JZAR www.jzar.org JOURNAL OF ZOO AND AQUARIUM RESEARCH

JZAR Research article

OPEN ACCESS

Research article

Plasma concentrations of corticosteroids associated with performance-based physical activities in bottlenose dolphins (*Tursiops truncatus*)

Don R. Bergfelt[1], Sydney Davis[1], Anne Conan[1], Michelle Martinez[2], Maria Vences[2], Rocio Canales[3], and Roberto Sanchez-Okrucky[3]

[1]Ross University School of Veterinary Medicine, Basseterre, Federation of St. Kitts and Nevis
[2]Dolphin Discovery, Basseterre, Federation of St. Kitts and Nevis
[3]Dolphin Discovery, Cancun, Mexico

Correspondence: Don R. Bergfelt; dbergfelt@rossvet.edu.kn

Keywords: aldosterone, bottlenose dolphin, cortisol, dolphin–human swim interactions, metabolism

Article history:
Received: 21 Aug 2019
Accepted: 25 Jan 2020
Published online: 31 Jul 2020

Abstract

This study was designed to evaluate metabolic-related changes in circulating concentrations of cortisol and aldosterone in bottlenose dolphins before vs. after performance-based physical activities associated with dolphin–human swim interactions. Secondarily, samples collected before the swim interactions were used to evaluate the effect of gender, age and aquarium location on corticosteroid concentrations. A total of 31 male and female dolphins 2–9 yr (juveniles) and 12–35 yr (adults) were evaluated from four aquarium locations in the eastern Caribbean: Anguilla (n=8), Grand Cayman (n=9), St Kitts (n=6), Tortola (n=8). Blood samples were collected between 0900 and 1200 after the first swim interaction of the day. Dual samples were collected: the first within 30 min before and the second within 30 min after the swim interaction, which lasted 30–60 min and involved 3–12 human participants for each dolphin. Plasma concentrations of cortisol and aldosterone were analysed using EIA kits validated for use with dolphins. Cortisol concentrations were not significantly affected by gender, age or location. Correspondingly, there was no effect of gender or age on aldosterone concentrations but there was an effect (P=0.02) of location: concentrations were 28% higher in dolphins from Grand Cayman and Tortola vs. Anguilla and St Kitts. Combined across genders, ages and locations, mean concentrations of cortisol after (1,355 pg/mL) vs. before (1,915 pg/mL) the interactive programmes were lower (P=0.01). While mean concentrations of aldosterone were also lower after (41 pg/mL) vs. before (46 pg/mL) the programme, they were not statistically different (P=0.8). The relative decrease in concentrations was 29% for cortisol and 11% for aldosterone. The novelty of these results continues to support the concept that short-term, performance-based physical activities are not necessarily metabolically challenging in bottlenose dolphins conditioned for dolphin–human swim interactions, but more comprehensive studies are required for clarification.

Introduction

Generally, performance-based activities associated with dolphin–human swim interactions involve a variety of programmes with different degrees of physical activity that conditioned dolphins perform, with and without human contact, and in relatively short durations (NMFS 1990). While behavioural changes have been evaluated in multiple studies involving dolphin–human swim interactions (Trone et al. 2005; Jensen et al. 2013; Sew and Todd 2013; Clegg et al. 2018; Brando et al. 2019), evaluation of physiological changes associated with the physical activity of short-term swim interactions are essentially nil (Jensen et al. 2013; Miller et al. 2017). In one study (Jensen et al. 2013), involving a combination of six male and female bottlenose dolphins, there was no statistical difference in mean breathing rate before vs. after an interactive swim programme. In a recent study (Miller et al. 2017), non-targeted proteomic analysis of plasma samples collected before and after the first morning swim interactive programme involving four young, male bottlenose dolphins indicated a statistically significant decrease in mean abundance of a metabolic-related protein (flavin reductase) and an immune-related protein (lysozyme) after the programme. In the same study, mean plasma concentrations of several metabolic-related biochemical constituents (e.g., glucose, creatinine, alkaline phosphatase) also significantly decreased after the morning swim interactive programme. Although few in number, these initial observations suggest that performance-based physical activities associated with dolphin–human swim interactions are not necessarily metabolically challenging. To clarify this concept, additional studies are required to broaden the scope of the potential impact of performance-based physical activities on metabolic-related changes in dolphins involved in dolphin–human swim interactions.

Metabolism encompasses both catabolism and anabolism, which are physiological processes primarily involved in growth and development in response to internal and external environmental changes (review Frayn and Evans 2019). Depending on the degree of physical activity, catabolism breaks down carbohydrates, lipids and proteins to produce energy, whereas anabolism uses the energy to produce many different substances required to maintain homeostasis and support growth and development. The endocrine system plays a major role in metabolism via the hypothalamic-pituitary-adrenal axis such that the adrenal glands produce major steroidogenic hormones involved in these metabolic processes (review Loriaux 2001). In response to adrenocorticotropic hormone (ACTH) from the pituitary, the adrenal glands secrete cortisol, a glucocorticoid involved in the mobilisation of glucose, fatty acids and amino acids as substrates for energy. Correspondingly, aldosterone is secreted from the adrenal glands also in response to ACTH, which is a mineralocorticoid involved indirectly with metabolism by maintaining systemic water and electrolyte balance. Together, cortisol and aldosterone are commonly referred to as corticosteroids.

