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Angiotensin II and Aldosterone Increase with Fasting in Breeding Adult Male Northern Elephant Seals (*Mirounga angustirostris*)

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ABSTRACT

The renin-angiotensin-aldosterone system (RAAS) appears to contribute significantly to osmoregulation of fasting northern elephant seal (*Mirounga angustirostris*) pups; however, RAAS has not been characterized in fasting adult seals. Therefore, this study examined the contribution of RAAS to water turnover rates in fasting adult male northern elephant seals. Blood samples were obtained twice during their breeding fast at an interval of 6.5 wk, and water efflux rate was estimated by isotopic dilution during the same period. Serum electrolytes (Na^+ , K^+ , Cl^-) and osmolality were unaltered between the two sampling periods, indicating ionic and osmotic homeostasis during the fast. Despite the lack of an increase in vasopressin, serum angiotensin II and aldosterone were increased and were significantly and positively correlated. Changes in aldosterone concentration and water efflux rate were significantly and negatively correlated, suggesting that the greater the increase in aldosterone, the smaller the loss of water. Adult male seals maintain ionic and osmotic homeostasis similar to that of fasting weaned pups, and this homeostasis appears to be mediated, at least in part, by RAAS, which probably contributes to increased water retention as well. The hormonal mechanisms by which northern elephant seals maintain water and electrolyte balance during fasting conditions appear to be similar regardless of age.

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Introduction

Few mammals have adapted to prolonged periods of fasting. Hibernators such as the American black bear (*Ursus americanus*; Brown et al. 1971), the hedgehog (*Erinaceus europaeus*; Clausen and Storesund 1971), the little brown bat (*Myotis lucifugus*; Gustafson and Belt 1981), prairie dogs (*Cynomys leucurus* and *Cynomys ludovicianus*; Harlow and Braun 1995), and the yellow-bellied marmot (*Marmota flaviventris*; Kastner et al. 1978) experience periods as long as 7 mo without food and water, but they are essentially metabolically inactive during this time. In contrast, camels (*Camelus dromedarius*) may withstand prolonged periods (2–3 wk) of food and water deprivation in arid and hot environments without exhibiting deleterious effects (Ben Goumi et al. 1993; Yagil 1993). As with the aforementioned species, fasting for protracted periods (1–3 mo) is a natural component of the life-history year of the northern elephant seal (*Mirounga angustirostris*), occurring twice a year, regardless of age or sex (Le Boeuf et al. 1972). Nonetheless, the contributions of the osmoregulatory hormones arginine vasopressin (AVP), angiotensin II (Ang II), and aldosterone to the maintenance of water and electrolyte homeostasis during periods of prolonged fasting in adapted mammals remain largely unresolved.

Pups of the northern elephant seal maintain fluid and ionic homeostasis throughout their postweaning fast by conserving the water derived from the oxidation of their large body fat stores (Ortiz et al. 1978). In fasting pups, body water is conserved by increasing urine osmolality (Ortiz et al. 1996) and decreasing urine output (Adams and Costa 1993; Ortiz et al. 1996). By increasing the tubular reabsorption of electrolytes, pups are able to reduce the excretion of Na^+ and K^+ and thus are able to maintain electrolyte homeostasis (Adams and Costa 1993; Ortiz et al. 1996). In addition, pups maintain a state of metabolic quiescence similar to that of hibernators during their postweaning fast, which allows them to further reduce energetic expenditures and metabolic water losses. In contrast, adult male seals are physically more active during their fasting period associated with the breeding season, exhibiting high rates of mass loss and energy expenditure (Deutsch et al. 1990). This increase in activity could potentially impede their ability to maintain ionic and osmotic homeostasis, which is observed in the relatively inactive fasting pups. However, the osmoregulatory ad-

justments made by fasting adult elephant seals, especially during the breeding season, when animals are physically more active, have yet to be examined.

