg) G5: HFD + MGL-3169 (0.5 mg/kg) G6: HFD + T3 (0.5 mg/kg)	MGL-3196 T3	0.5–5.0 mg/kg	G3: ↑ T3 and ↑ THR G4: ↑ T3 and ↑ THR G5: ↑ T3 and ↑ THR G6: ↑ T3 and ↑ THR	↑ Dio1 mRNA ↑ Me1 mRNA	↓ Serum lipid profile ↑ FFA oxidation
Bruinstroop et al. [54] (2021)	Mice	G1: Control NCD G2: Control WDF G3: Dio1 LKD WDF	—	—	G3: ↓ T3	↓ Dio1 mRNA ↓ Dio1 activity	↑ TAG ↑ Cholesterol ↑ Risk for NAFLD progression

Table 3. *Cont.*

Manuscript	Sample	Groups	Treatment	Dose	THs	TH Target	Effect on Liver
Caddeo et al. [33] (2021)	Mice	G1: C57BL/6 G2: C57BL/6 + HFD G3: C57BL/6 + HFD + MGL-3196 G4: C57BL/6 + HFD + TG68	MGL-3196 TG68	3 mg·kg^{-1} 2.8 mg·kg^{-1}	G3: ↑ T3 G4: ↑ T3	↑ Dio1 mRNA ↑ THRsp mRNA	↓ liver weight ↓ Serum TAG ↓ Plasma ALT ↓ Plasma AST
Kannt et al. [36] (2021)	Mice	G1: C57BL/6J + DP G2: C57BL/6J + HFD G3: C57BL/6J + HFD + Resmetirom	Resmetirom	3 mg·kg^{-1}	G3: ↑ THR	↑ Dio1 mRNA ↑ CYP7A1 mRNA ↑ Me1 mRNA	↓ Serum lipid profile ↓ Liver weight ↓ NAFLD Score
Ge et al. [41] (2022)	Mice	G1: C57BL/6 control G2: C57BL/6 LOP G3: C57BL/6 HOP G4: C57BL/6 LOP + Dityr G5: C57BL/6 Dityr	—	—	G2: ↓ T3 G3: ↓ T3 G4: ↓ T3 G5: ↓ T3	↓ Dio1 mRNA ↓ THRβ mRNA ↓ CPT-1α mRNA ↓ PPARα mRNA ↓ PGC-1α mRNA ↓ CYP7A1 mRNA ↑ MDA ↑ ROS ↓ CAT ↓ GSH/GSSG	↑ Risk NAFLD ↑ Inflammation ↑ Oxidative stress ↓ Hepatic energy metabolism ↑ Hepatic lipid synthesis ↓ Hepatic lipid catabolism ↓ Fatty-acid oxidation

G1 (Group 1); G2 (Group2); G3 (Group 3); G4 (Group 4); G5 (Group 5); FFA (free fatty acid); NAFLD (non-alcoholic fatty liver disease); NASH (non-alcoholic steatohepatitis); HTLG (hepatic triacyiglycerol lipase); PGC-1α (peroxisome proliferator-activated receptor-gamma coactivator-1alpha); CPT-1α (carnitine palmitoyltransferase-1alpha); SOD (superoxide dismutase); CAT (catalase); CYP7A1 (cholesterol 7-alpha-monooxygenase); G6PC (glucose-6-phosphatase); PCK1 (phosphoenolpyruvate carboxykinase 1); LXR-α (liver X receptor-alpha); PPAR-α (peroxisome proliferator-activated receptor-alpha); FGF21 (fibroblast growth factor 21); Dio1 (iodothyronine deiodinase 1); UCP2 (uncoupling protein 2); ROS (reactive oxygen species); GSH (reduced glutathione); GSSG (oxidized glutathione); TGF- β (transforming growth factor-beta); ERK (extracellular signal-regulated kinases); Akt (protein kinase B); THRβ (thyroid hormone receptor-beta); Me1 (malic enzyme 1); MDA (malondialdehyde); GPx (glutathione peroxidase); NO (nitric oxide); ↑ increases; ↓ decreases.

3. Discussion

3.1. THs Dependent Mechanisms in Hepatic Metabolism

The processes involving hepatic metabolism occur through the action of several genes, and the modulation of these genes is performed by the signaling of THs via THR-β isoform in the liver, generating signaling of coactivators and corepressors, co-regulators of gene transcription [3,57–59] (Figure 2A).
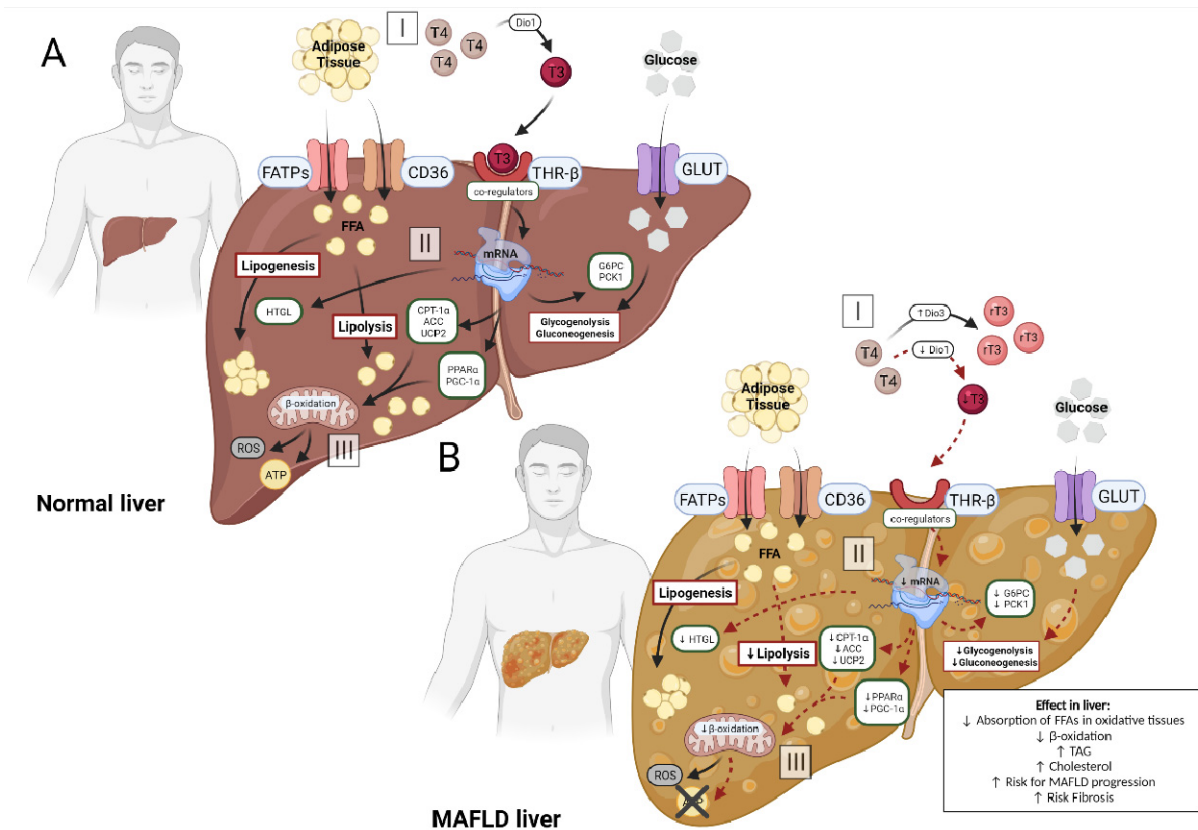

Figure 2. (**A**): In the normal liver, circulating adipose tissue enters the hepatocyte via specific receptors, FATPs and CD36 (A-I). At the same time, the circulating hormone T4 is converted into T3 by the enzyme Dio1, making it biologically active and enabling its binding to its THRβ receptor (A-I). This attachment generates the activation of co-regulators, which could stimulate the transcription of genes in the hepatocyte (A-II). The main genes activated in this process are CPT-1α, UCP2, PGC-1α and PPARs, increasing hepatic lipolysis and β-oxidation, and ATP production (A-III). Other genes that are indirectly stimulated are HTLG linked to the process of lipogenesis and genes linked to glycogenolysis and gluconeogenesis, G6PC and PCK-1, using the serum glucose that entered the hepatocyte via the specific transporter GLUT (A-II). (**B**): In the MAFLD liver, the process is changed. Circulating adipose tissue enters the hepatocyte via FATPs and CD36 (B-I). However, circulating T4 does not convert into T3, but into rT3 or T2, due to the reduction of Dio1 activity and the increase of Dio3, decreasing the binding of the hormone to the THRβ (B-I) receptor. With lower availability of T3, the activation of co-regulators does not occur effectively, reducing the transcription of genes in the hepatocyte (B-II). Among the affected genes are CPT-1α, UCP2, PGC-1α and PPARs, decreasing hepatic lipolysis and β-oxidation, reducing ATP production and increasing reactive oxygen species (ROS) (B-III). Other genes also affected alter the process of lipogenesis and decrease glycogenolysis and gluconeogenesis (B-II). This imbalance of hepatic metabolism is one of the main factors involved in the progression of MAFLD, with a high risk of fibrosis.

The signaling generated by THs is one of the central mechanism of changes in liver functions. The mobilization of proteins responsible for the absorption of free fatty acids (FFAs) is an important source of lipids in the liver. Its absorption is carried out via specific

transport proteins, such as fatty acid transport proteins (FATPs) and translocase protein (CD36) [60]. Studies have suggested that normal amounts of circulating THs facilitate the absorption of FFAs by tissues, thus establishing a direct association between FFA transporters and THs [16,61,62].

