The Journal of Experimental Biology 215, 1448-1455

doi:10.1242/jeb.067173

## RESEARCH ARTICLE

# Prolonged fasting increases purine recycling in post-weaned northern elephant seals

José Guadalupe Soñanez-Organis[1,*], José Pablo Vázquez-Medina[1], Tania Zenteno-Savín[2], Andres Aguilar[1], Daniel E. Crocker[3] and Rudy M. Ortiz[1]

[1]School of Natural Sciences, University of California, Merced, 5200 North Lake Road, Merced, CA 95343, USA, [2]Planeación Ambiental y Conservación, Centro de Investigaciones Biológicas del Noroeste, S.C., La Paz, Baja California Sur, C.P. 23090, México and [3]Department of Biology, Sonoma State University, Rohnert Park, CA 94928, USA

*Author for correspondence (jorganis@ucmerced.edu)

Accepted 4 January 2012

**SUMMARY**

**Northern elephant seals are naturally adapted to prolonged periods (1–2 months) of absolute food and water deprivation (fasting). In terrestrial mammals, food deprivation stimulates ATP degradation and decreases ATP synthesis, resulting in the accumulation of purines (ATP degradation byproducts). Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) salvages ATP by recycling the purine degradation products derived from xanthine oxidase (XO) metabolism, which also promotes oxidant production. The contributions of HGPRT to purine recycling during prolonged food deprivation in marine mammals are not well defined. In the present study we cloned and characterized the complete and partial cDNA sequences that encode for HGPRT and xanthine oxidoreductase (XOR) in northern elephant seals. We also measured XO protein expression and circulating activity, along with xanthine and hypoxanthine plasma content in fasting northern elephant seal pups. Blood, adipose and muscle tissue samples were collected from animals after 1, 3, 5 and 7 weeks of their natural post-weaning fast. The complete HGPRT and partial XOR cDNA sequences are 771 and 345 bp long and encode proteins of 218 and 115 amino acids, respectively, with conserved domains important for their function and regulation. XOR mRNA and XO protein expression increased 3-fold and 1.7-fold with fasting, respectively, whereas HGPRT mRNA (4-fold) and protein (2-fold) expression increased after 7 weeks in adipose tissue and muscle. Plasma xanthine (3-fold) and hypoxanthine (2.5-fold) levels, and XO (1.7- to 20-fold) and HGPRT (1.5- to 1.7-fold) activities increased during the last 2 weeks of fasting. Results suggest that prolonged fasting in elephant seal pups is associated with increased capacity to recycle purines, which may contribute to ameliorating oxidant production and enhancing the supply of ATP, both of which would be beneficial during prolonged food deprivation and appear to be adaptive in this species.**

Key words: hypoxanthine-guanine phosphoribosyl transferase, northern elephant seal, prolonged fasting, purine metabolism, xanthine oxidoreductase.

## INTRODUCTION

Northern elephant seals, *Mirounga angustirostris* (Gill 1866), are naturally exposed to prolonged periods of absolute food and water deprivation (fasting) while breeding, molting or weaning (Le Boeuf et al., 1972; Ortiz et al., 1978). In humans, rats and mice, prolonged fasting increases oxidant production, oxidative damage and inflammation (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). In elephant seals, prolonged fasting does not induce oxidative damage or inflammation despite promoting the chronic activation of the renin-angiotensin system (RAS) and increasing circulating cortisol, NADPH oxidase activity, Nox4 protein expression and insulin resistance (Ortiz et al., 2000; Ortiz et al., 2002; Vázquez-Medina et al., 2010; Viscarra et al., 2011a; Viscarra et al., 2011b).

The purines adenine and guanine are essential components of nucleic acids, high-energy phosphates and signaling molecules such as cyclic adenosine monophosphate and cyclic guanosine monophosphate (Elson, 1965; Verma and Eckstein, 1998). Dietary ingestion of purines contributes to the maintenance of the body pool whereas *de novo* synthesis of purines from non-purine precursors requires energy (Raivio et al., 2001). Therefore, under normal physiological conditions, intermediates of purine degradation are efficiently reutilized through purine salvage pathways (Raivio et al., 2001). Conditions characterized by increased ATP degradation and/or decreased ATP synthesis (e.g. starvation, tissue hypoxia or strenuous exercise) overload the purine catabolic pathway accumulating degradation products (Becker, 2001). Xanthine oxidoreductase (XOR) catalyzes the last two steps of purine degradation, the oxidation of hypoxanthine (HX) and xanthine to uric acid (Bray, 1975; Hille and Nishino, 1995; Vorbach et al., 2003). XOR exists predominantly as xanthine dehydrogenase (XDH), but under certain conditions (e.g. tissue ischemia), XDH is converted to xanthine oxidase (XO), which transfers electrons to molecular oxygen-producing oxidants such as superoxide ($O_2^{\bullet-}$) and mainly hydrogen peroxide ($H_2O_2$) and potentially increasing oxidative stress (McCord, 1985; Parks et al., 1983).

Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) catalyzes a salvage pathway in which HX is converted to inosine monophosphate and, ultimately, to ATP (Cherin et al., 2006; Krenitsky et al., 1969). The need for purine salvage is most evident after circumstances that increase nucleotide degradation, such as starvation/food deprivation or chronic sleep apnea, both of which constitute natural components of the life history of the elephant seal. Under such conditions, ATP hydrolysis may exceed ATP synthesis, compromising the energy status of the tissues and increasing the accumulation of HX and xanthine (Brault and Terjung, 2001;

Decking et al., 1997; Deussen et al., 1993; Hellsten-Westing et al., 1993). Formation of these metabolites represents a loss from the adenosine nucleotide pool and prevents *de novo* synthesis of ATP. A decrease in the intracellular process of purine salvage is associated with an increase in the biochemical reduction of HX by XOR (Becker, 2001; Raivio et al., 2001; Stone and Simmonds, 1991).

Along with being exposed to prolonged fasting while on land, seals are also exposed to ischemia during breath-holding while diving and sleeping (Castellini et al., 1994; Elsner, 1999; Meir et al., 2009; Stockard et al., 2007). Seal tissues accumulate HX after *ex vivo* exposure to ischemia (Elsner et al., 1998). Seal heart and kidney, however, produce less HX than pig tissues, suggesting that seals have a greater capacity to conserve or salvage purines (Elsner et al., 1995; Elsner et al., 1998). The potential participation of purine recycling as a mechanism to conserve energy and avoid oxidant production during fasting in seals has yet to be investigated. In the present study, full-length and partial HGPRT and XOR cDNA sequences were obtained. Also, HGPRT and XOR mRNA and protein expression was quantified in adipose tissue and muscle from fasting elephant seals, along with the plasma levels of HX and xanthine and the plasma activities of HGPRT and XO. We hypothesized that prolonged fasting upregulates XOR and HGPRT in a tissue-specific manner, and increases plasma HX, xanthine, XO and HGPRT activities in post-weaned northern elephant seals. The northern elephant seal is able to withstand long periods of prolonged fasting without deleterious consequences, making it an interesting model to understand the molecular and physiological mechanisms that allow adapted mammals to tolerate this potentially pathological condition.

## MATERIALS AND METHODS

All methods were reviewed and approved by the Institutional Animal Care and Use Committees of the University of California, Merced, and Sonoma State University. All work was realized under the National Marine Fisheries Service marine mammal permit no. 87-1743.

