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Prolonged fasting does not increase oxidative damage or inflammation in postweaned northern elephant seal pups

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SUMMARY

Elephant seals are naturally adapted to survive up to three months of absolute food and water deprivation (fasting). Prolonged food deprivation in terrestrial mammals increases reactive oxygen species (ROS) production, oxidative damage and inflammation that can be induced by an increase in the renin–angiotensin system (RAS). To test the hypothesis that prolonged fasting in elephant seals is not associated with increased oxidative stress or inflammation, blood samples and muscle biopsies were collected from early (2–3 weeks post-weaning) and late (7–8 weeks post-weaning) fasted seals. Plasma levels of oxidative damage, inflammatory markers and plasma renin activity (PRA), along with muscle levels of lipid and protein oxidation, were compared between early and late fasting periods. Protein expression of angiotensin receptor 1 (AT_1), pro-oxidant (Nox4) and antioxidant enzymes (CuZn- and Mn-superoxide dismutases, glutathione peroxidase and catalase) was analyzed in muscle. Fasting induced a 2.5-fold increase in PRA, a 50% increase in AT_1, a twofold increase in Nox4 and a 70% increase in NADPH oxidase activity. By contrast, neither tissue nor systemic indices of oxidative damage or inflammation increased with fasting. Furthermore, muscle antioxidant enzymes increased 40–60% with fasting in parallel with an increase in muscle and red blood cell antioxidant enzyme activities. These data suggest that, despite the observed increases in RAS and Nox4, an increase in antioxidant enzymes appears to be sufficient to suppress systemic and tissue indices of oxidative damage and inflammation in seals that have fasted for a prolonged period. The present study highlights the importance of antioxidant capacity in mammals during chronic periods of stress to help avoid deleterious systemic consequences.

Key words: antioxidant enzyme, cachexia, fasting, inflammation, oxidative stress, renin–angiotensin system.

INTRODUCTION

Food deprivation is a stressful physiological condition that activates the hypothalamic–pituitary–adrenal axis, leading to an increased release of adrenocorticotropic hormone and glucocorticoids and to subsequent alterations in fluid balance (Munck et al., 1984), cardio-respiratory function (Sapolsky et al., 2000) and pro-oxidant/antioxidant systems (Mitsui et al., 2002; Sorensen et al., 2006). While moderate caloric restriction has beneficial effects on animal health (Chandrasekar et al., 2001; Ahmet et al., 2005; Mattson and Wan, 2005), prolonged food deprivation leads to the depletion of antioxidant stores and to an increase in production of reactive oxygen species (ROS) and oxidative damage in a variety of animals, including humans (Martensson, 1986).

The harmful effects of prolonged food deprivation are consistent among various vertebrate species (Pascual et al., 2003; Morales et al., 2004). Prolonged food deprivation in the rat increases hepatic mitochondrial ROS production, protein oxidative damage, lipid peroxidation and lipoperoxidation-derived protein modifications (Sorensen et al., 2006). Food deprivation also increases the activity of tumor necrosis factor-α (TNF-α) in rat adipose tissue (Wu et al., 2004) and the oxidatively modified DNA content in peripheral blood mononuclear cells (De Souza Rocha et al., 2008). Likewise, prolonged food deprivation increases myocardial hydrogen peroxide production and hepatic lipid peroxidation and decreases liver and muscle glutathione (GSH) content and antioxidant enzyme activities (Crescimanno et al., 1989; Di Simplicio et al., 1997; Grattagliano et al., 2000; Kondoh et al., 2003).

Northern elephant seals (*Mirounga angustirostris* Gill 1866) maintain electrolyte and fluid homeostasis during prolonged food and water deprivation (fasting) by activating the renin–angiotensin system (RAS) (Ortiz et al., 2000; Ortiz et al., 2006). In human and rat, the chronic activation of RAS is associated with several pathological conditions, such as inflammatory disease, hypertension, heart and kidney disease, endothelial dysfunction and insulin resistance (Mancini et al., 1996; Kimi and Iwao, 2000; Wang et al., 2000; McFarlane et al., 2001; Cooper et al., 2007; Ortiz et al., 2007). Increased RAS stimulates production of ROS by increasing the expression/activity of NADPH oxidases. ROS react with lipids, proteins or nucleic acids, in a variety of tissues and cells, leading to oxidative damage and inflammation (Rajagopalan et al., 1996; Cai and Harrison, 2000; Landmesser et al., 2002; Lambeth, 2004; Bedard and Krause, 2007).

