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Differences in Body Water Turnover Rate Between *Naja kaouthia* Lesson, 1831 and *Malayopython reticulatus* (Schneider, 1801) Snakes

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ABSTRACT. – Total body water and the water turnover were studied in *Naja kaouthia* Lesson, 1831 and *Malayopython reticulatus* (Schneider, 1801) snakes by tritiated water dilution techniques. Both snakes species were injected intramuscularly with an aqueous solution carrier-free tritiated water (^3H_2O) at a single dose of 10 µCi/kg body mass. The equilibration time was determined by taking blood samples for 8 days after the injection. The results show that the absolute values of the rate of water turnover (WTO), water space (TOH) and total body water (TBW) of *M. reticulatus* were significantly higher than those of *N. kaouthia*. The relative values of WTO per fat-free, body mass of *N. kaouthia* were significantly higher than those *M. reticulatus*, while the biological half-life of tritiated water of *N. kaouthia* was significantly shorter than those of *M. reticulatus*. The relative values of both TOH and TBW (% body mass) showed no significant differences between two species. Hematocrit and plasma concentrations of protein, specific gravity, total solid, K^+ and Cl^- were similar in both snake species, while plasma concentration of Na^+ and plasma osmolality were significantly higher in *N. kaouthia* than *M. reticulatus*. These results suggest that a lower WTO as a percentage of body mass and a higher biological half-life of ^3H_2O of *M. reticulatus* in comparison with *N. kaouthia* could be attributed to relatively higher efficiency in the water retention mechanism, which *M. reticulatus* could be a well adaptation to the tropical environment.

KEYWORDS: total body water, water turnover rate, *Naja kaouthia*, *Malayopython reticulatus*

INTRODUCTION

The importance of body water regulation in a tropical environment is well established in vertebrate physiology. The body water turnover is the fundamental consideration dealing with body water intake and water loss. The characteristics of water turnover rates are modified to satisfy changing demands of the organism in terms of external balance and internal flux. External balance refers to the comparison between the water input and the water output to the external environment. Over any period of time, input equals output and the organism is in water balance. Water turnover alters heat transfer within the body, and changes in water turnover rate are an important thermoregulatory mechanism of all vertebrates (Seebacher and Franklin, 2005). There are a number of biodiversity of animals that are investigated in the tropic. Reptiles are usually considered as the first vertebrates capable of completing their life cycle on dry land. Reptiles, particularly, snake species, have fascinated to scientists for many decades.

In homothermic animals, many factors are known to influence body water turnover rate, such as species, physiological state, climatic condition and body weight. In mammals, it is known that the regulation of body temperature by directing the flow of body fluids between the surface and core of the body for convective heat dissipation to the environment (Terrien, et al., 2011). In poikilothermic vertebrates like snakes modify transient heat transfer between their body and the environment to facilitate thermoregulation via locomotion, tongue flicking and digestion (Stevenson, et al., 1985).

Many studies indicate a clear relationship between body water turnover and energy metabolism (MacFarlane and Howard, 1970). Food is the most important source of water in tropical snake and the food of snakes are primarily carnivorous while differences in behaviour and species-specific. As part of a study aimed to elucidate whether water metabolism alterations could be identified which might themselves be factors responsible for energy metabolism in comparison between *Malayopython reticulatus* (Schneider, 1801) non-venomous, and *Naja kaouthia* Lessen, 1831, venomous snakes. An understanding of its body water regulation of different species of snake in the tropic may indirectly improve husbandry for animal care and prolong animal life-spans in captivity, reducing the need for the frequent collection of the endangered species from the wild. Studies were performed simultaneously on water metabolism including the plasma compositions of these two species of snake, the results may be of wider interest.

MATERIALS AND METHODS

Animals and management

Two groups of wild-caught snakes of six *M. reticulatus* snakes (average 4-8 kg in body mass) and five *N. kaouthia* (average 0.4-0.6 kg in body mass) were collected from the wild in Thailand and used for this experiment. Both groups were individually captive in wire meshed cage for *M. reticulatus* or plastic boxes for *N. kaouthia* at the snake farm of Queen Saovabha Memorial Institute (QSMI), in a restricted-access room where they were acclimated to a normal ambient temperature (27±1°C), relative humidity of 65.3 ± 0.9%, and 12-h light:12-h dark photoperiod (lights on at 06:00 h). The ambient temperature and the relative humidity were recorded using a wet and dry bulb thermometer. The snakes were fed on the preference food such as a mouse for *N. kaouthia* and rabbit for *M. reticulatus* of which the weight of food was about 10% of the snake's body mass every 2 weeks-interval and had free access to tap water. Animal care and procedures used were approved by the Institutional Animal Care and Use Committee of QSMI (QSMI-ACUC-012-2021).

