

# Cytoprotective and suicidal signaling in oxidative stress\*

Luis A Videla

Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile

## ABSTRACT

Oxidative stress is an imbalance between pro-oxidants and antioxidants in favor of the pro-oxidants, leading to different responses depending on the level of pro-oxidants achieved and the duration of exposure. In this article, we discuss the cytoprotective or suicidal signaling mechanisms associated with oxidative stress by addressing: (i) the development of an acute and mild pro-oxidant state by thyroid hormone administration eliciting the redox upregulation of the expression of proteins affording cell protection as a preconditioning strategy against ischemia-reperfusion liver injury; and (ii) the role of prolonged and severe oxidative stress and insulin resistance as determinant factors in the pathogenesis of non-alcoholic fatty liver disease associated with obesity.

**Key terms:** oxidative stress, thyroid hormone, liver preconditioning, obesity, insulin resistance, non-alcoholic fatty liver disease.

## INTRODUCTION

Due to the aerobic nature of cellular metabolism, O<sub>2</sub> reduction is a major event in biological systems. This process proceeds through one-electron transfer reactions, considering the electronic structure of O<sub>2</sub> in the ground state with two unpaired electrons in the outer orbital. The result of one-electron reduction of O<sub>2</sub> is the generation of reactive oxygen species (ROS) including free radicals, peroxides, and excited states (Videla and Fernández, 1988). The detoxication of ROS is a major prerequisite of aerobic life (Sies, 1986), which is accomplished via several enzymatic and non-enzymatic antioxidant mechanisms that are available in different cell compartments (Sies, 1986; Fernández and Videla, 1996). Secondary mechanisms restoring used cofactors and repairing altered biomolecules are also required, in addition to those triggering the expression of proteins damaged by ROS or needed to attain cell survival (Sies, 1986; Fernández and Videla, 1996; Dröge, 2002). These mechanisms need to be coupled to the intermediary metabolism for ATP, NADPH, and precursors supply, and depend on the dietary replenishment of essential components to maintain pro-oxidant reactions and cellular damage at a minimum level under basal conditions.

As proposed by Sies (1986), oxidative stress is a redox disequilibrium in which the pro-oxidant/antioxidant balance is shifted in favor of the pro-oxidants. At the cellular level, oxidative stress leads to a wide spectrum of responses, depending on the cell type, the level of ROS achieved, and the duration of the exposure (Dröge, 2002; Poli et al., 2004). The moderate increase in ROS and reactive nitrogen species (RNS) in a defined time window can elicit an imbalance capable of redox regulation, as found for L-3,3',5-triiodothyronine (T<sub>3</sub>)-induced oxidative stress (Varela et al., 2006), which may involve important signals regulating either protein function, via reversible oxidation or nitrosation of

protein sulfhydryls, and/or gene expression, through modulation of specific kinases, phosphatases, and redox-sensitive transcription factors (Dröge, 2002; Poli et al., 2004). However, in the case of organs subjected to ischemia-reperfusion (IR) (Romanque et al., 2005) or in obesity (Videla, 2008), large levels of ROS are attained, which may induce loss of cell viability and death. The relevance of oxidative stress on human pathology is manifold. Oxidative stress can trigger an irreversible situation in which severe oxidation of biomolecules by ROS cannot be overcome by antioxidant, repair, and biosynthetic pathways. This condition may also trigger dysregulation of signal transduction and gene expression, leading to cell death through necrotic and/or apoptotic mechanisms (Dröge, 2002). The limit between irreversible and reversible oxidative stress is not clear (Boveris et al., 2008), however, the latter form of redox imbalance involves stimulation of signal transduction mechanisms leading to beneficial effects, which may contribute to avoiding pathological events or help to recover from an already present pathological condition. In humans, determination of oxidative stress markers in blood and plasma usually reflect the production of ROS in the vascular space or oxidative damage occurring in target organs. Thus, antioxidant therapy may improve the health of patients with chronic pathologies or that are subjected to chemotherapy or radiotherapy; in these cases, assessment of systemic oxidative stress provides diagnostic and treatment control (Boveris et al., 2008).

