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RESEARCH ARTICLE

Prolonged fasting increases glutathione biosynthesis in postweaned northern elephant seals

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SUMMARY

Northern elephant seals experience prolonged periods of absolute food and water deprivation (fasting) while breeding, molting or weaning. The postweaning fast in elephant seals is characterized by increases in the renin–angiotensin system, expression of the oxidant-producing protein Nox4, and NADPH oxidase activity; however, these increases are not correlated with increased oxidative damage or inflammation. Glutathione (GSH) is a potent reductant and a cofactor for glutathione peroxidases (GPx), glutathione-S transferases (GST) and 1-cys peroxiredoxin (PrxVI) and thus contributes to the removal of hydroperoxides, preventing oxidative damage. The effects of prolonged food deprivation on the GSH system are not well described in mammals. To test our hypothesis that GSH biosynthesis increases with fasting in postweaned elephant seals, we measured circulating and muscle GSH content at the early and late phases of the postweaning fast in elephant seals along with the activity/protein content of glutamate-cysteine ligase [GCL; catalytic (GCLc) and modulatory (GCLm) subunits], γ-glutamyl transpeptidase (GGT), glutathione disulphide reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), GST and PrxVI, as well as plasma changes in γ-glutamyl amino acids, glutamate and glutamine. GSH increased two- to four-fold with fasting along with a 40–50% increase in the content of GCLm and GCLc, a 75% increase in GGT activity, a two- to 2.5-fold increase in GR, G6PDH and GST activities and a 30% increase in PrxVI content. Plasma γ-glutamyl glutamine, γ-glutamyl isoleucine and γ-glutamyl methionine also increased with fasting whereas glutamate and glutamine decreased. Results indicate that GSH biosynthesis increases with fasting and that GSH contributes to counteracting hydroperoxide production, preventing oxidative damage in fasting seals.

Key words: glutathione, northern elephant seal pup, prolonged fasting.

INTRODUCTION

Northern elephant seals, *Mirounga angustirostris* (Gill 1866), naturally experience prolonged periods of absolute food and water deprivation (fasting) while breeding, molting or weaning, without apparent detrimental effects (Crocker et al., 1998; Ortiz et al., 1978; Ortiz et al., 2001). In terrestrial mammals, prolonged food deprivation is a stressful physiological condition that activates the hypothalamic–pituitary–adrenal axis, increases the release of adrenocorticotropic hormone and glucocorticoids, and leads to subsequent alterations in fluid balance and cardiorespiratory function (Munck et al., 1984; Sapolsky et al., 2000). Prolonged food deprivation also promotes production of superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and lipid hydroperoxides that contribute to oxidative damage, inflammation and antioxidant depletion in a variety of terrestrial mammals including humans, rats and mice (Crescimanno et al., 1989; Di Simplicio et al., 1997; Grattagliano et al., 2000; Kondoh et al., 2003; Mårtensson, 1986; Sorensen et al., 2006; Souza Rocha et al., 2008; Wu et al., 2004).

Glutathione (GSH; γ-glutamyl-cysteinyl-glycine) is the most important non-enzymatic endogenous antioxidant in animal cells (Forman et al., 2009). It is synthesized from glutamate, cysteine and glycine in a two-step enzymatic process catalyzed by the enzymes glutamate-cysteine ligase (GCL) and glutathione synthase (Griffith, 1999). The rate-limiting enzyme in GSH biosynthesis is GCL, which is composed of a heavy catalytic subunit (GCLc) and a light modifier subunit (GCLm) (Griffith, 1999). GSH is used as a cofactor in glutathione peroxidase (GPx) and glutathione-S transferase (GST) catalytic reactions that yield glutathione disulphide (GSSG). GSH homeostasis is partially maintained by γ-glutamyl transpeptidase (GGT), which breaks down extracellular GSH, providing cysteine, the rate-limiting substrate for intracellular GSH *de novo* synthesis (Zhang et al., 2005). Glutathione disulphide reductase (GR) restores GSH from GSSG at the expense of NADPH, which is recycled from $NADP^+$ by glucose-6-phosphate dehydrogenase (G6DPH) in an enzymatic process that maintains the reduced GSH pool (Kosower and Kosower, 1978; Meister and Anderson, 1983). GSH is also essential for the activity of 1-cys peroxiredoxin (peroxiredoxin VI, PrxVI), which – along with catalase, GPx and GST – protects cells against membrane oxidation because the GSH peroxidases and PrxVI eliminate H_2O_2, lipid and phospholipid hydroperoxides (Manevich et al., 2004). Thus, they remove the H_2O_2 produced by dismutation of $O_2^{\cdot-}$ by superoxide dismutases (SODs) and the hydroperoxides produced by lipid peroxidation.

