UCP3 Expression in Liver Modulates Gene Expression and Oxidative Metabolism in Response to Fatty Acids, and Sensitizes Mitochondria to Permeability Transition

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Key Words

Uncoupling protein-3 • Liver • Gene expression • Permeability transition • Mitochondria

Abstract

Background/Aims: Uncoupling protein-3 (UCP3) is expressed in liver only under conditions of high fatty acid catabolism. However, the specific role of UCP3 in liver mitochondria and overall hepatic function is still poorly known. **Methods:** A model of “in vivo” induction of UCP3 expression in mouse liver mitochondria via a tail-vein injection of a recombinant adenoviral vector was developed. The effects on liver mitochondrial bioenergetics and permeability transition, liver gene expression, and systemic metabolism were then determined. **Results:** UCP3 expression in liver did not cause basal, non-specific, uncoupling but led to a stimulation of palmitate-induced state 4 respiration. UCP3 expression in liver also caused an increase in the expression of certain genes involved in lipid catabolism and metabolic response to starvation (e.g. medium chain acyl-CoA-dehydrogenase or peroxisome proliferator-activated receptor- γ co-activator-1 α). UCP3 also conferred to liver mitochondria an enhanced sensitivity to classical inducers of per-

meability transition, such as calcium and carboxyatractylate. **Conclusion:** UCP3 expression in liver exerts direct actions on mitochondrial activity, favoring fatty acid-induced uncoupling and sensitizing mitochondria to permeability transition, as well as causing retrograde signaling to nuclear gene expression consistent with favoring lipid catabolism and oxidative metabolism.

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Introduction

Uncoupling protein-3 (UCP3) is a member of the mitochondrial inner membrane transporter family. It is expressed at high levels in skeletal muscle, brown adipose tissue and, to a lesser extent, in heart, white adipose tissue and some specific types of neurons [1-3]. UCP3 is highly homologous to UCP1, which is mostly expressed in brown adipose tissue, and especially to UCP2, which is expressed in most tissues.

UCP3 is usually not expressed in the liver. However, high levels of expression of UCP3 in liver take place in situations of enhanced hepatic fatty acid catabolism (e.g. a high-fat diet plus treatment with fenofibrate,

or fenofibrate alone) [4, 5]. The ability of fatty acids to induce the expression of the UCP3 gene in peripheral tissues such as adipose tissue or skeletal muscle, is mediated by activation of peroxisome proliferator-activated receptors (PPAR) response elements located in the UCP3 gene promoter, at least in muscle cells [6, 7]. The molecular mechanisms that support the suppression of the expression of UCP3 in hepatocytes, however, seem to be avoided only by treatments that involve high fatty acid availability and a high lipid oxidation in the liver.

The functional significance of high expression of UCP3 in the liver is not fully understood. The current hypothesis about the physiological function of UCP3 in the tissues in which it is commonly expressed suggests a role in favoring oxidation and/or transport of fatty acids, and a role in protecting cells against a high production of reactive oxygen species [8, 9]. Potential involvements of UCP3 in enhancing fatty acid peroxide translocation [10] or in protecting against fat storage after a long-term high-fat feeding, have also been reported [11]. Situations of forced lipid metabolism in liver, in which UCP3 expression is induced, are known to be potentially deleterious for hepatocytes and may lead to a progressive damaging process in liver (i.e. from initial hepatic steatosis and oxidative stress to the development of cirrhosis and liver failure) [12]. UCP3 expression in liver may actually be a physiological reaction to protect hepatic cells against metabolic damage in such situations. On the other hand, UCPs have also been shown to be involved in the regulation of cell death mediated by mitochondria [3, 13, 14]. Critical events in cell death are regulated by mitochondrial pathways, among them the increase of the permeability of the mitochondrial inner membrane to molecules of up to 1.5 kDa mediated by the opening of the mitochondrial permeability transition pore (MPTP). The MPTP can be opened in response to different stimuli, including a decrease in the mitochondrial membrane potential, a high concentration of intramitochondrial calcium, or an exposure to the adenine nucleotide translocase inhibitor, atractylate [15, 16]. The expression of UCP3 in HEK-293 cells, sensitizes the MPTP opening to calcium stimulation [14]. Recently, it has been proposed that UCP3 may also participate to mitochondrial calcium uptake [17], even though this statement remains controversial [18].

