Cold Stress Induces an Adrenocortical Response in Bottlenose Dolphins (*Tursiops truncatus*)

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COLD STRESS INDUCES AN ADRENOCORTICAL RESPONSE IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

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Abstract: Two adult bottlenose dolphins (*Tursiops truncatus*) were individually housed in aboveground pools over a 10-day period and exposed to decreasing water temperatures to determine whether cold stress activated the hypothalamic-pituitary-adrenal axis. To serve as controls, two additional adult dolphins were similarly housed for the same duration but at ambient water temperatures (16.8–19.6°C). Across all subjects, water temperatures ranged from 4.2 to 19.6°C. Voluntary blood draws were made from each dolphin every 2–3 days, and serum was analyzed via radioimmunoassay for cortisol and aldosterone. Dolphins exposed to cold water showed an increase in serum cortisol and aldosterone as temperature declined; at the coldest water exposure, cortisol was more than three times and aldosterone more than two times the levels measured at ambient temperature. Elevations occurred before the water temperature declined below the individual animal's lower critical temperature, the point at which the metabolic rate increases to compensate for the loss of body heat. Variations in corticosteroids were unrelated to the 10-day isolation period, suggesting that the response was related to the cold stress and not impacted by the isolation. Elevations in cortisol and aldosterone were lower than those observed in force captured and stranded dolphins. Although potentially related to the general adaptive stress response, elevations in cortisol and aldosterone may have other adaptive functions related to mitigating impacts resulting from cold environmental temperatures.

Key words: Bottlenose dolphin, *Tursiops truncatus*, cortisol, aldosterone, cold stress.

INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis can be activated in response to both exogenous and endogenous stressors. The result of this activation in mammals is an increase in glucocorticoids, hormones that serve to mobilize energy stores and regulate immune and inflammatory cell responses. In bottlenose dolphins (*Tursiops truncatus*), activation of the HPA axis during acute stress related to capture, handling, or transport that is behaviorally unconditioned results in the increased production of both the glucorticoid cortisol and the mineralocorticoid aldosterone. Mean cortisol levels observed during capture and forced restraint of groups of dolphins typically range between 70 and 150 nmol/L, whereas mean aldosterone levels range from 300 to 1,880 pmol/L.[5,25,35,37] The values show wide individual variability and a dependence on the duration of the capture and handling event and time of sampling.[25,35] Stranded dolphins have been observed with serum cortisol levels ranging from 210 to 490 nmol/L,[12] and levels are typically above those found in semidomesticated animals that are accustomed to handling ($\leq$50 nmol/L).[35] In addition, chronic stress has been linked to cortical hypertrophy and hyperplasia in several species of odontocete cetacean, suggesting a stress-induced increase in the production of adrenocorticotropic hormones.[4,17,18]

Bottlenose dolphins inhabit tropical and temperate waters around the world,[38] but seasonal temperature fluctuations and migratory behavior may potentially expose bottlenose dolphins to cold water temperatures. Indeed, extralimital occurrences of bottlenose dolphins have been observed as far as 76°N, and resident populations are known to exist throughout the year in the coastal regions of the United Kingdom.[3,13,21,39] Water temperatures in these regions can fall to $<$5.5°C. The lower critical temperature (LCT), the temperature at which the metabolic rate is increased to maintain the core body temperature, is ~5.5°C for adult male bottlenose dolphins in excess of 187 kg.[40] Because thermal inertia is affected in part by mass, smaller dolphins would presumably have a higher LCT. Based on these findings it might be expected that dolphins inhabiting waters with temperatures $<$5.5°C are thermally stressed and must engage physiologic mechanisms that permit them to continue using a habitat despite exposure to cold extremes.

The purpose of this study was to investigate the production of cortisol and aldosterone in bottlenose dolphins exposed to water temperatures

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that are near or below their LCT. Circulating levels of cortisol and aldosterone were measured to determine whether thermal stress activates the HPA axis and whether increasing thermal stress was correlated with increased hormone levels. Cortisol and aldosterone may contribute to allostasis during thermal stress through facilitating the mobilization of body stores for energy or maintaining blood pressure during cold-induced peripheral vasoconstriction. However, they also contribute to the allostatic load of the animal, potentially increasing the probability of tissue damage via dysregulation of normal physiologic function. Information from this study contributes to understanding the stressors experienced by dolphins which errantly or willfully occupy thermally challenging environments.