The effects of prolonged food deprivation on GSH metabolism have been scarcely investigated. In humans, food deprivation

depletes circulating GSH content after 1 week (Mårtensson, 1986) whereas in rats, it decreases liver GSH content after 18 h (Grattagliano et al., 2000). Beyond these studies, the effects of prolonged food deprivation on GSH biosynthesis, recycling and the activity of the enzymes that regulate its content have not been examined. In postweaned northern elephant seals, prolonged fasting activates the renin–angiotensin system (RAS), which is known to stimulate pro-oxidant pathways, and increases the expression of the $O_2^{\cdot-}$- and H_2O_2-producing protein Nox4, as well as NADPH oxidase activity, without increasing oxidative damage or inflammation (Ortiz et al., 2000; Vázquez-Medina et al., 2010). The postweaning fast in northern elephant seals also increases the activity and protein content of the antioxidant enzymes MnSOD, CuZnSOD, catalase and GPx, suggesting that fasting seals are responsive to increased antioxidant capacity, which likely contributes to their ability to avoid the detrimental effects of pro-oxidants (Vázquez-Medina et al., 2010). However, the associations among the activated RAS, the elevated antioxidant enzymes and the GSH system during prolonged food deprivation have not been examined. Because GSH is the most important non-enzymatic endogenous antioxidant in animal cells and because it plays a major role in the cellular defense against H_2O_2, lipid and phospholipid hydroperoxides, we hypothesize that prolonged fasting upregulates the enzymes involved in GSH biosynthesis, recycling and utilization in postweaned northern elephant seals. To test our hypothesis, we measured circulating and muscle GSH content at the early and late phases of their natural postweaning fast along with activity and protein content of selected enzymes involved in GSH metabolism and GSH-related metabolites in plasma to better evaluate the effects of prolonged food deprivation on the GSH system in mammals.

MATERIALS AND METHODS

Animal handling and sample collection

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee's of both the University of California Merced (AUP08-0002) and Sonoma State University (2008-37). All work was realized under National Marine Fisheries Service marine mammal permit no. 87-1743.

