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REVIEW

Recent advances in liver preconditioning: Thyroid hormone, n-3 long-chain polyunsaturated fatty acids and iron

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Abstract

Liver preconditioning (PC), defined as an enhanced tolerance to injuring stimuli induced by previous specific maneuvers triggering beneficial functional and molecular changes, is of crucial importance in human liver transplantation and major hepatic resection. For these reasons, numerous PC strategies have been evaluated in experimental models of ischemia-reperfusion liver injury, which have not been transferred to clinical application due to side effects, toxicity and difficulties in implementation, with the exception of the controversial ischemic PC. In recent years, our group has undertaken the assessment of alternate experimental liver PC protocols that might have application in the clinical setting. These include thyroid hormone (T_3), n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), or iron, which suppressed liver damage due to the 1 h ischemia-20 h reperfusion protocol. T_3, n-3 LCPUFA and iron are hormetic agents that trigger biologically beneficial effects in the low-dose range, whose multifactorial mechanisms of action are discussed in the work.

Key words: Liver preconditioning; Thyroid hormone; n-3 polyunsaturated fatty acids; Iron

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INTRODUCTION

Liver functioning is characterized by a multiplicity of processes that include most of the pathways for intermediary metabolism, biotransformation of xenobiotics, plasma protein biosynthesis, excretion and secretion of various types of molecules. With the exception of hyperglycemic conditions, the high energy requirements to support liver functions are primarily met by fatty acid oxidation, making the liver highly dependent on O_2 supply and susceptible to hypoxic or anoxic conditions. Liver damage underlying cellular death is associated with cholestasis, viral hepatitis, drug-induced injury, obesity[1] and ischemia-reperfusion (IR) episodes, including liver transplantation, hepatic resection, low-blood pressure conditions and abdominal surgery requiring hepatic vascular occlusion[2-5]. IR injury is a phenomenon in which cellular damage due to hypoxia is exacerbated following restoration of O_2 and nutrient supply[2-5]. In these situations, different types of ischemia can occur in the liver: namely, (1) warm ischemia inducing hepatocyte and sinusoidal endothelial cell (SEC) death, a feature of hepatic trauma, hypovolemic shock and inflow occlusion during

liver surgery; and (2) cold ischemia leading to SEC death observed in liver transplantation after harvesting and preservation, which might involve rewarming ischemia during vascular anastomosis[6]. IR liver injury is due to numerous contributory factors, including Kupffer cell activation, oxidative stress and up-regulation of pro-inflammatory cytokine signaling, which often determine hepatic failure[1-6]. Considering that IR liver injury is a major complication in clinical practice due to its complexity in terms of molecular and cellular mechanisms, strategies reducing IR injury have been extensively studied[4-8].

In general terms, organ preconditioning (PC) is defined as an increased tolerance to IR injury afforded by previous specific maneuvers triggering beneficial functional and molecular changes, a phenomenon initially described by Murry *et al*[9] in the heart. PC strategies evaluated in experimental models of IR liver injury include: (1) pharmacological approaches targeting tumor necrosis factor-α (TNF-α) response, mitochondrial dysfunction, reactive oxygen species (ROS) production, microcirculatory disorders or neutrophil infiltration; (2) gene therapy directed to up-regulation of proteins abrogating ROS production, apoptosis and nuclear factor-κB (NF-κB) activation or down-regulating of intercellular adhesion molecule-1 and P-selectin expression reducing neutrophil recruitment; and (3) surgical strategies such as ischemic preconditioning (IP) or other strategies underlying moderate oxidative stress development (for specific references see[4-8,10]). The latter group of PC maneuvers includes development of hyperthermia[11], hyperbaric oxygen therapy[12] or the administration of the model oxidants *tert-butyl* hydroperoxide[13], doxorubicin[14] and ozone[15]. However, due to toxicity, side effects and difficulties in implementation, these experimental PC strategies have not been transferred to clinical application, with the exception of IP[6,16]. Although IP proved to be useful in human liver resections[17-19] and in human liver transplantation[20-22], this PC maneuver remains controversial[23-25]. For these reasons, our group has recently undertaken the evaluation of alternate experimental liver PC strategies that might have application in the clinical setting; namely, administration of thyroid hormone (L-3,3',5-triiodothyronine, T_3)[26], n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA)[27] or iron[28], prior to an IR protocol.