MATERIALS AND METHODS

The study was conducted at the U.S. Navy Marine Mammal Program (MMP) and involved four adult Atlantic bottlenose dolphins (three males, one female; Table 1). All procedures were approved by the Institutional Animal Care and Use Committee of the Biosciences Division, Space and Naval Warfare Systems Center Pacific and followed all applicable U.S. Department of Defense guidelines for the care of laboratory animals. The dolphins were housed in floating net pens within San Diego Bay and were therefore acclimated to ambient bay water temperatures at the time of the study (~17–20°C). All dolphins were fed a mixed diet of herring (*Clupea harengus*), mackerel (*Scomber scombrus*), and capelin (*Mallotus villosus*), and each dolphin received daily vitamin supplements (Mazuri® Tabs, PMI Nutrition International, Richmond, Indiana, USA) in accordance with the standard operating procedures of the MMP.

All animals were previously trained for transport to and residency in aboveground pools as part of their participation in the MMP. The pools were spherical and measured 4.9 m in diameter × 1.2 m in depth (Vogue Pool Products, LaSalle, Quebec, Canada H8N 1V2).Two dolphins (D1 and D2) were individually exposed to increasingly cold water temperatures over a 10-day period. These dolphins were part of a previously published study to determine the LCT of the bottlenose dolphin.[40] Two other dolphins (D3 and D4) were individually housed according to the same procedure, but they were maintained at the ambient water temperatures of San Diego Bay (Table 2). These control animals were used to determine whether the HPA axis was activated in response to social isolation and whether this factor potentially influenced results obtained from cold-exposed dolphins. For cold-exposed animals, the pool water temperature (T_w) was controlled by an in-line water chiller (Trane, Cullen, Louisiana 71021, USA). The water chiller maintained T_w within ±1°C and achieved a minimum of approximately 0.2°C. Over a period of 10 days, the water temperature was dropped from near ambient to between 4.2 and 4.4°C, depending on the dolphin (Table 2). Water temperatures were gradually decreased by 1 to 2°C each night. On the 10th day of residency, before removing the dolphins and returning them to their pens in San Diego Bay, the water in the pool was exchanged with San Diego Bay water and returned to ambient temperature. The dolphins exposed to cold water were monitored for 5 min every 0.5 hr during the study for indications of cold stress (e.g., increased respiration rate, shivering). These dolphins received daily inspections from either veterinarians or veterinary technicians to ensure they remained in good health. The dolphins exposed only to ambient temperatures were monitored periodically throughout the day and night in accordance with MMP standard operating procedures for pool residency.

The dolphins were trained to voluntarily present their flukes for blood sampling, and blood samples were collected every 2–3 days over the duration of the pool residency. Samples were collected between 0745 and 1000 hr to minimize the potential for diel cycles in hormone production to affect circulating hormone levels. On the last day of residency, after the water had been returned to ambient temperature and before the dolphin being returned to its pen in San Diego Bay, a final blood sample was collected. Samples were drawn into chilled 7.5-ml serum tubes (BD Biosciences, San Jose, California 95131, USA) from vessels on the ventral surface of the dol-

Table 1. Age, sex, and mass of bottlenose dolphins participating in the cold and ambient temperature exposures.

Dolphin ID	Sex	Mass (kg)	Age (yr)
Cold exposure			
D1	Male	177	27
D2	Male	191	24
Ambient temperature			
D3	Male	175	39
D4	Female	163	35

Table 2. Pool water and air temperature on the days that blood samples were collected for corticosteroid analysis. Cortisol and aldosterone levels are given for each sample.

Dolphin ID	Days in pool	T_w (°C)	T_a (°C)	Cortisol (nmol/L)	Aldosterone (pmol/L)
Cold exposure					
D1	4	11.3	17.9	9.7	36.9
	7	7.9	17.6	43.9	73.5
	9	4.4	17.1	48.8	114.6
	10	13.4	18.8	43.9	43.8
D2	1	18.6	18.7	13.2	78.5
	4	12.2	16.3	32.6	145.7
	6	8.2	15.3	37.0	151.2
	8	4.2	16.1	40.3	175.9
	11	15.1	16.0	12.4	57.7
Ambient temperature					
D3	2	19.6	18.8	12.7	58.2
	4	18.1	17.7	39.3	78.4
	6	—[a]	—[a]	9.4	98.4
	8	18.1	18.4	20.6	87.5
	10	18.4	18	26.4	43.1
D4	2	17.9	20	9.4	39.1
	4	16.8	18	9.8	36.5
	6	18	17.1	—[b]	14.7
	8	17.8	16.7	4.2	—[b]
	10	18.3	16.8	7.0	14.7

[a] Missing data.
[b] Hormone levels were below the sensitivity of the radioimmunoassay.

phin's fluke. Samples were immediately centrifuged at 3,000 rpm for 10 min, and the serum was drawn off and frozen at –80°C. Serum samples were analyzed in duplicate for cortisol and aldosterone via RIA (kit TKCO1 and TKAL1, respectively, Siemens Healthcare Diagnostics, Deerfield, Illinois 60015-0778, USA). These kits have been previously validated;[25] however, the validation process was repeated here. The commercial RIA kits were validated by testing the equality of slopes to establish parallelism between a serially diluted pool of dolphin serum and established standard curves. Serially diluted pooled serum samples displayed significant parallelism with the standard curves. Recovery of exogenous hormone added to pooled serum samples was 98.4 ± 3.1 and 95 ± 2.2 % for cortisol and aldosterone, respectively. The intra-assay CV for both hormones was <5%.