Northern elephant seals naturally experience prolonged periods (up to three months) of absolute food and water deprivation while breeding, nursing, molting or weaning, with no apparent detrimental effects (Ortiz et al., 1978; Crocker et al., 1998; Ortiz et al., 2001; Ortiz et al., 2006). Unlike hibernators, elephant seals remain normothermic during their postweaning fast, with relatively high metabolic rates compared with mammals of similar size (Rea and Costa, 1992; Crocker et al., 1998). Because prolonged fasting is a natural component of the life history of the elephant seal, we hypothesized that fasting is not associated with increased oxidative damage or inflammation despite an increase in RAS. In order to test our hypothesis, we compared systemic and tissue levels of

oxidative stress and inflammation, along with selected RAS components, between early- and late-fasted northern elephant seal pups, in an effort to contribute to the elucidation of the mechanisms evolved by these animals to cope with this potentially detrimental behavior. Elephant seals have clearly evolved robust physiological mechanisms that have allowed them to adapt to extreme behaviors or environmental conditions, making them an ideal and interesting model to study the physiological mechanisms by which mammals contend with the detrimental effects of fasting-induced oxidative stress.

MATERIALS AND METHODS

All procedures were reviewed and approved by the Institutional Animal Care and Use Committees of both the University of California Merced and Sonoma State University (CA, USA). All work was carried out under the National Marine Fisheries Service marine mammal permit no. 87-1743.

Animals and sample collection

Postweaned northern elephant seal pups of known age (within 2–3 days of weaning) from Año Nuevo State Reserve (30 km north of Santa Cruz, CA, USA) were sampled at two periods: early (2–3 weeks postweaning; N=10, 5 males, 5 females) and late (7–8 weeks postweaning; N=9, 5 males, 4 females). Each sampling group was independent, but, because their age was known with relative accuracy, they represented early and late fasting. Pups were weighed at weaning, and the percentage of body mass loss over the fasting period was calculated. On the day of sampling, animals were initially sedated with 1 mg kg^{-1} telazol (tiletamine/zolazepam HCl, Fort Dodge Labs, Ft Dodge, IA, USA) administrated intramuscularly. Once immobilized, a 16-gauge, 3.5 inch spinal needle was inserted into the extradural spinal vein, and blood samples were collected in pre-chilled EDTA-treated vacutainer sample tubes and placed on ice. Immobilization was maintained with a 100 mg bolus of intravenous injections of ketamine, as needed. Muscle biopsies were collected by first cleaning a small region in the flank of the animal near the hind flipper, with alternating wipes of isopropyl alcohol and betadine, followed by a subcutaneous injection of 2–3 ml of lidocaine (Henry Schein, Melville, NY, USA). A small (<1.5 cm) incision was made using a sterile scalpel, and a muscle biopsy (*ca.* 50 mg) was collected with a sterile biopsy punch needle (Henry Schein). Biopsies were rinsed with ice-cold PBS, placed in cryogenic vials, frozen by immersion in liquid nitrogen and stored at –80°C until analyzed. Blood samples were centrifuged for 15 min at 3000 ***g*** at 4°C, plasma was transferred to cryo-vials, frozen by immersion in liquid nitrogen and stored at –80°C. Red blood cells (RBCs) were packed by adding two volumes of 0.9% saline solution and centrifuged for 10 min at 3000 ***g***. Buffy coat was discarded and the RBC pellet was lysed by vortexing in four volumes of HPLC-grade water for 1 minute. Lysed RBCs were centrifuged and the supernatant collected, frozen by immersion in liquid nitrogen and stored at –80°C. Frozen tissue samples were homogenized in two volumes of 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-Aldrich, St Louis, MO, USA). Total protein content in tissue samples was measured by using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

Western blot

Thirty µg of total protein were resolved in 4–15% Tris-HCl SDS gradient gels. Proteins were electroblotted using the Bio-Rad trans-blot, semi-dry 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 NADPH oxidase 4 (Nox4), angiotensin receptor type 1 (AT_1), catalase (Santa Cruz Biotechnology, Santa Cruz, CA, USA), CuZn-superoxide dismutase (CuZnSOD), Mn-superoxide dismutase (MnSOD), glutathione peroxidase (GPx) or actin (Assay Designs, Ann Arbor, MI, USA) diluted 1:500 to 1:5000. Membranes were washed, incubated with HRP-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 by using Quantity One software (Bio-Rad).

