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Original Contribution

Causal role of oxidative stress in unfolded protein response development in the hyperthyroid state

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ABSTRACT

L-3,3′,5-Triiodothyronine (T_3)-induced liver oxidative stress underlies significant protein oxidation, which may trigger the unfolded protein response (UPR). Administration of daily doses of 0.1 mg T_3 for three consecutive days significantly increased the rectal temperature of rats and liver O_2 consumption rate, with higher protein carbonyl and 8-isoprostane levels, glutathione depletion, and absence of morphological changes in liver parenchyma. Concomitantly, liver protein kinase RNA-like endoplasmic reticulum (ER) kinase and eukaryotic translation initiator factor 2α were phosphorylated in T_3-treated rats compared to controls, with increased protein levels of binding immunoglobulin protein and activating transcription factor 4. In addition, higher mRNA levels of C/EBP homologous protein, growth arrest and DNA damage 34, protein disulfide isomerase, and ER oxidoreductin 1α were observed, changes that were suppressed by *N*-acetylcysteine (0.5 g/kg) given before each dose of T_3. In conclusion, T_3-induced liver oxidative stress involving higher protein oxidation status has a causal role in UPR development, a response that is aimed to alleviate ER stress and promote cell survival.

Ischemia and reperfusion (IR) of the liver lead to severe injury (IRI), a feature observed in liver transplantation and liver resection that is associated with oxygen and nutrient deprivation occurring during the procedure in the former case and with vascular occlusion to prevent hemorrhage in the latter case [1,2]. The mechanisms underlying liver IRI gathered in experimental models led to the development of numerous surgical and pharmacological protective strategies; however, few have reached clinical practice [1,2]. Development of an acute, nonlethal burst of oxidative stress constitutes a major pharmacological liver preconditioning (PC) strategy [3,4], which includes the administration of thyroid hormone (L-3,3′,5-triiodothyronine; T_3) before an IR protocol [5], as evidenced by the reestablishment of liver injury after the administration of the antioxidant *N*-acetylcysteine (NAC) before T_3 [6].

T_3 is considered a hormetic agent that triggers biologically beneficial effects in the liver in the low-dose range, leading to cell proliferation and the induction of proteins affording cellular protection, namely, antioxidant, antiapoptotic, and acute-phase proteins [7]; phase II detoxification enzymes; and phase III transporters [8]; and downregulation of the mRNA and protein expression of cytokines and adhesion molecules related to liver inflammation [9]. Accordingly, liver PC is confronted with a major energy requirement to cope with ATP demands for operation of most of the PC mechanisms outlined above, plus those for repair and resynthesis of altered biomolecules during IR and repletion of ATP levels reduced in the ischemic phase [7]. In agreement with this view, T_3 administration upregulates liver AMP-activated protein kinase (AMPK) [10], a sensor of energy status supporting cellular energy homeostasis [11]. Liver AMPK upregulation by T_3 is associated with significant enhancements in (i) AMPK mRNA expression, (ii) AMPK phosphorylation coupled to the activation of the upstream kinases Ca^{2+}–calmodulin-dependent protein kinase kinase-β and transforming growth factor-β-activated kinase-1, and (iii) AMP/ATP ratios promoting allosteric AMPK activation [10]. Consequently, AMPK signaling induced by T_3 enhances hepatic fatty acid oxidation leading to a ketogenic response, representing a key molecular mechanism regulating energy dynamics to limit metabolic stresses such as IRI [12].

T_3-induced liver O_2 consumption is accompanied by an elevation in the rate of mitochondrial superoxide radical ($O_2^{\bullet -}$) [13] and hydrogen peroxide (H_2O_2) [13,14] generation. Liver production of reactive oxygen species (ROS) induced by T_3 is also elicited at microsomal, cytosolic, and peroxisomal levels in hepatocytes, with Kupffer cells playing a contributory role owing to enhancement of

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the respiratory burst activity [15]. Under these conditions, T_3 achieved a substantial enhancement in hepatic free radical-dependent protein oxidation, assayed by the reaction of protein carbonyls with 2,4-dinitrophenylhydrazine, in addition to a lipid peroxidation response [16]. Oxidized proteins undergo partial unfolding or misfolding due to the loss of secondary and tertiary structures within the domain of the oxidative impact, which results in proteome instability that may trigger endoplasmic reticulum (ER) stress [17]. The ER is primarily involved in the synthesis, folding, and maturation of secreted and transmembrane proteins, lipid biosynthesis, and Ca^{2+} homeostasis, and overload of these functions leads to ER stress and the unfolded protein response (UPR) [18]. Upon ER stress, ER-located transmembrane sensors evoke diverse protective responses through exposure of hydrophobic domains in oxidized proteins, which may be recognized by chaperones or by the proteasome, representing an important adaptive mechanism in secretory cells such as hepatocytes to attain homeostatic regulation [17,18]. Considering the role of T_3 in liver PC [5,15] and that the cellular UPR program is aimed to alleviate ER stress promoting cell survival [19], the aim of this work was to test the hypothesis that in vivo T_3 administration involving the development of oxidative stress with a protein oxidation response triggers UPR development in the liver. For this purpose, rats were subjected to a T_3 protocol after which parameters related to T_3 calorigenesis, liver viability, and hepatic indicators of oxidative stress (levels of reduced glutathione (GSH), 8-isoprotanes, and protein carbonyls) and ER stress (components of the protein kinase RNA-like ER kinase (PERK) axis and protein disulfide isomerase (PDI)–ER oxidoreductin 1α (ERO1α) couple) were measured, followed by assessments in separate groups of animals upon antioxidant (NAC) intervention.