In this study, we developed a model of “in vivo” ectopic expression of UCP3 in mouse liver using a recombinant adenoviral vector. This model system was then used to determine the consequences of a high expression of UCP3 on liver mitochondrial function, systemic metabolism and hepatic gene expression.

Materials and Methods

Induction of UCP3 expression in the liver mitochondria of mice

The mice used were of the strain C57Bl6/J (Harlan Interfauna) and were maintained in standard lighting conditions (12h cycles of light/dark) and temperature (21 ± 1 °C). Mice were fed a standard diet composed of 72% carbohydrates, 6% fat, 22% protein (% gross energy) (BK Universal, Spain). The care and use of mice were in accordance with the European Community Council Directive 86/609/EEC and approved by CEEA, University of Barcelona. The experiments were conducted with male mice 6.5 weeks of age. Mice were injected by the tail vein with 10^9 adenoviral pfu of a human UCP3 (AdCMV-UCP3) [19] or β -galactosidase (AdCMV-LacZ, used as a control) expression vectors. In preliminary studies, it was determined that 72h after injection was the time for optimal levels of expression of the transgene, and therefore this was the time used in all subsequent experiments. (data not shown). The experimental groups of mice subjected to fasting were starved during the 36h prior to sacrifice. After sacrifice of mice, liver was extracted, a fragment was frozen in liquid nitrogen for gene expression studies and the remaining was used for mitochondria isolation (see below). For comparative purposes, mitochondria were isolated from gastrocnemius muscle from mice under basal conditions or after 6-month high-fat diet treatment (45% calories as fat, BK Universal Spain) using standard procedures [20].

Immunodetection

Mitochondrial, nuclear and cytosolic fractions from mouse liver were separated by denaturing acrylamide gel electrophoresis (SDS-PAGE, 12.8%), transferred to a polyvinylidene fluoride membranes (PVDF membranes Immobilon-P, Millipore) and further incubated with antibodies against UCP3 (Chemicon AB3046 (1:1000)), cytochrome c (PharMingen clone 7H8.2C12 (1:500), histone H3 (H0164 (1:5000) and β -actin (Sigma clone AC-15 (1:10000)). The binding of antibodies was detected with a secondary anti-mouse antibody (Bio-Rad 170-6516 (1:3000)) or anti-rabbit (Santa Cruz sc-2004 (1:3000)), both horseradish peroxidase-conjugated, and using a chemiluminiscent enhanced detection kit (ECL, Amersham).

Subcellular fractionation and obtention of functional mouse liver mitochondria

The liver was removed from mice and immediately homogenized in a buffer containing 250 mM deionized saccharose, 1 mM EDTA, 50 mM KCl, 25 mM Tris, using a glass homogenizer. The homogenate was centrifuged at 700g for 10 min at 4 °C. The resulting supernatant was centrifuged at 7000g for 10 min at 4 °C. The pellet after the 700g centrifugation and the supernatant after the 7000g centrifugation corresponded to the crude nuclear and the crude cytosolic fractions, respectively. The final pellet, that was resuspended in a small volume (about 400 μ) of homogenization buffer, corresponded to the liver mitochondrial fraction. Part of these fractions was supplemented with 2 mM PMSF, 2 mg/ml leupeptine,

pepstatine, aprotinin and 2 mM benzamidine and kept for further immunoblotting studies. All processes were performed at 4 °C. Protein concentration assessment was performed after diluting the samples 1/10 in homogenization buffer with 0.3% Triton and further use of the BCA Protein Assay kit (Pierce).

Oxygen consumption assays

Respiration rates of the mitochondrial fraction obtained from mouse liver, were determined at 25 °C using a Clark oxygen electrode (Rank Brothers, Ltd.) connected to a micro-computer that allows a continuous readout of the concentration of oxygen in the medium. The medium composition was 250 mM deionized saccharose, 1 mM EDTA, 50 mM KCl, 25 mM Tris, 5 mM KH₂PO₄, and 0.3% delipidated BSA (Sigma). State 4 respiration measurements, which allowed an estimation of the extent of proton leakage, were obtained by incubating the mitochondria in the presence of 10 µg/ml oligomycin using either 6 mM glutamate/malate, or 6 mM succinate plus 5 µM rotenone as respiratory substrates. Oxygen consumption in the presence of one respiratory substrate and 1 mM ADP represented the state 3 respiration. The respiratory control index was calculated on the basis of the state 3/state 4 ratio for each experimental setting. In state 4 respiration conditions (6 mM succinate, 5 µM rotenone, 10 µg/ml oligomycin), the effects of the addition of 300 µM palmitate, or 3 µM CCCP were also determined.

