

T₃-induced liver AMP-activated protein kinase signaling: Redox dependency and upregulation of downstream targets

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Supported by National Commission for Scientific and Technological Research Grant No. 1120034

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Received: April 22, 2014 Revised: June 6, 2014

Accepted: July 29, 2014

Published online: December 14, 2014

Abstract

AIM: To investigate the redox dependency and promotion of downstream targets in thyroid hormone (T₃)-induced AMP-activated protein kinase (AMPK) signaling as cellular energy sensor to limit metabolic stresses in the liver.

METHODS: Fed male Sprague-Dawley rats were given a single ip dose of 0.1 mg T₃/kg or T₃ vehicle (NaOH 0.1 N; controls) and studied at 8 or 24 h after treatment. Separate groups of animals received 500 mg N-acetylcysteine (NAC)/kg or saline ip 30 min prior T₃. Measurements included plasma and liver 8-isoprostane and serum β -hydroxybutyrate levels (ELISA), hepatic

levels of mRNAs (qPCR), proteins (Western blot), and phosphorylated AMPK (ELISA).

RESULTS: T₃ upregulates AMPK signaling, including the upstream kinases Ca²⁺-calmodulin-dependent protein kinase kinase- β and transforming growth factor- β -activated kinase-1, with T₃-induced reactive oxygen species having a causal role due to its suppression by pretreatment with the antioxidant NAC. Accordingly, AMPK targets acetyl-CoA carboxylase and cyclic AMP response element binding protein are phosphorylated, with the concomitant carnitine palmitoyltransferase-1 α (CPT-1 α) activation and higher expression of peroxisome proliferator-activated receptor- γ co-activator-1 α and that of the fatty acid oxidation (FAO)-related enzymes CPT-1 α , acyl-CoA oxidase 1, and acyl-CoA thioesterase 2. Under these conditions, T₃ induced a significant increase in the serum levels of β -hydroxybutyrate, a surrogate marker for hepatic FAO.

CONCLUSION: T₃ administration activates liver AMPK signaling in a redox-dependent manner, leading to FAO enhancement as evidenced by the consequent ketogenic response, which may constitute a key molecular mechanism regulating energy dynamics to support T₃ preconditioning against ischemia-reperfusion injury.

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Key words: Liver; Thyroid hormone; N-acetylcysteine; AMP-activated protein kinase; Fatty acid oxidation

Core tip: This work investigated the redox dependency and promotion of downstream targets in thyroid hormone (T₃)-induced AMP-activated protein kinase (AMPK) signaling. T₃ upregulates AMPK with T₃-induced reactive oxygen species having a causal role due to its suppression by pretreatment with the antioxidant NAC. Accordingly, AMPK targets acetyl-CoA carboxylase and cyclic AMP response element binding protein

are phosphorylated, with the concomitant carnitine palmitoyltransferase-1 α activation and higher expression of peroxisome proliferator-activated receptor- γ co-activator-1 α and that of the fatty acid oxidation (FAO)-related enzymes. This lead to enhancement in the serum levels of β -hydroxybutyrate, a surrogate marker for hepatic FAO, which represent a key molecular mechanism regulating energy dynamics to limit metabolic stresses.

Videla LA, Fernández V, Cornejo P, Vargas R, Morales P, Ceballos J, Fischer A, Escudero N, Escobar O. T₃-induced liver AMP-activated protein kinase signaling: Redox dependency and up-regulation of downstream targets. *World J Gastroenterol* 2014; 20(46): 17416-17425 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v20/i46/17416.htm> DOI: <http://dx.doi.org/10.3748/wjg.v20.i46.17416>

INTRODUCTION

Genomic^[1] and nongenomic^[2] mechanisms underlying thyroid hormone action are responsible for cellular responses such as O₂ consumption of most tissues and the metabolic rate of individuals. The liver, a target organ for L-3,3,5-triiodothyronine (T₃) calorogenesis, exhibits a concomitant enhancement in reactive oxygen species (ROS) production, which is due to acceleration of mitochondrial respiration and activation of other ROS-generating systems^[3]. Under these conditions, activation of redox-sensitive transcription factors nuclear factor- κ B, activating protein-1, signal transducer and activator of transcription-3, and nuclear factor (erythroid-derived 2)-like 2 is achieved, with promotion of cell proliferation and the expression of cytoprotective proteins including antioxidant, anti-apoptotic, and acute-phase proteins, phase-II detoxification enzymes, and phase-III transporters^[3,4]. Thus, *in vivo* T₃ administration re-establishes redox homeostasis, promotes cell survival, and protects the liver against ischemia-reperfusion injury (IRI), which constitute the basis for T₃ liver preconditioning^[5,6] and an important issue in post-stress recovery and repair^[7].