1. Materials and methods

1.1. Animal treatment

Male Sprague–Dawley rats (animal facility of the Institute of Biomedical Sciences, Faculty of Medicine, University of Chile) weighing 180–200 g were housed on a 12 h light/dark cycle and were provided with rat chow and water ad libitum. Animals received an intraperitoneal dose of 0.1 mg of T_3/kg bw or equivalent volumes of hormone vehicle (0.1 N NaOH, controls) at time 0, 24, and 48 h, and studies were performed 24 h after hormone treatment. At this experimental time, T_3-induced calorigenesis was assessed by the rectal temperature of the animals by means of a thermocouple (Cole-Palmer Instrument Co., Chicago, IL, USA), and the rats were anesthetized with 1 ml/kg zolazepam chlorhydrate (25 mg/ml) and tiletamine chlorhydrate (25 mg/ml) ip (Zoletil 50; Virbac S/A, Carros, France) to obtain blood by cardiac puncture and liver samples. Blood serum was used to measure T_3 levels via ELISA (Monobind, Lake Forest, CA, USA), aspartate amino transferase (AST) (international units/L), and albumin (Valtek Diagnostics, Santiago, Chile). Liver samples were fixed in 10% buffered formaldehyde and paraffin embedded, and sections were stained with hematoxylin–eosin for light-microscopy studies [20]. Separate liver samples were taken, frozen in liquid nitrogen, and kept at −80 °C for protein carbonyl, 8-isoprostane, and GSH content measurements, in addition to those using Western blot and real-time quantitative PCR analyses. The rate of O_2 consumption was assessed polarographically in liver perfusion experiments in separate groups of animals as previously described [21].

Studies with NAC were carried out in separate groups of rats receiving 0.5 g/kg or saline (controls) ip, 0.5 h before each dose of T_3 administered, and studies were performed at 24 h after hormone treatment in the resulting four experimental groups, namely, (a) controls, (b) T_3, (c) NAC, and (d) NAC+T_3. Experimental animal protocols and animal procedures complied with the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, NIH Publication 86-23, revised 1985) and were approved by the Ethics Committee of the Faculty of Medicine, University of Chile (Protocol CBA 0440 (FMUCH)).

1.2. Oxidative stress-related parameters

Liver samples frozen in liquid nitrogen and stored at −80 °C were used for 8-isoprostanes evaluation (ELISA kit, Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's instructions. In separate groups of animals, livers were perfused in situ with a cold solution containing 150 mM KCl and 5 mM Tris (pH 7.4) to remove blood, and total GSH equivalents [22], protein carbonyl, and total protein contents were measured [23].

1.3. Western blot analysis

The cytosolic fraction was isolated from homogenized liver samples (300 mg) suspended in a buffer solution (pH 7.9), containing 10 mM Hepes, 1 mM EDTA, 0.6% NP-40, 150 mM NaCl, 0.5 mM phenylmethanesulfonyl fluoride, protease inhibitors (1 μg/ml aprotinin, 1 μg/ml leupeptin), and phosphatase inhibitor (1 mM orthovanadate), followed by centrifugation (3000 *g* for 5 min). Protein concentration was determined using the BCA kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's protocols. A 60 μg protein sample was separated on 12% polyacrylamide gels using SDS–PAGE [24] and transferred to nitrocellulose membranes [25], which were blocked for 1 h at room temperature with Tris-buffered saline (TBS) containing 5% bovine serum albumin. The blots were washed with TBS containing 0.1% Tween 20, and the following primary antibodies were added: rabbit polyclonal primary antibodies for pPERK, PERK, phosphorylated eukaryotic translation initiator factor 2α (peIF2α), eIF2α, BiP, ATF-4, α-tubulin, and lamin A/C (1:1000; Cell Signaling Technology, Danvers, MA, USA). Then the blots were incubated at 4 °C overnight. In all determinations, rabbit monoclonal antibody for anti-α-tubulin was used as internal control. After extensive washing, the antigen–antibody complexes were detected using horseradish peroxidase goat anti-rabbit IgG or goat anti-mouse IgG and the SuperSignal West Pico chemiluminescence kit detection system (Pierce). Bands were quantified by densitometry using a gel documentation system, Biosens SC-750 (Shanghai Bio-Tech Co., Ltd., China). Results are expressed as relative units (individual protein/α-tubulin or phosphorylated protein/total protein). Lamin A/C was used to control the purity of cytosolic fractions (data not shown).