In mammals, the reabsorption of solute-free water from the collecting duct is mediated by AVP (Wade et al. 1982). As part of the renin-angiotensin-aldosterone system (RAAS), the precursor hormone, angiotensinogen, is cleaved by renin to produce angiotensin I, which is further converted to Ang II by angiotensin-converting enzyme (Funder 1993). Ang II, in turn, stimulates the adrenal release of aldosterone (Morris 1981; Funder 1993). Both Ang II (Ichikawa and Harris 1991) and aldosterone (Morris 1981; Funder 1993) have been reported to possess an antidiuretic function in the distal tubule of the mammalian kidney, in addition to regulating Na^+ reabsorption. Under normal fasting conditions in elephant seal pups, the renal conservation of water and electrolytes appears to be regulated, at least in part, by an increase in the response of RAAS without an increase in AVP (Ortiz et al. 2000). Although water reabsorption does not appear to be mediated by AVP under natural fasting conditions in elephant seal pups, other studies in adult seals suggest that water retention may be associated with AVP (Bradley et al. 1954; Page et al. 1954; Hong et al. 1982; Skog and Folkow 1994). In addition, AVP concentrations are greater in adults of other species of pinnipeds compared to those of conspecific pups (Zenteno-Savin and Castellini 1998), suggesting that concentrations of AVP in adult elephant seals may also be greater than those previously measured in pups and may increase with the breeding fast. However, concentrations of AVP, Ang II, and aldosterone have not been previously reported in adult elephant seals during fasting conditions. In addition, data on RAAS in marine mammals, especially during fasting conditions, are scarce, which contributes to our lack of thorough understanding of osmoregulation in this group of animals.

Unlike the postweaning fast observed in pups of the northern elephant seal, in which pups minimize activity by slumbering for a majority of the fast, the fast of adult males during the breeding season is associated with levels of high activity, including combat with other males to establish dominance, copulations, and frequent periods of terrestrial locomotion. Such stark differences in physical activity between pups and actively breeding males could potentially be associated with differences in osmoregulatory capabilities, since increased physical activity could burden total body water stores. For that reason, in order for seals to adapt to increased levels of activity during fasting periods as they mature, renal function and capabilities, as well as hormonal content, may change with development and age to accommodate such an adaptation. Thus, the assumption that the osmoregulatory alterations observed in fasting pups under natural conditions are similar to those of conspecific adults would be inappropriate. Therefore, this study was conducted to determine whether breeding adult male elephant seals maintain ionic and osmotic homeostasis and to examine the changes

in levels of aldosterone, Ang II, and AVP during their natural prolonged fast.

Methods

All methods were reviewed and approved by the University of California, Santa Cruz, Chancellor's Animal Research Committee. This study was conducted in conjunction with another study on the metabolism and behavior of breeding adult male northern elephant seals.

Animals. Seventeen adult male seals from Año Nuevo State Reserve (approximately 30 km north of Santa Cruz, CA) were studied during the 1998 breeding season. Animals were considered adult based on body mass measurements and development of secondary sexual characteristics including proboscis and neck shield. Individuals were identified by marking them with hair dye (Clairol, Stamford, CT) and by reading preexisting flipper tags. Markings and tags facilitated identification of animals for subsequent sampling. Individuals were weighed and sampled twice during their nonmolting fast with a sampling interval of 45 ± 1 d. The initial sampling is referred to as "early" and the subsequent sampling as "late."

