Pharmacological Research 132 (2018) 168–175

Contents lists available at ScienceDirect

Pharmacological Research

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

Review

# Crosstalk mechanisms in hepatoprotection: Thyroid hormone-docosahexaenoic acid (DHA) and DHA-extra virgin olive oil combined protocols

Rodrigo Valenzuela[a], Luis A. Videla[b,*]

[a] Nutrition Department, Faculty of Medicine, University of Chile, Santiago, Chile
[b] Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile

ARTICLE INFO

*Article history:*
Received 17 October 2017
Received in revised form
27 November 2017
Accepted 12 December 2017
Available online 16 December 2017

*Keywords:*
Hepatoprotection
Thyroid hormone
Preconditioning
Docosahexaenoic acid
Extra virgin olive oil
Steatosis attenuation
AMP-activated protein kinase

ABSTRACT

Normal liver function includes a number of metabolic processes, secretion of cellular mediators and its role in immunobiology; these require a high energy supply, which is further enhanced under adverse conditions triggering hepatic disorders or injury due to the operation of counteracting mechanisms. Alterations in oxygen availability, such as ischemia-reperfusion (IR) leading to liver inflammation and high-fat diet (HFD)-induced hepatic steatosis, are noxious responses encountered in hepatic surgery and obesity, respectively. Several strategies have been developed to attenuate or prevent these disorders, including thyroid hormone ($T_3$), docosahexaenoic acid (DHA) and extra virgin olive oil (EVOO). These hormetic agents that exert beneficial effects in the low dose range were shown to abrogate IR-induced liver injury effectively in the case of $T_3$, DHA, or their combined administration, whereas DHA plus EVOO attenuate HFD-induced hepatic steatosis, although they can induce adverse effects in other experimental settings. The use of combined hepatoprotective protocols (DHA + $T_3$ or DHA + EVOO) using low doses or reduced supplementation periods is characterized by the stimulation of different types of molecular defensive mechanisms and similar signaling processes that exhibit synergism, thus constituting suitable experimental liver pharmacological preconditioning strategies with possible future clinical applications.


**Contents**



* Corresponding author.
*E-mail address:* lvidela@med.uchile.cl (L.A. Videla).

https://doi.org/10.1016/j.phrs.2017.12.013
1043-6618/

## 1. Introduction

Liver function includes a variety of metabolic processes such as the majority of the pathways of intermediary metabolism, biotransformation of xenobiotics and endobiotics, biosynthesis of plasma proteins, bile production, and the secretion of hepatokines, proteins that influence metabolic processes via autocrine, paracrine, and endocrine signaling [1,2]. In addition, functions of the liver in immunobiology that are carried out by several immunologically active cells including Kupffer cells involve innate or nonspecific and adaptive or specific mechanisms [3]. Therefore, normal liver functioning requires an adequate supply of oxygen and nutrients to energize essential processes, which is primarily met by fatty acid (FA) oxidation with secondary usage of carbohydrate. Alterations in oxygen availability (ischemia-reperfusion (IR) episodes) [4], nutrient supply (high fat and/or fructose diets [5,6]) or excessive alcohol consumption [7] induce adverse conditions in the liver that may trigger hepatic disorders.

### 1.1. Liver ischemia-reperfusion injury (IRI)

The use of vascular occlusion to prevent haemorrhage during liver resection leads to IR injury (IRI) [4], a feature that is also inherent to liver transplantation causing primary non-function or delayed function of grafts [8]. Hepatic IRI occurs in two phases, the first (up to 4 h after reperfusion) is characterized by Kupffer cell activation, with release of reactive oxygen species (ROS) and pro-inflammatory cytokines (tumour necrosis factor α (TNF-α), interleukin (IL)-1), which upregulate intercellular adhesion molecule (ICAM) expression in endothelial cells that adhere to neutrophils. In the second phase of IRI (from 6 h after reperfusion) neutrophils undergo extravasation, adhere to hepatocytes after ICAM induction and achieve activation with further ROS release, protease production and higher phagocytic capacity, causing parenchymal cell injury [4,9–11]. Under these conditions, major molecular mechanisms of liver IRI include (i) lethal oxidative stress due to higher ROS generation derived from NADPH oxidase, xanthine oxidase, and mitochondrial activity in Kupffer cells, neutrophils and/or hepatocytes, which trigger lipid peroxidation, protein carbonylation and DNA oxidative alterations that cause a necrotic response [11]; (ii) ROS-dependent endoplasmic reticulum (ER) stress inducing an apoptotic reaction that is suppressed by the antioxidant N-acetylcysteine [12]; whereas (iii) liver inflammation is associated with inflammasome NLRP3 upregulation, which is primed by TNF-α/nuclear factor-κB (NF-κB)-mediated expression of inflammasome components, followed by pro-IL-1β processing into IL-1β by caspase-1 activation [13,14], mechanisms that often produce hepatic failure [9–11]. Since liver IRI is a major complication in clinical practice, which can be exacerbated in the case of old liver donors or the presence of hepatic steatosis in relation to organ transplantation, several strategies attenuating liver IRI have been developed [8–10,15,16]. These preconditioning (PC) techniques increase the tolerance of the liver to IRI due to previous manoeuvres triggering beneficial molecular and functional changes, which include gene therapy, surgical strategies, and pharmacological approaches [11,15,16]. Pharmacological PC is based on direct interference with the injurious mechanisms underlying IRI or indirect induction of a low stress level able to trigger defence mechanisms. With the exception of methylprednisolone [4,17], pharmacological PC protocols have not been applied in the clinical setting due to toxicity, side effects, and/or difficulties in implementation [4,8,11]. Due to these observations, recent work by our group addressed the study of experimental liver pharmacological PC strategies with possible clinical applications. These include thyroid hormone (3,3′,5-L-triiodothyronine; $T_3$) [18], n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFAs) [19] and iron [20], essential biomolecules representing hormetic agents with dose-response relationships showing beneficial effects in the low dose range and harmful responses at high doses [21].

### 1.2. High-fat diet (HFD)-induced liver steatosis

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent metabolic hepatic disturbance in the world [22]. The development of NAFLD is directly associated with over-nutrition with excess of energy and concomitant presence of obesity, which underlies an important interaction between insulin resistance, oxidative stress and depletion in the levels of n-3 LCPUFAs [23,24]. NAFLD is characterized by an abnormal fat accumulation in the hepatic tissue (over 5% of total liver weight) [25]; the main lipid molecules present in liver steatosis are triacylglycerols (TAGs) and ceramides [26]. Saturated FAs, specially palmitic acid (C16:0), are in higher levels in liver steatosis (TAGs and ceramides), a condition that favours the development of oxidative stress and lipotoxicity [27,28]. Patients with NAFLD usually have diets with higher content of energy, FAs (saturated and n-6 LCPUFAs), and carbohydrates (glucose and fructose), accompanied by a very low intake of n-3 LCPUFAs and natural antioxidants (tocopherols and polyphenols) [29]. In accordance with these adaptive-pathological conditions observed in liver steatosis, today the use of high-fat diet (HFD) models is a good experimental proposal to understand the metabolic alterations induced and the possible novel dietary and/or nutritional interventions for prevention or treatment of NAFLD [30,31]. The HFD model is extensively used in experimental protocols related to NAFLD, considering that this diet has 60% of its energy as fat (principally saturated fat) and that is not deficient in macro (proteins and amino acids) and micronutrients (vitamins) [32,33]. Mice fed a HFD for 12 weeks develop metabolic disturbances similar to those observed in patients with NAFLD, specifically, overweight, insulin resistance, oxidative stress (systemic and hepatic), liver steatosis, pro-inflammatory status and decrease in the synthesis and levels of n-3 LCPUFA (liver and other tissues) [30,31]. In addition, NAFLD in animal models and human patients showed similar molecular alterations including upregulation of sterol regulatory element-binding protein 1c (SREBP-1c) and NF-κB, with concomitant downregulation of peroxisome proliferator-activated receptor-α (PPAR-α) and nuclear erythroid 2-related factor 2 (Nrf2), a condition that favours a pro-lipogenic, pro-inflammatory and pro-oxidant hepatic status [24,31,34,35].

N-3 LCPUFAs, which are depleted in the liver of NAFLD patients [24] and HFD-fed mice [36], are a family of nine FAs, the most relevant being eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA), since they have the capacity to modulate lipid metabolism in the liver, principally by activation of PPAR-α with concomitant downregulation of the activity of SREBP-1c [31,37]. EPA and DHA can be metabolised to eicosanoids and docosanoids (resolvins and protectins), molecules that regulate inflammatory responses inducing down-regulation of NF-κB and reduction in the expression of inflammatory genes (TNF-α, IL-1β and IL-6) [38,39]. In addition, these LCPUFAs can be metabolized into isoprostanes of the J series (specifically J3 isoprostanes), which can increase the activity of transcription factor Nrf2 improving the antioxidant response of the liver under steatotic conditions [40].