Seventeen northern elephant seal pups were sampled at Año Nuevo State Reserve at two periods during their natural postweaning fast: early (1–2 weeks postweaning; N=9, 5 males and 4 females) and late (7–8 weeks postweaning; N=8, 4 males and 4 females). Each sampling group was independent but, because their age was known with relative accuracy, they represented early and late fasting. Animals were sedated with 1 mg kg^{-1} Telazol® (tiletamine/zolazepam HCl, Fort Dodge Labs, Fort Dodge, IA, USA) administrated intramuscularly. Once immobilized, a 16 gauge, 3.5 inch spinal needle was inserted into the extradural spinal vein. Blood samples were collected in pre-chilled EDTA-treated vacutainer sample tubes and placed on ice. Immobilization was maintained with a 100 mg bolus (intravenous injection) of ketamine as needed. Muscle biopsies were collected as described previously (Vázquez-Medina et al., 2010). Biopsies were rinsed with ice-cold phosphate buffered saline (PBS) and divided into two subsamples. One subsample was placed in a cryogenic vial, frozen by immersion in liquid nitrogen and stored in dry ice and subsequently at –80°C until it was analyzed for enzyme activities or protein content. The other subsample was placed in a microcentrifuge tube containing ice-cold PBS and minced on ice. PBS was removed and 0.45 ml 10% perchloric acid (PCA) solution containing 7.5 nmol γ-glutamyl glutamate (GGA) was added. Samples were kept in ice until they were homogenized at the nearby Long Marine Laboratory (University of California Santa Cruz, Santa Cruz, CA, USA). Homogenates were centrifuged at 6000 ***g*** for 20 min at 4°C. Supernatants were removed, frozen by immersion in liquid nitrogen and stored at –80°C until they were analyzed for GSH content. Blood samples were centrifuged at 3000 ***g*** for 15 min at 4°C, plasma was frozen in liquid nitrogen and stored at –80°C until they were assayed for GGT activity or GSH-related metabolites. Red blood cell (RBC) lysates were prepared as described previously (Vázquez-Medina et al., 2010) and divided into two subsamples. One subsample was treated with 10% PCA containing 7.5 nmol GGA and centrifuged at 6000 ***g*** for 20 min at 4°C. Clear supernatants were transferred to cryovials, frozen by immersion in liquid nitrogen and stored at –80°C until GSH content analysis. The other subsample was mixed with Drabkin's reagent, frozen by immersion in liquid nitrogen and stored at –80°C until hemoglobin content analysis. Frozen tissue samples were homogenized in 50 mmol l^{-1} potassium phosphate buffer containing 1 mmol l^{-1} EDTA, 1% Triton X-100, 1% PMSF and 1% protease inhibitor cocktail (Sigma, St Louis, MO, USA), as described previously (Vázquez-Medina et al., 2006; Vázquez-Medina et al., 2007; Vázquez-Medina et al., 2010; Vázquez-Medina et al., 2011). Total protein content in tissue samples was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Hemoglobin content in RBC lysates was measured by the cyanmethemaglobin method using a commercially available kit (Cayman Chemical, Ann Arbor, MI, USA).

GSH measurement

Total glutathione equivalents (TGSH-eq) were measured by HPLC following the method of Fariss and Reed (Fariss and Reed, 1987) and using GGA as internal standard in RBC lysates and muscle samples. Extracted samples were mixed with 100 mmol l^{-1} iodoacetic acid and pH was adjusted to 8–9 using KOH (2 mol l^{-1})/KHCO3 (2.4 mol l^{-1}). After 15 min of incubation in the dark at room temperature, 1% dinitrobenzene was added. Samples were vortexed and stored overnight at 4°C. L-lysine (1 mol l^{-1}) was added and the precipitated salt was removed by centrifugation after incubating the samples at 4°C for 2 h. Supernatants were transferred to autosampler vials and analyzed by HPLC.

Western blot

Soluble proteins were extracted from untreated frozen muscle samples as described above. Twenty micrograms of total protein were resolved in 4–15% Tris-HCl gradient gels under denaturizing conditions. Proteins were electroblotted using the Bio-Rad Trans-Blot® SD semi-dry transfer cell onto 0.45 µm nitrocellulose membranes. Membranes were blocked with 3% bovine serum albumin in PBS containing 0.05% Tween 20 and incubated overnight with primary antibodies against mammalian GCLm, PrxVI, actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or GCLc (Liu et al., 1998) diluted 1:500 to 1:3000. Membranes were washed, incubated with horseradish-peroxidase-conjugated secondary antibodies (Pierce, Rockford, IL, USA), re-washed and developed by using the Immun-Star Western C kit (Bio-Rad). Blots were visualized using a Chemi-Doc XRS system (Bio-Rad) and quantified using Bio-Rad's Quantity One software.

Enzyme activities

GR, GST and G6PDH activities were measured in muscle tissue extracts by using commercially available kits (Cayman Chemical; Biovision Research Products, Mountain View, CA, USA). Results are expressed in mU enzyme mg^{-1} protein, as described previously (Vázquez-Medina et al., 2006; Vázquez-Medina et al., 2007). GGT

