Plasma concentration of ionized calcium in healthy iguanas

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Objective—To measure plasma concentration of ionized calcium in healthy green iguanas.

Design—Prospective study.

Animals—Nine juvenile and 21 (10 male, 11 female) adult iguanas.

Procedure—Blood samples were obtained from each iguana, and plasma calcium, glucose, phosphorus, uric acid, total protein, albumin, globulin, potassium, and ionized calcium concentrations, aspartate transaminase (AST) activity, and pH were measured. Heparinized blood was used for measurement of ionized calcium concentration and blood pH. A CBC was also performed to assess the health of the iguanas.

Results—Significant differences were not detected among the 3 groups (juveniles, males, and females) with regard to ionized calcium concentration. Mean ionized calcium concentration measured in blood was 1.47 ± 0.105 mmol/L. Significant differences were detected between juveniles and adults for values of phosphorus, glucose, total protein, albumin, globulin, and AST activity.

Conclusions and Clinical Relevance—Ionized calcium concentration provides a clinical measurement of the physiologically active calcium in circulation. Evaluation of physiologically active calcium in animals with suspected calcium imbalance that have total plasma calcium concentrations within reference range or in gravid animals with considerably increased total plasma calcium concentrations is vital for determining a therapeutic plan. Accurate evaluation of calcium status will provide assistance in the diagnosis of renal disease and seizures and allow for better evaluation of the health status of gravid female iguanas. (J Am Vet Med Assoc 2001;219:326–328)

The green iguana (Iguana iguana) is one of the most commonly kept reptiles in the United States. Veterinary care of these animals is challenging, because there is little published information available regarding hematologic and biochemical variables in iguanas. Additionally, assessment of disease in gravid females can be difficult, as they may have considerably increased serum calcium concentrations. This increase in serum calcium concentration may result from the binding of calcium by proteins associated with egg formation, as is seen in birds during egg formation. Although nutritional secondary hyperparathyroidism is a common illness in iguanas, diagnosis can be complicated by a variety of factors that affect serum calcium concentration. Renal disease is also difficult to diagnose in iguanas, because plasma calcium and phosphorus concentrations can vary greatly. The purpose of the study reported here was to establish a reference range for plasma ionized calcium concentration in healthy green iguanas, with the hypothesis that ionized calcium homeostasis is tightly controlled as it is in other species. Measurement of ionized calcium could provide a useful means of assessing the biologically active calcium of iguanas, which may aid in the diagnosis of various diseases seen in this species.

Materials and Methods

Iguanas—Thirty healthy green iguanas (9 juvenile and 21 [10 male, 11 female] adults) were examined and used in this study. Iguanas were housed outside at 2 locations in northern Florida. The juveniles (10 to 12 months of age) were offspring of the adult females in this study. All iguanas were fed a diet of chopped leafy greens and vegetables. The diet was considered to be adequate in calcium concentration based on the high content of dark leafy greens. The diet varied with availability of different vegetables but was composed of approximately 75 to 80% leafy greens (eg, turnip greens, mustard greens, collard greens, spinach, kale), 20 to 25% vegetables (eg, squash, zucchini, broccoli, green beans), and a small amount of fruit (eg, apples, bananas, grapes, tomatoes). Exact amounts of food consumed were not measured. Blood samples were collected during July and August. Health was assessed by physical examination, CBC, and plasma biochemical analyses. This project was approved by the University of Florida Institutional Animal Care and Use Committee.

Biochemical analyses—Blood samples were obtained by venipuncture of the ventral coccygeal vein, using a balanced sodium-lithium heparinized 2-ml syringe containing 80 units of heparin. The balanced syringe was used specifically to prevent binding of calcium by heparin, which would alter the measurement of ionized calcium. Plasma calcium, glucose, phosphorus, uric acid, total protein, albumin, globulin, potassium, and ionized calcium concentrations, aspartate transaminase (AST) activity, and pH were measured. Cloacal temperature of each iguana was measured and recorded immediately prior to blood collection. Temperature was measured by use of a thermometer capable of measuring temperatures of −40 to 150°C. Following blood collection, a portion of the blood was immediately analyzed by use of a blood gas analyzer to measure ionized calcium and pH. The remaining blood was placed into lithium heparin collection tubes adequate for volume. Blood smears for differential cell counts were prepared immediately and air-dried. Total WBC counts were performed manually, using a hemocytometer and Natt and Herrick staining solution in a 1:100 dilution. Plasma was frozen at −70°C until samples could be analyzed. All bio-
chemical assays except ionized calcium and pH were performed by use of spectrophotometric analysis with a chemistry analyzer. Total protein concentration was measured by the biuret method, albumin concentration was measured by the bromocresol green method, and globulin concentration was measured by the biuret method. Albumin concentration was measured by the biuret method, and globulin concentration was measured by the biuret method. Globulin concentration was measured by the biuret method. Globulin concentration was calculated as the difference between the 2 values.