Determination of liver mitochondria swelling

Mitochondrial swelling in response to MPTP activators and inhibitors was measured as an index of MPTP opening, as already described in [21]. Mitochondrial swelling was assessed by determining the decline of the absorbance at 540 nm of mitochondrial suspensions at 25 °C. The liver mitochondria were obtained according to the procedure described previously, but the final pellet was resuspended in a buffer containing 213 mM mannitol, 71 mM sucrose, 5 mM Hepes/Tris pH 7.8. Mitochondrial suspensions (0.25 mg protein/ml) were incubated in a buffer containing 250 mM deionized saccharose, 5 mM succinate, 10 µM EGTA, 2 µM rotenone, 1 mM KH₂PO₄ and 10 mM TrisMOPS pH 7.4 at 25 °C. At this time, 5 µM cyclosporine A (CsA) (Sigma) was added, when needed. After 2 minutes, a continuous readout of the absorbance at 540 nm was performed during 6 minutes using a spectrophotometer in kinetic mode (Shimadzu UV-160A). CaCl₂ (100-200 µM) or atractylate (150-300 µM) (Sigma) were added and mixed with the solution right before starting the experiment.

Analysis of Gene Expression by Quantitative Real-Time PCR

RNA was extracted from samples of mouse liver using the Rneasy kit (Qiagen). The quantitative analysis of the expression of mRNA was conducted in a PCR reaction in two steps: first, 1 µg RNA was retro-transcribed into cDNA in the GeneAmp PCR 2400 system (Applied Biosystems, USA), and then a Real-Time PCR was carried out on the ABI PRISM 7700HT sequence detection (Applied Biosystems, USA). The RT-PCR was performed in a final volume of 50 µl using a TaqMan Reverse Transcription Reagents kit (Applied Biosystems, USA), con-

Fig. 1. Induction of UCP3 expression in mouse liver mitochondria via adenoviral-mediated gene transfer. A, Immunoblot analysis of 30 µg of mitochondrial protein obtained from the liver of mice injected with an adenoviral vector driving either UCP3 (AdCMV-UCP3) or β-galactosidase (AdCMV-LacZ, used as a control) expressions, and probed with specific UCP3 and cytochrome c antibodies. Liver samples were obtained 72 h after adenovirus injections. For comparative purposes, immunoblots of 30 µg of mitochondrial protein from gastrocnemius skeletal muscle of mice under control conditions or after 6-month high-fat diet are shown. B, Immunoblot analysis of 20 µg of protein from mitochondria (M), crude nuclear (N) or crude cytosolic (C) fractions from liver of mice injected with AdCMV-UCP3. Detection was performed using specific UCP3, cytochrome c, histone H3 or β-actin antibodies.

taining the reverse transcriptase MultiScribe™, an inhibitor of RNase, a mixture of dNTP, Random Hexamers, 10X buffer for RT, and a solution of MgCl₂. The reaction of real-time PCR was carried out in a final volume of 25 µl using TaqMan Universal PCR Master Mix, and the following probes standardized for quantification of the corresponding transcripts: Mm00447183, peroxisome proliferator-activated receptor γ-coactivator-1α (PGC-1α); Mm00656767, glutathione peroxidase-1 (GPX1); Mm00515041, GPX4; Mm00449726, superoxide dismutase-2 (SOD2); Mm00448091, sequestosome-1; Mm00476361, acyl-Coenzyme A dehydrogenase, medium chain (MCAD); Mm00440939, PPARα; Mm00803186_g1, PPARδ; and Hs99999901, 18S ribosomal RNA (Applied Biosystems, USA). Each sample was analyzed in duplicate, and the average values of each duplicate were used to determine the level of mRNA

