Molecular Genetics and Metabolism xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

The higher susceptibility of congenital analbuminemic rats to Ca^{2+}-induced mitochondrial permeability transition is associated with the increased expression of cyclophilin D and nitrosothiol depletion

Tiago R. Figueira [a], Roger F. Castilho [a], Ângela Saito [a], Helena C.F. Oliveira [b], Anibal E. Vercesi [a,*]

[a] Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil
[b] Departamento de Fisiologia e Biofísica, Instituto de Biologia; Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 9 August 2011
Received in revised form 30 August 2011
Accepted 30 August 2011
Available online xxxx

Keywords:
Analbuminemia
Nagase rats
Calcium retention capacity
Hypoalbuminemia
Dyslipidemia

ABSTRACT

Congenital analbuminemia is a rare autosomal recessive disorder characterized by a trace level of albumin in blood plasma and mild clinical symptoms. Analbuminemic patients generally present associated abnormalities, among which dyslipidemia is a hallmark. In this study, we show that mitochondria isolated from different tissues (liver, heart and brain) from 3-month-old analbuminemic rats (NAR) present a higher susceptibility to Ca^{2+}-induced mitochondrial permeability transition (MPT), as assessed by either Ca^{2+}-induced mitochondrial swelling, dissipation of membrane potential or mitochondrial Ca^{2+} release. The Ca^{2+} retention capacity of the liver mitochondria isolated from 3-month-old NAR was about 50% that of the control. Interestingly, the assessment of this variable in 21-day-old NAR indicated that the mitochondrial Ca^{2+} retention capacity was preserved at this age, as compared to age-matched controls, which indicates that a reduced capacity for mitochondrial Ca^{2+} retention is not a constitutive feature. The search for putative mediators of MPT sensitization in NAR revealed a 20% decrease in mitochondrial nitrosothiol content and a 30% increase in cyclophilin D expression. However, the evaluation of other variables related to mitochondrial redox status showed similar results between the controls and NAR, *i.e.*, namely the contents of reduced mitochondrial membrane protein thiol groups and total glutathione, H_2O_2 release rate, and NAD(P)H reduced state. We conclude that the higher expression of cyclophilin D, a major component of the MPT pore, and decreased nitrosothiol content in NAR mitochondria may underlie MPT sensitization in these animals.

1. Introduction

Due to albumin's abundance in relation to total plasma proteins, albumin exerts a major contribution to total plasma coloidosmotic pressure and fluid distribution across body compartments under normal physiology [1]. Many other important roles of plasma albumin are usually less highlighted. At nearly 42 mg/mL, plasma albumin is quantitatively the main plasma free radical scavenger [2]. Nearly 80% of total plasma reduced thiol groups and nitrosothiols also reside in albumin molecules [3]. Owing to its binding properties, albumin can act as a sink, which confers protection against the toxicity of certain substances, including xenobiotics and also some endogenous metabolites such as bilirubin [1, 4]. Prominently, a low level of plasma albumin, which is a common feature of certain diseases, is an independent risk factor for cardiovascular disease and all-cause mortality [5, 6].

Astonishingly, despite the mentioned roles of plasma albumin, humans and rats are viable with only trace levels of plasma albumin (<1 mg/mL) [7, 8]. Human congenital or familiar analbuminemia is a rare autosomal recessive disorder where the primary defects are mutations in the albumin gene [10]. These mutations result in a lack of liver albumin synthesis and, consequently, very low levels of plasma albumin. Although congenital analbuminemia is rare among humans, the understanding of some of its features was aided by the establishment of a colony of mutant Nagase analbuminemic rats (NAR), which closely resemble the human disease [11]. Similar to human patients, albumin is not produced in the liver of NAR, which ultimately results in a severe plasma albumin deficiency (values as low as 0.042 mg/mL) [9, 12]. In both species, the total plasma protein level is slightly below the reference range as the lack of albumin is quite well-compensated by the secretion of other proteins, mainly globulins [7, 8, 11].

A hallmark of analbuminemia is an abnormal plasma lipid and lipoprotein profile. NAR and individuals with analbuminemia present high levels of triglycerides, cholesterol and lipoproteins [13-15]. A unique feature of analbuminemic dyslipidemia is a plasma free fatty acid (FFA) deficiency [13, 14, 16]. Hyperlipidemia is a major risk factor for many diseases that generally involve lipotoxicity and cellular

Abbreviations: SDR, Sprague-Dawley rats; NAR, Nagase analbuminemic rats; FFA, free-fatty acid; MPT, mitochondrial permeability transition.

* Corresponding author at: Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 13083–887, Campinas, SP, Brazil. Fax: +55 19 3521 9434.
E-mail address: anibal@unicamp.br (A.E. Vercesi).

1096-7192/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2011.08.031

stress [17]. Our group has recently reported on abnormal mitochondrial functions in primary hypertriglyceridemic (ApoCIII transgenic mice) and hypercholesterolemic (LDL receptor knockout mice) mice [18–22]. A common alteration between these two hyperlipidemic mouse models previously studied by our group is a higher susceptibility to Ca^{2+}-induced inner mitochondrial membrane permeability transition (MPT) [18, 21]. MPT is a process that can lead to cell death as it is followed by mitochondrial energy failure and the release of pro-apoptotic factors from this organelle [23]. MPT is involved in many pathological processes, which encompass cellular Ca^{2+} overload and oxidative stress (*e.g.*, ischemia–reperfusion) [23]. In Ca^{2+}-loaded mitochondria, the occurrence of MPT is favored by oxidative stress [24-30], and more recently nitric oxide and nitrosothiols have emerged as important players in MPT regulation as well [31–33].

In this paper, we aimed at studying mitochondrial (dys)function in dyslipidemic analbuminemic rats.

2. Material and methods

2.1. Chemicals and reagents

Unless otherwise stated, all utilized chemicals were of the highest grade and purchased from Sigma (St. Louis, MO, USA). Chemical abbreviations: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid). EDTA, 2,2′,2″,2‴-(ethane-1,2-diyldinitrilo)tetraacetic acid. EGTA, glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid. FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone. HEPES, 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid. SDS, sodium dodecyl sulfate. t-BOOH, *tert*-butyl hydroperoxide.

2.2. Animal housing and plasma variable assessments

NAR founders were kindly donated by Dr. Eder Quintão (University of São Paulo Medical School) and were bred and maintained in the animal facility of our department. Control rats (SDR, Sprague-Dawley rats) were from the breeding colony at our university. The experiments were approved by the Committee for Ethics in Animal Experimentation at the university and are in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences. Rats were housed at 22 ± 2 °C on a 12-h light-dark cycle with free access to a standard laboratory rodent chow diet (Nuvital CR1, Nuvital, Curitiba, PR, Brazil) and water. Groups of 3-month-old male rats were used for the measurements described below.

Plasma variable measurements were carried out after 20 h of fasting, and blood samples were taken from the tip of the tail. Triglycerides (Roche Diagnostics, Mannheim, Germany), total cholesterol (Roche Diagnostics, Mannheim, Germany), total proteins (Bradford reagent, St. Louis, MO, USA) and free fatty acids (Wako Chemicals, Osaka, Japan) were determined in the plasma using enzymatic-colorimetric methods according to the manufacturer's instructions. For the remaining experiments with isolated mitochondria, organs were harvested from fed rats after their sacrifice by decapitation in the morning (9–10 AM). Livers were also harvested from 21-day-old male NAR and SDR rats for mitochondrial isolation after sacrifice by cervical dislocation.