In addition, THs stimulate gene transcription of mitochondrial β-oxidation-linked proteins, such as carnitine palmitoyltransferase-1 (CPT-1), coactivator 1 α (PGC1-α) and peroxisome proliferator-activated receptor gamma (PPARs) [63–65]. Changes in T3 availability directly impact the ability to metabolize FFAs, reducing the mRNA of these proteins and increasing the risk of developing metabolic dysfunction associated with fatty liver disease (MAFLD). It is suggested that T3 plays a central role in the regulation of mitochondrial β-oxidation, which is an important step in energy (Tables 2 and 3). The hepatic glycolytic pathway is also regulated by THs, involving gluconeogenesis and glycogenolysis [61]. In this context, changes in hepatic glucose metabolism are observed with altered circulating levels of THs [62]. In situations of hyperthyroidism, there is a resistance to insulin status, probably due to increased expression of CPT-1 and reduced malonyl-CoA. It is known that malonyl-CoA in the liver, has the ability to inhibit CPT-1 by increasing circulating glucose-stimulated insulin release [66].

Another factor that may explain the increase in circulating glucose levels is the action of serum T3 in the activation of genes involved in gluconeogenesis. Increased activity of enzymes such as glucose-6-phosphatase (G6PC), along with phosphoenolpyruvate carboxykinase 1 (PCK1) and pyruvate carboxylase (PC) are associated with increased gluconeogenesis activity. Studies have shown that higher levels of serum T3 increase the transcription of the G6PC and PCK1 genes, involved in the homeostatic control of glucose levels via gluconeogenesis (Tables 2 and 3).

Some limitations were observed in this systematic review. First, clinical studies aimed at verifying the risk of developing MAFLD in patients with thyroid dysfunction that is already established. Well-designed studies on euthyroid patients can bring new answers about the role of THs on euthyroid and the possible causes of the onset of MAFLD. Another important point seen here is the lack of studies exploring the complexity of the involvement of THs on the progression of MAFLD, especially clinical studies that performed a biopsy for the diagnosis of the disease.

The data compiled in this systematic review may suggest that the availability of THs directly impacts hepatic metabolic capacity, given the importance of THs signaling in the regulation of metabolic pathways. The key role played by THs seems to be fundamental to the understanding of the triggering and progression of liver metabolic diseases and could be involved as part in the treatment of this disease.

3.2. Thyroid Hormone Metabolism Alterations and MAFLD

It is known that in normal situations hepatocytes show high expression of Dio1 and low expression of Dio3, maintaining adequate TH function and hepatic metabolism activity (Figure 2A). Recently, evidence has shown that alterations in these enzymes results in changes in the availability of hepatic T3 (Figure 2B). The study by He et al. (2017) found, in a meta-analysis, evidence of a direct and significant association of low T3 levels with a higher risk of developing MAFLD, when compared with normal thyroid function [13].

Table 2 presents the clinical studies selected for review. The design used in the studies is mainly cross-sectional or cohort. Studies with this design answer specific questions, generating limited data related to the mechanisms involved in thyroid metabolism in MAFLD. Randomized clinical trials [27,28] are more robust. However, studies that used the biopsy technique for the diagnosis of MAFLD somehow drew attention. The tissue fragment collected could have been better explored, answering questions at the molecular level that have not yet been answered in clinical studies. We believe that new studies using this approach should evaluate gene expression and quantification of proteins related to the mechanisms involved in the disease, thus creating new data related to this topic.

In general, all studies reached the same conclusion. All, including our work, confirmed a direct association between serum T3 levels and MAFLD progression. However, our study advances knowledge, bringing different insight from the selected studies. Our work was able to better understand and correlate the mechanisms that are influenced by a lower availability of T3 in the liver.

Our findings show that alterations in the expression of genes involved in T3 activation have a negative impact on hepatic metabolic capacity. A reduction of Dio1 expression [26] reduces the availability of T3, affecting the binding of the hormone with its THR-β receptor, which decreases the activation of other genes involved in hepatic metabolism. These changes generate a reduction in mitochondrial capacity and, consequently, a dysfunction in hepatic lipid metabolism, increasing fat deposits and increasing the risk of MAFLD progression. In contrast, other studies found that an increase in the amount of the THR-β receptor increases the sensitivity of the liver tissue to the T3 hormone, improving mitochondrial function, β-oxidation, and slowing the progression of the disease.

The findings of the studies listed in Table 3 present similar results. They reiterate the association between liver T3 availability and MAFLD development and progression. THs regulate genes linked to the functionality of several metabolic pathways, such as lipogenesis, lipid oxidation and hepatic gluconeogenesis [6]. Table 3, together with Figure 2B, clearly demonstrates that the reduction of T3-stimulated genes favors MAFLD progression. Reduced expression of PPAR-α reduces the transcription of genes involved in lipid homeostasis and, together with lower expression of CPT-1, generates a reduction in the translocation of FFA from the cytosol to the mitochondrial matrix, decreasing the metabolism of fat and the mitochondrial capacity [67]. In addition, other genes that are inhibited by T3 inactivity stimulate disease progression, as the Me1 gene, that decreases the encoding of cytosolic enzymes linked to fatty acid biosynthesis and the reduction of UCP2, decreases the decoupling of oxygen consumption from ATP synthesis.

The findings of this review support the hypothesis that as the disease progresses to more severe conditions such as NASH and fibrosis, Dio1 expression decreases while Dio3 expression increases, decreasing the availability of active T3 (Figure 2B-I). Local T3 reduction has a negative impact on the activation of co-regulators released by THRs, causing a cascade effect on genes involved in lipolytic processes: lower transcription of PPARs, CPT-1 and PGC1-α (genes involved in β-oxidation processes) (Figure 2B-II). In addition to the lipolytic pathway, the glycolytic pathway undergoes changes, with reduced transcription of G6PC and PCK1 (Figure 2B-II). It leads to an increase in the accumulation of triglycerides by hepatocytes and the reuptake of LDL, due to an impairment of the breakdown of triglycerides and a lower capacity for β-oxidation of FFAs, reducing energy production (Figure 2B-III).

Nevertheless, there are still several gaps in knowledge about MAFLD. For example, knowing that the pathophysiology of MAFLD is characterized by inflammatory changes and the REDOX state, it is possible to think that the use of antioxidants could improve the dysfunction in thyroid hormone metabolism, improving mitochondrial capacity and consequently lipid metabolism, probably stabilizing MAFLD.

4. Methods

4.1. Protocol and Registration

This systematic review adheres to the PRISMA guidelines and was registered in the International Prospective Register of Systematic Reviews (PROSPERO CRD42022323766).

4.2. Study Objectives

Our objective was to investigate the role of thyroid hormone metabolism in MAFLD. We focused on the following research question:

What are the mechanisms of THs that are associated with MAFLD progression?

4.3. Eligibility Criteria

We included only cohort studies (prospective or retrospective), clinical trials and experimental studies that related THs levels and/or mechanisms with MAFLD. Exclusion criteria were age younger than 18 years and articles that were not a cohort study, clinical trial or experimental. Only articles written in English were considered. No restrictions on publication date were applied.

4.4. Search Strategy and Study Selection

We performed a systematic search of the MEDLINE (via PubMed) and Embase databases from their inception to March 2022. Comprehensive search queries included text words and descriptors (MeSH and Entree) based on the phrases 'non-alcoholic fatty liver disease' and 'thyroid hormones'.

The complete search strategy for Embase and Pubmed was: Pubmed Exposition: ("Thyroid hormones" [Mesh] OR "Thyroid Hormone Receptors beta" [Mesh]) OR ("iodothyronine deiodinase type I" [Supplementary Concept] OR "iodothyronine de-iodinase type II" [Supplementary Concept] OR "iodothyronine deiodinase type III" [Supplementary Concept]) AND ("Non-alcoholic Fatty liver disease" [Mesh] OR "Fatty liver" [Mesh] OR "Diet, High-Fat" [Mesh]) AND ("Clinical Trial" [Publication Type] OR "Clinical Study" [Publication Type] OR "Observation" [Mesh] OR "Randomized controlled trial" [Publication Type] OR "Rats" [Mesh] OR "Mice" [Mesh] OR "Cells" [Mesh]) NOT ("Review" [Publication Type] OR "Systematic review" [Publication Type] OR "Meta-Analysis" [Publication Type]). Embase: Exposition: 'Thyroid hormones' OR 'Thyroid Hormone Receptors beta' OR 'deiodinase type 1' OR 'de-iodinase type 2' OR 'deiodinase type 3' AND 'Nonalcoholic Fatty liver/exp' OR 'Non-alcoholic Fatty liver' OR 'Fatty liver' OR lipid diet' AND 'Clinical Trial' OR 'Clinical Study' OR 'Observation study' OR 'Randomized controlled trial' OR 'Rat' OR 'Mouse' OR 'Cells'.

Three independent reviewers (RAM, FA and RTR) assessed records for inclusion based on titles and abstracts. Abstracts that did not meet the inclusion criteria or that met the exclusion criteria were discarded. The remaining records and abstracts that did not provide enough information to decide on their exclusion were selected for full-text evaluation, which was performed by the same reviewers independently. A fourth reviewer (SW) resolved the disagreements.

4.5. Data Collection and Extraction

Independent reviewers extracted the data using a standardized system. The following information was obtained: first author, year of publication, sample, study design, NAFLD assessment technique, groups, treatment, dose, THs, TH target, effect in liver. The research team verified and discussed the results of the extraction.

5. Conclusions

There have been advances in the understanding of the mechanisms involving THs and THRs in the maintenance of liver homeostasis in recent years. The interrelationship between the availability of tissue T3 and the signaling of THRs in the metabolic processes involved in liver diseases seems to be the key to better understand the disease. Recent findings have given us a basis of knowledge on lipid metabolism and its complexity in the processes of FFA regulation, although its relationship with THs in the disease is still unclear. Deepening knowledge of the mechanisms involved in MAFLD related to THs seems to be the focus for future studies.

Author Contributions: Conceptualization, R.A.M. and S.M.W.; material selection, R.A.M., F.A. and R.T.R. Writing—original draft preparation, R.A.M. and R.T.R.; writing—review and editing, S.M.W. All authors have read and agreed to the published version of the manuscript.

References

1. Peeters, R.P.; Visser, T.J. Metabolism of Thyroid Hormone. In *Endotext*; Feingold, K.R., Anawalt, B., Boyce, A., Chrousos, G., de Herder, W.W., Dhatariya, K., Dungan, K., Hershman, J.M., Hofland, J., Kalra, S., et al., Eds.; MDText.com, Inc.: South Dartmouth, MA, USA, 2000.