Organ preconditioning (PC) refers to the development of an increased tolerance to noxious stimuli such IRI due to previous maneuvers triggering beneficial molecular and functional changes. In the liver, numerous experimental PC and post-conditioning strategies have been proposed, however, difficulties with gene therapy and pharmacological maneuvers have hindered their transfer to clinical practice, whereas those based on surgical approaches are limited or controversial^[8]. In this respect, the T₃ PC strategy has potential clinical application, considering that T₃ is a widely employed and well-tolerated therapeutic agent whose side effects are readily controlled, and that its mechanisms of PC action are beginning to be understood^[3,5,6]. Among these, AMP-activated protein kinase (AMPK) may constitute the metabolic basis of T₃ liver PC, as effective PC involves high ATP demands to

power the expression of numerous protective proteins, oxidized biomolecules repair (phospholipids, DNA) or resynthesis (proteins), and hepatocyte and Kupffer-cell proliferation, in addition to energy requirements needed for normal hepatic metabolic, secretory, and excretory functions^[9-11]. AMPK is considered a key energy sensor able to limit anabolic pathways, to reduce ATP consumption, and to facilitate catabolic pathways, to increase ATP production^[9]. In the present study, we show that T₃ administration to rats significantly enhances liver (1) AMPK signaling in a redox-sensitive manner; (2) the phosphorylation of downstream AMPK targets; and (3) the expression of components associated with fatty acid (FA) oxidation (FAO), leading to a ketogenic response.

MATERIALS AND METHODS

Animal treatments

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-hour light/dark cycle and were provided with rat chow and water *ad libitum*. Animals received a single intraperitoneal (*ip*), dose of 0.1 mg T₃/kg body weight or equivalent volumes of hormone vehicle (0.1 mol/L NaOH, controls) at time zero and studies were done after 24 h of treatment. Studies with N-acetylcysteine (NAC) were carried out in the above described groups receiving either 0.5 g/kg or saline (controls) *ip*, 0.5 h before T₃ administration, and studies were performed at 8 or 24 h after treatment in four experimental groups, namely, (1) controls; (2) T₃; (3) NAC; and (4) NAC + T₃. T₃-induced calorogenesis was assessed by the rectal temperature of the animals by means of a thermocouple (Cole-Palmer Instrument Co., Chicago, IL). Blood samples to measure 8-isoprostanes in plasma and β -hydroxybutyrate in serum were obtained by cardiac puncture in rats anesthetized (1 mL/kg) with zolazepam chlorhydrate (25 mg/mL) and tiletamine chlodrihydrate (25 mg/mL) *ip* (Zoletil 50; Virbac S/A, Carros, France), and liver samples were taken, frozen in liquid nitrogen, and kept at -80 °C for measurements of mRNA and protein expression. All experiments and animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, NIH Publication 6-23, revised 1985) and were approved by the Bioethics Committee for Research in Animals, Faculty of Medicine, University of Chile (Protocol CBA 0381 FMUCH).

8-isoprostanes and β -hydroxybutyrate levels

Liver and plasma 8-isoprostane levels were measured in samples frozen in liquid nitrogen and kept at -80 °C, using a commercial kit (ELISA; Cayman Chemical Co., Ann Arbor, MI, United States) according to the manufacturer's instructions at 405-420 nm. Results were calculated from a standard curve and expressed as ng/g of liver or pg/mL of plasma, respectively. Serum β -hydroxybutyrate levels were assayed in samples frozen in liquid nitrogen and

Table 1 Primers for SYBR green based qRT-PCR assays

mRNA	Forward primer	Reverse primer
Rat ACC α	GAC GTT CGC CAT AAC CAA GT	CTG CAG GTT CTC AAT GCA AA
Rat ACC β	CCT GTA GAT GCC AGT CAG CA	AGT TCT GGG GAG GAA CAG GT
Rat ACOT2	TCA GGA TGA CCA CAACTG GA	ATG TTA GAC CCC ACC AGG AG
Rat ACOX1	CTG ATG AAA TAC GCC CAG GT	GGT CCC ATA CGT CAG CTT GT
Rat AMPK	TTT GCC TAG AAT CCC CAC AG	TAA GGA GCC CAG AAA ACA GC
Rat β -actin	AGC CAT GTA CGT AGC CAT CC	CTC TCA GCT GTG GTG GTG AA
Rat CaMKK β	GGA TTG TGG TGC CTG AAA TC	AGG CTG GGA ATG TGT TTG AC
Rat CPT-1 α	CAG CTC GCA CAT TAC AAG GA	TGC ACA AAG TTG CAG GAC TC
Rat CREB	TCA GCC GGG TAC TAC CAT TC	CCT CTC TCT TTC GTG CTG CT
Rat PGC-1 α	TCC ACA GAT TCA AGC CAG TG	TGA CCG AAG TGC TTG TTC AG
Rat RPS23	GTA GGG GTT GAA GCC AAA CA	CAC CTT AAA GCG GAC TCC AG
Rat TAK1	AAC AAG TCC CTG CCA CAA AC	CAT CCT CTT GCC CTC AGA AG

Sequences are listed in the 5' \rightarrow 3' direction. ACC α (β): Acetyl-CoA carboxylase α and β ; ACOT2: Acyl-CoA thioesterase; ACOX1: acyl-CoA oxidase 1; AMPK: AMP-activated protein kinase; CaMKK β : Ca²⁺-Calmodulin-dependent protein kinase β ; CPT-1 α : Carnitine palmitoyltransferase-1 α ; CREB: cAMP-response element-binding protein; PGC-1 α : Peroxisome proliferator-activated receptor- γ coactivator-1 α ; RPS23: Ribosomal protein S23; TAK1: Transforming growth factor- β -activated kinase-1.

kept at -80 °C, using a commercial kit (ELISA; BioVision Incorporated, CA, United States) according to the manufacturer's instructions at 450 nm. Results were calculated from a standard curve and expressed in μ mol/L units.

RNA isolation and cDNA synthesis

Total RNA was isolated using RNeasy[®] Lipid Tissue Mini Kit (QUIAGEN Sciences, Maryland, United States) according to the manufacturer's instructions. cDNA was synthesized using ThermoScript[™] RT-PCR System (Life Technologies Corporation, Carlsbad, California, United States) according to the manufacturer's instructions.