Oleic acid (C8:1n-9, OA) is the main FA present in extra virgin olive oil (EVOO), a monounsaturated FA that enhances the cellular antioxidant and FA oxidation capacities [41,42]. In addition, EVOO is rich in natural antioxidants, particularly tocopherols and polyphenols, bioactive compounds that induced the expression of antioxidant and anti-inflammatory genes [43,44]. Hydroxytyrosol (HT), a main antioxidant present in EVOO, induces downregulation of NF-κB activity and expression of inflammatory genes with upregulation of PPAR-α and Nrf2, improving the antioxidant

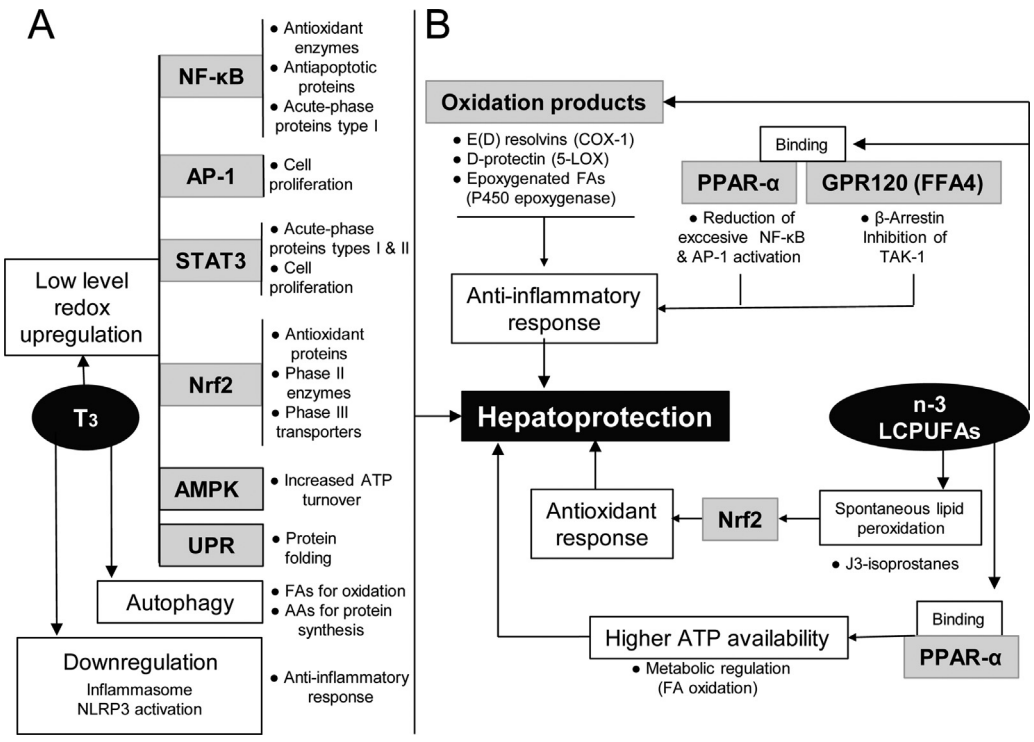


**Fig. 1.** Molecular mechanisms involved in hepatoprotection induced by thyroid hormone ($T_3$) and n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFAs). (A) $T_3$-dependent ROS production representing a non-lethal, moderate oxidative stress, activates (i) redox-sensitive transcription factors nuclear factor-κB (NF-κB: Mn-superoxide dismutase, inducible nitric oxide synthase, Bcl2 and haptoglobin induction), activating protein 1 (AP-1: cell proliferation), signal transducer and activator of transcription 3 (STAT3: upregulation of haptoglobin, β-fibrinogen and cell proliferation), nuclear factor erythroid-2-related factor-2 (Nrf2: higher expression of heme-oxygenase-1 (HO-1), glutamate-cysteine ligase (GCL), thioredoxin, NAD(P)H-quinone oxidoreductase, glutathione-S-transferases (GST) and multidrug resistance proteins 2 and 3); and (ii) AMP-activated protein kinase (AMPK), an ATP sensor regulating energy dynamics by reducing anabolism and increasing catabolism that enhances ATP availability. In addition to redox activation, AMPK upregulation by $T_3$ includes higher mRNA expression, threonine-172 phosphorylation by $Ca^{2+}$-calmodulin-dependent protein kinase kinase-β (CaMKKβ) and transforming growth factor-β-activated kinase-1 (TAK1) and the allosteric activation by increased cellular AMP/ATP ratios. In addition, $T_3$ also induces (iii) autophagy, a stress-related process degrading cellular components to fatty acids (FAs) for ATP generation, and amino acids (AAs) to synthesise proteins for cell survival; and (iv) the endoplasmic reticulum (ER) unfolded protein response (UPR), which is triggered by $T_3$-induced protein oxidation encompassing protein unfolding, with upregulation of the protein kinase RNA-like ER kinase (PERK) and induction of the protein disulphide isomerase-ER oxidoreductin-1α couple (PDI-ERO1α) that achieves proper folding. In addition to the hepatoprotective mechanisms related to redox regulation outlined above, $T_3$ confers protection against IRI by suppression of the inflammatory response associated with inflammasome NLRP3 and interleukin (IL)-1β upregulation, with AMPK playing a causal role controlling energy dynamics. (B) Liver protective mechanisms by n-3 LCPUFAs include antioxidant, anti-inflammatory and energy-providing responses. (i) Due to the high degree of unsaturations, n-3 LCPUFAs easily undergo peroxidation with formation of J3-isoprostanes that promote Nrf2 activation, with induction of HO-1, GCL, GST, catalase, glutathione peroxidase, glutathione reductase and glutathione repletion, thus inducing an antioxidant response; (ii) the anti-inflammatory actions of n-3 LCPUFAs include interactions with either NF-κB and AP-1 with n-3 LCPUFA-activated PPAR-α to form inactive complexes or with G protein-coupled receptor 120 (GPR120 or free-fatty acid receptor 4, FFA4) that inhibits TAK1, thus compromising excessive NF-κB activation, as well as oxidation products of n-3 LCPUFAs produced by cyclo-oxygenase-1 (COX-1), 5-lipoxygenase (5-LOX) and cytochrome 450 NADPH-dependent epoxygenase, attenuate pro-inflammatory mechanisms; and (iii) enhancement in energy availability is achieved through PPAR-α-dependent n-3 LCPUFA activation, mainly increasing FA oxidation pathways.

hepatic tissue defence [36]. Liver antioxidant responses prevent the decrease in the synthesis and levels of n-3 LCPUFA, jointly with attenuation in the reduction of the activity of PPAR-α and increment in the actions of SREBP-1c [45], metabolic effects that induce anti-inflammatory, antioxidant and anti-lipogenic actions that overcome those triggered by HFD. Considering these aspects, the use of n-3 LCPUFA, EVOO, or n-3 LCPUFA plus EVOO protocols represent an interesting and possible therapeutic strategy to prevent liver steatosis and other metabolic disturbances induced by HFD.

## 2. Crosstalk mechanisms in hepatoprotection by a docosahexaenoic acid (DHA) and thyroid hormone ($T_3$) combined protocol in ischemia-reperfusion liver injury

$T_3$-induced liver PC against 1 h ischemia–20 h reperfusion is associated with the calorigenic response elicited, which involves low-level ROS generation producing a moderate oxidative stress status within 48 h after $T_3$ treatment [1,18,21] that is able to trigger redox regulation [46]. The latter phenomenon promotes cell protection and survival upregulating antioxidant, anti-apoptotic, anti-inflammatory and cell proliferation responses, with parallel higher energy supply, detoxification and protein folding potentials (Fig. 1A) [14,47–49]. Additional $T_3$-dependent PC mechanisms include (i) acceleration of the differentiation of hepatic progenitor cells into hepatocytes [50]; and (ii) induction of hepatic autophagy that increases amino acid availability for protein synthesis and that of FAs for β-oxidation enhancement that support ATP production and cell survival [51]. Remarkably, beneficial effects of $T_3$ or thyroxin ($T_4$) are also observed against IRI in extrahepatic tissues (heart, kidney, and brain) or against other deleterious stimuli, as well upon restoration to normal thyroid hormone levels in clinical conditions exhibiting previous reduced $T_3/T_4$ serum levels (for specific references, see [49]). As shown in Fig. 1, $T_3$ and n-3 LCPUFAs exert hepatoprotection through different molecular mechanisms, but they coincide in antioxidant responses triggered by NF-κB and/or Nrf2 activation, anti-inflammatory effects and higher ATP availability associated with FA oxidation enhancement, to comply with the elevated energy demands of the processes involved in liver protection. The latter feature underlies PPAR-α and AMPK opera-

