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Cortisol, Lactate, and Ammonia Plasma Concentrations Associated with Performance-Based Physical Activities in Bottlenose Dolphins (*Tursiops truncatus*)

Don R. Bergfelt,[1] Maria Vences,[2] Danielle Merritt,[1] Marcus Machado,[1] Julienne Rawlins,[1] Luis Pablo Hervé-Claude,[1] Loveness Dzikiti,[1] Rocio Canales,[3] and Roberto Sanchez-Okrucky[3]

[1]*Ross University School of Veterinary Medicine, Basseterre, Federation of St. Kitts and Nevis, West Indies*
E-mail: dbergfelt@rossvet.edu.kn
[2]*Dolphin Discovery, Federation of St. Kitts and Nevis, West Indies*
[3]*Dolphin Discovery, Cancun, Mexico*

Abstract

Evaluation of metabolic characteristics encompassing short-term performance-based physical activities has fundamental and practical implications to enhance management practices associated with dolphin–human swim interactions. A total of four male and six female bottlenose dolphins (*Tursiops truncatus*) were involved in the study. All 10 animals were first assigned to a no-swim interaction group as a control group and, after a period of 5 to 77 days of routine training and performance activities, animals were assigned to a dolphin–human swim interaction group. For the swim interaction group, the first or early morning session (0842 to 1005 h) and second or late afternoon session (1354 to 1454 h) involved various degrees of physical activities with and without human participants. For the no-swim interaction group, dolphins swam freely in separate pens prior to feeding and collection of blood samples at corresponding times in the morning and afternoon. Blood samples were collected from both groups within 15 minutes before and after the morning and afternoon sessions. Plasma concentrations of cortisol, lactate, and ammonia were determined using enzyme immunoassay and clinical chemistry techniques. Mean cortisol concentrations were lower after the early morning session ($p < 0.021$) by 56% and after the late afternoon session ($p < 0.004$) by 43%. Conversely, and although not statistically significant, mean lactate concentrations were higher after the morning session by 18% and after the afternoon session by 19%. There was also a decrease in ammonia concentrations in the no-swim interaction group during the afternoon session. Regardless of groups and times, there was a temporal negative correlation (r = -0.359, $p < 0.0015$) between cortisol and lactate such that cortisol decreased ($p < 0.05$) by 59% from the morning to afternoon and, conversely, lactate increased ($p < 0.05$) by 68% from the morning to afternoon. While relevance of the inverse relationship between cortisol and lactate requires clarification, the results continue to support the concept that short-term, performance-based physical activities are not metabolically challenging in bottlenose dolphins that are trained and conditioned for dolphin–human swim interactions.

Key Words: dolphin–human swim interactions, cortisol, lactate, ammonia, bottlenose dolphin, *Tursiops truncatus*

Introduction

In a recent line of studies (Miller et al., 2017; Bergfelt et al., 2020; Matsushiro et al., 2021) evaluating metabolic changes associated with performance-based physical activities, a preliminary study (Miller et al., 2017) involving four young male bottlenose dolphins (*Tursiops truncatus*) was conducted using a non-targeted approach to determine the plasma proteome in blood samples collected approximately 30 min before and after the first morning dolphin–human swim interaction. Results identified 196 proteins of which 13% were related to metabolic activities. Mean abundance of a metabolic-related protein (flavin reductase) was significantly lower after the swim interaction. In the same study, mean plasma concentrations of several metabolic-related biochemical constituents (e.g., glucose, creatinine, alkaline phosphatase) were also significantly lower after the swim interaction. In a more comprehensive follow-up study (Bergfelt et al., 2020) involving a total of 31 juvenile and adult male and female bottlenose dolphins at several different aquariums in the Eastern Caribbean, a targeted approach was conducted to evaluate metabolic-related changes in plasma concentrations of corticosteroids (cortisol and aldosterone) in blood samples collected approximately 30 min before and after the first morning dolphin–human swim interactions. With little or no statistically significant effect of age, sex, or location on concentrations of corticosteroids, analysis of the combined data indicated a significant decrease in mean concentrations of cortisol (29%) and, although not significant, there was a decrease in aldosterone (11%) after the swim interaction. The reproducibility of these results was supported in a more recent study conducted by other investigators (Matsushiro et al., 2021) involving nine adult female aquarium-based bottlenose dolphins. Compared to plasma concentrations before the swim interactions, cortisol was significantly lower after the swim interactions during the morning and afternoon sessions. The basis for higher concentrations of cortisol before compared to after dolphin–human swim interactions is not known.