In this review article, we address the cytoprotective or suicidal signaling mechanisms associated with oxidative stress by discussing (i) the implications of the redox regulation of T<sub>3</sub>-induced gene expression as a preconditioning mechanism against IR liver injury; and (ii) the role of oxidative stress and insulin resistance as contributor factors in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) in obese patients.

Corresponding author: Prof. Luis A. Videla, Programa de Farmacología Molecular y Clínica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. E-mail: [videla@med.uchile.cl](mailto:videla@med.uchile.cl), Telephone: 56-2-9786256, fax: 56-2-7372783.

Received: June 9, 2010. In revised form: July 22, 2010. Accepted: August 11, 2010.

\* This review article was written as a tribute to Professor Gustavo Hoecker for his mastery in Cellular Biology, teaching and research achievements, representing outstanding efforts in the development of scientific research in Chile.

## OXIDATIVE STRESS AND CYTOPROTECTIVE SIGNALING: THYROID HORMONE LIVER PRECONDITIONING

### *Thyroid hormone-induced liver oxidative stress*

In mammals, thyroid hormones exert major effects on cellular oxygen consumption ( $QO_2$ ) and metabolic rates, leading to stimulation and maintenance of basal thermogenesis (Schwartz and Oppenheimer, 1978). This action of  $T_3$  is carried out via thyroid hormone receptors expressed in almost all tissues, which are recognized by specific thyroid hormone response elements in DNA, leading to ligand-dependent upregulation of the expression of respiratory, metabolic, and uncoupling protein genes (Fig. 1A) (Videla et al., 2007). In addition to the above classical genomic model of  $T_3$ -dependent calorogenesis, non-genomic mechanisms may also contribute to increasing cellular  $QO_2$ , with the consequent enhancement of the mitochondrial capacity for oxidative phosphorylation and ROS generation (Fernández and Videla, 1993).  $T_3$ -induced ROS production also involves (i) higher activities of microsomal NADPH-cytochrome P450 reductase (Ram and Waxman, 1992) and NADPH oxidase (Fernández et al., 1985), which is related to the induction of the highly pro-oxidant cytochrome P450E1 isoform by  $T_3$  (Fernández et al., 2003); (ii)  $O_2^-/H_2O_2$  generation by xanthine oxidase (Huh et al., 1998) or coupled to enhanced fatty acid  $\beta$ -oxidation due to liver peroxisomal proliferation (Fernández and Videla, 1993); and (iii) Kupffer-cell activation with increased respiratory burst activity due to NADPH oxidase (Tapia et al., 1997).

Liver depletion of antioxidant defenses is a major consequence of  $T_3$ -induced ROS generation, leading to enhancement in the oxidative stress status of the liver (Fig. 1A) (Varela et al., 2006; Venditti and Di Meo, 2006; Videla et al., 2007). This pro-oxidant state achieved in the liver by  $T_3$ -induced calorogenesis can be considered as a mild redox alteration, as suggested by the lack of occurrence of morphological changes in liver parenchyma, except for the significant hyperplasia and hypertrophy of Kupffer cells, the resident macrophages of the liver (Tapia et al., 1997) playing a central role in the homeostatic response to liver injury (Tsukamoto, 2002).

### *$T_3$ -induced Kupffer cell-dependent upregulation of cytokine expression and hepatocyte proteins related to cytoprotection*

Kupffer cell hyperplasia is a major finding after *in vivo*  $T_3$  administration, an effect that may involve the expansion of Kupffer cell precursors by means of circulating monocyte recruitment, the differentiation of pre-existing local Kupffer cell precursors into mature liver macrophages, or both (Gomes et al., 2004). Consequently, enhancement in Kupffer cell function is achieved as shown by the significant increases in the rate of carbon phagocytosis and the associated carbon-induced  $O_2$  uptake representing the respiratory burst activity of Kupffer cells. This process is largely dependent on the activity of the ROS-generator NADPH oxidase and abolished by pretreatment with the Kupffer cell inactivator gadolinium chloride ( $GdCl_3$ ) (Fig. 1A) (Tapia et al., 1997). Under these conditions, significant increases in the hepatic DNA binding of the transcription factors NF- $\kappa$ B (Tapia et al., 2003), STAT3 (Tapia et al., 2006),