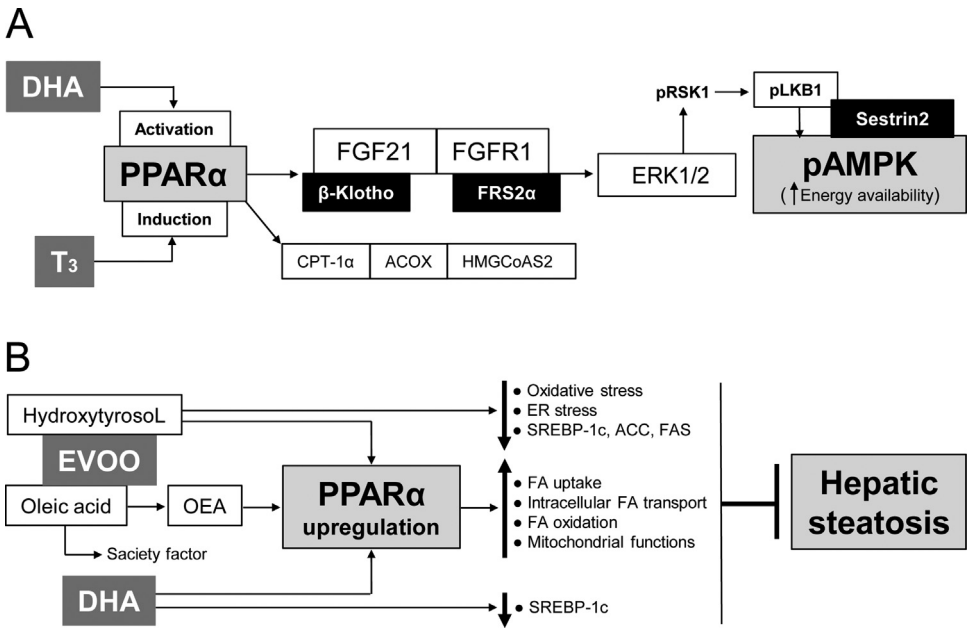


**Fig. 2.** Hepatic signaling mechanisms related to enhancement in energy expenditure via the PPAR-α-FGF21-AMPK cascade elicited by a combined docosahexaenoic acid-thyroid hormone (DHA-$T_3$) protocol involved in liver preconditioning (A) and in the anti-steatotic effect of DHA-extra virgin olive oil (DHA-EVOO) supplementation in high-fat diet fed animals (B). Abbreviations: ACC, acetyl-CoA carboxylase; ACOX, acyl-CoA oxidase; CPT-1α, carnitine palmitoyltransferase-1α; ER, endoplasmic reticulum; ERK1/2, extracellular-signal-regulated kinase 1/2; FA, fatty acid; FAS, fatty acid synthase; FGF21, fibroblast growth factor 21; FGFR1, FGF receptor 1; FRS2α, FGFR substrate 2α; HMGCoAS2, hydroxymethylglutaryl-CoA syntase 2; OEA, oleoylethanolamide; pAMPK, phosphorylated AMP-activated protein kinase; PPARα, peroxisome proliferator-activated receptor-α; pLKB1, phosphorylated liver kinase B1; pRSK1, phosphorylated ribosomal S6 kinase-1; SREBP-1c, sterol regulatory element-binding protein 1c.

tion (Fig. 1), signaling mediators that are interconnected through the functioning of hepatokine fibroblast growth factor 21 (FGF21) (Fig. 2A) [52].

Liver PC against IRI was achieved by either a single dose of 0.1 mg $T_3$/kg given 48 h before the IR protocol [18] or by daily amounts of encapsulated fish oil for 7 days, representing 270 mg EPA/kg plus 180 mg DHA/kg, 24 h before IR [19], shown by suppression of IR-induced oxidative stress, recovery of serum transaminase levels with minimal hepatic lesions and rescue of NF-κB functionality loss during IR. In order to reduce n-3 LCPUFA and $T_3$ dosages to achieve PC conditions suitable for clinical application, rats were given daily doses of 300 mg n-3 LCPUFA/kg for 3 consecutive days plus 0.05 mg $T_3$/kg on the fourth day, and the IR protocol was carried out 24 h later [53]. Under these conditions, lack of protection against liver IRI was observed either after separate n-3 LCPUFA supplementation or 0.05 mg $T_3$/kg administration; however combining the two agents elicited significant hepatoprotection. The synergistic action of the combined n-3 LCPUFA-$T_3$ protocol is related to the development of a suitable oxidative stress status and NF-κB recovery to enhance cytoprotective processes and reduce inflammatory responses, thus attaining PC [53]. Synergism in PC mechanisms may include (i) $T_3$-induced activation of the acute-phase response, recovery of the basal antioxidant and anti-apoptotic status of the liver and downregulation of inflammasome NLRP3 induction (Fig. 1A) [53–56]; and (ii) n-3 LCPUFA-mediated enhancement in antioxidant and anti-inflammatory potentials (Fig. 1B) [53], the latter action being possibly associated with resolvin production [57]. In addition to the synergistic effect of n-3 LCPUFA and $T_3$ in liver PC outlined above, a long-term diet rich in n-3 LCPUFA showed body weight, serum triglycerides and cholesterol reductions, with comparable serum $T_3$, $T_4$, and thyrotropin levels and normal hepatic 5′-deiodinase activity [58]. Under these conditions, greater protein expression of thyroid hormone receptor β1 (THRβ1) and THRβ1-dependent mitochondrial glycerophosphate dehydrogenase activity were observed; this enzyme is a target for $T_3$ genomic action and related to thermogenesis [58]. These data suggest that n-3 LCPUFA increase $T_3$ signaling in the liver leading to reduction in serum lipid levels [58], which may play an alternate role in the crosstalk mechanisms involved in the combined n-3 LCPUFA-$T_3$ PC protocol.

Recent studies by our group revealed that AMPK upregulation exhibits a causal role in liver PC against IRI by $T_3$, shown by suppression of $T_3$-induced liver mRNA and protein levels of AMPK, with concomitant loss of PC [14,59] upon pre-treatment with the specific AMPK inhibitor compound C [60]. AMPK activation is also accomplished by DHA, shown by the increase in glucose uptake by skeletal muscle through a GPR120-mediated AMPK pathway [61] and the inhibition of proteolytic processing of SREBP-1c in McA rat hepatoma cells by AMPK-dependent phosphorylation of SREBP-1c [62]. In agreement with these views, the combined DHA-$T_3$ protocol at low dosages elicited AMPK activation by Thr-172 phosphorylation compared to the respective controls, which is characterized by the upstream upregulation of PPAR-α-FGF21 signalling (Fig. 2A) [63]. The contention that liver AMPK activation plays a critical role in IR injury prevention (Fig. 2A) is strengthened by studies showing alleviation of alcoholic liver injury by betulin, a triterpene that decreases TAG accumulation by downregulation of lipogenic factor SREBP-1c via LKB1-AMPK activation *in vitro* and *in vivo* [64]. Mechanistically, DHA-$T_3$ supplementation resulted in higher hepatic PPAR-α mRNA expression and DNA binding capacity, with induction of PPAR-α target genes including *FGF21* by $T_3$ (Fig. 2A) [63,65], whereas DHA contribution relates to ligand activation of PPAR-α (Fig. 1B) [66]. The former mechanism is associated with the genomic action of $T_3$, which is supported by upregulation of the expression of thyroid hormone receptor and retinoic X receptor needed for full activation of gene transcription [63]. Liver FGF21 induction by DHA-$T_3$ could also be due to the ER stress-dependent unfolded protein response (UPR) [67,68], which is triggered by acute $T_3$ administration via activation of the PKR-like ER kinase (PERK) branch of the UPR (Fig. 1A) [48].