A correlation analysis was performed to determine whether variations in circulating aldosterone and cortisol concentrations were related. Relationships between hormone levels and T_w and residency time (in days) were then compared with a linear mixed effect model. In the model, T_w and residency time were set as fixed effects and the individual animals were set as a random effects subject term to control for individual variation. Model residuals were assessed for approximate normality.

RESULTS

The dolphins demonstrated no adverse behavioral reactions to the cold water exposures. Cortisol and aldosterone concentrations and the water and air temperatures at the time of blood collections are given in Table 2. Trends in the hormone concentrations and water temperature with time in the pools also are shown for dolphins D1 and D2 (Figs. 1, 2). Cortisol and aldosterone concentrations were highest in the animals exposed to cold water conditions, and a positive correlation existed between cortisol and aldosterone for these two dolphins (D1, $r = 0.66$; D2, $r = 0.99$). The correlation coefficient for one of the control animals, D3, was low and negative ($r = -0.23$). The correlation coefficient for D4 was 0.98; however, either serum aldosterone or cortisol was below the limits of detection in two of the samples, leaving only three samples for the correlation. Results of the linear mixed effects model indicated that hormone concentrations were significantly related to water temperature (cortisol: $F = 11.42$, $P < 0.01$; aldosterone: $F = 44.49$, $P < 0.001$), but they were not related to residency time in the pool (cortisol: $F = 0.2$, $P =$

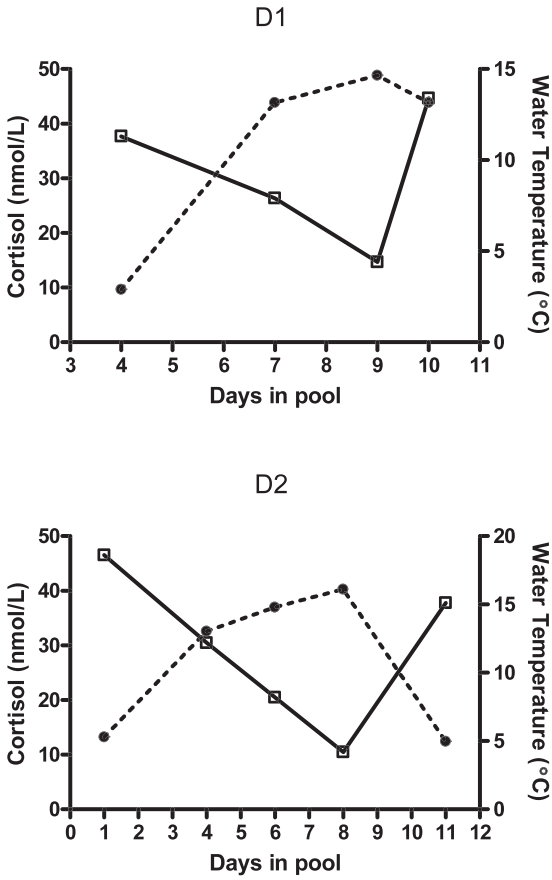

Figure 1. Cortisol (filled circles) and water temperature (open squares) as a function of time in the pool for dolphins D1 and D2.

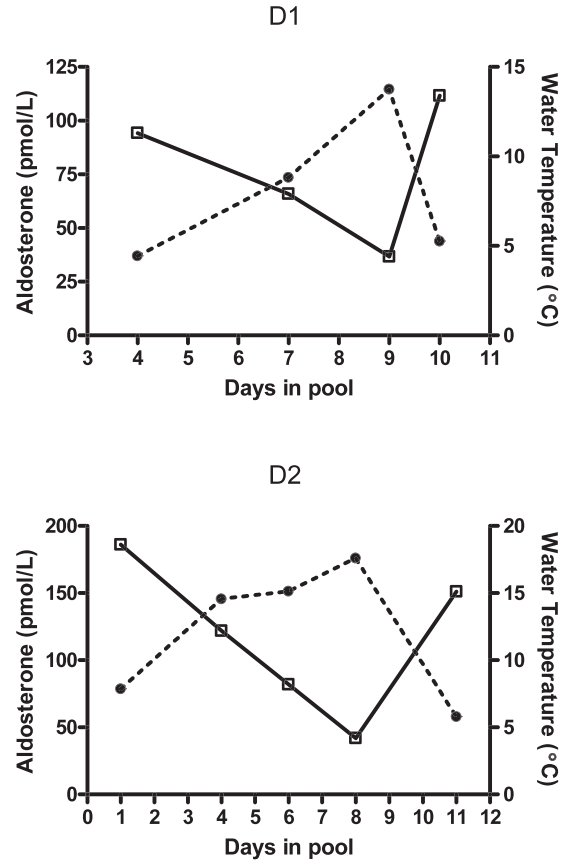

Figure 2. Aldosterone (filled circles) and water temperature (open squares) as a function of time in the pool for dolphins D1 and D2.

