J Physiol Biochem (2010) 66:93–103
DOI 10.1007/s13105-010-0014-x

ORIGINAL PAPER

Oxidative stress in mouse liver caused by dietary amino acid deprivation: protective effect of methionine

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Received: 14 December 2009 / Accepted: 30 March 2010 / Published online: 25 June 2010

Abstract The aim of this work was to evaluate the effects of a diet depleted of amino acids (protein-free diet, or PFD), as well as the supplementation with methionine (PFD+Met), on the antioxidant status of the female mouse liver. With this purpose, cytosolic protein spots from two-dimensional non-equilibrium pH gel electrophoresis were identified by several procedures, such as mass spectrometry, Western blot, gel matching and enzymatic activity. PFD decreased the contents of catalase (CAT), peroxiredoxin I (Prx-I), and glutathione peroxidase (GPx) by 67%, 37% and 45%, respectively. Gene expression analyses showed that PFD caused a decrease in CAT (−20%) and GPx (−30%) mRNA levels but did not change that of Prx-I. It was also found that, when compared to a normal diet, PFD increased the liver contents of both reactive oxygen species (+50%) and oxidized protein (+88%) and decreased that of glutathione (−45%). Supplementation of PFD with Met prevented these latter effects to varying degrees, whereas CAT, Prx-I and GPx mRNA levels resulted unmodified. Present results suggest that dietary amino acid deprivation deranges the liver antioxidant defences, and this can be, in part, overcome by supplementation with Met.

Keywords Mouse liver · Protein-free diet · Oxidative stress · Methionine · Antioxidant defences

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Introduction

In mammals, cellular protein turnover depends on the quality and amount of amino acid ingestion [15, 28, 58]. In addition, amino acids can either control or be a part of non-proteinogenic pathways [33]. Thus, amino acid-deprived diets cause significant physiological and biochemical changes in all tissues [3–5].

We previously found that feeding a diet depleted of amino acids [protein-free diet (PFD)] to mice for 5 days decreases the total protein content and alters the protein pattern of the liver [14, 38, 42, 47, 48]. Indeed, two-dimensional non-equilibrium pH gel electrophoresis tests reveal that PFD changes the mass of 192 out of 305 cytosolic protein spots [47]. Several of these proteins take part in cellular detoxification and antioxidant defence, such as glutathione *S*-transferases (GSTs), carbonic anhydrase III (CAIII) and CuZn-superoxide dismutase [42, 47, 48]. Interestingly, the supplementation of PDF with the amino acids Met or Cys prevents to different degrees the changes caused by PFD in

CAIII and GST contents [42]. Our previous observations suggest that a diet lacking amino acids causes oxidative stress, and this can be partly counteracted by Met and Cys.

The aim of this work was to contribute to the knowledge of the effects of both PFD and PFD+Met diets on the antioxidant status of the mouse liver. Here, we report that the contents of catalase (CAT), peroxiredoxin I (Prx-I) and glutathione peroxidase (GPx) decrease significantly with PFD, but not with PFD + Met. CAT and GPx mRNA levels correlated with their protein contents, but not those of Prx-I. In addition, feeding mice with PFD increases the content of both reactive oxygen species (ROS) and oxidized proteins and decreases that of Glutathione (GSH), whereas addition of Met to PFD reduces the extent of these changes.

Our results support the notion that a lack of dietary amino acids decreases the levels of enzymes involved in ROS removal in the liver. Methionine prevents these changes by acting on the antioxidant machinery.

Materials and methods

Animals and treatments

Two-month-old female BALB/C mice (body weight 24–27 g) obtained from INTA, Balcarce, Argentina, were kept for 1 week after arrival in a room at 22°C, illuminated from 07:00 to 19:00 hours. The specimens had an ad libitum access to normal diet and water. Feeding with control and test diets started at 19:00 hours and continued for 5 days. The local ethical committee for animal research approved the protocols used in this study.

Diets

All diets used were based on the PFD outlined by the USP XV Pharmacopoeia [16, 55]. Diet details are published by Ronchi et al. [42]; normal diet comprises PFD plus 23% (w/w) bovine β-casein, while PFD+Met contains Met as present in a normal diet (0.85%). Since carbohydrates replace proteins and amino acids, all diets are isocaloric. Daily food intake was 2.2±0.45 g per mouse for the normal diet, 2.7±0.3 g for PFD, and 2.57±0.25 g for PFD+Met.