Inflammatory markers and oxidative damage

Plasma TNF-α levels were measured using a commercially available EIA kit (Cayman Chemical). Plasma C-reactive protein (hs-CRP) concentration was quantified by using a Tina-quant high-sensitivity assay (Roche Diagnostics, Indianapolis, IN, USA). Circulating levels of 8-isoprostanes (8-iso-$PGF_{2\alpha}$) were quantified by GC-MS, as described previously (Morrow and Roberts, 1999). Muscle lipid peroxidation was evaluated by measuring the content of thiobarbituric acid reactive substances (TBARS) using a commercially available kit (Cayman Chemical, Ann Arbor, MI, USA). The relative concentration of 4-hydroxynonenal (4-HNE) in muscle samples as well as plasma and muscle levels of total nitrotyrosine (NT) were measured by slot blot. Thirty micrograms of total protein (for tissue samples) or 25 µl of plasma were loaded onto 0.45 µm nitrocellulose membranes using the Bio-Dot SF microfiltration apparatus (Bio-Rad). Membranes were blocked, probed with antibodies against 4-HNE (Calbiochem, San Diego, CA, USA) and NT (Cayman Chemical) and developed as described above. Protein carbonyl content in muscle samples was evaluated by using an Oxyblot™ protein oxidation detection kit (Millipore, Temecula, CA, USA). Fifteen micrograms of total protein were treated and loaded onto membranes by using the Bio-Dot SF. Membranes were blocked, probed and developed as described above.

Enzyme activities

Plasma renin activity (PRA) was measured using a commercially available RIA kit (DiaSorin, Stillwater, MN, USA), previously validated for its use with northern elephant seal plasma (Ortiz et al., 2000; Ortiz et al., 2003b; Ortiz et al., 2006). Total SOD and GPx activities were measured in tissue extracts and RBC lysates with commercially available kits (Cayman Chemical). Catalase activity was measured by monitoring the removal of 10 mmol l^{-1} H_2O_2 at 240 nm, as described previously (Vázquez-Medina et al., 2006). NADPH oxidase activity was measured by using the lucigenin-enhanced chemiluminescence (CL) assay (Griendling et al., 1994). Homogenates were incubated in the dark with 100 µmol l^{-1} NAPDH and 5 µmol l^{-1} lucigenin in PBS. Relative CL was measured for 10 minutes in a luminometer (Berthold, Oak Ridge, TN, USA). Basal activity was measured in the absence of NADPH. A buffer blank was subtracted from each reading. NADPH oxidase activity was expressed as RLU mg protein^{-1}.

Statistics

Means were compared between early and late fasting groups by two-sample t-tests with Bonferroni adjustment. Means (±s.e.m.) were considered significantly different at P<0.05. Statistical analyses were performed with the SYSTAT 11.0 software (SPSS, Richmond, CA, USA).

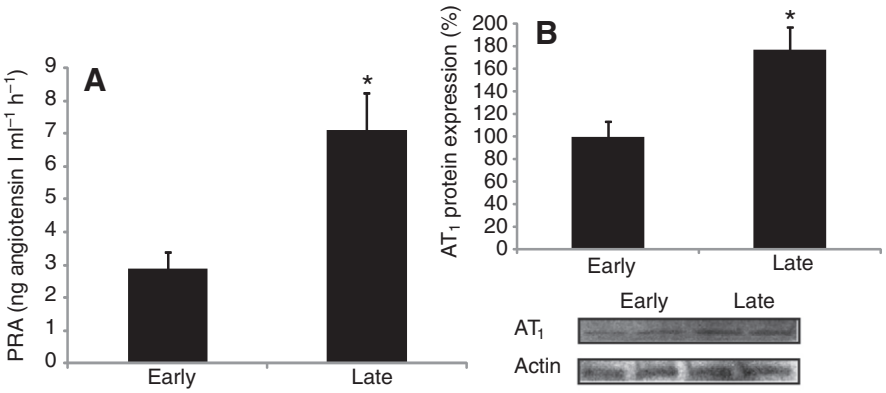

Fig. 1. Prolonged fasting activates the renin–angiotensin system in northern elephant seal pups. Mean (±s.e.m.) (A) plasma renin activity (PRA) and (B) muscle AT_1 protein content (expressed as the percentage change from 'early') between early (2–3 weeks postweaning) and late (7–8 weeks postweaning) fasting periods. *P<0.05.