Upregulation of liver PPAR-α-FGF21-AMPK signaling by the combined DHA-$T_3$ protocol is functionally operative due to the association with the scaffold proteins β-Klotho, FGFR substrate 2α (FRS2α) and sestrin2, which are induced by $T_3$ [69,70]. These scaffolds allow concentration and positioning in close vicinity to their regulatory proteins that improves signalling specifically [71]. Actually, FGF21 function involves the interaction with (i) FGF receptor-1 (FGFR1) acting as tyrosine kinase [72], which exhibits an absolute requirement for the scaffold co-factor β-Klotho, the essential component for propagation of FGF21 signals [73]; (ii) the docking/scaffold adaptor protein FRS2α acting as an amplifier for the FGF21-FGFR1-β-Klotho complex activity [74]; whereas (iii) sestrin2 favours AMPK activation by phosphorylation (Fig. 2A) [75]. Signalling transduction from the FGF21-FGFR1-β-Klotho complex exhibiting tyrosine phosphorylation activity to AMPK Thr172 phosphorylation for activation was proposed to involve an additional kinase cascade [70]. This proposal was based on the findings that DHA-$T_3$-induced FGF21 activity triggered higher phosphorylation of its target extracellular-signal-regulated kinase 1 (ERK1 at Tyr204)-ERK2 (at Tyr187) [70], ERK1/2 being able to activate downstream ribosomal S6 kinase-1 (pRSK1 at Ser363) [70,76], with pRSK1-depe1ndent phosphorylation of liver kinase B1 (pLKB1 at Ser431) [70,76] for further pLKB1-dependent Thr172 phosphorylation of AMPK [70,77], as shown in Fig. 2A. Although a single dose of 0.1 mg $T_3$/kg involving liver AMPK activation and AMPK-dependent phosphorylation of the downstream targets cAMP-response element-binding protein (CREB) and PPAR-γ coactivator-1α (PGC-1α) that coordinates with PPAR-α the induction of FA oxidation with a ketogenic response [78], assessment of these adaptive changes by the DHA-$T_3$ protocol remains to be studied.

## 3. Crosstalk mechanisms in hepatoprotection by extra virgin olive oil (EVOO) and docosahexaenoic acid (DHA) in high-fat diet (HFD)-induced liver steatosis

The combination of low doses of DHA (50 mg/kg/day) and EVOO (50 mg/kg/day) for 12 weeks concomitantly with a HFD protocol showed a significant decrease in hepatic steatosis compared to the respective controls [79]. The latter feature of the combined DHA-EVOO protocol may be attained by potentiation of anti-lipogenic mechanisms triggered by separate DHA and EVOO supplementations (Fig. 2B). N-3 LCPUFAs selectively incorporate into hepatic phospholipids, inhibit *de novo* lipogenesis and change the FA profile in the non-steatotic liver, which may help to reduce TAG accumulation after HFD [80]. DHA contribution to limit HFD-induced hepatic steatosis is related to repletion of n-3 LCPUFA levels that exerts anti-lipogenic effects through PPAR-α activation and SREBP-1c downregulation [31,37], whereas that of antioxidant-rich EVOO [81] seems to involve actions put forward by several of its components including HT and OA [82] (Figs. 1 and 2B). In fact, both EVOO components are able to activate PPAR-α as shown by (i) HT-induced liver PPAR-α mRNA levels and those of its target carnitine-palmitoyl transferase 1α (CPT-1α), with parallel enhancement in acetyl-CoA carboxylase (ACC) phosphorylation, suggesting AMPK activation via FGF21 upregulation (Fig. 2A), and in FA oxidation with reduced liver steatosis [83]; and (ii) OA-dependent synthesis of oleoylethanolamide (OEA) acting as a satiety factor reducing food intake and as a PPAR-α activator [84], with upregulation of the mRNA expression of PPAR-α and of its targets fatty acid translocase (FAT/CD36), liver fatty acid binding protein (L-FABP), intracellular FA transport and oxidation [85] (Fig. 2B).

In addition to PPAR-α activation (higher PPAR-α DNA binding with induction of acyl-CoA oxidase (ACOX) and CPT-1α involved in FA oxidation), HT exerts alternate anti-lipogenic actions in HFD-fed mice, including (i) reduction in the oxidative stress status of the liver (upregulation of the antioxidant enzymes glutathione-S-transferase (GST), γ-glutamyltranspeptidase (GGT), glutamyl-cisteine ligase (GCL), superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase via higher Nrf2 DNA binding) thus avoiding excessive n-3 LCPUFA oxidative loss [36,45]. The latter effect of HT is also observed in rats subjected to a HFD for 4 weeks, as shown by the decrease in plasma oxidative stress-related indicators, with concomitant enhancement in the antioxidant capacity of serum [86]; (ii) decrease in the pro-lipogenic potential induced by HFD (downregulation of SREBP-1c, ACC and fatty acid synthase (FAS)) [36,45] and (iii) normalization of the hepatic activity of Δ5 and Δ6 desaturases, thus supporting n-3 LCPUFA biosynthesis and recovery [45] (Fig. 2B).

Proteins undergoing unfolding cause ER stress that activates the UPR to regain proteome stability, which upon excessive and/or prolonged conditions reaches deleterious responses [87]. In the case of NAFLD, prolonged UPR is achieved in association with development of oxidative stress-dependent protein oxidation [23,88] and higher levels of saturated FAs such as palmitate, leading to protein palmitoylation and saturation of membrane phospholipids with a steatotic response [87]. Under these conditions, UPR induction activates two pro-lipogenic branches of ER stress, namely, (i) the PERK/eukaryotic translation initiation factor-1α (eIF2α) pathway upregulating SREBP-1c and activating transcription factor 4 (ATF4) which in turn induces the expression of the lipogenic enzymes ACC, FAS, stearyl-CoA desaturase 1 (SCD1) and diacylglycerol acetyltransferase 2 (DGAT2) [89]; and (ii) the inositol-requiring enzyme-1α (IRE1α)/X-box binding protein-1 (XBP1) branch upregulating PPAR-γ, CCAAT/enhancer binding protein β/δ (C/EBPβ/δ) and enzymes involved in TAG synthesis [90]. Interestingly, obesity with hepatic steatosis in association with fructose overload also induces ER stress with an UPR involving PERK/eIF2α activation and nuclear accumulation of lipogenic factors SREBP-1c and carbohydrate response element binding protein (ChREBP) leading to FAS, ACC, and SCD1 upregulation, which are suppressed by the chemical ER chaperone phenylbutyric acid [91]. *In vitro* studies with HT revealed a significant decrease in makers of ER stress and related apoptosis, thus improving ER homeostasis with decline in the associated steatotic response [92].

According to these considerations, combined DHA-EVOO supplementation attenuates HFD-induced liver steatosis in association with the central activation of PPAR-α by its components DHA, OA, and HT, with reinforcement of the downstream effects of PPAR-α enhancing FA oxidation and decreasing FA incorporation into TAGs, in view of the deactivating action of both DHA and HT on the lipogenic action of SREBP-1c (Fig. 2B). Liver PPAR-α upregulation by combined DHA-EVOO may involve activation of the energy sensor AMPK, as proposed for the combined DHA-$T_3$ protocol enhancing energy expenditure (Fig. 2A) [70], in agreement with studies of fish oil supplementation in a fat-free diet for 7 days revealing increases in AMPK and ACC phosphorylation compared to fat-free diet alone in the absence of changes in protein phosphatase 2A activity that can act on pAMPK [93]. Although these findings are consistent with a similar effect of DHA in mouse C2C12 and rat L6 myoblasts [61] or McA rat hepatoma cells [62], and of secoiridoid polyphenols in EVOO phenolic extracts on JIMT1 breast cancer cells [94], the effect of DHA-EVOO supplementation on liver AMPK activation in HFD-fed animals remains to be addressed. Although HT [95,96] and EVOO [97] have been shown to exert adverse effects in experimental studies, these were reported in models that are not related to the HFD-induced liver steatosis discussed here, exhibit controversial results or lack the underlying mechanisms. The lack of unanimous consensus regarding consumption recommendations of monounsaturated FAs such OA present in EVOO point to the need for further studies to clar-

ify their potential benefits in primary and secondary prevention [98].

## 4. Conclusions and perspectives

Genomic, redox-independent actions of a single dose of $T_3$ induce the expression of hepatic genes involved in energy metabolism, accelerating $O_2$ consumption with consequent ROS generation as a primary mechanism of $T_3$ action, a transient hyperthyroid state that is not associated with altered liver function [99]. Secondarily, low-level redox upregulation of defensive transcription factors, the energy-sensing AMPK and the proteome stabilizer UPR is achieved, representing non-genomic mechanisms of $T_3$ functioning affording hepatoprotection, with the concomitant contribution of $T_3$-induced autophagy, inflammasome NLRP3 deactivation and stimulation of hepatocyte proliferation needed to avoid IRI of the liver (Fig. 1A). Hepatoprotection against liver IRI is also achieved by DHA, either by binding to PPAR-α and GPR120 or by production of oxidation products that trigger antioxidant, anti-inflammatory and energy-yielding responses (Fig. 1B), which are mimicked by the EVOO components HT and OA attenuating HFD-induced hepatic steatosis (Fig. 2B). It is concluded that the use of combined protocols (DHA + $T_3$ or DHA + EVOO) employing lower doses of the hormetic agents than those in the single procedures and reduced supplementation periods, trigger both different molecular defensive mechanisms and similar signaling processes exhibiting synergism, thus constituting suitable experimental liver pharmacological hepatoprotective strategies with possible future clinical applications. This is particularly important in dietary liver steatosis, a condition in which lifestyle intervention considering diet and exercise practice remains the first treatment strategy. Nevertheless, long-term weight loss is not easily attained in NAFLD patients, primarily due to the low adherence to dietary restrictions of energy, fat and carbohydrate intake.