Water efflux calculations. Isotopic dilution of tritiated water ($^3\text{H}_2\text{O}$) was used to estimate total body water pool size and water turnover as previously described (Ortiz et al. 1978). Males were lured onto a platform truck scale (± 5 kg) and weighed. No initial radioactive enrichment was assumed, and each male that was successfully weighed was immediately given an intramuscular injection of 185–296 MBq $^3\text{H}_2\text{O}$ in 12 mL of sterile saline. The following day, after an equilibration period of 12–16 h, the animal was sedated with 0.3 mg/kg body mass tiletamine HCl and zolazepam HCl (Telazol, Fort Dodge Animal Health, Fort Dodge, IA) in order to obtain blood samples (early). Blood samples from the beginning and end of the immobilization procedure (20–30 min apart) were compared to ensure complete equilibration of isotope. For the second sample (late), males were weighed and then immediately immobilized and sampled. After the collection of blood samples, a second dose of $^3\text{H}_2\text{O}$ was administered. The animal was allowed to equilibrate for 12–16 h before being immobilized for a final blood collection. The interval from the time the animals were injected with the sedative to the time the blood sample was obtained was <10 min. Blood samples (20 mL) were obtained from the extradural spinal vein using an 18-gauge needle, collected into an untreated blood collection tube, and placed on ice in a portable ice chest until they could be returned to the lab to be centrifuged, which was typically within 6 h. Blood samples were then centrifuged for 15 min ($1,500$ g at 4°C), and serum was collected and frozen at -70°C for later analyses. Daily water efflux was calculated using equation (4) of Nagy and Costa (1980).

Electrolyte, osmolality, and hormone analyses. Hormone concentrations were measured by radioimmunoassay using

Table 1: Mean (\pm SE) serum electrolyte concentrations, osmolality, and hormone concentrations in fasting adult male northern elephant seals during early and late fasting periods

	N	Early Period	Late Period	P
Na ⁺ (mM)	16	156 \pm 1	152 \pm 1	NS
K ⁺ (mM)	16	4.4 \pm .1	4.5 \pm .1	NS
Cl ⁻ (mM)	16	109 \pm 1	107 \pm 1	NS
Osmolality (mOsm/L)	17	300 \pm 3	296 \pm 2	NS
Aldosterone (pg/mL)	17	145.2 \pm 10.7	254.0 \pm 38.5	<.0001
Angiotensin II (pg/mL)	16	56.6 \pm 9.0	110.8 \pm 14.9	.02
Vasopressin (pg/mL)	16	14.5 \pm 1.2	12.2 \pm 1.1	NS

Note. NS = not significant.

commercially available kits for Ang II and AVP (Phoenix Pharmaceuticals, Belmont, CA) and aldosterone (DPC, Los Angeles). Each assay had been validated previously for use with northern elephant seal samples (Zenteno-Savin and Castellini 1998; Ortiz et al. 2000, 2002a, 2002b, 2003; Houser et al. 2001). For measurements of Ang II and AVP, separate 1.0-mL aliquots were extracted using C18 columns (Prep-Sep, Fisher Scientific, Fair Lawn, NJ) as previously described (Zenteno-Savin and Castellini 1998). All samples were analyzed in duplicate and run in a single assay with intra-assay coefficient of variability percentage of less than 8% for all assays. Osmolality was measured using a vapor pressure osmometer (Wescor 5500, Logan, UT), and electrolytes were measured on a Cobas Mira auto-analyzer (Roche Diagnostic Systems, Montclair, NJ).

Statistics. Means for each parameter at early and late periods were compared by paired *t*-test. In the event that sufficient sample from an animal was not available from either the early or the late period, comparisons were made from a sample size of 16 (i.e., paired samples were available for at least 16 animals). Relationships between dependent and independent variables were evaluated by simple regression, and correlations were evaluated using Pearson correlation coefficients. Means, regressions, and correlations were considered significant at $P < 0.05$. Statistical analyses were made using Statview (SAS Institute 1998).

