The Significance of Serum **Ionized Calcium and 25-hydroxy-cholecalciferol (Vitamin D_3)** Assays in African Grey Parrots

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Products at a Glance

- ★ **High Potency** Organic Formula, Harrison's Bird Foods, www.harrisonsbirdfoods.com
- ★ **Oxyglobin** - Biopure Corp, Cambridge, MA, USA, www.biopure.com
- ★ **IDS OCTEIA** 25-hydroxycholecalciferol assay (IDS Ltd. 10 Didcot Way, Tyne and Wear, Newcastle upon Tyne)

A GROUP OF AFRICAN GREY parrots kept indoors on the premises of a parrot breeder in the UK are being studied longitudinally over 3 years to monitor the effects of staged changes in husbandry on calcium metabolism. The study group consists of 100 healthy adult African grey parrots of known sex that were fed a seed diet for 12 months prior to the beginning of the study. Fecal samples were taken from each bird for parasitology and Gram's stain examination. Blood samples taken from each bird anesthetized with isoflurane were submitted for routine hematological and serum biochemical tests as well as circovirus, polyomavirus and chlamydophila PCR. Each bird was examined clinically and by laparoscopy. On the basis of these tests, only healthy birds were included in the study. Additional serum was assayed for ionized calcium, 25-hydroxycholecalciferol and parathyroid hormone levels.

Forty birds were then randomly allocated to two groups (n=20 birds per group, 10 male and 10 female). One group was maintained on the original seed diet and was designated the control group; the other group was converted to a pelleted parrot diet (Harrison's High Potency). After an additional 12 months, blood was again sampled to assess the effect of dietary changes on serum levels of ionized calcium, 25-hydroxycholecalciferol and parathyroid hormone.

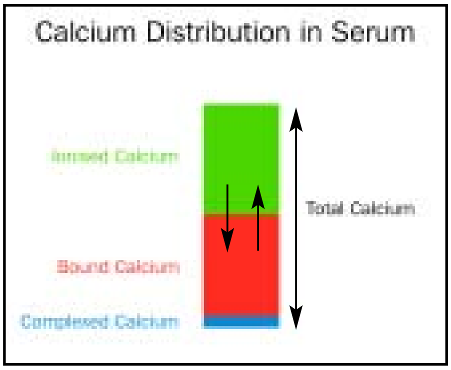
The birds were then placed under artificial ultraviolet light (UV-B; wavelength = 285-315 nm) for 12 hours daily, and the diets were kept constant. During the study, UV-B light levels have been consistently measured in the 285-315 nm spectrum. Blood samples will be collected in September, 2003 to evaluate the effect of artificial UV light on serum levels of ionized calcium, 25-hydroxycholecalciferol and parathyroid hormone. The breeding performance of the birds has also been monitored during the study.

Hypocalcemia in African Greys

Hypocalcemia is a recognized syndrome in captive African grey parrots (*Psittacus erithacus*), but is rarely reported in other psittacine birds. Although the etiology of this syndrome is unconfirmed, proposed mechanisms include primary hypoparathyroidism or a secondary nutritional hyperparathyroidism due to inadequate husbandry. Classically affected birds present clinically with a variety of neurological signs ranging from slight ataxia to seizures, which respond well to calcium therapy (Fig 1). Osteodystrophy is perceived as a common condition in young African grey parrots, which may require extensive corrective surgery (Figs 2,3). Hypocalcemia may remain undiagnosed in some African greys due to the tendency of pathology laboratories to measure total calcium rather than ionized calcium levels in routine serum biochemistry profiles.