While corticosteroids are produced by the adrenal gland in response to ACTH, cortisol and aldosterone are secreted by structurally different areas of the gland and, hormonally, have functionally different roles (Loriaux 2001; Frayn and Evans 2019). In a recent study to evaluate the hypothalamic-pituitary-adrenal axis in cetaceans, a standardised acute stress test was conducted where aquarium-based bottlenose dolphins voluntarily beached onto a padded platform and remained out of water for two hours (Champagne et al. 2018). Results indicated increases in circulating concentrations of ACTH, cortisol, and aldosterone during the stress test with each hormone returning to baseline within 2 hours after animals reentered the water. Although cortisol and aldosterone were correlated with ACTH under acute physiological stress, it is not known if the relationship between cortisol and aldosterone would be as strongly coupled under less strenuous conditions.

While there are no peer-reviewed publications available regarding systemic changes in corticosteroids in dolphins involved in dolphin–human swim interactions, there are studies available in other species involved in animal–human interactions. In a study involving horses conditioned to provide riding for pleasure and instruction (Kang and Lee 2016), saliva samples were collected in early morning as reference and, thereafter, in association with riding in late morning, early afternoon and late afternoon. Results indicated mean cortisol concentrations were consistently lower over time in horses involved in horse–rider interactions compared to those that were not involved. In another study involving horses conditioned to provide riding for human therapy, blood samples were collected before and after each one-hour session for 6 days (Malinowski et al. 2018). Results indicated no increase in heart rate variability or differences in cortisol concentrations over time in horses involved or not involved in horse–rider interactions. In contrast to relatively low performance physical activities, high performance sport horse–rider activities (jumping, eventing, trotting, racing, endurance) resulted in statistically significant increases in plasma concentrations of cortisol after vs. before; jumping horses produced the smallest and endurance horses the greatest increases (Linden et al. 1991). Combined, it appears that the level of change in systemic concentrations of cortisol associated with horse–human interactions are related to the strenuous nature of the physical activities. While previous studies in dolphins (Jensen et al. 2013; Miller et al. 2017) suggested that dolphin–human swim interactions are not necessarily metabolically challenging, the degree of change in cortisol and, perhaps, aldosterone in dolphins involved in dolphin–human swim interactions may be supportive of the relatively low or non-strenuous nature of this performance-based activity.

The present study was designed to evaluate metabolic-related changes in circulating concentrations of cortisol and aldosterone in bottlenose dolphins before vs. after short-term, performance-based, physical activities associated with dolphin–human swim interactions. Based on preliminary results of an earlier study (Miller et al. 2017), it was hypothesised that the level of performance activities associated with dolphin–human swim interactions would not result in increases in dolphin plasma cortisol or aldosterone after the swim interactive programmes. Secondarily, blood samples collected before the swim interactions were used to evaluate the effect of gender, age and aquarium location on corticosteroid concentrations.

Methods

Dolphins and dolphin management

The study involved bottlenose dolphins (*Tursiops trunactus*) under the care and management of Dolphin Discovery (Cancun, Mexico). A total of 31 male and female bottlenose dolphins of 2–9 years (juveniles) and 12–35 years (adults) participated in the study, which were located on four different islands in the Eastern Caribbean: The British territories of Anguilla (18.22° N, 63.07° W), Grand Cayman (19.32° N, 81.24° W), Federation of St. Christopher (Kitts) and Nevis (17.36° N, 62.78° W), and Tortola (18.43° N, 64.63° W). Dolphin Discovery-Anguilla (n=8 dolphins) consisted of an open-water enclosure to allow the flow of natural sea water with an estimated volume of 27,110 m^3 and depth that ranged from 3.5 to 6.2 m. Dolphin Discovery-Grand Cayman (n=9 dolphins) consisted of a semi-open enclosure with a concrete pool where natural sea water was constantly pumped in and exchanged at an estimated rate of 4.2 times a day. Estimated water volume was 9,309 m^3 with a depth that ranged from 3 to 3.7 m. Dolphin Discovery-St Kitts (n=6 dolphins) consisted of an open-water enclosure to allow the flow of natural sea water with an estimated volume of 18,614 m^3 and depth of approximately 5.0 m. Dolphin Discovery-Tortola (n=8 dolphins) consisted of an open-water enclosure to allow the flow of natural sea water with an estimated volume of 2,492 m^3 and depth that ranged from 3.5 to 4.0 m.

At each location, dolphins were prescribed an individual diet of wild-caught fish and squid, which was provided periodically throughout the day (early morning to late afternoon) and served, in part, as positive reinforcement for training or performance-based activities. Animals were managed and housed in compliance with the US Animal Welfare Act (AWA 2013), Standards and Guidelines of the Alliance of Marine Mammal Parks and Aquariums (AMMPA 2010), and International Marine Animal Trainers' Association (IMATA 2016). In addition, this study was approved by the Institutional Animal Care and Use Committee (IACUC 15-8-023) of Ross University School of Veterinary Medicine.