1.4. RNA isolation and cDNA synthesis

Total RNA was isolated from tissues with the RNeasy Lipid Tissue Mini Kit (Qiagen Sciences, Germantown, MD, USA) according to the manufacturer's instructions. Two micrograms of RNA was used for generation of cDNA using the ThermoScript RT-PCR System (Life Technologies Corp., Carlsbad, CA, USA) according to the manufacturer's protocols.

1.5. Real-time quantitative PCR

Real-time quantitative PCR was performed on a Stratagene Mx3005P thermocycler (Agilent Technologies, Santa Clara, CA, USA) using the Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies) following the manufacturer's protocols. The primer pair sequences used are shown in Table 1. Primers were optimized to yield 95–100% reaction efficiency with PCR products run on an

Table 1
Primers for qRT-PCR assays.

mRNA	Forward primer	Reverse primer
Rat CHOP	ACCACCACACCTGAAAGCAG	AGCTGGACACTGTCTCAAAG
Rat GADD34	TCCGGTCTCTGAAAATCACC	GAACTTTGGGCTTCCACATC
Rat PDI	TCCAAAGATTTTCGGAGGTG	TGCTCAATTTGCCATCGTAG
Rat ERO1α	ATTCCAAGGAAGCCAACCTC	TCGTGTTTCGTCCACTGAAG
Rat β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA

Sequences are listed 5′→3′.

agarose gel to verify the correct amplification length (data not shown). Melt-curve analyses verified the formation of a single desired PCR product in each PCR. The expression levels of each sample were normalized against β-actin (internal control). The relative expression levels were calculated using the comparative C_T method ($\Delta\Delta C_T$).

1.6. Statistical analyses

Data showing Gaussian distribution according to the Kolmogorov–Smirnov test are expressed as means ± standard error of the mean (SEM) for the number of separate experiments indicated. As required, one-way ANOVA and the Newman–Keuls test or Student *t* test for unpaired data were used to assess the statistical significance ($p < 0.05$) of differences between mean values. To analyze the association between variables, the Pearson correlation coefficient was used. All statistical analyses were computed employing GraphPad Prism version 2.0 (GraphPad Software, San Diego, CA, USA).

2. Results

2.1. T_3-induced calorigenesis is associated with liver oxidative stress involving protein oxidation

T_3 administration according to the protocol used resulted in a significant sixfold increment in serum T_3 levels over basal values (controls, 50 ± 10 ng/dl($n=4$); T_3-treated rats, 350 ± 20 ($n=4$); $p < 0.05$), concomitant with a 10% diminution in those of albumin (controls, 3.96 ± 0.05 g/dl ($n=3$); T_3-treated rats, 3.56 ± 0.14 ($n=3$); $p < 0.05$), in control rats (Fig. 1A) and T_3-treated animals (Fig. 1B) exhibiting normal liver morphology and comparable ($p > 0.05$) serum AST levels (Fig. 1C). Under these conditions, the rectal temperature of the animals (Fig. 1D), liver oxygen consumption rate (Fig. 1E), and contents of protein carbonyls (Fig. 1F) and 8-isoprostanes (Fig. 1G) were significantly enhanced by 4, 39, and 140% and 25.9-fold, respectively, whereas hepatic GSH levels (Fig. 1H) were diminished by 59% ($p < 0.05$). The analysis of correlations between the studied parameters shown in Fig. 1 exhibited significant associations of the rectal temperature with liver O_2 consumption ($r=0.67$; $p < 0.04$), protein carbonyls ($r=0.96$; $p < 0.0001$), and 8-isoprostanes ($r=0.80$; $p < 0.02$), whereas a