### Animal handling and sample collection

Twenty-eight elephant seal pups of known age were sampled at Año Nuevo State Reserve (30 km north of Santa Cruz, CA, USA), seven at a time, at four periods during their natural post-weaning fast (1, 3, 5 and 7 weeks post-weaned). Pups were initially sedated with 1 mg kg$^{-1}$ tiletamine hydrochloride and zolazepam hydrochloride (telazol; Fort Dodge Animal Health, Fort Dodge, IA, USA). Once immobilized, a 16 gauge, 3.5 inch spinal needle was inserted into the extradural vein. Sedation was maintained with 100 mg bolus intravenous injections of ketamine (Fort Dodge Animal Health) as needed. Blood samples were collected into EDTA-treated collection tubes containing 10 µl ml$^{-1}$ protease inhibitor cocktail and 0.005% butylated hydroxytoluene (Sigma, St Louis, MO, USA) and centrifuged on site for 15 min at 3000 ***g*** before plasma was aliquoted into separate cryovials. Adipose tissue and muscle biopsies (20–40 mg) were collected from a small region in the flank of the animal near the hind flipper, as described previously (Vázquez-Medina et al., 2010; Vázquez-Medina et al., 2011a). Plasma and tissue samples were frozen by immersion in liquid nitrogen immediately after collection and stored at –80°C until analyzed.

### HGPRT and XOR cloning

The complete HGPRT and partial XOR sequences were obtained using primers designed based on homolog mammalian nucleotide sequences using Primer3 software (http://frodo.wi.mit.edu) (Rozen and Skaletsky, 2000) (Table 1). The first PCR fragments of HGPRT and XOR were obtained using the following conditions. For a 30 µl final volume reaction, 25 µl of Platinum PCR SuperMix (Invitrogen, Carlsbad, CA, USA), 3 µl of cDNA and 1 µl (20 µmol l$^{-1}$) of each primer (for HGPRT, HGPRTF1 + HGPRTR1 and for XO, XOFw1 + XORv2) were mixed and subjected to the following conditions: 75°C for 5 min for one cycle; 94°C for 3 min for one cycle; 40 cycles of 94°C for 30 s, 58°C for 40 s and 68°C for 1 min; and an overextension step of 68°C for 7 min. Negative controls in which the template (cDNA) was omitted from PCR reactions were run concurrently. The PCR products were analyzed by electrophoresis on 1% agarose gels stained with SYBR Safe (Invitrogen) and visualized with ultraviolet light. PCR fragments of ~200 and ~345 bp were obtained, sequenced and identified as HGPRT (esealHGPRT) and XOR (esealXOR), respectively, by comparing them with GenBank data using the BLAST algorithm (Altschul et al., 1990).

Table 1. Primers used to obtain the cDNA sequences of hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and xanthine oxidoreductase (XOR)

| Primer name | Nucleotide sequence (5′–3′) |
|---|---|
| HGPRT | |
| HGPRTF1 | GATCCATTCCTATGACTGTAG |
| HGPRTF2 | GACTGAAGAGCTACTGTAATG |
| HGPRTF3 | CCGTTATGGCGACCCGCAG |
| HGPRTR1 | CACCAGCAAGCTTGCAACC |
| HGPRTR3 | GCTGGCCACAGAACTAGAAC |
| XOR | |
| XOFw1 | GAGCACTTCTACCTGGAGAC |
| XOFw2 | ACAGAACACCATGAAGACCC |
| XORv1 | CTGCCACCAGTTATCAGCATG |
| XORv2 | CCCAGTCTTCATGAAGCCAAC |

The 5′ and 3′ ends of HGPRT were obtained using primers designed based on their respective untranslated regions (UTRs) and internal regions of each homolog nucleotide sequence of HGPRT. In contrast, the UTRs for XOR were not obtained using primers designed based on esealXOR sequence and homolog nucleotide sequences of human *Homo sapiens* (GenBank accession no. NM_000379), domestic dog *Canis lupus familiaris* (XM_540143) and giant panda *Ailuropoda melanoleuca* (XM_002927017), probably due to the large size of the XOR gene (~5.7 kb). The HGPRTF3 + HGPRTR1 and HGPRTF1 + HGPRTR3 primers were used to obtain the 5′ and 3′ ends, respectively. The PCR reactions consisted of 20 µl of Platinum PCR SuperMix (Invitrogen), 3 µL of cDNA and 1 µl (20 µmol l$^{-1}$) of each primer (for a final reaction volume of 25 µl). This volume was mixed and subjected to the following conditions: 94°C for 3 min for 1 cycle; 40 cycles of 94°C for 30 s, 55°C for 40 s and 68°C for 2 min; and an overextension step of 68°C for 7 min. PCR products of 500 bp (for the 5′ end, esealHGPRT5′) and 300 bp (for the 3′ end, esealHGPRT3′) were obtained, cloned on pGEM-T Easy Vector System (Promega, San Luis Obispo, CA, USA) and sequenced. The full-length cDNA sequence for HGPRT (esealHGPRT, 771 bp) was obtained by overlapping esealHGPRT, esealHGPRT5′ and esealHGPRT3′. The predicted HGPRT and XOR amino acid sequence was obtained using a translation web site (http://arbl.cvmbs.colostate.edu/molkit/translate/), aligned with other HGPRT and XOR using Clustal W (Thompson et al., 1994) and compared with protein databases using BLAST (Altschul et al., 1990).

### HGPRT and XOR mRNA expression

Total RNA was isolated individually from frozen adipose tissue and muscle samples using TRIzol reagent (Invitrogen) following the

manufacturer's instructions. RNA integrity was confirmed by measuring the absorbance at 260 nm/280 nm and by 1% agorose gel electrophoresis (Sambrook and Russell, 2001). Genomic DNA in total RNA was eliminated by digestion using recombinant DNase I (Roche, Indianapolis, IN, USA), as specified by the manufacturer. Separate cDNAs from each tissue were synthesized from total DNA-free RNA (1 μg) using the QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA, USA) and oligo-dT (0.5 μg).

Specific primers for HGPRT and XOR were designed based on esealHGPRT and esealXOR sequences. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal standard to normalize HGPRT and XOR expression data. HGPRT, XO and GAPDH mRNA was measured by quantitative RT-PCR (qRT-PCR) using the HGPRTF2 + HGPRTR, XOFw2 + XORv1 and GAPDHFw + GAPDHRv primers, respectively. Two PCR reactions for each available tissue sample were run for qRT-PCR on an 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) in a final volume of 20 μl containing 10 μl of SYBR Green PCR Master Mix (Applied Biosystems), 6 μl of $H_2O$, 0.5 μl of each primer (20 μmol $l^{-1}$) and 3 μl of cDNA (equivalent to 150 ng of total RNA). After an initial denaturing step at 94°C for 5 min, amplifications were performed for 40 cycles at 94°C for 30 s, 60°C for 35 s and a final step of 55 s at 72°C, with a single fluorescence measurement and a final melting curve program decreasing 0.3°C each 20 s from 95 to 60°C. Positive and negative controls were included. Standard curves of HGPRT, XOR and GAPDH were run to determine the efficiency of amplification using dilutions from $5\times10^{-4}$ to $5\times10^{-8}$ ng $\mu l^{-1}$ of PCR fragment. For each measurement, expression levels (ng $\mu l^{-1}$) were normalized to GAPDH and expressed as the ratio of HGPRT or XOR to GAPDH.