The use of combined hepatoprotective protocols in clinical conditions underlying crosstalk mechanisms is further supported by recent studies showing additional beneficial effects of thyroid hormones and natural lipids on protective functions. First, 3,5-diiodothyronine ($T_2$), a metabolite of $T_3$ catabolism, was shown to increase basal metabolic rate (BMR) with no deleterious effects on the thyroid axis or at cardiac levels when given to experimental animals or humans [100]. $T_2$ decreases adiposity and increases FA oxidation, preventing $H_2O_2$ production, the related lipid peroxidation enhancement, and the consequent hepatic steatotic response induced by a HFD, with mechanisms involving AMPK coupled to Sirt1 upregulation and the resultant deacetylation of SREBP-1c and PGC-1α downregulating lipogenesis [100,101]. Second, antisense oligonucleotides and $T_3$ conjugates (ASO-$T_3$) in obese mice increase energy expenditure and weight loss, with concomitant higher $O_2$ consumption, $CO_2$ production and FA oxidation [102]. These effects of ASO-$T_3$ were observed in parallel with enhanced white adipose tissue (WAT) browning and decreased lipogenic gene expression in liver, thus providing a novel strategy for drug development for obesity treatment [102]. Third, higher serum baseline $T_3$ and $T_4$ levels were shown to predict more weight loss, but not weight regain, in overweight and obese patients with normal thyroid function subjected to weight loss diets [103]. Besides this, $T_3$ levels were significantly associated with changes in body weight, blood pressure and metabolic parameters such as blood glucose, TAGs, insulin, leptin and BMR at 6 and 24 months, suggesting a better benefit from a diet intervention for weight loss [103]. Finally, further strengthening of the data obtained in *in vivo* studies in animal models [31,37,79], human intervention trials with n-3 LCPUFAs exhibited beneficial effects on the severity of liver steatosis as demonstrated by laboratory parameters and imaging determinations [104], with positive clinical outcomes also being observed in paediatric NAFLD [105]. However, interventions with combined DHA-$T_3$ or DHA-EVOO protocols and $T_2$ or ASO-$T_3$ conjugates in humans require adequate randomized controlled trials with sufficient duration, size and histological endpoints to determine the long-term safety and efficacy of these proposed therapeutic approaches for attenuation or prevention of deleterious conditions in the liver. In agreement with this proposal, combined drug protocols have been considered in the case of uncontrolled hypertension as a therapeutic option over monotherapy treatments [106], in order to normalize blood pressure and enhance the adherence to therapy [107].

## Author contributions

All authors participated in the conception of the review as well as in the interpretation of data and drafting the manuscript.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

This work was supported by grant 1150104 from the National Fund for Scientific & Technological Development (FONDECYT, Chile, to LAV).

## References

[1] V. Fernández, G. Tapia, L.A. Videla, Recent advances in liver preconditioning: thyroid hormone, n-3 long-chain polyunsaturated fatty acids and iron, Word J. Hepatol. 4 (2012) 119–128.
[2] N. Stefan, H.-U. Häring, The role of hepatokines in metabolism, Nat. Rev. Endocrinol. 9 (2013) 144–152.
[3] G.A. Parker, C.A. Picut, Immune functioning in non-lymphoid organs: the liver, Toxicol. Pathol. 40 (2012) 237–247.
[4] R. Bahde, H.-U. Spiegel, Hepatic ischemia-reperfusion injury from bench to bedside, Brit. J. Surg. 97 (2010) 1461–1475.
[5] G. Svegliati-Baroni, C. Candelaresi, S. Saccomanno, G. Ferretti, T. Bachetti, M. Marzioni, S. De Minicis, L. Nobilli, R. Salzano, A. Omenetti, D. Pacetti, S. Sigmund, A. Benedetti, A. Casini, A model of insulin resistance and non-alcoholic steatohepatitis in rats. Role of peroxisome proliferator-activated receptor-α and n-3 polyunsaturated fatty acid treatment on liver injury, Am. J. Pathol. 169 (2006) 846–860.
[6] P.J. Havel, Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism, Nutr. Rev. 63 (2005) 133–157.
[7] A. Louvet, P. Mathurin, Alcoholic liver disease: mechanisms of injury and targeted treatment, Nat. Rev. Gastroenterol. Hepatol. 12 (2015) 231–242.
[8] N. Selzner, M. Boehnert, M. Selzner, Preconditioning, postconditioning, and remote conditioning in solid organ transplantation: basic mechanisms and translational applications, Transpl. Rev. 26 (2012) 115–124.
[9] H. Jaeschke, Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning, Am. J. Physiol. Gastrointest. Liver Physiol. 284 (2003) G15–G26.
[10] N.R. Banga, S. Homer-Vanniasinkam, A. Graham, A. Al-Mukhtar, S.A. White, K.R. Prasad, Ischaemic preconditioning in transplantation and major resection of the liver, Brit. J. Surg. 92 (2005) 528–538.
[11] A. Casillas-Ramírez, I.B. Mosbah, F. Ramalho, J. Roselló-Catafau, C. Peralta, Past and future approaches to ischemia-reperfusion lesion associated with liver transplantation, Life Sci. 79 (2006) 1881–1894.
[12] Y. Sun, L.-Y. Pu, L. Lu, X.-H. Wang, F. Zhang, J.-H. Rao, N-acetylcysteine attenuates reactive-oxygen-species-mediated endoplasmic reticulum stress during liver ischemia-reperfusion injury, World J. Gastroenterol. 20 (2014) 15289–15298.
[13] H.-Y. Kim, S.-J. Kim, S.-M. Lee, Activation of NLRP3 and AIM2 inflammasomes in Kupffer cells in hepatic ischemia/reperfusion, FEBS J. 282 (2015) 259–270.
[14] R. Vargas, L.A. Videla, Thyroid hormone suppresses ischemia-reperfusion-induced liver NLRP3 inflammasome activation: role of AMP-activated protein kinase, Immunol. Lett. 184 (2017) 92–97.
[15] M. Das, D.K. Das, Molecular mechanisms of preconditioning, IUBMB Life 60 (2008) 199–203.
[16] O. de Rougemont, K. Lehmann, P.A. Clavien, Preconditioning, organ preservation, and postconditioning to prevent ischemia-reperfusion injury to the liver, Liver Transpl. 15 (2009) 1172–1182.
[17] L. Aldrighetti, C. Pulitano, M. Arru, R. Finazzi, M. Catena, L. Soldini, L. Comotti, G. Ferla, Impact of preoperative steroids administration on