Statistical analyses—Statistical analyses were performed, using a statistical computer software program. To test differences between 2 groups, a paired Student t-test was used. To test differences among 3 groups, 1-way ANOVA was performed. When data did not have normal distribution, a Kruskal-Wallis 1-way ANOVA on ranks was used, followed by the Tukey key for identification of pair-wise differences.

Results
Significant differences were not detected among males, females, and juveniles for body temperature, Hct, pH, and potassium concentration. Significant differences were not detected in mean (± SD) ionized calcium (1.47 ± 0.105 mmol/L; range, 1.22 to 1.62) or plasma calcium (12.8 ± 0.93 mg/dL; range, 10.9 to 14.4) concentrations among groups. Significant \( P \leq 0.001 \) differences were detected between juveniles and adults for phosphorus, glucose, total protein, albumin, and globulin concentrations and AST activity \( (P = 0.008 \) for AST; Table 1). Significant differences were not detected in blood pH at the normal body temperature of iguanas, compared with blood pH at 37°C \( (P = 0.509) \).

Discussion
Measurement of total plasma calcium concentration has been the primary means of assessing calcium status of iguanas in which calcium abnormalities are suspected. Total plasma calcium is comprised of 3 forms: ionized calcium, protein bound calcium, and calcium complexed to various anions (ie, bicarbonate, lactate, citrate). In humans, there is a significant linear correlation between total serum calcium and albumin concentrations such that equations and nomograms have been developed to correct total calcium concentrations for protein binding. A similar correlation has been found in dogs, and adjustment formulae have been suggested. Results of a study of African grey parrots \( (Psittacus erithacus) \) revealed a linear correlation between total plasma calcium and albumin concentrations. Results of a similar study of peregrine falcons \( (Falco peregrinus) \) revealed a linear correlation between total protein, albumin, and total plasma calcium concentrations. Studies in other species, such as cats and horses, have failed to reveal similar correlations. Total plasma calcium concentration, even if corrected for the effects of protein binding, does not provide information regarding the ionized calcium, which is the physiologically active fraction of the plasma calcium.

The distribution of calcium into the various fractions is influenced by several factors. Acidosis decreases the protein binding capacity for calcium and causes an increase in the ionized calcium fraction, whereas alkalosis causes a decrease in the ionized calcium. Ionized calcium and pH are typically measured concurrently because of the dependence of calcium on pH.

Temperature must also be monitored when measuring ionized calcium concentration, because changes in temperature alter blood pH. Most poikilotherms alter blood and tissue pH when environmental temperature changes occur. Poikilotherms regulate ventilation in such a way that the arterial partial pressure of carbon dioxide is adjusted to maintain a constant fractional dissociation of imidazole groups. Acid-base regulation is a function of regulation of α-imidazole groups of histidyl residues of proteins; this results in a stable hydroxide/hydrogen ion ratio and the observed change in blood pH with temperature: change in pH/change in temperature = –0.015 to 0.020 pH/C. Theoretically, this type of regulation suggests that with changes in the body temperature of the iguanas there would be changes in blood pH and associated changes in ionized calcium. In our study, significant differences were not detected in body temperatures, blood pH, or ionized calcium concentrations among groups. Further studies are necessary to determine whether variations in body temperature result in significant changes in plasma ionized calcium concentration.

In humans, while a decrease in plasma protein concentration can result in a decreased total calcium value, the ionized calcium concentration usually does not change. This was evident in the iguanas of our study when results from juveniles and adults were compared, in that total protein concentration differed significantly with age, but ionized calcium concentration did not differ between juveniles and adults.

Age-related variation is often seen in plasma biochemical values among different species. Studies in storks \( (Ciconia ciconia) \), houbara \( (Chlamydotis undulata macqueenii) \), and kori bustard \( (Anastomus kori) \), mallard ducks \( (Anas platyrhynchos) \), lechwe waterbucks \( (Kobus lechwe) \), and Scimitar-horned oryx
have revealed that juveniles of these species have lower total protein concentrations and higher AST, phosphorus, and glucose concentrations than adults. Significant variations in these variables were evident between the juvenile and adult iguanas in our study.

Measurement of ionized calcium concentration provides a useful means of assessing the biologically active calcium of iguanas. Results of our study suggest that calcium homeostasis is tightly controlled in iguanas, as it is in other species. The notable variations in biochemical values between juvenile and adult iguanas indicate the importance of distinguishing the age of an iguana when evaluating results of blood analyses in a clinical situation.

References