In numerous species (Chung et al., 2011), including bottlenose dolphins (Suzuki et al., 2003; Matsushiro et al., 2021), a diurnal rhythm of cortisol has been observed such that systemic concentrations are higher in the morning than in the afternoon, which may be influenced by overnight fasting (Venn-Watson & Ridgway, 2007). The potential confounding effects of a diurnal rhythm on cortisol was not addressed in previous studies (Miller et al., 2017; Bergfelt et al., 2020; Matsushiro et al., 2021) where concentrations were higher before compared to after dolphin–human swim interactions. Thus, a more robust study design is proposed herein to include collection of blood samples before and after the first or early morning and second or late afternoon dolphin–human swim interactions.

Voluntary muscle movement associated with locomotion or physical activity requires energy (adenosine triphosphate [ATP]) which involves a series of integrated biochemical changes and bodily responses from major systems such as the metabolic, endocrine, respiratory, and cardiovascular pathways to maintain homeostasis (Yap et al., 2017). Generally, physical activity is predominantly an aerobic glycolytic process involving glucose and oxygen to produce ATP. As intensity, duration, or frequency of physical activities increase, there may be an excessive consumption or shortage of ATP that leads to a more predominant anaerobic glycolytic process to maintain homeostasis where, under conditions of reduced oxygen availability, glucose or glycogen is converted to lactate and subsequently to ATP (Nalbandian & Takeda, 2016). Correspondingly, excess consumption ATP under intense physical activity can lead to homeostatic imbalance and deamination of adenosine monophosphate (AMP) and catabolism of branched-chain amino acids in skeletal muscles. Unlike lactate, ammonia is a waste product primarily from the metabolism of AMP. Nonetheless, changes in blood concentrations of lactate and ammonia in human and animal athletes are considered well-known biomarkers to assess the intensity of training regimens and to monitor performance activities (Finsterer, 2012). While blood concentrations of lactate have been evaluated in cetaceans for assessing the physiological status associated with open-water swimming and diving (Williams et al., 1993, 1999; Shaffer et al., 1997), there is no apparent documentation for using blood concentrations of ammonia in dolphins as a biomarker to assess performance-based physical activities involved in dolphin–human swim interactions.

The present study was designed as a more comprehensive approach to evaluate the effect of dolphin–human swim interactions on blood concentrations of cortisol, lactate, and ammonia in samples collected before and after the first or early morning and second or late afternoon swim interactions. In addition, the design included collection of samples at corresponding times from animals not involved in dolphin–human swim interactions.

Methods

Dolphins and Dolphin Management

The study involved bottlenose dolphins under the care and management of Dolphin Discovery-St. Kitts (Federation of St. Kitts and Nevis [officially the Federation of Saint Christopher and Nevis], 17.36° N, 62.78° W). A total of 10 bottlenose dolphins, male ($n = 4$) and female ($n = 6$) as juveniles (6 to 9 y, $n = 6$) and adults (11 to 38 y, $n = 4$), participated in the study. Dolphin Discovery-St. Kitts is an ocean-water enclosure that allows free flow of water from the Atlantic Ocean with an estimated volume of 18,614 m^3 and maximum depth of approximately 5.0 m.

Dolphins were prescribed individual diets of fresh/frozen imported wild-caught fish and squid which were provided in respective buckets and fed periodically throughout the day and served, in part, as positive reinforcement for performance or training activities. Animals were managed and housed in compliance with the Standards and Guidelines of the Alliance of Marine Mammal Parks and Aquariums (AMMPA) (2017), U.S. Animal Welfare Act (AWA) (U.S. Department of Agriculture, 2019), and International Marine

Animal Trainers' Association (IMATA) (2020). 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 (RUSVM).