Cytosol isolation

Four livers for each nutritional condition were pooled and homogenized at 4°C in 4 ml/g fresh weight of a buffer containing 0.15 M NaCl, 1 mM EDTA, 5 mM β-mercaptoethanol and 20 mM Tris–HCl, pH 7.4. The supernatant obtained by centrifugation at 100,000 × *g* for 60 min represents the cytosol [47].

Protein determination

Protein contents were estimated according to Bradford [7] using bovine serum albumin as a standard.

Two-dimensional non-equilibrium pH gel electrophoresis

Two dimensional non-equilibrium pH gel electrophoresis (2D-NEPHGE) separation of cytosolic proteins was performed as suggested by O'Farrel et al. [36]. Cytosols from four pooled livers per nutritional condition were analysed as triplicates in three independent experiments. This approach has the purpose of ensuring reproducibility in 2D-NEPHGE analyses and overcoming interindividual variabilities [39]. The first gel contained 6.8% (w/v) ampholytes (Pharmacia pharmalyte: pH 3–10, 1%; pH 5.8, 1.75%; pH 8–10.5, 4.08%). Cytosolic protein samples containing 25 μg of protein in 10 μl of 2% (w/v) 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate hydrate (CHAPS), 0.1 M dithiothreitol (DTT) and 9 M urea were deposed under 30 μl of pharmalyte pH 8–10.5 5.4% in capillary tubes (1×60 mm). After separation for 100 min at 750 V and 10°C, gels were extruded and treated for 4 min with a 3% sodium dodecyl sulfate (SDS), 50 mM DTT, 0.5 mM Tris–HCl, and pH 6.8 solution. After equilibration with 3% SDS, 0.2 M iodoacetamide, 0.5 mM Tris–HCl, pH 6.8, gels were layered over a 12.5% SDS-polyacrylamide slab gel (1 mm thick, 90 mm wide and 75 mm long) and proteins further separated at 10°C [20]. Then, protein spots were revealed with either Coomassie blue [35] or silver [34]. The images were analysed with the computer image-analysis system Visage 2000 (Bioimage, Millipore Corporation, Bedford, MA, USA), equipped with the Electrophoresis Quantifier Software. Protein spots were identified by using both the 2DWG-meta database and the Flicker program to compare 2D gel electrophoresis images created by Lemkin [29].

Identification of Prx-I using mass spectrometry

Within two Prx-I isoforms identified by gel matching in silver-stained 2D-NEPHGE, only that of major p*I* was visualised in Coomassie blue-stained gels. This Coomassie blue-stained 2D-NEPHGE spot excised from three runs was pooled and in-gel trypsin digested as described by Rosenfeld et al. [44]. Tryptic fragments were subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry in a Finnigan MAT (San Jose, CA, USA) equipment. Mass values for all the samples were the average of at least four analyses, each involving 20–50 pulses. The oxidized β-chain of bovine insulin (Boehringer, Mannheim, Germany) was used for calibration of the mass spectrometer. Peptide mass fingerprints were searched using ProFound (Rockefeller University NY, USA, version 4.10.5), with restrictions to proteins from 0 to 300 kDa and mass tolerance for the peptides of ±2 Da. Partial enzymatic digest involving one cleavage site per molecule, oxidation of methionine and modification of cysteine with iodoacetamide were considered in these searches (Table 1). Experimental p*I* and Mr were compared with the theoretical values available in UniProtKB. Protein identity was confirmed using the UniProtKB protein database.

Western blot test for CAT

After 2D-NEPHGE separation, proteins were transferred to nitrocellulose membrane as described by Ey and Ashman [17]. CAT was identified using polyclonal antibodies raised against bovine CAT at a 1:1500 dilution. Blots were incubated with a secondary antibody conjugated to alkaline phosphatase (Sigma, ST. Louis, MO, USA) and developed with 5-bromo-4-chloro-3′-indolyphosphate *p*-toluidine salt and nitro-blue tetrazolium chloride (Pierce, Rockford, IL, USA; according to manufacturer's manual). Alternatively, a secondary antibody conjugated to horseradish peroxidase was used. Bands were developed using the Super Signal West Pico Chemiluminiscent Substrate for Western blot (Pierce). Signal quantification was done by densitometry and data were referred to the normal diet condition.

Catalase activity

Total CAT activity was measured at 20°C by following H_2O_2 disappearance at 214 nm [1]. One milliliter of reaction mix contained 20 μl cytosol, 50 mM sodium phosphate pH 7.4 and the amount of H_2O_2 able to produce a shift at OD at 214 nm equal to 0.55. One enzymatic unit was defined as an OD at 214 nm equal to 0.05; the activity was expressed as units produced by 1 ml of cytosol during 1 min.