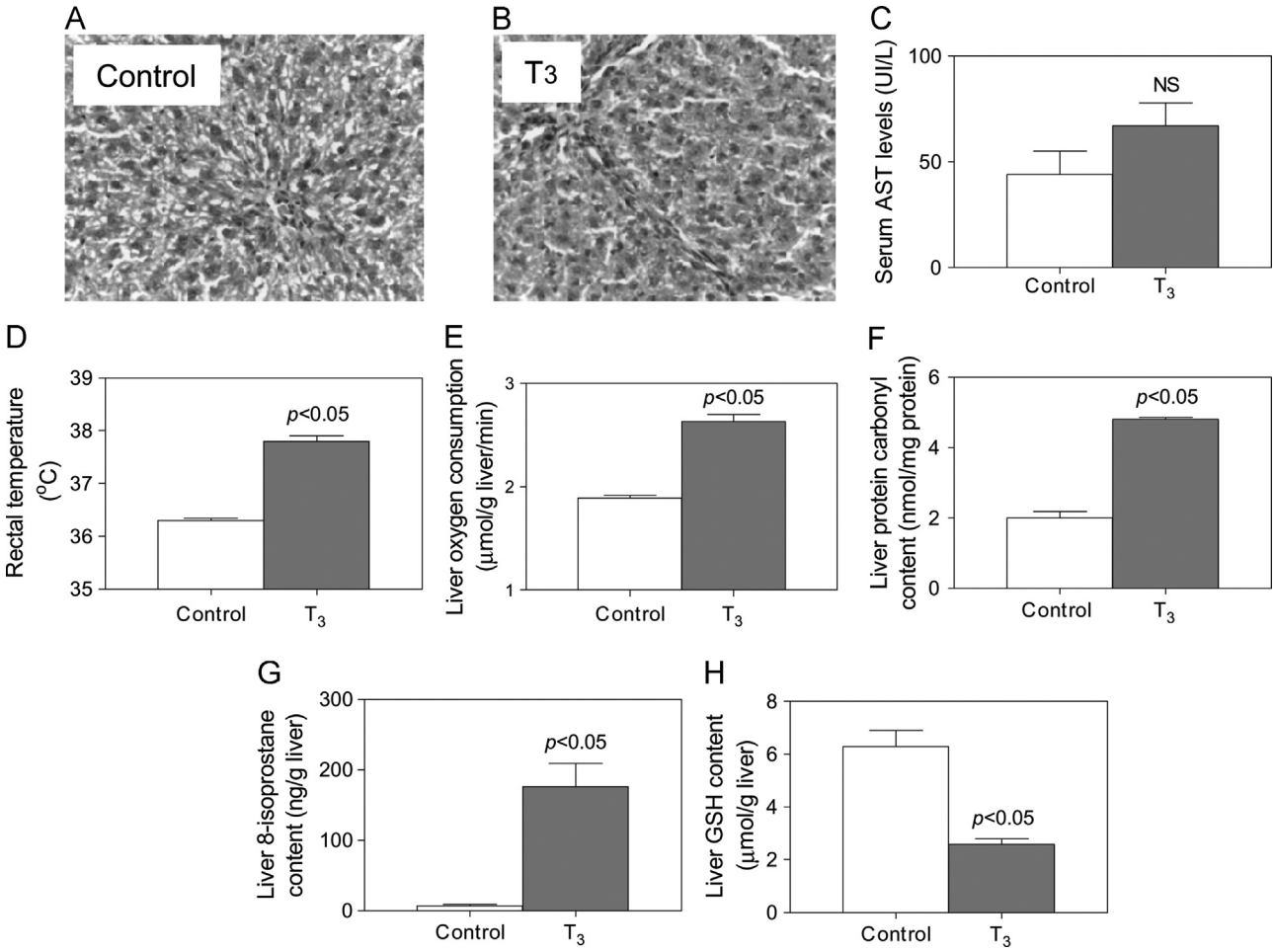

Fig. 1. Liver morphological characteristics in (A) control and (B) T_3-treated rats, (C) serum aspartate transaminase (AST) levels, (D) rectal temperature of the animals, (E) liver O_2 consumption rate, (F) liver contents of protein carbonyls, (G) 8-isoprostanes, and (H) GSH. Values shown are means ± SEM for four animals per experimental group. A significance ($p < 0.05$) study for the differences between control rats and T_3-treated animals was assessed by Student's *t* test for unpaired data (NS, not significant).

