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Physiological and Behavioral Adjustments Relative to Catecholamine Levels During Nesting in Olive Ridley (*Lepidochelys Olivacea*) and Hawksbill (*Eretmochelys Imbricata*) Sea Turtles in Masirah Island, Oman.

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التكيفات الفيزيولوجية والسلوكية بواسطة معدلات الأدرينالين والنورادرينالين للسلاحف البحرية الزيتونية وصقرية النقار في جزيرة مصيرة بسلطنة عمان

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خلاصة : تم في هذه الدراسة لأول مرة قياس الأدرينالين والنورادرينالين في الزيتونية وصقرية المنقار من السلاحف البحرية في جزيرة مصيره على بحر العرب وذلك في مجموعات السلاحف بعد التعشيش. لقد تم جمع عينات من الدم من مجموعات السلاحف التي أنهت التعشيش وأخرى لم تعشش بعد. ولقد تفاوتت مدة التبويض بين الساعة والنصف والساعتين في كلا النوعين من السلاحف. ولوحظت حالة من الضنى تعم هذه السلاحف وهي تقوم بجهد متواصل مضني تتخلله فترات من الاسترخاء. وفي هذا الإطار من النشاط أظهرت هذه السلاحف ارتفاعاً في معدلات ثاني أكسيد الكربون والحمضية ونقص الأكسجين في الدم وهذه كلها خصائص تستتبع التنفس اللاهوائي. أما معدلات الأدرينالين والنورادرينالين فقد كانت مستقره في كلا النوعين، ولم تتغير معدلات الجلوكوز في النوعين أيضاً. وفي هذا الإطار فإنه يبدو أن استراتيجية التكاثر في كلا النوعين من السلاحف تتشابه إلى حد بعيد.

ABSTRACT: Plasma adrenaline (ADR) and noradrenaline (NA) levels were measured for the first time in natural populations of hawksbill (*Eretmochelys imbricata*) and olive ridley (*Lipedochelys olivacea*) in Masirah Island, Arabian Sea; one of the few protected nesting grounds remaining in the world for these two endangered species. Plasma ADR and NA levels were assessed in individuals after they oviposited eggs and completed nesting exercises, and in individuals which were still searching for suitable nesting sites. Blood samples were taken from the cervical sinuses from two groups (oviposited and non-oviposited), which spent at least 1.5 h on the nesting grounds. The duration of the nesting period varied between 1.5 and 2.0 h for both species. There was no significant difference between oviposited and non-oviposited turtles in both species. As the turtles move onto the nesting grounds, their heavy weight compresses the thoracic region making terrestrial breathing laborious and difficult. During phases of nesting, the turtles undergo brief bursts of strenuous and exhaustive exercise which usually lasts less than one minute followed by a brief recovery period which is less than the exercise phase. Reptiles in general, particularly turtles, are intermittent breathers and after bursts of exercise, they appear to develop hypoxia, hypercapnia and acidemia, which are characteristic of anaerobic metabolism. The data reveals that catecholamine levels remain stable in both species during phases of nesting and may play an important role in combating stress as well as mobilizing energy reserves. The high plasma lactate and CO_2 levels in olive and hawksbill turtles may signify anaerobic metabolism during exercise. Glucose levels remain unchanged throughout nesting in both species. There was no significant difference in the lactate and glucose values in the two species. The physiological and the behavioral adjustments in this study showed remarkable similarities in the two species and may be an important factor in the reproductive strategy for species survival.

KEYWORDS: Nesting, Sea Turtles, Catecholamines, Glucose, Lactate, CO_2.

1. Introduction

The stress system in vertebrates, under the influence of the environmental factors, triggers the neuroendocrine system to secrete stress hormones such as catecholamines and glucocorticoids (Stratakis and Chrousos, 1995; AlKindi *et al.,* 1996; AlKindi *et al.,* 2000a; Denver, 1997). In addition to hormones, there is also an increase in glucose, lactate and blood electrolytes (Chrousos and Gold, 1992).

The nesting process is a difficult ordeal, and frequently the turtle makes several unsuccessful attempts before oviposition (AlKindi *et al*., 2000b). As the turtles emerge from the sea, they remain on the nesting grounds for a long time (2.5– 4 h in green and 1.5-2.5 h in the olive ridley turtles) and thus undergo a tremendous stress and energy expenditure (AlKindi *et al*., 2000b). Valverde *et al.,* (1999) speculated a lower hypothalamo-pituitary-adrenal axis (HPA) in olive ridley arribada and solitary nesters than in basking and non-nesting turtles. They concluded that the slow increase in corticosterone levels during nesting may indicate a hyporesponsiveness of HPA to turning stress which may be a physiological adaptation during the nesting process.