RESULTS

Fasting induces loss of body mass

Loss of body mass in elephant seals was calculated in order to evaluate the effects of prolonged fasting on the overall body composition of elephant seals. The average body mass of elephant seals at weaning was 124±6 kg and at sampling 92±5 kg. Elephant seals lost 26±2% (P<0.001) of their body mass during the course of fasting (*ca*. 8 weeks). By contrast, muscle total protein content did not change with fasting (early: 4.2±0.5 mg ml^{-1} *vs* late: 4.5±0.6 mg ml^{-1}). These results suggest that, despite losing nearly one-quarter of their body mass, elephant seals possess robust physiological mechanisms to tolerate this potentially detrimental condition.

Fasting activates RAS

PRA and AT_1 protein expression were measured to evaluate the effects of prolonged fasting on RAS activation in elephant seals. Fasting increased PRA nearly 2.5 fold (early: 2.8±0.5 *vs* late: 7.1±1.1 ng Ang I ml^{-1} h^{-1}, P<0.01) and muscle AT_1 protein expression by 77% (early: 100±13 *vs* late: 177±19, P<0.05) (Fig. 1), indicating that RAS is activated as a response to prolonged fasting in elephant seals.

Fasting increases Nox4 protein expression and NADPH oxidase activity

Nox4 muscle protein expression and NADPH oxidase activity were measured to evaluate whether prolonged fasting stimulates ROS-producing proteins in elephant seals. Fasting induced a twofold increase in Nox4 muscle protein expression (early: 100±8 *vs* late: 201±26, P<0.01) and a 74% increase in NADPH oxidase activity (early: 686±31 *vs* late: 1194±172 RLU mg protein^{-1}, P<0.05) (Fig. 2). These results demonstrate that fasting stimulates the pro-oxidant system, which can potentially contribute to increased ROS production and oxidative damage.

Neither oxidative damage nor inflammation increases with fasting

Circulating levels of 8-iso-$PGF_{2\alpha}$, NT, TNF-α and hs-CRP along with muscle levels of 4-HNE, TBARS, NT and protein carbonyls were measured to evaluate the effects of prolonged fasting on local and systemic indices of oxidative damage and inflammation in elephant seals. None of this suite of markers of oxidative damage and inflammation in plasma or muscle increased over the course of fasting (Table 1, Fig. 3). Moreover, muscle protein carbonyls (early: 100±3 *vs* late: 81±4) and NT levels (early: 100±1 *vs* late: 95±1) decreased (P<0.05) with fasting (Fig. 3). These results show that, despite the fasting-induced activation of RAS and Nox4, neither systemic nor local oxidative damage or inflammation increased with fasting, suggesting that these animals have evolved robust physiological mechanisms to avoid oxidative stress and inflammation during this potentially detrimental condition.

Fasting increases antioxidant enzymes

In order to explore the mechanisms elephant seals have evolved to cope with fasting-induced RAS and Nox4 activation, we compared muscle protein content of the antioxidant enzymes CuZnSOD, MnSOD, GPx and catalase, as well muscle and RBC SOD, catalase and GPx activities, between early and late fasted seals. CuZnSOD (early: 100±9 *vs* late: 158±17), MnSOD (early: 100±9 *vs* late: 140±8), GPx (early: 100±7 *vs* late: 151±14) and catalase (early: 100±10 *vs* late: 142±2) protein expression increased 40–60% (P<0.05) with fasting (Fig. 4). Similarly, the activities of muscle catalase (early: 29±4 *vs* late: 69±15 U mg protein^{-1}) and GPx (early:

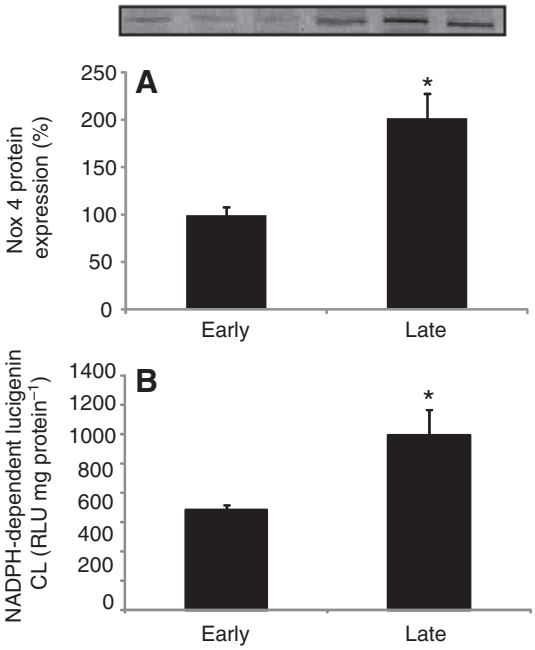

Fig. 2. Nox4 protein expression and NADPH oxidase activity increase with fasting in northern elephant seal pups. Mean (±s.e.m.) (A) muscle Nox4 protein content (expressed as the percentage change from 'early') and (B) muscle NADPH oxidase activity between early (2–3 weeks postweaning) and late (7–8 weeks postweaning) fasting periods. *P<0.05.

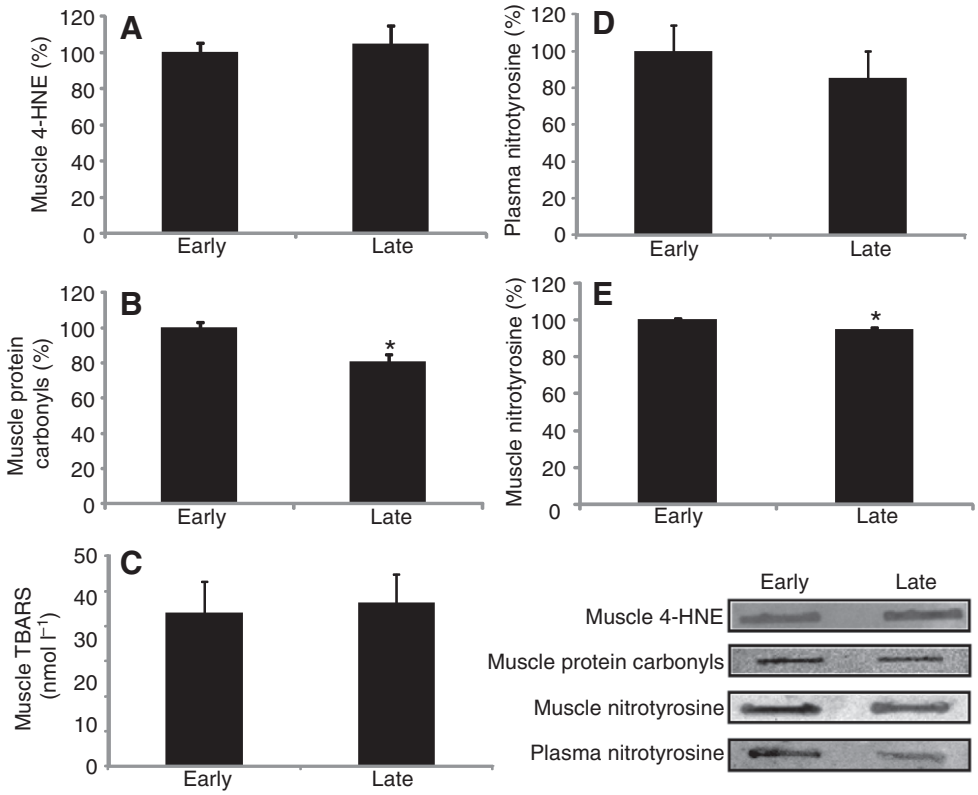

Fig. 3. Neither systemic nor muscle levels of oxidative damage increase with fasting in northern elephant seal pups. Mean (±s.e.m.) expressed as the percentage change from 'early' of (A) muscle 4-hydroxynonenal (4-HNE), (B) muscle protein carbonyls, (C) muscle thiobarbituric acid reactive substances (TBARS), (D) plasma nitrotyrosine and (E) muscle nitrotyrosine between early (2–3 weeks postweaning) and late (7–8 weeks postweaning) fasting periods. *P<0.05.