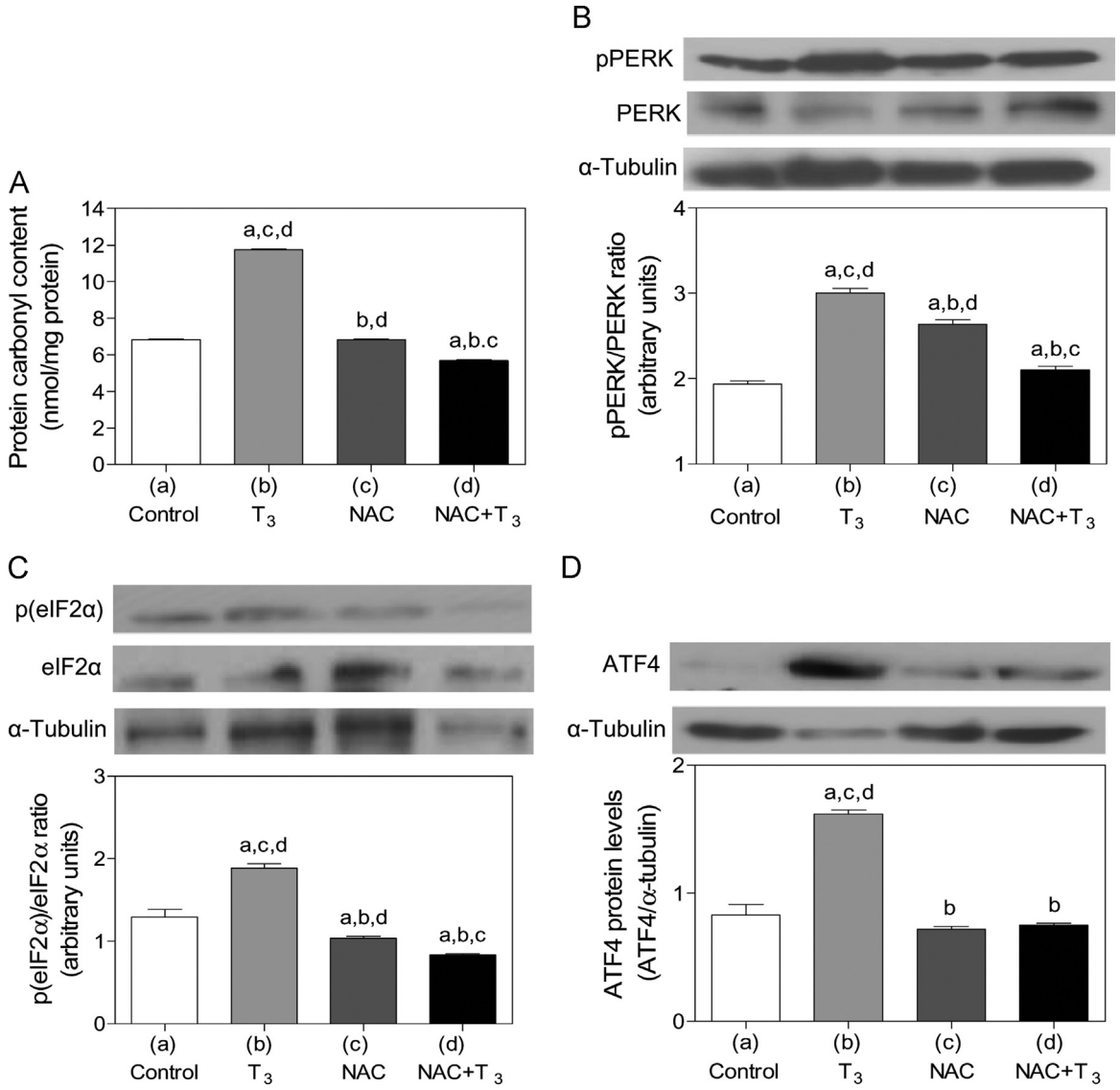

Fig. 2. Influence of NAC pretreatment on (A) T_3-induced liver protein carbonylation, (B) pPERK/PERK ratios, (C) peIF2α/eIF2α ratios, and (D) ATF4 protein expression. Animals were treated as described under Materials and methods. Values shown are means ± SEM for three animals per experimental group. Significance ($p < 0.05$; one-way ANOVA and the Newman–Keuls test) is indicated by the letters identifying each experimental group. For abbreviations, see legend to Fig. 4.

negative correlation was obtained for the rectal temperature of the animals and liver GSH content ($r = -0.89$; $p < 0.03$).

2.2. T_3-induced liver UPR is dependent on oxidative stress development

T_3-dependent liver protein oxidation (Fig. 2A) was associated with activation of ER stress, as evidenced by the enhancement in the phosphorylation status of the ER stress sensor PERK [18,19], which showed 61% increase in pPERK/PERK ratios over control values (Fig. 2B). In addition, the downstream target of PERK, eIF2α, exhibited 106% enhancement in peIF2α/eIF2α ratios (Fig. 2C), with a concurrent increase in activating transcription factor 4 (ATF4) translation, as shown by the 95% increment in its protein levels compared to controls (Fig. 2D). Under these conditions, NAC pretreatment before T_3 administration eliminated the T_3-induced protein carbonylation response (Fig. 2A), with suppression of the enhancement in liver pPERK/PERK (Fig. 2B) and peIF2α/eIF2α (Fig. 2C) ratios and ATF4 induction (Fig. 2D) elicited by T_3. In this experimental setting, liver protein carbonyl levels were significantly associated with pPERK/PERK ($r = 0.81$; $p < 0.0008$) and peIF2α/eIF2α ($r = 0.94$; $p < 0.0001$) ratios and ATF4 protein levels ($r = 0.95$; $p < 0.0001$).

T_3-induced liver UPR involving the PERK–eIF2α–ATF4 regulatory axis was characterized by 54% increase ($p < 0.05$) in the protein levels of the ER-located chaperone binding immunoglobulin protein (BiP) compared to control values (Fig. 3A). These findings were observed concomitant with upregulation of the ATF4-dependent mRNA expression of C/EBP homologous protein (CHOP) (Fig. 3B; 275% increase; $p < 0.05$) and the CHOP-dependent expression of growth arrest and DNA damage 34 (GADD34) (Fig. 3C; 555% increase; $p < 0.05$). In addition, T_3 administration also enhanced the mRNA expression of liver PDI (Fig. 3D; 118% increase, $p < 0.05$) and that of ERO1α (Fig. 3E; 100% increase; $p < 0.05$) over control levels. The enhancements in liver BiP protein content and in the mRNA levels of CHOP, GADD34, PDI, and ERO1α achieved by T_3 were abolished by the combined NAC–T_3 protocol studied (Fig. 3). Under these conditions, significant correlations were found between the levels of liver ATF4 protein and CHOP mRNA ($r = 0.96$; $p < 0.0001$), CHOP and GADD34 mRNA contents ($r = 0.77$; $p < 0.002$), and PDI and ERO1α mRNA levels ($r = 0.92$; $p < 0.0001$).