and AP-1 (Fernández et al., 2007a) (Fig. 1A) are observed. Activation of these transcription factors by  $T_3$  administration is suppressed by the *in vivo* pretreatment with  $GdCl_3$  or antioxidants prior to hormone treatment (Tapia et al., 2003; 2006), thus supporting the view that  $T_3$  induces the redox activation of hepatic NF- $\kappa$ B, STAT3, and AP-1 by actions primarily exerted at the Kupffer cell level (Fig. 1A).  $T_3$  administration involving significant NF- $\kappa$ B and AP-1 activation induced mRNA expression of the NF- $\kappa$ B/AP-1-responsive genes for TNF- $\alpha$ , with increased serum levels of the cytokine (Fernández et al., 2002; Tapia et al., 2003).  $T_3$  also elicited an increase in the serum levels of IL-6 (Tapia et al., 2006) and in the hepatic mRNA expression and serum levels of IL-1 (Tapia et al., 2003). In addition to NF- $\kappa$ B and AP-1 activation, the enhancement in STAT3 DNA binding by  $T_3$  administration (Tapia et al., 2006) may be associated with the proliferation of macrophage precursors and their differentiation into Kupffer cells (Gomes et al., 2004), considering the central role of STAT3 in gp130-mediated cell growth, differentiation, and survival (Hirano et al., 2000).

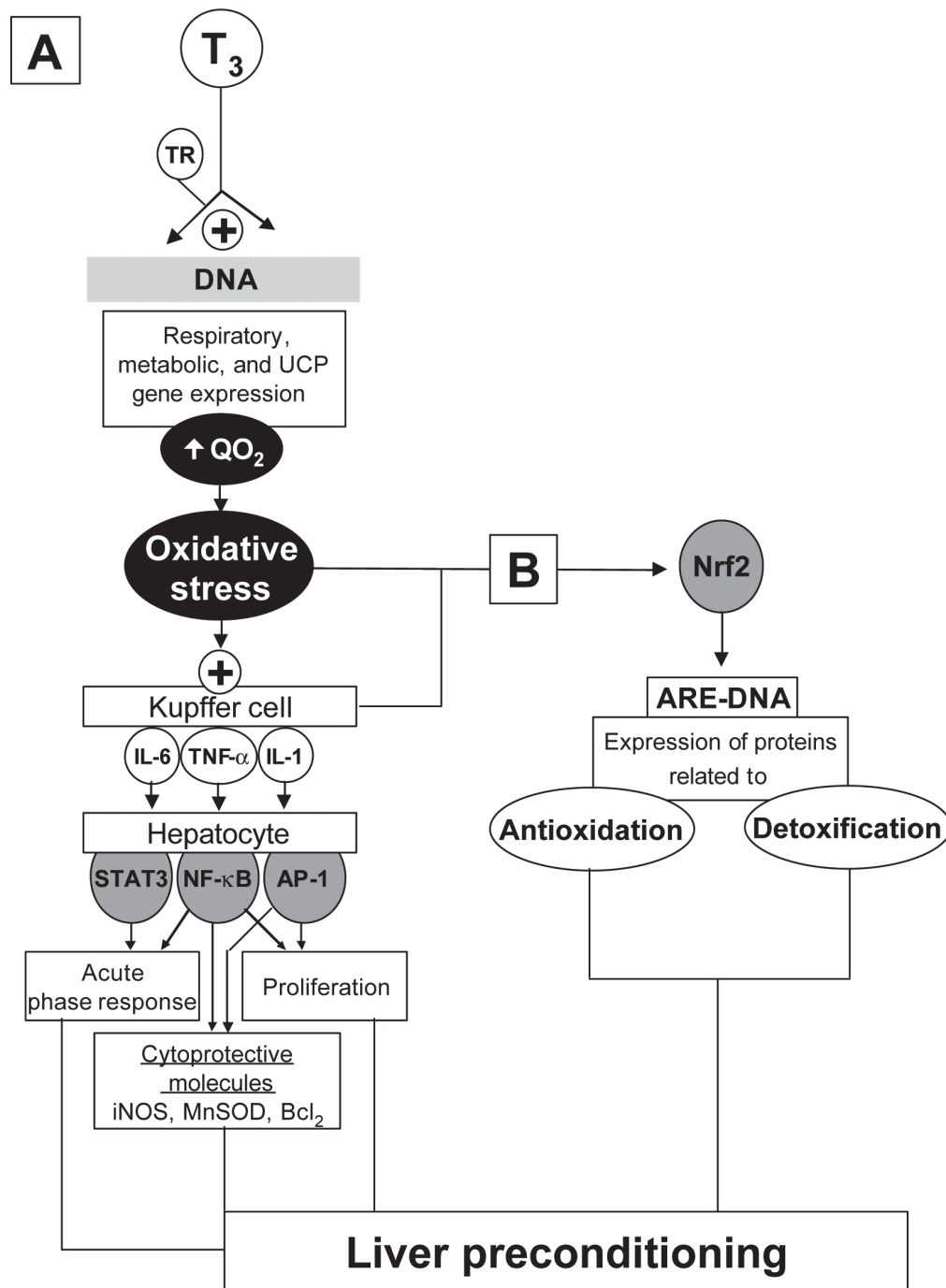
The effects of cytokines released from Kupffer cells are exerted through their interaction with specific surface receptors in target cells within the liver to mediate signaling from cell membrane to the nucleus (Tsukamoto, 2002). In agreement with the above view, the transient TNF- $\alpha$  response elicited by  $T_3$  administration correlates with the substantial increase in liver I $\kappa$ B- $\alpha$  phosphorylation (Fernández et al., 2005a; 2005b), evidencing the activation of the IKK complex that activates NF- $\kappa$ B (Gloire et al., 2006).  $T_3$ -induced TNF- $\alpha$  response, liver IKK phosphorylation, and NF- $\kappa$ B activation are abolished by pretreatment with either  $\alpha$ -tocopherol or  $GdCl_3$ , supporting the role of ROS production and Kupffer-cell activation in  $T_3$ -dependent signaling (Fernández et al., 2005a; 2005b). This is evidenced by upregulation of the NF- $\kappa$ B-responsive genes encoding for the hepatoprotective proteins inducible NOS (iNOS) (Fernández et al., 2005a), manganese superoxide dismutase (MnSOD), and the anti-apoptotic protein Bcl-2 (Fernández et al., 2005b) (Fig. 1A).