2. Bianco, A.C.; Salvatore, D.; Gereben, B.; Berry, M.J.; Larsen, P.R. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* **2002**, *23*, 38–89. [CrossRef] [PubMed]

3. Ritter, M.J.; Amano, I.; Hollenberg, A.N. Thyroid Hormone Signaling and the Liver. *Hepatology* **2020**, *72*, 742–752. [CrossRef] [PubMed]

4. Duntas, L.H. Thyroid Disease and Lipids. *Thyroid* **2002**, *12*, 287–293. [CrossRef]

5. Raftopoulos, Y.; Gagné, D.J.; Papasavas, P.; Hayetian, F.; Maurer, J.; Bononi, P.; Caushaj, P.F. Improvement of Hypothyroidism after Laparoscopic Roux-en-Y Gastric Bypass for Morbid Obesity. *Obes. Surg.* **2004**, *14*, 509–513. [CrossRef] [PubMed]

6. Sinha, R.A.; Singh, B.K.; Yen, P.M. Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. *Trends Endocrinol. Metab.* **2014**, *25*, 538–545. [CrossRef]

7. Sinha, R.A.; Bruinstroop, E.; Singh, B.K.; Yen, P.M. Nonalcoholic Fatty Liver Disease and Hypercholesterolemia: Roles of Thyroid Hormones, Metabolites, and Agonists. *Thyroid* **2019**, *29*, 1173–1191. [CrossRef]

8. Than, N.N.; Newsome, P.N. A concise review of non-alcoholic fatty liver disease. *Atherosclerosis* **2015**, *239*, 192–202. [CrossRef]

9. Arab, J.P.; Arrese, M.; Trauner, M. Recent Insights into the Pathogenesis of Nonalcoholic Fatty Liver Disease. *Annu. Rev. Pathol.* **2018**, *13*, 321–350. [CrossRef]

10. Masarone, M.; Federico, A.; Abenavoli, L.M.; Loguercio, C.; Persico, M. Non Alcoholic Fatty Liver: Epidemiology and Natural History. *Rev. Recent Clin. Trials* **2015**, *9*, 126–133. [CrossRef]

11. Eshraghian, A.; Jahromi, A.H. Non-alcoholic fatty liver disease and thyroid dysfunction: A systematic review. *World J. Gastroenterol.* **2014**, *20*, 8102–8109. [CrossRef]

12. Guo, Z.; Li, M.; Han, B.; Qi, X. Association of non-alcoholic fatty liver disease with thyroid function: A systematic review and meta-analysis. *Dig. Liver Dis.* **2018**, *50*, 1153–1162. [CrossRef] [PubMed]

13. Zeng, X.; Li, B.; Zou, Y. The relationship between non-alcoholic fatty liver disease and hypothyroidism: A systematic review and meta-analysis. *Medicine* **2021**, *100*, e25738. [CrossRef] [PubMed]

14. Klieverik, L.P.; Coomans, C.P.; Endert, E.; Sauerwein, H.P.; Havekes, L.M.; Voshol, P.J.; Rensen, P.C.N.; Romijn, J.A.; Kalsbeek, A.; Fliers, E. Thyroid Hormone Effects on Whole-Body Energy Homeostasis and Tissue-Specific Fatty Acid Uptake in vivo. *Endocrinology* **2009**, *150*, 5639–5648. [CrossRef] [PubMed]

15. Bano, A.; Chaker, L.; Plompen, E.P.C.; Hofman, A.; Dehghan, A.; Franco, O.; Janssen, H.L.A.; Murad, S.D.; Peeters, R.P. Thyroid Function and the Risk of Nonalcoholic Fatty Liver Disease: The Rotterdam Study. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 3204–3211. [CrossRef]

16. Du, J.; Chai, S.; Zhao, X.; Sun, J.; Zhang, X.; Huo, L. Association between thyroid hormone levels and advanced liver fibrosis in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease. *Diabetes Metab. Syndr. Obes. Targets Ther.* **2021**, *14*, 2399–2406. [CrossRef]

17. Chaves, C.; Bruinstroop, E.; Refetoff, S.; Yen, P.M.; Anselmo, J.D. Increased Hepatic Fat Content in Patients with Resistance to Thyroid Hormone Beta. *Thyroid* **2021**, *31*, 1127–1134. [CrossRef]

18. Liangpunsakul, S.; Chalasani, N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? *J. Clin. Gastroenterol.* **2003**, *37*, 340–343. [CrossRef]

19. Chung, G.E.; Kim, D.; Kim, W.; Yim, J.Y.; Park, M.J.; Kim, Y.J.; Yoon, J.H.; Lee, H.S. Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. *J. Hepatol.* **2012**, *57*, 150–156. [CrossRef]

20. Kim, D.; Kim, W.; Joo, S.K.; Bae, J.M.; Kim, J.H.; Ahmed, A. Subclinical Hypothyroidism and Low-Normal Thyroid Function Are Associated With Nonalcoholic Steatohepatitis and Fibrosis. *Clin. Gastroenterol. Hepatol.* **2018**, *16*, 123–131. [CrossRef]

21. Manka, P.; Bechmann, L.; Best, J.; Sydor, S.; Claridge, L.C.; Coombes, J.D.; Canbay, A.; Moeller, L.; Gerken, G.; Wedemeyer, H.; et al. Low Free Triiodothyronine Is Associated with Advanced Fibrosis in Patients at High Risk for Nonalcoholic Steatohepatitis. *Am. J. Dig. Dis.* **2019**, *64*, 2351–2358. [CrossRef]

22. Kim, D.; Vazquez-Montesino, L.M.; Escober, J.A.; Fernandes, C.T.; Cholankeril, G.; Loomba, R.; Harrison, S.A.; Younossi, Z.M.; Ahmed, A. Low Thyroid Function in Nonalcoholic Fatty Liver Disease Is an Independent Predictor of All-Cause and Cardiovascular Mortality. *Am. J. Gastroenterol.* **2020**, *115*, 1496–1504. [CrossRef]

23. D'Ambrosio, R.; Campi, I.; Maggioni, M.; Perbellini, R.; Giammona, E.; Stucchi, R.; Borghi, M.; Degasperi, E.; De Silvestri, A.; Persani, L.; et al. The relationship between liver histology and thyroid function tests in patients with non-alcoholic fatty liver disease (NAFLD). *PLoS ONE* **2021**, *16*, e0249614. [CrossRef] [PubMed]

24. Wang, B.; Wang, B.; Yang, Y.; Xu, J.; Hong, M.; Xia, M.; Li, X.; Gao, X. Thyroid function and non-alcoholic fatty liver disease in hyperthyroidism patients. *BMC Endocr. Disord.* **2021**, *21*, 27. [CrossRef] [PubMed]

25. Moustafa, A.H.A.; Ali, E.M.; Mohamed, T.; Abdou, H.I. Oxidative stress and thyroid hormones in patients with liver diseases. *Eur. J. Intern. Med.* **2009**, *20*, 703–708. [CrossRef] [PubMed]

26. Krause, C.; Grohs, M.; El Gammal, A.T.; Wolter, S.; Lehnert, H.; Mann, O.; Mittag, J.; Kirchner, H. Reduced expression of thyroid hormone receptor β in human nonalcoholic steatohepatitis. *Endocr. Connect.* **2018**, *7*, 1448–1456. [CrossRef]

27. A Harrison, S.; Bashir, M.R.; Guy, C.D.; Zhou, R.; A Moylan, C.; Frias, J.P.; Alkhouri, N.; Bansal, M.B.; Baum, S.; A Neuschwander-Tetri, B.; et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: A multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* **2019**, *394*, 2012–2024. [CrossRef]

28. Harrison, S.A.; Bashir, M.; Moussa, S.E.; McCarty, K.; Frias, J.P.; Taub, R.; Alkhouri, N. Effects of Resmetirom on Noninvasive Endpoints in a 36-Week Phase 2 Active Treatment Extension Study in Patients With NASH. *Hepatol. Commun.* **2021**, *5*, 573–588. [CrossRef]

29. Grasselli, E.; Voci, A.; Canesi, L.; De Matteis, R.; Goglia, F.; Cioffi, F.; Fugassa, E.; Gallo, G.; Vergani, L. Direct effects of iodothyronines on excess fat storage in rat hepatocytes. *J. Hepatol.* **2011**, *54*, 1230–1236. [CrossRef]

30. Grasselli, E.; Voci, A.; Canesi, L.; Salis, A.; Damonte, G.; Compalati, A.D.; Goglia, F.; Gallo, G.; Vergani, L. 3,5-Diiodo-L-Thyronine Modifies the Lipid Droplet Composition in a Model of Hepatosteatosis. *Cell. Physiol. Biochem.* **2014**, *33*, 344–356. [CrossRef]

31. Nozaki, S.; Shimomura, I.; Funahashi, T.; Menju, M.; Kubo, M.; Matsuzawa, Y. Stimulation of the activity and mRNA level of hepatic triacylglycerol lipase by triiodothyronine in HepG2 cells. *Biochim. Biophys. Acta (BBA)-Lipids Lipid Metab.* **1992**, *1127*, 298–302. [CrossRef]

32. Zhang, Y.; Ma, K.; Song, S.; Elam, M.B.; Cook, G.A.; Park, E.A. Peroxisomal proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1 alpha) enhances the thyroid hormone induction of carnitine palmitoyltransferase I (CPT-I alpha). *J. Biol. Chem.* **2004**, *279*, 53963–53971. [CrossRef] [PubMed]

33. Caddeo, A.; Kowalik, M.A.; Serra, M.; Runfola, M.; Bacci, A.; Rapposelli, S.; Columbano, A.; Perra, A. TG68, a Novel Thyroid Hormone Receptor-β Agonist for the Treatment of NAFLD. *Int. J. Mol. Sci.* **2021**, *22*, 13105. [CrossRef] [PubMed]