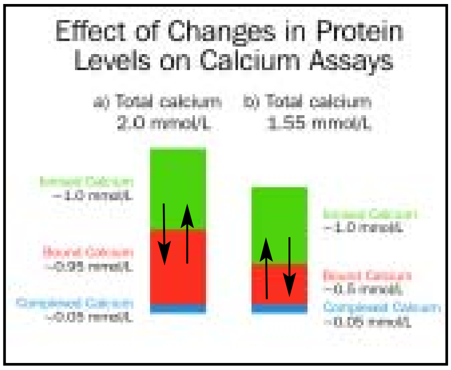
Calcium Distribution

The mechanism of calcium distribution in the avian subject must be understood in order to fully appreciate disorders of its metabolism. Calcium exists as three fractions in avian serum: ionized as a salt, protein bound (primarily with albumin), and complexed with a variety of anions such as citrate. Ionized calcium is the physiologically active fraction of serum calcium, which is important in bone homeostasis, muscle and nerve conduction, blood coagulation and



control over the secretion of hormones such as vitamin D_3 and parathyroid hormone. The protein-bound calcium fraction is physiologically inactive, and any increase in this fraction would not be considered pathophysiologically significant. The complexed fraction is insignificantly small in the healthy patient, although levels may become important in severely ill birds with acid-base imbalances. Therefore, in order to accurately diagnose disorders of calcium metabolism, serum ionized calcium levels should be measured as part of any health profile.

Any condition affecting serum albumin levels will affect the total serum calcium concentration, due to alterations in the protein-bound calcium fraction, but the serum ionized calcium level will not necessarily change.



The classic example of this is in the egg-laying hen. Albumin levels in egg-laying birds may rise by up to 100% and produce an inflated total calcium concentration due to an increase in the protein-bound fraction while not affecting the ionized calcium level. Similarly, a bird could be clinically hypocalcemic with low ionized calcium levels but still exhibit a normal total calcium level. If only total calcium levels are measured in egg-bound birds, hypocalcemia will be frequently underdiagnosed. Indeed, the total calcium levels in these birds may often suggest a hypercalcemia, discouraging routine treatment with calcium supplements and leading to unnecessary surgery to remove impacted eggs. The same situation would be found in many reptiles (Fig 4).

Fig 1. Seizuring African grey parrot

Fig 2. Osteodystrophy in a young African grey parrot

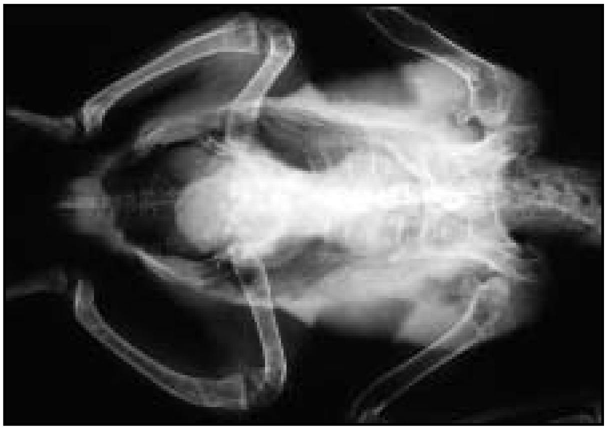
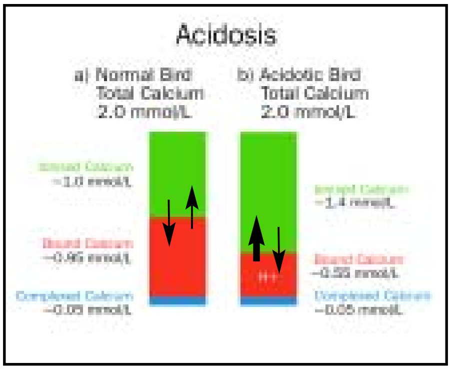
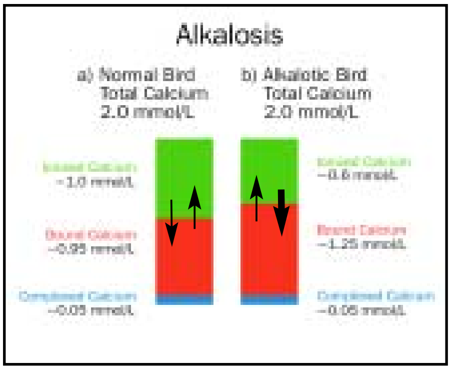


Fig 3. Radiograph of an osteodystrophic African grey parrot





The binding reaction between calcium ions and albumin is strongly pH dependent, so acid-base imbalances will affect serum ionized calcium levels. An increase or decrease in blood pH will respectively increase or decrease the serum protein-bound calcium fraction. The level of ionized calcium is inversely proportional to the level of protein-bound calcium in the blood. Most of the birds seen in practice might be expected to have some degree of acid-base imbalance. Therefore, a patient with metabolic acidosis would be expected to show an ionized hypercalcemia level due to decreased protein binding. The author commonly sees this situation with severe diseases, such as circovirus infection.