$T_3$  administration also upregulated the acute-phase response (APR) of the liver and hepatocyte proliferation. The APR is a major pathophysiologic reaction, in which normal homeostatic mechanisms are replaced by new set points contributing to defensive or adaptive capabilities against inflammation and oxidative stress (Ramadori and Christ, 1999). In fact,  $T_3$  induced the Kupffer-cell-dependent release of IL-6 and activation of hepatic STAT3 controlling both type I (haptoglobin) and type II ( $\beta$ -fibrinogen) acute-phase protein (APP) genes (Tapia et al., 2006), a response that may be contributed to by upregulation of the TNF- $\alpha$ /IKK/NF- $\kappa$ B pathway (Fernández et al., 2005a; 2005b) controlling type I APP genes (Agrawal et al., 2003). Furthermore, the *in vivo* effects of  $T_3$  as a primary hepatic mitogen leading to hepatocyte proliferation in intact liver are well established (Fig. 1A) (Fernández et al., 2007a), a process involving a large number of genes that requires the concurrence of cytokine, growth factor, and metabolic networks (Fausto et al., 2006). Resting hepatocytes in the G0 phase of the cell cycle need to be primed by TNF- $\alpha$  and IL-6 before they can respond to growth factors, with the concomitant activation of NF- $\kappa$ B, STAT3, AP-1, and E/EBPb, to enter the G1 phase and initiate cell cycle progression.  $T_3$  administration was

associated with increased liver cyclin-dependent kinase 2 expression and hepatocyte proliferation, as evidenced by the enhancement in Ki-67, a nuclear cell proliferation-associated protein expressed in all active parts of the cell cycle, and in the proliferating cell nuclear antigen (PCNA) (Fernández et al., 2007a).