Real-time quantitative PCR for AMPK, CaMKK β , TAK1, ACC- α (β), CREB, PGC-1 α , CPT-1 α , ACOX1, and ACOT2

Real-time quantitative PCR was carried out in a Stratagene Mx3005P (Agilent Technologies, California, United States) using Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies, California, United States) following the manufacturer's protocols. Gene specific primer sequences used are shown in Table 1. Primers were optimized to yield 95%-100% reaction efficiency with PCR products run on agarose gel to verify the correct amplification length. Melt curve analyses verified the formation of a single desired PCR product in each PCR reaction. The expression levels of each sample were normalized against RPS23 and β -actin (internal controls). The relative expression levels were calculated using the comparative C_T method ($\Delta\Delta C_T$) and values were normalized to RPS23 level or β -actin level as internal control genes.

Western Blot Analysis for CaMKK β , TAK1 and pTAK1, ACC and pACC, CREB and pCREB, CPT-1 α , ACOX1, and ACOT2

Liver samples (100-500 mg) frozen in liquid nitrogen were homogenized and suspended in 1.5 mL of a buffer solution (pH 7.9), containing 10 mmol/L Hepes, 1 mmol/L EDTA, 0.6% NP-40, 150 mmol/L NaCl, 0.5

mmol/L PMSF, protease inhibitors (1 μ g/mL aprotinin, 1 μ g/mL leupeptin) and fosfatase inhibitor (1 mmol/L orthovanadate) followed by centrifugation (3000 *g* for 5 min). Cytosolic soluble protein fractions (70 μ g) were separated on 12% polyacrylamide gels using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)^[12] and transferred to nitrocellulose membranes^[13], which were blocked for 1 h at room temperature with Tris buffer saline (TBS) containing 5% bovine serum albumin. The blots were washed with TBS containing 0.5% Tween 20 and hybridized with rabbit polyclonal primary antibodies for CaMKK β , TAK1, pTAK1, pACC, and CPT-1 α (1:1000; Abcam, Cambridge, MA, United States), PGC-1 α , ACC, α -tubulin, and lamin A/C (1:1000; Cell Signalling Technology, Inc, MA, United States), pCREB and CREB (1:1000; EMD Millipore Corporation, Billerica, MA, United States); acyl-coenzyme A thioesterase 2 (ACOT2) and acyl-coenzyme A oxidase 1 (ACOX1) (1:1000, Santa Cruz Biotechnology, Inc, Santa Cruz, CA, United States). In all determinations, rabbit monoclonal antibody for anti- α -tubulin was used as internal control and anti-lamin A/C was employed as control for purity. After extensive washing, the antigen-antibody complexes were detected using horseradish peroxidase goat anti-rabbit IgG or goat anti-mouse IgG and SuperSignal West Pico Chemiluminescence kit detection system (Pierce, Rockford, IL, United States). Bands were quantified by densitometry using Gel Documentation System Biosens SC-750 (Shanghai Bio-Tech Co., Ltd., China). Results are expressed as relative units (individual protein/ α -tubulin), with further normalization by the average values obtained in the control groups.

AMPK α [pT172] ELISA kit

AMPK α [pT172] concentration was determined by AMPK α [pT172] ELISA kit (Invitrogen Corporation, CA, United States) according to the manufacturer's instructions and read the plate at a wavelength at 450 nm. Results were calculated from a standard curve.

Statistical analysis

Data showing Gaussian distribution according to the Kolmogorov-Smirnov test are expressed as means \pm SEM for the number of separate experiments indicated. As required, one-way ANOVA analysis of variance and the Newman-Keuls test or the Student's *t*-test for unpaired data assessed the statistical significance ($P < 0.05$) of differences between mean values. To analyze the association between different variables, the Pearson correlation coefficient was used. All statistical analyses were computed employing GraphPad Prism™ version 2.0 (GraphPad Software, Inc., San Diego, CA, United States).

RESULTS

Liver AMPK upregulation by T₃ administration is redox-sensitive

The administration of a single dose of T₃ to fed rats elicited a calorogenic response, with (1) increases in the rectal temperature of the animals (8 h after treatment, controls, 36.5 ± 0.04 ($n = 4$) °C; T₃-treated rats, 38.0 ± 0.2 ($n = 5$); $P < 0.05$; 24 h after treatment, controls, 36.3 ± 0.06 ($n = 13$); T₃-treated rats, 37.7 ± 0.17 ($n = 13$); $P < 0.05$); and (2) the associated development of liver oxidative stress as evidenced by increases in O₂ consumption in the time range of 2–24 h^[14], with enhanced protein carbonylation at 12 h and glutathione depletion at 24 h after T₃^[5], and higher levels of 8-isoprostanes in plasma (112% higher) and liver (41% higher) over control values at 24 h (Figure 1A). The enhancement in 8-isoprostane levels as index related of oxidative stress was suppressed by 0.5 g of NAC/kg given 0.5 h before T₃ (Figure 1A). Under these conditions, NAC treatment did not modify the mRNA expression of AMPK (Figure 1B) or that of the upstream kinases Ca²⁺-calmodulin-dependent protein kinase kinase- β (CaMKK β) (Figure 1C) and transforming growth factor- β -activated kinase-1 (TAK1) (Figure 1D), or the protein levels of CaMKK β (Figure 1E). TAK1 activation by phosphorylation was significantly increased by NAC over controls (Figure 1F). Consistent with a previous report showing liver T₃-induced AMPK upregulation in the period of 8–36 h after treatment^[15], the enhancement in AMPK activation observed at 24 h was abolished by NAC (Figure 1B). Furthermore, early (8 h) CaMKK β upregulation (Figure 1C and E) and late (24 h) enhancement in TAK1 mRNA expression and phosphorylation (Figure 1D and F) induced by T₃ were either suppressed or significantly diminished by NAC pretreatment.