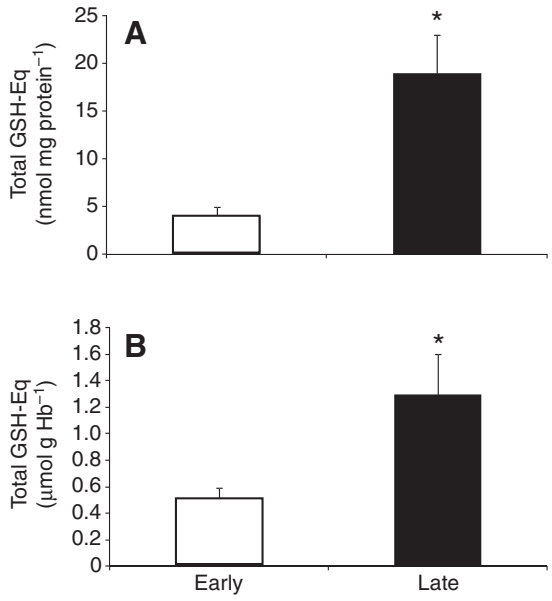

Fig. 1. Mean (±s.e.m.) total glutathione equivalents (TGSH-Eq) in northern elephant seal (A) muscle and (B) red blood cells during early (1–2 weeks postweaning, *N*=9) and late (7–8 weeks postweaning, *N*=8) fasting. Asterisks indicate a significant (P<0.05) difference from early fasting.

activity was measured in plasma using a commercially available kit (Bioo Scientific, Austin, TX, USA) and is expressed in U l^{-1}.

Metabolomics

Fasting-induced changes in GSH-related metabolites (γ-glutamyl amino acids, glutamate and glutamine) in plasma were assessed using a metabolomics approach. Samples were compared between early and late fasting periods in postweaned pups and opportunistically, in lactating females (day 5 and day 22 post-partum, *N*=10). Metabolite analysis was performed by Metabolon, Inc. (Raleigh, NC, USA). Samples were divided into two fractions for analysis by liquid chromatography/mass spectrometry-mass spectrometry (LC-MS, LC-MS2) and gas chromatography/mass spectroscopy (GC-MS). Mean ratios of the median scaled data from two samples for each seal were calculated.

Statistics

Means were compared between early and late fasting groups using two-sample *t*-tests with Bonferroni adjustment. Means (±s.e.m.) were considered significantly different at P<0.05. For the metabolomics data, peak heights were scaled to the median of the early samples and comparisons with late samples were made using a paired *t*-test. Statistical analyses were performed with SYSTAT® 11.0 software (SPSS, Richmond, CA, USA).

RESULTS

GSH content

Total GSH content was measured in muscle and RBC lysates to evaluate whether this non-enzymatic antioxidant thiol increases with fasting in elephant seals and thus has a potential participatory role in the cellular defenses against fasting-induced hydroperoxide production and oxidative damage. Fasting increased (P<0.05) mean TGSH-Eq content fourfold in muscle and over twofold in RBCs (Fig. 1).