Results

Body mass decreased by $23.2\% \pm 1.4\%$ ($P < 0.0001$) between early ($1,479 \pm 65$ kg) and late ($1,135 \pm 53$ kg) periods. Over the 6.5-wk period, water efflux rate averaged 4.04 ± 0.20 mL/kg/d (range: 2.80–6.15 mL/kg/d). Mean Ang II increased nearly twofold ($P = 0.02$), and mean aldosterone increased 75% ($P < 0.0001$) between the two sampling periods (Table 1). During the early period, Ang II and aldosterone did not exhibit a significant relationship or correlation; however, during the late period, aldosterone concentration increased significantly with increasing Ang II concentration (aldosterone = $99.9 + 1.2$ Ang II; $r = 0.612$; $P = 0.02$; Fig. 1). Water efflux rate declined sig-

nificantly with change in aldosterone concentration (Δ aldosterone; water efflux = $4.3 - 0.003 \Delta$ aldosterone; $r = 0.492$; $P = 0.04$; Fig. 2). The relationships between Δ Ang II and Δ AVP and water efflux rate were not significant ($P > 0.10$). In addition, concentrations of AVP and Ang II did not exhibit a significant correlation ($P > 0.10$) at either sampling period. Electrolytes, osmolality, and AVP remained unchanged between the two measurement periods (Table 1).

Discussion

Deprivation of food and water for extended periods (months) has the potential to induce deleterious effects on water and electrolyte homeostasis. However, a number of hibernating and nonhibernating mammals have adapted various physiological mechanisms to survive such extreme conditions. For example, pups of the northern elephant seal rely on the metabolic water derived from the oxidation of their large fat stores (Ortiz et al. 1978) to maintain fluid and ionic homeostasis (Costa and Ortiz

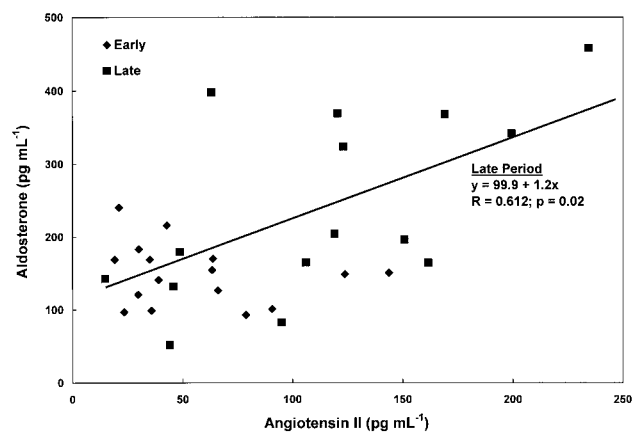


Figure 1. Correlation between circulating angiotensin II and aldosterone during early and late fasting periods in adult male northern elephant seals. Line and equation describe the relationship during only the late fasting period because the relationship during the early period was not significant. Regression was considered significant at $P < 0.05$.

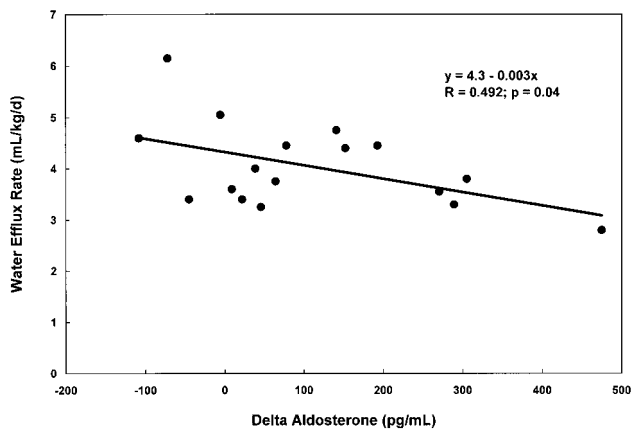


Figure 2. Correlation between the change in aldosterone concentration (Δ aldosterone) and water efflux rate in fasting adult male northern elephant seals. Regression was considered significant at $P < 0.05$.