### Plasma measurements

Plasma XO activity, xanthine and HX concentrations were measured using an Amplex® Red assay kit (Molecular Probes, Eugene, OR, USA). Plasma HGPRT activity was measured using a PRECICE® HPRT assay kit (NovoCIB SAS, Lyon, France).

### HGPRT and XO protein expression

Cytosolic protein fractions were prepared from frozen adipose and muscle tissues using the NE-PER cytoplasmic protein extraction kit (Pierce, Rockford, IL, USA). Total protein content was measured using the Bio-Rad Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Twenty micrograms of protein were mixed with Laemmli sample buffer, boiled and resolved in 4–15% Tris-HCl gradient gels. Proteins were electroblotted onto 0.45 μm nitrocellulose membranes using the Trans-Blot Turbo transfer system (Bio-Rad Laboratories). Membranes were blocked 1 h at room temperature and incubated overnight with primary antibodies against HGPRT and XO (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:1000 and 1:100, respectively. Membranes were washed, incubated with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology) diluted 1:5000, re-washed and developed with the Immun-Star™ WesternC™ kit (Bio-Rad Laboratories). Blots were visualized using a Chemi-Doc XRS system (Bio-Rad Laboratories) and quantified by using Bio-Rad's Quantity One software. Percent change from 1 week was calculated after band densities were normalized against GAPDH.

### Statistics

Differences across the fast were detected by one-way ANOVA with Bonferroni *post hoc* tests. Means (±s.e.m.) were considered statistically different at $P<0.05$. Statistical analyses were performed using STATISTICA 8 software (StatSoft Inc., Tulsa, OK, USA).

## RESULTS

### HGPRT and XO coding sequences from the northern elephant seal

The complete HGPRT (esealHGPRT; GeneBank accession no. JN820130) and partial XOR (esealXOR; GeneBank accession no. JN820131) coding sequences for *M. angustirostris* were obtained from muscle cDNA (Fig. 1). The esealHGPRT sequence is 771 bp long with a 654 bp open reading frame and encodes a predicted protein of 218 amino acids and a calculated molecular weight of 24.4 kDa (Fig. 1A,B). The partial esealXOR is 345 bp and encodes a predicted protein fragment of 115 amino acids (Fig. 1C,D). The translated esealHGPRT sequence shows high identity to HGPRT from the dog (99%; NP_001003357), humans (97%, NP_000185) and giant panda (94%, EFB15658). The translated esealXOR shows high identity to XOR from the giant panda (97%, EFB16086), the African elephant *Loxodonta africana* (96%, XP_003416107), humans (95%, AAA75287) and the mouse *Mus musculus* (92%, NP_035853). The predicted esealHGPRT protein shows only 2, 7 and 12 differences with the dog, human and giant panda HGPRT amino acid sequences, respectively (Fig. 1B). Conserved regions that encode functional domains of HGPRTs and XORs were found in esealHGPRT and esealXOR predicted protein sequences. The binding sites for purine ($^{63}$VALCVLKGGYKFFADLL$^{79}$) and 5′-phos-phoribosyl-1-pyrophosphate (PRPP) ($^{128}$KNVLIVEDIIDTGKTMQTL$^{146}$ and $^{186}$KFVVGYALDY$^{195}$) were found in esealHGPRT (Fig. 1B), whereas the domain essential for the binding of NAD and FAD ($^{56}$GGGFGGKETRST$^{67}$) was found in esealXOR (Fig. 1D).

### HGPRT and XOR mRNA expression is tissue-specific and influenced by fasting

HGPRT and XOR mRNA expression levels were measured in adipose tissue and muscle from elephant seal pups to determine tissue-specific expression and the effects of fasting. HGPRT transcripts increased ($P<0.05$) 4-fold in adipose tissue (Fig. 2A) and muscle (Fig. 2B) after 7 weeks of fasting but did not change after 3 or 5 weeks compared with week 1. XOR transcripts increased ($P<0.05$) 3-fold in adipose tissue after 7 weeks of fasting but did not change after 3 or 5 weeks compared with week 1 (Fig. 2C). In contrast, XOR transcripts decreased ($P<0.05$) 4-fold and 22-fold in muscle after 3 and 5 weeks of fasting, respectively, and increased to basal levels after 7 weeks (Fig. 2D).

### Fasting increases HGPRT and XO protein expression

HGPRT and XO protein expression was measured in adipose tissue and muscle to assess the tissue-specific relationships between changes in transcript and protein levels in response to fasting. Protein expression of HGPRT increased ($P<0.05$) approximately 2-fold in both adipose tissue (193±14%) and muscle (204±21%) after 7 weeks of fasting, without any changes after 3 (80±14% and 113±15%, respectively) or 5 weeks (88±18 and 84±11%, respectively; Fig. 3A,B). Adipose XO protein expression increased 74% (174±28%) after 7 weeks of fasting ($P<0.03$; Fig. 3C), and muscle XO expression increased 55% (155±7%) and 97% (197±36%) after 3 and 7 weeks, respectively ($P<0.03$; Fig. 3D).

### Purine metabolism increases with fasting

The circulating content of the ATP degradation products – xanthine and HX – was measured to determine the effects of prolonged fasting on purine conservation and recycling and to translate the