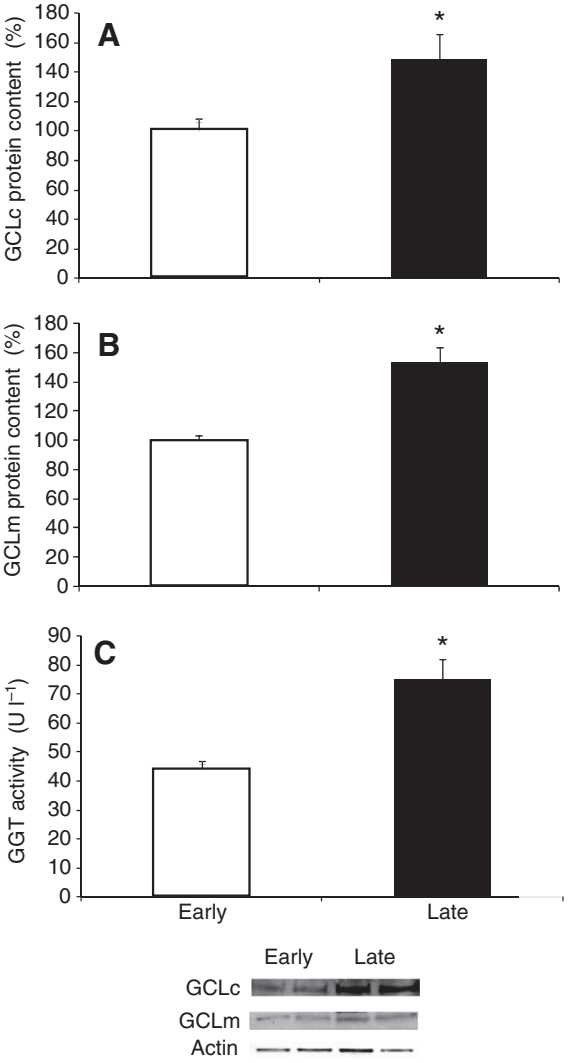

Fig. 2. Mean (±s.e.m.) protein expression of northern elephant seal muscle (A) glutamate-cysteine ligase catalytic (GCLc) and (B) glutamate-cysteine ligase modulatory (GCLm) subunits, and (C) plasma γ-glutamyl transpeptidase (GGT) activity during early (1–2 weeks postweaning, *N*=9) and late (7–8 weeks postweaning, *N*=8) fasting. A representative western blot of GCLc and GCLm expression is shown. Results are expressed in % change from early for GCL protein expression and in U l^{-1} for GGT activity. One unit of GGT activity is defined as the amount of enzyme that catalyzes the transformation of 1 μmol substrate min^{-1} at 37°C. Asterisks indicate a significant (P<0.05) difference from early fasting.

GSH biosynthesis

GCLc and GCLm muscle protein content was measured to evaluate whether prolonged fasting increases the levels of GCL, the rate-limiting enzyme in GSH biosynthesis. Plasma GGT activity was also measured to evaluate whether this enzyme, which breaks down extracellular GSH – thus providing cysteine, the rate-limiting substrate for intracellular GSH *de novo* synthesis – also increases with fasting. Fasting increased GCLc by 40%, GCLm by 50% and plasma GGT activity by ~75% (P<0.05; Fig. 2). In the same way, the metabolomics study revealed that the plasma content of several γ-glutamyl amino acids increased with fasting in pups but not in lactating females (Table 1). Likewise, plasma content of glutamate and glutamine decreased with fasting in pups but remained

Table 1. Changes in glutathione-related metabolites in plasma samples from postweaning northern elephant seal pups and fasting lactating females

Stage	Metabolite	*P*	Change
Postweaning pups	Glutamate	0.0001	↓
	Glutamine	0.01	↓
	γ-Glutamyl glutamine	0.01	↑
	γ-Glutamyl isoleucine	0.003	↑
	γ-Glutamyl methionine	0.02	↑
Lactating females	Glutamate	0.15	↔
	Glutamine	0.07	↔
	γ-Glutamyl glutamine	0.002	↓
	γ-Glutamyl isoleucine	0.046	↓
	γ-Glutamyl methionine	0.25	↔

↑, Increase; ↓, decrease; ↔, unchanged.

unchanged in females (Table 1), suggesting that GSH biosynthesis increases with fasting in postweaned elephant seal pups but not in lactating females.

GR and G6PDH activity

Muscle GR and G6PDH activities were measured to evaluate whether GSSG recycling to GSH increases with fasting in elephant seal pups. Because the direct measurement of GSSG is not feasible in a field setting, the activity measurements of GR and G6PDH provide an indirect assessment of GSH recycling. Fasting increased mean GR and G6PDH activities two- and 2.5-fold, respectively ($P<0.05$; Fig. 3), suggesting that GSSG recycling to GSH increases with fasting and the reduced GSH pool is maintained in postweaned northern elephant seals.

PrxVI expression and GST activity

Muscle PrxVI protein content and GST activity were measured to evaluate whether these peroxide-removing enzymes increase with fasting in elephant seals. Fasting increased mean PrxVI protein content by 30% and GST activity nearly twofold ($P<0.05$; Fig. 4), suggesting that peroxide removal increased with fasting in postweaned northern elephant seals.