In-gel GPx activity

Cytosols [25 μg protein in 25 μl of 50% (v/v) glycerol, 10 mM Tris–HCl 10 mM, pH 7] were separated in nondenaturing 1D polyacrylamide gel electrophoresis (PAGE) with 10% acrylamide. After electrophoresis, the gel was incubated at 20°C with 1 mM GSH for 10 min, and 1 mM GSH plus 0.003% (v/v) cumene hydroperoxide 0.003% for 10 min. Then, it was washed with bidistilled water and stained with 2% (w/v) potassium ferricyanide plus 2% (w/v) ferric chloride [53]. Achromatic bands show removal of hydrogen peroxide by GPx. Densitometric analyses of data were referred to

Table 1 Results of peptide mass fingerprint search for Prx-I

	Mass (exp.)	Mass (Theor.)	Residues[a]	Peptide sequence
Prx-I (coverage 45%)	1005.532	1005.528	8–16	IGYPAPNFK
	1163.562	1163.564	17–27	ATAVMPDGQFK
	1651.862	1651.859	94–109	QGGLGPMNIPLISDPK
	1106.602	1106.597	111–120	TIAQDYGVLK
	893.422	893.424	121–128	ADEGISFR
	919.502	919.501	129–136	GLFIIDDK
	1224.682	1224.682	141–151	QITINDLPVGR
	830.452	830.449	152–158	SVDEIIR
	1195.622	1195.623	159–168	LVQAFQFTDK

The expectation value calculated from Profound was 1.6×10^{-9}. Nine peptides matched and one unmatched with monoisotopic masses: 2106.190

[a] Position of amino acid inside Prx-I sequence

the normal fed condition. Control samples were done in the presence of 10 mM iodoacetic acid, which suppresses GPx action (not shown).

Measurement of tissue ROS content

The oxidation-sensitive fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (from Invitrogen, Carlsbad, CA, USA) was used to analyse the total content of ROS. After treatment, cytosols were incubated with 2.5 μM DCFH-DA (30 min, 37°C) in PBS. Fluorescence (Ex 485 nm–Em 510 nm) was measured in a Microplate Fluorescence Reader Fluoroskan Ascent & Ascent, related to protein mass and expressed as a percentage of the normal fed control.

Measurement of tissue GSH content

Cytosols were extracted in a buffer containing 0.2% Triton X-100 and 2.5% sulfosalicylic acid. Aliquots of the supernatant recovered after centrifugation at 15000 × *g* for 15 min were used for total glutathione content assessment by the method of Griffith [22], with some changes introduced by Sánchez et al. [46]. Measurements were related to protein mass and expressed as percentages of the normal fed control.

Measurement of protein carbonyl content

The reactive carbonyl content of liver proteins was measured according to Oliver et al. [37]. Briefly, livers were homogenized at 4°C in 10 ml/g fresh weight of cold 0.15 M NaCl, 1 mM EDTA, and 20 mM Tris–HCl, pH 7.4. Two 0.75-ml aliquots per sample containing nearly 1.0 mg protein were mixed with 0.75 ml of 20% (w/v) trichloroacetic acid (TCA). The pellets were separated by centrifugation at 6000 × *g* for 5 min, mixed with either 0.75 ml of 2 N HCl (blank) or 0.75 ml of 2 N HCI containing 0.2% (w/v) dinitrophenylhydrazine (DNPH) and agitated for 1 h at 25°C in the dark. Then, they were re-precipitated with 0.75 ml of 20% (w/v) TCA, washed three times with ethanol/ethyl acetate (l:l, v/v), dried and mixed with 0.75 ml of 6 M guanidine HCl at 25°C. After removing the debris by centrifugation, absorbance at 370 nm of DNPH-treated samples after subtraction of blanks was assessed. This value was used to calculate the nanomoles of DNPH incorporated per milligram of protein based on an average absorption of 21.0 mM-1 cm-1 for aliphatic hydrazones [27].

Northern blotting

For each assay, total RNA was extracted from liver using 4 M guanidinium thiocyanate followed by extraction with phenol [45]. After the extraction, RNAs were resuspended in diethyl pyrocarbonate (DEPC)-treated water, quantified and stored at −80°C for further analysis. Thirty micrograms of RNAs for each condition was denatured in GLYOXAL at 55°C for 1 h [56]. Extracted RNAs were separated and analysed on agarose gels 1.5% (w/v) and transferred onto a nylon membrane (Amersham, UK).