2.3. Mitochondrial isolation, incubation conditions and respirometry

Mitochondria from liver, heart, and brain were isolated by conventional differential centrifugation, as detailed and described elsewhere [34–36]. The protein concentration of final mitochondrial suspensions was determined using a modified Biuret assay. Unless otherwise stated, all the experiments with isolated mitochondria were performed at 28 °C in a standard reaction medium (125 mM sucrose, 65 mM KCl, 2 mM K_2HPO_4, 1 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.2)) containing a cocktail of NADH-linked substrate (3.4 mM malate, 1.86 mM α-ketoglutarate, 2.1 mM pyruvate, 2.1 mM glutamate). Oxygen consumption was measured using a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA) in a 1.3-mL glass chamber equipped with a magnetic stirrer and filled with standard reaction medium supplemented with EGTA (200 μM). Stimulated respiration by oxidative phosphorylation (State 3 respiration) was elicited by the addition of ADP to a final concentration of 300 μM. Phosphorylation efficiency (ADP/O) was calculated as the molar ratio between the amount of added ADP and the oxygen consumed during State 3. For all experiments with functioning mitochondria, the equipments employed had magnetic stirrers and a temperature-controlled water bath. The final concentration of mitochondrial proteins was 0.5 mg/mL in the respiratory and MPT assays.

2.4. Assessment of Ca^{2+}-induced Mitochondrial Permeability Transition (MPT)

In suspensions of isolated mitochondria, cyclosporin A-sensitive Ca^{2+}-induced MPT results in osmotic swelling, disruption of transmembrane electrical potential, and Ca^{2+} release to the medium; all of which can be spectrophotofluorometrically followed over time.

Mitochondrial swelling was accessed by incubating organelles in 2 mL of standard reaction medium, and the decrease in the absorbance of the mitochondrial suspension (measured at 540 nm in a Hitachi U-3000 spectrophotometer, Tokyo, Japan) was taken as mitochondrial swelling. Specifically for brain mitochondria, the light scattering (excitation and emission wavelength set at 540 nm) of the suspension was measured on a Hitachi 4010 spectrofluorometer (Hitachi, Tokyo, Japan) instead of taking absorbance measurements.

Disruption of the transmembrane electrical potential ($\Delta\psi$) was evaluated by incubating mitochondria in 2 mL of standard reaction medium supplemented with safranine (5 μM), and $\Delta\psi$ was assessed by following safranine O fluorescence changes on a spectrofluorometer (Shimadzu RF-5301 PC, Kyoto, Japan) operated at excitation and emission wavelengths of 495 and 586 nm, respectively, and slit widths of 3 nm [37].

Ca^{2+} retention capacity was determined in mitochondria incubated in 2 mL of standard reaction medium supplemented with 0.2 μM Calcium Green-5N (Molecular Probes, Invitrogen, Carlsbad, CA). Levels of external free Ca^{2+} were measured by recording the fluorescence of Calcium Green-5N on a spectrofluorometer (Shimadzu RF-5301 PC, Kyoto, Japan) operated at excitation and emission wavelengths of 506 and 532 nm, respectively, and slit widths of 5 nm. Three min after the addition of mitochondria (0.5 mg/mL) to the cuvette, boluses of 5 nmol of $CaCl_2$ were sequentially added every third min until the mitochondria began to release Ca^{2+} into the medium. The amount of $CaCl_2$ added prior to mitochondrial Ca^{2+} release was taken as the mitochondrial Ca^{2+} retention capacity, a quantitative approach to compare MPT between groups [38].

2.5. Mitochondrial releasable endogenous Ca^{2+}

Isolated mitochondria were suspended to a final concentration of 1.0 mg/mL in 2 mL of standard reaction buffer supplemented with 2 μM ruthenium red (an inhibitor of the calcium uniporter and mitochondrial Ca^{2+} uptake) and 40 μM arsenazo III (an indicator of external free Ca^{2+}). The differential absorbance of arsenazo III (685–665 nm) was measured on a dual wavelength spectrophotometer (DW 2000 Aminco, SLM Instruments, Urbana, IL, USA). After recording the initial differential absorbance of energized mitochondria, mitochondria were deenergized by FCCP to promote the release of endogenous Ca^{2+} into the medium. The amount of released Ca^{2+} was estimated by EGTA titration considering a 1:1 stoichiometry under this condition [39, 40].

Please cite this article as: T.R. Figueira, et al., The higher susceptibility of congenital analbuminemic rats to Ca2+-induced mitochondrial permeability transition is associated wit..., Mol. Genet. Metab. (2011), doi:10.1016/j.ymgme.2011.08.031

2.6. Citrate synthase (CS) activity

The conversion of oxaloacetate and acetyl-CoA to citrate and SH-CoA catalyzed by citrate synthase was monitored by measuring the colorimetric product thionitrobenzoic acid [41]. Liver homogenates (16–20 μg/mL) were incubated at 30 °C in a buffer containing 50 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 250 μM oxaloacetate, 50 μM acetyl-CoA, and 100 μM 5,5′-dithiobis(2-nitrobenzoic acid). The increase in absorbance at 412 nm was monitored for 6 min using a microplate reader (PowerWave XS 2, BioTek Instruments, Winooski, VT, USA).

2.7. Mitochondrial hydrogen peroxide release (H_2O_2)

Mitochondrial suspensions (0.5 mg/mL) were incubated in standard reaction medium plus Amplex red (10 μM; Molecular Probes, Invitrogen, Carlsbad, CA) and horseradish peroxidase 1 U/mL. The fluorescence was monitored over time on a spectrofluorometer (Shimadzu RF-5301 PC, Kyoto, Japan) operated at excitation and emission wavelengths of 563 and 587 nm, respectively, and slit widths of 5 nm. The slope of the linear increase in fluorescence over time represents the rate of H_2O_2 released from isolated mitochondria. A standard curve was built with known concentrations of hydrogen peroxide.

2.8. Redox state of mitochondrial pyridine nucleotides

The autofluorescence of reduced pyridine nucleotides (NAD(P)H) in mitochondrial suspensions (1 mg/mL) was followed on a spectrofluorometer (Hitachi F-4500, Tokyo, Japan) operated at excitation and emission wavelengths of 366 and 450 nm, respectively, and slit widths of 5 nm. For this assay, rotenone (2 μM) was added to the incubation medium, and 5 mM of succinate was used as an energizing substrate instead of complex I-linked substrates [22]. As experimental controls, the prooxidant *tert*-butyl hydroperoxide (20 μM) was added to promote the oxidation of NADPH, and the substrate isocitrate (1 mM) was added at the end of each trace to feed isocitrate dehydrogenase and re-reduce $NAD(P)^+$ if oxidation had occurred. Known amounts of NADPH were added as a reference [22].