34. Grasselli, E.; Voci, A.; Demori, I.; Canesi, L.; De Matteis, R.; Goglia, F.; Lanni, A.; Gallo, G.; Vergani, L. 3,5-Diiodo-l-thyronine modulates the expression of genes of lipid metabolism in a rat model of fatty liver. *J. Endocrinol.* **2011**, *212*, 149–158. [CrossRef] [PubMed]

35. Iannucci, L.F.; Cioffi, F.; Senese, R.; Goglia, F.; Lanni, A.; Yen, P.M.; Sinha, R.A. Metabolomic analysis shows differential hepatic effects of T(2) and T(3) in rats after short-term feeding with high fat diet. *Sci. Rep.* **2017**, *7*, 2023. [CrossRef]

36. Kannt, A.; Wohlfart, P.; Madsen, A.N.; Veidal, S.S.; Feigh, M.; Schmoll, D. Activation of thyroid hormone receptor-β improved disease activity and metabolism independent of body weight in a mouse model of non-alcoholic steatohepatitis and fibrosis. *J. Cereb. Blood Flow Metab.* **2021**, *178*, 2412–2423. [CrossRef]

37. Luong, X.G.; Stevens, S.K.; Jekle, A.; Lin, T.-I.; Gupta, K.; Misner, D.; Chanda, S.; Mukherjee, S.; Williams, C.; Stoycheva, A.; et al. Regulation of gene transcription by thyroid hormone receptor β agonists in clinical development for the treatment of non-alcoholic steatohepatitis (NASH). *PLoS ONE* **2020**, *15*, e0240338. [CrossRef]

38. Mollica, M.P.; Lionetti, L.; Moreno, M.; Lombardi, A.; De Lange, P.; Antonelli, A.; Lanni, A.; Cavaliere, G.; Barletta, A.; Goglia, F. 3,5-diiodo-l-thyronine, by modulating mitochondrial functions, reverses hepatic fat accumulation in rats fed a high-fat diet. *J. Hepatol.* **2009**, *51*, 363–370. [CrossRef]

39. Senese, R.; Cioffi, F.; de Lange, P.; Leanza, C.; Iannucci, L.F.; Silvestri, E.; Moreno, M.; Lombardi, A.; Goglia, F.; Lanni, A. Both 3,5-Diiodo-L-Thyronine and 3,5,3′-Triiodo-L-Thyronine Prevent Short-term Hepatic Lipid Accumulation via Distinct Mechanisms in Rats Being Fed a High-Fat Diet. *Front. Physiol.* **2017**, *8*, 706. [CrossRef]

40. Xia, S.F.; Qiu, Y.Y.; Chen, L.M.; Jiang, Y.Y.; Huang, W.; Xie, Z.X.; Tang, X.; Sun, J. Myricetin alleviated hepatic steatosis by acting on microRNA-146b/thyroid hormone receptor b pathway in high-fat diet fed C57BL/6J mice. *Food Funct.* **2019**, *10*, 1465–1477. [CrossRef] [PubMed]

41. Ge, Y.; Yang, Y.; Jiang, Y.; Feng, C.; Li, B.; Sun, J.; Tang, X.; Shi, Y.; Le, G. Oxidized Pork Induces Hepatic Steatosis by Impairing Thyroid Hormone Function in Mice. *Mol. Nutr. Food Res.* **2021**, *66*, e2100602. [CrossRef]

42. Cavallo, A.; Priore, P.; Gnoni, G.V.; Papa, S.; Zanotti, F.; Gnoni, A. 3,5-Diiodo-L-Thyronine Administration To Hypothyroid Rats Rapidly Enhances Fatty Acid Oxidation Rate and Bioenergetic Parameters in Liver Cells. *PLoS ONE* **2013**, *8*, e52328. [CrossRef] [PubMed]

43. Feng, X.; Jiang, Y.; Meltzer, P.; Yen, P.M. Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. *Mol. Endocrinol.* **2000**, *14*, 947–955. [CrossRef]

44. Huang, C.; Freake, H.C. Thyroid hormone regulates the acetyl-CoA carboxylase PI promoter. *Biochem. Biophys. Res. Commun.* **1998**, *249*, 704–708. [CrossRef] [PubMed]

45. Lopez, D.; Socarrás, J.F.A.; Bedi, M.; Ness, G.C. Activation of the hepatic LDL receptor promoter by thyroid hormone. *Biochim. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids* **2007**, *1771*, 1216–1225. [CrossRef]

46. Ness, G.C.; Pendleton, L.C.; Li, Y.C.; Chiang, J.Y. Effect of thyroid hormone on hepatic cholesterol 7α hydroxylase, LDL receptor, HMG-CoA reductase, farnesyl pyrophosphate synthetase and apolipoprotein A-I mRNA levels in hypophysectomized rats. *Biochem. Biophys. Res. Commun.* **1990**, *172*, 1150–1156. [CrossRef]

47. Santana-Farré, R.; Mirecki-Garrido, M.; Bocos, C.; Henríquez-Hernández, L.A.; Kahlon, N.; Herrera, E.; Norstedt, G.; Parini, P.; Flores-Morales, A.; Fernández-Pérez, L. Influence of Neonatal Hypothyroidism on Hepatic Gene Expression and Lipid Metabolism in Adulthood. *PLoS ONE* **2012**, *7*, e37386. [CrossRef]

48. Souza, L.L.; Cordeiro, A.; Oliveira, L.S.; de Paula, G.S.M.; Faustino, L.C.; Ortiga-Carvalho, T.M.; Oliveira, K.J.; Pazos-Moura, C.C. Thyroid hormone contributes to the hypolipidemic effect of polyunsaturated fatty acids from fish oil: In vivo evidence for cross talking mechanisms. *J. Endocrinol.* **2011**, *211*, 65–72. [CrossRef]

49. Noguchi-Yachide, T.; Aoyama, A.; Makishima, M.; Miyachi, H.; Hashimoto, Y. Liver X receptor antagonists with a phthalimide skeleton derived from thalidomide-related glucosidase inhibitors. *Bioorganic Med. Chem. Lett.* **2007**, *17*, 3957–3961. [CrossRef]

50. Adams, A.C.; Astapova, I.; Fisher, F.M.; Badman, M.K.; Kurgansky, K.E.; Flier, J.S.; Hollenberg, A.N.; Maratos-Flier, E. Thyroid Hormone Regulates Hepatic Expression of Fibroblast Growth Factor 21 in a PPARα-dependent Manner. *J. Biol. Chem.* **2010**, *285*, 14078–14082. [CrossRef]

51. Alonso-Merino, E.; Martin Orozco, R.; Ruiz-Llorente, L.; Martinez-Iglesias, O.A.; Velasco-Martin, J.P.; Montero-Pedrazuela, A.; Fanjul-Rodriguez, L.; Contreras-Jurado, C.; Regadera, J.; Aranda, A. Thyroid hormones inhibit TGF-beta signaling and attenuate fibrotic responses. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3451–3460. [CrossRef]

52. Liu, Y.Y.; Heymann, R.S.; Moatamed, F.; Schultz, J.J.; Sobel, D.; Brent, G.A. A mutant thyroid hormone receptor alpha antagonizes peroxisome proliferator-activated receptor alpha signaling in vivo and impairs fatty acid oxidation. *Endocrinology* **2007**, *148*, 1206–1217.

53. Jackson-Hayes, L.; Song, S.; Lavrentyev, E.N.; Jansen, M.S.; Hillgartner, F.B.; Tian, L.; Wood, P.A.; Cook, G.A.; Park, E.A. A Thyroid Hormone Response Unit Formed between the Promoter and First Intron of the Carnitine Palmitoyltransferase-Iα Gene Mediates the Liver-specific Induction by Thyroid Hormone. *J. Biol. Chem.* **2003**, *278*, 7964–7972. [PubMed]

54. Bruinstroop, E.; Zhou, J.; Tripathi, M.; Yau, W.W.; Boelen, A.; Singh, B.K.; Yen, P.M. Early induction of hepatic deiodinase type 1 inhibits hepatosteatosis during NAFLD progression. *Mol. Metab.* **2021**, *53*, 101266. [PubMed]

55. Cable, E.E.; Finn, P.D.; Stebbins, J.W.; Hou, J.; Ito, B.R.; van Poelje, P.D.; Linemeyer, D.L.; Erion, M.D. Reduction of hepatic steatosis in rats and mice after treatment with a liver-targeted thyroid hormone receptor agonist. *Hepatology* **2009**, *49*, 407–417. [PubMed]

56. Bruinstroop, E.; Dalan, R.; Cao, Y.; Bee, Y.M.; Chandran, K.; Cho, L.W.; Soh, S.B.; Teo, E.K.; Toh, S.-A.; Leow, M.K.S.; et al. Low-Dose Levothyroxine Reduces Intrahepatic Lipid Content in Patients With Type 2 Diabetes Mellitus and NAFLD. *J. Clin. Endocrinol. Metab.* **2018**, *103*, 2698–2706.

57. Mendoza, A.; Hollenberg, A.N. New insights into thyroid hormone action. *Pharmacol. Ther.* **2017**, *173*, 135–145.

58. Ishizuka, T.; Lazar, M.A. The Nuclear Receptor Corepressor Deacetylase Activating Domain Is Essential for Repression by Thyroid Hormone Receptor. *Mol. Endocrinol.* **2005**, *19*, 1443–1451.

59. Shimizu, H.; Astapova, I.; Ye, F.; Bilban, M.; Cohen, R.N.; Hollenberg, A.N. NCoR1 and SMRT Play Unique Roles in Thyroid Hormone Action in Vivo. *Mol. Cell. Biol.* **2015**, *35*, 555–565.

60. Coburn, C.T.; Hajri, T.; Ibrahimi, A.; Abumrad, N.A. Role of CD36 in Membrane Transport and Utilization of Long-Chain Fatty Acids by Different Tissues. *J. Mol. Neurosci.* **2001**, *16*, 117–122.

61. Brenta, G. Why can insulin resistance be a natural consequence of thyroid dysfunction? *J. Thyroid Res.* **2011**, *2011*, 152850.