Liver AMPK phosphorylation targets are enhanced by T₃ administration

T₃-induced liver AMPK upregulation (Figure 1B and 2A) was found to trigger two major downstream signaling pathways, namely, acetyl-CoA carboxylase α and β (ACC α and ACC β) and cyclic AMP response element binding protein (CREB), either through mRNA expression or protein phosphorylation (Figure 2C and D), parameters that were significantly correlated (Figure 2E). These

changes occurred with the concomitant increase in the mRNA expression of hepatic peroxisome proliferator-activated receptor (PPAR)- γ co-activator-1 α (PGC-1 α) (Figure 2B).

Liver AMPK-dependent metabolically operative targets are increased by T₃ administration

T₃ administration enhanced hepatic mRNA expression of PGC-1 α over control values by 2.15-fold, which correlated with the 37% increase in CPT-1 α protein content (Figure 3A) ($r = 0.86$; $P < 0.02$). Under these conditions, PGC-1 α upregulation by T₃ was associated with increased expression of the FAO-related enzymes ACOX1 (Figure 3B and ACOT2 (Figure 3C), both at transcriptional and translational levels, with 72% enhancement in serum β -hydroxybutyrate levels over control values (Figure 3D).

DISCUSSION

Data presented indicate that T₃-induced liver AMPK upregulation is accomplished through enhancement in AMPK transcription and AMPK phosphorylation associated with early CaMKK β and late TAK1 activation, as upstream AMPK kinases^[9], mechanisms subjected to redox signaling due to their abrogation by the ROS suppressive action of NAC. The NAC protocol used results in significant circulating levels of the antioxidant^[16], which decline within 24 h due to the elimination half-life of 1–4.3 h^[17], an agent having a potent antioxidant activity due to its direct ROS scavenging action and the stimulation of hepatocellular glutathione synthesis^[18]. Although NAC did not alter the mRNA expression of CaMKK β , TAK1, and AMPK, TAK1 phosphorylation was significantly increased, an effect that could be ascribed to the maintenance of relevant sulfhydryl groups in proteins in the reduced state^[19], which may lead to modifications of signal transduction pathways by favoring the phosphorylation process. In agreement with the causal role of ROS in AMPK upregulation induced by T₃, ROS trigger cellular AMPK activation under different conditions including (1) *in vitro* hydrogen peroxide (H₂O₂) addition to cell cultures^[20–23]; (2) *in vitro* and *in vivo* conditions underlying ROS production in hepatocytes, heart, or skeletal muscle^[24–26]; and (3) ROS generation associated with chain reactions induced by polyphenols^[27]. In this respect, H₂O₂ is considered as a major ROS able to achieve redox signaling that can be achieved by reversible oxidation of cysteine residues in signaling proteins into cysteine-sulfenate derivatives, glutathionylation, or intramolecular disulfide formation^[28], H₂O₂ being significantly enhanced in hyperthyroid state^[3].

Upregulation of liver AMPK by T₃, as evidenced by the increase in hepatic AMPK mRNA levels, is in agreement with the observed higher Thr172-phosphorylated AMPK levels, which in turn correlate with the activation of CaMKK β and TAK1 as upstream kinases. These data suggest that T₃ substantially enhances the phosphorylating potential of hepatic AMPK, considering the higher

