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# Body water handling in response to hypertonic-saline induced diuresis in fasting northern elephant seal pups (*Mirounga angustirostris*)

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## Abstract

During natural fasting conditions in postweaned northern elephant seal (NES) (*Mirounga angustirostris*) pups, urinary water loss is minimized and percent total body water (TBW) is maintained constant. However, following infusion of hypertonic saline, glomerular filtration rate (GFR) and urine output increased in fasting pups. Therefore, we quantified the magnitude of the hypernatremia-induced diuresis relative to the animal's total body water (TBW) pool and the percentage of filtered water reabsorbed. Following a 24 h control period, naturally fasting NES pups ( $n=7$ ) were infused ( $4 \text{ ml min}^{-1}$ ) with hypertonic saline (16.7%) at a dose of  $3 \text{ mmol NaCl kg}^{-1}$  body mass. Total body water was estimated prior to infusion by tritium dilution, GFR was estimated by standard creatinine clearance, and urine output (V) was measured for 24 h during the control and post infusion periods. Percentage of filtered water reabsorbed was calculated as  $(1 - (V/\text{GFR})) \times 100$ . Twenty-four hours following the infusion, GFR (control:  $69 \pm 12 \text{ ml min}^{-1}$  and post-infusion:  $118 \pm 19 \text{ ml min}^{-1}$ ; mean  $\pm$  S.E.) increased  $77 \pm 28\%$  above control and the percentage of filtered water reabsorbed was decreased  $0.4 \pm 0.1\%$ . The increase in urine output (control:  $218 \pm 47 \text{ ml d}^{-1}$  and post-infusion:  $883 \pm 92 \text{ ml d}^{-1}$ ) accounted for  $1.7 \pm 0.2\%$  of the pups' TBW. The hypernatremia-induced diuresis was accompanied by the loss of body water indicating the lack of water retention. Although the 77% increase in GFR was only associated with a 0.4% decrease in the percentage of filtered water reabsorbed, this decrease was significant enough to result in a 4-fold increase in urine output. Despite the observed diuresis, fasting NES pups appear to possess an efficient water recycling mechanism requiring only a small percentage of body water to excrete an excess salt load. This water recycling mechanism may allow pups to avoid negative perturbations in body water as they initiate feeding in a marine environment following the fast.

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## 1. Introduction

Pups of the northern elephant seal (*Mirounga angustirostris*) (NES) naturally fast for 8–12 weeks postweaning. Although the pups begin venturing into nearby shallow waters within the sec-

ond or third week of their postweaning fast, ingestion of water is undetectable suggesting the lack of drinking (Ortiz et al., 1978). During this fasting period, water balance is maintained by the production of metabolically derived water from the oxidation of their large fat stores (Ortiz et al., 1978). However, in order for fluid and electrolyte homeostasis to be achieved during the postweaning

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fast, mechanisms must exist to conserve the water produced from the metabolism of fat as well as the circulating electrolytes. Among the renal mechanisms employed by fasting pups to conserve body water are a reduction in GFR and excreted urea (Adams and Costa, 1993), and an increase in urine osmolality (Ortiz et al., 1996). Collectively, these mechanisms result in a decrease in urinary water loss (Adams and Costa, 1993; Ortiz et al., 1996). Increasing tubular reabsorption of electrolytes results in a reduction in excreted  $\text{Na}^+$  and  $\text{K}^+$ , which allows fasting pups to maintain electrolyte homeostasis (Ortiz et al., 2000). Clearly, fasting pups have well-developed renal conservatory mechanisms that allow them to withstand a prolonged period of fasting without exhibiting any deleterious effects on water and electrolyte homeostasis.

However, renal excretory mechanisms in fasting pups by employing infusion techniques have only recently been examined (Ortiz et al., 2002a,b). Pups infused with  $\text{Na}^+$  in an isotonic solution exhibited an increase in urine output, but only 30% of the water and 20% of the salts were excreted within 24 h of the infusion indicating a state of water and electrolyte retention (Ortiz et al., 2002b). However, an equimolar amount of  $\text{Na}^+$  presented as a hypertonic solution induced a diuresis, which was greater than the volume of water infused (Ortiz et al., 2002b) indicating a loss of body water. In humans, an acute infusion of hypertonic saline (Kimura et al., 1986) or increased dietary- $\text{Na}^+$  intake (Heer et al., 2000) results in a decrease or no change in urine output, usually associated with no change in glomerular filtration rate (GFR) (Luft et al., 1983). Despite the lack of an increase in GFR and urine output, urinary excretion of  $\text{Na}^+$  may remain elevated for days (Drummer et al., 1992). In dogs and swine, hypertonic saline infusions may induce an acute (<30 min) increase in urine volume, which may (Sandgaard et al., 2000) or may not be associated with an increase in GFR (Sondeen et al., 1990).