2.9. Glutathione levels

Glutathione (GSH) and oxidized glutathione (GSSG) were measured in isolated mitochondria by following the enzymatic recycling method described by Teare et al. [42]. Briefly, 250 μg of mitochondrial proteins were suspended to a final volume of 25 μL in water, and then a solution (1:1 ratio) of sulfosalicilic acid (11%) and Triton X-100 (0.11%) was added to these samples. After a brief incubation for 5 min at 4 °C under continuous shaking, the samples were centrifuged at 10,000 *g* for 10 min (4 °C), and the supernatant was saved for subsequent analyses of glutathione levels. To measure only oxidized glutathione, 10 μL of this supernatant were added to 110 μL of a reduced glutathione masking buffer (phosphate buffer (100 mM), EDTA (1 mM), 2-vinylpyridine (1.1%), pH 7.4) and incubated for 1 h at room temperature. The samples prepared for GSH and GSSG were then subjected to enzymatic recycling analyses in a recycling buffer system containing NADPH (300 μM), DTNB (225 μM), glutathione reductase (1.6 U/mL) and EDTA (1 mM) in 100 mM phosphate buffer (pH 7.4). The linear increase in absorbance at 412 nm over time was monitored using a microplate reader (PowerWave XS 2, BioTek Instruments, Winooski, VT, USA). A standard curve was built with known amounts of GSH and GSSG.

2.10. Reduced thiols of mitochondrial membrane proteins

The content of reduced thiols of mitochondrial membrane proteins was measured using the DTNB method, as detailed and described elsewhere [43, 44].

Table 1
General characteristics of SDR and NAR

	SDR	NAR
Body mass (g)	437 ± 47	357 ± 50*
Liver mass (g/100 g)	3.6 ± 0.4	4.5 ± 0.6*
Plasma protein (g/L)	60.7 ± 3.9	45.3 ± 6.5*
Triglycerides (mg/dL)	70.8 ± 30.2	112.1 ± 33.3*
Cholesterol (mg/dL)	76.7 ± 24.0	161.1 ± 30.9*
FFA (mEq/L)	1.09 ± 0.31	0.30 ± 0.05*

Mean ± SD. Liver mass was normalized by total body mass (g/100 g). Triglycerides and cholesterol levels were measured in blood plasma. FFA: plasma free fatty acid (mEq/L). N = 10 to 20.
* $P<0.05$.

2.11. S-nitrosothiols (S-NO)

The content of mitochondrial S-NO was determined using a modified Saville assay, as detailed by Park and Kostka [45] and Leite et al. [32].

2.12. Cyclophilin D expression

The abundance of cyclophilin D in mitochondrial suspensions was analyzed by western blotting. Ten micrograms of sample proteins were electrophoretically resolved in 12% acrylamide SDS-PAGE gel (150 V for 70 min). The proteins were then cold electrotransferred to nitrocellulose membranes (100 V for 90 min). Thereafter, we strictly followed the western blot protocol provided for the primary cyclophilin-D monoclonal antibody (MitoScience, Eugene, OR, USA). Briefly, the membrane was incubated with the primary antibody (1:1000 dilution in Tris-base saline plus 1% defatted milk) for 2 h at room temperature. The membrane was developed by a colorimetric method (amplified alkaline phosphatase immunoblot kit, Bio-Rad, Hercules, CA, USA) after incubation with the secondary biotinylated anti-mouse antibody (1:1000 dilution; ab6788, Abcam, Cambridge, MA, USA). The relative optical densities of bands were analyzed using the gel tool of the software Image J (N.I.H., USA). As a loading control, the samples were electrophoresed, and the gel was silver stained (Pierce Silver Stain Kit, Thermo Scientific, Rockford, IL, USA) for total proteins.

2.13. Statistical analyses

Data are expressed as mean ± SD. The normality of data was assessed using Shapiro–Wilk's test, and the differences between the groups were analyzed using Student's *t*-test. The significance level was set at $P<0.05$. All tests were performed using the software SigmaStat 3.1 (Systat, San Jose, CA, USA).

3. Results

Some characteristics of NAR and SDR are shown in Table 1. NAR presented a lower body mass, total plasma proteins and plasma FFA levels while liver masses, plasma cholesterol and triglycerides were

Table 2
Liver mitochondrial respiration and phosphorylation efficiency.

	SDR	NAR
V4 (nmol O/mg/min)	9.8 ± 3.6	8.9 ± 3.2
V3 (nmol O/mg/min)	62.1 ± 26.3	67.4 ± 27.3
RCR	6.9 ± 2.3	7.7 ± 2.1
ADP/O	2.52 ± 0.38	2.56 ± 0.38

Mean ± SD. The maximal respiration rate (V3) was elicited by ADP 300 μM. The resting respiration rate (V4) was considered the state after all of the added ADP had been phosphorylated. RCR, ratio between V3 and V4. Phosphorylation efficiency (ADP/O) is the ratio between the amount of added ADP and the amount of oxygen consumed to phosphorylate all the added ADP. N = 14.

Please cite this article as: T.R. Figueira, et al., The higher susceptibility of congenital analbuminemic rats to Ca2+-induced mitochondrial permeability transition is associated wit..., Mol. Genet. Metab. (2011), doi:10.1016/j.ymgme.2011.08.031

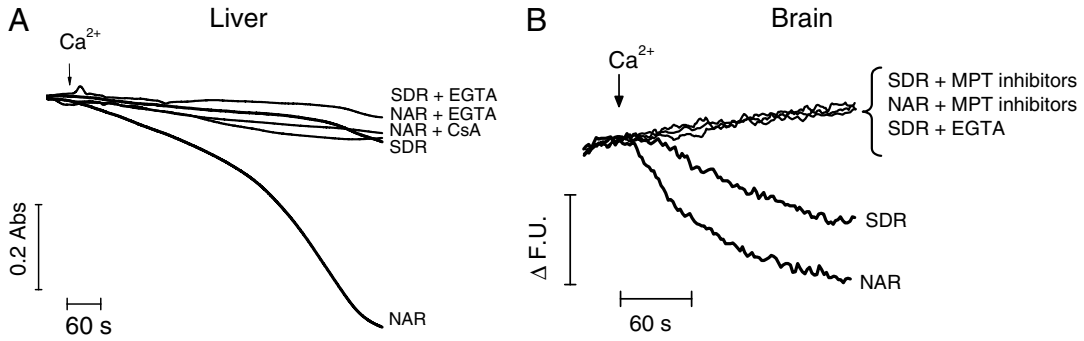

Fig. 1. Representative traces of Ca^{2+}-induced swelling in isolated mitochondria from liver and brain. SDR, Sprague-Dawley control rats. NAR, Nagase analbuminemic rats. A, calcium-induced swelling in liver mitochondria, N = 13. B, calcium-induced swelling in brain mitochondria, N = 5. Where indicated, EGTA (200 μM) or cyclosporin A (1 μM) was present as an experimental control. In A and B, the lower the ordinate value is, the higher the mitochondrial volume is.

increased. These characteristics are typical for the NAR phenotype and have also been reported by others [11, 46]. Table 2 depicts resting (V4) and ADP-stimulated (V3) liver mitochondrial respiration, the respiratory control ratio (RCR), and the phosphorylation efficiency (ADP/O ratio). None of these mitochondrial respiratory variables significantly differed between the NAR and SDR. Citrate synthase activity was assayed in liver homogenates as a marker of mitochondrial density in the tissue. Nearly identical values for citrate synthase activity were observed between the SDR and NAR, respectively, 131.2 ± 8.84 and 123.0 ± 9.0 mU/mg.