THYROID HORMONE LIVER PRECONDITIONING

Liver PC by *in vivo* T_3 administration is based on the calorigenic action of thyroid hormones leading to stimulation and maintenance of basal thermogenesis[29], a response that is carried out through genomic and non-genomic signaling mechanisms[1,30]. In the liver, this effect is evidenced by enhancement in the rate of O_2 consumption, with consequent increment in ROS generation[31,32] by mechanisms primarily triggered in hepatocytes and in Kupffer cells (Figure 1). ROS produced in Kupffer cells activate redox-sensitive transcription factors such as NF-κB, signal transducer and activator of transcription 3 (STAT3) or activating protein 1 (AP-1), as shown by suppression of T_3-induced DNA binding of these proteins by *in vivo* pretreatment with the antioxidants α-tocopherol and N-acetylcysteine or the Kupffer cell inactivator gadolinium chloride[1,30]. Under these conditions, activation of NF-κB and AP-1 in Kupffer cells is associated with up-regulation of the expression of genes for the cytokines TNFα, interleukin (IL)-1 and IL-6[33], with enhanced synthesis and release into hepatic sinusoids (Figure 1). Interaction of Kupffer cell-derived TNF-α with TNF receptor 1 may trigger two responses in hepatocytes[33]: namely, (1) NF-κB activation *via* inhibitor of κB kinase (IKK) phosphorylation leading to the expression of antioxidant (manganese superoxide dismutase, inducible nitric oxide synthase), anti-apoptotic (Bcl2) and type I acute-phase (haptoglobin) proteins[34,35]; and (2) AP-1 activation *via* c-Jun N-terminal kinase (JNK) phosphorylation leading to up-regulation of hepatocyte proliferation[36] (Figure 1). In addition, interaction of Kupffer cell-derived IL-6 with IL-6 receptor through its binding to the gp130 receptor subunit[37] may activate Janus kinase (JAK)/STAT3 system and the transcription of both type I (haptoglobin) and type II (β-fibrinogen) acute-phase protein genes[35] (Figure 1). Activation of NF-κB, STAT3 and AP-1 by Kupffer cell-derived TNF-α and IL-6 may be reinforced by ROS generated within hepatocytes by different enzymatic mechanisms triggered by T_3 (Figure 1). These cytoprotective responses could be contributed by additional processes triggered by T_3 administration: including (1) post-transcriptional up-regulation of the acute-phase protein ferritin through increased iron-induced displacement of iron regulatory protein from the iron-responsive element in ferritin mRNA[38]; and (2) transcriptional up-regulation of uncoupling proteins *via* the classical genomic pathway[39], which have been proposed to decrease the pro-oxidant potential of the liver[38-40].

Recently, *in vivo* T_3 administration to rats was shown to activate hepatic nuclear factor erythroid 2-related factor 2 (Nrf2), as evidenced by the increased cytosol-to-nuclear translocation observed[41]. Liver Nrf2 activation induced by T_3 appears to be a redox-dependent process due to its abolishment by N-acetylcysteine pretreatment, which may be contributed by Nrf2 phosphorylation related to p38 activation[41]. This would represent a novel and alternate cytoprotective mechanism of T_3 action against free-radical and electrophile toxicity[41], in addition to that afforded by NF-κB, STAT3 and AP-1 up-regulation[34-36] (Figure 1), considering that Nrf2 controls the expression of antioxidant components, detoxification enzymes or membrane transporters (Figure 1) and interplays with NF-κB affording anti-inflammatory responses[42].

Redox activation of NF-κB, STAT3, AP-1 and Nrf2 up-regulating transcription of protective genes represents an additional non-genomic mechanism of T_3 action to those reported for different cellular processes[43], which is dependent upon the genomic pathway enhancing energy metabolism with ROS production. These observations