This study is part of ongoing projects related to the behavioral and physiological studies on the natural population of sea turtles in Oman. The purpose of this study is to compare the behavioral and the physiological adjustments during nesting in two species that choose the same nesting grounds.

2. Methods

2.1 Study area

This investigation was conducted at Masirah Island, Sultanate of Oman, off the east coast of the Arabian Sea.

2.2 Animals

Twelve normal healthy nesting olive ridley and hawksbill turtles without any physical defects or injuries were studied during the nesting season (n=12). The observations were conducted late in March and April. Both the hawksbill and olive ridley turtles share the same nesting grounds on the island.

Behavioral observations were recorded during different phases of nesting. Carapace length, carapace width and turtle weight were recorded. Air, water, ground and cloacal temperatures were taken during the time of observations. Measurements of carapace length, width and body weight were recorded after nesting.

Blood samples (10 ml) were taken during nesting exercise with a syringe and needle from the dorsal cervical sinuses according to the method of Owens and Ruiz (1980). Two groups were sampled oviposited and non-oviposited which spent at least 1.5 h on the nesting grounds. Several trips were made to the study area during each period for the data collection.

2.3 Blood analysis

Catecholamine analysis: Mixed standards (5ng/ml) of adrenaline, noradrenaline, 3-hydroxytyramine (dopamine) and the internal standard 3,4-dihydroxybenzylamine-HBr (DHBA were used for calibration of HPLC instrument to quantify adrenaline, noradrenaline and dopamine in plasma samples. All reagents were from Chromsystems.

Preparation of the sample involved the extraction of 500 μl of plasma and 100 μl of internal standard with 50 mg of acid washed alumina neutral type in a 1.5 ml polypropylene microcentrifuge tube. After mixing for 20 min. on an autovortex set at medium speed, the tube was centrifuged at 2800g for two min. The supernatant was withdrawn and the residue was treated with 0.5 ml of washing buffer, mixed using an autovortex for two minutes, centrifuged, and discarded. This washing procedure was repeated three times. The catecholamines were then recovered from the alumina by shaking with 200 µl of elution buffer for five minutes. After centrifugation (11,500g) for two minutes, the supernatant was transferred to a clean vial of polyprophyne for storage at 2-4°C (maximum storage time of two days).

HPLC analysis consisted of loop injection of 50 µl of the prepared sample. The mobile phase (Chromsystems cat. # 5001) is specific for electrochemical detection of catecholamines. Instrument conditions were constant flow of 1.2 ml/min., electrochemical detection at 600 mV, current range 10 nA and damping of 1 sec. Readouts in ng/ml of standards and samples were corrected for extraction efficiency to obtain direct values for plasma noradrenaline, adrenaline and dopamine concentrations.

Plasma glucose, lactate and CO_2 analyses: The Beckman Synchron CX Systems were used to determine plasma glucose, lactate and CO_2 in this study. The system automatically proportions appropriate samples and reagent volumes relative to each plasma parameter. Plasma glucose was measured using amperometric method utilizing glucose oxidase production of O_2 and detection of Δ potential. Plasma lactate was measured using enzymatic method utilizing lactate dehydrogenase with conversion to pyruvate and the production of peroxide. Peroxidase was added to produce quinoneimine and was detected at wavelength of 520nm. Plasma CO_2 was measured using potentiometric method that utilizes ion selective electrode which measures change in potential. The standard error of the mean (SEM) was calculated for the parameters.

3. Results

Plasma adrenaline and noradrenaline levels showed no significant differences in the two species (Figure 1), and also between the individuals of the same species. There was no significant difference between oviposited turtles and non-oviposited turtles. The hormonal levels remained stable throughout the nesting period.

Values of glucose and lactate (Figure 2), and CO_2 (Figure 3), showed no significant differences among the individuals of the same species or the two species.

There was no significant difference between the cloacal temperatures, sand, air or water temperatures.