DISCUSSION

Prolonged food and water deprivation is a potentially pathological condition that, in most mammalian species, increases $O_2^{\cdot-}$ and hydroperoxide production, oxidative damage and inflammation. In elephant seals, prolonged food and water deprivation stimulates RAS, upregulates Nox4 and increases NADPH oxidase activity, circulating cortisol and non-esterified fatty acid content without increasing local or systemic oxidative damage or inflammation (Ortiz et al., 2000; Ortiz et al., 2001; Ortiz et al., 2003; Ortiz et al., 2006; Vázquez-Medina et al., 2010). This sequence of events suggests that elephant seals have evolved mechanisms that allow them to cope with conditions characterized by increased $O_2^{\cdot-}$ and hydroperoxide production. However, the contributing cellular responses that help alleviate the potential detriments associated with prolonged fasting are not well described in mammals.

We have shown that expression and activity of the antioxidant enzymes SOD, GPx and catalase increase with fasting in elephant seals, likely contributing to the alleviation of the fasting-induced RAS stimulation, Nox4 upregulation and increased NADPH oxidase activity (Vázquez-Medina et al., 2010). In the present study, we found that GSH biosynthesis along with GR, G6PDH, GST and PrxVI activity/protein content increased with fasting, suggesting that

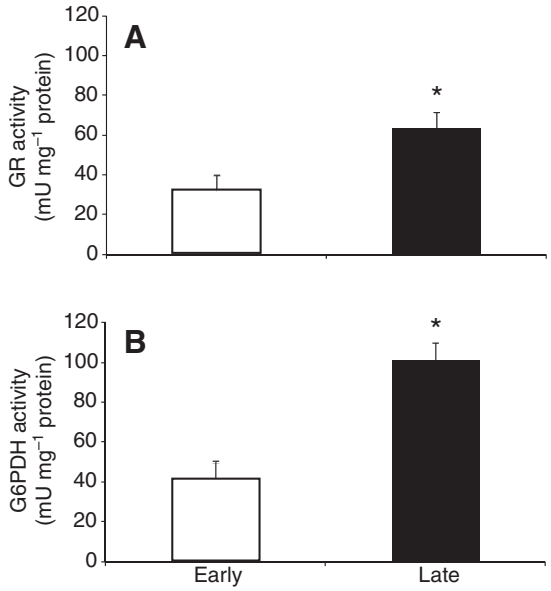

Fig. 3. Mean (±s.e.m.) activities of muscle (A) glutathione disulfide reductase (GR) and (B) glucose-6-phosphate dehydrogenase (G6PDH) during early (1–2 weeks postweaning, $N=9$) and late (7–8 weeks postweaning, $N=8$) fasting in northern elephant seals. One unit of GR is defined as the amount of enzyme that will cause the oxidation of 1 nmol NADPH min^{-1} at 25°C. One unit of G6PDH defines the amount of enzyme that catalyzes the conversion of 1 μmol NAD^+ to NADH per minute at 37°C. Asterisks indicate a significant ($P<0.05$) difference from early fasting.

GSH plays a key role in the elephant seal's defense against the pro-oxidant conditions associated with their natural, prolonged fast. GSH is crucial for the adaptive response against oxidative stress because it is a potent reductant and cofactor for GPx, GST and PrxVI catalytic reactions, and thus contributes significantly to the removal of hydroperoxides (Forman et al., 2009).

The observed increases in the expression of both GCLc and GCLm, along with the increase in circulating and tissue GSH-Eq content, indicate that GSH biosynthesis increases with fasting in postweaned northern elephant seals. Increased plasma GGT activity, increased plasma content of γ-glutamyl amino acids and decreased circulating content of glutamate and glutamine support this idea because GGT breaks down extracellular GSH conjugates into γ-glutamyl groups and cysteinylglycine conjugates providing cysteine, the rate-limiting substrate for intracellular GSH synthesis (Zhang et al., 2005). The GSH system has been proposed to be a key component of protection against diving-induced ischemia/reperfusion in seals (Murphy and Hochachka, 1981; Vázquez-Medina et al., 2007; Wilhelm Filho et al., 2002), a condition that increases $O_2^{\cdot-}$ and hydroperoxide production (Hermes-Lima and Zenteno-Savín, 2002; Johnson et al., 2004; Johnson et al., 2005; Vázquez-Medina et al., 2006; Zenteno-Savin et al., 2002; Zenteno-Savin et al., 2010). Because the postweaning fast of elephant seals is the developmental stage that immediately precedes the beginning of their diving lifestyle (Le Boeuf et al., 1972) and is characterized by adjustments that lead to pro-oxidant conditions (Ortiz et al., 2001; Vázquez-Medina et al., 2010) that can activate the cellular adaptive oxidative stress response (Franco et al., 1999; Sen and Packer, 1996), it is likely an anticipatory mechanism that stimulates the seal's antioxidant system, thus preventing systemic oxidative damage. The observed differences in the circulating content of γ-