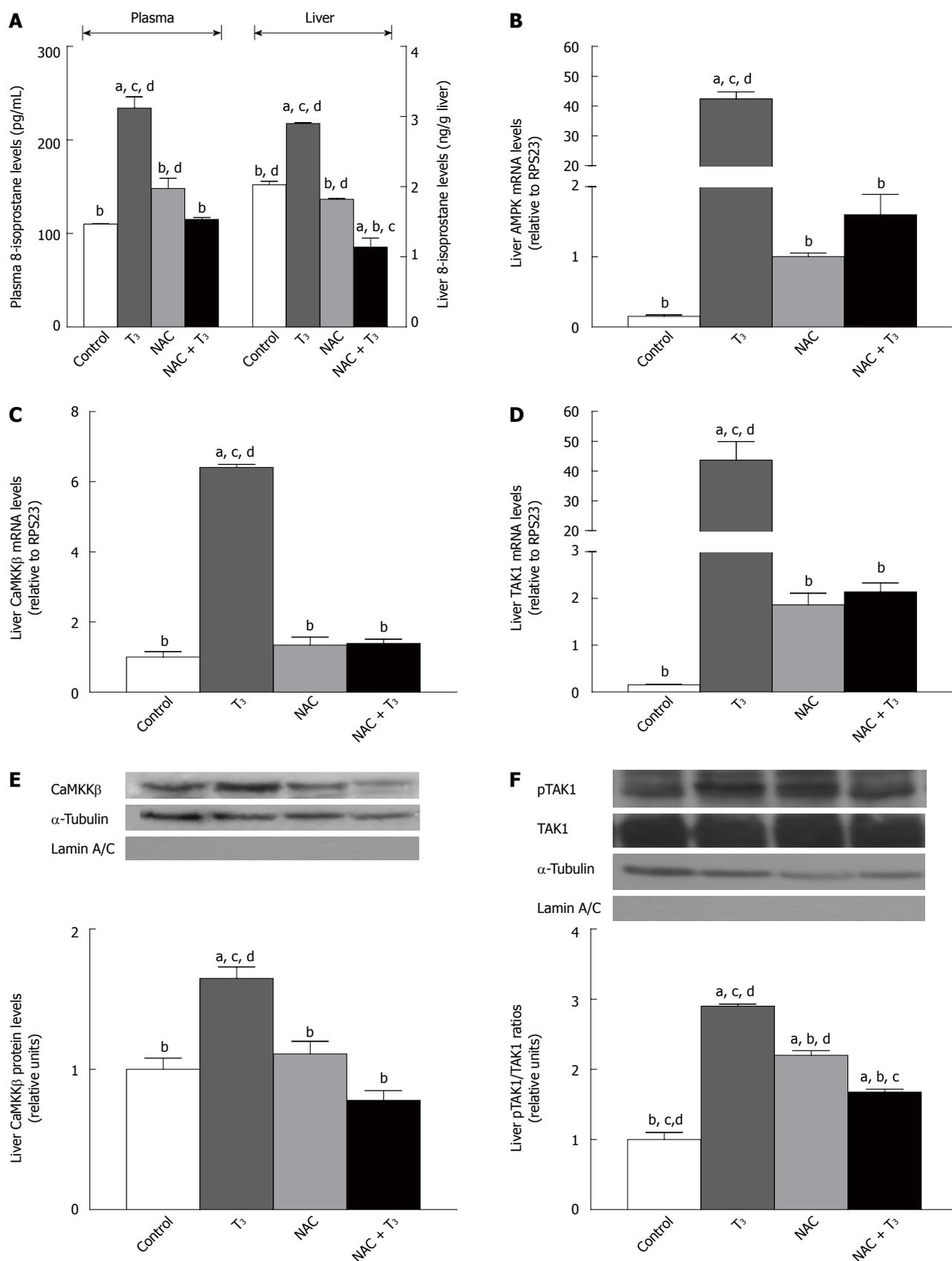


Figure 1 Effect of N-acetylcysteine on T₃-induced liver CaMKKβ/TAK1-AMPK cascade. (A) Rat plasma and liver 8-isoprostane levels, liver mRNA levels of AMPK (B) and CaMKKβ (C, E) hepatic CaMKKβ protein content, and liver expression of TAK1 mRNA (D) and pTAK1/TAK1 ratios (F). Values shown are means ± SEM (n = 3-4). Statistical significance (one-way ANOVA and the Newman-Keuls' test; P < 0.05) is indicated by the letters identifying each experimental group. AMPK: AMP-activated protein kinase; CaMKKβ: Ca²⁺-calmodulin-dependent protein kinase kinase-β; NAC: N-acetylcysteine; (p)TAK1, (phosphorylated) transforming growth factor-β-activated kinase-1.