62. Liu, Y.-Y.; Brent, G.A. Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. *Trends Endocrinol. Metab.* **2010**, *21*, 166–173. [PubMed]

63. Yang, L.; Li, P.; Fu, S.; Calay, E.S.; Hotamisligil, G.S. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab.* **2010**, *11*, 467–478. [PubMed]

64. Singh, R.; Kaushik, S.; Wang, Y.; Xiang, Y.; Novak, I.; Komatsu, M.; Tanaka, K.; Cuervo, A.M.; Czaja, M.J. Autophagy regulates lipid metabolism. *Nature* **2009**, *458*, 1131–1135. [PubMed]

65. Wierzbicki, M.; Chabowski, A.; Zendzian-Piotrowska, M.; Górski, J. Differential effects of in vivo PPAR alpha and gamma activation on fatty acid transport proteins expression and lipid content in rat liver. *J. Physiol. Pharmacol.* **2009**, *60*, 99–106.

66. Crunkhorn, S.; Patti, M.-E. Links between Thyroid Hormone Action, Oxidative Metabolism, and Diabetes Risk? *Thyroid* **2008**, *18*, 227–237.

67. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Group, P. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ* **2009**, *339*, b2535.

CAPÍTULO 2

Role of Type 3 Deiodinase in the progression of non-alcoholic liver disease:
Interconnections between Oxidative Stress, Respiratory Changes and Macrophage
Activation

2. PERSPECTIVAS

Tendo em vista os resultados encontrados nos estudos apresentados, é possível acrescentar o metabolismo dos hormônios tireoidianos e seus respectivos mecanismos como fatores contribuintes na fisiopatologia da MAFLD. Além disso, os achados obtidos nesses estudos abrem novas possibilidades terapêuticas no tratamento da doença, visto que ainda não existe um tratamento farmacológico liberado para o controle da mesma e que ainda são utilizados mudanças no estilo de vida (alimentação e exercício físico) no combate a MAFLD.

Avaliar abordagens que visem melhorar o estado REDOX, no fígado, parece ser uma boa alternativa para reestabelecer a função das desiodases e consequentemente a disponibilidade do T3 no fígado. Em estudos anteriores do nosso grupo [41-43] verificamos que em situações de injuria aguda o uso de medicamentos com capacidade antioxidante mostraram bons resultados, tanto no tecido alvo quanto nos periféricos, reequilibrando o estado REDOX e aumentando a atividade do hormônio T3.

Neste sentido, realizar estudos com este enfoque de tratamento pode trazer novos avanços junto ao controle da MAFLD como também potencializar tratamento que já estão em teste.

3. REFERENCIAS

1. Than, N.N. and P.N. Newsome, A concise review of non-alcoholic fatty liver disease. *Atherosclerosis*, **2015**. 239(1): p. 192-202.
2. Arab, J.P., M. Arrese, and M. Trauner, Recent Insights into the Pathogenesis of Nonalcoholic Fatty Liver Disease. *Annu Rev Pathol*, **2018**. 13: p. 321-350.
3. Jahn, D., S. Kircher, H.M. Hermanns, and A. Geier, Animal models of NAFLD from a hepatologist's point of view. *Biochim Biophys Acta*, **2018**.
4. Pardhe, B.D., S. Shakya, A. Bhetwal, J. Mathias, P.R. Khanal, R. Pandit, J. Shakya, H.O. Joshi, and S.B. Marahatta, Metabolic syndrome and biochemical changes among non-alcoholic fatty liver disease patients attending a tertiary care hospital of Nepal. *BMC Gastroenterol*, **2018**. 18(1): p. 109.
5. Bieghs, V. and C. Trautwein, Innate immune signaling and gut-liver interactions in non-alcoholic fatty liver disease. *Hepatobiliary Surg Nutr*, **2014**. 3(6): p. 377-85.
6. Meli, R., G. Mattace Raso, and A. Calignano, Role of innate immune response in non-alcoholic Fatty liver disease: metabolic complications and therapeutic tools. *Front Immunol*, **2014**. 5: p. 177.
7. Saltzman, E.T., T. Palacios, M. Thomsen, and L. Vitetta, Intestinal Microbiome Shifts, Dysbiosis, Inflammation, and Non-alcoholic Fatty Liver Disease. *Front Microbiol*, **2018**. 9: p. 61.
8. Kikuchi, L., C.P. Oliveira, M.R. Alvares-da-Silva, C.M. Tani, M.A. Diniz, J.T. Stefano, A.L. Chagas, R.S. Alencar, D.C. Vezozzo, G.R. Santos, et al., Hepatocellular Carcinoma Management in Nonalcoholic Fatty Liver Disease Patients: Applicability of the BCLC Staging System. *Am J Clin Oncol*, **2016**. 39(5): p. 428-32.
9. Cotrim, H.P., C.P. Oliveira, H.S. Coelho, M.R. Alvares-da-Silva, L. Nabuco, E.R. Parise, C. Ivantes, A.L. Martinelli, J. Galizzi-Filho, and F.J. Carrilho, Nonalcoholic steatohepatitis and hepatocellular carcinoma: Brazilian survey. *Clinics (Sao Paulo)*, **2016**. 71(5): p. 281-4.
10. Poeta, M., L. Pierri, and P. Vajro, Gut-Liver Axis Derangement in Non-Alcoholic Fatty Liver Disease. *Children (Basel)*, **2017**. 4(8).
11. Chao, C.Y., R. Battat, A. Al Khoury, S. Restellini, G. Sebastiani, and T. Bessissow, Co-existence of non-alcoholic fatty liver disease and inflammatory bowel disease: A review article. *World J Gastroenterol*, **2016**. 22(34): p. 7727-34.
12. Brunt, E.M. and D.G. Tiniakos, Histopathology of nonalcoholic fatty liver disease. *World J Gastroenterol*, **2010**. 16(42): p. 5286-96.
13. Masarone, M., A. Federico, L. Abenavoli, C. Loguercio, and M. Persico, Non alcoholic fatty liver: epidemiology and natural history. *Rev Recent Clin Trials*, **2014**. 9(3): p. 126-33.
14. Younossi, Z.M., A.B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, and M. Wymer, Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, **2016**. 64(1): p. 73-84.
15. Goulart, A.C., I.R. Oliveira, A.P. Alencar, M.S. Santos, I.S. Santos, B.M. Martines, D.P. Meireles, J.A. Martines, G. Misciagna, I.M. Benseñor, et al., Diagnostic accuracy of a noninvasive hepatic ultrasound score for non-alcoholic fatty liver disease (NAFLD) in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). *Sao Paulo Med J*, **2015**. 133(2): p. 115-24.
16. Wong, R.J., M. Aguilar, R. Cheung, R.B. Perumpail, S.A. Harrison, Z.M. Younossi, and A. Ahmed, Nonalcoholic steatohepatitis is the second leading etiology of liver disease

among adults awaiting liver transplantation in the United States. *Gastroenterology*, **2015**. 148(3): p. 547-55.

17. Cholankeril, G., R.J. Wong, M. Hu, R.B. Perumpail, E.R. Yoo, P. Puri, Z.M. Younossi, S.A. Harrison, and A. Ahmed, Liver Transplantation for Nonalcoholic Steatohepatitis in the US: Temporal Trends and Outcomes. *Dig Dis Sci*, **2017**. 62(10): p. 2915-2922.

18. Younossi, Z., M. Stepanova, J.P. Ong, I.M. Jacobson, E. Bugianesi, A. Duseja, Y. Eguchi, V.W. Wong, F. Negro, Y. Yilmaz, et al., Non-alcoholic Steatohepatitis is the Fastest Growing Cause of Hepatocellular Carcinoma in Liver Transplant Candidates. *Clin Gastroenterol Hepatol*, **2018**.

19. Estes, C., H. Razavi, R. Loomba, Z. Younossi, and A.J. Sanyal, Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*, **2018**. 67(1): p. 123-133.

20. Estes, C., Q.M. Anstee, M.T. Arias-Loste, H. Bantel, S. Bellentani, J. Caballeria, M. Colombo, A. Craxi, J. Crespo, C.P. Day, et al., Modeling NAFLD Disease Burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol*, **2018**.

21. Van Herck, M.A., L. Vonghia, and S.M. Francque, Animal Models of Nonalcoholic Fatty Liver Disease-A Starter's Guide. *Nutrients*, **2017**. 9(10).

22. Azzam, H. and S. Malnick, Non-alcoholic fatty liver disease - the heart of the matter. *World J Hepatol*, **2015**. 7(10): p. 1369-76.

23. Francque, S.M., D. van der Graaff, and W.J. Kwanten, Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *J Hepatol*, **2016**. 65(2): p. 425-43.

24. Day, C.P. and O.F. James, Steatohepatitis: a tale of two "hits"? *Gastroenterology*, **1998**. 114(4): p. 842-5.

25. Day, C.P. and O.F. James, Hepatic steatosis: innocent bystander or guilty party? *Hepatology*, **1998**. 27(6): p. 1463-6.

26. Lau, J.K., X. Zhang, and J. Yu, Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. *J Pathol*, **2017**. 241(1): p. 36-44.

27. Peeters, R.P. and T.J. Visser, *Metabolism of Thyroid Hormone*, in *Endotext*, K.R. Feingold, et al., Editors. 2000: South Dartmouth (MA).

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

29. Raftopoulos, Y., D.J. Gagne, P. Papasavas, F. Hayetian, J. Maurer, P. Bononi, and P.F. Caushaj, Improvement of hypothyroidism after laparoscopic Roux-en-Y gastric bypass for morbid obesity. *Obes Surg*, **2004**. 14(4): p. 509-13.

30. Sinha, R.A., B.K. Singh, and P.M. Yen, Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. *Trends Endocrinol Metab*, **2014**. 25(10): p. 538-45.

31. Huang, Y.Y., A.M. Gusdon, and S. Qu, Cross-talk between the thyroid and liver: a new target for nonalcoholic fatty liver disease treatment. *World J Gastroenterol*, **2013**. 19(45): p. 8238-46.

32. Sinha, R.A., S.H. You, J. Zhou, M.M. Siddique, B.H. Bay, X. Zhu, M.L. Privalsky, S.Y. Cheng, R.D. Stevens, S.A. Summers, et al., Thyroid hormone stimulates hepatic lipid catabolism via activation of autophagy. *J Clin Invest*, **2012**. 122(7): p. 2428-38.