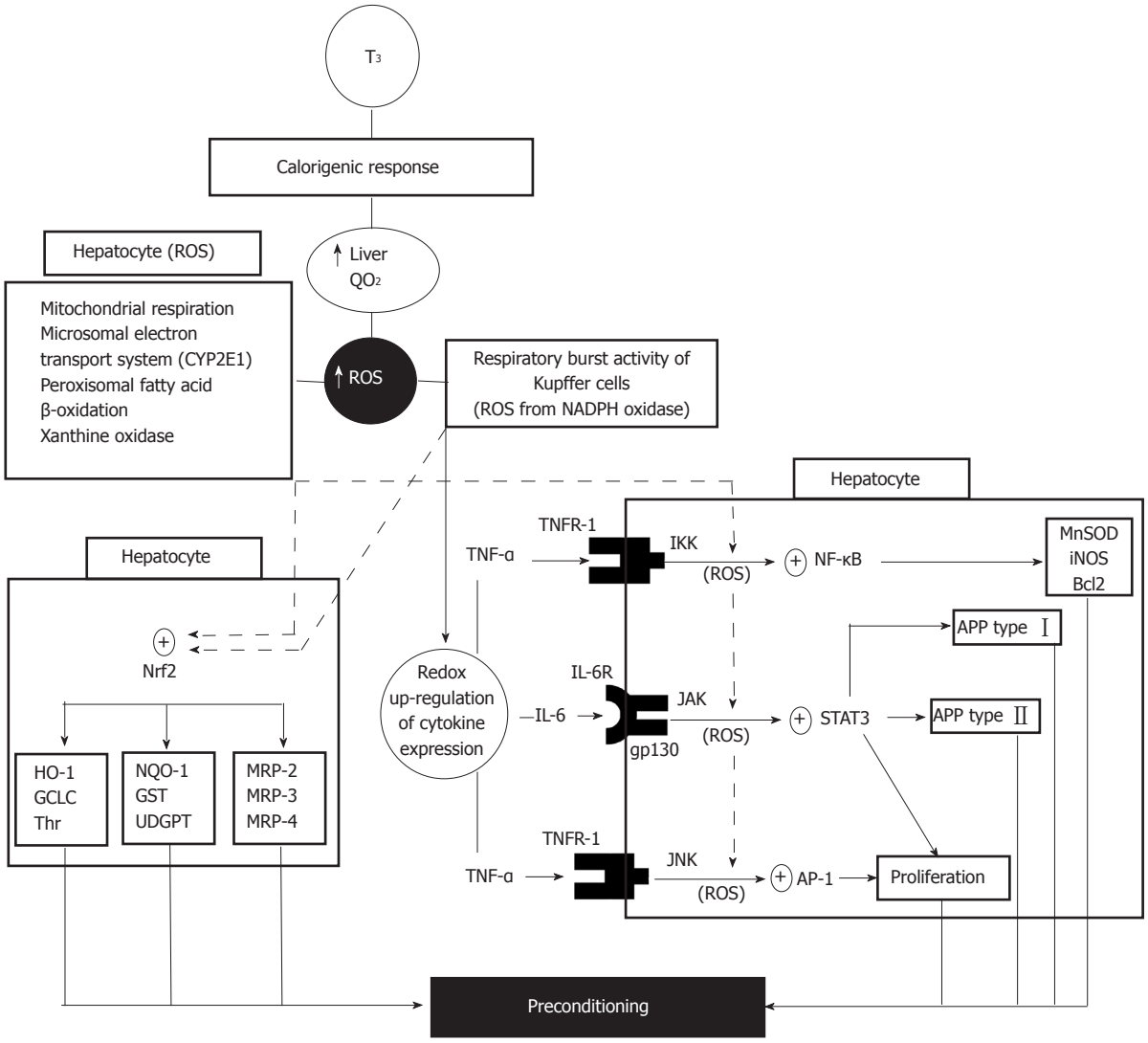

Figure 1 Redox signaling in T_3 liver preconditioning is mediated by activation of transcription factors nuclear factor-κB, activating protein 1, signal transducer and activator of transcription 3, and nuclear factor erythroid 2-related factor 2 triggering antioxidant, anti-apoptotic, acute-phase and proliferative responses. AP-1: Activating protein 1; APP: Acute-phase protein; CYP2E1: Cytochrome P450 isoform 2E1; GCLC: Glutamate cysteine ligase catalytic subunit; GST: Glutathione-S-transferase; HO-1: Heme-oxygenase 1; IKK: Inhibitor of IκB kinase; iNOS: Inducible nitric oxide synthase; IL-6: Interleukin-6; IL-6R: Interleukin-6 receptor; JAK: Janus kinase; JNK: c-Jun N-terminal kinase; MnSOD: Manganese superoxide dismutase; MRP: Multidrug resistance protein; NF-κB: Nuclear factor-κB; NQO-1: NAD(P)H quinone oxidoreductase 1; Nrf2: Nuclear factor-erythroid 2 related factor 2; QO_2: Rate of oxygen consumption; ROS: Reactive oxygen species; STAT3: Signal transducer and activator of transcription 3; Thr: Thioredoxin; TNF-α: Tumor necrosis factor-α; TNFR1: Tumor necrosis factor-α receptor 1; UDPGT: UDP-glucuronyl transferase.

constitute the basis for liver PC by T_3, with integration of different T_3-signaling inputs to achieve metabolic and redox balance (Figure 1) that are required to deal with the cytotoxic mechanisms underlying IR liver injury. Increased hepatocyte proliferation compensating for liver cells lost due to IR-induced necrosis[36] is an additional PC response due to the mitogenic action of T_3, leading to direct hyperplasia[44]. In agreement with these views, IR-induced (1) drastic enhancement in liver oxidative stress status and TNF-α response; (2) loss of DNA binding capacity of NF-κB and STAT3, implying loss of cytoprotective potential; and (3) major increment in hepatic AP-1 activation, which constitutes a crucial determinant of hepatotoxicity under conditions of reduced NF-κB activation and enhanced TNF-α response, are normalized by T_3 treatment[26]. Similar T_3 actions involving other physiological functions have been described, including (1) NF-κB activation in lymphocytes from thyroxin-treated rats[45] or hyperthyroid patients[46] in association with higher oxidative stress status and potentiation of humoral immune response; and (2) JNK/STAT3 activation by T_3 in a nutritional model of non-alcoholic steatosis in rats, with complete regression of fat accumulation[47].