Experimental Groups

Bottlenose dolphins have been behaviorally and physically trained and conditioned in accord with IMATA's (2020) guidelines to do various swim activities when cued by a trainer. In the context of this study, performance-based physical activities were defined in accord with dolphin–human swim interactions described by the National Marine Fisheries Service (NMFS) (1990). Generally, swim interactions involved various degrees of physical activities with and without human participants such as vocalizing, splashing, partial and complete breech, and pulling and pushing participants.

Each of the 10 dolphins participated in a 1-d event that involved dolphin–human swim interactions (swim interaction group) or no-swim interactions (no-swim interaction group) where the latter served as controls. Typically, four dolphin–human swim interactions are conducted in a day. In the present study, the first or early morning and second or late afternoon swim interactions were under study. The number of participants in the dolphin–human swim interactions during the early morning session ranged from six to 15 with a duration from 38 to 65 min (mean: 51.1 min); and during the late afternoon session, participants ranged from two to seven with a duration from 25 to 48 min (mean: 37.9 min), all of which included children (> 8 y) and adults. The second or late morning and first or early afternoon swim interactions not under study generally involved a corresponding number of participants for about the same duration. The early morning dolphin–human swim interactions under study occurred at the beginning of the day between 0842 to 1005 h (range: 38 to 65 min), and the late afternoon swim interactions occurred at the end of the day between 1354 to 1454 h (range: 25 to 48 min).

As a control group, all dolphins were first assigned to a no-swim interaction group and, after a period of 5 to 77 d (mean: 32 d) of routine training and performance activities, all dolphins were assigned to a dolphin–human swim interaction group. On the day of study, dolphins in the no-swim interaction group were separated from the swim interaction group and swam freely within the oceanarium until they engaged with trainers for feeding and collection of blood samples at corresponding times to those dolphins in the swim interaction group.

Blood Collections

Blood samples were collected from both groups in the early morning between 0851 to 1125 h (range: 38 to 76 min) and late afternoon between 1345 to 1510 h (range: 25 to 67 min). Samples were collected within 15 min before and 15 min after the morning and afternoon sessions. Preconditioned behavior facilitated the voluntary collection of blood samples which consisted of trainers cueing 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 venipuncture 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 1,350 × g for 10 min. Thereafter, plasma was pipetted into Eppendorf cryotubes (2 mL; Eppendorf, Hamburg, Germany), labelled, and stored at -80°C until analysis.

Cortisol, Lactate, and Ammonia Analysis

Analysis of total plasma concentrations of cortisol (pg/mL) was conducted using a commercial enzyme immunoassay (EIA) kit (K003-H1; Arbor Assay, Ann Arbor, MI, USA). The kit was previously validated for use with dolphin plasma (Bergfelt et al., 2020). Briefly, an extraction process involved a solvent:sample ratio of 5:1 (v/v) where 5 mL of diethyl ether and one mL of plasma were combined in a set of glass tubes, vortexed (approximately 1 min), set aside at ambient temperature for solvent:sample separation, and placed in a -80°C freezer for approximately 10 min. After freezing, the solvent layer was decanted into a corresponding set of 2-mL conical Eppendorf tubes and evaporated using a Speed-vac (CentriVap Centrifugal Vacuum Concentrator; Labconco Corp., Kansas City, MO, USA) for 2 to 3 h or until dry. Thereafter, the extracted and dried samples were resuspended initially with 100% ethanol (5% v/v) followed by assay buffer to yield a 1:1 dilution. Assay performance as assessed over two assays indicated intra- and inter-assay CVs of 6.8 and 6.4%, respectively, with a mean sensitivity of 51.96 pg/mL at 85 to 90% specific binding.