DNA probes were obtained by PCR amplification with specific primers (see below) using DNA as template. Then, the probes were labelled by random priming (Invitrogen, Life Technologies, Carlsbad, CA, USA, DNA labelling System) using [γ-^{32}P]-dCTP. Probes were purified using G-50 Sephadex columns. Sequential hybridization with the different probes was performed using standard methods. The intensity of the bands was detected by Scanner Storm (Amersham Biosciences, Piscataway, NJ, USA) and quantified using ImageQ TL v2005. All the bands were normalised to actin to account for uneven gel loading.

Mouse specific primer sequences used for PCR reactions:

Actin	FOR: 5′-AGT ACT TGC GCT CAG GAG GA-3′
	REV: 5′-TCC TCC CTG GAG AAG AGC TA-3′
Prx-I	FOR: 5′-TTA AAG GCT GAT GAA GGT AT-3′
	REV: 5′-GAA TTC ACG TTT AAT AGA TAC T-3′
CAT	FOR: 5′-AGG CGG GAA CCC AAT AGG AGA TAA-3′
	REV: 5′-ATG GAT AAA GGA TGG AAA CAA TA-3′
GPx	FOR: 5′-CCA CGA TCC GGG ACT ACA CC-3′
	REV: 5′-GTC GGG GCC CAC CAG GAA CT-3′

Statistical analysis

All the measurements were subjected to one-way analysis of variance followed by the Dunnett test of mean comparison with references (InStat, Graph Pad software). *P* values lower than 0.05 were considered as significant. Data from at least three separate experiments were analysed.

Results

Cytosolic proteins affected by protein depletion

In view that PFD changes mouse liver glutathione *S*-transferase (mGST), glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase III and Zn superoxide dismutase (CuZn-SOD) contents [42, 47, 48], we wished to explore whether it affects other cellular antioxidative enzymes. Figure 1 shows a 2D-NEPHGE silver-stained gel from normal liver [47], in which new and previously identified spots were labelled in black and gray, respectively. Table 2 summarises the identification data for CAT, Prx-I and GPx. This table also shows that levels of these enzymes decreased after feeding mice with PFD for 5 days compared with the normal diet control. In 2D-NEPHGE Western blot tests (Fig. 2a–b), CAT displayed four isoforms whose proportions changed with PFD. Thus, CAT total content was assessed by 1D Western blot. Compared with the control, CAT amount decreased by a 67% with PFD (Fig. 2c), in agreement with a 46±5.0% decrease in its enzymatic activity.

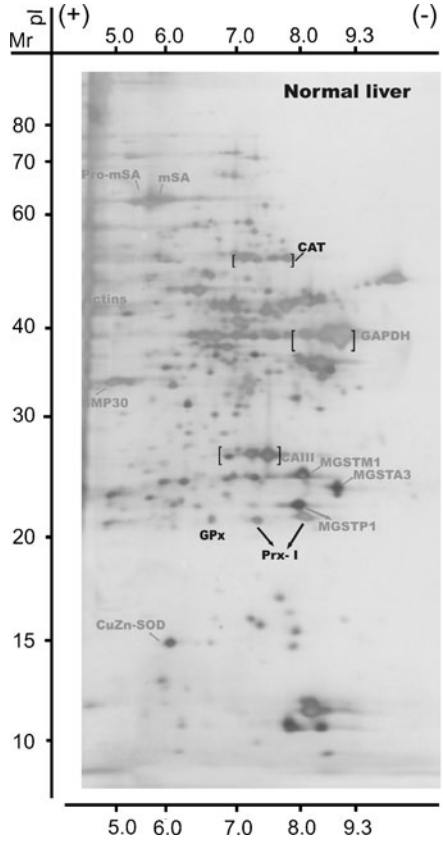

Fig. 1 2D-NEPHGE silver-stained pattern of liver cytosolic proteins from normal fed mouse. Twenty-five micrograms of protein was loaded. Proteins changed by PFD identified either in this study or in previous works are marked by *gray* and *black letters*, respectively. A representative image of three independent experiments is shown

Mass spectrometric and gel-matching tests revealed two Prx-I isoforms that, taken together, decreased 37% with PFD (Table 2 and Fig. 3).

Comparison of gels with a 2D-NEPHGE database evidenced that a spot with a p*I* of 6.7+0.2 and a molecular mass of 22.8 kDa that decreased with PFD could be GPx (Table 2). With the purpose of confirming this, the in-gel enzymatic activity of GPx was analysed. Figure 4 shows that GPx activity decreased significantly with PFD (45±2.0%; $P<0.01$).