Performance-based physical activity

Bottlenose dolphins involved in the present study have been behaviourally and physically conditioned in accord with the International Marine Animal Trainers' Association (IMATA 2016) to do various swim activities when cued by a trainer. In this regard, performance-based activities were defined in accord with dolphin–human swim interactive programmes. Generally, swim interactive programmes involved various degrees of physical activity with and without a human participant such as vocalising, splashing, partial and complete breech, and pulling and pushing human participants. In regard to the present study, overall mean duration of a swim interactive programme was 40 min, which ranged from

https://doi.org/10.19227/jzar.v813.441

Table 1. Mean (±SE) plasma concentrations of cortisol and aldosterone in samples collected in association with the first dolphin–human swim interaction of the day between 0900 and 1200. Samples were collected from each of 30–31 dolphins within 30 min before and, again from the same dolphins, within 30 min after the swim interaction for a total of 60–62 samples. [1]Effect of gender, age and aquaria location on corticosteroid concentrations was evaluated separately using respective hormone concentrations before the dolphin–human swim interactions. [2]Effect of location on aldosterone before the swim interactions was different (P=0.02). [3]Overall, mean cortisol but not aldosterone concentrations before vs after ([xy]) the swim interactions was different (P=0.01). [ab]Mean aldosterone concentrations with uncommon superscripts among locations before the swim interactions are different (P<0.05).

Factors	Cortisol (pg/mL)			Aldosterone (pg/mL)		
Gender	n	Before	After	n	Before	After
Male	16	1743.8±277.1	1330.5±154.2	16	49.9±14.1	48.0±10.8
Female	14	2110.5±297.9	1382.4±178.1	15	42.5±14.7	34.2±5.8
Age						
Juvenile (2–9 years)	15	1999.1±280.2	1337.4±129.7	17	39.4±13.2	43.3±10.2
Adult (12–35 years)	15	1830.6±299.9	1372.1±194.6	14	54.7±15.5	8.9±6.7
Location[2]						
Anguilla	6	1965.0±241.5	1820.3±77.6	8	[a]22.7±3.6	46.5±19.5
Grand Cayman	9	1802.9±180.1	1643.0±176.9	9	[b]39.1±4.8	40.0±6.9
St Kitts	6	2661.3±717.2	1464.8±251.0	6	[a]16.8±5.1	21.8±6.7
Tortola	9	1495.9±391.2	682.6±117.2	8	[b]100.0±32.0	52.3±11.3
Overall[3]	30	[x]1914.9±202.2	[y]1354.7±115.0	31	46.3±10.0	41.3±6.3

30–60 min. Correspondingly, mean number of human participants involved in a programme was eight for each dolphin, which ranged from 3–12, inclusive of children (>8 years) and adults.

Blood collection

Blood samples were collected in association with the first dolphin–human swim interaction of the day during the morning between 0900 and 1200 to avoid or minimise the potential confounding effects of the diurnal rhythm of cortisol as reported in numerous species (Chung et al. 2011), including dolphins (Suzuki et al. 2003). Dual samples were collected from each of 31 dolphins. The first sample was collected within 30 min before and the second sample within 30 min after the swim interaction for a total of 62 samples. Due to management constraints, dolphins not participating in dolphin–human swim interactions were not included in this preliminary study. Hence, samples collected before the swim interactive programmes served as controls for comparison of results after the programmes. Preconditioned behaviour facilitated the voluntary collection of blood samples which consisted of trainers cuing the dolphins to move into a dorsal-down or ventral-up position with the flukes presented to the veterinarian. Blood was collected (approximately 10 mL) via venous-puncture of vessels of the tail flukes using a 21-ga winged BD Vacutainer® Safety-Lok™ Blood Collection Set attached to a Vacutainer® tube containing sodium citrate (BD Company, Franklin Lakes, NJ, USA). Subsequent to collection, samples were placed on crushed ice, transported to the laboratory, and centrifuged at approximately 1350xg for 10 min. Thereafter, plasma was pipetted into Eppendorf cryotubes (2 mL), labelled, and initially stored at −20 or −80°C depending on location. Plasma samples from Anguilla, Grand Cayman, and Tortola were transported frozen to Ross University School of Veterinary Medicine in St Kitts where all samples were stored at −80°C until analysis. Export and import of dolphin plasma samples were in accord with the Convention on International Trade in Endangered Species (CITES Appendix II).

Corticosteroid analysis

Analysis of total concentrations of plasma cortisol was conducted using a commercial enzyme immunoassay kit (EIA; Arbor Assay, K003-H5; Ann Arbor, MI, USA). The kit was validated for use with dolphin plasma using a pool of plasma comprised from several dolphins across genders, ages and aquarium locations. A steroid-liquid sample extraction protocol provided by the kit manufacturer was adapted. Briefly, a solvent:sample ratio of 5:1 (v/v) with 5 mL of ethyl acetate (polar solvent) and 1 mL of the plasma pool were combined in glass tubes, vortexed, set aside at ambient temperature for solvent:sample separation, and placed in a −80°C freezer for approximately 10 min. Subsequent to freezing of the aqueous layer, the solvent layer was pipetted into a second set of glass tubes and evaporated (Thermo Scientific™ Reacti-Vap™ Evaporator, Waltham, MA, USA) under a gently stream of compressed, non-reactive nitrogen gas. Thereafter, extracted samples were resuspended with assay buffer to yield 1:1, 1:2, and 1:4 dilutions to check for parallelism, which is necessary to determine if the immunoreactive response of anti-cortisol to cortisol in the dolphin plasma is similar to the cortisol reference standards (Andreasson et al. 2015). A spike and recovery evaluation was also conducted to determine if the concentration–response relationship was similar between cortisol in the dolphin plasma and cortisol reference standards (Andreasson et al. 2015). Extracts of the plasma pool were spiked with low, medium and high cortisol reference standards provided in the kit. Success of the validation was indicated by visual assessment of linearity of the dilutions that paralleled the standard curve between 20 and