1982; Ortiz et al. 1996, 2000). This study demonstrates that despite the increased physical activity and high rates of energy expenditure observed in breeding adult male elephant seals, the seals are still able to maintain ionic and osmotic homeostasis during their nonmolting fast similar to that observed in relatively inactive conspecific pups. The lack of change in serum osmolality and electrolytes between the early and late sampling periods in adult male seals provides an indication of their ability to maintain electrolyte balance, suggesting that these animals, regardless of their activity level, possess rigorous physiological mechanisms to tightly regulate circulating electrolyte concentrations. Serum osmolality and electrolyte concentrations in this study are similar to those measured in fasting seal pups (Costa and Ortiz 1982; Ortiz et al. 1996, 2000) and fasting lactating females (D. E. Crocker, unpublished data). In contrast, water deprivation for 10 or 14 d in camels increased mean plasma Na^+ (3.3% or 24%, respectively) and osmolality (10.5% or 33.5%, respectively; Ben Goumi et al. 1993; Yagil 1993), suggesting that camels exhibit signs of dehydration during their bouts of water deprivation, unlike elephant seals. Hibernating marmots have also been shown to increase mean plasma Na^+ 3.4%–3.8% after 9 d (Zatzman and South 1972; Kastner et al. 1978), further demonstrating the impressive feat by elephant seals of maintaining electrolyte homeostasis, especially in the face of increased activity levels observed in breeding adults.

Previous studies have shown that RAAS in elephant seal pups responds to various stimuli (fasting [Ortiz et al. 2000], altered salinity [Ortiz et al. 2002a, 2002b], AVP infusion [Ortiz et al. 2003]), and in West Indian manatees (*Trichechus manatus*), it responds to changes in water salinity (Ortiz et al. 1998). However, to date, no studies of mammals adapted to prolonged fasting have demonstrated an association between changes in some component of the RAAS and changes in some parameter of water conservation, such as reduced water efflux rate. The

negative relationship between Δ aldosterone and water efflux rate (water loss) suggests that increases in aldosterone contribute to conservation of water in fasting adult seals. This correlation implies that the greater the change in circulating aldosterone, the smaller the rate of water efflux or loss, and thus it supports the contention that the increase in aldosterone significantly contributes to osmotic homeostasis in fasting adult northern elephant seals.

The correlation between Δ aldosterone and water efflux also supports the suggestion that increases in aldosterone and Ang II play a significant role in the conservation of electrolytes during this period in adult seals. In mammals, Na^+ and K^+ are regulated by aldosterone via RAAS (Morris 1981; Funder 1993). Aldosterone induces the increased reabsorption of Na^+ in the distal tubule that is accompanied by the reabsorption of water (Morris 1981; Funder 1993). Ang II has also been reported to have antidiuretic activity in the kidney (Ichikawa and Harris 1991). Therefore, the observed increases in Ang II and aldosterone between the early and late periods, coupled with a positive correlation between the two hormones during the late period, indicate that RAAS is active and contributing to electrolyte homeostasis during the fast in adult males. Plasma renin activity (PRA), an indicator of circulating angiotensin I generation, and aldosterone have been shown to be positively correlated in fasting elephant seal pups during their postweaning fast (Ortiz et al. 2000), as well as in other marine mammals (Malvin et al. 1978; Ortiz et al. 1998), further implicating the presence of an active RAAS in this group. Also, hibernating marmots exhibited an increase in PRA and a concomitant increase in aldosterone after 9 d. The contribution of RAAS in water-deprived camels is not so definitive. After 10 d of water deprivation, PRA increased nearly fourfold, associated with a 61% increase in aldosterone (Yagil 1993). However, in a similar study, 14 d of water deprivation stimulated a nearly 10-fold increase in PRA without the requisite increase in plasma aldosterone. The authors of that study suggest that the lack of increase in plasma aldosterone may be attributed to the hypernatremia also observed in these animals during the same period (Ben Goumi et al. 1993), which is possible because treatment with the natriuretic furosemide alleviated the hypernatremia and resulted in an increase in PRA and concomitant increase in aldosterone (Riad et al. 1994). Nonetheless, the existing data on RAAS and electrolyte balance in mammals adapted to prolonged periods of water deprivation suggest that discrepancies exist in the response of these animals to such an osmotic challenge.