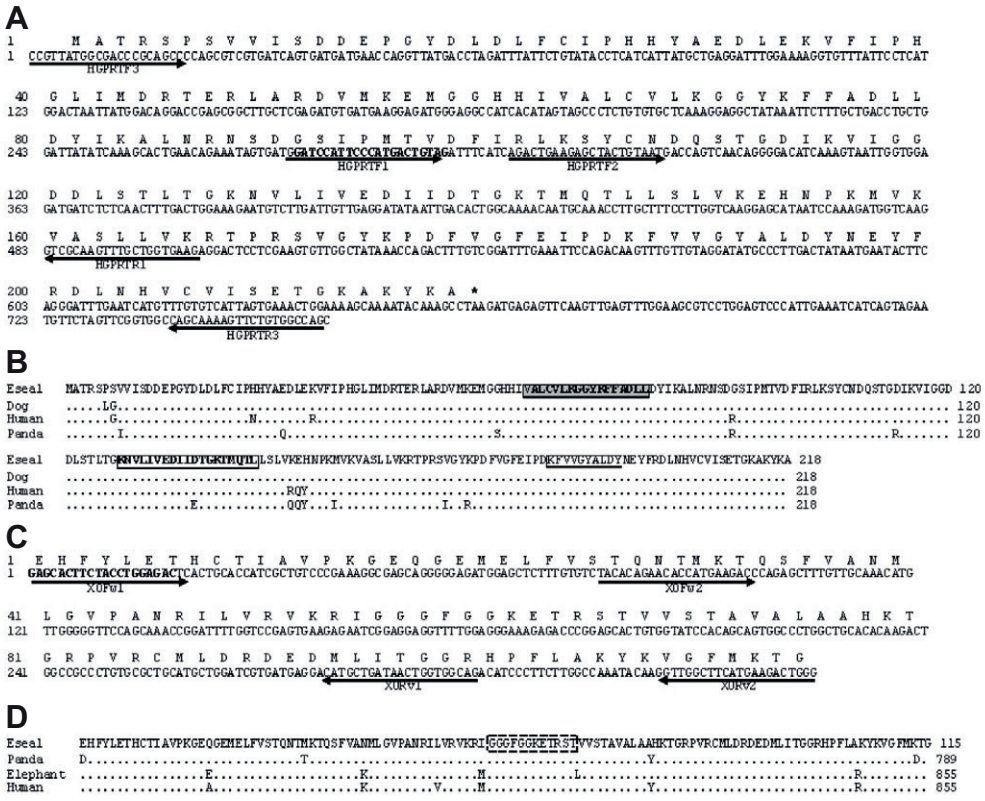


Fig. 1. (A) Nucleotide and (B) predicted amino acid sequences and multiple amino acid alignment of complete hypoxanthine-guanine phosphoribosyl transferase (HGPRT), and (C) nucleotide and (D) predicted amino acid sequences and multiple amino acid alignment of partial xanthine oxidoreductase (XOR) from the northern elephant seal, *Mirounga angustirostris*. Primer sequences for HGPRT (A) and XOR (C) are shown in bold and underlined. The amino acid positions for the different domains are indicated for HGPRT (B) and include the binding domains for purines (gray shading) and 5′-phosphoribosyl-1-pyrophosphate (PRPP) (white shading), and for XOR (D) include the NAD and FAD (dashed box) binding domain. Arrows indicate primer location and direction.

physiological relevance of the observed changes in transcript and protein expression of HGPRT and XO (Fig. 4). Plasma HX content decreased 1.6-fold after 3 weeks (3.1±0.3 μmol $l^{-1}$; $P$<0.05) and increased 2.5-fold after 5 (13.0±1.0 μmol $l^{-1}$) and 7 weeks (13.0±2.0 μmol $l^{-1}$) compared with week 1 (5.0±0.9 μmol $l^{-1}$; $P$<0.02; Fig. 4A). Plasma xanthine content decreased 1.9-fold after 3 weeks of fasting (36±4 μmol $l^{-1}$; $P$<0.05), and increased 3-fold and 2.8-fold after 5 (204±23 μmol $l^{-1}$) and 7 weeks (191±33 μmol $l^{-1}$), respectively, compared with week 1 (68±12 μmol $l^{-1}$; $P$<0.02; Fig. 4B).

Activities of plasma HGPRT and XO were measured to assess the relationship between the changes in transcript and protein expression in tissues with plasma activities during fasting. Plasma HGPRT and XO activities increased ($P$<0.05) at each week across the fasting over the 7 week measurement period (Fig. 4C,D).

## DISCUSSION

Unlike terrestrial mammals, prolonged fasting in northern elephant seals does not induce oxidative damage or inflammation despite increasing RAS, Nox4, NADPH oxidase activity, circulating

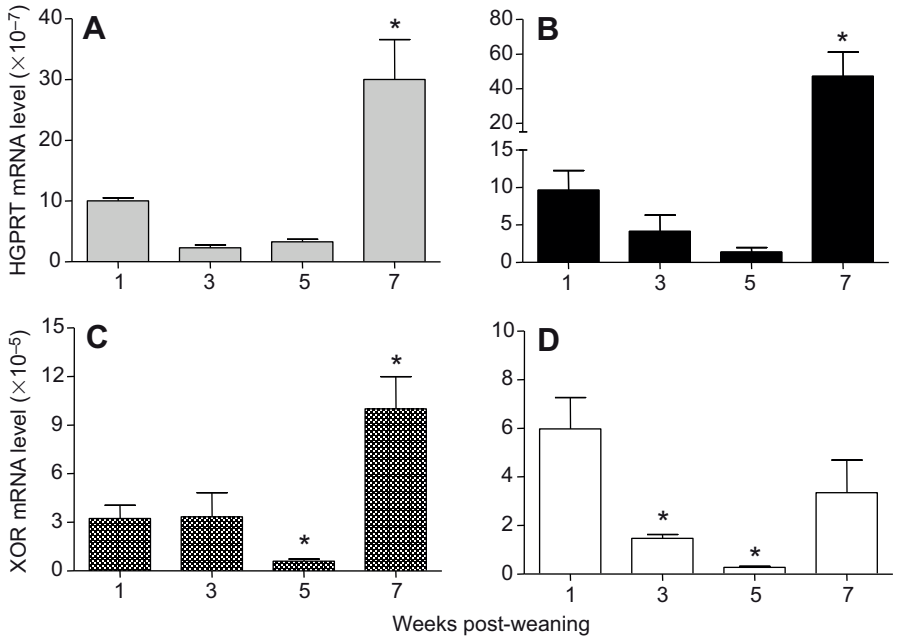


Fig. 2. Mean (±s.e.m.) mRNA levels of (A) adipose hypoxanthine-guanine phosphoribosyl transferase (HGPRT), (B) muscle HGPRT, (C) adipose xanthine oxidoreductase (XOR) and (D) muscle XOR from fasted elephant seal pups. Asterisks denote significant ($P$<0.05) differences from week 1 post-weaned values.

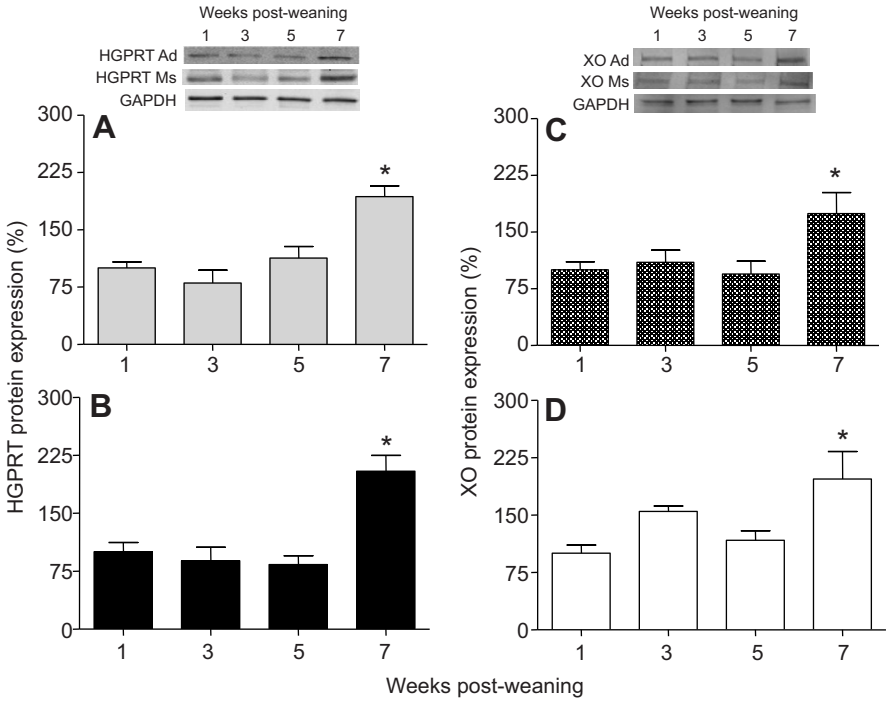


Fig. 3. Mean (±s.e.m.) protein expression of (A) adipose (Ad) hypoxanthine-guanine phosphoribosyl transferase (HGPRT), (B) muscle (Ms) HGPRT, (C) adipose xanthine oxidase (XO) and (D) muscle XO from fasted elephant seal pups. Asterisks denote significant ($P$<0.05) differences from week 1 post-weaned values. A representative western blot of HGPRT and XO expression is shown.