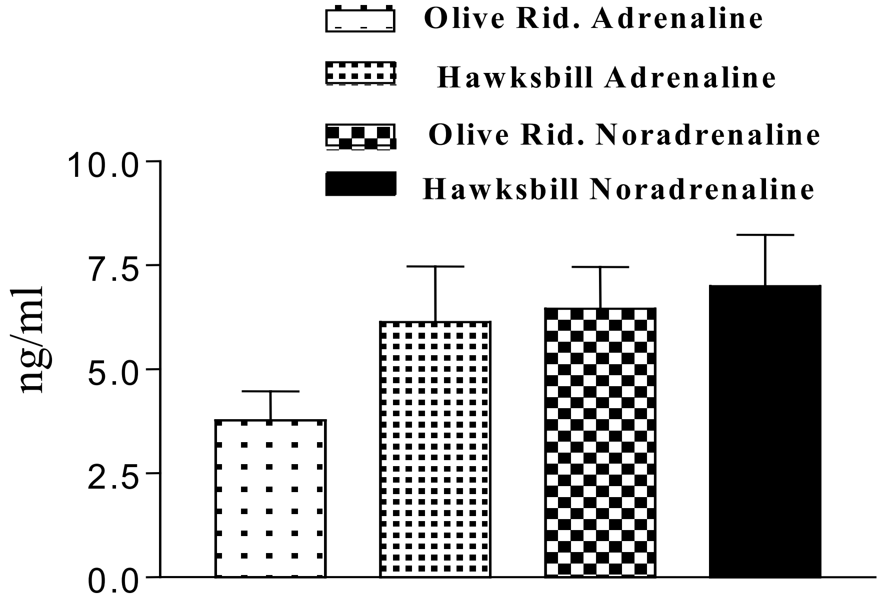

Figure 1. Adrenalin and noradrenalin levels
(n=12; ± SEM;* = significant difference)

The mean values (ng/ml) for olive ridley for noradrenaline (6.46 ± 1.00, n=11) and adrenaline (3.78 ± 0.70, n=13), and for hawksbill noradrenaline (7.00 ± 1.24, n=11) and adrenaline (6.14 ±

1.34, n=11). The mean values (mmol/L) for olive ridley for glucose (5.38 ± 0.25, n=12) and, for hawksbill glucose (5.94 ± 0.27, n=11). The mean values (mmol/L) for olive ridley for lactate (15.43 ± 1.98, n=12), and for hawksbill lactate (19.61 ± 1.44, n=11). The mean values (mmol/L) for olive ridley for CO_2 (26.50 ± 1.36, n=12), and for hawksbill CO_2 (23.72 ± 0.78, n=11). There were no significant differences in all parameters between oviposited and non-oviposited turtles both intra-specifically and inter-specifically (Figures 1-3).

The mean values for temperatures (^oC) for olive ridley and hawksbill turtles, respectively, are: water (25.6 ± 0.26, n=10) (26.0 ± 0.0, n=5), sand (26.4 ± 1.18, n=10) (28.2 ± 0.86, n=5), air (25.15 ± 0.72, n=10) (26.5 ± 0.31, n=5) and cloacal (27.5 ± 0.45, n=10) (27.4 ± 0.4, n=5). There was no significant difference between the cloacal temperatures, sand, air or water temperatures.

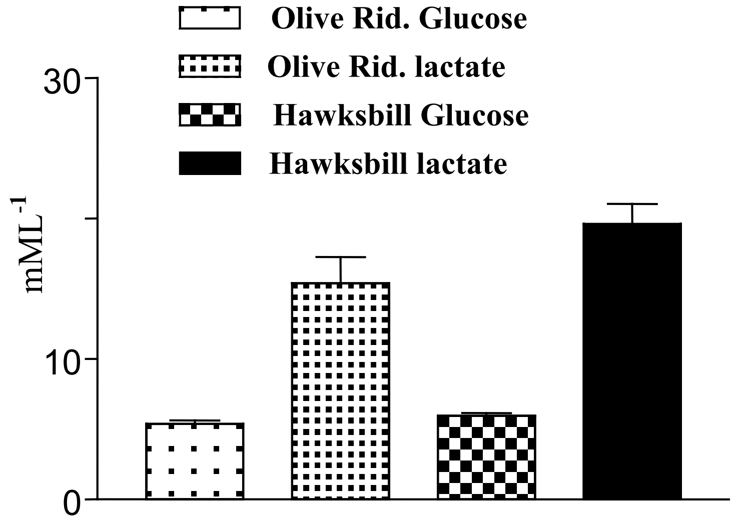

Figure 2. Mean glucose and lactate levels (n=12;± SEM; * = significant difference)