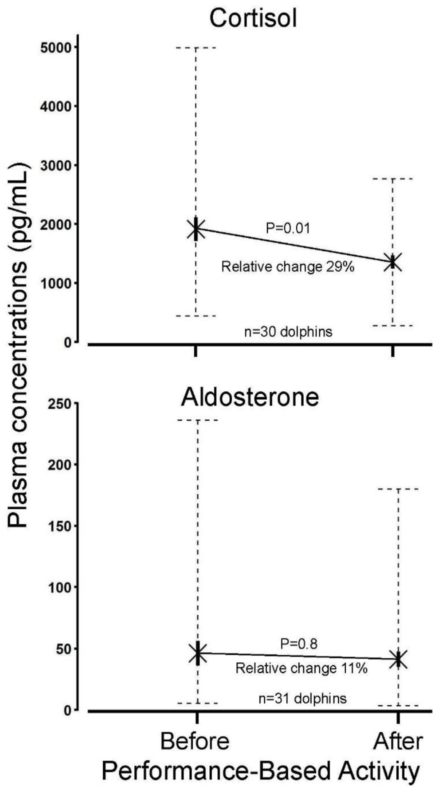

Figure 1. Mean±SE and range (dashed line) in plasma concentrations of cortisol (upper panel) and aldosterone (lower panel) in samples collected in association with the first dolphin–human swim interaction of the day between 0900 and 1200. Samples were collected from each of 30–31 dolphins within 30 min before and, again from the same dolphins, within 30 min after the swim interaction for a total of 60–62 samples. Combined across genders, ages and aquaria locations and compared to corticosteroid concentrations before the swim interactive programmes as reference, mean cortisol decreased by 29% (P=0.01) after the programmes. Although aldosterone concentrations were not significantly different after the interactive programmes, mean concentrations decreased by 11% (P=0.8).

60% specific binding with approximately 16% CV among dilutions and an expected vs. observed recovery rate of approximately 90%.

An analysis of total concentrations of plasma aldosterone was conducted using a commercial enzyme immunoassay kit (EIA; Arbor Assay, K052-H5; Ann Arbor, MI, USA). The kit was validated for use with dolphin plasma using the same plasma pool and extraction process as that used for cortisol. Subsequent to extraction, samples were resuspended and concentrated or diluted at a ratio of plasma:assay buffer 1:0.3 (3x), 1:0.7 (1.5x), and 1:1.3 to check for parallelism. A spike and recovery evaluation was also conducted using extracts of the dolphin plasma pool spiked with low, medium and high aldosterone reference standards provided in the kit. Success of the validation was indicated by visual assessment of linearity of the dilutions that paralleled the standard curve between 68 and 86% specific binding with approximately 22% CV among dilutions and an expected vs. observed recovery rate of approximately 105%.

In accord with the validation results, analysis of corticosteroids in individual dolphin plasma samples were first subjected to extraction and, second, resuspended in assay buffer, diluted two-fold for cortisol and concentrated three-fold for aldosterone. Extracted plasma samples were subsequently analysed in accord with the manufacturer's protocol for respective kits. Based on differences between duplicate samples within assays and differences between corresponding reference standards between assays, intra- and inter-assay CVs were approximately 19% for cortisol and aldosterone. Assay sensitivities were 17.30 pg/mL for cortisol and 4.97 pg/mL for aldosterone.

Statistical analysis

Data analyses were done using the R statistical software (R Core Team 2017). Initially, data were checked for outlying values using Dixon's outlier test and normality using Shapiro–Wilk's test and transformed as necessary. Thereafter, a Kruskal-Wallis test was used to compare cortisol and aldosterone concentrations between genders (male and female) and ages (juvenile and adult) and among aquarium locations (Anguilla, Grand Cayman, St Kitts, Tortola) using hormone concentrations before the dolphin–human swim interactions as reference. If a statistically significant (P≤0.05) effect was detected, a Wilcoxon rank sum test was used to determine mean differences in corticosteroid concentrations between and among variables. A multivariate analysis with a generalised linear mixed regression model was used to compare cortisol and aldosterone concentrations before vs. after the dolphin–human swim interactions. The model was completed with gender and age as fixed effects and aquarium location and dolphin within location as nested random effects. Fitting the regression model involved evaluating multicolinearity among factors with a Variance Inflation Factor and backward elimination of factors. If two or more main effects were detected (P≤0.05), then respective interactions among factors were evaluated.