Collectively, the data discussed indicate that  $T_3$  triggers cytoprotection in the liver through redox- and Kupffer cell-

dependent signaling mechanisms, leading to (i) antioxidant responses (iNOS, MnSOD); (ii) anti-apoptosis (Bcl-2); (iii) immune, transport, and antioxidant (haptoglobin, ceruloplasmin, ferritin) functions fulfilled by APR induction; and (iv) hepatocyte proliferation (Fig. 1A) being the associated metabolic demands met by acceleration of energy metabolism due to  $T_3$ -induced calorigenesis (Varela et al., 2006; Videla et al., 2007).



**Figure 1:** Oxidative stress signaling in thyroid hormone ( $T_3$ ) liver preconditioning as mediated by redox-sensitive transcriptional factors NF- $\kappa$ B, AP-1, and STAT3 (A) or Nrf2 (B). Abbreviations: AP-1, activating protein 1; ARE, antioxidant responsive element; GdCl<sub>3</sub>, gadolinium chloride; IL, interleukin; iNOS, inducible nitric oxide synthase; MnSOD, manganese superoxide dismutase; NF- $\kappa$ B, nuclear factor- $\kappa$ B; Nrf2, nuclear factor-erythroid 2-related factor 2; QO<sub>2</sub>, rate of oxygen consumption; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; STAT3, signal transducer and activator of transcription 3; TR, thyroid hormone receptor; UCP, uncoupling protein.

*Thyroid hormone-induced liver preconditioning*

In general terms, IR injury refers to tissue damage produced by blood perfusion to the previously ischemic organ, which in the liver is associated with the clinical settings of hepatic resection, transplantation, low-blood pressure states, and abdominal surgery requiring hepatic vascular occlusion (Romanque et al., 2005; Casillas-Ramírez et al., 2006). IR liver injury assessed in a model involving 1 hour of partial ischemia induced by vascular clamping followed by reperfusion for 20 h elicited substantial liver damage, concomitantly with (i) a drastic increase in the oxidative stress status of the liver and TNF- $\alpha$  response; (ii) loss in the DNA binding of NF- $\kappa$ B and STAT3 implying loss of cytoprotective potential; and (iii) enhancement in the hepatic AP-1 DNA binding activity, which may constitute a major determinant of hepatotoxicity under conditions of reduced NF- $\kappa$ B activation and TNF- $\alpha$  response (Fernández et al., 2007b; 2008). These changes were normalized by T<sub>3</sub> treatment given 48 h before the IR protocol, a preconditioning effect that was sensitive to the antioxidant N-acetylcysteine given prior to T<sub>3</sub> (Fernández et al., 2008), with enhanced hepatocyte proliferation compensating for liver cells lost due to IR-induced hepatocellular necrosis (Fernández et al., 2007a).

In conclusion, redox regulation of gene transcription induced by T<sub>3</sub> involves antioxidant-sensitive NF- $\kappa$ B, AP-1, and STAT3 activation and upregulation of the expression of cytoprotective proteins affording liver preconditioning (Fig. 1A) (Fernández and Videla, 2009). T<sub>3</sub> liver preconditioning may also involve the activation of the Nrf2-Keap1 defense pathway up-regulating antioxidant proteins and phase-2 detoxifying enzymes (Fig. 1B) (Kobayashi and Yamamoto, 2005), which is currently under study in our laboratory.

#### OXIDATIVE STRESS AND SUICIDAL SIGNALING: NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) ASSOCIATED WITH OBESITY

##### *Oxidative stress and insulin resistance as pathogenic factors in steatosis in obese NAFLD patients*

NAFLD includes simple triacylglycerol (TAG) accumulation in hepatocytes (hepatic steatosis) or steatosis with inflammation, fibrosis, and cirrhosis (non-alcoholic steatohepatitis, NASH), with oxidative stress, insulin resistance, and nutritional factors playing major contributory roles (Angulo, 2002; Videla et al., 2004a). Fatty acids (FAs) are the major oxidative fuel in the liver under most circumstances, however, carbohydrate and lipid affluence induce significant changes in hepatic intermediary metabolism. In fact, high glucose and insulin levels stimulate FA synthesis from glucose and inhibit FA  $\beta$ -oxidation, re-directing FAs towards the formation of TAG (Videla, 2008). Upon consumption of calorie-enriched diets, FAs in excess are likely converted to TAGs and stored as lipid droplets within hepatocytes due to limitations in VLDL synthesis and export. Since non-adipose tissue has limited capacity for TAG storage, the lipids in excess that accumulate under conditions of over-nutrition determine high intracellular levels of saturated FAs, which can induce cell dysfunction and/or cell death, a phenomenon known as lipotoxicity