33. Bohinc, B.N., G. Michelotti, G. Xie, H. Pang, A. Suzuki, C.D. Guy, D. Piercy, L. Kruger, M. Swiderska-Syn, M. Machado, et al., Repair-related activation of hedgehog signaling in

stromal cells promotes intrahepatic hypothyroidism. *Endocrinology*, **2014**. 155(11): p. 4591-601.

34. Jaruvongvanich, V., A. Sanguankeo, and S. Upala, Nonalcoholic Fatty Liver Disease Is Not Associated with Thyroid Hormone Levels and Hypothyroidism: A Systematic Review and Meta-Analysis. *Eur Thyroid J*, **2017**. 6(4): p. 208-215.

35. Tahara, K., T. Akahane, T. Namisaki, K. Moriya, H. Kawaratani, K. Kaji, H. Takaya, Y. Sawada, N. Shimozato, S. Sato, et al., Thyroid-stimulating hormone is an independent risk factor of non-alcoholic fatty liver disease. *JGH Open*, **2020**. 4(3): p. 400-404.

36. Piantanida, E., S. Ippolito, D. Gallo, E. Masiello, P. Premoli, C. Cusini, S. Rosetti, J. Sabatino, S. Segato, F. Trimarchi, et al., The interplay between thyroid and liver: implications for clinical practice. *J Endocrinol Invest*, **2020**. 43(7): p. 885-899.

37. Vallejo, C.G., A.M. Seguido, P.S. Testillano, and M.C. Risueno, Thyroid hormone regulates tubulin expression in mammalian liver. Effects of deleting thyroid hormone receptor-alpha or -beta. *Am J Physiol Endocrinol Metab*, **2005**. 289(1): p. E87-94.

38. Kwakkel, J., H.C. van Beeren, M.T. Ackermans, M.C. Platvoet-Ter Schiphorst, E. Fliers, W.M. Wiersinga, and A. Boelen, Skeletal muscle deiodinase type 2 regulation during illness in mice. *J Endocrinol*, **2009**. 203(2): p. 263-70.

39. Dentice, M., R. Ambrosio, V. Damiano, A. Sibilio, C. Luongo, O. Guardiola, S. Yennek, P. Zordan, G. Minchiotti, A. Colao, et al., Intracellular inactivation of thyroid hormone is a survival mechanism for muscle stem cell proliferation and lineage progression. *Cell Metab*, **2014**. 20(6): p. 1038-48.

40. Ogawa-Wong, A., C. Carmody, K. Le, R.A. Marschner, P.R. Larsen, A.M. Zavacki, and S.M. Wajner, Modulation of Deiodinase Types 2 and 3 during Skeletal Muscle Regeneration. *Metabolites*, **2022**. 12(7).

41. Lehnen, T.E., R. Marschner, F. Dias, A.L. Maia, and S.M. Wajner, Oxidative remote induction of type 3 deiodinase impacts nonthyroidal illness syndrome. *J Endocrinol*, **2020**. 246(3): p. 237-246.

42. Lehnen, T.E., M.V. Santos, A. Lima, A.L. Maia, and S.M. Wajner, N-Acetylcysteine Prevents Low T3 Syndrome and Attenuates Cardiac Dysfunction in a Male Rat Model of Myocardial Infarction. *Endocrinology*, **2017**. 158(5): p. 1502-1510.

43. Vidart, J., S.M. Wajner, R.S. Leite, A. Manica, B.D. Schaan, P.R. Larsen, and A.L. Maia, N-acetylcysteine administration prevents nonthyroidal illness syndrome in patients with acute myocardial infarction: a randomized clinical trial. *J Clin Endocrinol Metab*, **2014**. 99(12): p. 4537-45.
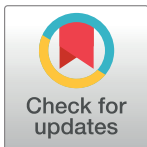
PRODUÇÃO CIENTÍFICA

(2018-2022)

Short-term exercise training improves cardiac function associated to a better antioxidant response and lower type 3 iodothyronine deiodinase activity after myocardial infarction

Rafael Aguiar Marschner[1,2], Patrícia Banda[1], Simone Magagnin Wajner[2], Melissa Medeiros Markoski[3], Maximiliano Schaun[1], Alexandre Machado Lehnen[1] *

1 Institute of Cardiology of Rio Grande do Sul/University Foundation of Cardiology, Porto Alegre, Rio Grande do Sul, Brazil, **2** Thyroid Division, Endocrinology Service, Hospital de Clínicas de Porto Alegre/Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, **3** Federal University of Health Sciences of Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil

* amlehnen@gmail.com

Abstract

Aims

We assessed the effects of a short-term exercise training on cardiac function, oxidative stress markers, and type 3 iodothyronine deiodinase (D3) activity in cardiac tissue of spontaneously hypertensive rats (SHR) following experimental myocardial infarction (MI).

Methods

Twenty-four SHR (aged 3 months) were allocated to 4 groups: sham+sedentary, sham+trained, MI+sedentary and MI+trained. MI was performed by permanent ligation of the coronary artery. Exercise training (treadmill) started 96 hours after MI and lasted for 4 weeks (~60% maximum effort, 4x/week and 40 min/day). Cardiac function (echocardiography), thioredoxin reductase (TRx), total carbonyl levels, among other oxidative stress markers and D3 activity were measured. A Generalized Estimating Equation was used, followed by Bonferroni's test (p<0.05).

Results

MI resulted in an increase in left ventricular mass (p = 0.002) with decreased cardiac output (~22.0%, p = 0.047) and decreased ejection fraction (~41%, p = 0.008) as well as an increase in the carbonyl levels (p = 0.001) and D3 activity (~33%, p<0.001). Exercise training resulted in a decrease in left ventricular mass, restored cardiac output (~34%, p = 0.048) and ejection fraction (~20%, p = 0.040), increased TRx (~85%, p = 0.007) and reduced carbonyl levels (p<0.001) and D3 activity (p<0.001).

RESEARCH

Oxidative remote induction of type 3 deiodinase impacts nonthyroidal illness syndrome

Tatiana Ederich Lehnen*, Rafael Marschner*, Fernanda Dias, Ana Luiza Maia and **Simone Magagnin Wajner**

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

Correspondence should be addressed to S M Wajner: simonewajner@gmail.com

*(T E Lehnen and R Marschner contributed equally to this work)

Abstract

Imbalances in redox status modulate type 3 deiodinase induction in nonthyroidal illness syndrome. However, the underlying mechanisms that lead to D3 dysfunction under redox imbalance are still poorly understood. Here we evaluated D3 induction, redox homeostasis, and their interrelationships in the liver, muscle, and brain in an animal model of NTIS. Male Wistar rats were subjected to left anterior coronary artery occlusion and randomly separated into two groups and treated or not (placebo) with the antioxidant N-acetylcysteine. Sham animals were used as controls. Animals were killed 10 or 28 days post-MI induction and tissues were immediately frozen for biochemical analysis. D3 activity, protein oxidation and antioxidant defenses were measured in liver, muscle, and brain. Compared to those of the sham group, the levels of D3 expression and activity were increased in the liver ($P = 0.002$), muscle ($P = 0.03$) and brain ($P = 0.01$) in the placebo group. All tissues from the placebo animals showed increased carbonyl groups ($P < 0.001$) and diminished sulfhydryl levels ($P < 0.001$). Glutathione levels were decreased and glutathione disulfide levels were augmented in all examined tissues. The liver and muscle showed augmented levels of glutathione peroxidase, glutathione reductase and thioredoxin reductase activity ($P = 0.001$). NAC prevented all the alterations described previously. D3 dysfunction in all tissues correlates with post-MI-induced protein oxidative damage and altered antioxidant defenses. NAC treatment prevents D3 dysfunction, indicating that reversible redox-related remote D3 activation explains, at least in part, the thyroid hormone derangements of NTIS.

Introduction

Nonthyroidal illness syndrome (NTIS), also known as low T3 syndrome, is a clinical condition observed in ill patients characterized by a rapid decrease in serum triiodothyronine (T3) levels accompanied by increased reverse T3 (rT3) and a subsequent decrease in plasma thyroxine (T4), without alteration in thyroid stimulating hormone (TSH) levels (Wajner & Maia 2012). NTIS is observed in patients with several diseases, and low serum T3 levels are inversely correlated with mortality in this condition (Iervasi *et al.* 2003, Peeters *et al.* 2005, Alevizaki *et al.* 2007). The pathophysiology of NTIS includes profound changes in peripheral thyroid hormone metabolism. This process is carried by a set of enzymes, deiodinase type 1, 2 and 3 (D1, D2 and D3, respectively). D1 and D2 convert T4 to T3, while D3 exclusively inactivates both T4 and T3. Deiodinases are

SCIENTIFIC REPORTS

natureresearch

Check for updates

OPEN

Different exercise training modalities produce similar endothelial function improvements in individuals with prehypertension or hypertension: a randomized clinical trial Exercise, endothelium and blood pressure

Marinei L. Pedralli [1], Rafael A. Marschner[2], Daniel P. Kollet[1], Salvador G. Neto[1], Bruna Eibel [1], Hirofumi Tanaka[3] & Alexandre M. Lehnen [1]✉

Endothelial dysfunction is a characteristic of systemic arterial hypertension (SAH) and an early marker of atherosclerosis. Aerobic exercise training (AT) improves endothelial function. However, the effects of resistance training (RT) and combined training (CT) on endothelial function remain controversial in individuals with SAH. We determined the effects of AT, RT, and CT on endothelial function and systolic (SBP)/diastolic blood pressure (DBP) in individuals with prehypertension or hypertension. Forty-two participants (54 ± 11 y, resting SBP/DBP $137 \pm 9/86 \pm 6$ mmHg) were randomly allocated into AT (n = 14, 40 min of cycling, 50–75% heart rate reserve), RT (n = 14, 6 resistance exercises, $4 \times$ 12 repetitions, 60% maximum strength) and CT (n = 14, $2 \times$ 12 repetitions of RT + 20 min of AT). All participants performed a 40-minute exercise session twice a week for 8 weeks. Endothelial function was evaluated by brachial artery flow-mediated dilation (FMD). Blood pressure was evaluated through ambulatory monitoring for 24 hours. After 8 weeks of exercise training, blood pressure was reduced in all 3 groups: -5.1 mmHg in SBP (95%CI -10.1, 0.0; $p = 0.003$) in AT; -4.0 mmHg in SBP (95%CI -7.8, -0.5; $p = 0.027$) in RT; and -3.2 mmHg in DBP (95%CI -7.9, 1.5; $p = 0.001$) in CT. All 3 exercise training modalities produced similar improvements in FMD: $+3.2\%$ (95%CI 1.7, 4.6) ($p < 0.001$) in AT; $+4.0\%$ (95%CI 2.1, 5.7) ($p < 0.001$) in RT; and $+6.8\%$ (95%CI 2.6, 11.1) ($p = 0.006$) in CT. In conclusion, different exercise training modalities were similarly effective in improving endothelial function but impacts on ambulatory blood pressure appear to be variable in individuals with prehypertension or hypertension.