n-3 LONG-CHAIN-POLYUNSATURATED FATTY ACID LIVER PRECONDITIONING

Dietary fatty acids, especially LCPUFA, are essential for growth and development in mammals including man,

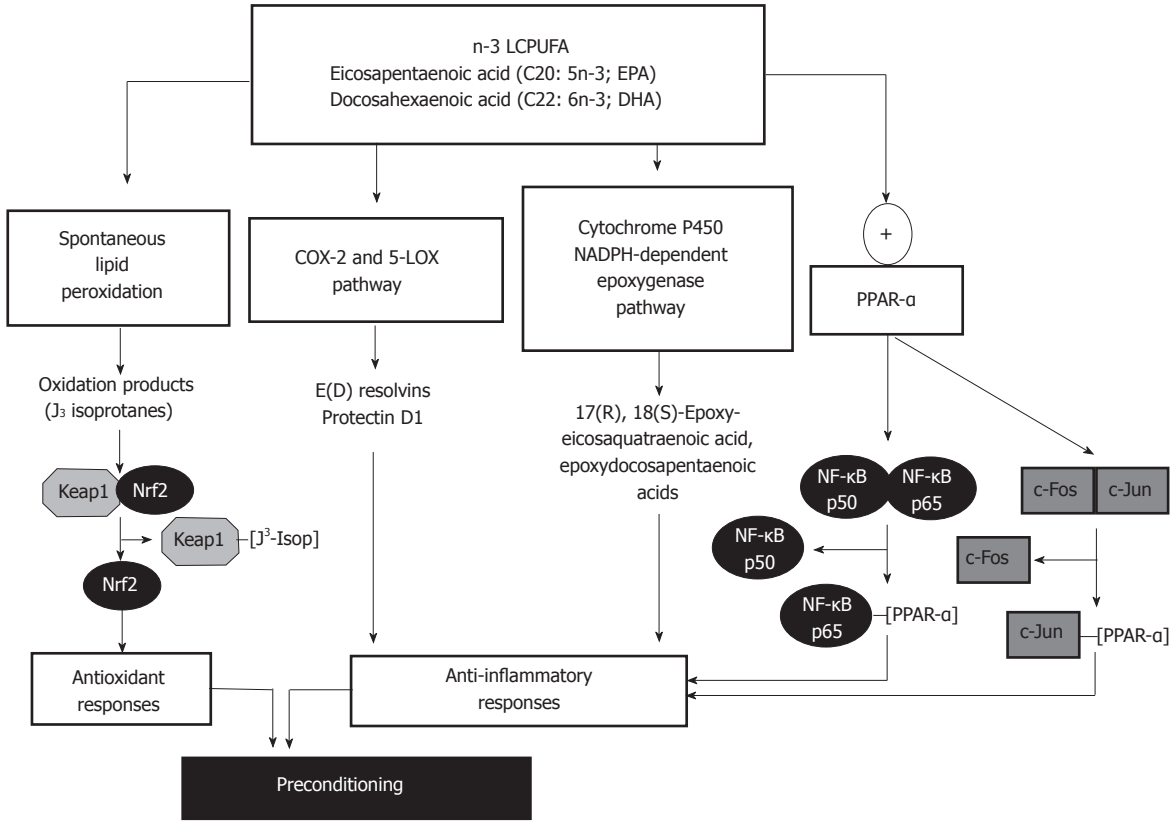

Figure 2 *n-3* long-chain polyunsaturated fatty acid -induced liver preconditioning is associated with antioxidant and anti-inflammatory responses triggered by oxidative products and peroxisome proliferator-activated receptor -α activation. n-3 LCPUFA: n-3 long-chain polyunsaturated fatty acid; **COX-2: Cyclo-oxy-**genase 2; Keap1: **Kelch-like ECH-associated protein 1; 5-LOX: 5-lipoxygenase; NF-κB: Nuclear factor-κB; Nrf2: Nuclear factor-erythroid 2 related factor 2; PPAR-α:** Peroxisome proliferator-activated receptor-α.

both n-6 and n-3 LCPUFAs being important as structural components of cellular lipids and substrates for the synthesis of physiological mediators[48]. Among the n-3 series of LCPUFAs, eicosapentaenoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3; DHA), produced from α-linolenic acid (C18:3n-3), have been associated with multiple positive health effects[48,49] and proposed for the prevention of non transmissible chronic diseases[50] or against heart[51] and liver[27] IR injury. It is considered that attainment of a given n-6/n-3 ratio is crucial for prevention and treatment of several diseases, as a potential sensor for the activation of mechanisms involved in inflammatory processes[52] such as liver IR injury[1-6].