Determination of water metabolism

Total body water and the water turnover rate were determined in each snake by tritiated water dilution techniques. On the specified day, the snake was captured in tube and weighed and bred (1 ml) from the ventral tail vein via the heparinized syringe. The snake was then injected intramuscularly with an aqueous solution carrier-free tritiated water (^3H_2O) in normal saline at a single dose of 10 μCi/kg body weight. The equilibration time was determined by taking blood samples for 8 days after the injection. Blood samples were collected at days 0, 1, 2, 3, 4, 5, 6, 7 and 8 subsequent to the injection. Preparation of samples for counting by Beta counter was achieved by the internal standardization technique as described by Vaughan and Boling (1961). The corrected activity of samples, in disintegrating per minute (d.p.m.), were plotted on semi-logarithmic paper against time. The dilution curve of tritiated water in plasma was described by an exponential equation using the compartment system model (Shipley and Clark, 1972) for determinations of the water turnover rate and total body water.

The exponential equation describing the one compartmental model was calculated:

$$Y1 = Ae^{-k1t},$$

where Y is the concentration of tritium in plasma at time t (nci/ml); A is plasma concentration intercept 1 in nci/ml.

The extrapolated activity at theoretical zero time of complete mixing of radio-isotope was used to determine the total body water space (TOH). The TOH was calculated:

TOH (ml) = [standard count (dis/min) x dose (ml)] / [radio activity counts at zero time (dis/min)].

The biological half-life of tritium-labelled water (Tl/2) was determined from the slope of the linear regression line obtained from a plot on a semi-logarithmic paper of the activity of the samples taken over 8 days against time. The water turnover rate was calculated from the equation (Holleman, et al., 1982):

WTO (l/day) = 0.693 x TOH space / Tl/2.

Total body water (TBW) was calculated by using the corrected factor (1 – a fraction of plasma solids) x TOH (Chaiyabutr *et al.* 1997).

Determination of hematocrit and plasma osmolality and electrolyte concentrations

The composition of electrolytes in plasma was measured as following: sodium and potassium concentration by a flame photometer (Clinical flame photometer 410C, Corning Ltd.) and chloride concentration by a chloridometer (Chloride Analyzer 925, Corning Ltd.), The plasma solids concentration was determined by a refractometer (American Optical CORP. Keene, N.H., USA). Plasma osmolality was determined using the freezing point depression method (Advance Osmometer model 3, USA).

Statistical analysis

All data were reported as mean ± S.D. The unpaired t-test was to estimate the significant difference in values between a group.

RESULTS

Table 1. Total body water, water turnover rate, the biological half-life of tritiated water of *M. reticulatus* and *N. kaouthia*

The data in Table 1 show that the absolute values of the water turnover rate (WTO), water space (TOH) and total body water (TBW) of *M. reticulatus* were significantly higher than those of *N. kaouthia* snakes. The values of WTO per fat-free, wet, body mass ($kg^{0.82}$) (MacFarlane and Howard 1972) of *N. kaouthia* (33.22± 3.19 ml/$kg^{0.82}$/d) were significantly higher ($P<0.001$) than those of *M. reticulatus* (18.47 ± 4.67 ml/$kg^{0.82}$/d), while the biological half-life of tritiated water of *N. kaouthia* (13.7± 3.8 days) was significantly shorter than those of *M. reticulatus* (30.3± 3.3 days) ($P<0.001$). The relative values of both TOH and TBW (percentage of body mass) for *N. kaouthia* and *M. reticulatus snakes* showed no significant differences during studies. Body mass of *N. kaouthia* used in the present study was markedly lower than that of *M. reticulatus.*

TABLE 1. Body mass, water turnover rate (WTO), total body water (TBW), total body water space (TOH), and the biological half-life of tritiated water of *Malayopython reticulatus* and *Naja kaouthia* snakes. (mean ±SD).