Effect of PFD on mRNA levels

As feeding with PFD caused a decrease in the protein levels of CAT, Prx-I and GPx, we further examined whether these changes were due to a decrease in gene expression. Figure 5 shows that PFD decreased both CAT and GPx mRNA levels compared with the normal fed control [20% ($P<0.05$) and 30% ($P<0.001$), respectively], whereas Prx-I mRNA levels were unmodified.

Influence of dietary Met

We examined whether supplementing PFD with Met had any impact on the steady-state levels of antioxidant enzymes. Indeed, Met counteracted to some extent the effect of PFD on CAT, Prx-I and GPx levels. Feeding mice with PFD+Met partially prevented the decrease caused by PFD in the contents of CAT and Prx-I, and GPx activity (Figs. 2c, 3 and 4). It must be also remarked that PFD+Cys prevent the decrease caused by PFD in CAT content and GPx activity [43]. In contrast, the supplementation of PFD with Met did not modify the effect of PFD on the mRNA levels of the three proteins (Fig. 5).

ROS and GSH content

ROS production increased in the liver of mice fed with PFD (50%, $P<0.05$), and this happened to lower extent with PDF+Met (35%, $P<0.05$) (Fig. 6a). Conversely, liver GSH content decreased with PFD

Table 2 Identification of antioxidative enzymes affected by PFD after separation by 2D-NEPHGE

No.	Accession number[a]	Protein description	Experimental		Sequence covered by peptides (%)	PFD effect (% of normal)	Identification procedure
			p*I*	kDa			
1	P35700	Prx-I	8.3	22	45	63±23% [b]	MS, PMF, GM
2	P24270	CAT (four spots)	7.0–8.2	56–51	–	81±2.5% 15±4.5%[b] 57±18% [b] 71±40%	ID, GM
3	P11352	GPx	~6.7	22.8	–	61±17%	GM

PFD effect: average % of spot intensity compared to normal fed control ± SEM (n=3)

MS mass spectrometry, *MALDI-TOF* matrix-assisted laser desorption ionization time-of-flight, *PMF* peptide mass fingerprinting, *ID* immunodetection, *GM* gel matching of experimental data compared with 2D gel database

[a] Accession number refers to the UniProtKB (http://www.uniprot.org)

[b] Comparison of means by Student's *t* test. Differences verified at 5% level, $P<0.05$

(50%, $P<0.05$) and, to a slightly lower degree (30%, $P<0.05$), with PFD+Met (Fig. 6b). It is noteworthy that PFD+Cys caused similar effects than PFD+Met on GSH content [43].

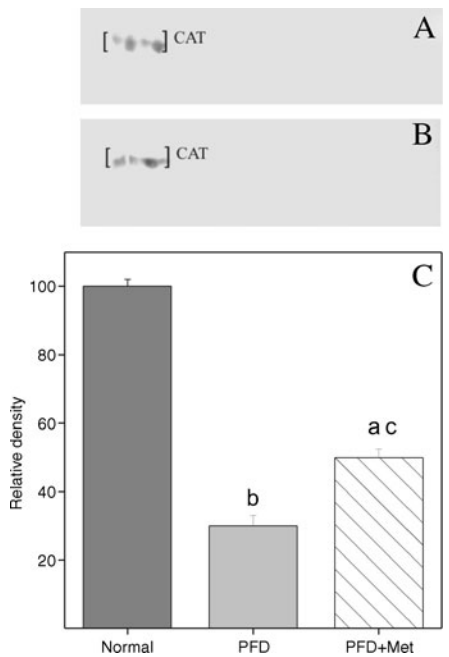

Fig. 2 Influence of the diet on liver CAT content. Groups of mice were fed with normal diet, PFD and PFD+Met for 5 days. Cytosolic proteins (25 μg) were separated on 2D-NEPHGE and analysed for CAT by Western blot: **A** normal diet; **B** PFD. **C** Cytosols from normal, PFD and PFD+Met fed mice separated by 1D-SDS-PAGE were tested for CAT by Western blot. *Bars* represent the immunoreaction intensity as percentage of the normal diet control. Data are from three independent tests. *Bars* show SEM. Letters *a* and *b* indicate differences from normal at $P<0.05$ and $P<0.01$, respectively; *c* indicates differences from PFD at $P<0.001$

Carbonyl-group content of protein

The content of carbonyl groups in liver proteins was evaluated by their reaction with DNPH. Figure 7 shows that PFD increased the levels of oxidized proteins (88%; $P<0.001$), whereas feeding with PFD+Met partially neutralised the increase caused by PFD (53%; $P<0.01$).