Recently, liver PC against IR injury was reported in rats subjected to fish oil (270 mg/kg EPA plus 180 mg/kg DHA) or saline (controls) administration for 7 d, prior to the 1 h ischemia-20 h reperfusion protocol[27]. *In vivo* n-3 LCPUFA supplementation significantly enhanced liver n-3 LCPUFA content and decreased n-6/n-3 LCPUFA ratios, with prevention of IR-induced liver injury, suppression of oxidative stress, recovery of pro-inflammatory cytokine homeostasis, and NF-κB functionality lost during IR[27]. Several molecular mechanisms can be invoked to explain liver PC by n-3 LCPUFA, including antioxidant and anti-inflammatory responses (Figure 2).

Considering the high susceptibility of n-3 LCPUFAs to free-radical attack with further decomposition[53], these fatty acids readily undergo *in vitro* non-enzymatic lipid peroxidation with formation of cyclopentenone-containing J-ring isoprostanes (J_3-isoprostanes)[54]. Gao *et al*[54] reported that J_3-isoprostanes from EPA and DHA oxidation react with sulfhydryl groups in recombinant Keap1, a Cul-containing E3 ubiquitin ligase (Cul3)-Ring box 1 complex responsible for Nrf2 ubiquitination and degradation[55]. This interaction alters Keap1 structure, leading to loss of binding to Cul3, Nrf2 stabilization and nuclear translocation, with expression of the antioxidant enzymes heme-oxygenase-1 and glutamate cysteine ligase, as assessed in cultured HepG2 cells[54] (Figure 2). In agreement with these findings, *in vivo* EPA supplementation in mice was shown to up-regulate the expression of other Nrf2-dependent antioxidant proteins, namely, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and catalase, with significant increases in liver glutathione content and diminution in lipid peroxidation rate[56].

Both EPA and DHA have been reported as effective anti-inflammatory and tissue protective mediators[48-50], effects that may underlie different mechanisms of action (Figure 2). These include various aspects of eicosanoid metabolism generating n-3 LCPUFA-derived mediators produced in the resolution phase following acute inflam-

mation. EPA and DHA can be metabolized by cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) to generate E-series and D-series of resolvins *in vivo* and *in vitro*, respectively (Figure 2), which exhibit anti-inflammatory effects compared with those derived from arachidonic acid[57]. DHA can also be metabolized by 5-LOX to produce protectins, protectin D1 being the most potent anti-inflammatory isomer[58] (Figure 2). Resolvins E1, E2 and protectin D1 exert their anti-inflammatory action mainly through inhibition of neutrophil infiltration in target tissues[57,59]. In the case of resolving E1, its binding to G-protein-coupled receptors chemokine-like receptor-1 and leukotriene B_4 receptor attenuates the pro-inflammatory effects of NF-κB and leukotriene B_4 signaling, respectively[60]. Although the influence of resolvins and protectins has not been evaluated in IR liver injury, mouse kidney subjected to bilateral IR leads to endogenous mobilization and higher blood levels of DHA, with enhanced production of D-series of resolvins and protectins[61]. Moreover, pretreatment with exogenous resolvins was able to protect from IR kidney injury[61]. Interestingly, *in vivo* DHA supplementation is protective against liver necroinflammatory injury in mice subjected to carbon tetrachloride intoxication, a condition enhancing hepatic formation of the DHA-derived metabolites 17S-hydroxy-DHA (17S-HDHA) and protectin D1[62]. These findings and the protective effect of DHA and 17S-HDHA against *in vitro* hydrogen peroxide toxicity in hepatocytes establish a significant protective role of n-3 LCPUFA supplementation in well-known animal models of liver injury, which amplifies formation of DHA-derived anti-inflammatory lipid mediators in the liver[62] (Figure 2). In addition to EPA and DHA metabolism by the COX2/5-LOX pathway, these fatty acids may undergo oxygenation by cytochrome P450 NADPH-dependent epoxygenases (Figure 2), with production of multiple epoxyeicosaquatraenoic acid and epoxydocosapentaenoic acid regioisomers, respectively[57,63], which might have anti-inflammatory effects[57]. The finding that IR elicited a net decrease in the content of n-3 LCPUFA in the liver of EPA plus DHA supplemented rats over that in non-supplemented animals[27], support the contention that *in vivo* n-3 LCPUFA protection may be related to utilization of the fatty acids in lipid peroxidation, COX-2/5-LOX and cytochrome P450-dependent epoxygenation pathways. However, n-3 LCPUFA β-oxidation and replacement for n-6 LCPUFA in membrane phospholipids cannot be discarded.