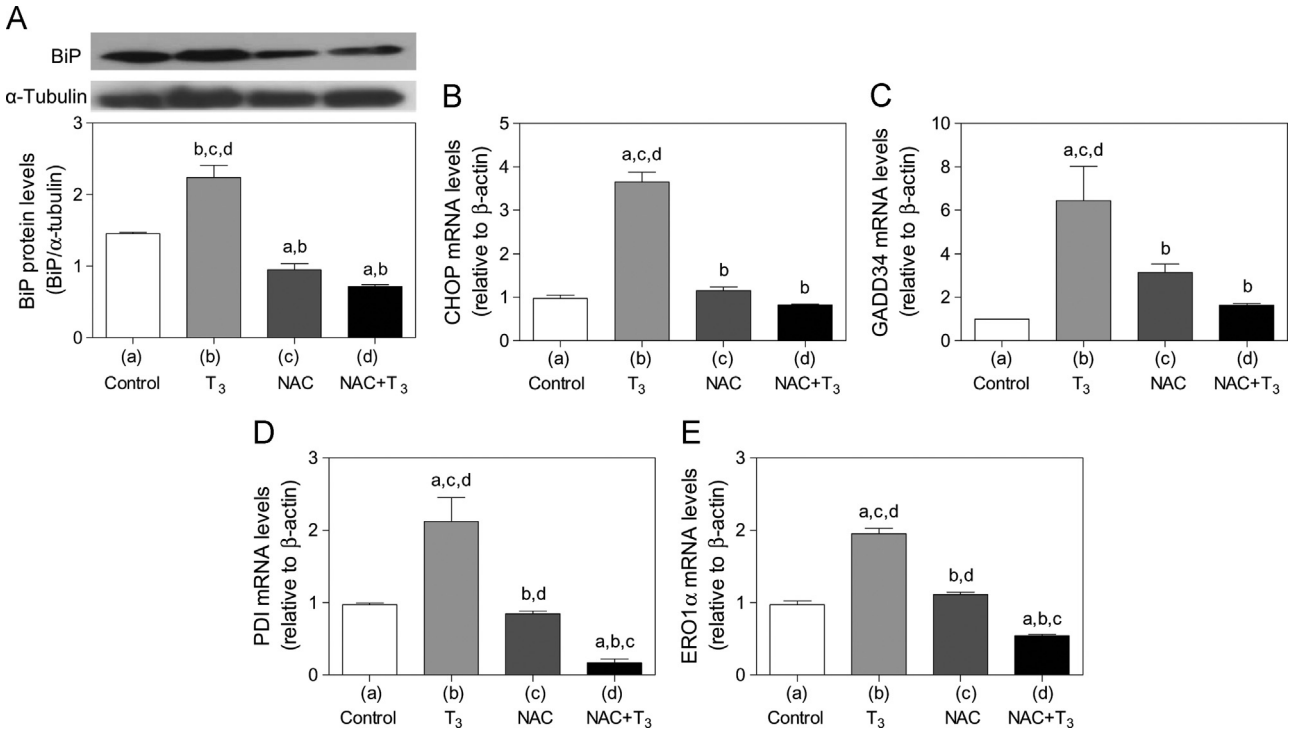

Fig. 3. Influence of NAC pretreatment on (A) T_3-induced liver BiP protein contents and mRNA levels of (B) CHOP, (C) GADD34, (D) PDI, and (E) ERO1α. Animals were treated as described under Materials and methods. Values shown are means ± SEM for three animals per experimental group. Significance ($p < 0.05$; one-way ANOVA and the Newman–Keuls test) is indicated by the letters identifying each experimental group. For abbreviations, see legend to Fig. 4.

3. Discussion

Cell protection, survival, and functional recovery are elicited by the administration of low doses of T_3 to experimental animals [7]. In the liver, these features are coupled to the activation of the redox-sensitive transcription factors nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3), activating protein 1, or nuclear factor-erythroid 2-related factor 2 (Nrf2) upon oxidative stress development, with concurrence of AMPK upregulation for energy supply (Fig. 4). These mechanisms are effective in protecting the liver against IRI [5,6], the beneficial actions of thyroid hormones being also observed in ischemic