Parameters	***M. reticulatus* (n=6)**	***N. kaouthia* (n=5)**	**P-value***
Body mass (Kg)	6.5 ± 2.0	0.5 ± 0.1	0.001
WTO (ml/d)	103.7 ± 66.5	16.7 ± 1.4	0.018
WTO ($ml/kg^{0.82}/d$)	18.47 ± 4.67	33.22 ± 3.19	0.001
Biological half-life of tritium (d)	30.3 ± 3.3	13.7 ± 3.8	0.001
TOH (ml)	5293.0 ± 283.8	399.7 ± 67.3	0.001
TOH (L/100 kg)	81.6 ± 3.1	78.5 ± 2.7	NS
TBW (ml)	4846.2 ± 107.6	364.7 ± 3.5	0.001
TBW (L/100kg)	74.5 ± 1.8	72.9 ± 0.7	NS

Values are mean ± SD. The number of animals is given in brackets.
*P-value by unpaired t-test; significant differences at level P< 0.05 between *M. reticulatus* and *N. kaouthia* ; NS, not significant.

Table 2. Haematocrit and plasma concentrations of electrolytes, specific gravity, total solid, total water and total protein and plasma osmolality.

As shown in Table 2, haematocrit and plasma concentrations of protein, specific gravity, total solid, total water and total protein and plasma concentrations of K^+ and Cl^- were similar in *N. kaouthia* and *M. reticulatus snakes*. Plasma concentration of Na^+ and plasma osmolality were significantly higher in *N. kaouthia* than *M. reticulatus (*P<0.004).

DISCUSSION

The absolute values of total body water, body water space and water turnover rate of *M. reticulatus* snakes differed significantly with *N. kaouthia* snakes having higher of these parameters in *M. reticulatus* snakes (Table 1). This was due to differences in snake body size used in the present study in which *M. reticulatus* snakes were larger (Table 1). However, when correcting for body mass in both snake groups (volume of either TBW or TOH per unit body mass), the relative values per body mass showed no significant differences between *M. reticulatus* snakes and *N. kaouthia* snakes. The present results demonstrated that the mean value of TBW (74.5 % body mass) of the *M. reticulatus* corresponded well with the previous report in *M. reticulatus* (78.9 % body weight) (Sitprija et al., 2016) and other two species of snakes: 77.1% of the Black Rat Snake, *Elaphe obsolete obsolete*, and 77.2% of the Pacific Rattle Snake, *Crotalus viridis helleri* (Smits and Lillywhite, 1985). However, the relative values of both TBW and TOH of the *M. reticulatus* tended to be higher than those of *N. kaouthia*. It is possible that the relatively expanded TBW of the *M. reticulatus* may reflect on the common incidence of hydration relating to its existence on living nearly water in the tropical environment.

It is well known that water homeostasis is essential for the normal living organism. Body water turnover plays an essential role in the process of body temperature regulation, especially during heat dissipates via an evaporative mechanism in mammal. In the present results, mean values of the water turnover rate were adjusted to the value of WTO per fat free body mass weight ($kg^{0.82}$) (MacFarlane and Howard 1972), we found significant differences (P<0.001) between the two species of snakes, *i.e.* for snake *M. reticulatus* was 18.47 ± 4.67 $ml/kg^{0.82}/d$; and it was 33.22± 3.19 $ml/kg^{0.82}/d$) for *N. kaouthia*. Thus, the differences in WTO between *M. reticulatus* and *N. kaouthia* would not be influenced by the effect of changes in environmental conditions, since both captive snakes were kept in the individual cage under the same environment, although snakes were poikilotherms and no sweat glands. In the present results, the biological haft-life of tritiated water was significantly shorter while the relative value of water turnover rate was higher in *N. kaouthia* than that of *M. reticulatus.* These differences may be explained, in part, based on the differences in the species-specific of the snake.