Figs. 1 and 2 show representative experiments conducted to assess MPT in mitochondria isolated from liver, brain and heart. In Fig. 1A and B, the swelling assay for MPT revealed that NAR liver and brain mitochondria undergo more extensive swelling than organelles from SDR when incubated in the presence of micromolar Ca^{2+} concentrations. Under similar incubation conditions, the safranine fluorescence assay for mitochondrial transmembrane electrical potential depicts an earlier disruption of transmembrane electrical potential for NAR heart mitochondria compared to SDR heart mitochondria (Fig. 2). The MPT inhibitor cyclosporin A (CsA) and the Ca^{2+} chelator EGTA were used as experimental controls, and these conditions are shown in the figures. Full inhibition of MPT in brain mitochondria requires the use of cyclosporin A plus ADP [38]. Under our experimental conditions, MPT results in minor changes in absorbance or light scattering of heart mitochondrial suspensions, so the safranine fluorescence assay for mitochondrial transmembrane electrical potential was conducted instead. Thus, these assays indicate that NAR mitochondria from different tissues were more susceptible to Ca^{2+}-induced MPT.

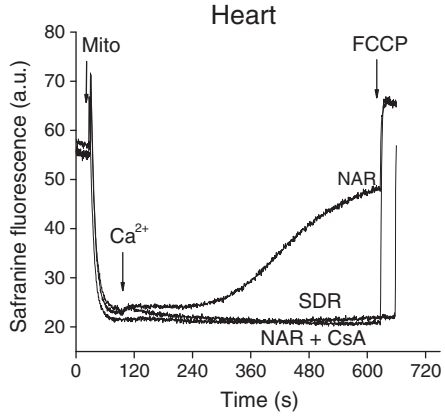

Fig. 2. Representative traces of Ca^{2+}-induced dissipation of heart mitochondrial transmembrane potential ($\Delta\Psi$), N = 4. Where indicated, EGTA (200 μM) or cyclosporin A (1 μM) was present as an experimental control. In this assay, the lower the safranine fluorescence signal was, the higher the mitochondrial $\Delta\Psi$ was. The uncoupler FCCP was added at the end to dissipate the mitochondrial $\Delta\Psi$.

After the initial screening for MPT susceptibility across tissues, more in depth studies on the mechanisms of MPT sensitization in NAR were conducted with isolated liver mitochondria. The mitochondrial Ca^{2+} retention capacities are shown in Fig. 3. A representative raw experiment is depicted in Fig. 3A. A nearly 40% lower Ca^{2+} retention capacity was observed for NAR liver mitochondria compared to SDR (Fig. 3B). Under basal incubation conditions, the amount of releasable endogenous Ca^{2+} upon mitochondrial deenergization by FCCP did not significantly differ between the NAR and SDR liver mitochondria (2.64 ± 0.67 and 3.24 ± 0.69 nmol/mg, respectively), which indicated that the difference in the Ca^{2+} retention capacity between the groups upon exogenous Ca^{2+} challenging is not associated with different levels of preloaded Ca^{2+} into the organelles. To address the question of whether a plasma albumin-bound substance could leak into NAR cells and promote mitochondrial toxicity, experiments were also conducted in the presence of exogenous albumin. Under these experimental conditions, the NAR mitochondria still displayed a lower Ca^{2+} retention capacity (Fig. 3C). To investigate the hypothesis that the lower Ca^{2+} retention capacity was not a constitutive alteration present in NAR, we assessed this variable in 21-day-old rats (Fig. 3D), and no significant difference was observed at this age when compared to age-matched SDR.

Because young NAR (21-day-old) did not present MPT sensitization and oxidative stress is strictly involved in the MPT process [25-30], we evaluated whether MPT sensitization in NAR could result from a more oxidizing mitochondrial environment. Fig. 4A shows representative traces of mitochondrial NAD(P)H autofluorescence over time. Neither mitochondria from NAR nor SDR displayed spontaneous NAD(P)H oxidation over time, and a similar recovery of NAD(P)H fluorescence was also observed when mitochondria were challenged with *tert*-butyl hydroperoxide, which is metabolized by the glutathione peroxidase/reductase system at the expense of NADPH [47]. The rate of H_2O_2 release from mitochondria did not differ between organelles isolated from NAR and SDR (Fig. 4B). Also, the content of mitochondrial membrane-protein reduced thiols and total mitochondrial glutathione were not different between the NAR and SDR, Figs. 4C and D, respectively. Fig. 4E depicts the significantly lower content of nitrosothiols in NAR mitochondria compared to the control (~20%, $P<0.05$).

Last, we assessed mitochondrial cyclophilin D (CyD) protein expression, a major protein involved in the MPT process [48, 49]. Western blot analyses, which are shown in Fig. 5, revealed that CyD expression was approximately 30% greater ($P<0.05$) in NAR mitochondria compared to SDR.

Please cite this article as: T.R. Figueira, et al., The higher susceptibility of congenital analbuminemic rats to Ca2+-induced mitochondrial permeability transition is associated wit..., Mol. Genet. Metab. (2011), doi:10.1016/j.ymgme.2011.08.031

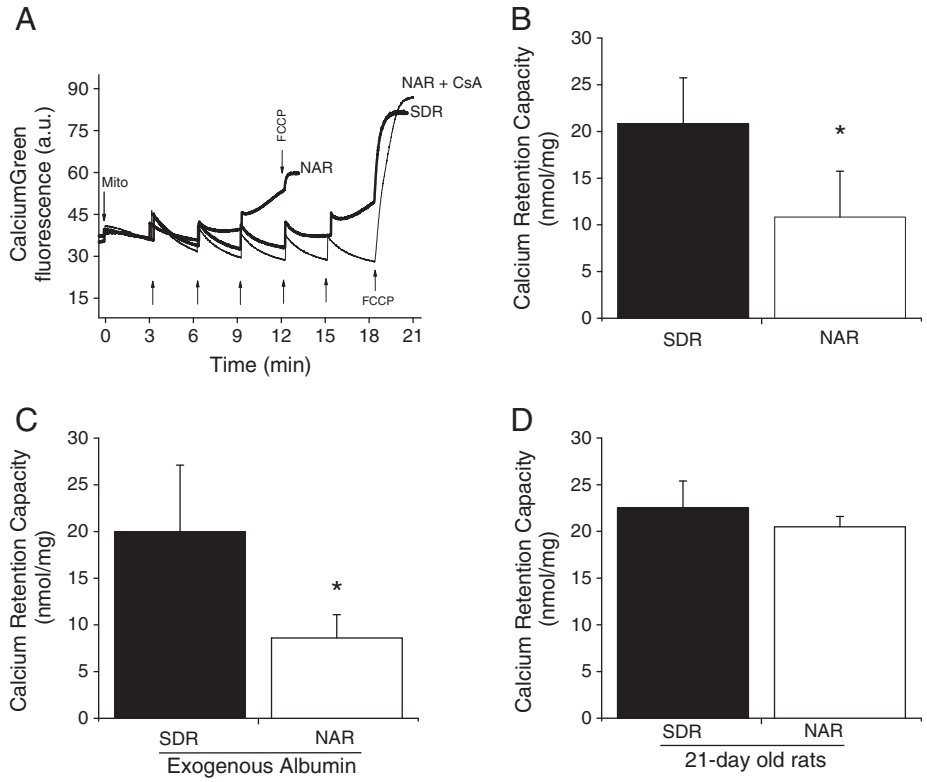

Fig. 3. Ca^{2+} retention capacity in liver mitochondria. A, representative traces showing the dynamics of the external Ca^{2+} concentration in response to sequential additions of Ca^{2+} (5 nmol every 3 min). The higher the fluorescence signal is, the higher the external Ca^{2+} concentration is. The sum of added Ca^{2+} over the steps before the spontaneous Ca^{2+} release from mitochondria was taken as the Ca^{2+} retention capacity and is shown as mean ± SD (panels B to D). B, Ca^{2+} retention capacity in mitochondria isolated from 3-month-old adult rats, N = 4. C, Ca^{2+} retention capacity in mitochondria from 3-month-old adult rats was assayed in the presence of exogenous albumin (0.2 mg/mL), N = 5. D, The Ca^{2+} retention capacity in mitochondria isolated from 21-day-old weaning rats, N = 5.