Fig. 2. Effects of UCP3 on liver mitochondria respiratory rates. A, Respiratory rate of liver mitochondria in state 4 (in the presence of 10 μ g/ml oligomycin) and state 3 (in the presence of 1mM ADP) using either 6 mM succinate or 6mM glutamate/malate as respiratory substrates B, Respiratory rate in state 4 after addition of 300 μ M palmitate or 3 μ M CCCP. Data are means $\pm$ SEM of oxygen consumption rate in natO/min/mg mitochondrial protein in 4-6 experiments from independent mitochondrial preparations from distinct mice. * p <0.05 shows statistical significance respect to state 4 oxygen consumption levels for each condition (panel A), or respect to control in each condition (panel B). # p <0.05 indicates statistical significance of differences due to UCP3 expression.

expression of each study. The amount of transcripts of interest in each sample was normalized in relation to a reference control using the method of comparison (2- Δ CT) according to manufacturer's instructions (Applied Biosystems, USA). In our case, the 18S ribosomal RNA was used as reference control.

Assessment of the concentration of circulating metabolites

Quantification of plasma metabolite levels was performed with Accu-Chek Sensor devices for glucose, triacilglycerides and lactate (Roche) according to the manufacturer instructions. The ketone bodies and non-sterified fatty acids (NEFA) were measured by the colorimetric kits β -hydroxybutyrate liquid Color (Stanbio) and NEFA C ACS-ACOD Method (Wako), respectively.

Statistical Analysis

The data are shown as mean $\pm$ standard error, and were analyzed with the Student's t-test. Differences were considered significant for p-values less than 0.05.

Results

Establishment of UCP3 ectopic expression in mouse liver mitochondria

Gene transfer "in vivo" via adenoviral vectors allowed the expression of high levels of UCP3 in liver mi-

tochondria, at levels comparable to those present in skeletal muscle under conditions of mice treated with a high fat diet (Fig 1A). The highest expression of UCP3 was achieved 72h after the injection of the vector in our experimental model (data not shown). We found that UCP3 expressed in the liver was properly directed to the mitochondria as it could only be detected in the mitochondrial, but not in the nuclear or the cytosolic fraction (Fig 1B). This result is in agreement with the specific detection of adenoviral-driven expressed UCP3 in mitochondrial fractions of other model systems as human muscle cells differentiated *in vitro* [19]. Immunoblot analysis of tissues other than liver (skeletal muscle, brown fat, spleen) indicated a negligible effect on UCP3 levels as a consequence of Ad-CMV-UCP3 injection to mice (not shown). This is in agreement with the known preferential adenoviral gene transfer to mouse liver after direct injections through the tail vein [22].

Effects of UCP3 on liver mitochondria bioenergetics

Expression of UCP3 in liver mitochondria did not induce a significant change of state 4 or state 3 respiratory rates obtained when either glutamate/malate (which provides electrons preferentially to the respiratory chain

Fig. 3. Effects of UCP3 expression in liver on the expression of genes related to oxidative stress and metabolic response to starvation. Data are means $\pm$ SEM of 5-6 mice/groups and are expressed as the relative values of expression respect to AdCMV-LacZ-injected mice under fed conditions (controls). Statistically significant differences due to UCP3 expression in fed or fasted conditions are shown as * $p < 0.05$, and those due to fasting respect to fed conditions either in AdCMV-LacZ-injected or AdCMV-UCP3-injected mice, as # $p < 0.05$.

complex I) or succinate (which provides the electrons directly to complex II) were used as respiratory substrates (Fig 2A). Consequently, the respiratory control index (i.e. the ratio between state 4 and state 3 respiratory rates) was also unchanged in both conditions (i.e. 9.0 ± 0.4 in UCP3-expressing versus 8.9 ± 2.7 in controls; and 8.2 ± 1.7 in UCP3-expressing versus 8.1 ± 1.8 in controls when glutamate/malate or succinate were used as respiratory substrate, respectively).

The effect of palmitate on state 4 respiration was then determined on both UCP3-expressing and control mouse liver mitochondria using succinate as respiratory substrate. Fig 2B shows that the presence of UCP3 specifically enhanced the increase of state 4 mitochondrial respiratory activity in the presence of 300 μ M of palmitate. This result indicates that back-leakage of protons due to UCP3 was only occurring after the addition of the fatty acid. The differential effect of palmitate on respiratory rates in mitochondria expressing UCP3 was specific, and did not affect the respiratory maximal capacity of the mitochondria which was attained after addition of the chemical uncoupler CCCP.