Therefore, the present study was conducted in conjunction with our previous study (Ortiz et al., 2002b) in order to quantify the magnitude of the hypernatremia-induced diuresis relative to the animal's total body water (TBW) pool and to determine if this diuresis was associated with a reduction in the percentage of filtered water reabsorbed.

## 2. Methods

All methods were reviewed and permitted by the University of California, Santa Cruz Chancellor's Animal Care Committee.

### 2.1. Animals and catheterization procedures

Seven (4 males, 3 females) pups ( $109 \pm 14$  kg; 90–135 kg; approx. 8–11 weeks old; 4–7 weeks postweaning) were captured at Año Nuevo State Park and transported to Long Marine Lab, University of California, Santa Cruz. Upon arrival at the marine lab, pups were weighed using a hanging-load cell and placed in a sand pit. The following morning, a pup was sedated with 1 mg tiletamine HCl and zolazepam HCl (Telazol; Fort Dodge Animal Health, Fort Dodge, IA) per kg body mass and a catheter was inserted into the extradural spinal-vein. Immediately following the catheterization and every day until the catheter was removed, each pup received a prophylactic dose of one-gram cefazolin sodium (Fort Dodge Animal Health, Fort Dodge, IA). The catheterized pup was then placed in a metabolic cage with a urine collection pan underneath attached to a collection flask. Urine was collected over two 24-h periods (control and post infusion) and daily urine output (V) was recorded following each period. Prior to the initiation of the control period each pup was allowed to recover from the catheterization procedure for 20–23 h in the metabolic cage.

### 2.2. Estimate of total body water

Total body water was measured during the control period prior to infusion of  $\text{Na}^+$ . After catheterization each pup received 0.3 mCi of tritiated water (HTO) in 3 ml sterile saline to estimate TBW. The catheter line was flushed once with 20 ml of sterile saline to insure complete delivery of HTO. Calculation of isotopic dilution space was similar to that previously determined for NES pups (Ortiz et al., 1978) using an over-estimation correction of 4% in the determination of TBW (Nagy and Costa, 1980).

### 2.3. Sodium infusion

The morning following the control period, pups were infused with sterile hypertonic saline (16.7%) at a dose of 1 ml kg body mass<sup>-1</sup> (3 mmol NaCl

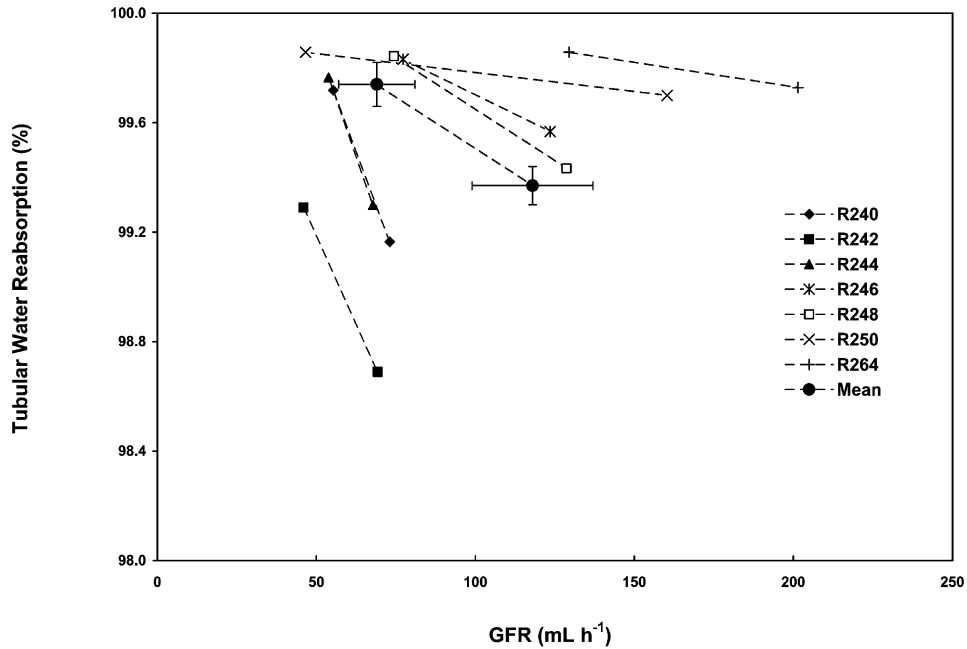


Fig. 1. Relationship between glomerular filtration rate (GFR) and percentage of filtered water reabsorbed demonstrates that efficiency of reabsorption decreases as GFR increases for each individual, fasting northern elephant seal pup. For each relationship the symbol on the left represents control and the symbol to the right represents post-hypertonic saline (16.7%) infusion. Mean ( $\pm$ S.E.) for control and post-infusion periods is represented by the sole large symbol.

kg<sup>-1</sup>) as described previously (Ortiz et al., 2002b). Warmed (35 °C) saline was drawn into sterilized 60 ml syringes and infused at a rate of 4 ml min<sup>-1</sup>.