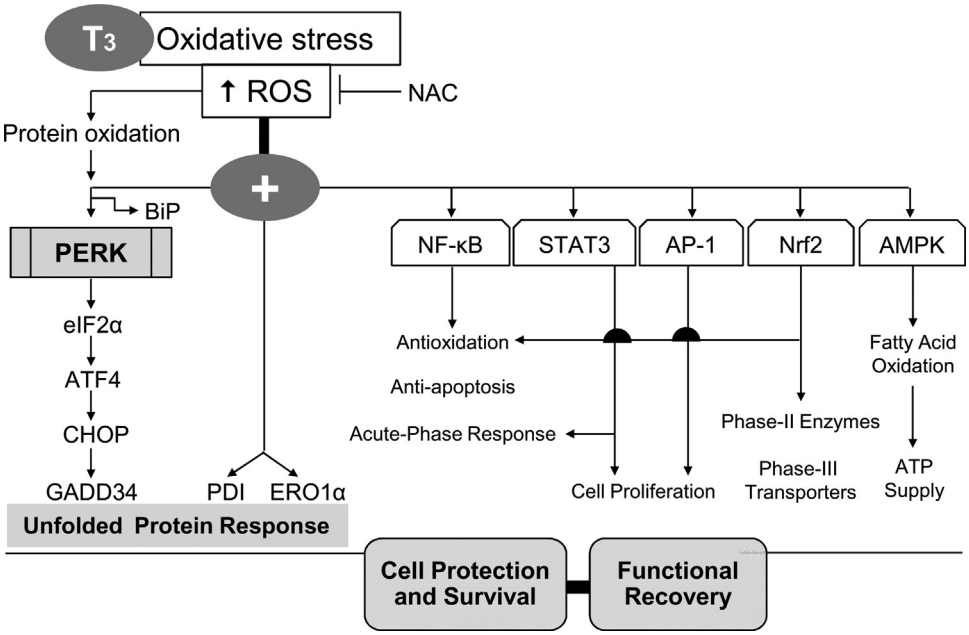

Fig. 4. Relationships between thyroid hormone (T_3)-induced liver reactive oxygen species (ROS) production involving a protein oxidation response and upregulation of the unfolded protein response and alternate signaling pathways affording cell protection, survival, and functional recovery. (⊣) Inhibition by N-acetylcysteine; NAC). *Abbreviations:* AP-1, activating protein-1; ATF4, activating transcription factor 4; AMPK, AMP-activated protein kinase; BiP, binding immunoglobulin protein; CHOP, C/EBP homologous protein; ERO1α, endoplasmic reticulum oxidoreductin-1α; eIF2α, eukaryotic translation initiator factor 2α; GADD34, growth arrest and DNA damage 34; NF-κB, nuclear factor-κB; Nrf2, nuclear factor-erythroid 2-related factor 2; PERK, protein kinase RNA-like ER kinase; PDI, protein disulfide isomerase; STAT3, signal transducer and activator of transcription 3.

injury of the heart [26], kidney [27], and brain [28] or in the repair of organs subjected to several types of injury other than IRI [29]. Data presented indicate that a protocol comprising the administration of single daily doses of 0.1 mg T_3/kg to rats on three consecutive days underlies an enhanced liver ROS generation coupled to T_3-induced increase in oxygen consumption as a result of the calorigenic effect achieved, leading to significant lipid peroxidation and protein oxidation responses and concomitant GSH depletion. These changes induced by T_3 point to the development of an acute, nonlethal oxidative stress status, considering the lack of damaging effects on the liver as evidenced by the presence of normal liver morphology and serum AST levels.

Protein oxidation by ROS is a complex and irreversible process involving several oxidative modifications of amino acid residues in proteins, which favor protein unfolding, degradation [23,30,31], and UPR activation [17–19], a signaling mechanism that may be of importance in the PC and repairing effects of T_3 (Fig. 4). Concomitant with the enhancement in liver protein oxidation induced by T_3, the ER-localized signal transducer PERK and its downstream components are activated, responses that are abolished by the use of NAC (0.5 g/kg) previous to each dose of T_3. This protocol of NAC administration was adopted considering the rapid onset and offset in the circulating levels of the antioxidant observed [6], which exhibits an elimination half-life of 1 to 4.5 h in the rat [32]. Abolishment of liver T_3-induced UPR by NAC can be ascribed to the direct free radical-scavenging activity of NAC and its function as a precursor for GSH biosynthesis [33,34], thus establishing a causal role for ROS in UPR development in the hyperthyroid state.