ischemia-reperfusion injury and systemic responses in liver surgery: a prospective randomized study, Liver Transpl. 12 (2006) 941–949.
[18] V. Fernández, I. Castillo, G. Tapia, P. Romanque, S. Uribe-Echevarría, M. Uribe, D. Cartier-Ugarte, G. Santander, M.T. Vial, L.A. Videla, Thyroid hormone preconditioning: protection against ischemia-reperfusion liver injury in the rat, Hepatology 45 (2007) 170–177.
[19] J. Zúñiga, F. Venegas, M. Villarreal, D. Núñez, M. Chandía, R. Valenzuela, G. Tapia, P. Varela, L.A. Videla, V. Fernández, Protection against *in vivo* liver ischemia-reperfusion injury by n-3 long-chain polyunsaturated fatty acids in the rat, Free Radic. Res. 44 (2010) 854–863.
[20] M. Galleano, G. Tapia, S. Puntarulo, P. Varela, L.A. Videla, V. Fernández, Liver preconditioning induced by iron in a rat model of ischemia/reperfusion, Life Sci. 89 (2011) 221–228.
[21] L.A. Videla, Hormetic responses of thyroid hormone calorigenesis in the liver: association with oxidative stress, IUBMB Life 62 (2010) 460–466.
[22] Z.M. Younossi, A.B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, M. Wymer, Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes, Hepatology 64 (2016) 73–84.
[23] L.A. Videla, R. Rodrigo, J. Araya, J. Poniachik, Insulin resistance and oxidative stress interdependency in non-alcoholic fatty liver disease, Trends Mol. Med. 12 (2006) 555–558.
[24] P. Pettinelli, T. Del Pozo, J. Araya, R. Rodrigo, A.V. Araya, G. Smok, A. Csendes, L. Gutierrez, J. Rojas, O. Korn, F. Maluenda, J.C. Diaz, G. Rencoret, I. Braghetto, J. Castillo, J. Poniachik, L.A. Videla, Enhancement in liver SREBP-1c/PPAR-α ratio and steatosis in obese patients: correlations with insulin resistance and n-3 long-chain polyunsaturated fatty acid depletion, Biochim. Biophys. Acta 1792 (2009) 1080–1086.
[25] K.G. McCormick, E. Scorletti, L. Bhatia, P.C. Calder, M.J. Griffin, G.F. Clough, C.D. Byrne, Impact of high dose n-3 polyunsaturated fatty acid treatment on measures of microvascular function and vibration perception in non-alcoholic fatty liver disease: results from the randomised WELCOME trial, Diabetologia 8 (2015) 1916–1925.
[26] A.K. Leamy, R.A. Egnatchik, J.D. Young, Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease, Prog. Lipid Res. 52 (2013) 165–174.
[27] A. Lim, J. Zhou, R.A. Sinha, B.K. Singh, S. Ghosh, K.H. Lim, P.K. Chow, E.C.Y. Woon, P.M. Yen, Hepatic FTO expression is increased in NASH and its silencing attenuates palmitic acid-induced lipotoxicity, Biochem. Biophys. Res. Commun. 479 (2016) 476–481.
[28] L. Martínez, S. Torres, A. Baulies, C. Alarcón-Vila, M. Elena, G. Fabriàs, J. Casas, J. Caballeria, J.C. Fernandez-Checa, C. García-Ruiz, Myristic acid potentiates palmitic acid-induced lipotoxicity and steatohepatitis associated with lipodystrophy by sustaining *de novo* ceramide synthesis, Oncotarget 6 (2015) 41479–41496.
[29] M.C. Hernandez-Rodas, R. Valenzuela, L.A. Videla, Relevant aspects of nutritional and dietary interventions in non-alcoholic fatty liver disease, Int. J. Mol. Sci. 16 (2015) 25168–25198.
[30] R. Valenzuela, C. Barrera, A. Espinosa, P. Llanos, P. Orellana, L.A. Videla, Reduction in the desaturation capacity of the liver in mice subjected to high fat diet: relation to LCPUFA depletion in liver and extrahepatic tissues, Prostaglandins Leukot. Essent. Fatty Acids 98 (2015) 7–14.
[31] G. Tapia, R. Valenzuela, A. Espinosa, P. Romanque, C. Dossi, D. Gonzalez-Mañán, L.A. Videla, A. D'Espessailles, N-3 long-chain PUFA supplementation prevents high fat diet induced mouse liver steatosis and inflammation in relation to PPAR-α upregulation and NF-κB DNA binding abrogation, Mol. Nutr. Food Res. 6 (2014) 1333–1341.
[32] J. Willebrords, I.V. Pereira, M. Maes, S. Crespo Yanguas, I. Colle, B. Van Den Bossche, T.C. Da Silva, C.P. de Oliveira, W. Andraus, V.A. Alves, B. Cogliati, M. Vinken, Strategies, models and biomarkers in experimental non-alcoholic fatty liver disease research, Prog. Lipid Res. 59 (2015) 106–125.
[33] J.K. Lau, X. Zhang, J. Yu, Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances, J. Pathol. 241 (2017) 36–44.
[34] S.S. Chambel, A. Santos-Gonçalves, T.L. Duarte, The dual role of Nrf2 in nonalcoholic fatty liver disease: regulation of antioxidant defenses and hepatic lipid metabolism, Biomed. Res. Int. 2015 (2015), 597134.
[35] S. Spahis, E. Delvin, J.M. Borys, E. Levy, Oxidative stress as a critical factor in nonalcoholic fatty liver disease pathogenesis, Antioxid. Redox Signal. 10 (2017) 519–541.
[36] R. Valenzuela, P. Illesca, F. Echeverría, A. Espinosa, M.A. Rincón-Cervera, M. Ortiz, M.C. Hernandez-Rodas, A. Valenzuela, L.A. Videla, Molecular adaptations underlying the beneficial effects of hydroxytyrosol in the pathogenic alterations induced by a high-fat diet in mouse liver: PPAR-α and Nrf2 activation, and NF-κB down-regulation, Food Funct. 8 (2017) 1526–1537.
[37] C.G. Dossi, G.S. Tapia, A. Espinosa, L.A. Videla, A. D'Espessailles, Reversal of high-fat diet-induced hepatic steatosis by n-3 LCPUFA: role of PPAR-α and SREBP-1c, J. Nutr. Biochem. 25 (2014) 977–984.
[38] J. Dalli, S. Ramon, P.C. Norris, R.A. Colas, C.N. Serhan, Novel proresolving and tissue-regenerative resolvin and protectin sulfido-conjugated pathways, FASEB J. 29 (2015) 2120–2136.
[39] C.N. Serhan, J. Dalli, R.A. Colas, J.W. Winkler, N. Chiang, Protectins and maresins. New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome, Biochim. Biophys. Acta 2015 (1851) 397–413.
[40] L. Gao, J. Wang, K.R. Sekhar, H. Yin, N.F. Yared, S.N. Schneider, S. Sasi, T.P. Dalton, M.E. Anderson, J.Y. Chan, J.D. Morrow, M.L. Freeman, Novel n-3 fatty acid oxidation products activate Nrf2 by destabilizing the association between Keap1 and Cullin3, J. Biol. Chem. 282 (2007) 2529–2537.