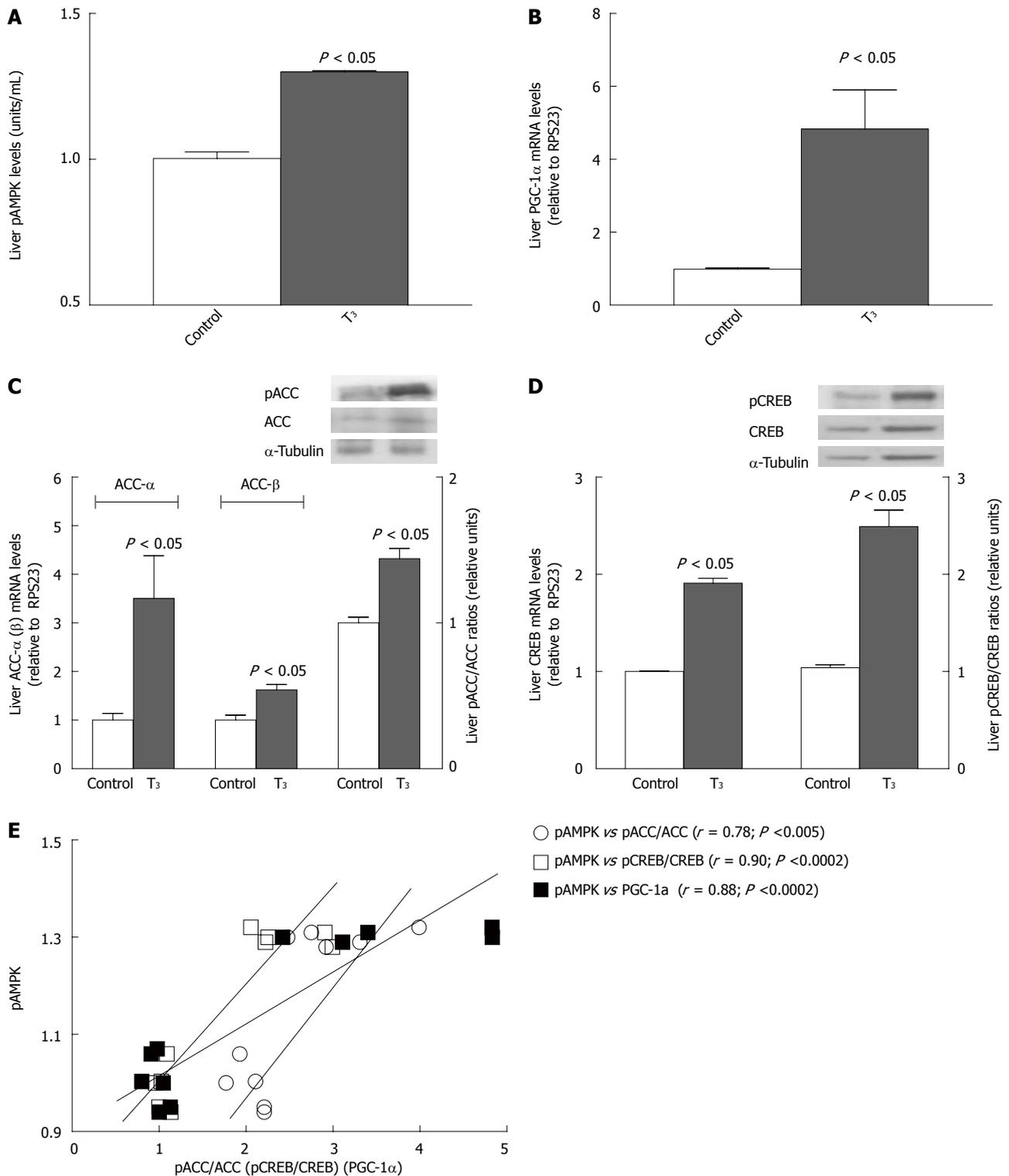


Figure 2 T₃-induced changes in liver direct AMP-activated protein kinase targets. A: pAMPK levels; B: PGC-1 α mRNA content; C: Contents of ACC- α mRNA and pACC- α ; D: Levels of CREB mRNA and pCREB/CREB ratios; E: Correlations between pAMPK and pACC- α , pCREB/CREB ratios, and PGC-1 α . Values shown are means \pm SEM ($n = 3-6$). Statistical significance was performed by one-way ANOVA and the Newman-Keuls' test (C, D) or Student's *t*-test for unpaired data (A, B) ($P < 0.05$). Associations between variables were analyzed by the Pearson correlation coefficient. pAMPK: Phosphorylated AMP-activated protein kinase; (p) ACC- α : (Phosphorylated) acetyl-CoA carboxylase- α ; (p)CREB: (phosphorylated) cAMP-response element-binding protein; PGC-1 α : Peroxisome proliferator-activated receptor- γ coactivator-1 α .

transcriptional activity of the *AMPK* gene and the activation of the enzyme by both phosphorylation (Figure 2A) and by the concomitant increase in the hepatocellular AMP/ATP ratio previously observed^[15]. Phosphoryla-

tion of hepatic ACC by activated AMPK is known to result in ACC inhibition, with a substantial diminution in the levels of malonyl-CoA, the potent allosteric inhibitor of CPT-1 α that favors the entry of acyl-CoAs into