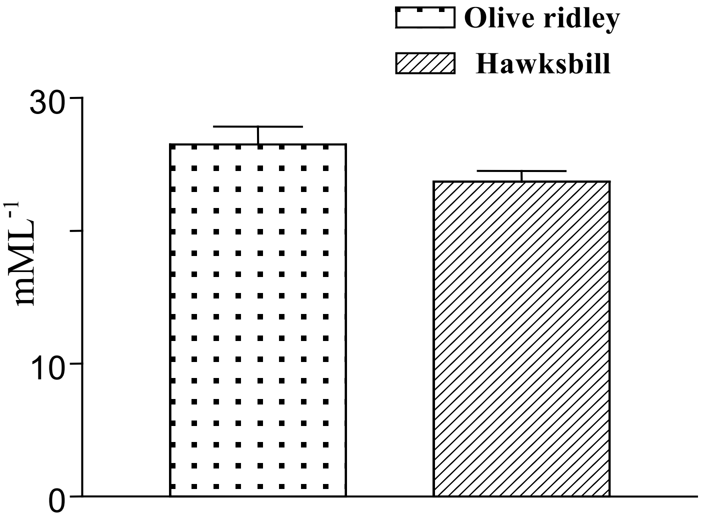

Figure 3. Mean CO_2 levels (n=12;± SEM;* = significant difference)

The mean values (cm) for olive ridley for carapace length and width (72.75 ± 0.55, n=12; 50.91 ± 1.33, n=12) and for hawksbill (78.12 ± 1.17, n=8; 60.62 ± 2.33, n=8), for body weight

(g) for olive ridley (72.33 ± 0.47, n=12) and for hawksbill (75.63 ± 0.42, n=8). There were no significant differences in values between adrenaline, noradrenaline, glucose, lactate and CO_2 in olive ridley and hawksbill turtles of both ovipositors and non-ovipositors, therefore, all the data were pooled (Figures 1-3).

4. Discussion

The data on olive ridley and hawksbill reveals that there is no significant differences in catecholamine, glucose, lactate and CO_2 values in turtles that had already oviposited their eggs and completed all phases of nesting, and turtles which had not oviposited their eggs and had not completed their nesting phases.

The stable levels of catecholamines in this study may be an important physiological adjustment to combat stress. Matt *et al.* (1997) reported that in the male tree lizards (*Urosaurus ornatus*), when captured within a short time, there was no apparent rise in catecholamines. However, restraint stress caused a significant increase in nordrenaline, adrenaline and dopamine plasma levels.

Based on the lack of intra-specific or inter-specific significant differences, it seems that both species undergo the same physiological adjustments, we suggest that the catecholamines release into the blood stream occurs in surges and the amount depends on the magnitude of the physical exercise and related physiological conditions. This assumption is based on the fact that the present data reveals a wide variation in the catecholamine values among the individuals of the same nesting group.

It appears that the rise in the catecholamine values may facilitate O_2 uptake, increase heart rate and mobilizes glycogen metabolism by stimulating glycogenolysis and/or gluconeogenesis.

In both species, there was a large accumulation of plasma lactate and CO_2 which is in conformity with other reptiles. Lizards that rely on anaerobic metabolism during territorial encounters produce large amounts of lactate (Bennet *et al.*, 1981; Pough and Andrew, 1985; Wilson *et al.*, 1990).

Gleeson *et al.* (1993) reported that in the lizard (*Dipsosaurus dorsalis*) the skeletal muscles utilize mainly lactate under the influence of adrenaline as a gluconeogenic substrate for glycogen production. Moreover, plasma adrenaline and corticosterone increased after 5 min. of exhaustive exercise; and adrenaline facilitates lactate removal by skeletal muscles while corticosterone has no effect on lactate metabolism in the lizard skeletal muscles. Other studies have also confirmed that skeletal muscles in lizards are capable of resynthesizing muscle glycogen *in situ* shortly after strenuous exercise (Gleeson, 1982, 1986, 1991; Gleeson and Dalessio, 1989, 1990).