6.9±1.4 *vs* late: 10.7±0.5 U mg protein^{-1}), and RBC SOD (early: 839±16 *vs* late: 920±24 U mL^{-1}), catalase (early: 5192±753 *vs* late: 8185±973 U ml^{-1}) and GPx (early: 430±90 *vs* late: 935±149 U ml^{-1}) increased (P<0.05) with fasting (Fig. 5). These results indicate that fasting stimulates the antioxidant enzymatic system in elephant seal pups, which likely contributes to the suppression of oxidative stress and inflammation, despite the increases in RAS and Nox4.

DISCUSSION

Prolonged food deprivation is a potentially detrimental behavior, which stimulates ROS production and oxidative damage that, when chronically elevated, can contribute to a host of complications, including cardiovascular, renal and hepatic diseases and multi-organ failure (Mancini et al., 1996; Kimi and Iwao, 2000; McFarlane et al., 2001; Cooper et al., 2007). The present study demonstrates that

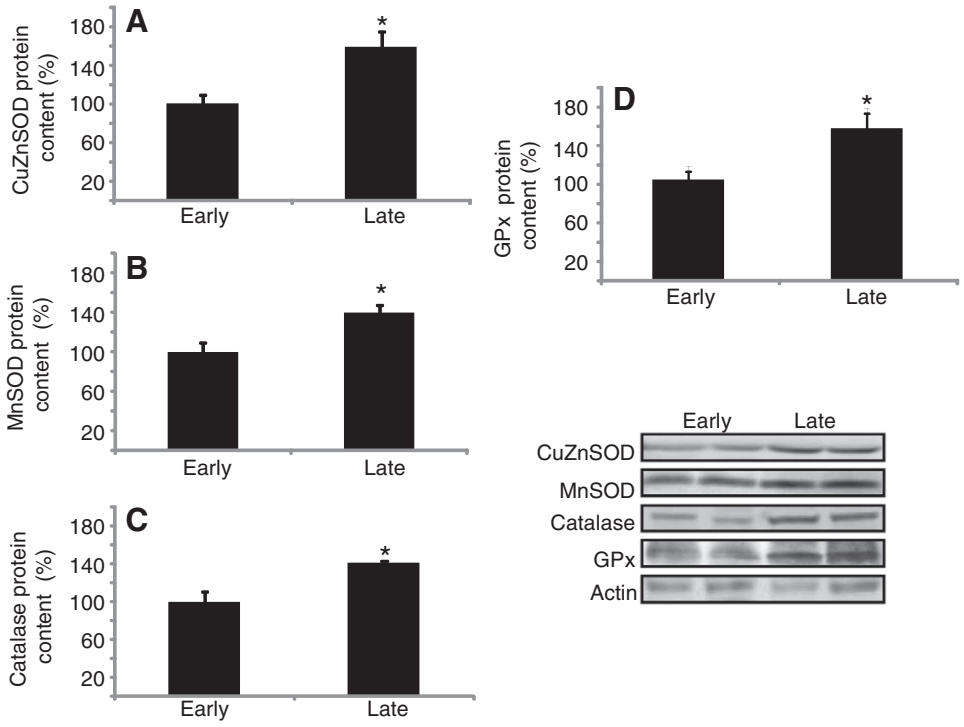

Fig. 4. Expression levels of antioxidant enzymes increase with fasting in northern elephant seals. Mean (±s.e.m.) content expressed as the percentage change from 'early' of (A) copper-zinc superoxide dismutase (CuZnSOD), (B) manganese superoxide dismutase (MnSOD), (C) catalase and (D) glutathione peroxidases (GPx) between early (2–3 weeks postweaning) and late (7–8 weeks postweaning) fasting periods. *P<0.05.