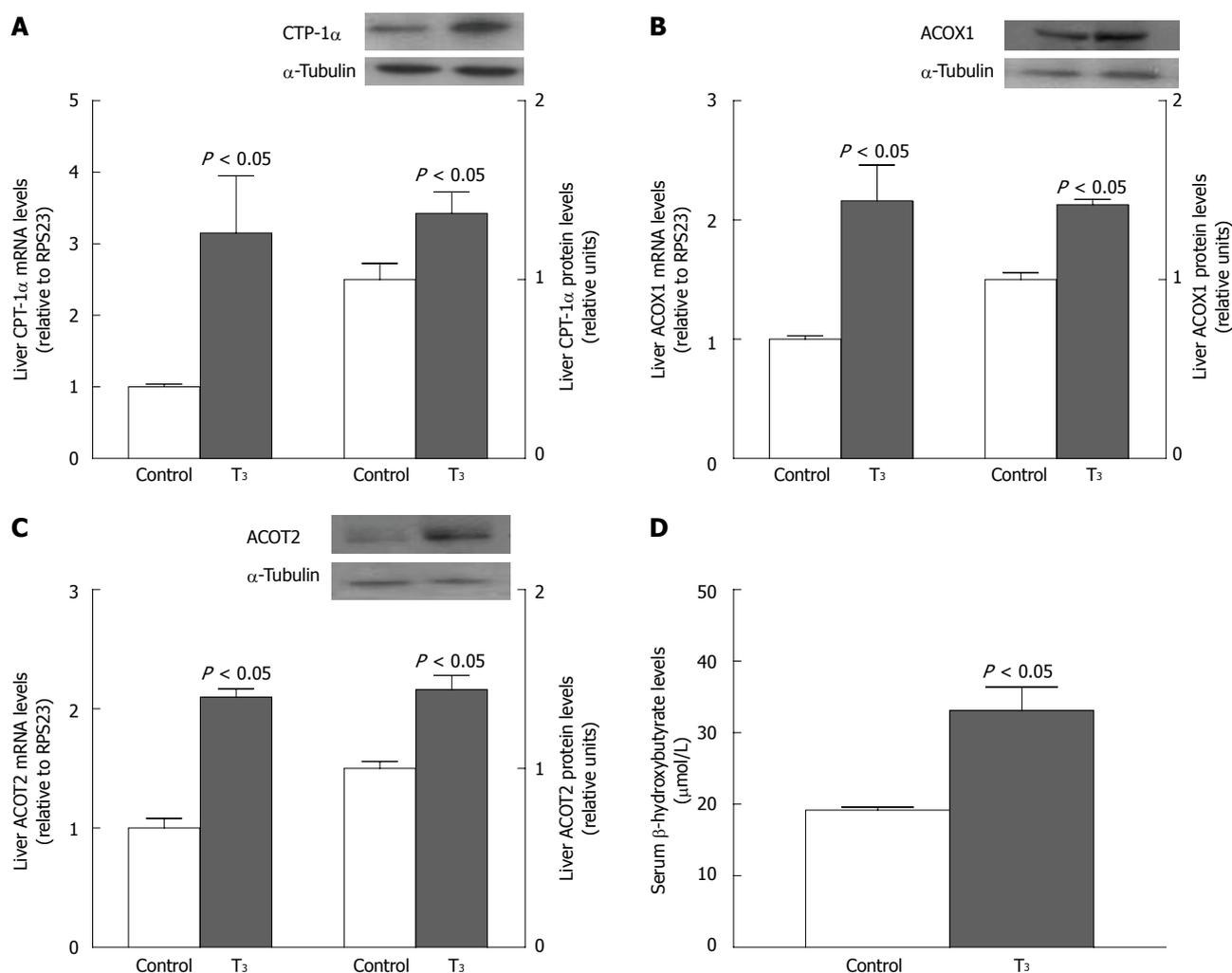


Figure 3 T₃-induced changes in liver AMPK-dependent targets related to fatty acid oxidation. mRNA and protein levels of CPT-1 α (A), ACOX1 (B), and ACOT2 (C), and circulating levels of β -hydroxybutyrate (D). Values shown are means \pm SEM ($n = 3-5$). Statistical significance was performed by Student's t-test for unpaired data ($P < 0.05$). AMPK: AMP-activated protein kinase; ACOT2: Acyl-CoA thioesterase 2; ACOX1: Acyl-CoA oxidase 1; CPT-1 α : Carnitine palmitoyltransferase-1 α .

mitochondria thus enhancing the FAO potential of the liver^[9,29]. In addition to ACC, T₃-induced AMPK activation is associated with increased CREB mRNA expression and CREB phosphorylation, a transcription factor that is central to diverse cellular responses including those related to T₃ signaling. In fact, after inducing the dissociation of the repression complex, T₃ stimulates the recruitment of several nuclear co-activators including CREB-binding protein (CBP/p300), which facilitates T₃/thyroid hormone receptor-dependent transcription activation^[30]. This is accomplished by AMPK-dependent CREB phosphorylation at Ser133^[31], which is known to increase its association with co-activator CBP/p300^[32]. Interestingly, T₃ administration significantly increased the mRNA expression of hepatic PGC-1 α , which co-activates several transcription factors associated with mitochondrial biogenesis that may also enhance the capacity of the cell for FAO^[29]. Furthermore, activated AMPK is able to elicit PGC-1 α activation by direct phosphorylation^[33], whereas control of PGC-1 α transcription is partially mediated by CREB^[29]. Taken as a whole, these data suggest that T₃ triggers liver AMPK upregulation confronting FAO,

which appears to be associated with (1) phosphorylation of its target enzyme ACC; and (2) phosphorylation and transcriptional regulation of CREB and PGC-1 α , signaling components that may undergo reinforcing mechanisms.

Transcriptional activation of nuclear receptor target genes is known to be triggered by co-activator molecules. These include PGC-1 α that upon activation coordinates induction of hepatic FA oxidation *via* co-activation of transcription factors PPAR- α , nuclear respiratory factors 1 and 2, and estrogen receptor-related- α ^[34]. Accordingly, PGC-1 α plays a role in the transduction of the T₃ stimulus to the transcriptional regulation of genes involved in liver energy metabolism, as proposed for cold exposure or fasting^[35]. Thus, T₃-induced liver AMPK signaling may involve CREB-CBP/p300 interaction with PGC-1 α upregulation leading to the PPAR- α -dependent expression of FAO-related enzymes (Figure 4). These include (1) CPT-1 α facilitating the entry of FAs into mitochondria for FAO; (2) ACOX1, a peroxisomal enzyme catalyzing the FAD-dependent desaturation of long-chain acyl-coenzyme A derivatives; and (3) ACOT2, a mitochondrial