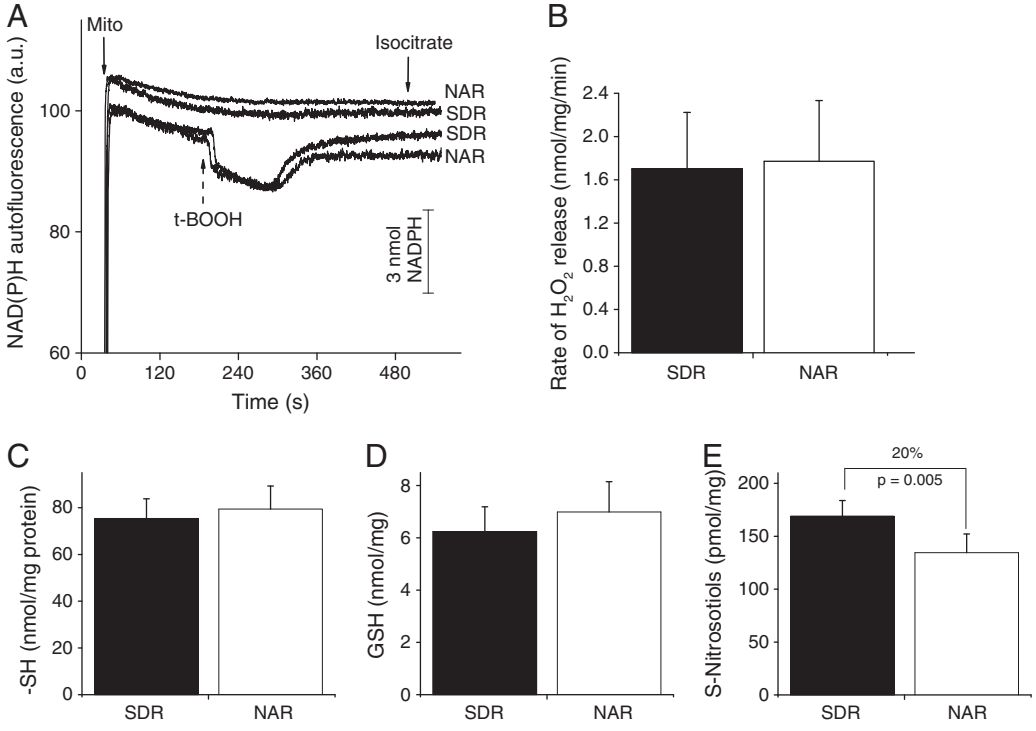

Fig. 4. Mitochondrial redox status. A, representative traces of reduced mitochondrial pyridines nucleotides (NAD(P)H) autofluorescence followed over time. Isocitrate was added at the end to feed isocitrate dehydrogenase and re-reduce NADP+ if oxidation had occurred. As an experimental control, the oxidant *tert*-butyl hydroperoxide (t-BOOH) was added to elicit NADPH oxidation. These experimental control curves were displaced downwards for better visualization. The addition of isocitrate did not evoke an increase in autofluorescence in any of the experiments. N = 6. B, mitochondrial release of hydrogen peroxide, N = 5. C, mitochondrial membrane proteins reduced thiol content, N = 5. D, mitochondrial content of total glutathione, N = 8; The GSH/GSSG ratio is not shown but was not different between the groups. E, mitochondrial content of S-nitrosothiols (S-NO), N = 6.

Please cite this article as: T.R. Figueira, et al., The higher susceptibility of congenital analbuminemic rats to Ca2+-induced mitochondrial permeability transition is associated wit..., Mol. Genet. Metab. (2011), doi:10.1016/j.ymgme.2011.08.031

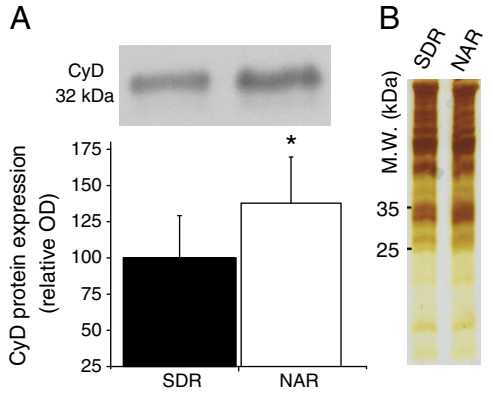

Fig. 5. Mitochondrial cyclophilin D (CyD) protein expression. Protein (10 μg) from isolated liver mitochondria was electrophoresed in SDS-PAGE gel and subsequently western blotted for CyD. A, means ± SD of relative optical densities of CyD bands, N = 4. B, as sample loading controls, samples were electrophoresed in SDS-PAGE gel. The gel was subsequently silver stained for total proteins (means ± SD of relative densities of the silver stained lanes were 100 ± 13 and 92 ± 21% for the SDR and NAR, respectively).

4. Discussion

In this work, we show that mitochondria from analbuminemic rats present a higher susceptibility to Ca^{2+}-induced permeability transition, which was associated with a nearly 20% depletion of nitrosothiols and overexpression of mitochondrial CyD in liver mitochondria (Figs. 4E and 5). A higher propensity for MPT opening was also observed in mitochondria isolated from NAR brain and heart (Figs. 1 and 2). Although MPT is a well-characterized process in *in vitro* systems (*e.g.*, isolated mitochondria and cells), its relevance in physiology and diseases has gained experimental support only in the last few years [49]. There is a growing body of evidence that MPT opening contributes to tissue degeneration/dysfunction in a variety of diseases [49–51]. However, little is known about the molecular events through which some physiological and pathological conditions (*e.g.*, heart failure or physical exercise) affect the Ca^{2+} threshold for MPT opening [52-55], which make mitochondria more or less susceptible to Ca^{2+}-induced MPT. CyD is a well- established component of MPT, and its genetic ablation or pharmacological inhibition (*e.g.*, cyclosporin A or analogs) greatly increases the Ca^{2+} threshold for MPT pore opening [48, 49]. Similar to our results, a higher CyD expression in experimental models for alcoholism [56], aromatase deficiency [55], heart failure [54] and in subpopulations of neuronal mitochondria [57, 58] correlates with a higher susceptibility to Ca^{2+}-induced MPT. Moreover, the depletion of the nitrosothiol content in NAR liver mitochondria (Fig. 4E) may also contribute to a higher susceptibility to Ca^{2+}-induced MPT in NAR. The status of mitochondrial nitrosothiols has been recently demonstrated to play a role in the MPT process and in mitochondrial and tissue integrity under challenging conditions [32, 59]. Indeed, a recent report indicates that S-nitrosylation of CyD on cysteine 203 partially inhibits MPT opening in H_2O_2-treated cells [33]. Therefore, an alteration of these two variables, known to be critical for MPT regulation, might explain mitochondrial dysfunction in NAR.

