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Biological Trace Element Research

ISSN 0163-4984

Biol Trace Elem Res
DOI 10.1007/s12011-016-0734-z

Springer

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Reference Intervals of Mineral Elements in Plasma of Anesthetized Free-Ranging Adult Females of South American Sea Lion, *Otaria flavescens*

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Received: 24 February 2016 / Accepted: 2 May 2016

Abstract Levels of zinc (Zn), copper (Cu), potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) in plasma of *Otaria flavescens* females (n = 29) were evaluated. Reference intervals were established for each element, being the first report for this species.

Keywords *Otaria flavescens* · Blood · Minerals elements

Short communication

Mineral elements are inorganic substances some present in body tissues and fluids necessary for the maintenance of certain physicochemical processes essential to life. Pinnipeds, like other marine mammal species, obtain their required minerals primarily from the diet [1]. Often, diseases are associated with an imbalance of the electrolyte homeostasis causing changes in blood chemistry [1, 2]. Therefore, reference intervals are a critical diagnostic tool used in the care of stranded animals and for population health investigations. In marine mammals from natural colonies, the information about essential elements in blood fractions is limited, and most reports refer to whole blood [1, 3–6].

There are no reports about mineral elements in Southern sea lions (*Otaria flavescens*, *SSL*) blood and due to females are key elements of population dynamics, the aims of this study were to assess the levels of Zn, Cu, K, Ca, Mg, and P in plasma and establish their reference intervals for SSL females.

The study was conducted in Isla de Lobos (35°01′ 50″ S, 54°53′ 00″ W, Uruguay) in May 2007 and 2010. Fourteen (2007) and thirteen (2010) SSL females were captured by the DINARA (National Aquatic Research, Uruguay) staff and held in a big corral under veterinary observation during 24 h.

The animals were physically restrained using a squeeze cage and anesthetized with isoflurane using a mask (5–10 L min^{-1} and 5 % of isoflurane) and afterwards with an endotracheal tube with a constant oxygen flow (2-L min^{-1} oxygen and isoflurane 0.75–1.5 %). Animals breathed constantly and regularly by themselves. Heart and breathing rates, body temperature, oxygen, electrocardiographic recording and oral mucosa condition were monitored each 5 min with both manual techniques and a multi-parametric monitor (Guoteng, Science and Technology). All females were classified as ASA 1 (American Society Anesthesiologist, www.asahq.org/clinical/physicalstatus.html) by a veterinarian, meaning that individuals were young, healthy and with no sign of diseases. Likewise, the total length vs total weight

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Published online: 07 May 2016

relationship presented a good fit in both surveys, 2007 ($r^2 = 0.9665835$, $p < 0.01$ to a potential curve W (kg) = $1.8708899^{e-05 * Lt (cm) 3.0149994}$) and 2010 ($r^2 = 0.9743778$, $p < 0.05$ to a potential curve W (kg) = $4.3265166^{e-05 * Lt (cm) 2.839149}$), indicating a normal relationship between both morphometric parameters. The SSL total weight and length were determined for all animals (Table 1). Age was estimated according to the curve described by Grandi et al. [7], allowing to determine the sexual maturity of individuals (mature and immature).

Blood was collected from an interdigital vein of flipper using an intravenous catheter (BD Angiocath 18G X 1.16″) and syringe with sodium heparin. Blood was centrifuged at 3000 rpm during 10 min; plasma was frozen in liquid nitrogen and stored at −80 °C until analysis.

Concentrations of Cu, Zn, Ca, P, K, and Mg were determined by Atomic Absorption Spectrometry (GBC 906, Scientific Equipment, Victoria, Australia). An aliquot of plasma was deproteinized in equal quantities with 10 % (*v*/*v*) trichloroacetic acid [8]. Standard Reference Material (SRM 956c, Frozen Human Serum from the National Institute of Standards and Technology, USA) was used to validate results. Blanks of reagent were performed and they were treated under the same conditions of samples and SRM. The curves of calibrations were carried out with a standard solution (Mallinckrodt) of each element. The addition of standard solution was performed and the recoveries were between 97 and 102 % ($p < 0.01$). The detection limits were Zn 0.033 mg L^{-1}, Cu 0.023 mg L^{-1}, 0.097 mg L^{-1}, P 0.200 mg L^{-1}, Ca 0.100 mg L^{-1}, Mg 0.010 mg L^{-1}, and K 0.400 mg L^{-1}. The sample concentrations were expressed in mg L^{-1} in wet weight.

Mean values and standard deviation (SD) were calculated. Homoscedasticity of data was checked with Levene test ($p < 0.05$) and normality was checked with Shapiro-Wilk's test ($p < 0.01$) [9]. Statistical differences for each element between groups 2007 and 2010 females were checked by Student's *t* test, as well as the differences between mature vs immature females. As no differences were found between any groups for all studied elements ($p < 0.05$), all females were analyzed as an only data set. Reference intervals were developed for each element and are defined as 95 % central range comprising between 2.5th and 97.5th percentiles (mean ± 1.96 SD) of the data distribution.