Results

The effects of gender, age and aquarium location on plasma corticosteroid concentrations were evaluated in samples taken in the morning before the first swim interaction of the day as shown in Table 1. Mean concentrations of cortisol were not significantly different between male vs. female and juvenile vs. adult dolphins or among aquarium locations. Correspondingly, mean aldosterone concentrations were not different between genders and ages but there was an effect among aquarium locations (P=0.02). Mean concentrations were higher in dolphins from Grand Cayman and Tortola vs. Anguilla and St Kitts (P<0.03).

Considering there was only one main effect of aquarium location regarding aldosterone, the need to evaluate interactions with gender and age was not warranted. Hence, combined across genders, ages and locations, circulating concentrations of corticosteroids in samples before vs. after dolphin–human swim interactions were evaluated with the overall results shown in Table 1 and Figure 1. Overall results indicated lower (P=0.01) mean concentrations of cortisol after vs. before the interactive programmes, whereas mean concentrations of aldosterone encompassing the programmes were not different (P=0.8). The relative decrease in mean concentrations after vs. before the swim interactions was 29% for cortisol and 11% for aldosterone (Figure 1).

Discussion

The novelty of the current study involving aquarium-based bottlenose dolphins indicated that, regardless of dolphin genders, ages and aquarium locations in the eastern Caribbean, performance-based, physical activities involving dolphin–human swim interactions were not associated with metabolic-related

Journal of Zoo and Aquarium Research 8(3) 2020
https://doi.org/10.19227/jzar.v813.441

increases in corticosteroids. Instead, there was a significant decrease (29%) in mean plasma concentrations of cortisol and, although not significant, a decrease (11%) in aldosterone after vs. before the swim interactions.

To determine if circulating concentrations of corticosteroids differed between male and female and juvenile and adult bottlenose dolphins as well as among various open-water enclosures located in the eastern Caribbean, blood samples were collected in the morning (0900 and 1200) before the first dolphin–human swim interaction of the day. Without the potential confounding effects of a swim interaction and diurnal rhythm of cortisol as reported in numerous species (Chung et al. 2011), including dolphins (Suzuki et al. 2003), mean plasma concentrations of cortisol were not significantly different between genders and ages or among aquariaum locations. Correspondingly, mean concentrations of aldosterone were not significantly different between genders and ages but were significantly different among locations. The apparent lack of differences in circulating concentrations of corticosteroids between male and female, and juvenile and adult dolphins concurs with that documented in several studies involving free-ranging and aquarium-based dolphins (Thomson and Geraci 1986; St Aubin et al. 1996; Fair et al. 2014; Hart et al. 2015). However, concerning urinary concentrations of corticosteroids in free-ranging bottlenose dolphins, cortisol was higher in females vs. males and both cortisol and aldosterone were higher in juveniles vs. adults (Fair et al. 2014). In the present study, despite relatively close proximity of aquariums in the eastern Caribbean and comparable conditions of blood collection, management and housing, mean aldosterone concentrations were approximately 28% higher in dolphins from Grand Cayman and Tortola vs. Anguilla and St Kitts. The basis for the discrepancy is not known, but differences in circulating concentrations of aldosterone of unknown origin have also been reported in different populations of free-ranging bottlenose dolphins located along the southeast Atlantic coast between North Carolina and Florida (Fair et al. 2014; Hart et al. 2015).

The physical environment of the Caribbean Sea is spatially heterogeneous due, in part, to river plumes, runoffs, upwellings and bathymetric effects (review Chollett et al. 2012). In this regard, the physicochemical environment is highly dynamic with aquatic changes (e.g., salinity) that can influence biological processes (e.g., metabolism) of marine life. In cetaceans, the kidneys have evolved and adapted morphologically and physiologically to accommodate a saline-rich aquatic environment (review Ortiz 2001; Xu et al. 2013). Reportedly, there is a positive correlation between plasma renin activity and aldosterone concentrations in bottlenose dolphins (Malvin et al. 1978). Perhaps, therefore, the renin-angiotensin-aldosterone axis in dolphins is a more sensitive mode of action relative to terrestrial mammals (Loriaux 2001; Frayn and Evans 2019) for the regulation and balance of water and electrolytes needed to maintain homeostasis. In a retrospective study (Ewing et al. 2017), displaced, out-of-habitat bottlenose dolphins were exposed to various low-salinity ocean environments along the southeast Atlantic coast, including the northern coast of the Gulf of Mexico. Results of serum biochemistry indicated sodium, chloride and osmolality were significantly lower, and below reference ranges, in displaced compared to free-ranging case control animals. Although there appears to be no direct evidence, indirectly it is speculated that circulating concentrations of aldosterone would have been elevated to accommodate the imbalance. Nonetheless, in the present study, the observation that circulating concentrations of aldosterone varied among populations of bottlenose dolphins housed in open-water enclosures at different locations in the eastern Caribbean suggests that regional seawater salinity varied among locations. Future studies are required to clarify this concept.