It is insightful that a lack of plasma albumin, an abnormality primarily confined to the extracellular compartment, affects mitochondria. This may comprise a novel finding in the sense that a benign extracellular disturbance (*i.e.*, elicits only mild clinical symptoms) can over time impair mitochondrial function. To note, 21-day-old weaning NAR do not present an increased susceptibility to MPT compared to their age-matched controls (Fig. 3D). In the case of NAR, it is known that several alterations occur secondarily to analbuminemia, as aforementioned. These alterations along with the primary defect (*i.e.*, lack of plasma albumin) may interact to predispose NAR tissues to degeneration, as evidenced by plasma oxidative stress [60], increased plasma liver enzymatic activities [4], and by microscopic liver pigmentation and glomerulosclerosis with aging [61]. It is important to highlight that albumin has antioxidant and cytoprotective properties [62, 63], and a lack of albumin by itself may comprise an important loss for redox homeostasis not only in plasma. Recent findings indicate that albumin plays a key role as a nitrosothiol trafficking protein, aiding in the transnitrosation of cellular proteins [64, 65] and conferring cytoprotection [64, 65]. Because albumin molecules comprise ~80% of the total pool of thiols in blood plasma [2], this finding may have important implications for NAR because these animals also present very low levels of plasma nitrosothiols [66]. Thus, the depletion of nitrosothiols in NAR mitochondria may be related to the likely compromised nitrosothiol trafficking in these animals.

The redox regulation of MPT sensitivity to Ca^{2+} load has long been studied by our group [26–30, 67] (for a comprehensive overview see Kowaltowski et al. [25]). The oxidation of membrane protein thiols by mitochondrial reactive oxygen species promotes disulfide bonds and protein aggregation in the inner mitochondrial membrane, which results in the assembling of the MPT pore [24, 25, 28, 29, 67]. The redox state of pyridine nucleotides is especially critical because they provide the reducing power for H_2O_2 detoxification in mitochondria [26, 27, 30, 47]. Therefore, we evaluated these and other important variables related to the mitochondrial redox state, but none of them differed between NAR and SDR (Fig. 4). The redox profile of NAR mitochondria greatly differs from that of hypercholesterolemic mice (LDL receptor knockout) mitochondria, which have been previously studied by our group [21, 22]. The mitochondria from the hypercholesterolemic mice present an inability to sustain a reduced state of mitochondrial NADPH, and consequently a higher generation rate of mitochondrial H_2O_2 and susceptibility to Ca^{2+}-induced MPT [21, 22].

With respect to hypertriglycerolemia in NAR, we previously showed that ApoCIII transgenic hypertriglyceridemic mice display an increased resting mitochondrial respiration (*i.e.*, State 4 respiration) and whole body metabolic rate due to mild mitochondrial uncoupling mediated by redox activation of mitochondrial ATP-sensitive K^+ channels [20]. Despite the hypertriglyceridemia of NAR (Table 1), we did not find significant differences for mitochondrial respiratory variables between the NAR and SDR (Table 2). Similar results were also observed for female NAR, which have nearly 5-fold higher plasma triglycerides levels than controls (data not shown). In this context, the main difference between NAR and ApoCIII transgenic hypertriglyceridemic mice is that the latter also present high levels of circulating FFA [18] while NAR instead display a FFA deficiency (Table I). It is important to note that triglycerides are not a substrate readily available for peripheral tissues, and their breakdown to glycerol and FFA is a critical step for the tissue uptake of plasma triglyceride-derived FFA [68]. Thus, rather than hypertriglyceridemia *per se*, plasma FFA seem to be the real cause of cellular lipotoxicity. In addition, we recently found that intravascular lipolysis is hampered in NAR [16], which presumably further limits FFA flux into peripheral tissues.

Overall, our data indicate that the higher susceptibility of NAR liver mitochondria to Ca^{2+}-induced MPT is associated with an increased expression of CyD and lower content of mitochondrial nitrosothiols. These alterations sum to the already described subclinical comorbidities in NAR. The association between the overexpression of cyclophilin-D and MPT opening propensity is an emerging paradigm in some diseases and deserves further investigation.

Role of funding source

The funding sources have no involvement with the content of this paper nor with the decision to submit this work to this journal.

Acknowledgments

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto Nacional de Obesidade e Diabetes (INCT). Figueira TR is a PhD student currently supported by a FAPESP fellowship. We are grateful to Sergio Catanozzi from the University of São Paulo Medical School, who maintains a colony of Nagase rats.