UCP3 effect on gene expression in liver

We determined the impact of the expression of UCP3 on the mRNA levels of genes related to oxidative stress and lipid catabolism in liver (Fig 3). For oxidative stress, the expression of superoxide dismutase-2 (SOD2) and glutathione peroxidase-4 (GPX4) were determined, as they are major enzyme systems for scavenging reac-

tive oxygen species in mitochondria and are also genes highly susceptible to be induced by oxidative stress-related signals [23]. The study was expanded to glutathione peroxidase-1 (GPX1), a major enzymatic system contributing to scavenging reactive oxygen species in the cytosol [24]. The presence of UCP3 did not change the expression of genes encoding for these components of the cellular antioxidant machinery when the mice were regularly fed. However, fasting of mice for 36h caused a statistically significant induction of gene expression for SOD2 and GPX4 (the mitochondrial components of the antioxidant machinery) only in UCP3-expressing mouse livers. We also considered the transcript levels of the sequestosome-1 gene, which encodes for a protein involved in both pro-inflammatory processes and lipid catabolism in liver [25]. These levels are therefore potentially informative on the cross-talk status of these pathways in the experimental conditions tested. Results indicated that sequestosome-1 (SQM1) gene expression was also significantly induced by fasting in the liver of UCP3-expressing mice.

About genes related to fatty acid catabolism, we first considered the mRNA levels of the medium-chain acyl-CoA dehydrogenase (MCAD) gene, which encodes for a main component of the mitochondrial fatty acid β -oxidation pathway [26]. Transcription of the MCAD gene was induced by fasting in mouse liver, as expected. The MCAD mRNA levels were significantly higher in liver from mice expressing UCP3 by respect to controls in the fasted conditions. The expression levels of PGC-1 α

Table 1. Serum metabolite levels in mice expressing UCP3 in liver (AdCMV-UCP3-injected) and controls (AdCMV-LacZ-injected), in fed and fasted conditions.

	AdCMV-La		AdCMV-UCP3	
	Fed	Fasted	Fed	Fasted
Glucose (mg/dl)	189 ± 55	112 ± 15*	171 ± 15	122 ± 10*
Triglycerides (mg/dl)	374 ± 61	329 ± 29	445 ± 32	287 ± 73
NEFA (mM)	1.61 ± 0.25	2.73 ± 0.44*	1.64 ± 0.27	3.36 ± 0.65*
β-hydroxybutyrate (μM)	162 ± 40	1516 ± 297*	153 ± 19	1264 ± 324*

mRNA were also significantly over-induced in UCP3-expressing liver versus controls in response to starvation. This finding was particularly relevant considering that PGC-1α is a transcriptional co-activator acting as a master controller of the adaptations in hepatic gene expression in response to fasting [27].

Expression of UCP3 in liver does not cause major changes in the levels of circulating metabolites

The presence of high levels of UCP3 in mice liver did not cause a significant change in the concentration of any of the circulating metabolites tested, i.e. glucose, non-esterified fatty acids (NEFA), triglycerides, or β-hydroxybutyrate) (Table 1). After subjecting the mice to fasting during the 36h prior to sacrifice, circulating metabolite levels changed as expected (decrease in glucose levels, increased levels of β-hydroxybutyrate and NEFA), indicating enhanced lipolysis and NEFA availability as well as enhanced lipid catabolism in liver (Table 1). In fasted mice, the expression of UCP3 did not significantly modify the levels of these circulating metabolites.

UCP3 sensitizes liver mitochondria to the opening of MPTP

Opening of the MPTP was assessed by measuring the decrease in absorbance at 540 nm of mitochondrial suspensions that could be inhibited by cyclosporine A (CsA). Mitochondria isolated from liver of mice expressing UCP3 showed a more rapid decline in absorbance in basal conditions. Increasing concentrations of calcium caused a dose-response increase in the rate of mitochondrial swelling that was always more pronounced in mitochondria containing UCP3. Such swelling process was fully inhibited by CsA (Fig 4). In order to determine whether the effects observed were specific for the calcium-dependent stimulus of MPTP opening, the effects of a distinct agent, atractylate (an inhibitor of the adenine nucleotide translocase known to cause the opening of MPTP) were determined. Atractylate caused also MPTP opening as evidenced by progressive swelling and this effect was also more pronounced in mitochondria containing UCP3. Finally, CsA also inhibited atractylate-in-

duced swelling (data not shown).

