Determinination of plasma osmolality and agreement between measured and calculated values in healthy adult Hispaniolan Amazon parrots (*Amazona ventralis*)

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Objective—To determine plasma osmolality in healthy adult Hispaniolan Amazon parrots (*Amazona ventralis*) and validate osmolality equations in these parrots.

Animals—20 healthy adult Hispaniolan Amazon parrots.

Procedures—A blood sample (0.5 mL) was collected from the right jugular vein of each parrot and placed into a lithium heparin microtainer tube. Samples were centrifuged, and plasma was harvested and frozen at –30°C. Samples were thawed, and plasma osmolality was measured in duplicate with a freezing-point depression osmometer. The mean value was calculated for the 2 osmolality measurements.

Results—Plasma osmolality values were normally distributed, with a mean ± SD of 326.0 ± 6.878 mOsm/kg. The equations (2 × [Na^+ + K^+]) + (glucose/18), which resulted in bias of 2.3333 mOsm/kg and limits of agreement of –7.0940 to 11.7606 mOsm/kg, and (2 × [Na^+ + K^+]) + (uric acid concentration/16.8) + (glucose concentration/18), which resulted in bias of 5.8117 mOsm/kg and limits of agreement of –14.6640 to 3.0406 mOsm/kg, yielded calculated values that were in good agreement with the measured osmolality.

Conclusions and Clinical Relevance—IV administration of large amounts of hypotonic fluids can have catastrophic consequences. Osmolality of the plasma from parrots in this study was significantly higher than that of commercially available prepackaged fluids. Therefore, such fluids should be used with caution in Hispaniolan Amazon parrots as well as other psittacines. Additional studies are needed to determine whether the estimation of osmolality has the same clinical value in psittacines as it does in other animals. (*Am J Vet Res* 2009;70:1151–1154)

Although most measures of solute concentration take into account the number and weight of the molecules dissolved in a solvent, osmolality measures only the number of particles in solution without regard to size, weight, or charge.[1] In biological systems, the relative osmolality of the intracellular and extracellular spaces determines the fluid volume in each compartment.[2,3] Therefore, it is extremely important that osmolality be closely regulated. In mammals, the release of vasopressin in response to an increase in plasma osmolality is one of the body's primary mechanisms for controlling osmolality.[4,5] Abnormalities in this system can quickly lead to life-threatening fluid and electrolyte imbalances.[6]

Knowledge of the plasma osmolality of a particular species is of paramount importance when formulating fluid treatment for a patient. Commonly used prepackaged fluids, such as lactated Ringer's solution and physiologic saline (0.9% NaCl) solution, are specifically designed for human patients who have an osmolality of approximately 301 mOsm/L.[7] Veterinary-specific solutions have a similar osmolality. These fluids may be isotonic, hypotonic, or hypertonic for other species, depending on the specific plasma osmolality of each species. The plasma osmolality of some commonly kept pets (eg, cats and dogs) have been determined[8,9]; however, to our knowledge, there is no published information regarding plasma osmolality in psittacine species.

Plasma osmolality is routinely measured in clinical laboratories by use of freezing-point osmometers. These automated devices rely on the principle that each mole of dissolved solute will decrease the freezing point of a liquid by 1.86°C.[3] Osmolality can also be estimated by use of calculations.[2] The difference between the measured and calculated osmolality is the osmolar gap and is of clinical importance when exposure to a

Received October 7, 2008.
Accepted November 14, 2008.
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toxin is suspected. Whereas an osmolar gap of up to 10 mOsm/kg is considered normal in humans and dogs, gaps with values higher than this suggest the presence of unmeasured solutes.[1,10] Toxins such as ethanol, isopropanol, methanol, acetone, ethyl ether, mannitol, or ethylene glycol (as well as their metabolites) can attain high plasma concentrations with relatively low molecular weights, which causes high osmolar gaps.[1,10]

Several equations have been proposed for calculating plasma osmolaltiy.[11–14] Nevertheless, because there are many plasma solutes in relatively small amounts (ie, glucose)[2] or that move freely between the extracellular and intercellular space (ie, urea),[5,15] equations for use in mammalian species are often reduced to (2 × Na^+) + (BUN concentration/2.8) + (glucose concentration/18), (2 × Na^+) + (glucose concentration/18), or (2 × Na^+).[2,5] Because osmolality values have not been determined for clinically normal psittacines, equations for calculating plasma osmolality in these birds have not been developed.