In addition to the above discussed mechanisms related to the anti-inflammatory responses of n-3 LCPUFA involving oxidative processes, EPA and DHA may directly alter intracellular signaling pathways associated with transcription factors peroxisome proliferator-activated receptors (PPAR)-α/PPAR-γ and NF-κB/AP-1. The mechanism is based on the findings that LCPUFA, fatty acid derivatives and eicosanoids act as natural ligands for PPARs leading to their activation[64], which physically interact with both the p65 component of NF-κB and the c-Jun component of AP-1 (Figure 2), thus interfering with NF-κB and AP-1 transactivation of inflammatory genes[65]. Alternate mechanisms triggered by PPAR-α activation include: (1) enhancement of IκB-α mRNA and protein expression and its nuclear abundance, with diminution in NF-κB DNA binding activity[66]; (2) decreased IκB-α degradation, probably due to diminished phosphorylation[67]; and (3) up-regulation of antioxidant enzymes[56,68] (Figure 2) with reduction of the oxidative stress status, leading to loss of NF-κB activation and inflammatory cytokine production[68]. n-3 LCPUFA-induced re-establishment of inflammatory cytokine homeostasis under IR conditions[27,69] is accompanied by improvement of hepatic microcirculation, as a contributory factor protecting the liver against IR injury[70,71]. Although the relevance of n-3 LCPUFA supplementation in conditions underlying IR liver injury in humans has not been evaluated, several clinical studies have reported that supplementation with fish oil, seal oil or purified n-3 LCPUFA reduces hepatic lipid content in obese non-alcoholic fatty liver disease patients[72-76], exhibiting substantial depletion of n-3 LCPUFA content[77]. In addition, n-3 LCPUFA administration improved circulating liver function markers[72-76], serum triacylglycerol (TAG)[73,74] and tumor necrosis factor-α[73] levels, and hepatic microcirculatory function[72].

IRON LIVER PRECONDITIONING

Iron is an essential micronutrient and bio-catalyst of oxidation-reduction reactions that are related to its chemistry promoting electron exchange under aerobic conditions, being crucial for mitochondrial oxidative phosphorylation and other processes requiring enzymes/proteins with iron as a cofactor[78,79]. At the different cell compartments, iron is bound to low-molecular-weight molecules, giving a steady-state concentration of labile iron within the cell. This labile iron pool corresponds to a low-molecular-weight pool of weakly chelated iron that readily passes through the cell, representing a minor fraction of total cellular iron (3%-5%)[79]. The cellular labile iron pool is in equilibrium with (1) iron taken from the diet, delivered into bloodstream, and incorporated into cells through transferring-receptors; (2) iron export; (3) iron reversibly incorporated into heme and non-heme proteins; and (4) iron stored in ferritin, which constitutes a major and safe fraction of the iron that entered into the cell (Figure 3)[79].

The intracellular labile iron pool has been associated with physiological, pharmacological and toxicological iron functions. Iron is able to catalyze the conversion of by-products of respiration [superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2)] into hydroxyl radical ($HO^{\bullet}$) *via* the Fenton reaction or the Fe^{2+}-assisted Haber-Weiss reaction (Figure 3)[79], thus enhancing the oxidative stress status of the cell. Rats subjected to a sub-chronic iron administration protocol (six doses of 50 mg iron-dextran/kg, ip every second day during 10 d) showed significant enhancement in total iron and in the labile iron pool of