[41] H. Haeiwa, T. Fujita, Y. Saitoh, N. Miwa, Oleic acid promotes adaptability against oxidative stress in 3T3-L1 cells through lipohormesis, Mol. Cell. Biochem. 386 (2014) 73–83.
[42] J.H. Lim, Z. Gerhart-Hines, J.E. Dominy, Y. Lee, S. Kim, M. Tabata, Y.K. Xiang, P. Puigserver, Oleic acid stimulates complete oxidation of fatty acids through protein kinase A-dependent activation of SIRT1-PGC1α complex, J. Biol. Chem. 288 (2013) 7117–7126.
[43] M.A. Rosillo, M.J. Alcaraz, M. Sánchez-Hidalgo, J.G. Fernández-Bolaños, C. Alarcón-de-la-Lastra, M.L. Ferrándiz, Anti-inflammatory and joint protective effects of extra-virgin olive-oil polyphenol extract in experimental arthritis, J. Nutr. Biochem. 25 (2014) 1275–1281.
[44] M. Piroddi, A. Albini, R. Fabiani, L. Giovannelli, C. Luceri, F. Natella, P. Rosignoli, T. Rossi, A. Taticchi, M. Servili, F. Galli, Nutrigenomics of extra-virgin olive oil: a review, Biofactors 43 (2017) 17–41.
[45] R. Valenzuela, F. Echeverria, M. Ortiz, M.A. Rincón-Cervera, A. Espinosa, M.C. Hernandez-Rodas, P. Illesca, A. Valenzuela, L.A. Videla, Hydroxytyrosol prevents reduction in liver activity of Δ-5 and Δ-6 desaturases, oxidative stress, and depletion in long chain polyunsaturated fatty acid content in different tissues of high-fat diet fed mice, Lipids Health Dis. 16 (1) (2017) 16.
[46] G. Leonarduzzi, B. Sottero, G. Testa, F. Biasi, G. Poli, New insights into redox-modulated cell signaling, Curr. Pharm. Des. 17 (2011) 3994–4006.
[47] A. Taki-Eldin, L. Zhou, H.-Y. Xie, K.-J. Chen, D. Yu, Y. He, S.-S. Zheng, Triiodothyronine attenuates hepatic ischemia/reperfusion injury in a partial hepatectomy model through inhibition of proinflammatory cytokines transcription factors, and adhesion molecules, J. Surg. Res. 178 (2012) 646–656.
[48] L.A. Videla, V. Fernández, P. Cornejo, R. Vargas, J. Carrasco, J. Fernández, N. Varela, Causal role of oxidative stress in unfolded protein response development in the hyperthyroid state, Free Radic. Biol. Med. 89 (2015) 401–408.
[49] L.A. Videla, Redox signaling associated with thyroid hormone action: perspectives in medical sciences, Oxid. Antioxid. Med. Sci. 5 (2016) 8–14.
[50] V. Laszló, K. Dezso, K. Baghy, V. Papp, I. Kovalszky, G. Sáfráni, S.S. Thorgeirsson, P. Nagy, S. Paku, Triiodothyronine accelerates differentiation of rat liver progenitor cells into hepatocytes, Histochem. Cell Biol. 130 (2008) 1005–1014.
[51] R.A. Sinha, 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, C.B. Newgard, M.A. Lazar, P.M. Yen, Thyroid hormone stimulates hepatic lipid catabolism via activation of autophagy, J. Clin. Invest. 122 (2012) 2428–2438.
[52] R. Mullur, Y.-Y. Liu, G.A. Brent, Thyroid hormone regulation of metabolism, Physiol. Rev. 94 (2014) 355–382.
[53] M. Mardones, R. Valenzuela, P. Romanque, N. Covarrubias, F. Anghileri, V. Fernández, L.A. Videla, G. Tapia, Prevention of liver ischemia reperfusión injury by a combined thyroid hormone and fish oil protocol, J. Nutr. Biochem. 23 (2012) 1113–1120.
[54] G. Tapia, V. Fernández, C. Pino, R. Ardiles, L.A. Videla, The acute-phase response of the liver in relation to thyroid hormone –induced redox signaling, Free Radic. Biol. Med. 40 (2006) 1628–1635.
[55] V. Fernández, G. Tapia, P. Varela, I. Castillo, C. Mora, F. Moya, M. Orellana, L.A. Videla, Redox up-regulated expression of rat liver manganese superoxide dismutase and Bcl-2 by thyroid hormone is associated with inhibitor of κB-α phosphorylation and nuclear factor-κB activation, J. Endocrinol. 186 (2005) 539–547.
[56] R. Vargas, L.A. Videla, Thyroid hormone suppresses ischemia-reperfusion-induced liver NLRP3 inflammasome activation: role of AMP-activated protein kinase, Immunol. Lett. 184 (2017) 92–97.
[57] C.N. Serhan, N. Chiang, T.E. Van Dyke, Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators, Nat. Rev. Immunol. 323 (2010) 292–297.
[58] L.L. Souza, M.O. Nunes, G.S.M. Paula, A. Cordeiro, V. Penha-Pinto, J.F.N. Neto, K.J. Oliveira, M.G. Tavares do Carmo, C.C. Pazos-Moura, Effects of dietary fish oil on thyroid hormone signaling in the liver, J. Nutr. Biochem. 21 (2010) 935–940.
[59] L.A. Videla, V. Fernández, P. Cornejo, R. Vargas, I. Castillo, Thyroid hormone in the frontier of cell protection, survival and functional recovery, Expert Rev. Mol. Med. 17 (2015) e10.
[60] N. Handa, T. Takagi, S. Saijo, S. Kishishita, D. Takaya, M. Toyama, T. Terada, M. Shirouzu, A. Suzuki, S. Lee, T. Yamauchi, M. Okada-Iwabu, M. Iwabu, T. Kadowaki, Y. Minokoshi, S. Yokoyama, Structural basis for compound C inhibition of the human AMP-activated protein kinase α2 subunit kinase domain, Acta Cryst. D67 (2011) 480–487.
[61] N. Kim, J.O. Lee, H.J. Lee, H.I. Kim, J.K. Kim, Y.W. Lee, S.K. Lee, S.J. Kim, S.H. Park, H.S. Kim, Endogenous ligand for GPR120 docosahexaenoic acid, exerts benign metabolic effects on the skeletal muscles via AMP-activated protein kinase pathway, J. Biol. Chem. 290 (2015) 20438–20447.
[62] X. Deng, Q. Dong, D. Bridges, R. Raghow, E.A. Park, M.B. Elam, Docosahexaenoic acid inhibits proteolytic processing of sterol regulatory element-binding protein-1c (SREBP-1c) via activation of AMP-activated kinase, Biochim. Biophys. Acta 1851 (2015) 1521–1529.
[63] L.A. Videla, V. Fernández, R. Vargas, P. Cornejo, G. Tapia, N. Varela, R. Valenzuela, A. Arenas, J. Fernández, M.C. Hernández-Rodas, B. Riquelme, Upregulation of rat liver PPARα-FGF21 signaling by a docosahexaenoic acid and thyroid hormone combined protocol, Biofactors 42 (2016) 638–646.