Discussion

Ectopic expression of UCP3 in mitochondria of liver provides an experimental model for a direct investigation of the physiological role of UCP3 expression in liver, as well as it constitutes a unique tool for analyzing the impact of UCP3 in mitochondria bioenergetics. As liver mitochondria in basal conditions is almost devoid of any UCP protein (UCP2 protein expression is almost negligible in liver [5]), ectopic expression of UCP3 in liver occurs in an almost “UCPs-free” mitochondrial environment and this facilitates the study of what changes are elicited specifically by UCP3. The behaviour of liver mitochondria expressing UCP3 indicated no changes in basal respiration rate, either in state 3 or state 4. There were also no changes in the respiratory control index, all this indicating no signs of uncontrolled uncoupling of mitochondria due to a strong adenoviral-driven expression of UCP3. However, the presence of UCP3 conferred to liver mitochondria a higher sensitivity to increase state 4 respiration in response to palmitate, in agreement with the known positive action of fatty acids on UCPs activity [28]. The analysis of liver mitochondria from rats treated with fenofibrate, a situation in which hepatic UCP3 expression is induced [5], also indicated a lack of major alterations in basal uncoupling activity. However, in this situation, the bioenergetic behaviour of mitochondria could be affected by changes in the abundance of other mitochondrial proteins elicited by fenofibrate effects. Fenofibrate is known to modify the expression of a plethora of genes related to lipid metabolism in liver, mainly via PPARα activation and subsequent induction of its target genes [29]. The previous studies by Lanni and collaborators [4, 5] established that fenofibrate can even overcome the basal repression of the UCP3 gene, a target of PPARα, in liver cells, thus suggesting the involvement of UCP3 in liver metabolism when a high fat oxidation takes place. In our present experimental setting, we wanted to establish whether an increase in UCP3 levels in mito-

Fig. 4. Effects of UCP3 on MPTP opening. Representative example of time-dependent swelling of UCP3-expressing or non-UCP3-expressing mouse liver mitochondria. Data show the profile of decrease in absorbance at 540 nm of mitochondrial suspensions after exposure to the indicated concentrations of CaCl_2 (A) or atractylate (B), and the action of cyclosporine A (CsA). The figure is representative of 4-6 measurements from independent preparations of mitochondria from distinct mice.

chondria could be a primary cause of adaptive modulation of gene expression, regardless of the independent action of fenofibrate on the regulation of other genes. We observed that UCP3 expression in liver did not cause major changes in the expression of the marker genes of oxidative stress or lipid catabolism analyzed in fed mice. Considering the dependency of UCP3 activity on the action of fatty acids and the enhanced availability and oxidation of fatty acids in liver during fasting conditions, the study of the effects of UCP3 expression in liver was expanded to mice subjected to long-term fasting (36h). This condition was chosen to maximize the delivery of fatty acid coming from white fat lipolysis to the liver. In fact, UCP3 has been recently reported to be essential for fasting-induced enhancement of fatty acid oxidation rate, although the mechanism remains unclear because mitochondrial fatty acid oxidation as such and fatty acid anion export appear to be unaltered [30].

The gene expression of MCAD, an enzyme playing a key role in mitochondrial fatty acid oxidation machinery [26], was over-induced due to fasting in UCP3-expressing mice. The same happened for the expression of PGC-1 α , a transcriptional co-activator essential for the induction of gluconeogenic and fatty acid oxidation genes in response to starvation in liver [31]. These changes occurred without modifications in the expression of the PPAR α or PPAR δ , which are nuclear receptors controlling lipid oxidative genes in liver. It is known that PPARs can mediate changes in gene expression without changes in their own levels, either via ligand-dependent activation or through interaction with co-activators such as PGC-1 α [32]. A milder UCP3-dependent induction of gene expression was observed during fasting for SOD2 and GPX4; two genes marker of oxidative stress in mitochondria. The same happened for the mRNA of sequestosome-1, a protein related to the ubiquitin system, required for