cortisol, non-esterified fatty acids and promoting insulin resistance (Ortiz et al., 2000; Ortiz et al., 2001; Ortiz et al., 2003; Ortiz et al., 2006; Vázquez-Medina et al., 2010; Viscarra et al., 2011a; Viscarra et al., 2011b). Unfortunately, the molecular and physiological mechanisms adopted by seals to cope with prolonged fasting are not fully elucidated. In the present study, the full-length and partial cDNA sequences of HGPRT and XO from the northern elephant seal were identified and characterized. The amino acid sequences of both proteins are nearly identical (>92% homology) to other mammalian homologs and contain conserved functional and regulatory domains, suggesting that their function and regulation are similar to those in terrestrial mammals. Moreover, HGPRT and XO mRNA and protein expression are upregulated in a tissue-specific manner during fasting, associated with parallel increases in plasma HGPRT and XO activities, and circulating HX and xanthine content, suggesting that: (1) increased expression of these proteins translates into increased enzymatic activity and thus, plasma HGPRT and XO activities may serve as surrogate measures for their tissue levels, and (2) increased purine recycling is an adaptive strategy to cope with prolonged fasting in post-weaned northern elephant seals.

The increase in XOR mRNA in adipose tissue at 7 weeks and the decrease in muscle at 3 and 5 weeks demonstrate that fasting is associated with tissue-specific changes in XOR gene expression. Moreover, the tissue-specific expression of XOR mRNA indicates the ability of these animals to base their purine metabolism through the changes on XOR gene expression during fasting. Post-transcriptional mechanisms that regulate gene expression in eukaryotes are well known and include the control of splicing, mRNA stability, localization and translation. Therefore, the lack of

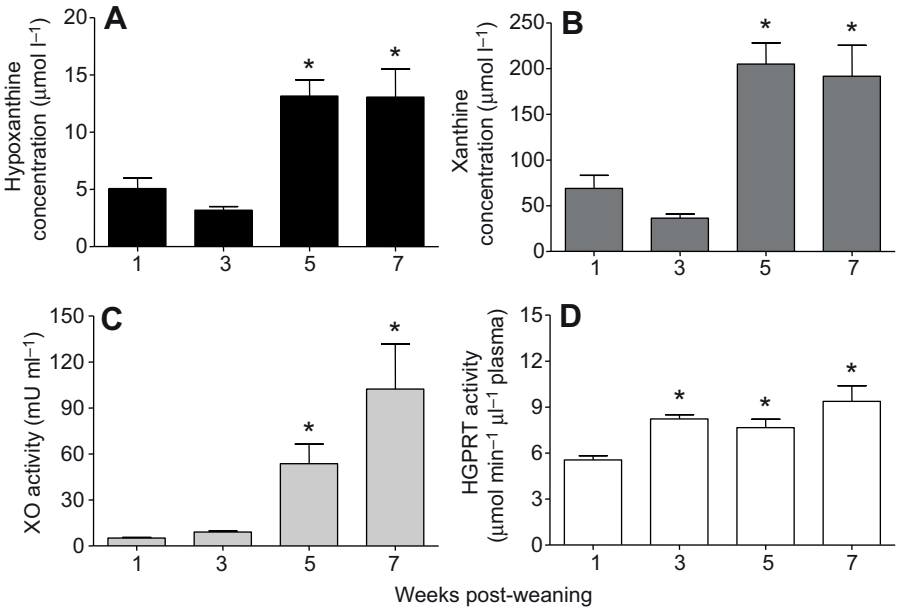


Fig. 4. Mean (±s.e.m.) (A) plasma hypoxanthine concentration, (B) plasma xanthine concentration, (C) plasma xanthine oxidase (XO) activity, and (D) plasma hypoxanthine-guanine phosphoribosyl transferase (HGPRT) activity in fasting elephant seal pups. Asterisks denote significant ($P$<0.05) differences from week 1 post-weaned values.

a correlation between the decrease of XOR mRNA after 3 and 5 weeks of fasting and the increase of XO protein expression at 5 and 7 weeks in muscle, but not in adipose tissue, suggests post-transcriptional modifications or changes in transcript stability in muscle. Several studies have demonstrated tissue-specific distribution and regulation of XOR enzyme activity, protein and mRNA in humans (Bruder et al., 1984; Cook et al., 1997; Hellsten-Westing et al., 1993; Linder et al., 1999; Moriwaki et al., 1993; Sarnesto et al., 1996). Hypoxia has been reported as the main transcriptional and post-translational regulator of XOR in several animal models. Putative binding sites for the oxygen-regulated transcription factors NF-Y, NF-κB, AP-1 and HIF-1 have been identified in the promoter for the human XOR gene (Hoidal et al., 1997; Xu et al., 1996). Interferon-γ is also a potent upregulator of XOR mRNA and activity in rat lung endothelium, in part because of increased transcription XOR (Dupont et al., 1992). In mouse fibroblast and human bronchial epithelial cells, XOR activity is transcriptionally induced by intracellular iron but post-translationally inactivated by iron chelation (Martelin et al., 2002). Also, angiotensin II (Ang II) induces XO activation in cultured endothelial cells (Landmesser et al., 2007). Plasma Ang II and other RAS components increase with fasting in elephant seals (Ortiz et al., 2006; Ortiz et al., 2001), suggesting that the increases in XO protein expression and plasma XO activity may be induced by increased Ang II.

The observed increases in xanthine and HX in fasting pups are also consistent with those demonstrated in fasted Arabian camels (*Camelus dromedaries*) (Mohamed, 2006; Mura et al., 1986), hibernating mammals (Kennaway, 1911; Mura et al., 1986), rats (Gross et al., 1988), buffalo (*Bubalis bubalis*) (Chen et al., 1996) and llamas (*Lama glama* and *L. guanaco*) (Bakker et al., 1996). During food deprivation and hypoxic conditions in mammals, ATP degradation is increased, purine catabolism is overloaded and degradation products are accumulated (Becker, 2001; Raivio et al., 2001; Stone and Simmonds, 1991). During the post-weaning fast, fatty acid oxidation is the primary source of energy, contributing to as much as 95% of the elephant seal's metabolic rate (Adams and Costa, 1993; Castellini et al., 1987; Ortiz et al., 1978), whereas glucose and protein catabolism contribute 10% at most (Keith, 1984; Pernia et al., 1980). Protein catabolism also decreases with fasting progression (Adams and Costa, 1993; Houser and Costa, 2001; Pernia et al., 1980) while blood glucose levels remain high (Costa and Ortiz, 1982; Ortiz et al., 2003), likely *via* a mechanism involving the Cori cycle (Champagne et al., 2005). Decreased purine synthesis has been demonstrated during amino acid starvation in human lymphoblasts (Boss and Erbe, 1982; Bowen et al., 1987). Dietary ingestion and *de novo* synthesis of purines contribute to maintain the body pool stimulating, ATP synthesis. Because the energetic cost of synthesizing a purine nucleotide *de novo* is six times higher (in ATP equivalents) than that of recycling an intact purine ring, cells have efficient mechanisms for the salvage of degraded purines (Becker, 2001; Raivio et al., 2001; Stone and Simmonds, 1991). Plasma uric acid, the end-product of purine metabolism, has previously been reported to remain relatively low and unchanged throughout the fast in weaned pups (Costa and Ortiz, 1982; Adams and Costa, 1993), providing further evidence that purine recycling is increased. The observed increase in HGPRT mRNA, protein and activity along with the increased levels of plasma xanthine and HX suggest that purine recycling enhances the supply of ATP to match the energetic demands, allowing pups to sustain metabolism throughout the duration of the post-weaning fast.