Metabolic-related changes in plasma concentrations of corticosteroids were analysed in dual samples collected before and after the first dolphin–human swim interaction of the day between 0900 and 1200. The first sample was collected within 30 min before the start of the swim interaction and, following a 30–60 min interaction, a second sample was collected from the same animal. Results indicated mean plasma concentrations of cortisol were significantly lower after (1,355 pg/mL) vs. before (1,915 pg/mL) the interactions. Correspondingly, while mean concentrations of aldosterone decreased, they were not significantly different after (41 pg/mL) vs. before (46 pg/mL) the programmes. The decreases in cortisol (29%) and aldosterone (11%) encompassing the swim interactive programmes supported the hypothesis that performance-based, physical activities associated with dolphin–human swim interactions would not result in increases in dolphin plasma corticosteroids. The present results are also supportive of previous results from studies (Jensen et al. 2013; Miller et al. 2017) of similar design involving dolphin–human swim interactions where the performance-based activities of the swim interactive programmes were thought not to be metabolically challenging. In those studies, there was no increase in respiratory rates after vs. before the swim interactions (Jensen et al. 2013) and plasma abundance of flavin reductase and concentrations of glucose, creatinine and alkaline phosphatase were significantly lower after vs. before the swim interactions (Miller et al. 2017). Correspondingly, in horse–human interactions that provide riding for pleasure and instruction (Kang and Lee 2016), mean cortisol concentrations were consistently lower over time in horses involved in horse–rider interactions compared to those that were not involved. In another horse–human interaction that provides riding for human therapy (Malinowski et al. 2018), there was no increase in heart rate variability or differences in cortisol concentrations over time in horses involved or not involved in horse–rider interactions.

In bottlenose dolphins under acute physiological stress, the increases in circulating concentrations of cortisol and aldosterone were highly correlated (Champagne et al. 2018). In the present study, under less strenuous conditions, plasma concentrations of cortisol and aldosterone were coupled with decreases after the dolphin–human swim interactions; however, the decline of 29% for cortisol was more pronounced than the decline of 11% for aldosterone. Concerning cortisol, the degree of change may be related to inherently high concentrations in the morning before the first swim interactive programme of the day. In numerous species (Chung et al. 2011), including bottlenose dolphins (Suzuki et al. 2003), the diurnal rhythm of cortisol is such that systemic concentrations are higher in the morning than afternoon. Other possible influences on the present results might include absence of overnight feeding. As reported, overnight fasting of bottlenose dolphins for 10–14 hours resulted in morning elevations of serum concentrations of glucose, creatinine, alkaline phosphatase, gamma-glutamy transpeptidase, and creatinine kinase (Venn-Watson et al. 2007). In addition, anticipatory responses prior to feeding dolphins leads to excitatory increases such as spy-hopping, more time spent on the water surface, and enhanced attentiveness to trainers (Jensen et al. 2013; Sew and Todd 2013; Clegg et al. 2018; Brando et al. 2019). Concerning aldosterone, the degree of change may have been influenced by the variability of low and high concentrations among dolphins within locations and potentially different environmental conditions of seawater salinity among locations as previously discussed. Thus, considering the influence of these potential factors on the corticosteroid results herein is unknown, future studies are required to clarify their impact.

Multiple studies (Trone et al. 2005; Jensen et al. 2013; Sew and Todd 2013; Clegg et al. 2018; Brando et al. 2019) have evaluated dolphin behaviour in association with dolphin–human swim interactions. Apart from anticipatory behaviour before the start of the swim interactions, essentially each of the previous studies reported the lack of any abnormal or aversive animal behaviour during or after the swim interactive programmes. The relationship of the physiological aspects of cortisol coupled with behavioural aspects associated with dolphin–human swim interactions has apparently not been documented. However, there are reports in other species where cortisol and other hormones (oxytocin, vasopressin) have been used to assess behavioural aspects associated with various animal–human interactions (Shiverdecker et al. 2013; Glenk et al. 2014; Ng et al. 2014; MacLean et al. 2017; Petersson et al. 2017). Although dolphin behaviour was not evaluated in the present study, indirectly, differential changes in plasma concentrations of cortisol before vs. after the dolphin–human swim interactions in the present study appears to support the behavioural observations encompassing the swim interactive programmes in past studies. Future, more comprehensive studies that include both behavioural and physiological aspects of cortisol and, perhaps, other hormones within a study are required to further clarify this concept.

In conclusion, while the results of this study are considered preliminary, the novelty that plasma concentrations of corticosteroids (cortisol and aldosterone) declined in bottlenose dolphins after participating in dolphin–human swim interactive programmes continues to support the concept that short-term, performance-based, physical activities are not necessarily metabolically challenging in conditioned animals.

Acknowledgements

The authors want to thank management, resident veterinarians, animal trainers, and staff at the various Dolphin Discovery locations in Mexico and Eastern Caribbean for providing professional and technical support and availability of dolphins to support this research. In addition, we acknowledge the professional and technical assistance provided by Ms. Silvia Marchi of the research laboratory at Ross University School of Veterinary Medicine (RUSVM) for in-lab validation of the cortisol and aldosterone EIA kits. Portions of this research were supported by an intramural grant from RUSVM and Veterinary Student Scholars Program grant (Project #: D16ZO-612) from Morris Animal Foundation awarded to Dr Sydney Davis.