In the study reported here, plasma osmolality was measured in a population of healthy adult Hispaniolan Amazon parrots. We also evaluated the ability of various mathematic equations to accurately estimate osmolality in this population. Our hypothesis was that there would be good agreement between the calculated and measured osmolality of the parrots.

Materials and Methods

Animals—Twenty healthy adult Hispaniolan Amazon parrots (*Amazona ventralis*) were used for the study. The parrots were part of a research flock at the Louisiana State University School of Veterinary Medicine. This population was sexually monomorphic, so sex of the 20 parrots was not known. The study was conducted so that it coincided with the annual hematologic and physical examinations of the flock. Hispaniolan Amazon parrots were selected because their size, dietary requirements, and physiologic characteristics are comparable with those of many other popular pet psittacines. The study was performed in accordance with the regulations set forth by the Institutional Animal Care and Use Committee at Louisiana State University.

Procedures—Each parrot was examined to ensure that it was in good health. A 26-gauge needle and 3-mL syringe were used to collect approximately 0.5 mL of blood from the right jugular vein of each parrot. Immediately after collection, blood samples were placed into a lithium heparin microtainer tube and centrifuged at 500 × *g* for 15 minutes. Plasma was harvested, separated into aliquots, and frozen at –30°C. Within the next 5 days, plasma samples were thawed and plasma osmolality was measured in duplicate by use of a commercial freezing-point depression osmometer.[a] Samples were assayed in batches, and all measurements were performed by one of the authors (MJA) who was trained in use of the osmometer. The osmometer was calibrated by use of a 2-point calibration technique in accordance with the manufacture's instructions. The 2 osmolality measurements from each parrot were used to calculate a single mean value. When the difference between the duplicate measurements was ≥ 5%, the values were discarded and the parrot was removed from the study. Plasma biochemical values were determined by use of a commercial chemistry analyzer.[b] For electrolyte concentrations, plasma samples were diluted 1:2 and concentrations were determined by use of the indirect method. Uric acid concentration was determined by use of the direct method.

On the basis of equations commonly used to calculate osmolality in humans and other animals, 4 equations for determination of calculated plasma osmolality were developed and tested. The equations were 2 × Na^+, 2 × (Na^+ + K^+), (2 × [Na^+ + K^+]) + (glucose concentration/18), and (2 × [Na^+ + K^+]) + (uric acid concentration/16.8) + (glucose concentration/18). In these equations, plasma concentrations of uric acid and glucose were divided by 16.8 and 18, respectively, to convert milligrams per deciliter to millimoles per liter. Thus, results of these calculations were in milliosmoles per liter of fluid (osmolarity); however, in body fluids, there is not a substantial difference between osmolarity and osmolality. Therefore, in human and veterinary medicine,

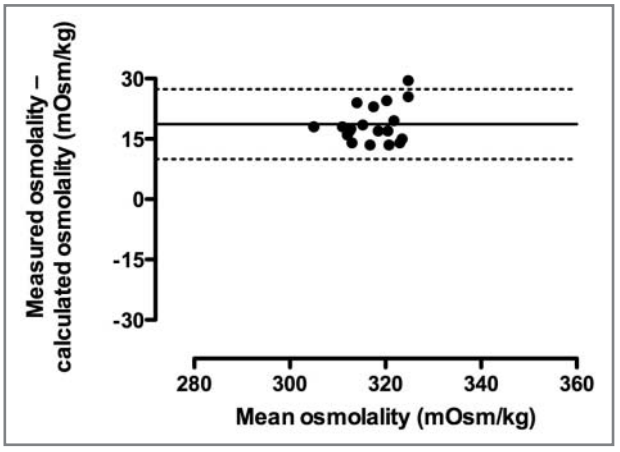

Figure 1—Bland-Altman plot of the difference between plasma osmolality measured in samples obtained from 20 Hispaniolan Amazon parrots (*Amazona ventralis*) and the osmolality calculated by use of the equation 2 × Na^+. Bias (solid horizontal line) was 18.65 mOsm/kg, and limits of agreement (dotted horizontal lines) were 9.95 to 27.35 mOsm/kg.