The progression of fasting in elephant seals is also characterized by the increase in number and duration of sleep apneas (breath-holding episodes) that normally last between 8 and 12 min and constitute 80% of the seals' time on land (Blackwell and Le Boeuf, 1993; Castellini et al., 1988; Castellini et al., 1994). In addition, the post-weaning fast is associated with dramatic increases in time spent submerged in nearshore waters. By week seven, pups spend an average of 12 h per day in the water, exhibiting submergences similar in duration to the terrestrial apneas while swimming (Thorson and Le Boeuf, 1994). Cardiovascular adjustments during apnea in seals result in ischemia to peripheral tissues, which can induce tissue hypoxia (Ponganis et al., 2006; Ponganis et al., 2008; Stockard et al., 2007). The increase in the number and duration of sleep apneas is associated with an increase in antioxidants (Vázquez-Medina et al., 2010; Vázquez-Medina et al., 2011b) over the course of the post-weaning fast. Furthermore, it was recently demonstrated that plasma XO activity, HX and xanthine content increase in elephant seal pups in response to rest- and voluntary submersion-associated apneas (Vázquez-Medina et al., 2011c). Therefore, fasting and sleep apnea may stimulate ATP degradation, increasing intracellular and circulating oxidant production, potentially modulated by increased purine salvage/recycling pathways (Elsner et al., 1998; Zenteno-Savín et al., 2002).

In summary, the results demonstrate that prolonged fasting upregulates XOR gene expression in a tissue-specific manner and increases purine catabolism in elephant seal pups. The increase in HGPRT mRNA and protein expression in adipose tissue and muscle along with the increase in plasma HGPRT activity during prolonged fasting suggests that fasting seals possess a high capacity for purine salvage/recycling and that the increase in purine recycling contributes to ATP supply and the amelioration of oxidant production. The timing of the events observed in late fasting (week 7) may also be associated with an increase in number and duration of sleep apneas. Thus, increased HGPRT-mediated purine recycling and increased antioxidant enzyme activities and protein content (Vazquez-Medina et al., 2011a; Vazquez-Medina et al., 2011b) provide two of the principal cellular mechanisms that elephant seals have evolved to tolerate potentially deleterious consequences of their naturally adapted behaviors, such as prolonged fasting and sleep-induced apnea.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| $H_2O_2$ | hydrogen peroxide |
| HGPRT | hypoxanthine-guanine phosphoribosyl transferase |
| HX | hypoxanthine |
| RAS | renin-angiotensin system |
| $O_2^{\bullet-}$ | superoxide radical |
| XDH | xanthine dehydrogenase |
| XO | xanthine oxidase |
| XOR | xanthine oxidoreductase |

## ACKNOWLEDGEMENTS

We thank M. Tift, J. Viscarra and R. Rodriguez for their help with animal handling.

## FUNDING

J.G.S.-O. was supported by a postdoctoral fellowship from the University of California Institute for Mexico and the United States and Mexico's National Council for Science and Technology (UC MEXUS-CONACYT). J.P.V.-M. was supported by fellowships from UC MEXUS-CONACYT and the University of California. R.M.O. was partially supported by a Career Development Award from the National Institutes of Health National Heart, Lung and Blood Institute (NIH NHLBI) (K02HL103787). Research was funded by grants from NIH NHLBI (R01HL91767 to R.M.O.) and UC-MEXUS-CONACYT (to R.M.O. and T.Z.-S.). Deposited in PMC for release after 12 months.

## REFERENCES


**Adams, S. H. and Costa, D. P.** (1993). Water conservation and protein metabolism in northern elephant seal pups during the postweaning fast. *J. Comp. Physiol.* **163B**, 367-373.

**Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J.** (1990). Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410.

**Bakker, M. L., Chen, X. B., Kyle, D. J., Orskov, E. R. and Bourke, D. A.** (1996). Urinary and plasma purine derivatives in fed and fasted llamas (*Lama glama* and *L. guanacoe*). *Comp. Biochem. Physiol.* **113B**, 367-374.

**Becker, M. A.** (2001). Hyperuricemia and gout. In *The Metabolic & Molecular Bases of Inherited Disease*, Vol. 2 (ed. C. R. Scriver, A. L. Beaudet, W. S. Sly and D. Valle), pp. 2513-2535. New York: McGraw-Hill.

**Blackwell, S. B. and Le Boeuf, B. J.** (1993). Developmental aspects of sleep apnoea in northern elephant seals, *Mirounga angustirostris*. *J. Zool.* **231**, 437-447.

**Boss, G. R. and Erbe, R. W.** (1982). Decreased purine synthesis during amino acid starvation of human lymphoblasts. *J. Biol. Chem.* **257**, 4242-4247.

**Bowen, W. D., Boness, D. J. and Oftedal, O. T.** (1987). Mass transfer from mother to pup and subsequent mass loss by the weaned pup in the hooded seal, *Cystophora cristata*. *Can. J. Zool.* **65**, 1-8.

**Brault, J. J. and Terjung, R. L.** (2001). Purine salvage to adenine nucleotides in different skeletal muscle fiber types. *J. Appl. Physiol.* **91**, 231-238.

**Bray, R. C.** (1975). Molybdenum iron-sulfur flavin hydroxylases and related enzymes. In *The Enzymes* (ed. P. D. Boyer), pp. 299-419. New York, NY: Academic Press.

**Bruder, G., Jarasch, E. D. and Heid, H. W.** (1984). High concentrations of antibodies to xanthine oxidase in human and animal sera. Molecular characterization. *J. Clin. Invest.* **74**, 783-794.

**Castellini, M. A., Costa, D. P. and Huntley, A. C.** (1987). Fatty acid metabolism in fasting elephant seal pups. *J. Comp. Physiol.* **157B**, 445-449.

**Castellini, M. A., Davis, R. W. and Kooyman, G. L.** (1988). Blood chemistry regulation during repetitive diving in Weddell seals. *Physiol. Zool.* **61**, 379-386.

**Castellini, M. A., Milsom, W. K., Berger, R. J., Costa, D. P., Jones, D. R., Castellini, J. M., Rea, L. D., Bharma, S. and Harris, M.** (1994). Patterns of respiration and heart rate during wakefulness and sleep in elephant seal pups. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **266**, R863-R869.

**Champagne, C. D., Houser, D. S. and Crocker, D. E.** (2005). Glucose production and substrate cycle activity in a fasting adapted animal, the northern elephant seal. *J. Exp. Biol.* **208**, 859-868.

**Chen, X. B., Samaraweera, L., Kyle, D. J., Orskov, E. R. and Abeygunawardene, H.** (1996). Urinary excretion of purine derivatives and tissue xanthine oxidase (EC 1.2.3.2) activity in buffaloes (*Bubalis bubalis*) with special reference to differences between buffaloes and *Bos taurus* cattle. *Br. J. Nutr.* **75**, 397-407.