(Aronis et al., 2005). Consequently, higher rates of FA oxidation and ROS generation are achieved, which might explain the increases in the oxidative stress-related parameters and antioxidant depletion found in the liver of obese NAFLD patients with steatosis (Fig. 2) (Videla et al., 2004b; Videla, 2008). Prolonged oxidative stress might, in turn, favour: (i) liver *n*-3 LCPUFA depletion, which may be compounded by dietary imbalance and defective desaturation activity (Araya et al., 2004; Elizondo et al., 2008); and (ii) insulin resistance, in association with the redox activation of multiple stress-sensitive serine/threonine kinases that alters insulin signaling (Fig. 2) (Evans et al., 2005), a phenomenon is a membrane-mediated process that might be also compromised by *n*-3 LCPUFA depletion due to loss of membrane polyunsaturation. Both IR and liver *n*-3 LCPUFA depletion can determine hepatic steatosis by different mechanisms, namely, (i) insulin resistance-dependent higher peripheral mobilization of FA and glycerol to the liver; and (ii) *n*-3 LCPUFA depletion-induced changes in the DNA binding activity of the peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ )/sterol regulatory element binding protein-1c (SREBP-1c) couple, determining a metabolic imbalance between FA oxidation and lipogenesis in favor of the latter (Fig. 2). This contention is based on the findings that *n*-3 LCPUFA are signaling biomolecules regulating hepatic lipid metabolism through (i) downregulation of the expression of SREBP-1c and its processing, with inhibition of the transcription of lipogenic and glycolytic genes; and (ii) upregulation of the expression of genes encoding enzymes of FA oxidation, acting as ligand of PPAR- $\alpha$  (Clarke, 2004).

##### *Hepatic oxidative stress enhancement and progression from steatosis to steatohepatitis*

In steatohepatitis, the alterations in oxidative stress indexes found in steatosis are observed concomitantly with (i) low catalase activity (Videla et al., 2004b); (ii) high immunohistochemical reactivity to 8-hydroxydeoxyguanosine and 4-hydroxy-2-nonenal as markers of oxidative DNA damage and lipid peroxidation, respectively (Seki et al., 2002); (iii) a further increment in 3-nitrotyrosine immunoreactivity and in the production of O<sub>2</sub><sup>-</sup> and malondialdehyde by Kupffer cells; (iv) induction of inducible nitric oxide synthase; and (v) upregulation of cytochrome P450 2E1 (CYP2E1) as evidenced by the higher protein expression of CYP2E1 and *in vivo* chlorzoxazone hydroxylation, an indicator of CYP2E1 activity (Fig. 2) (Videla, 2008).

Exacerbation of the oxidative stress status of the liver in steatohepatitis compared to steatosis seems to involve several mechanisms (Fig. 2). Upregulation of liver CYP2E1 (Videla et al., 2004b; Orellana et al., 2006) is of particular importance in the pathogenesis of NASH due to its large capacity for ROS generation with the consequent lipid peroxidation response (Caro and Cederbaum, 2004). Hepatic mitochondrial dysfunction is an alternate contributory factor to the genesis of lesions in steatohepatitis, considering the lower levels of mitochondrial DNA and decreased expression of mitochondrial DNA-encoded proteins, with reduced activity of respiratory complexes I, III, and IV, and ATP synthase complex V and enhanced ROS generation