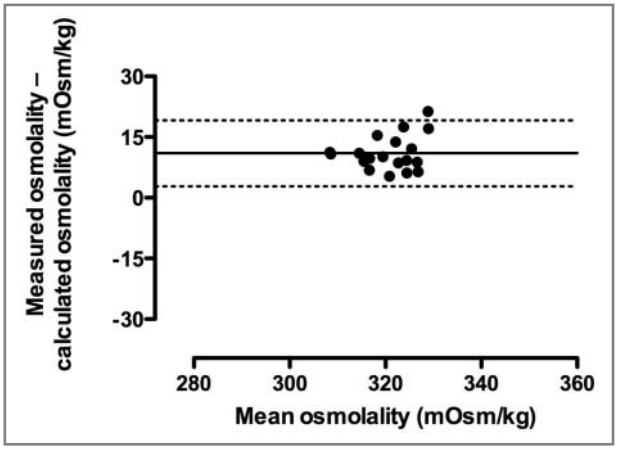

Figure 2—Bland-Altman plot of the difference between plasma osmolality measured in samples obtained from 20 Hispaniolan Amazon parrots and the osmolality calculated by use of the equation 2 × (Na^+ + K^+). Bias (solid horizontal line) was 11.01 mOsm/kg, and limits of agreement (dotted horizontal lines) were 2.83 to 19.19 mOsm/kg.

calculated osmolality is used and the units milliosmoles per kilogram are substituted.[1,10,15,16]

Statistical analysis—Mean values for the duplicate osmolality measurements were tested for a Gaussian distribution by use of a Shapiro-Wilk test.[17] Agreement between the measured and calculated plasma osmolality was determined by use of the Bland-Altman method.[18] Bias was defined as the mean difference between the 2 methods, and limits of agreement were calculated as the bias ± (1.96 × SD). Good agreement was defined as a bias and limits of agreement that varied by < 5% of the mean of the measured plasma osmolality. Statistical analysis was performed by use of a commercially available statistical program.[c]

Results

Valid osmolality measurements were available for all 20 parrots. Mean values of the duplicate osmolality measurements were normally distributed. Overall mean ± SD for all 20 parrots was 326.0 ± 6.88 mOsm/kg. Calculated osmolality determined by use of the equation 2 × Na^+ had a bias of 18.65 mOsm/kg and limits of agreement of 9.95 to 27.35 mOsm/kg (**Figure 1**). Calculated osmolality determined by use of the equation 2 × (Na^+ + K^+) had a bias of 11.01 mOsm/kg and limits of agreement of 2.83 to 19.19 mOsm/kg (**Figure 2**). Calculated osmolality determined by use of the equation (2 × [Na^+ + K^+]) + (glucose concentration/18) had a bias of 2.33 mOsm/kg and limits of agreement of –7.09 to 11.76 mOsm/kg (**Figure 3**). Calculated osmolality determined by use of the equation (2 × [Na^+ + K^+]) + (uric acid concentration/16.8) + (glucose concentration/18) had a bias of –5.8 mOsm/kg and limits of agreement of –14.67 to 3.04 mOsm/kg (**Figure 4**). On the basis of the aforementioned criteria, the equations (2 × [Na^+ + K^+]) + (glucose concentration/18) and (2 × [Na^+ + K^+]) + (uric acid concentration/16.8) + (glucose concentration/18) both had calculated values in good agreement with the measured osmolality.

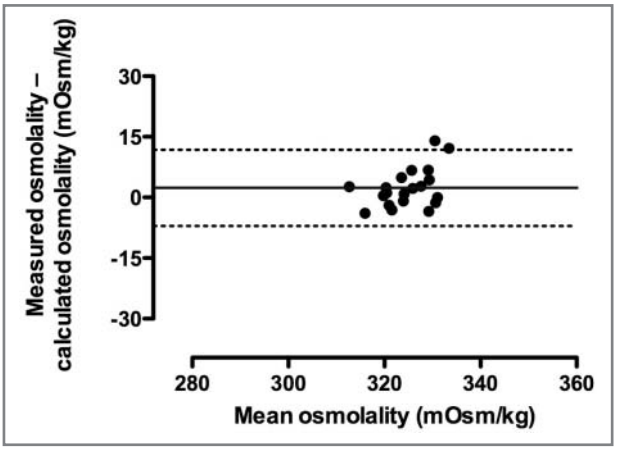

Figure 3—Bland-Altman plot of the difference between plasma osmolality measured in samples obtained from 20 Hispaniolan Amazon parrots and the osmolality calculated by use of the equation (2 × [Na^+ + K^+]) + (glucose concentration/18). Bias (solid horizontal line) was 2.33 mOsm/kg, and limits of agreement (dotted horizontal lines) were –7.09 to 11.76 mOsm/kg.