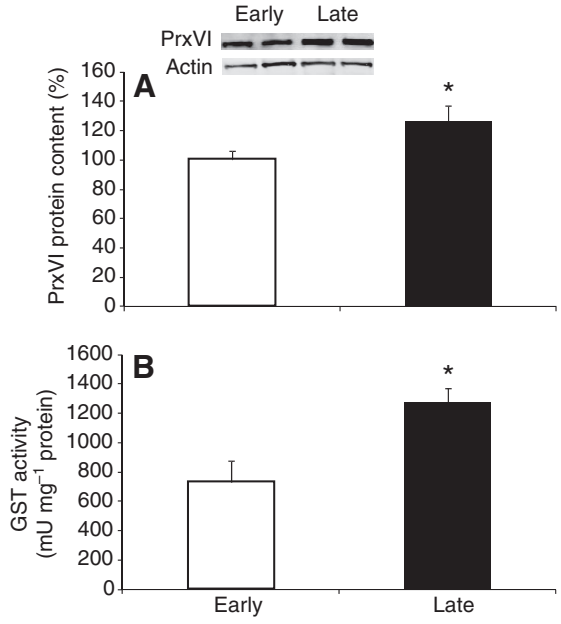

Fig. 4. Mean (±s.e.m.) muscle (A) 1-cys peroxiredoxin (PrxVI) protein expression and (B) glutathione S-transferase (GST) activity during early (1–2 weeks postweaning, *N*=9) and late (7–8 weeks postweaning, *N*=8) fasting in northern elephant seals. A representative western blot of PrxVI expression is shown. Changes in protein content are expressed as % change from early. One unit of GST is defined as the amount of enzyme needed to conjugate 1 nmol chlorodinitrobenzene with glutathione per minute at 25°C. Asterisks indicate a significant (P<0.05) difference from early fasting.

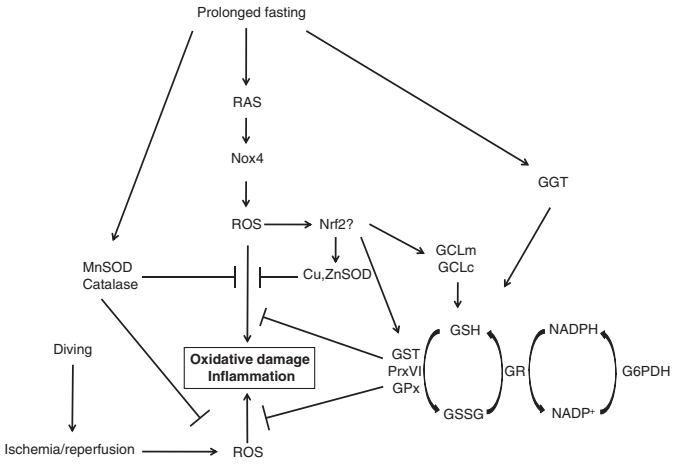

Fig. 5. Schematic representation of proposed molecular mechanisms leading to increased glutathione biosynthesis and antioxidant enzyme upregulation in response to prolonged fasting in northern elephant seals. Arrows indicate stimulation; capped lines indicate inhibition. See List of abbreviations for definitions.

glutamyl amino acids between fasting pups and adult lactating females also suggests that the enhancement of the elephant seal's antioxidant system is a crucial component of their postweaning development as they prepare to enter a diving lifestyle (Vázquez-Medina et al., 2010). Although the adult female's lactating fast is also followed by a feeding/diving phase, existing evidence suggests that the antioxidant system in pinnipeds develops with maturation in parallel with the development of their diving capacity (Burns et al., 2007; Burns et al., 2010; Lestyk et al., 2009; Vázquez-Medina et al., 2011).