In the present studies, the WTO of *N. kaouthia* was higher during a short period of study. These results indicate that the energy metabolism of *N. kaouthia* was higher than that of *M. reticulatus*, since a high relationship between water turnover and energy metabolism has been noted (MacFarlane and Howard, 1970). The significant shorter of biological half-life of tritiated water of the *N. kaouthia* reflected in the higher water requirement and more loss of water of the *N. kaouthia* in comparison to *M. reticulatus.*

TABLE 2. Haematocrit and plasma osmolality, protein, specific gravity, total solid, total water and electrolyte concentrations of *Malayopython reticulatus* and *Naja kaouthia* snakes. (mean ±SD)

Parameters	*M. reticulatus* (n=5)	*N. kaouthia* (n=5)	P-value*
Haematocrit (%)	21.4 ± 5.0	20.6 ± 4.5	NS
Osmolality (mOsm/kg)	274.4 ± 6.9	316.0 ± 11	0.001
Plasma Na^+(mM)	148.5 ± 4.2	161.5 ± 5.9	0.004
Plasma K^+(mM)	3.78 ± 0.52	4.38 ± 1.47	NS
Plasma Cl^-(mM)	103.8 ± 7.19	108 ± 21.89	NS
Specific gravity	1.0251 ± 0.005	1.0257 ± 0.002	NS
Total solid (g %)	8.44 ± 2.03	8.74 ± 0.90	NS
Total water (g %)	93.88 ± 1.55	93.68 ± 0.67	NS
Total protein (g %)	6.92 ± 1.87	7.18 ± 0.85	NS

Values are mean ± SD. The number of animals is given in brackets.
*P-value by unpaired t-test; significant differences at level P< 0.05 between *M. reticulatus* and *N. kaouthia* ; NS, not significant.

On the period of study, the effect of food intake on increase in energy metabolism and WTO rate in both snakes were not expected, since food was withheld prior to the short period of study. Changes in WTO rate could simply reflect a part of the process of adaptation to maintain normal body temperature. However, a lower WTO as a percentage of body mass and a higher biological half-life of tritiated water of *M. reticulatus* in comparison with *N. kaouthia* may be attributed to relatively higher efficiency in the water retention mechanism, which *M. reticulatus* may be related to the well adaptation to the tropical environment. A higher water reserve in *M. reticulatus* would not only provide a higher reservoir but is also useful in slowing down the elevation in body temperature of this snake species in hot humid conditions. In the present study, both captive snakes were housed in the same environment. This indicates that *M. reticulatus* probably do not require greater amounts of water, but could restore their body fluids to equilibrium in the tropical environment.

If, as Macfarlane and Howard (1970) suggest, water turnover and energy metabolism are closely related, then a low relative water turnover in *M. reticulatus* might be expected to have low energy metabolism which would relate to its feeding habits for example waiting motionlessly to ambush prey, expending little energy in hunting, and regulatory spans of digestion (Secor and Diamond,1998). However, if the relative water turnover in *N. kaouthia* is higher than *M. reticulatus* and the snake is compensating to maintain homeostasis, then it might be argued that such differences in high relative water turnover and high energy metabolism in *N. kaouthia* might be due to its actively foraging snakes and expending high energy in hunting. The differences in an ecophysiological co-adaptation relating to water and energy metabolism between these two species of snakes, venomous and nonvenomous snakes would be further investigated.

The present results provide further evidence that the plasma chemistry of snakes between *M. reticulatus* and *N. kaouthia* were in similar ranges. However, both plasma osmolality and the plasma concentration of Na^+ of *N. kaouthia* were significantly higher than those *M. reticulatus,* while plasma protein was not different (Table 2). It indicates that the plasma osmolality is regulated through crystalloid osmotic pressure which plasma concentration of Na^+ plays a role as osmotic skeleton in exertion through its contribution to the plasma osmotic pressure. Similar hematocrit and plasma protein concentrations between *M. reticulatus* and *N. kaouthia* support report which has suggested a high capacity of snakes to restore blood volume and to transfer fluid between the vascular and extravascular compartments (Smits and Lillywhite, 1985).

The significant differences in the high levels of both plasma osmolality and the plasma concentration of Na^+ in *N. kaouthia* in comparison to *M. reticulatus* in the present study, whether these differences confer any energy metabolism (e.g. actively foraging snakes) or body fluid regulation (e.g. osmoregulation) advantages or disadvantages to snakes during the elevations of these physiological parameters. Such differences could be attributable to disparities in species and the question then arises as to whether high plasma Na^+ and plasma osmolality influence body water metabolism and homeostasis in relating to a hormone produced in hypothalamus, for example, vasotocin (Silveira et al. 1998) under the influence of volume and osmotic stimuli in *N. kaouthia* should be further clarified.

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