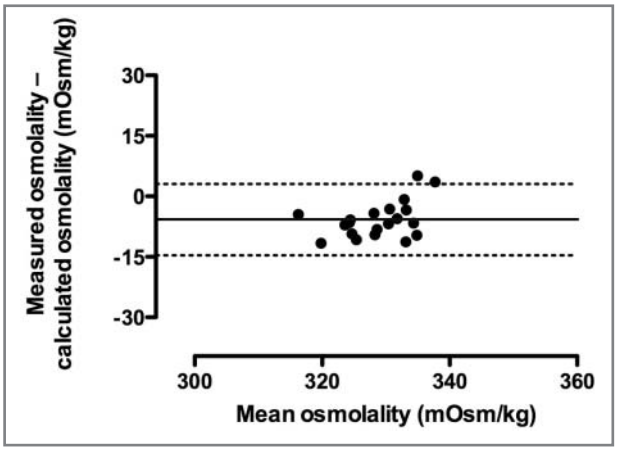

Figure 4—Bland-Altman plot of the difference between plasma osmolality measured in samples obtained from 20 Hispaniolan Amazon parrots and the osmolality calculated by use of the equation (2 × [Na^+ + K^+]) + (uric acid concentration/16.8) + (glucose concentration/18). Bias (solid horizontal line) was –5.8 mOsm/kg, and limits of agreement (dotted horizontal lines) were –14.67 to 3.04 mOsm/kg.

Discussion

In living systems, the relative intracellular and extracellular osmolality determines the fluid balance between these 2 spaces. A decrease in plasma osmolality causes fluid to shift from the extracellular to the intracellular space, which results in cell swelling and damage. An increase in plasma osmolality causes fluid to leave the cells and results in cellular dehydration. Both conditions can have catastrophic consequences. Therefore, knowledge of the plasma osmolality of a species as well as that of any fluids to be administered is critical when developing a fluid treatment plan.

Mean plasma osmolality of the Hispaniolan Amazon parrots in this study was 326.0 ± 6.88 mOsm/kg. This is higher than the osmolality of cats and dogs (308 and 300 mOsm/kg, respectively). The osmolality of commercially available prepackaged fluids, such as physiologic saline solution and lactated Ringer's solution, is 308 and 272 mOsm/kg, respectively. A study[19] conducted in animals revealed that large doses of lactated Ringer's solution can lead to a decrease in plasma osmolality and intracranial swelling. The difference between the osmolality of plasma of the parrots in the study reported here and the osmolality of commonly available prepackaged fluids is even greater.

The secondary objective of our study was to evaluate equations used to calculate plasma osmolality. The primary use of these equations in other species is as a screening test for certain low–molecular-weight toxins. In the study reported here, the equation (2 × [Na^+ + K^+]) + (uric acid concentration/16.8) + (glucose concentration/18) yielded values that had the highest agreement with the measured osmolality. The primary limitation of this study was that plasma from only healthy Hispaniolan Amazon parrots was evaluated. To more fully evaluate the ability of this or any other equation to be used for calculating osmolality, studies involving birds with various illnesses should be conducted. In addition, the agreement in this study was determined on the basis of osmolality measured by use of freezing-point depression and hematologic variables measured on a specific laboratory analyzer. Although freezing-point depression appears to be the method most commonly used for measuring osmolality in clinical labratories,[13]

there are other methods that could be used, and these may yield different results.

The need to avoid large shifts in plasma osmolality has been established. Analysis of the data reported in this study suggested that commonly available prepackaged fluids should be used with caution in Hispaniolan Amazon parrots as well as other avian species. For these patients to receive appropriate treatment in critical care settings, fluids with an appropriate osmolality must be developed.

a. Osmette, Precision Systems, Natick, Mass.
b. Olympus AU640e chemistry analyzer, Olympus America Center, Valley, Pa.
c. Prism 5.0 for Mac, GraphPad Software, La Jolla, Calif.

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