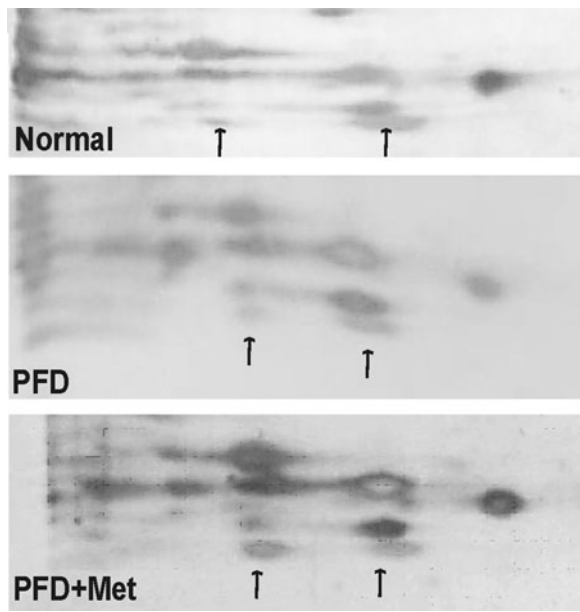

Fig. 3 Influence of the diet on Prx-I content. Cytosolic proteins (25 μg) from normal diet, PFD and PFD+Met fed mice were separated on 2D-NEPHGE and silver stained. The *arrows* show Prx-I spots as distinguished in Fig. 1. Typical images of three independent experiments are shown

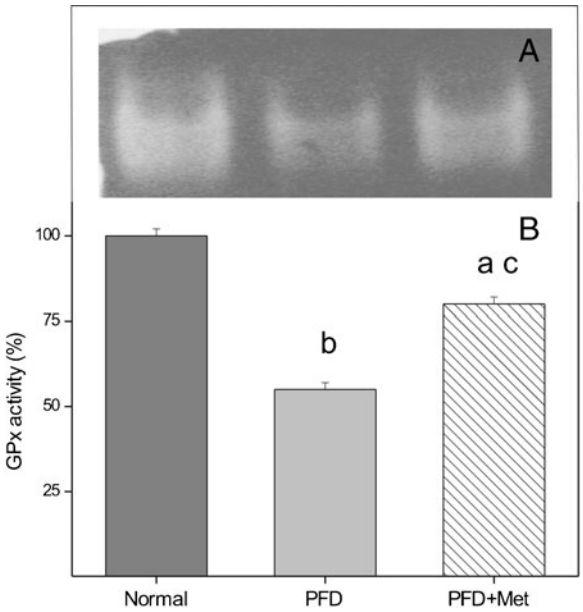

Fig. 4 Influence of the diet on GPx activity. Groups of mice were fed with normal diet, PFD and PFD+Met for 5 days. Cytosolic proteins (25 μg) were separated by non-dissociating 10% PAGE and tested for in gel GPx actvity. **A** Representative image of activity. **B** Densitometric analysis of zymograms expressed as percentage of the normal diet control. Values are means ± SEM of three independent tests. Letters *a* and *b* indicate differences from normal at $P<0.05$ and $P<0.01$, respectively; *c* indicate differences from PFD at $P<0.001$

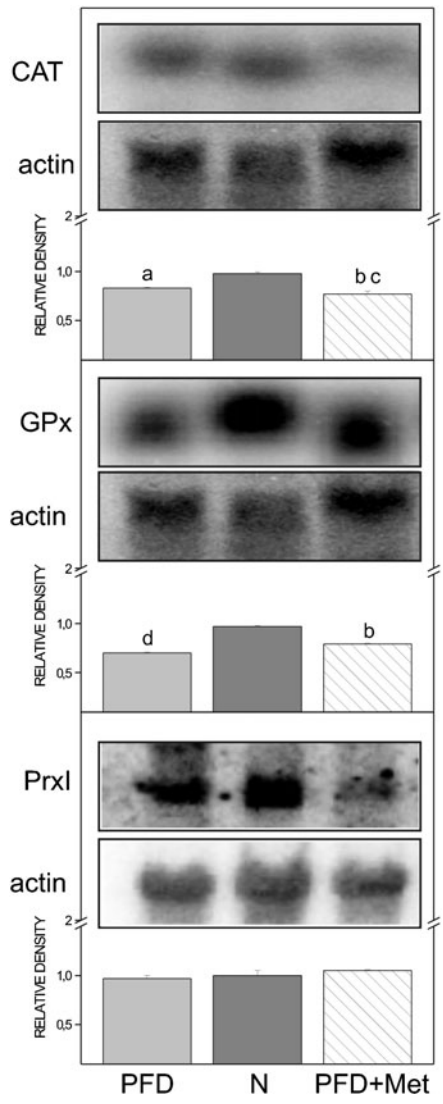

Fig. 5 Effect of the diet on mRNA levels. After different dietary treatments, total RNAs were isolated. Aliquots of 25 μg were subjected to Northern blot analysis using specific cDNA probes for CAT, GPx, Prx-I mRNAs. Actin cDNA probe was used for RNA normalisation. A representative image from three independent experiments is shown. The radioactive signal intensities obtained with the different probes were measured by scanning densitometry. Data from three independent tests are related to the normal diet group. *Bars* mark SEM; *a*, *b* and *d* indicate values that differ from normal at $P<0.05$, $P<0.01$ and $P<0.001$ respectively; *c* indicates differences from PFD at $P<0.001$