#### 2.4. Analyses and calculations

For the present study, blood samples were collected prior to the control period (Pre-control), 24 h following (Post-control), which was the same sample immediately prior to the infusion (Pre-infusion), and 24 h after infusion (Post-infusion). Plasma and urine were analyzed for creatinine. Creatinine concentrations were measured colorimetrically using a clinical autoanalyzer (Roche Diagnostics, Somerville, NJ). Glomerular filtration rate (GFR) was estimated by standard creatinine clearance method. Percentage of filtered water reabsorbed was calculated as  $(1 - (V/GFR)) \times 100$ .

#### 2.5. Statistics

Means ( $\pm$ S.E.) were compared by paired *t*-test and considered significantly different at  $P < 0.05$ .

Statistical analyses were made using Statview (SAS, 1998).

### 3. Results

Infusion of hypertonic saline induced a  $77 \pm 28\%$  increase ( $P < 0.001$ ) in GFR above control ( $69 \pm 12$  and  $118 \pm 19$  ml min<sup>-1</sup>) associated with a reduction in plasma creatinine ( $100 \pm 5$  and  $89 \pm 5$  mM) ( $P < 0.05$ ). Following the infusion, urine output increased 4-fold ( $218 \pm 47$  to  $883 \pm 92$  ml d<sup>-1</sup>) ( $P < 0.0001$ ). The observed diuresis occurred at the expense of  $1.70 \pm 0.19\%$  of estimated total body water ( $42.8 \pm 2.5$  l or  $38.1 \pm 1.8\%$  of body mass). Percentage of filtered water reabsorbed was significantly reduced by  $0.37 \pm 0.07\%$  ( $P < 0.01$ ) between control ( $99.74 \pm 0.08\%$ ) and post-infusion ( $99.37 \pm 0.07\%$ ) periods. Each pup exhibited an increase in GFR associated with a decrease in percentage of filtered water reabsorbed (Fig. 1).

### 4. Discussion

Northern elephant seal pups have evolved a number of robust physiological mechanisms to

Table 1  
Summary of plasma osmolalities and electrolytes, glomerular filtration rate (GFR), and excreted solutes, electrolytes, and urine during control and post-infusion periods

	Control	<sup>a</sup> Post-infusion
Plasma		
Osmolality (mOsm l <sup>-1</sup> )	304	312
Na <sup>+</sup> (mM)	147	145
Cl <sup>-</sup> (mM)	107	103
GFR (l d <sup>-1</sup> )	99	170
Excreted		
Solutes (mOsm d <sup>-1</sup> )	247	862
Na <sup>+</sup> (mmol d <sup>-1</sup> )	36	331
Cl <sup>-</sup> (mmol d <sup>-1</sup> )	31	319
Urine (ml d <sup>-1</sup> )	218	883

<sup>a</sup> Values presented are 24 h post-infusion.

Values amended from Ortiz et al., 2002b.

conserve Na<sup>+</sup> while the present study demonstrates that they have also evolved a unique mechanism for handling body water in response to a hypertonic-associated Na<sup>+</sup> load. Despite a fasting state in which exogenous water is not available, pups excrete excess Na<sup>+</sup> rapidly and efficiently (Ortiz et al., 2002b) at the expense of only a small percentage of their total body water. Clearly, kidneys of NES pups are well developed by the start of their postweaning fast and are capable of extreme Na<sup>+</sup> conservation and excretion depending on the circumstance.

Increased Na<sup>+</sup> intake may lead to an increase in the retention of body water and a decrease in urine output as a means to avoid or abate dehydration (Krieger et al., 1990) with excess salt excreted in days (Drummer et al., 1992). The hypertonic salt load administered in the present study induced a level of Na<sup>+</sup> excretion equivalent to approximately 10 times the basal urinary excretion of Na<sup>+</sup> (Table 1). However, urinary water loss only increased by 4 times the control level indicating a disproportional Na<sup>+</sup> load-to-water loss ratio favoring Na<sup>+</sup> excretion. Although the 4-fold increase in urine output was statistically significant, this increase accounted for less than 2% of the pups' total body water pool suggesting that the net loss of water to accomplish this diuresis was probably not physiologically significant. The body water required to excrete this Na<sup>+</sup> load could have been replenished by the subsequent oxidation of only 600–700 g of fat (approx. 1% of estimated body fat). Dogs provided with a high-Na<sup>+</sup> diet that was more than twice the mass-specific salt-

load than that infused in the present study decreased urine volume and retained water and Na<sup>+</sup> after the first day of treatment (Krieger et al., 1990) indicating a stark contrast in renal handling of excess Na<sup>+</sup> between a terrestrial and marine mammal. This contrast in renal handling of Na<sup>+</sup> may reflect the differences in the adaptation to the environments in which terrestrial and marine mammals have evolved. Unlike other mammals, fasting pups possess an ability to excrete excess Na<sup>+</sup> rapidly (within 24 h) and efficiently (Ortiz et al., 2002b) without retention at the expense of a small amount (2%) of body water. Indicators of hydration state such as hematocrit, total proteins, and glucose were not different 24 h following the Na<sup>+</sup> load (Ortiz et al., 2002b) suggesting that this small loss of body water did not impact hydration state of the animals.