Although urinary excretion data are not available for adult elephant seals, pups have been shown to exhibit a decrease in urinary Na^+ concentration over 10 wk of fasting (Ortiz et al. 1996), coinciding with the time in which RAAS is increased (Ortiz et al. 2000). This evidence further suggests that the increase in RAAS contributes to an increase in renal reabsorption of Na^+ in elephant seals.

Unfortunately, reports of aldosterone (Sangalang and Freeman 1976; Engelhardt and Ferguson 1980; St. Aubin and Geraci 1986; Ferreira et al. 2005) and Ang II (Zenteno-Savin and Castellini 1998) concentrations in adult or juvenile pinnipeds are scarce. Mean aldosterone concentrations during the early period (145 ± 11 pg/mL) were most similar to those measured (139 ± 75 pg/mL) in wild adult (values for males and females combined as reported) southern elephant seals (*Mirounga leonina*) during their molting fast (Ferreira et al. 2005) and were approximately half those reported for captive juvenile ringed seals (*Phoca hispida*) during normonatremic, salt-replete conditions (St. Aubin and Geraci 1986). Samples obtained from wild adult male gray seals (*Halichoerus grypus*) ranged from 1,390 to 3,300 pg/mL; however, it should be noted that these samples were obtained from animals that were shot to death, which may explain the comparatively high concentrations (Sangalang and Freeman 1976). A single sample from a wild adult male harp seal (*Phoca groenlandica*; physically restrained for sampling) had an aldosterone concentration (1,200 pg/mL) similar to that reported for gray seals. These relatively higher concentrations of aldosterone may reflect the impact of the mode of collection rather than the actual physiological status, especially when compared to the values in our study, in which animals were sedated during sample collection. Mean aldosterone concentrations from nursing and postnursing adult female harp seals (300 pg/mL) were similar to those reported here during the late period (254 ± 39 pg/mL) and for lactating adult female northern elephant seals (380 pg/mL; D. E. Crocker, unpublished data). In elephant seal pups, mean aldosterone concentrations increased from 200 to 1,500 pg/mL during the first 7 wk of the postweaning fast (Ortiz et al. 2000, 2002a, 2002b, 2003; Houser et al. 2001), representing a 7.5-fold increase, whereas the increase in fasting adult males over a comparable duration was only 1.7-fold. An age-related change in the sensitivity of aldosterone response to fasting may be but one potential explanation for the discrepancy in the degree of change of aldosterone between pups and adults. Interestingly, aldosterone concentrations in hibernating marmots and water-deprived camels remain below 100 pg/mL (Kastner et al. 1978; Ben Goumi et al. 1993), which is two- to 15-fold lower than concentrations observed in pinnipeds, providing an indication of aldosterone's importance in osmoregulation of marine mammals.

Mean Ang II concentrations during the early period (57 ± 9 pg/mL) are consistent with those measured (56 ± 12 pg/mL) in adult Steller sea lions (*Eumetopias jubatus*; Zenteno-Savin and Castellini 1998). These mean values are two- to 6.5-fold greater than those measured in other adult pinniped species (Zenteno-Savin and Castellini 1998), suggesting that distinct physiological differences with respect to RAAS, possibly dictated by their environment or breeding cycles, may exist among various species of pinnipeds.