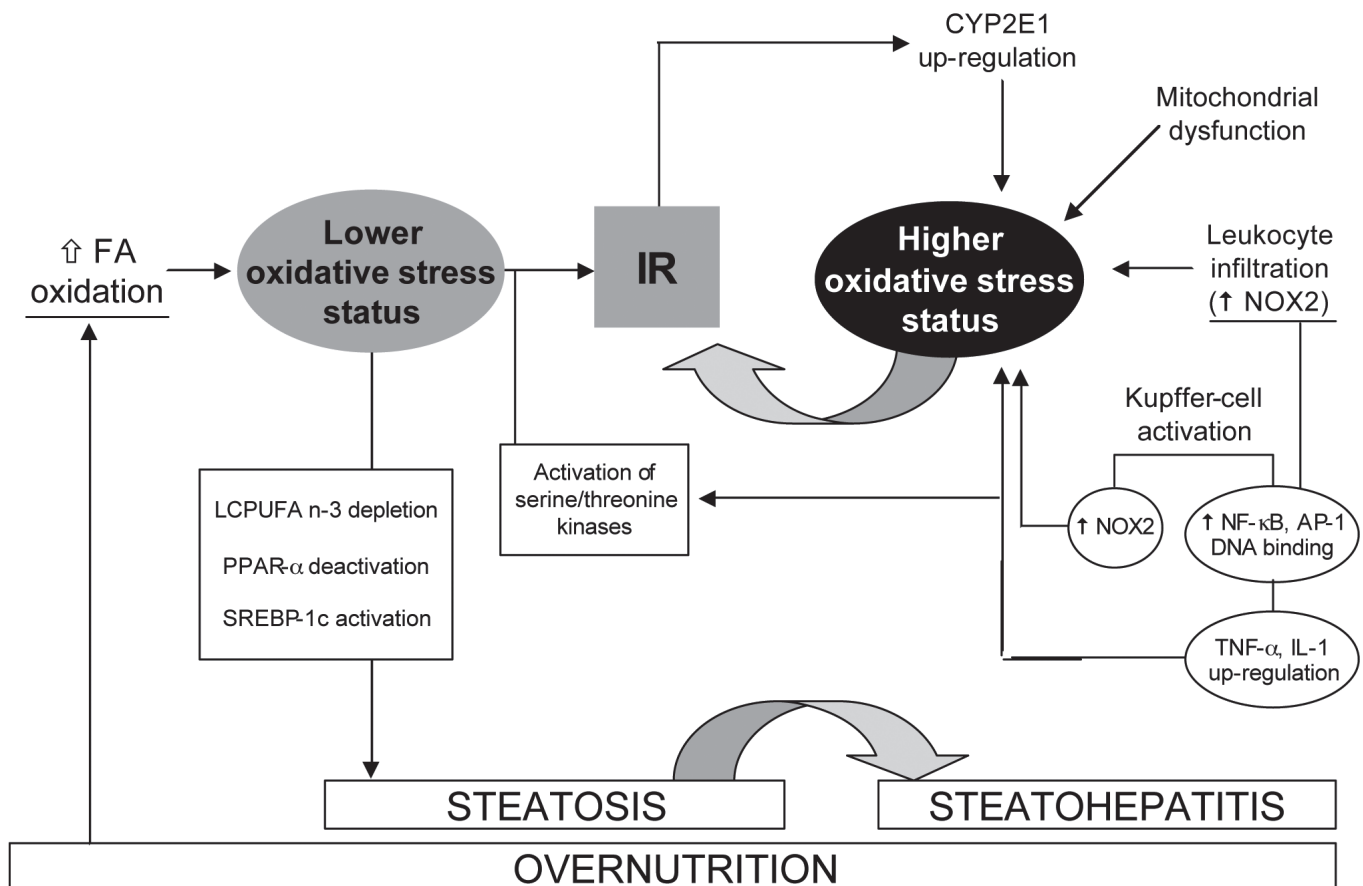
(Angulo, 2002). In addition, mixed inflammatory-cell infiltration is a characteristic feature of NASH including mononuclear cells, polymorphonuclear cells, or both (Angulo, 2002), which may represent an additional mechanism of ROS generation due to the expression and activation of NADPH oxidase (NOX2), an enzyme producing large amounts of ROS (Babior, 2004). NOX2 is also expressed in Kupffer cells, which in patients with steatohepatitis produce ROS at rates that are 20-fold higher than normal, with a substantial increase in Kupffer cell-dependent lipid peroxidation in steatohepatitis patients (Malaguarnera et al., 2006). Under these conditions, the oxidative stress status of the liver achieved in steatohepatitis might promote hepatocellular damage by inducing (i) severe oxidative alteration of biomolecules with loss of their functions and impairment of cell viability; and (ii) prolonged activation of redox-sensitive transcription factor such as NF- $\kappa$ B and AP-1, with consequent upregulation of the expression of pro-inflammatory mediators at the Kupffer cell level (Fig. 2) (Videla et al., 2009).