The results obtained for each element are presented in Table 2. The analytical results for all elements follow the Gaussian distribution pattern, showing very low variability, with CV values lower than 18.9 %.

The Zn and Cu concentrations found in SSL were similar to those reported in serum of *Leptonychotes weddellii* adult females [10]. In the same way, Ca, Mg, and K levels in SSL were similar to those reported in serum of *Zalophus californianus* [6] and in seals like *Hydrurga leptonyx* and *L. weddelli* [10] and *Phoca vitulina* [11, 12]. This information, coupled with the low CV of each element suggest that the values presented as reference intervals correspond to normal physiological values from SSL anesthetized. However, it should be noted that it has been reported that isoflurane in humans decreases Zn plasma levels [13], having no information for the other elements. Therefore, the values of this element in SSL could be overestimated, although to confirm it, other studies would be needed with anesthetized and non-anesthetized animals.

Table 1 Biological parameters of free-ranging females of *Otaria flavescens* (South American sea lion SSL) from 2007 and 2010 sampling surveys. *EA* estimated age

Year 2007				Year 2010			
SSL	Weight (kg)	Length (cm)	EA (year)	SSL	Weight (kg)	Length (cm)	EA (year)
01	83.1	168.0	9.0	15	62.0	145.5	4.5
02	77.6	146.0	4.5	16	71.4	151.0	5.0
03	77.1	159.0	6.5	17	145.8	193.0	13.0
04	132.2	185.0	>13.0	18	79.0	159.0	6.5
05	132.2	190.0	>13.0	19	109.6	184.0	13.0
06	76.5	160.0	6.5	20	64.1	153.0	5.0
07	61.3	148.0	4.5	21	121.0	189.0	13.0
08	81.7	160.0	6.5	22	69.2	162.0	7.0
09	61.6	149.0	4.5	23	67.4	151.0	5.0
10	64.7	146.0	4.5	24	64.8	152.0	5.0
11	54.9	141.0	4.0	25	61.0	142.0	4.0
12	113.9	175.0	>13.0	26	56.4	142.0	4.0
13	130.3	181.0	>13.0	27	55.3	137.0	4.0
14	51.9	139.0	4.0	–	–	–	–
Mean ± SD	85.6 ± 29.2	160.5 ± 16.9	–	–	80.6 ± 27.7	154.4 ± 23.5	–

Table 2 Mineral element concentrations (mg L^{-1}) in plasma of free-ranging female *Otaria flavescens* ($n = 27$)

	Zn	Cu	P	Ca	Mg	K	Cu/Zn
Mean ± SD	0.36 ± 0.06	0.98 ± 0.15	79.19 ± 14.95	92.44 ± 6.22	21.50 ± 2.02	186.18 ± 30.69	2.75 ± 0.62
Reference intervals	0.24–0.47	0.69–1.29	55.0–117.4	74.9–107.7	19.6–25.2	144.3–249.0	–
CV %	16.6	15.3	18.9	6.7	9.4	16.5	–

Reference intervals, 95% central range

SD standard deviation, *CV* coefficient of variation

The element concentrations reported represent a new contribution to the knowledge of the blood chemistry of marine mammals and are the first report about blood chemistry in *O. flavescens*. The reference intervals established can be used to assess clinical health of the species and would contribute to the captive management and reintroduction programs to the wild life.

Acknowledgments We thank the fieldwork assistance of DINARA personnel (César Barreiro, Nelson Veiga, Leonardo Olivera, Miguel Casella, Fernando Area) during capturing and handling of the sea lions. We also thank Jessica D'Imperio, Eduardo Juri, Meica Valdivia, Sabrina Riverón, Mariela Dassis, Agustina Mandiola, Ippei Suzuki, and Ian Davis for their assistance during sampling in the field. Anesthesia and veterinary monitoring were provided by the DVM Bruce Heath, Eduardo Mateos and Valeria Ruopollo. The animal care was according to OCA 1499/12. All field research was conducted under DINARA permits issued to APL and DR (A/009/2006; Ministerial Resolution 001/1383/2007, Expedientes DINARA No. 1136/ 2006, 503/2007 y 1378/2008). Grants: Agencia Nacional de Promoción Científica y Tecnológica of Argentina (Projects PICT 2007-01763 and 2011-1834), Universidad Nacional de Mar del Plata, Argentina (Projects 547/11), NSF-CONICET Cooperation Grant (CONICET Resolution 1340/10) and Alaska SeaLife Center (R-1972-01 and R-2972-01).

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