Systemic arterial hypertension (SAH) is a highly prevalent cardiovascular risk factor[1] and is associated with endothelial dysfunction[2,3]. Endothelial dysfunction is a phenotypic alteration in the endothelium characterized by prothrombotic, pro-inflammatory, and pro-constrictor conditions[4]. A reduction of 0.62% in endothelial function quantified by flow-mediated dilation (FMD) is associated with an increase of 20 mmHg in systolic blood pressure (SBP)[2]. Thus, given the relationship between SAH and endothelial dysfunction and high cardiovascular risk associated with SAH[5], improving endothelial function and decreasing blood pressure (BP) are crucial for management and prevention strategies in individuals with prehypertension and hypertension[5].

[1]Institute of Cardiology of Rio Grande do Sul/University Foundation of Cardiology, Porto Alegre, Brazil. [2]Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, Brazil. [3]Cardiovascular Aging Research Laboratory, Department of Kinesiology & Health Education, The University of Texas at Austin, Austin, TX, USA. ✉e-mail: amlehnen@gmail.com

scientific reports

Check for updates

OPEN

Individuals with controlled hypertension show endothelial integrity following a bout of moderate-intensity exercise: randomized clinical trial

Gustavo Waclawovsky[1], Liliana F. C. Boll[1], Bruna Eibel[1], Ana Paula Alegretti[2], Fabiane Spagnol[2], Juliana De Paoli[2], Simone Wajner[3], Rafael A. Marschner[3], Maximiliano I. Schaun[1] & Alexandre Machado Lehnen[1]✉

To examine the acute effects of aerobic exercise (AE), resistance exercise (RE) or combined exercise (CE) on flow-mediated dilation (FMD), progenitor cells (PCs), endothelial progenitor cells (EPCs), oxidative stress markers and endothelial-cell derived microvesicles (EMVs) in patients with hypertension. This is a randomized, parallel-group clinical trial involving an intervention of one session of three different modalities of exercise. Thirty-three males (43 ± 2y) were randomly divided into three groups: a session of AE (n = 11, 40 min, cycle ergometer, 60% HRR); a session of RE (n = 11, 40 min, 4 × 12 lower limb repetitions, 60% 1-RM); or a session of CE (n = 11, 20-min RE + 20-min AE). FMD was assessed 10 min before and 10, 40 and 70 min post-intervention. Blood samples were collected at the same time points (except 40 min). FMD were similar in all groups and from baseline (within each group) after a single exercise bout (AE, RE or CE). At 70 min, RE group showed higher levels of PCs compared to the AE (81%) and CE group (60%). PC levels were reduced from baseline in all groups (AE: 32%, $p = 0.037$; RE: 15%, $p = 0.003$; CE: 17%, $p = 0.048$). The levels of EPCs, EMVs and oxidative stress were unchanged. There were no acute effects of moderate-intensity exercise on FMD, EPCs, EMVs and oxidative stress, but PCs decreased regardless of the exercise modality. Individuals with controlled hypertension do not seem to have impaired vascular function in response to a single exercise bout.

Systemic arterial hypertension is a multifactorial clinical condition characterized by sustained high blood pressure (BP) levels[1]. An increase of 20 mmHg in systolic blood pressure (SBP) has been associated with a two-fold increased risk of death from ischemic heart disease due to vascular disease[2]. It has been proposed that exacerbated sympathetic activity plays an important role in the development and maintenance of hypertension[3].

The endothelium plays a central role in the modulation of angiogenesis, inflammatory response, regulation of vascular tone and peripheral vascular resistance[4]. It is well known that cardiovascular events are directly associated with impaired endothelial function[5] characterized by decreased production and bioavailability of nitric oxide (NO) and/or insufficient vasomotor response. Flow-mediated dilation (FMD) is an important non-invasive method for measuring vascular function[6], and FMD results from a single exercise session may predict adaptive training changes[7].

Endothelial-cell derived microvesicles (EMVs) are located in the membrane of endothelial cells and are released after cellular activation or apoptosis of these cells. Thus, EMVs are biomarkers of endothelial damage by increased circulating EMVs. In the long term, aerobic exercise training may decrease resting levels of EMVs in healthy individuals, which may reflect reduced vascular injury[8]. In an acute setting (i.e., short-term after a

[1]Institute of Cardiology of Rio Grande do Sul/University Foundation of Cardiology, Av. Princesa Isabel, 395 Santana, Porto Alegre, RS 90620-001, Brazil. [2]Clinical Pathology Laboratory, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. [3]Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. ✉email: amlehnen@gmail.com

RESEARCH

Non-thyroidal illness syndrome predicts outcome in adult critically ill patients: a systematic review and meta-analysis

Josi Vidart[1,2], Paula Jaskulski[3], Ana Laura Kunzler[3], Rafael Aguiar Marschner[1], André Ferreira de Azeredo da Silva[3] and **Simone Magagnin Wajner** [1,3]

[1]Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil
[2]Intensive Care Unit, Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil
[3]Internal Medicine Division, Hospital de Clínicas de Porto Alegre, Internal Medicine Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Correspondence should be addressed to S M Wajner: simone.wajner@ufrgs.br

Abstract

We performed a systematic review and meta-analysis to comprehensively determine the prevalence and the prognostic role of non-thyroidal illness syndrome (NTIS) in critically ill patients. We included studies that assessed thyroid function by measuring the serum thyroid hormone (TH) level and in-hospital mortality in adult septic patients. Reviews, case reports, editorials, letters, animal studies, duplicate studies, and studies with irrelevant populations and inappropriate controls were excluded. A total of 6869 patients from 25 studies were included. The median prevalence rate of NTIS was 58% (IQR 33.2–63.7). In univariate analysis, triiodothyronine (T3) and free T3 (FT3) levels in non-survivors were relatively lower than that of survivors (8 studies for T3; standardized mean difference (SMD) 1.16; 95% CI, 0.41–1.92; $I^2 = 97\%$; $P < 0.01$). Free thyroxine (FT4) levels in non-survivors were also lower than that of survivors (12 studies; SMD 0.54; 95% CI, 0.31–0.78; $I^2 = 83\%$; $P < 0.01$). There were no statistically significant differences in thyrotropin levels between non-survivors and survivors. NTIS was independently associated with increased risk of mortality in critically ill patients (odds ratio (OR) = 2.21, 95% CI, 1.64–2.97, $I^2 = 65\%$ $P < 0.01$). The results favor the concept that decreased thyroid function might be associated with a worse outcome in critically ill patients. Hence, the measurement of TH could provide prognostic information on mortality in adult patients admitted to ICU.

Key Words
▶ thyroid hormone
▶ low T3 levels
▶ critically ill patients

Introduction

Thyroid hormones (TH) are essential for cellular growth, differentiation, and energetic regulation. Critical illness is frequently associated with alterations in TH metabolism not caused by abnormalities of the hypothalamic–pituitary–thyroid function. These changes, collectively known as 'non-thyroidal illness syndrome' (NTIS) or 'low T3 syndrome' are characterized by low plasma concentrations of the biologic active hormone triiodothyronine (T3), low or normal plasma concentrations of thyroxine (T4), and elevated plasma levels of the inactive hormone reverse T3

(rT3) in the presence of normal thyrotropin (TSH) levels (1). The pathophysiology of NTIS is multifactorial. In the early phase, the peripheral TH metabolism is impaired, with reduced hormonal bioavailability consequent to the consumption of carrier proteins, as acute-phase proteins, and changes in the expression of transmembrane hormone transporters. Additionally, deranged iodothyronine deiodinases function causes a decrease of T4 to T3 conversion with a further raise in the inactivation of T4 to rT3. In the chronic course of the disease, inhibition of the

Article

Modulation of Deiodinase Types 2 and 3 during Skeletal Muscle Regeneration

Ashley Ogawa-Wong [1], Colleen Carmody [1], Katherine Le [1], Rafael Aguiar Marschner [2], P. Reed Larsen [1], Ann Marie Zavacki [1] and Simone Magagnin Wajner [1,2,*]

1 Division of Endocrinology, Diabetes, and Hypertension, Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA; anogawa05@gmail.com (A.O.-W.); ccarmody46@gmail.com (C.C.); katherine_le@meei.harvard.edu (K.L.); plarsen@partners.org (P.R.L.); annmarie.zavacki@gmail.com (A.M.Z.)
2 Endocrine Division, Department of Internal Medicine, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre 9000335, Brazil; rafamarschner@gmail.com
* Correspondence: simone.wajner@ufrgs.br

Abstract: The muscle stem-cell niche comprises numerous cell types, which coordinate the regeneration process after injury. Thyroid hormones are one of the main factors that regulate genes linked to skeletal muscle. In this way, deiodinase types 2 and 3 are responsible for the fine-tuning regulation of the local T3 amount. Although their expression and activity have already been identified during muscle regeneration, it is of utmost importance to identify the cell type and temporal pattern of expression after injury to thoroughly comprehend their therapeutic potential. Here, we confirmed the expression of Dio2 and Dio3 in the whole tibialis anterior muscle. We identified, on a single-cell basis, that Dio2 is present in paired box 7 (PAX7)-positive cells starting from day 5 after injury. Dio2 is present in platelet derived growth factor subunit A (PDGFA)-expressing fibro-adipogenic progenitor cells between days 7 and 14 after injury. Dio3 is detected in myogenic differentiation (MYOD)-positive stem cells and in macrophages immediately post injury and thereafter. Interestingly, Dio2 and Dio3 RNA do not appear to be present in the same type of cell throughout the process. These results provide further insight into previously unseen aspects of the crosstalk and synchronized regulation of T3 in injured muscle mediated by deiodinases. The set of findings described here further define the role of deiodinases in muscle repair, shedding light on potential new forms of treatment for sarcopenia and other muscular diseases.