References

AMMPA (Alliance of Marine Mammal Parks and Aquariums) (2017) AMMPA Accreditation Standards & guidelines. Alexandria, VA. Available at: https://www.ammpa.org/sites/default/files/files/Resource%20 Library/AMMPA-StandardsAndGuidelines-2017.pdf

Andreasson U., Perret-Liaudet A., van Waalwijk van Doorn L.J., Blennow K., Chiasserini D., Engelborghs S., Fladby T., Genc S., Kruse N., Kuiperij H.B., Kulic L., Lewczuk P., Mollenhauer B., Mroczko B., Parnetti L., Vanmechelen E., Verbeek M.M., Winblad B., Zetterberg H., Koel-Simmelink M., Teunissen C.E. (2015) A Practical Guide to Immunoassay Method Validation. *Frontiers in Neurology* 6(179): 1–8. doi: 10.3389/ fneur.2015.00179.eCollection 2015.

AWA (Animal Welfare Act) (2019) Washington, DC: U.S. Department of Agriculture. Available at: https://www.aphis.usda.gov/animal_ welfare/downloads/AC_BlueBook_AWA_508_comp_version.pdf

Brando S., Kooistra, N., Hosey, G. (2019) Pre and post session behaviour of captive bottlenose dolphins Tursiops truncatus involved in "Swim-with-Dolphin" events. J*ournal of Zoo and Aquarium Research 7*(4):195–202.

Champagne C.D., Kellar N.M., Trego M.L., Delehanty B., Boonstra R., Wasser S.K., Booth R.K., Crocker D.E., Houser D.S. (2018) Comprehensive endocrine response to acute stress in the bottlenose dolphin from serum, blubber, and feces. *General and Comparative Endocrinology* 15(266): 178–193. doi: 10.1016/j.ygcen.2018.05.015.

Chollett I., Mumby P.J., Muller-Karger F.E., Chuanmin Huc C. (2012) Physical environments of the Caribbean Sea. *Limnology and Oceanography* 57(4): 1233–1244.

Chung S., Son G.H., Kim K. (2011) Circadian rhythm of adrenal glucocorticoid: Its regulation and clinical implications. *Biochimica et Biophysica Acta* 1812: 581–591.

CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). "*The CITES Appendices*". Retrieved 13 September 2013. https://cites.org/eng/app/index.php.

Clegg I.L.K., Rödel H.G., Boivin X., Delfour F. (2018) Looking forward to interacting with their caretakers: dolphins' anticipatory behaviour indicates motivation to participate in specific events. *Applied Animal Behaviour Science* 202: 85–93.

Ewing R.Y., Mase-Guthrie B., McFee W., Townsend F., Manire C.A., Walsh M., Borkowski R, Bossart G.D., Schaefer A.M. (2017) Evaluation of Serum for Pathophysiological effects of prolonged low salinity water exposure in displaced bottlenose dolphins (*Tursiops truncatus*). *Frontiers in Veterinary Science* 4: 80. doi: 10.3389/fvets.2017.00080.

Fair P.A., Schaefer A.M., Romano T.A., Bossart G.D., Lamb S.V., Reif J.S. (2014) Stress response of wild bottlenose dolphins (Tursiops truncatus) during capture-release health assessment studies. *General and Comparative Endocrinology* 206: 203–12.

Frayn K.N., Evans R.D. (2019) *Human metabolism: A regulatory perspective.* New Jersey: Wiley-Blackwell, 365.

Glenk L.M., Kothgassner O.D., Stetina B.U., Palme R., Kepplinger B., Baran H. (2014) Salivary cortisol and behavior in therapy dogs during animal-assisted interventions: A pilot study. *Journal of Veterinary Behavior* 9(3): 98–106.

Hart L.B., Wells R.S., Kellar N., Balmer B.C., Hohn A.A., Lamb S.V, Rowles T., Zolman E.S., Lori H., Schwacke L.H. (2015) Adrenal Hormones in Common Bottlenose Dolphins (*Tursiops truncatus*): Influential Factors and Reference Intervals. *PLoS ONE* 10(5): e0127432. doi:10.1371/ journal. pone.0127432.

Hill E.E., Zack E., Battaglini C., Viru M., Viru A., Hackney A.C. (2008) Exercise and circulating cortisol levels: The intensity threshold effect. *Journal of Endocrinological Investigation* 31: 587–591.

IMATA (International Marine Animal Trainers' Association). (2020) IMATA's mission statement. Available at: https://www.imata.org/mission_ values

Jensen A.L.M., Delfour F., Carter T. (2013) Anticipatory Behavior in Captive Bottlenose Dolphins (*Tursiops truncatus*): A Preliminary Study. *Zoo Biology* 32: 436–444.

Kang O.D., Lee, W.S. (2016) Changes in salivary cortisol concentration in horses during different types of exercise. Asian Australas. *Journal of Animal Science* 29(5): 747–752.

Linden A., Art T., Amory H., Desmecht D., Lekeux P. (1991) Effect of 5 different types of exercise, transportation and ACTH administration of plasma cortisol concentration in sport horses. *Equine Exercise Physiology* 3: 391–396.

Loriaux D.L. (2002) The adrenal gland. In: Becker, K.L, Kahn, C.R., Rebar, R.W. (eds). *Principles and practice of endocrinology and metabolism.* Philadelphia, Pennsylvania: Lippincott Williams and Wilkins, 2477.

MacLean E.L., Gesquiere L.R., Gee N.R., Levy K., Martin W.L., Carter C.S. (2017) Effects of Affiliative Human–Animal Interaction on Dog Salivary and Plasma Oxytocin and Vasopressin. *Frontiers in Psychology* 8: 1–9. doi.org/10.3389/fpsyg.2017.01606.