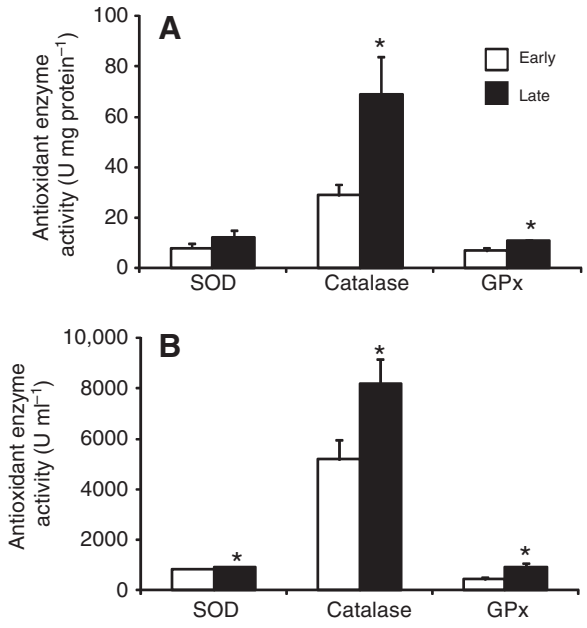

Fig. 5. Activities of antioxidant enzymes increase with fasting in northern elephant seal pups. Mean (±s.e.m.) activities of total superoxide dismutase (SOD), catalase and glutathione peroxidases (GPx) in (A) muscle and (B) red blood cells (RBCs) between early (2–3 weeks postweaning) and late (7–8 weeks postweaning) fasting periods. *P<0.05.

some mammals are uniquely adapted to counteract the detrimental effects of extreme behaviors or environmental conditions such as prolonged food and water deprivation. Our results show that, while prolonged fasting increases the expression of the ROS-producing protein Nox4, along with NADPH oxidase activity, an increase in antioxidant enzymes is sufficient to avoid the oxidative damage and inflammation commonly associated with fasting in terrestrial mammals.

Prolonged food and water deprivation can be detrimental by promoting oxidative stress and inflammation through activation of RAS. While northern elephant seals have adapted to tolerate such a situation, this behavior is associated with a number of chronic metabolic adjustments that include the activation of RAS (Ortiz et al., 2000; Ortiz et al., 2006). Elevated RAS increases the expression/activity of ROS-producing proteins in rat and human (Rajagopalan et al., 1996; Cai and Harrison, 2000; Landmesser et al., 2002; Lambeth, 2004; Bedard and Krause, 2007) and, thus, has been associated with increased concentrations of tissue and systemic markers of oxidative damage and inflammation (Mancini et al., 1996; Kimi and Iwao, 2000; McFarlane et al., 2001; Cooper et al., 2007). Elevated RAS has been shown to increase Nox4 protein expression *in vitro* and *in vivo* in rat and human (Wingler et al., 2001; Lapante et al., 2005). Other than these examples, data on the effects of elevated RAS on ROS-generating proteins during prolonged fasting are scarce. The present study demonstrates that prolonged fasting increases the expression of the ROS-producing protein Nox4, along with NADPH oxidase activity, in the presence of increased PRA and AT_1, suggesting that elevated RAS might contribute to the generation of ROS through Nox4. Alternatively, the previously observed increases in cortisol in fasting pups (Ortiz et al., 2001; Ortiz et al., 2003a; Ortiz et al., 2003b) might have contributed to the increase in Nox4 and NADPH oxidase activity as elevated glucocorticoids increase ROS production and oxidative damage as well (Mitsui et al., 2002; Caro et al., 2007).

Table 1. Means (±s.e.m.) of plasma markers of inflammation and oxidative damage between early (2–3 weeks postweaning) and late (7–8 weeks postweaning) fasting periods

	Early	Late
hs-CRP (μg ml^{-1})	0.35±0.10	0.33±0.08
TNF-α (pg ml^{-1})	20±4	35±8
8-Iso-$PGF_{2\alpha}$ (pg ml^{-1})	88±6	83±5

hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; 8-iso-$PGF_{2\alpha}$, 8-isoprostane $PGF_{2\alpha}$ (15-F_{2t}-IsoP).

More interestingly, despite the increases in Nox4 and RAS, neither local nor systemic oxidative damage or inflammation indices increase with fasting in elephant seals, indicating that these animals have evolved robust physiological mechanisms to alleviate the potentially detrimental consequences of this extreme condition. Further elucidation of these mechanisms can provide significant advancement of our understanding of diseases related to oxidative stress and RAS.