Keywords: skeletal muscle; thyroid hormone; deiodinases; muscle injury; FAPs

1. Introduction

Tissue regeneration allows damaged tissues to repair and remodel upon injury. Skeletal muscle is composed of multinucleated mature muscle cells (myofibers), a resident pool of muscle stem cells (MuSCs, also called muscle satellite cells), and other populations such as fibro-adipogenic progenitors (FAPs), endothelial cells, tenocytes, and resident immune cells [1–3]. Mammalian skeletal muscle exhibits the capacity for extensive regeneration in response to injury [4]. It is critical to understand the cell types and processes that mediate skeletal muscle healing to improve regenerative efficiency while limiting scar formation. Muscle repair, which mainly depends on satellite cells, counteracts skeletal muscle loss and supplies new myofibers [5,6].

Muscle is a major target of TH action [7,8]. T3 potentiates satellite cell differentiation and muscle regeneration upon injury [9,10]. THs also regulates oxygen consumption, fiber composition, calcium mobilization, and glucose uptake [11,12]. While the role of thyroid hormones is well established as part of muscle recovery after injury [7], the precise cells in which types 2 and 3 deiodinases are located, as well as the time course of their expression during regeneration, are still not well defined. Type 2 deiodinase (Dio2) converts thyroxine to 3,3,5-triiodothyronine (T3), while type 3 deiodinase (Dio3) inactivates T3, thus

Disruption of mitochondrial functions involving mitochondrial permeability transition pore opening caused by maleic acid in rat kidney

Ana Cristina Roginski[1] · Ângela Beatris Zemniaçak[1] · Rafael Aguiar Marschner[2] · Simone Magagnin Wajner[2] · Rafael Teixeira Ribeiro[1] · Moacir Wajner[1,3,4] · Alexandre Umpierrez Amaral[1,5]

Abstract

Propionic acid (PA) predominantly accumulates in tissues and biological fluids of patients affected by propionic acidemia that may manifest chronic renal failure along development. High urinary excretion of maleic acid (MA) has also been described. Considering that the underlying mechanisms of renal dysfunction in this disorder are poorly known, the present work investigated the effects of PA and MA (1–5 mM) on mitochondrial functions and cellular viability in rat kidney and cultured human embryonic kidney (HEK-293) cells. Mitochondrial membrane potential ($\Delta\psi m$), NAD(P)H content, swelling and ATP production were measured in rat kidney mitochondrial preparations supported by glutamate or glutamate plus malate, in the presence or absence of Ca^{2+}. MTT reduction and propidium iodide (PI) incorporation were also determined in intact renal cells pre-incubated with MA or PA for 24 h. MA decreased $\Delta\psi m$ and NAD(P)H content and induced swelling in Ca^{2+}-loaded mitochondria either respiring with glutamate or glutamate plus malate. Noteworthy, these alterations were fully prevented by cyclosporin A plus ADP, suggesting the involvement of mitochondrial permeability transition (mPT). MA also markedly inhibited ATP synthesis in kidney mitochondria using the same substrates, implying a strong bioenergetics impairment. In contrast, PA only caused milder changes in these parameters. Finally, MA decreased MTT reduction and increased PI incorporation in intact HEK-293 cells, indicating a possible association between mitochondrial dysfunction and cell death in an intact cell system. It is therefore presumed that the MA-induced disruption of mitochondrial functions involving mPT pore opening may be involved in the chronic renal failure occurring in propionic acidemia.

Keywords Maleic acid · Propionic acid · Propionic acidemia · Chronic renal failure · Mitochondrial permeability transition

✉ Alexandre Umpierrez Amaral
alexandreamaral@uricer.edu.br

1 Programa de Pós-Graduação Em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, RS, Brazil

2 Departamento de Medicina Interna, Faculdade de Medicina, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, RS, Brazil

3 Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, RS, Brazil

4 Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil

5 Departamento de Ciências Biológicas, Universidade Regional Integrada Do Alto Uruguai E das Missões, Avenida Sete de Setembro, 1621, Erechim, RS CEP 99709-910, Brazil

Introduction

Maleic acid (MA) is a dicarboxylic organic acid, the *cis isomer* of the citric acid cycle intermediate fumaric acid, that has been largely demonstrated to be nephrotoxic. Several studies have demonstrated that in vitro and in vivo treatments of proximal tubules with MA provoke acute renal dysfunction (Roth et al. 1978; Al-Bander et al. 1982), resembling Fanconi syndrome, which is associated with global dysfunction of the proximal tubule, leading to high urinary excretion of amino acids, glucose, phosphate, bicarbonate, uric acid, among others (Roth et al. 1981; Foreman 2019).

Of note, besides the predominant excretion of propionic (PA), 3-hydroxypropionic and 2-methylcitric acids, the presence of MA was observed in the urine of patients affected by propionic acidemia (PAcidemia, OMIM #606,054) (Bergstrøm et al. 1981). This disease is caused

Article

Ventilatory Muscle Training for Early Cardiac Rehabilitation Improved Functional Capacity and Modulated Vascular Function of Individuals Undergoing Coronary Artery Bypass Grafting: Pilot Randomized Clinical Trial

Bruna Eibel [1,*], **Juliana R. Marques** [1], **Thiago Dipp** [2], **Gustavo Waclawovsky** [1], **Rafael A. Marschner** [3], **Liliana C. Boll** [1], **Renato A. K. Kalil** [1,4], **Alexandre M. Lehnen** [1], **Allan R. K. Sales** [5,6] **and Maria Claudia Costa Irigoyen** [1,6]

[1] Instituto de Cardiologia/Fundação, Universitária de Cardiologia (IC/FUC), Porto Alegre Postcode, RS, Brazil; julianarm08@gmail.com (J.R.M.); gwsaude@yahoo.com.br (G.W.); lilianacboll@gmail.com (L.C.B.); kalil.renato@gmail.com (R.A.K.K.); amlehnen@gmail.com (A.M.L.); hipirigoyen@gmail.com (M.C.C.I.)

[2] Programa de Pós-Graduação em Saúde Coletiva, Universidade do Vale do Rio dos Sinos (UNISINOS), São Leopoldo Postcode, RS, Brazil; thdipp@hotmail.com

[3] Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre Postcode, RS, Brazil; rafamarschner@gmail.com

[4] Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre Postcode, RS, Brazil

[5] Institute DOR for Research and Education (IDOR), São Paulo Postcode, SP, Brazil; allan.sales@idor.org

[6] Instituto do Coração (InCor), Universidade de São Paulo (USP), São Paulo Postcode, SP, Brazil

[*] Correspondence: brunaeibel@gmail.com; Tel.: +55-51-32303600

Abstract: Background: Cardiac rehabilitation with aerobic exercises is the first strategy for non-pharmacological treatment in the postoperative period of individuals undergoing coronary artery bypass grafting (CABG) to improve functional capacity and vascular health. However, other exercise modalities remain uncertain regarding the same benefits. Objectives: Evaluation of the effect of different modalities of exercise, such as early cardiac rehabilitation on subjects submitted to CABG in the six-minute walk test (6-MWT) and on the percentage of flow-mediated dilatation (FMD) of the brachial artery. Methods: A randomized clinical trial in which 15 patients (62.7 ± 6.7 years) who underwent CABG were randomly assigned to the following groups: isometric (IG, Handgrip Jamar®), ventilatory muscle training (VG, PowerBreathe®) and control (CG, conventional respiratory and motor physiotherapy). All patients were attended to physically twice a day (20 min/session) for a consecutive week after the CABG (hospital admission). Functional capacity was assessed by 6-MWT and endothelial function was assessed through the technique of FMD, before and after (~7 days) admission to CABG. The doppler ultrasound videos were analyzed by Cardiovascular Suite® software (Company's name, City, Country) to measure %FMD. Statistics: Generalized estimation equation, followed by Bonferroni post hoc ($p < 0.05$). Results: Systolic, diastolic and mean arterial pressure (SBP/DBP/MAP, respectively) were 133, 76 and 95 mmHg. The groups presented walking meters (m) distance before and after intervention of: IG_{basal} 357.80 ± 47.15 m vs. IG_{post} 306.20 ± 61.63 m, $p = 0.401$ (+51 m); VG_{basal} 261.50 ± 19.91 m vs. VG_{post} 300.75 ± 26.29 m, $p = 0.052$ (+39 m); CG_{basal} 487.83 ± 83.23 m vs. CG_{post} 318.00 ± 31.08 m, $p = 0.006$ (−169 m). %FMD before and after intervention was IG_{basal} 10.4 ± 4.8% vs. IG_{post} 2.8 ± 2.5%, $p = 0.152$; VG_{basal} 9.8 ± 5.1% vs. VG_{post} 11.0 ± 6.1%, $p = 0.825$; CG_{basal} 9.2 ± 15.8% vs. CG_{post} 2.7 ± 2.6%, $p = 0.710$ and resting mean basal blood flow was IG_{basal} 162.0 ± 55.0 mL/min vs. IG_{post} 129.9 ± 63.7 mL/min, $p = 0.662$; VG_{basal} 83.74 ± 12.4 mL/min vs. VG_{post} 58.7 ± 17.1 mL/min, $p = 0.041$; CG_{basal} 375.6 ± 183.7 mL/min vs. CG_{post} 192.8 ± 115.0 mL/min, $p = 0.459$. Conclusions: Ventilatory muscle training for early cardiac rehabilitation improved acute functional capacity and modulated mean flow of individuals undergoing CABG.