**Cherin, T., Catbagan, M., Treiman, S. and Mink, R.** (2006). The effect of normothermic and hypothermic hypoxia-ischemia on brain hypoxanthine phosphoribosyl transferase activity. *Neurol. Res.* **28**, 831-836.

**Cook, W., Chu, R., Saksela, M., Raivio, K. and Yeldandi, A.** (1997). Differential immunohistochemical localization of xanthine oxidase in normal and neoplastic human breast epithelium. *Int. J. Oncol.* **11**, 1013-1017.

**Costa, D. P. and Ortiz, C. L.** (1982). Blood chemistry homeostasis during prolonged fasting in the northern elephant seal. *Am. J. Physiol.* **242**, R591-R595.

**Crescimanno, M., Armata, M. G., Rausa, L., Gueli, M. C., Nicotra, C. and D'Alessandro, N.** (1989). Cardiac peroxisomal enzymes and starvation. *Free Radic. Res.* **7**, 67-72.

**Decking, U. K., Schlieper, G., Kroll, K. and Schrader, J.** (1997). Hypoxia-induced inhibition of adenosine kinase potentiates cardiac adenosine release. *Circ. Res.* **81**, 154-164.

**Deussen, A., Bading, B., Kelm, M. and Schrader, J.** (1993). Formation and salvage of adenosine by macrovascular endothelial cells. *Am. J. Physiol.* **264**, H692-H700.

**Di Simplicio, P., Rossi, R., Falcinelli, S., Ceserani, R. and Formento, M. L.** (1997). Antioxidant status in various tissues of the mouse after fasting and swimming stress. *Eur. J. Appl. Physiol. Occup. Physiol.* **76**, 302-307.

**Dupont, G. P., Hueckstead, T. P., Marshall, B. C., Ryan, U. S., Michael, J. R. and Hoidal, J. R.** (1992). Regulation of xanthine dehydrogenase and xanthine oxidase activity and gene expression in cultured rat pulmonary endothelial cells. *J. Clin. Invest.* **89**, 197-202.

**Elsner, R.** (1999). Living in water: solutions to physiological problems. In *Biology of Marine Mammals* (ed. J. E. I. Reynolds and S. A. Rommel), pp. 73-116. Washington, DC: Smithsonian Institution Press.

**Elsner, R., Oyaster, S., Saugstad, O. D. and Blix, A. S.** (1995). Seal adaptations for long dives: recent studies of ischemia and oxygen radicals. In *Developments in Marine Biology* (ed. A. S. Blix, L. Walløe and Ø. Ulltang). *Whales, Seals, Fish and Man – Proceedings of the International Symposium on the Biology of Marine Mammals in the Northeast Atlantic*, Vol. 4, pp. 371-376. Amsterdam: Elsevier.

**Elsner, R., Oyaster, S., Almaas, R. and Saugstad, O. D.** (1998). Diving seals, ischemia-reperfusion and oxygen radicals. *Comp. Biochem. Physiol.* **119A**, 975-980.

**Elson, D.** (1965). Metabolism of nucleic acids (macromolecular DNA and RNA). *Annu. Rev. Biochem.* **34**, 449-486.

**Grattagliano, I., Vendemiale, G., Caraceni, P., Domenicali, M., Nardo, B., Cavallari, A., Trevisani, F., Bernardi, M. and Altomare, E.** (2000). Starvation impairs antioxidant defense in fatty livers of rats fed a choline-deficient diet. *J. Nutr.* **130**, 2131-2136.

**Gross, C. J., Stiles, J. E. and Savaiano, D. A.** (1988). Effect of nutritional state and allopurinol on purine metabolism in the rat small intestine. *Biochim. Biophys. Acta* **966**, 168-175.

**Hellsten-Westing, Y., Balsom, P. D., Norman, B. and Sjodin, B.** (1993). The effect of high-intensity training on purine metabolism in man. *Acta Physiol. Scand.* **149**, 405-412.

**Hille, R. and Nishino, T.** (1995). Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. *FASEB J.* **9**, 995-1003.

**Hoidal, J. R., Xu, P., Hueckstead, T., Sanders, K. A. and Pfeffer, K.** (1997). Transcriptional regulation of human xanthine dehydrogenase/xanthine oxidase. *Biochem. Soc. Trans.* **25**, 796-799.

**Houser, D. S. and Costa, D. P.** (2001). Protein catabolism in suckling and fasting northern elephant seal pups (*Mirounga angustirostris*). *J. Comp. Physiol.* **171B**, 635-642.

**Keith, E. O.** (1984). *Glucose Metabolism in Fasting Northern Elephant Seal Pups*. PhD thesis, University of California, Santa Cruz, CA, USA.

**Kennaway, E. L.** (1911). A note on purine metabolism in hibernating animals. *Biochem. J.* **5**, 188-192.

**Kondoh, M., Kamada, K., Kuronaga, M., Higashimoto, M., Takiguchi, M., Watanabe, Y. and Sato, M.** (2003). Antioxidant property of metallothionein in fasted mice. *Toxicol. Lett.* **143**, 301-306.

**Krenitsky, T. A., Papioannou, R. and Elion, G. B.** (1969). Human hypoxanthine phos phoribosyltransferase. I. Purification, properties and specificity. *J. Biol. Chem.* **244**, 1263-1277.

**Landmesser, U., Spiekermann, S., Preuss, C., Sorrentino, S., Fischer, D., Manes, C., Mueller, M. and Drexler, H.** (2007). Angiotensin II induces endothelial xanthine oxidase activation: role for endothelial dysfunction in patients with coronary disease. *Arterioscler. Thromb. Vasc. Biol.* **27**, 943-948.

**Le Boeuf, B. J., Whiting, R. J. and Gantt, R. F.** (1972). Perinatal behavior of northern elephant seal females and their young. *Behaviour* **43**, 121-156.

**Linder, N., Rapola, J. and Raivio, K. O.** (1999). Cellular expression of xanthine oxidoreductase protein in normal human tissues. *Lab. Invest.* **79**, 967-974.

**Martelin, E., Lapatto, R. and Raivio, K. O.** (2002). Regulation of xanthine oxidoreductase by intracellular iron. *Am. J. Physiol. Cell Physiol.* **283**, C1722-C1728.

**Martensson, J.** (1986). The effect of fasting on leukocyte and plasma glutathione and sulfur amino acid concentrations. *Metabolism* **35**, 118-121.

**McCord, J.** (1985). Oxygen-derived free radicals in postischemic tissue injury. *N. Engl. J. Med.* **312**, 159-163.

**Meir, J. U., Champagne, C. D., Costa, D. P., Williams, C. L. and Ponganis, P. J.** (2009). Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R927.

**Mohamed, H. E.** (2006). Purine derivatives in the plasma and urine and tissue xanthine oxidase (XO) in Sudanese camels (*Camelus dromedarius*). *J. Anim. Veter. Adv.* **5**, 310-312.

**Moriwaki, Y., Yamamoto, T., Suda, M., Nasako, Y., Takahashi, S., Agbedana, O. E., Hada, T. and Higashino, K.** (1993). Purification and immunohistochemical tissue localization of human xanthine oxidase. *Biochim. Biophys. Acta* **1164**, 327-330.