Analysis of plasma concentrations of lactate (mmol/L) and ammonia (µmol/L) were determined using an IDEXX Catalyst Dx Chemistry Analyzer (IDEXX Laboratories, Westbrook, ME, USA), with dry, single slide technology based on 100 uL of plasma (IDEXX Laboratories, 2020).

Statistical Analysis

Data analyses were done using *Stata 16* statistical software (StataCorp LLC, College Station, TX, USA). Linear regression for clustered data with "animal" as the clustering variable was used to adjust for using the same animals under different experimental conditions. Univariate linear regression analysis for clustered data inclusive of the dolphin–human swim and no-swim interaction groups was used to evaluate the effect of sex (male vs female) and age (juvenile vs adult) on cortisol, lactate, and ammonia concentrations in samples collected before the swim and no-swim interactions during the first or early morning and second or late afternoon sessions. Univariate linear regression analysis for clustered data inclusive of groups (swim and no-swim interactions) and times (before vs after the swim and no-swim interactions) was used to evaluate the effect of morning vs afternoon on cortisol, lactate, and ammonia. Multivariable linear regression for clustered data was used to evaluate the main effects of group, time, and group-by-time interactions on cortisol, lactate, and ammonia concentrations. Spearman's rank correlation was used to evaluate any temporal relationships between concentrations of cortisol, lactate, and ammonia. A p value of $p \leq 0.05$ indicated that a difference was statistically significant and, unless otherwise indicated, data are presented as the mean ± SE.

Results

All blood sample collections were completed for each of 10 dolphins in the no-swim interaction group (control) and nine dolphins in the dolphin–human swim interaction group; one animal was unwilling to volunteer for blood collection. Samples were collected before and after the swim and no-swim interactions during the early morning and late afternoon sessions for a total of 76 individual samples or 38 paired samples.

Combining the data for the swim and no-swim interaction groups and focusing on samples collected before the swim and no-swim interactions, there was an effect of age ($p < 0.033$) on cortisol concentrations during the early morning sessions such that mean concentrations were higher in juveniles (4,107.17 ± 719.59 pg/mL) vs adults (2,415.75 ± 122.59 pg/mL). Conversely, there was no effect ($p > 0.05$) of sex and age on lactate and ammonia concentrations during the early morning sessions or on cortisol, lactate, and ammonia concentrations during the late afternoon sessions. Although there was an effect of age on cortisol during the morning but not afternoon sessions and no other effects of age and sex on cortisol, lactate, and ammonia, these variables were combined in subsequent analyses.

During the late afternoon but not the early morning sessions, there was a main effect of group ($p < 0.027$) on cortisol such that mean concentrations were lower in the swim interaction group (994.91 ± 104.59 pg/mL) vs the no-swim interaction group (1,497.28 ± 158.29 pg/mL) as indicated in Figure 1 (upper left panel). Regardless of group, there were main effects of time on cortisol during the morning and afternoon sessions such that mean cortisol concentrations were higher ($p < 0.021$) before (4,258.63 ± 717.70 pg/mL) vs after (1,856.14 ± 157.47 pg/mL) the morning sessions and, correspondingly, higher ($p < 0.004$) before (1,608.84 ± 166.73 pg/mL) vs after (909.78 ± 56.96 pg/mL) the afternoon sessions (Figure 1, upper left panel).

Although there were no main effects of group ($p = 0.572$ to $p = 0.182$) or time ($p = 0.069$ to $p = 0.413$) on lactate concentrations during the early morning and late afternoon sessions, respectively (Figure 1, middle left panel), mean concentrations were lower before (0.92 ± 0.09 mmol/L) vs after (1.09 ± 0.09 mmol/L) the morning sessions and, correspondingly, lower before (1.55 ± 0.12 mmol/L) vs after (1.85 ± 0.14 mmol/L) the afternoon sessions.

The main effect of time ($p < 0.004$) on ammonia during the late afternoon sessions corresponded with a group-by-time interaction ($p < 0.005$) which was attributed to the differential changes in concentrations in samples collected before vs after in the swim and no-swim interaction groups (Figure 1, lower left panel).