Discussion

This study adds new information on the antioxidant system of the female mouse liver under an isocaloric diet deprived of amino acids (PFD), together with the role of Met. Despite published studies on low-protein diet [31, 54], high-protein diet [31], caloric restriction [2, 21, 49] and starvation [30], the effects of an isocaloric diet absolutely depleted of amino acids on the three enzymes studied here were unknown. To our knowledge, Carrillo et al. [8] have reported that both GSH and GST activities are significantly decreased by a PFD in mouse liver.

2D-NEPHGE patterns from mouse liver show the presence of four CAT isoforms with typical sizes but p*I*s slightly higher than those reported [26, 41]. The decrease in both CAT content and activity caused by PFD agree with that shown on mouse liver under a caloric restriction diet [21]. Also, these results are consistent with the decrease in CAT activity in the cortex and cerebellum of young rats caused by a low-protein diet [6]. Conversely, the prevention of CAT decrease by supplementation of PFD with Met is in agreement with the increment in mouse myocardium CAT activity caused by a hypocaloric diet enriched with Met [49].

Both the mass and p*I* of Prx-I determined here agree with previous reports [23, 62]. This enzyme

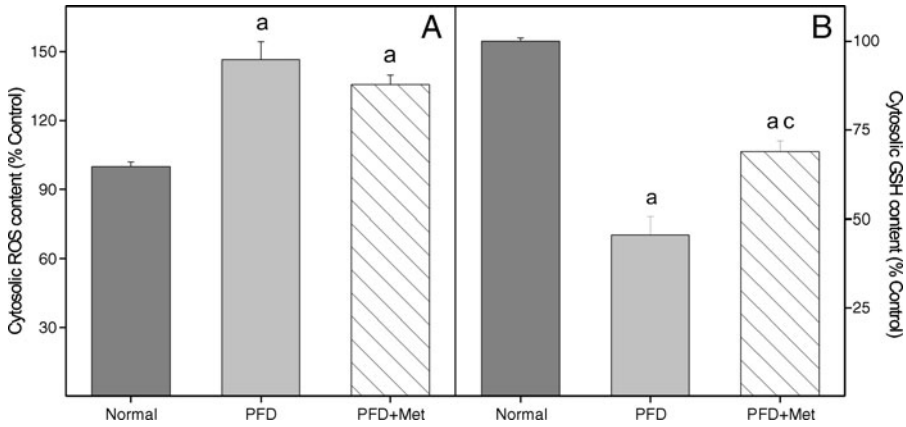

Fig. 6 Effects of the diet on liver ROS and GSH content. Mouse groups were fed with normal, PFD and PFD+Met diets for 5 days. Average levels were expressed as percentages of the normal fed control±SEM (three independent experiments); *a* indicates differences from normal at $P<0.05$; *c* indicates differences from PFD at $P<0.001$. **A** ROS; **B** GSH

belongs to the 2-Cys-Prx family of enzymes that uses thioredoxin as an electron donor for converting H_2O_2 and alkyl-hydroperoxide into water and alcohol, respectively [10]. Over-oxidation reduces both the mass and activity of Prx-I; this raises the levels of H_2O_2 which, in turn, acts as intracellular messenger to alter the overall redox potential of the cell [52]. Thus, the Prx-I decrease caused by PFD provides evidence for the activation of H_2O_2-dependent oxidative stress in amino acid-depleted conditions. Conversely, since the affinity of thioredoxin for its substrates depends on its glutathionylation level [9], the addition of Met to PFD probably contributes to Prx-I protection by increasing GSH availability.