Malinowski K., Yee C., Tevlin J.M., Birks E.K., Durando M.M., Pournajafi-Nazarloo H., Cavaiola A.A., McKeever K.H. (2018) The Effects of Equine Assisted Therapy on Plasma Cortisol and Oxytocin Concentrations and Heart Rate Variability in Horses and Measures of Symptoms of Post-Traumatic Stress Disorder in Veterans. *Journal of Equine Veterinary Science* 64: 17–26. https://doi.org/10.1016/j.jevs.2018.01.011.

Malvin R.L., Ridgway S.H., Cornell L. (1978) Renin and aldosterone levels in dolphins and sea lions. *Proceedings of the Society of Experimental Biology and Medicine* 157: 665–668.

McCullough A., Jenkins M., Ruehrdanz A., Gilmer M.J., Olson J., Pawar A., Holley L., Sierra-Rivera S., Linder D.E., Pinchette D., Grossman N.J., Hellman C., Guérin N., O'Haire M. (2018) *Physiological and behavioral effects of animal-assisted interventions for therapy dogs in pediatric oncology settings.* Department of Comparative Pathobiology Faculty Publications. Paper 37. https://docs.lib.purdue.edu/cpbpubs/37.

Miller B.A., Nanni P., Fortes C., Grossmann J., Arreola M.R., Vences M., Canales R., Sanchez-Okrucky R., de Almeida A.M., Bergfelt D.R. (2017) Plasma proteome and clinical biochemistry associated with performance-based physical activity in bottlenose dolphins (*Tursiops truncatus*). *Aquatic Mammals* 43: 453–464.

Petersson M., Uvnäs-Moberg K., Nilsson A., Gustafson L.L.,Hydbring-Sandberg E., Handlin L. (2017) Oxytocin and Cortisol Levels in Dog Owners and Their Dogs Are Associated with Behavioral Patterns: An Exploratory Study. *Frontiers in Psychology* 8: 1–8. doi.org/10.3389/fpsyg.2017.01796.

Ng Z.Y., Pierce B.J., Otto C.M., Buechner-Maxwell V.A., Siracusa C., Were S.R. (2014) The effect of dog–human interaction on cortisol and behavior in registered animal-assisted activity dogs. *Applied Animal Behaviour Science* 159: 69–81.

NMFS (National Marine Fisheries Service) (1990) *The Use of Marine Mammals in Swim-with-the-Dolphin Programs.* Final Environmental Impact Statement. Silver Spring, Maryland: NMFS Office of Protected Resources, 264.

Ok-Deuk K., Wang-Shik L. (2016) Changes in salivary cortisol concentration in horses during different types of exercise. Asian-Australas. *Journal of Animal Sciences* 29(5): 747–752. http://dx.doi.org/10.5713/ajas.16.0009.

Ortiz R.M. (2001) Review: Osmoregulation in marine mammals. The *Journal of Experimental Biology* 204: 1831–1844.

R Core Team (2017) "R: A Language and Environment for Statistical Computing" Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org/.

Shiverdecker M.D., Schiml P.A., Hennessy M.B. (2013) Human interaction moderates plasma cortisol and behavioral responses of dogs to shelter housing. *Physiology and Behavior* 109(17): 75–79.

Sew G., Todd P.A. (2013) The effects of human-dolphin interaction programmes on the behaviour of three captive indo-pacific humpback dolphins (*Sousa chinensis*). *The Raffles Bulletin of Zoology* 61(1): 435–442.

St. Aubin D.J., Ridgway S.H., Wells R.S., Rhinehart H.L. (1996) Dolphin thyroid and adrenal hormones: circulating levels in wild and semidomesticated *Tursiops truncatus*, and influence of sex, age, and season. *Marine Mammal Science* 12(1): 1–13.

Suzuki M., Uchida S., Ueda K., Tobayama T., Katsumata E., Yoshioka M., Aida K. (2003) Diurnal and annual changes in serum cortisol concentrations in Indo-Pacific bottlenose dolphins *Tursiops aduncus* and killer whales *Orcinus orca. General and Comparative Endocrinology* 132(3): 427–33.

Thomas C.A., Geraci J.R. (1986) Cortisol, aldosterone and leucocytes in the stress response of bottlenose dolphins, *Tursiops truncatus. Canadian Journal of Fisheries and Aquatic Sciences* 43: 1010–1016.

Trone M., Kuczaj S., Solangi M. (2005) Does participation in Dolphin–Human Interaction Programs affect bottlenose dolphin behaviour? *Applied Animal Behaviour Science* 93: 363–374.

Venn-Watson S.K., Ridgway S.H. (2007) Big Brains and Blood Glucose: Common Ground for Diabetes Mellitus in Humans and Healthy Dolphins. *Comparative Medicine* 57(4): 390–395.

Xu S., Yang Y., Zhou X., Xu J., Zhou K., Yang G. (2013) Adaptive evolution of the osmoregulation-related genes in cetaceans during secondary aquatic adaptation. *BMC Evolutionary Biology* 13: 189 http://www.biomedcentral.com/1471-2148/13/189.