With an alkalotic patient, such as a bird with severe diarrhea or regurgitation, an ionized hypocalcemia would occur as the level of serum protein binding increases.

Measuring Ionized Calcium

The measurement of ionized calcium levels is therefore considered the most accurate reflection of the patient's calcium status, especially in a diseased animal. Unfortunately, most veterinary pathology laboratories report total calcium concentrations, reflecting all three of the blood's calcium fractions. Measurement of total calcium levels in an avian patient with abnormal protein levels or acid-base abnormalities does not accurately reflect the calcium status of the animal. Of the 394 samples submitted from African grey parrots to the practice laboratory in 2001, 54 samples (13.17%) had low serum ionized calcium levels despite having normal serum total calcium concentrations, suggesting that hypocalcemia is underdiagnosed in this species. Precise measurement of ionized calcium levels involves the ion selective electrode (ISE) measurement principle.

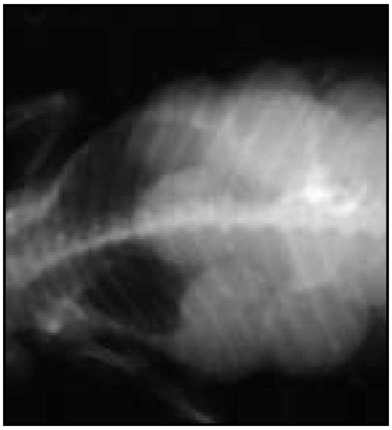


Fig 4. Egg-bound reptile

Fig 5. Acidotic African grey parrot with circovirus

Fig 6. Most ISE analyzers measure electrolytes in addition to ionized calcium, which, in the author's opinion, is information that is also potentially as useful in working with diseased psittacine birds as in mammals.