Inclusive of the swim and no-swim interaction groups and times before vs after the swim and no-swim interactions, mean concentrations of cortisol were lower ($p < 0.05$) during the late afternoon (1,259.31 ± 104.18 pg/mL) vs higher during the early morning (3,057.39 ± 412.70 pg/mL) sessions as indicated in Figure 1 (upper right panel). Conversely, mean concentrations of lactate were higher ($p < 0.05$) during the afternoon (1.70 ± 0.10 mmol/L) vs lower during the morning (1.01 ± 0.01 mmol/L) sessions (Figure 1, middle right panel). Inclusive of groups, times, and sessions, there was a negative correlation (r = -0.359, $p < 0.0015$) between a decrease in cortisol and an increase in lactate concentrations among individual dolphins.

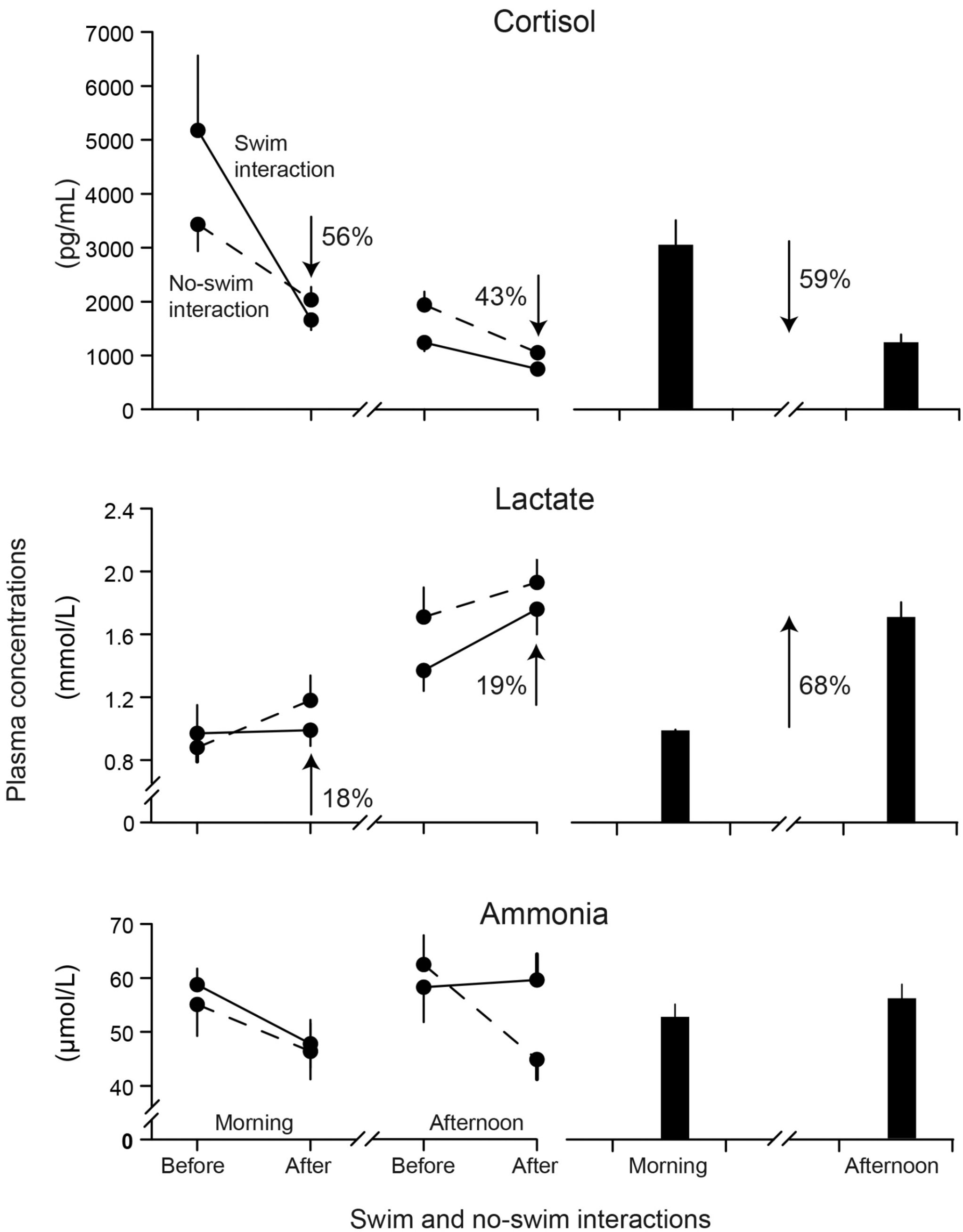

Figure 1. Mean (± SE) concentrations of cortisol, lactate, and ammonia in bottlenose dolphins (*Tursiops truncatus*) first assigned to a no-swim interaction group as controls (n = 10) and, after 5 to 77 d, assigned to a dolphin–human swim interaction group (n = 9). Blood samples were collected 15 min before and after the swim interactions and at corresponding times in association with no-swim interactions during the early morning and late afternoon sessions. Statistical analyses are as follows: For cortisol, upper left panel, there was a main effect of group ($p < 0.027$) in the afternoon, and main effect of time in the morning ($p < 0.021$) and afternoon ($p < 0.004$); and, in the upper right panel, overall cortisol concentrations in the afternoon were lower ($p < 0.05$) than in the morning. For lactate, middle right panel, overall concentrations in the afternoon were higher ($p < 0.05$) than in the morning. For ammonia, lower left panel, there was a main effect of time ($p < 0.004$) and group-by-time interaction ($p < 0.005$) in the afternoon.

Discussion

Prior to the analysis of the effect of dolphin–human swim interactions on plasma concentrations of cortisol, lactate, and ammonia, results indicated there was no significant effects of age (juvenile vs adult) or sex on these hormonal and biochemical factors during the early morning or late afternoon sessions except for significantly higher (52%) mean concentrations of cortisol in juvenile compared to adult dolphins in the morning but not afternoon sessions. While the effect of age on cortisol may require clarification in association with the diurnal rhythm of cortisol in bottlenose dolphins (Suzuki et al., 2003; Matsushiro et al., 2021), the present results generally concur with previous results in aquarium-based, free-ranging, and semidomesticated bottlenose dolphins that cortisol is not affected by age or sex (St. Aubin et al., 1996).