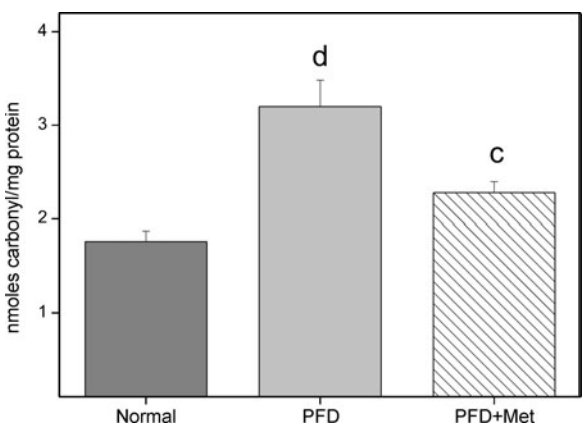

Fig. 7 Quantification of dietary-dependent protein oxidation in the mouse liver. The levels of carbonyl groups in liver proteins were determined for each nutritional condition: normal, PFD and PFD+Met. Data are from three independent experiments. *Bars* mark SEM; *d* indicates value different from normal at $P<0.001$ and *c* indicates value different from PFD at $P<0.01$

The 2D-NEPHGE spot of p*I* 6.7 and 22 kDa match the GPx isoform of p*I* 6.73 and 22.3 kDa previously reported [11, 19]. Both its content and activity decrease with PFD, while feeding with PFD+Met partially preserves its activity. These results agree with the decrease in rat liver GPx caused by a low-protein diet, which is minor when it is enriched with either Met or Ala [25]. Also, in rat hepatocytes, GPx activity increases when either Met or Cys is added to culture media [60].

While Prx-I is close to H_2O_2 signalling, GPx acts "hand-to-hand" with CAT. This is owing to the low rate constant for H_2O_2 removal of Prx-I, which allows for sensing low H_2O_2 concentrations [52, 61]. Conversely, based on K_m values, GPx and CAT might be in charge of removing low and high H_2O_2 concentrations, respectively [61]. Thus, the decline of GPx, CAT and Prx-I caused by PFD possibly increase both the content of oxidant molecules and oxidized proteins in the cells.

Our data show that feeding with PFD lowers both CAT and GPx mRNA levels, which point to decelerated protein synthesis as one of the reasons for reduced protein levels. The presence of Met seems enough to attenuate CAT and GPx protein decrease induced by PFD, but not to prevent the decay in the mRNA levels of these enzymes. This suggests that feeding with PFD+Met possibly preserves CAT and GPx content reducing their breakdown. In support of this is the fact that amino acids are inhibitors of protein degradation [59]. On the other hand, since

both diets did not change Prx-I mRNA levels, PFD and PFD + Met possibly control Prx-I content by increasing and decreasing its breakdown, respectively. Alternatively, both CAT and Prx-I content could depend on changes in their isoform patterns.

To summarise, under dietary depletion of most amino acids, Met is a major responsible for the control of CAT, GPx and Prx-I content.

It has been shown that malnutrition causes oxidative stress in liver [12, 13, 31, 40, 51]. We report here that PFD decreases the content of mouse liver CAT, GPx and Prx-I. Also, we previously found that PFD decreases the content of mouse liver CuZn-SOD, mGSTA3, cytochrome P450 proteins (cyt P450) and CA III [47, 48]. A common feature among these enzymes is their link with the redox status of cell. In addition, similarly to low-protein diets [12, 24], we report here that feeding with PFD decreases hepatic GSH content. These changes explain the increased hepatic ROS content, which led to protein oxidation. Conversely, supplementation of PFD with Met increases GSH level, but not to reach that of the controls. This produces a hepatic ROS content lower than that caused by PFD but higher than the control. Furthermore, supplementation with Met was not enough to neutralise the oxidative stress caused for PFD. Thus, because of its involvement in GSH synthesis [31, 32], dietary Met partly prevents the changes caused by PDF in the redox status of the liver. This has also been confirmed by PFD+Cys administration, which causes similar effects to PFD+Met [43].

Increasing evidence supports the idea that changes produced by PFD on several of the proteins examined in this and previous works occur in the same fashion of either precancerous or cancerous conditions [50, 57]. However, the precise mechanisms by which dietary Met controls the content of several of these proteins remain to be characterised. Current and previous data suggest that amino acids act on several metabolic points [3, 5, 18].

Acknowledgements This work was supported by grants to RDC from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de Mar del Plata (UNMDP), Argentina. RDC, ANC and AMG are CONICET career researchers. VJC and JRM are funded by fellowships from CONICET.

We are grateful to Dr. Jean Claude Guillemot for his support to analyse samples by MS in the Laboratoire de Biochimie des Protéines, Sanofi-Synthelabo Recherche, Labège Innopole, Labège Cedex, France. We also thank Dra. Verónica Beligni for critical reading of the manuscript and Ms. Liliana Bordoni for revision of English spelling and grammar.

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