In the lizard, *Dipsosaurus dorsalis*, red and white skeletal muscles *in vitro*, under the influence of adrenaline, are capable of synthesizing glycogen directly from lactate at many times greater than the rate of synthesizing glycogen from glucose. Thus, adrenaline is not effective on glucose metabolism (Gleeson *et al.*, 1993).

Our data indicate that both adrenaline and noradrenaline are effective in facilitating the conversion of lactate to glycogen but not effective in conversion of glucose to glycogen, as the glucose plasma levels remain stable throughout the nesting exercise, which is in conformity with the above investigations.

The olive ridley and hawksbill sea turtles undergo long and arduous exercises at different phases of nesting, lasting several hours, which includes ascent on to the beach, wandering, digging body chamber, digging egg chamber, laying eggs, filling egg chamber, filling and camouflaging body chamber, then they return to the sea.

Most reptiles, especially turtles including olive ridley and hawksbill, ventilate intermittently when they are subjected to severe hypoxia, acidemia and overall acid-base disturbance during exhaustive exercise periods (Shelton *et al.*, 1986; Wasser and Jackson, 1991; Comeau and Hicks, 1994). There is a general trend of adjustments in heart rate, pulmonary vascular resistance, and pulmonary blood flow associated with hypoxia and intermittent ventilation (Shelton and Burggren, 1976; Hicks, 1994; Comeau and Hicks, 1994). An increase in heart rate and pulmonary blood flow and a decrease in the pulmonary vascular resistance occurs during deep ventilation while the turtle is resting.

However, during exercise, there is a decrease in heart rate and pulmonary blood flow and an increase in pulmonary vascular resistance that leads to hypoxia. In addition, there is a major shift in blood flow in the heart, affecting the pulmonary and systemic circulation (Shelton and Burggren, 1976). To compensate for intermittent ventilation, the heart adjusted to shunts with blood flow from right to left shunt, where the systemic venous blood bypasses the lungs and enters the systemic circulation directly, which is usually associated with shallow ventilation during the exercise phase leading to hypoxia. In the left to right shunt, the pulmonary venous blood predominately enters the pulmonary circulation relative to deep ventilation and O_2 uptake during the resting phase (Shelton and Burggren, 1976).

Our observations of ridleys and hawksbills during phases of nesting showed that they go through bursts of exhaustive and forceful exercise which usually last less than one minute, but occasionally exceeded that. During this time, the turtles breathe very shallow as the throat muscles are not strongly contracting, evidenced by the lack of deep breathing movements. The extended turtle neck during exercise gives the observers the advantage of watching muscle movements. In addition, the heavy weight of the turtle compresses the thoracic region, making terrestrial breathing laborious and difficult, particularly during digging and burying activities. At rest, which is usually extended between a few seconds to over one minute, the turtle forcefully exhales a large air volume followed by ventilations. The exercise-rest cycles which occur in all phases of nesting activities are typical of intermittent breathers.

Stress, caused by rapid changes in environmental factors, can disrupt homeostasis and alter hormonal levels as well as other physiological and behavioral patterns through the neuroendocrine system. These changes are means of adjustment which eventually combat stress and restore homeostasis. More specifically, stress can activate the hypothalamo-pituitary-adrenal (HPA) axis that results in an increase in catecholamines, glucocorticoids, glucose and other blood components (Chrousos and Gold, 1992).

The olive ridley and the hawksbill develop apnea, accompanied by hypercapnia, acidemia and hypoxia, which are associated with anaerobic metabolism. These conditions were also reported in the nesting green turtles, *Chelonia mydas* (Al Kindi *et al*., 2000b). These physiological conditions are found in vertebrates, which undergo short bursts of exhaustive exercises, especially in intermittent ventilators and have been reported in fishes (Jensen, 1987; Bouissou *et al*., 1987; Tang and Boutilier, 1988; Hughes *et al.,* 1988; Van Raaij *et al*., 1996), in birds (Le Maho *et al*., 1992) and in turtles (Wasser and Jackson, 1991; West *et al*., 1992; Comeau and Hicks, 1994; Johnson *et al*., 1998).

The remarkable similarities between the two species in all of the parameters suggest that both species require the same physiological adjustments during the nesting process. The behavioral adjustments such as the short burst of strenuous exercise followed by recovery may be an important survival strategy, under the control of the neuroendocrine system. Moreover, both species require the same ecological conditions during nesting, as is reflected in their behavioral and physiological requirements.

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