0.66; aldosterone: $F = 3.79$, $P = 0.08$). The model did not indicate any significant contribution to the measured relationships resulting from variability across individuals.

DISCUSSION

Dolphins exposed to declining water temperatures that extend below their LCT show an increase in circulating levels of cortisol and aldosterone. Parallel elevations in both hormones occurred as temperatures declined, before T_w falling below either dolphin's LCT (D1 = 7.8°C, D2 = 5.6°C).[40] This suggests a mounted activation of both the glucocorticoid and mineralocorticoid pathways of the HPA-axis in response to declining environmental temperatures. The production of both hormones in the adrenal gland is affected by anterior pituitary stimulation with adrenocorticotropic hormone (ACTH), although the action is secondary to the renin-angiotensin-aldosterone system (RAAS) for production of aldosterone, and cold-induced elevations in aldosterone have not been conclusively linked to elevations in ACTH.[8] Elevations of both hormones may reflect the general adaptive response, but they also may serve other functions that mitigate the potential effects of current and future environmental temperatures.

Cortisol serves several functions, including the up-regulation of proteolysis and lipolysis in extrahepatic tissues, elevation of gluconeogenesis and glycogen synthesis in the liver, and inhibition of peripheral glucose use.[22,24,34] Elevations in cortisol in response to declining temperature could potentially induce foraging effort and energy acquisition in an animal. Elevated glucocorticoids have been associated with increased food intake in human, avian, and rodent models, possibly by exerting permissive effects on food intake.[1,11,19,33,36] Elevated cortisol may have adaptive value by inciting dolphins to increase energy

acquisition (feeding) in anticipation of future temperature declines. Energy acquisition in excess of that required for maintenance metabolism could be directed toward blubber deposition and increasing thermal insulation. Hyperphagia by wildlife in anticipation of hibernation or limitations in winter food sources may be induced by low doses of glucocorticoids, whereas high levels may inhibit feeding.[7] However, such observations may show considerable species variability.[28] Dolphins exposed to cold water in this study had elevated cortisol levels relative to levels measured at the start of the study, but values were well below those observed in animals that have stranded or that were force captured over a period that permitted up-regulation of cortisol production.[12,35,37] Additional work is required to determine whether there is adaptive significance to the cold-induced expression of cortisol outside of the general adaptive response.

Aldosterone primarily functions in the Na^+/K^+ balance, and the regulation of the extracellular fluid volume. It has been observed to increase in rats and humans in response to cold stress, arguably as a counter to cold-induced diuresis.[8,9,16,31,32] Alternatively, elevations of aldosterone may be secondary to the up-regulation of the RAAS system and the stress-mediated release of angiotensin II, recognized as an important hormonal component of the stress response.[30] Angiotensin II induces peripheral vasoconstriction by interaction with AT 1 receptors[14,15,20] that when activated in the adrenal cortex also serve to stimulate the production of aldosterone.[2] Whereas vasoconstriction and hypertension associated chronic activation of the RAAS have been linked to cardiac damage in humans,[6,10,26,29] it may be adaptive to dolphins that can undergo pronounced peripheral vasoconstriction in response to diving[23,27] and, presumably, cold exposure. Prolonged peripheral vasoconstriction may be a necessary response to atypical fluctuations in water temperature that enables core body temperature stability when blubber stores are insufficient for adequate insulation from heat loss to the environment.

The relationship between both corticosteroids and water temperature was independent of pool residency time, indicating that the isolation did not contribute to up-regulation of the HPA axis. The finding is not surprising given that the animals participating in the study have a long history of training for transport and residency in pools as part of their participation at the MMP. The animals probably have been desensitized to the pools through this conditioning process. Furthermore, although elevated, the levels of cortisol and aldosterone were substantially lower than those observed in animals under capture and handling stress,[5,25,35,37] suggesting that the cold exposure was not as significant a stressor as was the capture and handling process and that levels were only moderately elevated. Whether elevations in cortisol and aldosterone reflect the general adaptive response to a stressor or indicate a specific adaptive response to cold stress cannot be determined from the results of this study; however, the results are sufficiently intriguing to warrant further investigation into their potential adaptive function.

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