correct lipid catabolism in liver and playing also an important role in membrane-receptor mediated signal transduction in relation to inflammatory pathways and oxidative stress [25]. Overall, these observations suggest that UCP3 expression may elicit some type of “retrograde” signaling on nuclear gene expression, eliciting an enhancement of fatty acid catabolism pathways. It also suggests that such mechanism involve the induction of PGC-1 α , a transcriptional co-activator acting as a master controller of the metabolic response of liver to starvation, including the activation of lipid catabolism via co-activation of PPARs [27, 31]. Genes such as MCAD or SOD2 are targets of PGC-1 α [33, 34] and their higher expression in fasted mice expressing UCP3 in liver may be secondary to the over-induction of PGC-1 α . It is worth to mention that UCP3 expression in liver did not cause however massive changes in systemic metabolism. This indicates that, although UCP3 could be involved in activating part of the gene program associated with enhancement of lipid metabolism, UCP3 could also have a protective role against lipotoxicity when there is an overflow of fatty acid to be oxidized. The mechanisms by which the expression of UCP3 may affect PGC-1 α gene expression and elicit a subsequent cascade of gene regulatory events should deserve further research. It is worth noting that, in a distinct tissue system (adipose tissue), changes in the content of UCP2 in mitochondria exert such “retrograde” regulation of nuclear gene expression via the modulation of reactive oxygen species generation [35].

A further observation of the effects of UCP3 on mitochondrial behavior was that UCP3 sensitized mitochondria to the opening of the permeability transition pore, and this sensitization occurred not only in the MPTP opening response to calcium but also in response to a distinct inducer such as the adenine nucleotide translocator inhibitor, atractylate. It has been recently proposed that UCP3 (and also UCP2) could act as favoring calcium transport into mitochondria [17], although this statement has been the subject of a recent controversy [18]. The fact that the UCP3-dependent sensitization to MPTP opening occurs also in response to atractylate may indicate that it is not exclusive to the direct response of mitochondria to calcium, although several authors propose that MPTP opening in response to adenine nucleotide translocase inhibition also occurs via alterations in calcium permeability [16]. The functional significance of this role of UCP3 in relation to overall cellular functions is unclear. MPTP opening is often associated with the induction of cellular cell death, but this is an unlikely role

for UCP3 in liver considering that the actions of UCP3 are often associated with cellular protective effects (i.e. reduction of oxidative stress and reduction of lipotoxicity) [36]. In HEK-293 cells in culture, UCP3 favors MPTP opening in association with sensitization to apoptosis [14] whereas no sensitization of apoptotic cell death occurs in response to UCP3 over-expression in myogenic cells [37]. On the other hand, recent data indicate that MPTP transient opening may have physiological roles in addition to the control of cell death, in relation to energy metabolism, calcium homeostasis and reactive oxygen species generation [16, 38]. Further research will be needed to establish the physiological significance of UCP3-dependent sensitization to MPTP opening for hepatic cell functions. Moreover, the mechanisms by which UCP3 favors this phenomenon should also be further investigated, and either indirect effects (via modulation of the mitochondrial membrane potential) or direct effects could be hypothesised. In this last sense, members of the mitochondrial transporter family such as the adenine nucleotide translocase or the phosphate carrier, and even UCPs, have been shown to be capable of acquiring a pore conformation under certain conditions thus favoring permeability transition [16, 39, 40], and it cannot be excluded that UCP3, as a member of this protein family, was also directly involved. Finally, the observation that the presence of UCP3 induces changes in nuclear gene expression lead to the consideration that some of the effects observed in UCP3-expressing mitochondria could be indirect. For instance, it cannot be discarded that the presence of UCP3 in mitochondria lead to changes in the expression of any of the multiple proteins involved in mitochondrial permeability transition, and this indirect effect accounts for MPTP sensitization and not necessarily the presence of UCP3 itself. Further research will be required to clarify these issues.

In summary, the expression of UCP3 in liver confers to hepatic metabolism several features: it increases fatty acid-mediated mitochondrial oxidation, enhances gene expression associated to lipid catabolism pathways in starvation, and increases sensitivity to opening the mitochondrial permeability transition pore. It is proposed that UCP3 expression may be part of the liver adaptations to exposure of liver to high fatty acid availability, and contribute to favor mitochondrial oxidative capacity and enhancement of the enzyme machinery for lipid catabolism. In this sense, UCP3 may be viewed as a protective system against lipotoxicity in liver in front to the challenge of high fat exposure of dietary or other origin.

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