There was a significant main effect of time on concentrations of cortisol during both the early morning and late afternoon sessions. Mean concentrations were lower compared to before the swim and no-swim interactions. Mean concentrations of cortisol consistently decreased 56 and 43% during the morning and afternoon sessions, respectively; and overall, concentrations significantly decreased (59%) from early morning to late afternoon. The present results support previous results (Bergfelt et al., 2020) of a decrease in cortisol after dolphin–human swim interactions during the morning sessions and, in a more recent study by other investigators (Matsushiro et al., 2021), during the morning and afternoon sessions. Furthermore, as in other mammalian species (Chung et al, 2011), including dolphins (Suzuki et al., 2003; Matsushiro et al., 2021), the present results concur that cortisol has a diurnal rhythm in bottlenose dolphins. The novelty of the present results indicated that the decrease in cortisol is not related to dolphin–human swim interactions since cortisol decreased comparably in both the swim and no-swim interaction groups. While the results suggest that the decrease in cortisol may be, in part, due to higher concentrations of cortisol before the early morning session in association with diurnal changes (Suzuki et al., 2003; Matsushiro et al., 2021), this concept does not necessarily provide a basis for higher concentrations of cortisol before the afternoon sessions. Dolphin behavioral anticipatory responses such as increased spy-hopping, more time spent on the water surface, and enhanced attentiveness to trainers preceding performance or training activities have been well documented (Trone et al., 2005; Jensen et al., 2013; Sew & Todd, 2013; Clegg et al., 2018; Brando et al., 2019), but apparently not in relation to cortisol. In other animals, saliva or blood cortisol concentrations increased prior to competition to obtain food in male bonobos or pygmy chimpanzees (Wobber et al., 2010), training and sport racing in horses (Bohák et al., 2018), and competitive sports in human males and females (van Paridon et al., 2017). Therefore, perhaps the relatively high concentrations of cortisol before the early morning sessions in the swim and no-swim interaction groups was attributed to a combination of diurnal changes (Suzuki et al., 2003; Matsushiro et al., 2021), overnight fasting (Venn-Watson & Ridgway, 2007), and anticipatory responses (Trone et al., 2005; Jensen et al., 2013; Sew & Todd, 2013; Clegg et al., 2018; Brando et al., 2019), whereas the relatively high concentrations of cortisol before the late afternoon sessions in both groups was primarily attributed to anticipatory responses preceding feeding, performance, or training. While this concept needs to be clarified in future studies, there is no evidence indicated herein or in previous studies (Bergfelt et al., 2020; Matsushiro et al., 2021) that circulating concentrations of cortisol are elevated after dolphin–human swim interactions.