[64] T. Bai, Y. Yang, Y.-L. Yao, P. Sun, L.-H. Lian, Y.-L. Wu, J.-X. Nan, Betulin alleviated ethanol-induced alcoholic liver injury via SIRT1/AMPK signaling pathway, Pharmacol. Res. 105 (2016) 1–12.
[65] T. Lundasen, M.C. Hunt, L.M. Nilsson, S. Sanyal, B. Angelin, S.E.H. Alexson, M. Rudling, PPARa is a key regulator of hepatic FGF21, Biochem. Biophys. Res. Commun. 360 (2007) 437–440.
[66] B.M. Forman, J. Chen, R.M. Evans, Hypolipidemic drugs polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 4312–4317.
[67] F.G. Schaap, A.E. Kremer, W.H. Lamers, P.L. Jansen, I.C. Gaemers, Fibroblast growth factor 21 is induced by endoplasmic reticulum stress, Biochimie 95 (2013) 692–699.
[68] S. Jiang, C. Yan, M. Shao, Y. Zhang, Y. Liu, Y. Deng, B. Shan, J. Liu, H. Li, L. Yang, J. Zhoi, Z. Dai, Y. Liu, W. Jia, Fibroblast growth factor 21 is regulated by the IRE1α-XBP1 branch of the unfolded protein response and counteracts endoplasmic reticulum stress-mediated hepatic steatosis, J. Biol. Chem. 289 (2014) 29751–29765.
[69] L.A. Videla, R. Vargas, B. Riquelme, J. Fernández, V. Fernández, Thyroid hormone-induced expression of the hepatic scaffold proteins sestrin2, β-Klotho, and FRS2α in relation to FGF21-AMPK signaling, Exp. Clin. Endocrinol. Diabetes (2017), http://dx.doi.org/10.1055/s-0043-115533.
[70] R. Vargas, B. Riquelme, J. Fernández, L.A. Videla, Combined docosahexaenoic acid-thyroid hormone protocol upregulates rat liver β-Klotho expression and downstream components of FGF21 signaling as a potential novel approach to metabolic stress conditions, Food Funct. 8 (2017) 3980–3988.
[71] L.K. Langeberg, J.D. Scott, Signalling scaffolds and local organization of cellular behaviour, Nat. Rev. Mol. Cell. Biol. 16 (2015) (2016) 232–244.
[72] Y. Ogawa, H. Kurosu, M. Yamamoto, A. Nandi, K.P. Rosenblatt, R. Goetz, A.V. Eliseenkova, M. Mohammadi, M. Kuro-o, β-Klotho is required for metabolic activity of fibroblast growth factor 21, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 7432–7437.
[73] F.M. Fisher, J.L. Estall, A.C. Adams, P.J. Antonellis, H.A. Bina, F.S. Fisher, A. Kharitonenko, B.M. Spiegelman, E. Maratos-Flier, Integrated regulation of hepatic metabolism by fibroblast growth factor 21 (FGF21) in vivo, Endocrinology 152 (2011) 2996–3004.
[74] N. Gotoh, Regulation of growth factor signaling by FRS2 family docking/scaffold adaptor proteins, Cancer Sci. 99 (2008) 13119–13125.
[75] A. Morrison, L. Chen, J. Wang, M. Zhang, H. Yang, Y. Ma, A. Budanov, J.H. Lee, M. Karin, J. Li, Sestrin2 promotes LKB1-mediated AMPK activation in the ischemic heart, FASEB J. 29 (2015) 408–417.
[76] Y. Romeo, X. Zhang, P.P. Roux, Regulation and function of RSK family of protein kinases, Biochem. J. 441 (2012) 553–569.
[77] A. Salminen, A. Kauppinen, K. Kaarniranta, FGF21 activates AMPK signaling: impact on metabolic regulation and the aging process, J. Mol. Med. 95 (2017) 123–131.
[78] L.A. Videla, V. Fernández, P. Cornejo, R. Vargas, P. Morales, J. Ceballo, A. Fischer, N. Escudero, O. Escobar, $T_3$-induced liver AMP-activated protein kinase signaling: redox dependency and upregulation of downstream targets, World J. Gastroenterol. 20 (2014) 17416–17425.
[79] M.C. Hernández-Rodas, R. Valenzuela, F. Echeverría, M.A. Rincón-Cervera, A. Espinosa, P. Illesca, P. Muñoz, A. Corbari, N. Romero, D. González-Mañán, L.A. Videla, Supplementation with docosahexaenoic acid and extra virgin olive oil prevents liver steatosis induced by a high-fat diet in mice through PPAR-α and Nrf2 upregulation with concomitant SREBP-1c and NF-κB downregulation, Mol. Nutr. Food Res. 61 (2017) 1700479, http://dx.doi.org/10.1002/mnfr.201700479.
[80] A. Lamaziere, C. Wolf, U. Barbe, P. Bausero, F. Visioli, Lipidomics of hepatic lipogenesis inhibition by omega 3 fatty acids, Prostaglandins Leukot. Essent. Fatty Acids 88 (2013) 149–154.
[81] M.A. Rincón-Ververa, R. Valenzuela, M.C. Hernández-Rodas, M. Marambio, A. Espinosa, S. Mayer, N. Romero, C. Barrera, A. Valenzuela, L.A. Videla, Supplementation with antioxidant-rich extra virgin olive oil prevents hepatic oxidative stress and reduction of desaturation capacity in mice fed a high-fat diet: effects on fatty acid composition in liver and extrahepatic tissues, Nutrition 32 (2016) 1254–1267.
[82] S.A. Soto-Alarcón, R. Valenzuela, A. Valenzuela, L.A. Videla, Liver protective effects of extra virgin olive oil: interaction between its chemical composition and the cell-signaling pathways involved in protection, Endoc. Metab. Immune Disord. Drug Targets (2018), http://dx.doi.org/10.2174/1871530317666171114120552.
[83] C. Pirozzi, A. Lama, R. Simeoli, O. Paciello, T.B. Pagano, M.P. Mollica, F. Di Guida, R. Russo, S. Magliocca, R.B. Canani, G.M. Raso, A. Calignano, R. Meli, Hydroxytyrosol prevents metabolic impairment reducing hepatic inflammation and restoring duodenal integrity in a rat model of NAFLD, J. Nutr. Biochem. 30 (2016) 108–115.
[84] K.J. Bowen, P.M. Kris-Etherton, G.C. Shearer, S.G. West, L. Reddivari, P.J.H. Jones, Oleic acid-derived oleoylethanolamide: a nutritional science perspective, Progr. Lipid Res. 67 (2017) 1–15.
[85] J. Fu, F. Oveisi, S. Gaetani, E. Lin, D. Piomelli, Oleoylethanolamide, an endogenous PPAR-α agonist, lowers body weight and hyperlipidemia in obese rats, Neuropharmacology 48 (2005) 1147–1153.
[86] S. Hmimed, M. Belarbi, F. Visioli, Hydroxytyrosol augments the redox status of high fat diet-fed rats, PharmaNutrition 4 (2016) 139–142.
[87] J. Han, R.J. Kaufman, The role of ER stress in lipid metabolism and lipotoxicity, J. Lipid Res. 57 (2016) 1329–1338.
[88] K. Niforou, C. Cheimonidou, I.P. Trougakos, Molecular chaperones and proteostasis regulation during redox imbalance, Redox Biol. 2 (2014) 323–332.
[89] G. Chiao, T. Zhang, S. Yu, S. Lee, V. Calabuig-Navarro, J. Yamauchi, S. Ringquist, H.H. Dong, ATF4 protein deficiency protects against high fructose-induced hypertriglyceridemia in mice, J. Biol. Chem. 288 (2013) 25350–25361.
[90] A.H. Lee, E.F. Scapa, D.E. Cohen, L.H. Glimcher, Regulation of hepatic lipogenesis by the transcription factor XBP1, Science 320 (2008) 1494–1496.
[91] C. Zhang, X. Chenc, R.M. Zhu, Y. Zhang, T. Yu, H. Wang, H. Zhao, M. Zhao, Y.L. Ji, Y.H. Chen, X.H. Meng, W. Weib, D.X. Xu, Endoplasmic reticulum stress is involved in hepatic SREBP-1c activation and lipid accumulation in fructose-fed mice, Toxicol. Lett. 212 (2012) 229–240.
[92] E. Giordano, A. Davalos, N. Nicod, F. Visioli, Hydroxytyrosol attenuates tunicamycin- induced endoplamic reticulum stress in human hepatocarcinoma cells, Mol. Nutr. Food Res. 58 (2014) 954–962.
[93] G. Suchankova, M. Takle, A.K. Saha, N.B. Ruderman, S.D. Clarke, T.W. Gettys, Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats, Biochem. Biophys. Res. Commun. 326 (2005) 851–858.
[94] J.A. Menendez, J. Joven, G. Aragonès, E. Barrajón-Catalán, R. Beltrán-Debón, I. Borrás-Linares, J. Camps, B. Corominas-Faja, S. Cufí, S. Fernández-Arroyo, A. Garcia-Heredia, A. Hernández-Aguilera, M. Herranz-López, C. Jiménez-Sánchez, E. López-Bonet, J. Lozano-Sánchez, F. Luciano-Mateo, B. Martin-Castillo, V. Martin-Paredero, A. Pérez-Sánchez, C. Oliveras-Ferraros, M. Riera-Borrull, E. Rodríguez-Gallego, R. Quirantes-Piné, A. Rull, L. Tomás-Menor, A. Vazquez-Martin, C. Alonso-Villaverde, V. Micol, A. Segura-Carretero, Xenohormetic and anti-aging activity of secoiridoid polyphenols present in extra virgin olive oil, ABBV Cell Cycle 12 (2013) 555–578.
[95] S. Acín, M.A. Navarro1, J.M. Arbonés-Mainar, N. Guillén, A.J. Sarría, R. Carnicer, J.C. Surra, I. Orman, J.C. Segovia, R. de la Torre, M.I. Covas, J. Fernández-Bolaños, V. Ruiz-Gutiérrez, Jesús Osada, Hydroxytyrosol administration enhances atherosclerotic lesión development in apo E deficient mice, J. Biochem. 140 (2006) 383–391.
[96] J. Tomé-Carneiro, M.C. Crespo, E. Iglesias-Gutiérrez, R. Martín, J. Gil-Zamorano, C. Tomas-Zapico, E. Burgos-Ramos, C. Correa, D. Gómez-Coronado, M.A. Lasunción, E. Herrera, F. Visioli, A. Dávalos, Hydroxytyrosol supplementation modulates the expression of miRNAs in rodents and in humans, J. Nutr. Biochem. 34 (2016) 146–155.
[97] J.M. Arbones-Mainar, K. Ross, G.J. Rucklidge, M. Reid, G. Duncan, J.R. Arthur, G.W. Horgan, M.A. Navarro, R. Carnicer, C. Arnal, J. Osada, B. de Roos, Extra virgin olive oils increase hepatic fat accumulation and hepatic antioxidant protein levels in APOE-/- mice, J. Proteome Res. 6 (2007) 4041–4054.
[98] L. Schwingshackl, G. Hoffmann, Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses, Nutrients 4 (2012) 1989–2007.
[99] A.F. Ajayi, R.E. Akhigbe, Implication of altered thyroid state on liver function, Thyroid Res. Pract. 9 (2012) 84–87.
[100] M. Coppola, F. Cioffi, M. Moreno, F. Goglia, E. Silvestri, 3,5-Diiodo-L-thyronine: a possible pharmacological agent? Curr. Drug Deliv. 13 (2016) 330–338.
[101] R. Senese, F. Cioffi, P. de Lange, C. Leanza, L.F. Iannucci, E. Silvestri, M. Moreno, A. Lombardi, F. Goglia, A. Lanni, 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. 8 (2017) 706.
[102] Y. Cao, T. Matsubara, C. Zhao, W. Gao, L. Peng, J. Shan, Z. Liu, F. Yuan, L. Tang, P. Li, Z. Guan, Z. Fang, X. Lu, H. Huang, Q. Yang, Antisense oligonucleotide and thyroid hormone conjugates for obesity treatment, Sci. Rep. 7 (2017) 9307.
[103] G. Liu, L. Liang, G.A. Bray, L. Qi, F.B. Hu, J. Rood, F.M. Sacks, Q. Sun, Thyroid hormones and changes in body weight and metabolic parameters in response to weight loss diets: the POUNDS LOST trial, Int. J. Obes. 41 (2017) 878–886.
[104] M.N.D. Di Minno, A. Russolillo, R. Lupoli, P. Ambrosino, A. Di Minno, G. Tarantino, Omega-3 fatty acids for the treatment of non-alcoholic fatty liver disease, World J. Gastroenterol. 18 (2012) 5839–5847.
[105] C. Mameli, G. Zuccotti, C. Carnovale, E. Galli, P. Nannini, D. Cervia, C. Perrotta, An update on the assessment and management of metabolic syndrome, a growing medical emergency in paediatric populations, Pharmacol. Res. 119 (2017) 99–117.
[106] C. Tsioufis, C. Thomopoulos, Combination drug treatment in hypertension, Pharmacol. Res. 125 (2017) 266–271.
[107] M. Burnier, Drug adherence in hypertension, Pharmacol. Res. 125 (2017) 142–149.