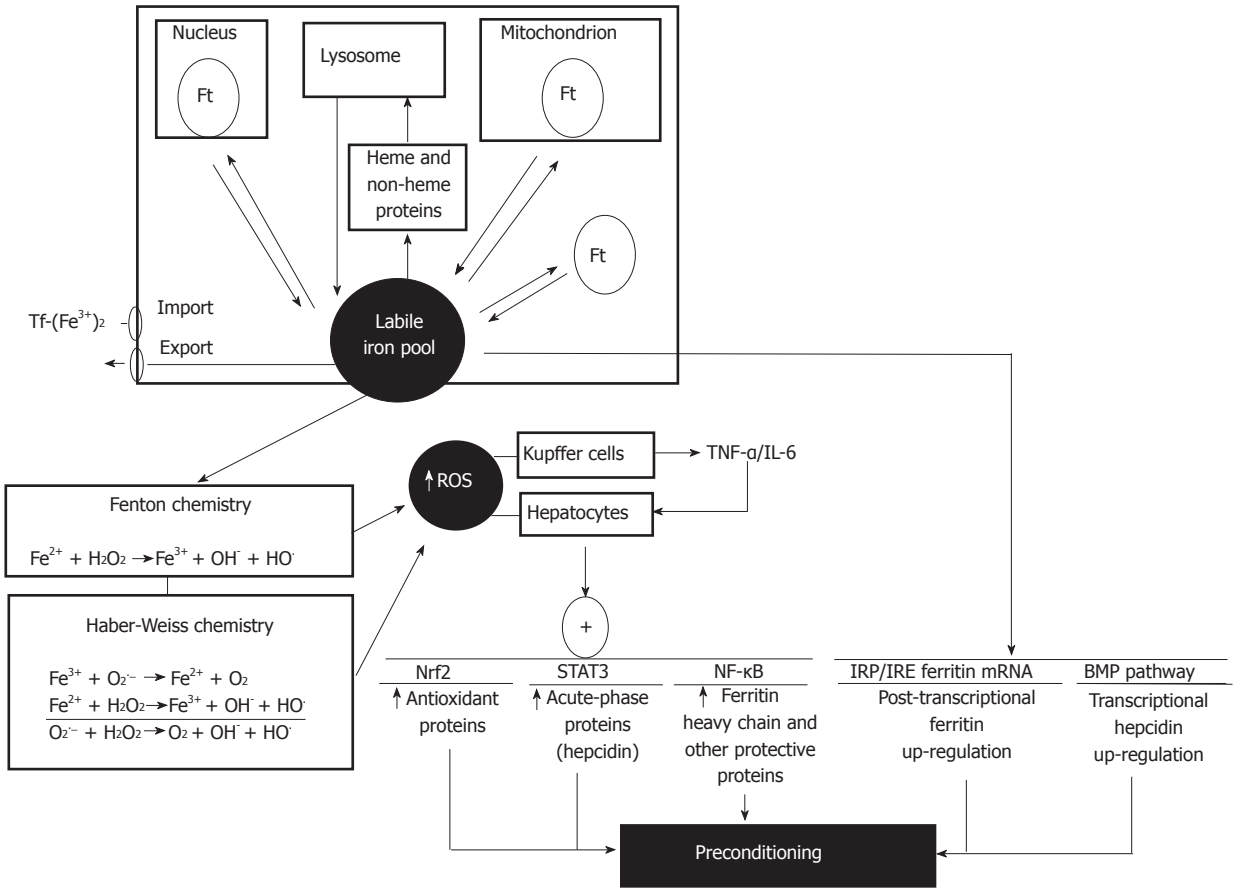

Figure 3 Redox signaling in iron (Fe)-induced liver preconditioning is elicited by the cellular labile Fe pool triggering nuclear factor-erythroid 2, signal transducer and activator of transcription 3, and nuclear factor-κB activation and iron-regulatory protein / iron-responsive element post-transcriptional up-regulation with antioxidant and acute-phase responses. BMP: Bone morphogenetic protein; Ft: Ferritin; H_2O_2: Hydrogen peroxide; $HO^{\cdot}$: hydroxyl radical; IL-6: Interleukin-6; IRE: Iron-responsive element; IRP: Iron-regulatory protein; Nrf2: Nuclear factor-erythroid 2 related factor 2; ROS: Reactive oxygen species; STAT3: Signal transducer and activator of transcription 3; $O_2^{\cdot-}$: Superoxide radical; Tf: Transferring; TNF-α: Tumor necrosis factor-α.

the liver, with consequent up-regulation of ferritin content, thus establishing a transient oxidative stress condition without development of hepatotoxicity[28]. Under these conditions, a significant protection was afforded by iron administration against liver IR injury, as evidenced by diminution in serum transaminase levels and normal liver architecture observed in iron supplemented animals subjected to IR compared to non-supplemented rats[28]. Iron liver preconditioning against IR could be due to cellular iron metabolism over the 72 h time-period between *in vivo* iron administration and the settlement of IR *in vitro*, with consequent ferritin up-regulation sequestering large amounts of administered iron to avoid liver injury, and expansion of the labile iron pool increasing the oxidative stress status that limits the further pro-oxidant challenge of IR. In addition, suppression of the TNF-α response and reversion of the changes in signal transduction and gene expression induced by IR were achieved by *in vivo* iron administration, with recovery of NF-κB activation and NF-κB-related expression of haptoglobin lost during IR (Figure 3)[28]. Haptoglobin is an anti-inflammatory and antioxidant acute-phase protein participating in the acute-phase response of the liver, a reaction restoring homeostasis by contributing to defensive and adaptive capabilities[80].