T_3-dependent activation of liver UPR involves the PERK regulatory axis, in which accumulation of oxidized, unfolded proteins in the ER lumen releases PERK bound to the ER chaperone BiP; PERK then dimerizes and autophosphorylates to achieve maximal activity [18,35]. This is evidenced by significant increases in the hepatic pPERK/PERK ratios and in BiP protein levels by T_3 over control values, which correlate with the enhanced eIF2α phosphorylation on Ser51. This response attenuates global mRNA translation [18,35,36], which is known to include that of the inhibitor of κB [37], a finding that may contribute to liver NF-κB activation elicited by T_3 (Fig. 4) [38]. Furthermore, eIF2α-mediated decrease in type 2 deiodinase (DIO2) synthesis and activity is induced in cell cultures by exposure to ER stress inducers [39], which may play a role in the recovery of normal T_3 levels under conditions of exogenous administration, DIO2 being responsible for cellular T_4 to T_3 conversion, thus exerting local control of T_3 action [40]. Under conditions of higher eIF2α phosphorylation, however, selective translation of specific mRNAs is accomplished [35,36], as found for liver ATF4 mRNA leading to elevated ATF4 protein levels in response to T_3 administration. ATF4 is a transcription factor induced not only by ER stress, but also by hypoxia, amino acid deprivation, or oxidative stress that allows cellular adaptation to noxious conditions [41,42]. In agreement with these views, T_3-induced liver ATF4 upregulation led to higher CHOP transcription, with consequent GADD34 induction (Fig. 4). Under these conditions, GADD34 upregulation by T_3 may constitute a negative feedback mechanism resuming mRNA translation after UPR development, a process that underlies GADD34 binding to protein phosphatase 1 to achieve eIF2α dephosphorylation [35,36]. Other genes that are induced by ATF4 include those for chaperones, metabolic stress, mitochondrial functions, and detoxification and redox processes [42], interaction with Nrf2 being related to the induction of antioxidant enzymes [43], a pathway that may contribute to T_3-induced liver Nrf2 activation (Fig. 4) [8]. In addition, PERK–eIF2α-reliant ATF4 upregulation has been shown to stimulate expression of the autophagy gene *ATG12*[44]. This is in agreement with the T_3-dependent induction of hepatic autophagy, a stress-related process degrading cellular components to produce fatty acids to generate ATP and amino acids to synthesize proteins for cell survival [45].

To ensure adequate transition from unfolded polypeptide to tertiary structure, the UPR involves other enzymatic modifications including disulfide bond formation [46,47], a process that is also upregulated by T_3 administration. This response is characterized by enhancements in the mRNA expression of hepatic PDI and ERO1α, which are significantly correlated and occurred in a NAC-sensitive manner (Fig. 4). Thus, T_3 may augment the hepatic capacity for oxidative protein folding coupled to the oxidoreductase activity of PDI, an enzyme also exhibiting the additional functions of disulfide isomerase and redox-dependent chaperone [46–48]. This suggestion is in agreement with the upregulation of liver ERO1α induced by T_3, an ER-located enzyme that assists PDI function when the regulatory disulfides of ERO1α are reduced, thus allowing the use of O_2 to achieve PDI and substrate protein oxidation, with concomitant ROS (H_2O_2) formation [49]. Although this feature led to the proposal that oxidative protein folding in the ER is responsible for a significant proportion of cellular ROS generation [35,49], estimations are based on theoretical calculations [50]; however, actual rates of H_2O_2 production have not been reported.

In conclusion, data presented indicate that T_3-induced liver oxidative stress involving a significant protein oxidation status has a causal role in UPR development, as evidenced by antioxidant intervention. Induction of UPR by T_3 is associated with upregulation of both the PERK regulatory axis and the PDI–ERO1α couple, even though changes in the other ER transducers, namely, inositol-requiring enzyme-1 and/or activating transcription factor 6 [18,35,36], cannot be discarded, an aspect that is currently being evaluated in our laboratory. Thyroid hormone-induced liver UPR is in agreement with the enhanced synthesis and release of the positive acute-phase proteins (APPs) α_1-acid glycoprotein [51], haptoglobin [51,52], and those involved in coagulation, inflammatory response, and lipoprotein metabolism [52]. Higher liver mRNA levels and serum activity of ceruloplasmin by thyroxine (T_4) administration were also reported [53], which comply with the direct involvement of the ER in the biosynthesis and maturation of secreted proteins by hepatocytes [18]. This contention is further supported by the T_3-induced upregulation of the hepatic expression of the APP ferritin posttranscriptionally [54] and that of haptoglobin and β-fibrinogen, which are associated with the redox activation of NF-κB and STAT3, respectively [55]. In agreement with the upregulation of positive APPs, T_3 led to a decrease in the serum levels of albumin, a negative APP that under acute stress conditions is downregulated to achieve a redistribution of liver protein pools [56,57]. Considering that the UPR confers rapid adaptation to the increase in unfolded proteins in the ER lumen by expanding its protein-folding capacity, T_3-induced liver UPR may represent a favorable mechanism against deleterious stimuli [5,6,9,29] in addition to those studied in experimental models (Fig. 4). In humans, readjusting thyroid hormones to normal levels is also beneficial in specific cases, as shown by (i) the improvement in mental, motor, and neurological outcomes in infants of <28 weeks' gestation [58]; (ii) the lower cognitive side effects of lithium and electroconvulsive therapy in patients with bipolar disorders [59]; and (iii) the higher number of organs from brain-dead donors that are acceptable for transplantation and exhibit better graft survival [60]. Recently, low-dose T_3 replacement was proposed as a therapy for diabetic vascular complications in humans, as it preserves coronary microvasculature and attenuates cardiac dysfunction in an experimental model [61], whereas T_3 supplementation in rats substantially recovered hypothyroidism-induced liver apoptosis [62], supporting the role of T_3 in cytoprotection and functional recovery.

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