References

[1] T. Peters, All about Albumin: Biochemistry, Genetics, and Medical Applications, Academic Press, San Diego, 1996.
[2] L. Turell, S. Carballal, H. Botti, R. Radi, B. Alvarez, Oxidation of the albumin thiol to sulfenic acid and its implications in the intravascular compartment, Braz. J. Med. Biol. Res. 42 (2009) 305–311.
[3] L. Turell, H. Botti, S. Carballal, R. Radi, B. Alvarez, Sulfenic acid—a key intermediate in albumin thiol oxidation, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 3384–3392.
[4] M. Takahashi, K. Kusumi, S. Shumiya, S. Nagase, Plasma lipid concentrations and enzyme activities in Nagase analbuminemia rats (NAR), Jikken Dobutsu 32 (1983) 39–46.
[5] A.G. Shaper, S.G. Wannamethee, P.H. Whincup, Serum albumin and risk of stroke, coronary heart disease, and mortality: the role of cigarette smoking, J. Clin. Epidemiol. 57 (2004) 195–202.
[6] B.W. Schalk, M. Visser, M.A. Bremmer, B.W. Penninx, L.M. Bouter, D.J. Deeg, Change of serum albumin and risk of cardiovascular disease and all-cause mortality: Longitudinal Aging Study Amsterdam, Am. J. Epidemiol. 164 (2006) 969–977.
[7] S. Nagase, K. Shimamune, S. Shumiya, Albumin-deficient rat mutant, Science 205 (1979) 590–591.
[8] E. Kallee, Bennhold's analbuminemia: a follow-up study of the first two cases (1953–1992), J. Lab. Clin. Med. 127 (1996) 470–480.
[9] E.J. Kim, A.K. Lee, S.H. Kim, S.G. Kim, M.G. Lee, Pharmacokinetics and pharmacodynamics of intravenous azosemide in mutant Nagase analbuminemic rats, Drug Metab. Dispos. 31 (2003) 194–201.
[10] L. Minchiotti, M. Galliano, U. Kragh-Hansen, T. Peters Jr., Mutations and polymorphisms of the gene of the major human blood protein, serum albumin, Hum. Mutat. 29 (2008) 1007–1016.
[11] G. Baldo-Enzi, M.R. Baiocchi, G. Vigna, C. Andrian, C. Mosconi, R. Fellin, Analbuminaemia: a natural model of metabolic compensatory systems, J. Inherit. Metab. Dis. 10 (1987) 317–329.
[12] H. Esumi, M. Okui, S. Sato, T. Sugimura, S. Nagase, Absence of albumin mRNA in the liver of analbuminemic rats, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 3215–3219.
[13] A. Van Tol, E.H. Jansen, H.A. Koomans, J.A. Joles, Hyperlipoproteinemia in Nagase analbuminemic rats: effects of pravastatin on plasma (apo)lipoproteins and lecithin:cholesterol acyltransferase activity, J. Lipid Res. 32 (1991) 1719–1728.
[14] B.G. Koot, R. Houwen, D.J. Pot, J. Nauta, Congenital analbuminaemia: biochemical and clinical implications. A case report and literature review, Eur. J. Pediatr. 163 (2004) 664–670.
[15] J. Newstead, S.E. Card, A.W. Lyon, Low serum albumin and abnormal body shape in a young Canadian First Nations woman, Laboratory Medicine 35 (2004) 350-+.
[16] T.R. Figueira, A.E. Vercesi, H.C. Oliveira, Lack of plasma albumin impairs intravascular lipolysis and explains the associated free fatty acids deficiency and hypertriglyceridemia, Lipids Health Dis 9 (2010) 146.
[17] J.M. Weinberg, Lipotoxicity, Kidney Int. 70 (2006) 1560–1566.
[18] L.C. Alberici, H.C. Oliveira, E.J. Bighetti, E.C. de Faria, G.R. Degaspari, C.T. Souza, A.E. Vercesi, Hypertriglyceridemia increases mitochondrial resting respiration and susceptibility to permeability transition, J. Bioenerg. Biomembr. 35 (2003) 451–457.
[19] L.C. Alberici, H.C. Oliveira, P.R. Patricio, A.J. Kowaltowski, A.E. Vercesi, Hyperlipidemic mice present enhanced catabolism and higher mitochondrial ATP-sensitive K+ channel activity, Gastroenterology 131 (2006) 1228–1234.
[20] L.C. Alberici, H.C. Oliveira, B.A. Paim, C.C. Mantello, A.C. Augusto, K.G. Zecchin, S.A. Gurgueira, A.J. Kowaltowski, A.E. Vercesi, Mitochondrial ATP-sensitive K(+) channels as redox signals to liver mitochondria in response to hypertriglyceridemia, Free Radic. Biol. Med. 47 (2009) 1432–1439.
[21] H.C. Oliveira, R.G. Cosso, L.C. Alberici, E.N. Maciel, A.G. Salerno, G.G. Dorighello, J.A. Velho, E.C. de Faria, A.E. Vercesi, Oxidative stress in atherosclerosis-prone mouse is due to low antioxidant capacity of mitochondria, FASEB J. 19 (2005) 278–280.
[22] B.A. Paim, J.A. Velho, R.F. Castilho, H.C. Oliveira, A.E. Vercesi, Oxidative stress in hypercholesterolemic LDL (low-density lipoprotein) receptor knockout mice is associated with low content of mitochondrial NADP-linked substrates and is partially reversed by citrate replacement, Free Radic. Biol. Med. 44 (2008) 444–451.
[23] J.J. Lemasters, T.P. Theruvath, Z. Zhong, A.L. Nieminen, Mitochondrial calcium and the permeability transition in cell death, Biochim. Biophys. Acta 1787 (2009) 1395–1401.
[24] R.F. Castilho, A.J. Kowaltowski, A.R. Meinicke, E.J. Bechara, A.E. Vercesi, Permeabilization of the inner mitochondrial membrane by Ca2+ ions is stimulated by t-butyl hydroperoxide and mediated by reactive oxygen species generated by mitochondria, Free Radic. Biol. Med. 18 (1995) 479–486.
[25] A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Mitochondrial permeability transition and oxidative stress, FEBS Lett. 495 (2001) 12–15.
[26] J.L. Coelho, A.E. Vercesi, Retention of Ca2+ by rat liver and rat heart mitochondria: effect of phosphate, Mg2+, and NAD(P) redox state, Arch. Biochem. Biophys. 204 (1980) 141–147.
[27] A.L. Lehninger, A. Vercesi, E.A. Bababunmi, Regulation of Ca2+ release from mitochondria by the oxidation-reduction state of pyridine nucleotides, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 1690–1694.
[28] A.E. Vercesi, Possible participation of membrane thiol groups on the mechanism of NAD(P)+−stimulated Ca2+ efflux from mitochondria, Biochem. Biophys. Res. Commun. 119 (1984) 305–310.
[29] V.G. Valle, M.M. Fagian, L.S. Parentoni, A.R. Meinicke, A.E. Vercesi, The participation of reactive oxygen species and protein thiols in the mechanism of mitochondrial inner membrane permeabilization by calcium plus prooxidants, Arch. Biochem. Biophys. 307 (1993) 1–7.
[30] A.E. Vercesi, The participation of NADP, the transmembrane potential and the energy-linked NAD(P) transhydrogenase in the process of Ca2+ efflux from rat liver mitochondria, Arch. Biochem. Biophys. 252 (1987) 171–178.
[31] P.S. Brookes, E.P. Salinas, K. Darley-Usmar, J.P. Eiserich, B.A. Freeman, V.M. Darley-Usmar, P.G. Anderson, Concentration-dependent effects of nitric oxide on mitochondrial permeability transition and cytochrome c release, J. Biol. Chem. 275 (2000) 20474–20479.
[32] A.C. Leite, H.C. Oliveira, F.L. Utino, R. Garcia, L.C. Alberici, M.P. Fernandes, R.F. Castilho, A.E. Vercesi, Mitochondria generated nitric oxide protects against permeability transition via formation of membrane protein S-nitrosothiols, Biochim Biophys Acta 1797 (2010) 1210–1216.
[33] T.T.M. Nguyen, M. Stevens, M. Kohr, S. Steenbergen, E. Murphy, S-nitrosylation of cyclophilin D alters mitochondrial permeability transition pore, FASEB J. 25 (2011) 1031–1033.
[34] R.S. Kaplan, P.L. Pedersen, Characterization of phosphate efflux pathways in rat liver mitochondria, Biochem. J. 212 (1983) 279–288.
[35] R.E. Rosenthal, F. Hamud, G. Fiskum, P.J. Varghese, S. Sharpe, Cerebral ischemia and reperfusion: prevention of brain mitochondrial injury by lidoflazine, J. Cereb. Blood Flow Metab. 7 (1987) 752–758.
[36] A.J. Kowaltowski, S. Seetharaman, P. Paucek, K.D. Garlid, Bioenergetic consequences of opening the ATP-sensitive K(+) channel of heart mitochondria, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H649–H657.
[37] T.R.M. Figueira, A.E. D.R.,Vercesi, R.F. Castilho, Safranine as a fluorescent probe for the evaluation of mitochondrial membrane potential in isolated organelles and permeabilized cells, Methods Mol. Biol. (in press), doi:10.1007/978-1-61779-382-0_7.
[38] A. Saito, R.F. Castilho, Inhibitory effects of adenine nucleotides on brain mitochondrial permeability transition, Neurochem Res 35 (2010) 1667–1674.
[39] E. Zaidan, N.R. Sims, The calcium content of mitochondria from brain subregions following short-term forebrain ischemia and recirculation in the rat, J. Neurochem. 63 (1994) 1812–1819.
[40] R.A. Vacca, L. Moro, G. Caraccio, F. Guerrieri, E. Marra, M. Greco, Thyroid hormone administration to hypothyroid rats restores the mitochondrial membrane permeability properties, Endocrinology 144 (2003) 3783–3788.
[41] D. Shepherd, P.B. Garland, The kinetic properties of citrate synthase from rat liver mitochondria, Biochem. J. 114 (1969) 597–610.
[42] J.P. Teare, N.A. Punchard, J.J. Powell, P.J. Lumb, W.D. Mitchell, R.P. Thompson, Automated spectrophotometric method for determining oxidized and reduced glutathione in liver, Clin. Chem. 39 (1993) 686–689.
[43] P.C. Jocelyn, Spectrophotometric assay of thiols, Methods Enzymol. 143 (1987) 44–67.
[44] A.J. Kowaltowski, A.E. Vercesi, R.F. Castilho, Mitochondrial membrane protein thiol reactivity with N-ethylmaleimide or mersalyl is modified by Ca2+: correlation with mitochondrial permeability transition, Biochim. Biophys. Acta 1318 (1997) 395–402.
[45] J.K. Park, P. Kostka, Fluorometric detection of biological S-nitrosothiols, Anal. Biochem. 249 (1997) 61–66.
[46] J.A. Joles, N. Willekes-Koolschijn, A. van Tol, M.M. Geelhoed-Mieras, L.H. Danse, E. van Garderen, W. Kortlandt, D.W. Erkelens, H.A. Koomans, Hyperlipoproteinemia in one-year-old analbuminemic rats, Atherosclerosis 88 (1991) 35–47.
[47] H.R. Lotscher, K.H. Winterhalter, E. Carafoli, C. Richter, Hydroperoxides can modulate the redox state of pyridine nucleotides and the calcium balance in rat liver mitochondria, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 4340–4344.
[48] C.P. Baines, R.A. Kaiser, N.H. Purcell, N.S. Blair, H. Osinska, M.A. Hambleton, E.W. Brunskill, M.R. Sayen, R.A. Gottlieb, G.W. Dorn, J. Robbins, J.D. Molkentin, Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, Nature 434 (2005) 658–662.
[49] V. Giorgio, M.E. Soriano, E. Basso, E. Bisetto, G. Lippe, M.A. Forte, P. Bernardi, Cyclophilin D in mitochondrial pathophysiology, Biochim Biophys Acta 1797 (2010) 1113–1118.
[50] C. Piot, P. Croisille, P. Staat, H. Thibault, G. Rioufol, N. Mewton, R. Elbelghiti, T.T. Cung, E. Bonnefoy, D. Angoulvant, C. Macia, F. Raczka, C. Sportouch, G. Gahide, G. Finet, X. Andre-Fouet, D. Revel, G. Kirkorian, J.P. Monassier, G. Derumeaux, M. Ovize, Effect of cyclosporine on reperfusion injury in acute myocardial infarction, N. Engl. J. Med. 359 (2008) 473–481.
[51] P.L. Leger, D. De Paulis, S. Branco, P. Bonnin, E. Couture-Lepetit, O. Baud, S. Renolleau, M. Ovize, A. Gharib, C. Charriaut-Marlangue, Evaluation of cyclosporine A in a stroke model in the immature rat brain, Exp Neurol 230 (2011) 58–66.
[52] M. Fernstrom, M. Tonkonogi, K. Sahlin, Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle, J. Physiol. 554 (2004) 755–763.
[53] K. Csukly, A. Ascah, J. Matas, P.F. Gardiner, E. Fontaine, Y. Burelle, Muscle denervation promotes opening of the permeability transition pore and increases the expression of cyclophilin D, J. Physiol. 574 (2006) 319–327.
[54] J. Matas, N.T. Young, C. Bourcier-Lucas, A. Ascah, M. Marcil, C.F. Deschepper, Y. Burelle, Increased expression and intramitochondrial translocation of cyclophilin-D associates with increased vulnerability of the permeability transition pore to stress-induced opening during compensated ventricular hypertrophy, J. Mol. Cell. Cardiol. 46 (2009) 420–430.