From the mechanistic viewpoint, development of transient oxidative stress in the liver of iron supplemented animals may be related to stimulation of different processes in Kupffer cells and hepatocytes. *In vivo* iron overload alters the functional status of Kupffer cells by increasing the respiratory burst activity without modifying phagocytosis, an effect that is probably related to O_2 equivalents used by NADPH oxidase to produce $O_2^{\bullet-}$ and H_2O_2, which may be further subjected to Fenton/Haber-Weiss reactions (Figure 3)[81]. Promotion of biomolecules oxidation and activation of nitric oxide synthase[82] may also contribute to this effect of iron. Iron-induced respiratory burst of Kupffer cells with enhanced ROS production[81] may have a role in NF-κB signaling, as shown by the activation of IKK and NF-κB DNA binding, leading to enhanced TNF-α promoter activity and TNF-α release from cultured Kupffer cells[83] (Figure 3). As proposed for T_3 liver preconditioning (Figure 1), TNF-α released from Kupffer cells may trigger protective signaling cascades in hepatocytes, thus achieving protection against IR liver injury. Interestingly, ferritin heavy

chain was identified as an essential mediator of the antioxidant and protective actions of NF-κB, as assessed in cultured NIN-3T3 cells[84]. This protein is induced downstream of NF-κB, providing a transcriptional regulatory mechanism for ferritin induction through iron-mediated ROS generation[85] (Figure 3), which represents a potential approach for anti-inflammatory therapy[84]. Up-regulation of ferritin expression by iron is also under post-transcriptional regulation, a mechanism involving the interaction of iron regulatory proteins with the iron-responsive elements in ferritin mRNA, to enhance ferritin synthesis and concentrate excess iron[84,86], avoiding cytotoxicity (Figure 3). Besides, iron overload up-regulates hepcidin expression, an acute-phase protein produced by hepatocytes that controls the dietary absorption, storage and tissue distribution of iron, which exhibits a significant correlation with serum ferritin levels[87]. The mechanism of hepcidin action involves internalization and degradation of ferroportin, a hepcidin-receptor and iron channel, that diminishes intestinal iron absorption, iron mobilization from hepatocytes, and iron recycling by macrophages, leading to iron entrapment in ferritin at enterocyte, macrophage and hepatocyte levels[87]. Although regulation of liver hepcidin transcription by iron involving the bone morphogenetic protein (BMP) pathway is not completely understood[87], IL-6/STAT3 signaling is a key effector of hepcidin expression during inflammatory conditions[88], a redox-sensitive pathway controlling the expression of several other acute-phase proteins (Figure 3). In addition to NF-κB and STAT3, liver Nrf2 signaling may also contribute to iron-induced preconditioning, considering (1) the enhancement in the expression of liver Nrf2 protein and catalase, glutathione-S-transferase and heme-oxygenase-1 mRNA in mice subjected to iron overloading[89]; and (2) the significant diminution in hepatic glutathione levels and in glutamate cysteine ligase activity observed in Nrf2$^{(-/-)}$ mice treated with ferric nitrilotriacetate over wild-type animals[90] (Figure 3).

CONCLUDING REMARKS

T_3, n-3 LCPUFA and iron can be considered as hormetic agents[91,92], which are defined as compounds inducing a dose-response phenomenon characterized by biologically beneficial effects in the low-concentration (dose) range (organ preconditioning)[26-28] and harmful responses at high concentrations (doses) or after prolonged exposure (thyrotoxicosis[93], gastrointestinal upset/increase bleeding time[48] and hemochromatosis[94], respectively). Organ preconditioning by these hormetic agents is not restricted to the liver[26-28], considering that (1) thyroid hormone-induced preconditioning against IR injury is also observed in the heart[95,96], with a pattern of protection comparable to that of ischemic preconditioning[97]; (2) beneficial effects of n-3 LCPUFA have been demonstrated in rheumatoid arthritis, inflammatory bowel disease, coronary artery disease, asthma and sepsis, conditions with inflammation as a key component of their pathology[48], in addition to neuroinflammation in all major central nervous system diseases[98]; and (3) protective effects of iron are reported in cardiomyocytes and heart[99-101], oligodendroglia cells[102] and neurones[103]. T_3, n-3 LCPUFA or iron liver preconditioning are suitable to be introduced in the clinical setting, considering that these hormetins are known to be well tolerated in the treatment of hypothyroidism[104], non-alcoholic fatty liver disease[72-76] and other diseases[48], or anemia[105,106], respectively. Interestingly, there is evidence to conclude that n-3 LCPUFAs potentiate the effects of certain drugs, thereby allowing a reduction of their required dose, thus avoiding adverse effects[48]. In agreement with this view, combined n-3 LCPUFA (300 mg/kg for 3 consecutive days) plus T_3 (0.05 mg/kg) administration prevented rat liver IR injury, whereas separate protocols lack protection[107], when compared with the preconditioning action afforded by separate n-3 LCPUFA (300 mg/kg for 7 consecutive days)[27] or T_3 (0.1 mg/kg)[26]. Data discussed in this article warrants further experimental and clinical research in the future, to support the incorporation of T_3, n-3 LCPUFA and iron preconditioning strategies or their combinations in human liver resections and in human liver transplantation using reduced-size grafts from living donors.

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