There were no significant main effects of group or time on concentrations of lactate; nonetheless, mean concentrations increased during the early morning and late afternoon sessions by 18 and 19%, respectively. Overall, mean concentrations of lactate were significantly higher (68%) in the afternoon (1.70 mmol/L) compared to the morning (1.01 mmol/L). Previous studies (Williams et al., 1993, 1999; Shaffer et al., 1997) have evaluated plasma concentrations of lactate in association with exercise physiology in adult male and female bottlenose dolphins and whales during open-water swimming, diving, and static swims pushing against a loaded, padded cell. In Atlantic bottlenose dolphins (Williams et al., 1993), mean concentrations of lactate increased from approximately 2.5 mmol/L at rest to about 2.9 mmol/L (16%) and 3.4 mmol/L (36%) after swimming at 2.1 and 2.9 m/s, respectively, for 10 to 25 min. In Pacific bottlenose dolphins (Williams et al., 1999), mean concentrations of lactate increased from approximately 1.1 mmol/L at rest to about 1.9 mmol/L (72%) and 3.8 mmol/L (245%) after diving 60 and 210 m, respectively. Again, in Atlantic bottlenose dolphins involved in static exercise with a load of 202 kg (Williams et al., 1993), results indicated an increase in mean concentrations of lactate from approximately 2.5 mmol/L at rest to about 12.4 mmol/L (396%) after 4 min. In white whales or belugas (*Delphinapterus leucas*; Shaffer et al., 1997), mean concentrations of lactate increased from approximately 0.7 mmol/L at rest to about 1.8 mmol/L (157%) after swimming at 2.1 to 2.8 m/s for 10 min. According to the authors (Williams et al., 1993), while there was a reliance on the anaerobic process, the increases in lactate concentrations were not exceptionally high for exercising dolphins.

In the present study, although there were four dolphin–human swim interactions throughout the day, only the first or early morning and second or late afternoon swim interactions were studied. Comparably, dolphins in the no-swim interaction group swam freely in the oceanarium except for periods of feeding and blood collections. While there was an apparent difference in the extent of physical activities between the swim and no-swim interaction groups, concentrations of lactate increased among individual dolphins, ranging from 0.01 to 1.89 mmol/L in 28 of 38 (74%) paired samples encompassing both groups during the morning and afternoon sessions. Although the environmental conditions, management of the dolphins, and other aspects were vastly different, absolute concentrations of lactate and percentage increases in concentrations herein are within the range of resting concentrations or low range percentage increases reported in previous studies (Williams et al., 1993, 1999; Shaffer et al., 1997). Hence, by inference to previous results (Williams et al., 1993, 1999; Shaffer et al., 1997) involving lactate physiology in exercising dolphins and whales, the present results with slight increases in lactate within the morning and afternoon sessions of 18 and 19%, respectively, or an overall increase of 68% from the morning to the afternoon were considered minimal or not exceptionally high for these juvenile and adult bottlenose dolphins trained and conditioned for performance-based physical activities.