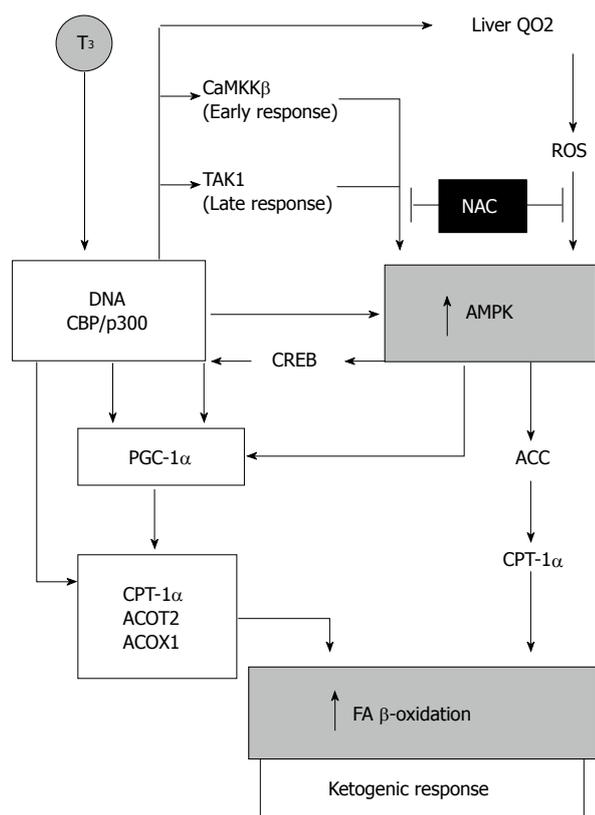


Figure 4 Schematic representation of T₃ signaling related to AMPK up-regulation and consequent fatty acid oxidation enhancement. ACC: Acetyl-CoA carboxylase; ACOT2: acyl-CoA thioesterase 2; ACOX1: acyl-CoA oxidase 1; AMPK: AMP-activated protein kinase; CaMKKβ: Ca²⁺-calmodulin-dependent protein kinase kinase-β; CBP/p300: CREB-binding protein; CPT-1α: Carnitine palmitoyltransferase-1α; CREB: cAMP-response element-binding protein; FA: Fatty acid; PGT-1α: Peroxisome proliferator-activated receptor-γ coactivator-1α; QO₂: Rate of oxygen consumption; ROS: Reactive oxygen species; TAK1: Transforming growth factor-β-activated kinase-1.

enzyme hydrolyzing acyl-coenzyme A thioesters to release free coenzyme A for FAO^[9,36,37] (Figure 4). In agreement with this proposal, T₃ administration resulted in a significant 72% enhancement in serum β-hydroxybutyrate levels over control values, a liver-derived ketone body that is considered a surrogate marker for hepatic FAO^[9].

In conclusion, data presented indicate that T₃-induced ROS production, revealed by higher 8-isoprostane levels in liver and plasma, have a causal role in upregulating rat liver AMPK signaling, resulting in FAO enhancement to support energy-demanding processes such as T₃-PC or tissue repair^[5-7] (Figure 4). This is evidenced by suppression of T₃-induced hepatic AMPK mRNA levels and those of the upstream kinases CaMKKβ and TAK1, as well as their protein expression, by pretreatment with the antioxidant NAC previous to T₃, findings that agree with higher pAMPK levels observed over control values. In addition, the higher AMPK phosphorylation potential elicited by T₃ is associated with enhancement in hepatic pACC/ACC and pCREB/CREB ratios as direct targets of activated AMPK (Figure 4). The latter changes induced by T₃ trigger FAO as assessed by the consequent ketogenic response, which may involve both ACC phos-

phorylation, with CPT-1α activation, and CREB phosphorylation, with enhanced expression of co-activator PGC-1α and that of the FAO-related enzymes CPT-1α, ACOX1, and ACOT2 (Figure 4). Enhancement in FAO-related energy metabolism by T₃ is in accordance with the enhanced liver ATP turnover reported^[15,38], which may comply with high-energy requiring processes such as liver PC^[5,6]. In agreement with this contention, T₃ was recently shown to stimulate hepatic FAO coupled with the induction of autophagy, a stress-related process degrading cellular components to produce FAs to generate ATP or amino acids to synthesize proteins for cell survival^[39]. Thyroid hormone PC is not restricted to the liver, considering that protective effects against IRI are also observed in the heart^[40,41], kidney^[42,43], and brain^[44]. In addition to ischemia-reperfusion injury, thyroid hormones have a critical role in the repair in several tissues subjected to other types of injury, namely, mechanical injury, nerve transection, chemotherapy-induced toxicity, hyperoxia injury, serum starvation, or wound^[7].

COMMENTS

Background

Liver preconditioning (PC) increases the tolerance to noxious stimuli such as ischemia-reperfusion injury due to previous maneuvers triggering beneficial changes, as shown for thyroid hormone (T₃) administration.

Research frontiers

T₃ liver PC involves promotion of cell proliferation and the expression of cytoprotective proteins including antioxidant, anti-apoptotic, and acute-phase proteins, phase-II detoxification enzymes, and phase-III transporters. In this research article, the authors addressed the study of AMP-activated protein kinase (AMPK) signaling as the metabolic basis for T₃ liver PC providing energy to power cytoprotective mechanisms.

Innovations and breakthroughs

T₃ administration to rats upregulates liver AMPK in a redox-dependent manner, as evidenced by its abrogation by pretreatment with the antioxidant N-acetylcysteine prior to T₃. The resulting AMPK activation is associated with phosphorylation of downstream targets leading to fatty acid oxidation enhancement, as shown by the increased ketogenic response, representing a molecular mechanism regulating energy dynamics.

Applications

Numerous experimental liver PC strategies have not been transferred to clinical practice due to major drawbacks and conflicting results. In this context, T₃ liver PC has potential clinical application as it is a well-tolerated therapeutic agent whose side effects are readily controlled.

Terminology

AMPK is considered a key energy sensor able to limit anabolic pathways, to diminish ATP consumption, and to facilitate catabolic pathways, to increase ATP production, thus limiting metabolic stresses in the liver.

Peer review

This is a detailed report on the mechanisms of T₃ in mediating AMPK signaling. The authors covered a range of early and late kinases in detail and showed that T₃ mediates AMPK signaling in a redox dependent manner.

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P- Reviewer: Ikura Y, Lu WY S- Editor: Qi Y L- Editor: A
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