The contributions of AVP to water and electrolyte balance

in species adapted to prolonged food deprivation remain ill defined. For example, neurohypophyseal content of AVP in the hibernating garden dormouse (*Eliomys quercinus*) increased fourfold; however, AVP content remained unchanged in the hibernating mouse-eared bat (*Myotis myotis*; Hudson and Wang 1979). In water-deprived camels, plasma AVP increased 4.4-fold and sixfold after 10 and 14 d, respectively (Ben Goumi et al. 1993; Yagil 1993). Only a few studies in pinnipeds have demonstrated an association between AVP (either infused as pitressin or quantified by changes in concentration) and reduced urine output or calculated free water clearance (Bradley et al. 1954; Page et al. 1954; Hong et al. 1982; Ortiz et al. 2002b). If the observed concentrations of AVP are physiologically significant, then the relatively higher concentrations of AVP in adults versus pups (Ortiz et al. 1996, 2000, 2002a, 2002b, 2003; Zenteno-Savin and Castellini 1998) suggest that the renal collecting ducts may be hyposensitive to AVP during the fasting period and thus necessitate higher circulating concentrations to elicit a physiological response. Thus, the lack of an increase in AVP does not necessarily preclude AVP from contributing to water and osmotic homeostasis in fasting adult seals. In addition, mean AVP concentrations in this study are still two-fold higher than in water-deprived camels (Ben Goumi et al. 1993; Yagil 1993). Alternatively, the sampling schedule may not have captured the initial increase in circulating concentrations of AVP, and thus, the observed levels would be indicative of maximally increased concentrations. Nonetheless, this trend toward higher concentrations of AVP in adults versus pups is consistent with that reported for other pinnipeds, including harbor (*Phoca vitulina*) and Weddell (*Leptonychotes weddellii*) seals (Zenteno-Savin and Castellini 1998). Mean AVP concentrations in our study are similar to those measured in adult Steller sea lions (14.2 ± 1.5 pg/mL), harbor seals (15.9 ± 2.5 pg/mL), and Weddell seals (12.0 ± 0.1 pg/mL) and are in the range of adult California sea lions (*Zalophus californianus*; 10.2 pg/mL; gender not reported; Zenteno-Savin and Castellini 1998).

We have previously shown that an acute intravenous infusion of AVP induced a rapid (within 15 min) increase in circulating cortisol in fasting pups, suggesting that AVP stimulated a neuroendocrine response in fasting seals (Ortiz et al. 2003). In the current study, cortisol was measured simultaneously (data not reported here) to address the potential concern that the observed concentrations of AVP were the product of a neuroendocrine response induced by the sampling protocol and did not necessarily reflect an osmotic response. The relatively rapid acquisition of blood and the lack of a positive correlation between AVP and cortisol concentrations at either of the sampling periods suggest that the concentrations of AVP measured here were not the product of neuroendocrine stimulation and probably reflected circulating concentrations at that time.

In summary, the negative correlation between Δ aldosterone and water efflux (water loss) indicates that water loss is abated

by increasing aldosterone. This provides some of the most definitive evidence to date that RAAS contributes significantly to water as well as electrolyte conservation in this species and probably in most mammals adapted to prolonged periods of fasting. Increases in Ang II and aldosterone in adult elephant seals during their breeding fast, when animals are active, are similar to those observed in pups during their postweaning fast, when animals are relatively inactive. The positive correlation between Ang II and aldosterone is consistent with the positive correlation between PRA and aldosterone observed in fasting pups (Ortiz et al. 2000), suggesting that RAAS is active and probably contributing to the maintenance of ionic and osmotic homeostasis. The discrepancy in the degree of change in aldosterone concentration during equivalent fasting durations between adult males and pups suggests that the response of RAAS to fasting varies with age in elephant seals. Despite the lack of a change in AVP, the relatively higher concentrations in adults versus those in pups suggest that an age-dependent shift in the dynamics of AVP metabolism and function may exist in elephant seals, similar to that observed with RAAS. Alternatively, these concentrations of AVP may reflect maximally increased levels. The contribution of AVP to osmoregulation in marine mammals continues to be ambiguous. However, this study implies the importance of RAAS in regulating water and electrolyte balance in mammals adapted to prolonged fasting, especially marine mammals.

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