The lack of increases in local and systemic indices of oxidative stress and inflammation despite the observed increases in Nox4 and RAS can be explained, at least in part, by the observed increases in antioxidant enzymes. It has been suggested that diving seals possess an enhanced antioxidant system that contributes to the protection against ROS produced in response to recurrent episodes of dive-induced ischemia/reperfusion (Elsner et al., 1998; Hermes-Lima and Zenteno-Savín, 2002; Zenteno-Savín et al., 2002; Johnson et al., 2004; Johnson et al., 2005). Higher activities of the antioxidant enzymes SOD, catalase, GPx, glutathione S-transferase and glutathione disulfide reductase, as well as higher GSH content, have been found in seal tissues than in tissues of terrestrial mammals (Vázquez-Medina et al., 2006; Vázquez-Medina et al., 2007). In the present study, protein expression of CuZnSOD, MnSOD, catalase and GPx, as well as their activity levels, increased with fasting, which likely contributed to the avoidance of oxidative damage and inflammation. These data suggest that fasting seals are responsive to increased antioxidant capacity, which contributes to their ability to avoid the detrimental effects of pro-oxidants. Furthermore, the end of the fasting period in northern elephant seal pups immediately precedes the beginning of their diving lifestyle (Le Boeuf et al., 1972). Therefore, it is possible that the accumulation of antioxidants during this period prepares elephant seals to cope with the potential ROS overproduction associated with diving-induced ischemia/reperfusion. The increase in NADPH oxidase activity likely stimulated the upregulation of antioxidant enzymes because ROS production can be one of the main factors that regulate antioxidant enzyme expression and activity (Warner et al., 1996; Franco et al., 1999; Pahl and Baeuerle, 2005).

Fasting seals utilize their fat stores as the primary source of energy, resulting in extensive lipid mobilization and increased concentrations of circulating non-esterified fatty acids (NEFA) (Ortiz et al., 1978; Castellini et al., 1987; Rea and Costa, 1992; Houser and Costa, 2001; Ortiz et al., 2003b). In humans, elevated NEFA concentrations are associated with a higher content of circulating 8-iso-$PGF_{2\alpha}$ (Stojiljkovic et al., 2001; Stojiljkovic et al., 2002), a widely used marker of systemic lipid peroxidation (Morrow and Roberts, 1999; Morrow and Roberts, 2000). In the rat, elevated RAS increases circulating levels of 8-iso-$PGF_{2\alpha}$ (Aizawa et al., 2002). In the present study, circulating concentrations of 8-iso-$PGF_{2\alpha}$ remained unchanged with fasting.

Moreover, in contrast to the previously reported increases in protein oxidation/nitration induced by fasting and increased RAS activation in terrestrial mammals (Di Simplicio et al., 1997; Sorensen et al., 2006; Cooper et al., 2007), muscle and plasma NT levels remained unchanged, and muscle protein carbonyls decreased, suggesting that fasting seals possess robust cellular mechanisms to protect lean tissue from the potentially damaging effects of oxidation associated with elevated RAS. The decrease in protein carbonyls is likely attributable to the fact that protein catabolism contributes to less than 4% of their average daily metabolic rate and that protein turnover decreases over the fasting period (Adams and Costa, 1993; Houser and Costa, 2001).

In summary, our results show that, despite the fasting-induced increases in RAS and Nox4, neither local nor systemic oxidative damage and inflammation indices were increased. The observed increases in antioxidant enzymes likely contributed to the suppression of oxidative damage and inflammation. Thus, the increased antioxidant response likely alleviated the potentially deleterious effects of fasting-derived ROS production. Elucidating the mechanism by which animals evolved to be uniquely and naturally adapted to extreme environmental and behavioral conditions will prove to be fruitful as we strive to gain a better understanding of how oxidative stress and inflammation promote a number of pathophysiological conditions.

LIST OF ABBREVIATIONS

AT_1	angiotensin receptor type 1
GPx	glutathione peroxidase
GSH	glutathione
4-HNE	4-hydroxynonenal
hs-CRP	high-sensitivity C-reactive protein
NT	nitrotyrosine
RAS	renin–angiotensin system
ROS	reactive oxygen species
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances

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