In an earlier study by Miller et al. (2017) involving early morning dolphin–human swim interactions, mean plasma concentrations of glucose were significantly lower (11%) after the swim interaction. In white whales (Shaffer et al., 1997), glucose decreased 5 to 15% from resting values following an open water swim for 10 min at 2.1 to 2.8 m/s. In the present study, dolphins were fed periodically throughout the day and, although glucose was not evaluated, the increase in lactate suggests there were shifts from aerobic to anaerobic ATP production (Nalbandian & Takeda, 2016) in the swim and no-swim interaction groups throughout the day to maintain homeostasis. The basis for the overall increase (68%) in lactate by late afternoon is not known. Speculatively, however, lactate production may have exceeded metabolism in the swim and no-swim interaction groups such that there was a gradual accumulation of residual lactate starting from early morning that resulted in higher concentrations by late afternoon. Other future studies are required to clarify this concept as well as to address various physiological adaptations (e.g., lactate dehydrogenase activity) that might be associated with training and conditioning dolphins for performance-based physical activities.

There was a slight but significant negative correlation ($r = -0.359$) between a temporal decrease in cortisol and an increase in lactate concentrations from early morning to late afternoon. The physiological relevance of the inverse relationship associated with performance-based physical activities in bottlenose dolphins has not been previously observed and does not appear to be supported from results in other species. In young female thoroughbred horses that were beginning training to be mounted for racing (Nogueira et al., 2002) and adult physically active human males in response to incremental exercise testing (Port, 1991), a significant positive correlation was observed between serum cortisol and lactate concentrations. In endurance- and power-trained human athletes (Paccotti et al., 2005), no significant correlation was observed between serum cortisol and lactate concentrations. Thus, until the basis for the decrease in cortisol and increase in lactate associated with dolphin–human swim interactions are fully clarified, physiological relevance of the apparent inverse relationship between cortisol and lactate observed herein in association with dolphin–human interactions remains unknown.

There was a significant main effect of time and group-by-time interaction associated with circulating concentrations of ammonia during the late afternoon sessions which were attributed to a differential decrease in mean concentrations in the no-swim interaction group. While no documented studies were found on blood concentrations of ammonia associated with locomotion, exercise, or physical activity in dolphins or, more broadly, marine mammals, results are interpreted in reference to other species. In performance standardbred horses (McGowan et al., 2002), mice (Chen et al., 2020), and human sprinters and triathletes (Kantanista et al., 2016), progressive or intensive training activities resulted in a linear increase in blood concentrations of ammonia. In addition, during intense leg press exercises in men, the increase in blood ammonia concentrations were positively correlated with an increase in blood lactate concentrations (Gorostiaga et al., 2014). While blood concentration of ammonia is considered a well-known biomarker to assess the intensity of training regimens and monitor performance activities in animal and human athletes (Finsterer, 2012; Nalbandian & Takeda, 2016), it appears dolphin–human swim interactions are not at a level of intensity that results in homeostatic imbalance and deamination of AMP and catabolism of skeletal muscle amino acids.

Future studies are required to clarify this concept and determine the value of blood ammonia as a relevant biomarker to assess and monitor performance-based physical activities in aquarium-based dolphins.

Conclusions

The present results continue to support previous results by the same authors (Bergfelt et al., 2020) and others (Matsushiro et al., 2021) that dolphin–human swim interactions are not accompanied by an increase in circulating concentrations of cortisol; instead, concentrations decrease. A novelty of this study is the increase in circulating concentrations of lactate at the end of the day in animals involved in dolphin–human swim and no-swim interactions, which may be reflective of an accumulation of lactate in association with periodic shifts from an aerobic to an anaerobic glycolytic pathway to support physical activities throughout the day. Another novelty of this study was the evaluation of circulating concentrations of ammonia as a metabolite of intense physical activity which were unchanged or decreased in dolphins involved in swim and no-swim interactions. Thus, the present results continue to support the concept that short-term, performance-based physical activities are not physiologically or metabolically challenging in bottlenose dolphins trained and conditioned for dolphin–human swim interactions.

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