The observed increase in GR and G6PHD activity suggests that the recycling of GSSG to GSH is increased and the reduced GSH pool is maintained over the course of elephant seal's postweaning fast (Kosower and Kosower, 1978; Meister and Anderson, 1983). The maintenance of the reduced GSH pool is crucial for cell survival during oxidative stress as the reactions of GSH with peroxides and reactive electrophiles, which are catalyzed by GPx, PrxVI and GST, use reduced GSH and yield GSSG (Kosower and Kosower, 1978; Manevich et al., 2004; Meister and Anderson, 1983). The maintenance of the GSH pool is crucial for providing available co-substrate to support the observed increase in GST activity and PrxVI protein expression. The fasting-related increases in GST activity, PrxVI expression, and GPx and catalase activity and protein content (Vázquez-Medina et al., 2010) suggest that there is an increase in the rate of the removal of H_2O_2, which is the principal substrate for those antioxidant enzymes and is also the main oxidant produced in response to ischemia/reperfusion due to the hypoxanthine/xanthine oxidase pathway (Brown et al., 1988). This pathway has been shown to be activated after experimental ischemia in seal organs (Elsner et al., 1998).

The postweaning fast in elephant seals is associated with increases in RAS and Nox4 (Ortiz et al., 2001; Ortiz et al., 2006; Vázquez-Medina et al., 2010), which collectively have been shown to increase intracellular H_2O_2 production (Dikalov et al., 2008). The modulation of H_2O_2 levels by peroxide-removing enzymes is essential for the actions of H_2O_2 as a second messenger in the activation of the NF-E2-related factor 2 (Nrf2) pathway, which leads to the upregulation of proteins involved in GSH synthesis, antioxidant defense and phase II detoxification, *via* the antioxidant response element (Immenschuh and Baumgart-Vogt, 2005; Jaiswal, 2004; Rhee et al., 2005; Sen and Packer, 1996). Thus, the observed increases in PrxVI and GST, along with the previously reported increases in catalase and GPx (Vázquez-Medina et al., 2010), suggest that H_2O_2 is actively modulated during the postweaning fast in elephant seals, which likely results in the upregulation of enzymes involved in GSH biosynthesis such as GCLc, GCLm, GST and GR (Fig. 5).

In summary, our results show that GSH biosynthesis increases with fasting in postweaned northern elephant seals, suggesting that the GSH system contributes to the cellular defense against fasting-derived pro-oxidant conditions. Our results also suggest that fasting pups require this postweaning developmental phase for the activation of their antioxidant systems. Increased protein content and activities of the enzymes associated with the GSH system contribute to the antioxidant mechanisms that have evolved in elephant seals and permit them to tolerate potentially pathological conditions characterized by increased hydroperoxide production.

LIST OF ABBREVIATIONS

Cu,ZnSOD	copper, zinc-dependent superoxide dismutase
G6PDH	glucose-6-phosphate dehydrogenase
GCL	glutamate-cysteine ligase
GCLc	glutamate-cysteine ligase catalytic subunit
GCLm	glutamate-cysteine ligase modulatory subunit
GGA	γ-glutamyl glutamate
GGT	γ-glutamyl transpeptidase
GPx	glutathione peroxidase
GR	glutathione disulfide reductase
GSH	glutathione
GSSG	glutathione disulfide
GST	glutathione S-transferase
H_2O_2	hydrogen peroxide

MnSOD	manganese-dependent superoxide dismutase
Nox4	NADPH oxidase 4
Nrf2	NF-E2-related factor 2
$O_2^{\cdot-}$	superoxide radical
PCA	perchloric acid
PrxVI	1-cys peroxiredoxin
RAS	renin–angiotensin system
RBC	red blood cell
SOD	superoxide dismutase
TGSH-eq	total glutathione equivalents

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