Maximum urine flow during water diuresis in other seals has been shown to represent between 10 and 12% of GFR, indicating that only 88–90% of filtered water is reabsorbed following fresh water infusions (Hong et al., 1982; Ladd et al., 1951). Fresh water infusion in fasting elephant seals also induced a diuresis associated with an increase in GFR and a 0.2% decrease in percentage of filtered water reabsorbed (Ortiz et al., 2002a). These increases in fractional clearance of water suggest that efficiency of tubular water reabsorption decreases as GFR increases. Similarly, in the present study, hypertonic saline-induced diuresis was associated with an increase in GFR and decrease in percentage of filtered water reabsorbed suggesting that GFR negatively impacted the efficiency of tubular water reabsorption. Although the 1.7-fold increase in GFR resulted in less than half of one percent (<0.5%) decrease in efficiency, this decrease was sufficient to result in 4-fold increase in urine output suggesting that small changes in tubular reabsorption can magnify urinary water loss in these fasting seals.

The mean GFR value measured during the control period in the present study is 41 and 16% less than that reported by Pernia et al. (1989) and Houser et al. (2001), respectively, in which GFR was measured by single <sup>3</sup>H-inulin injection technique in fasting pups of similar age and body mass. Conversely, the control value is 28% greater than that reported by Adams and Costa (1993) for fasting pups of similar age and body mass, wherein these investigators measured GFR by creatinine clearance. Although inulin clearance is regarded

as a more accurate technique for estimating GFR than creatinine clearance because of the kidneys ability to secrete creatinine into the tubules, inulin-determined GFR values between Pernia et al. (1989) and Houser et al. (2001) differed by approximately 20% suggesting that quite a bit of variability exists within different studies and within different groups of animals, regardless of technique. Aside from the discrepancies in fasting pups, GFR measurements appear to be quite labile among marine mammals. For example, in fasting adult elephant seals absolute and mass-specific GFR were increased between mid and late lactation in nursing mothers (Crocker et al., 1998), while data for fasting pups suggests either a decrease (Adams and Costa, 1993) or no change (Houser et al., 2001). In harbor seals (*Phoca vitulina*), feeding induced an increase in GFR (Hiatt and Hiatt, 1942), while postprandial hyperfiltration was not observed in postweaned elephant seal pups (Houser et al., 2001; Pernia et al., 1989). Therefore, quantitative comparisons of GFR among independent studies in marine mammals can not be made unconditionally. Nonetheless, the present study used the same technique to quantify the response to a stimulus by comparing post-infusion values to their own controls. Although not discernible in the present study, a number of factors such as blood pressure and vasopressin (i.e. Bouby et al., 1996) may have been responsible for the observed increase in GFR. A comparison between inulin- and creatinine-determined GFR measurements made in the same animals at different times of the postweaning fast as well as a more detailed examination of the factors affecting GFR in fasting pups are warranted.

Mammals adapted to living at sea do not appear to be any more tolerant of the toxic effects of sea water drinking than terrestrial mammals (Albrecht, 1950). However, these marine- adapted mammals excrete excess  $\text{Na}^+$  more efficiently than terrestrial mammals in regards to the time and the volume of body water required. Although hypernatremia resulted in an increase in urine output, this diuresis does not appear to be physiologically significant since it required less than 2% of the pups' TBW pool. Although the 77% increase in GFR resulted in only a 0.37% decrease in efficiency of tubular water reabsorption, urine output increased 4-fold. The maintenance of efficient water recycling was responsible for abating the loss of body water required to achieve the degree of natriuresis

observed (Ortiz et al., 2002b). Fasting pups excreted excess  $\text{Na}^+$  by increasing GFR, which is a unique response when compared to terrestrial mammals. This novel mechanism for excreting excess  $\text{Na}^+$  rapidly and efficiently may have been partly responsible for allowing terrestrial mammals to take advantage of marine resources. Results of the present study suggest that the transition of terrestrial mammals to a marine habitat necessitated a means to efficiently loose excess salt at the expense of a small percentage (<2%) of TBW. Although elephant seals during natural conditions would not likely be subjected to a  $\text{Na}^+$  load equivalent to that presented here, the present study demonstrates this species' renal capability to handle an extreme osmotic challenge.

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