**Mura, U., Osman, A. M., Mohamed, A. S. and Ipata, P. L.** (1986). Studies on purine turnover in the camel (*Camelus dromedarius*) and zebu (*Bos indicus*). *Comp. Biochem. Physiol.* **84B**, 589-593.

**Ortiz, C. L., Costa, D. and Le Boeuf, B. J.** (1978). Water and energy flux in elephant seal pups fasting under natural conditions. *Physiol. Zool.* **51**, 166-178.

**Ortiz, R. M., Wade, C. E. and Ortiz, C. L.** (2000). Prolonged fasting increases the response of the renin-angiotensin-aldosterone system, but not vasopressin levels, in postweaned northern elephant seal pups. *Gen. Comp. Endocrinol.* **119**, 217-223.

**Ortiz, R. M., Wade, C. E. and Ortiz, C. L.** (2001). Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R790-R795.

**Ortiz, R. M., Wade, C. E., Costa, D. P. and Ortiz, C. L.** (2002). Renal responses to plasma volume expansion and hyperosmolality in fasting seal pups. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, R805-R817.

**Ortiz, R. M., Noren, D. P., Ortiz, C. L. and Talamantes, F.** (2003). GH and ghrelin increase with fasting in a naturally adapted species, the northern elephant seal (*Mirounga angustirostris*). *J. Endocrinol.* **178**, 533-539.

**Ortiz, R. M., Crocker, D. E., Houser, D. S. and Webb, P. M.** (2006). Angiotensin II and aldosterone increase with fasting in breeding adult male northern elephant seals (*Mirounga angustirostris*). *Physiol. Biochem. Zool.* **79**, 1106-1112.

**Parks, D., Bulkley, G. and Granger, D.** (1983). Role of oxygen-derived free radicals in digestive tract diseases. *Surgery* **94**, 415-422.

**Pernia, S. D., Hill, A. and Ortiz, C. L.** (1980). Urea turnover during prolonged fasting in the northern elephant seal. *Comp. Biochem. Physiol.* **65B**, 731-734.

**Ponganis, P., Stockard, T., Levenson, D., Berg, L. and Baranov, E.** (2006). Cardiac output and muscle blood flow during rest-associated apneas of elephant seals. *Comp. Biochem. Physiol.* **144B**, 105-111.

**Ponganis, P. J., Kreutzer, U., Stockard, T. K., Lin, P. C., Sailasuta, N., Tran, T. K., Hurd, R. and Jue, T.** (2008). Blood flow and metabolic regulation in seal muscle during apnea. *J. Exp. Biol.* **211**, 3323-3332.

**Raivio, K., Saksela, M. and Lapatto, R.** (2001). *Xanthine Oxidoreductase – Role in Human Pathophysiology and in Hereditary Xanthinuria*. New York: McGraw Hill.

**Rozen, S. and Skaletsky, H.** (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* **132**, 365-386.

**Sambrook, J. and Russell, D. W.** (2001). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

**Sarnesto, A., Linder, N. and Raivio, K. O.** (1996). Organ distribution and molecular forms of human xanthine dehydrogenase/xanthine oxidase protein. *Lab. Invest.* **74**, 48-56.

**Sorensen, M., Sanz, A., Gomez, J., Pamplona, R., Portero-Otin, M., Gredilla, R. and Barja, G.** (2006). Effects of fasting on oxidative stress in rat liver mitochondria. *Free Radic. Res.* **40**, 339-347.

**Souza Rocha, G., Fonseca, A. S., Rodrigues, M. P., Dantas, F. J. S., Caldeira-de-Araujo, A. and Santos, R.** (2008). Comet assay to determine DNA damage induced by food deprivation in rats. *Acta Biol. Hung.* **59**, 315-325.

**Stockard, T., Levenson, D., Berg, L., Fransioli, J., Baranov, E. and Ponganis, P.** (2007). Blood oxygen depletion during rest-associated apneas of northern elephant seals (*Mirounga angustirostris*). *J. Exp. Biol.* **210**, 2607-2617.

**Stone, T. W. and Simmonds, H. A.** (1991). *Purines: Basic and Clinical Aspects*. Dordrecht: Kluwer Academic Publishers.

**Thompson, J. D., Higgins, D. G. and Gibson, T. J.** (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673-4680.

**Thorson, P. H. and Le Boeuf, B. J.** (1994). Developmental aspects of diving in northern elephant seal pups. In *Elephant Seals: Population Ecology, Behavior, and Physiology* (ed. B. J. Le Boeuf and R. M. Laws), pp. 271-289. Berkeley, CA: University of California Press.

**Vázquez-Medina, J. P., Crocker, D. E., Forman, H. J. and Ortiz, R. M.** (2010). Prolonged fasting does not increase oxidative damage or inflammation in postweaned northern elephant seal pups. *J. Exp. Biol.* **213**, 2524-2530.

**Vázquez-Medina, J. P., Zenteno-Savín, T., Crocker, D. E., Forman, H. J. and Ortiz, R. M.** (2011a). Prolonged fasting increases glutathione biosynthesis in postweaned northern elephant seals. *J. Exp. Biol.* **214**, 1294-1299.

**Vázquez-Medina, J. P., Soñanez-Organis, J. G., Burns, J. M., Zenteno-Savín, T. and Ortiz, R. M.** (2011b). Antioxidant capacity develops with maturation in the deep diving hooded seal. *J. Exp. Biol.* **214**, 2903-2910.

**Vázquez-Medina, J. P., Zenteno-Savín, T., Tift, M. S., Forman, H. J., Crocker, D. E. and Ortiz, R. M.** (2011c). Apnea stimulates the adaptive response to oxidative stress in elephant seal pups. *J. Exp. Biol.* **214**, 4193-4200.

**Verma, S. and Eckstein, F.** (1998). Modified oligonucleotides: synthesis and strategy for users. *Annu. Rev. Biochem.* **67**, 99-134.

**Viscarra, J. A., Champagne, C. D., Crocker, D. E. and Ortiz, R. M.** (2011a). 5′AMP-activated protein kinase activity is increased in adipose tissue of northern elephant seal pups during prolonged fasting-induced insulin resistance *J. Endocrinol.* **209**, 317-325.

**Viscarra, J. A., Vázquez-Medina, J. P., Crocker, D. E. and Ortiz, R. M.** (2011b). Glut4 is upregulated despite decreased insulin signaling during prolonged fasting in northern elephant seal pups. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **300**, R150-R154.

**Vorbach, C., Harrison, R. and Capecchi, M. R.** (2003). Xanthine oxidoreductase is central to the evolution and function of the innate immune system. *Trends Immunol.* **24**, 512-517.

**Wu, G., Brouckaert, P. and Olivecrona, T.** (2004). Rapid downregulation of adipose tissue lipoprotein lipase activity on food deprivation: evidence that TNF-α is involved. *Am. J. Physiol. Endocrinol. Metab.* **286**, E711.

**Xu, P., Hueckstedt, T. P. and Hoidal, J. R.** (1996). Molecular cloning and characterization of the human xanthine dehydrogenase gene (XDH). *Genomics* **34**, 173-180.

**Zenteno-Savín, T., Clayton-Hernandez, E. and Elsner, R.** (2002). Diving seals: are they a model for coping with oxidative stress? *Comp. Biochem. Physiol.* **133C**, 527-536.