Please cite this article as: T.R. Figueira, et al., The higher susceptibility of congenital analbuminemic rats to Ca2+-induced mitochondrial permeability transition is associated wit..., Mol. Genet. Metab. (2011), doi:10.1016/j.ymgme.2011.08.031

[55] L. Moro, A.A. Arbini, J.T. Hsieh, J. Ford, E.R. Simpson, A. Hajibeigi, O.K. Oz, Aromatase deficiency inhibits the permeability transition in mouse liver mitochondria, Endocrinology 151 (2010) 1643–1652.
[56] A.L. King, T.M. Swain, D.A. Dickinson, M.J. Lesort, S.M. Bailey, Chronic ethanol consumption enhances sensitivity to Ca(2+)-mediated opening of the mitochondrial permeability transition pore and increases cyclophilin D in liver, Am J Physiol Gastrointest Liver Physiol 299 (2010) G954–966.
[57] K.K. Naga, P.G. Sullivan, J.W. Geddes, High cyclophilin D content of synaptic mitochondria results in increased vulnerability to permeability transition, J. Neurosci. 27 (2007) 7469–7475.
[58] J.L. Hazelton, M. Petrasheuskaya, G. Fiskum, T. Kristian, Cyclophilin D is expressed predominantly in mitochondria of gamma-aminobutyric acidergic interneurons, J. Neurosci. Res. 87 (2009) 1250–1259.
[59] E.T. Chouchani, T.R. Hurd, S.M. Nadtochiy, P.S. Brookes, I.M. Fearnley, K.S. Lilley, R.A. Smith, M.P. Murphy, Identification of S-nitrosated mitochondrial proteins by S-nitrosothiol difference in gel electrophoresis (SNO-DIGE): implications for the regulation of mitochondrial function by reversible S-nitrosation, Biochem J 430 (2010) 49–59.
[60] Y. Yamamoto, K. Wakabayashi, E. Niki, M. Nagao, Comparison of plasma levels of lipid hydroperoxides and antioxidants in hyperlipidemic Nagase analbuminemic rats, Sprague-Dawley rats, and humans, Biochem. Biophys. Res. Commun. 189 (1992) 518–523.
[61] J.A. Joles, H. van Goor, M.L. van der Horst, A. van Tol, J.D. Elema, H.A. Koomans, High lipid levels in very low density lipoprotein and intermediate density lipoprotein may cause proteinuria and glomerulosclerosis in aging female analbuminemic rats, Lab. Invest. 73 (1995) 912–921.
[62] E. Bourdon, N. Loreau, D. Blache, Glucose and free radicals impair the antioxidant properties of serum albumin, FASEB J. 13 (1999) 233–244.
[63] E. Bourdon, D. Blache, The importance of proteins in defense against oxidation, Antioxid. Redox Signal. 3 (2001) 293–311.
[64] Y. Ishima, U. Kragh-Hansen, T. Maruyama, M. Otagiri, Albumin as a nitric oxide-traffic protein: characterization, biochemistry and possible future therapeutic applications, Drug Metab. Pharmacokinet. 24 (2009) 308–317.
[65] Y. Ishima, T. Akaike, U. Kragh-Hansen, S. Hiroyama, T. Sawa, A. Suenaga, T. Maruyama, T. Kai, M. Otagiri, S-nitrosylated human serum albumin-mediated cytoprotective activity is enhanced by fatty acid binding, J. Biol. Chem. 283 (2008) 34966–34975.
[66] Y. Minamiyama, S. Takemura, M. Inoue, Albumin is an important vascular tonus regulator as a reservoir of nitric oxide, Biochem. Biophys. Res. Commun. 225 (1996) 112–115.
[67] M.M. Fagian, L. Pereira-da-Silva, I.S. Martins, A.E. Vercesi, Membrane protein thiol cross-linking associated with the permeabilization of the inner mitochondrial membrane by Ca2+ plus prooxidants, J. Biol. Chem. 265 (1990) 19955–19960.
[68] B. Teusink, P.J. Voshol, V.E. Dahlmans, P.C. Rensen, H. Pijl, J.A. Romijn, L.M. Havekes, Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake, Diabetes 52 (2003) 614–620.

Please cite this article as: T.R. Figueira, et al., The higher susceptibility of congenital analbuminemic rats to Ca2+-induced mitochondrial permeability transition is associated wit..., Mol. Genet. Metab. (2011), doi:10.1016/j.ymgme.2011.08.031