Collectively, the discussed evidence supports the view of a functional interdependence between oxidative stress and insulin resistance (Fig. 2). This proposal might involve (i) the onset of ROS production due to lipotoxicity and the consequent development of insulin resistance in steatosis;

and (ii) a further enhancement in ROS generation due to CYP2E1 induction, mitochondrial dysfunction, and phagocyte NOX2 activity characterizing steatohepatitis. Dysregulation of pro-inflammatory cytokine, adipokine, and chemokine signaling in NAFLD may reinforce the initial mechanisms of ROS production and IR, representing key factors in the progression from stable steatosis to steatohepatitis, in the setting of oxidative stress-mediated hepatocyte sensitization (Videla et al., 2004a; Videla et al., 2006). In this context, antioxidants can act as insulin sensitizers by lowering ROS levels, a condition that might abrogate free-radical-mediated activation of signaling serine/threonine kinases and damage to biomolecules, as shown in cell-culture studies (Houstis et al., 2006). However, these findings remain to be confirmed in obese NAFLD patients.

#### CONCLUDING REMARKS

The development of extreme levels of oxidative stress in the liver determines opposite cellular responses, depending on the period of exposure to ROS. Following T<sub>3</sub> administration, induction of a mild pro-oxidant state within a time window of 48 h triggers liver preconditioning (Fernández et al., 2007b). This preconditioning strategy has clinical potential,



**Figure 2:** Interrelationships between the level of oxidative stress and insulin resistance achieved leading to hepatic steatosis and its progression to steatohepatitis associated with over-nutrition. Abbreviations: AP-1, activating protein 1; CYP2E1, ethanol inducible form of cytochrome P450; FA, fatty acid; IL-1, interleukin-1; LCPUFA; long-chain polyunsaturated fatty acid; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NOX2, NADPH oxidase in phagocytic cells; PPAR- $\alpha$ , peroxisome proliferator-activated receptor- $\alpha$ ; SREBP-1c, sterol regulatory element binding protein-1c; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

considering that (i) pharmacological and other liver preconditioning maneuvers have not been transferred to clinical application (Casillas-Ramírez et al., 2006); and (ii) T<sub>3</sub> is an endobiotic substance, widely used and a well-tolerated therapeutic agent, which at low doses has either no significant adverse effects or minimal side actions than can be readily controlled. However, prevention of IR injury in humans during liver surgery and liver transplantation using reduced-size grafts from living donors await further experimental and clinical studies. Development of a progressive and severe pro-oxidant state in the liver of obese NAFLD patients is associated with the onset of steatosis and its progression to steatohepatitis as a chronic model of nutritional oxidative stress. The molecular pathogenesis of NAFLD in obese patients seems to be multifactorial, with oxidative stress and insulin resistance as major pathophysiological mechanisms that might be interdependent (Videla et al., 2006). Considering the lack of an effective drug therapy for NAFLD at present (Angulo, 2002), further studies of potentially attractive therapeutic targets are required. These might include the expression and activation status of metabolic (PPAR- $\alpha$  and SREBP-1c) and pro-inflammatory (NF- $\kappa$ B and AP-1) transcription factors, and the activity of enzymes associated with insulin resistance such as serine/threonine stress kinases and protein tyrosine phosphatases, which will undoubtedly contribute to understanding the role of chronic and progressive oxidative stress and insulin resistance in determining steatosis and its progression to NASH. It is suggested that weight loss, as a central therapeutic issue, might be combined with antioxidants, to minimize or prevent the onset of oxidative stress-induced inflammatory response and insulin resistance, and/or *n*-3 LCPUFA, to improve the efficiency of signaling cascades related to hepatic lipid metabolism and insulin resistance (Videla, 2008).

#### ACKNOWLEDGEMENTS

The author is grateful to FONDECYT (grant 1090020) for financial support. Thanks are due to Drs. V. Fernández, G. Tapia, P. Varela, P. Romanque, P. Cornejo, R. Rodrigo, J. Araya, and J. Poniachik for valuable discussions. Their contributions are cited in this review.

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