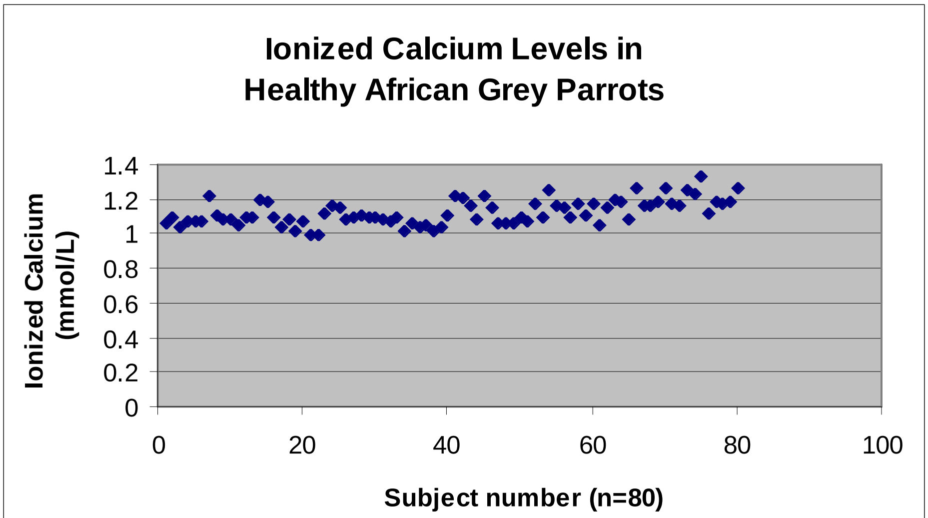


Table 1. Serum ionized calcium graph

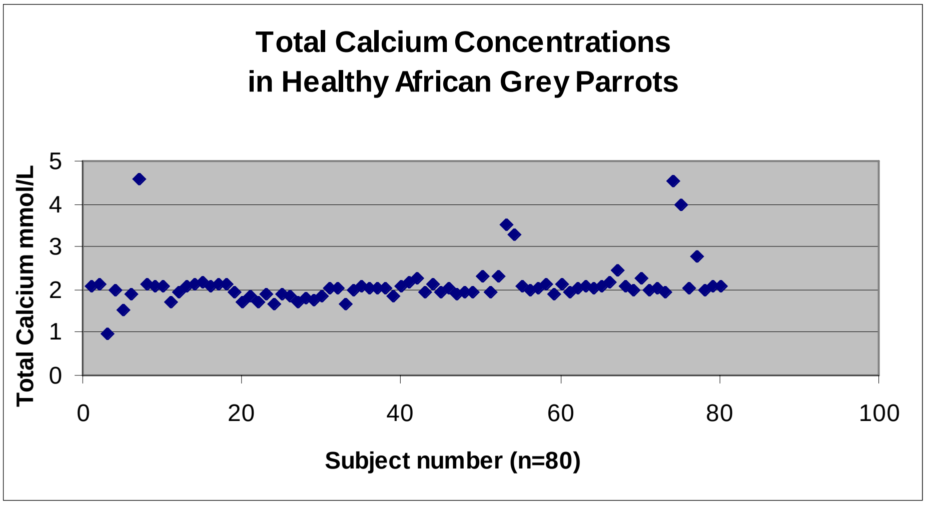


Table 2. Graph of serum total calcium levels

Table 1 indicates the results obtained from 80 healthy seed-fed African grey parrots from the initial group of 100 birds. Using a modified t2 test with 95% confidence limit, the normal range for serum ionized calcium in African grey parrots was found to be 0.96-1.22 mmol/L (3.84-4.88 mg/dl). This indicates that, as in mammals, ionized calcium levels are kept within a tight homeostatically controlled range. Any deviation in the ionized calcium level outside this range would suggest a severe abnormality.

Table 2 indicates the serum total calcium levels from the same 80 seed-fed African grey parrots as measured by spectophotometer. As demonstrated, total calcium levels fluctuate dramatically with protein levels. Blood samples were collected outside the breeding season in order to reduce the effects of estrogen and fluctuating serum protein levels.

Despite all the birds having normal serum ionized calcium levels, 26.2% had abnormal serum total calcium levels based on a normal range of 1.96-3.0 mmol/L (7.84-12.0) compiled by the commercial laboratory used by the author.

In humans, formulas have been developed to reflect the correlation between serum albumin and total calcium levels, thus allowing for correction of total calcium levels in the presence of fluctuations in albumin levels. A statistically significant correlation between albumin and total calcium levels was not present at any stage in this study with African grey parrots. Albumin levels were measured using protein electrophoresis, due to the poor accuracy of traditional spectrophotometric assays for determining protein levels in birds.

In humans, as a person becomes more significantly ill, the relationship between albumin and total calcium levels has a more linear correlation, so the clinician can use correction formulas to allow for fluctuations in protein levels. We did not find a relationship between serum albumin and total calcium levels in greys, perhaps because the birds in this study were healthy. Recent research in humans advises against the use of such adjustment formulas, recommending instead the use of ion selective electrodes to measure ionized calcium levels.[2] Analyzers using ion selective electrodes are also increasingly available for use in veterinary clinics, making the test simple and affordable.

Care of Blood Sample

Blood samples for serum ionized calcium assays should be stored in heparin and analyzed as soon as possible after venipuncture, because changes in the pH of the blood sample will affect the accuracy of the ionized calcium levels. For example, contact with carbon dioxide in the air will reduce the pH of the sample. In this study, blood samples were analyzed within 60 minutes of venipuncture. However, a study in dogs suggests that samples will not be adversely affected if not assayed for as long as 72 hours after collection.[10] This has also been the author's experience with avian samples if blood collection tubes are filled to minimize contact with air. Therefore, if properly packaged, samples can be sent to external laboratories. Hand-held analyzers are also available for in-practice use.

Vitamin D_3 Metabolism

Vitamin D_3 metabolism has been extensively reviewed in domestic fowl. Birds secrete 7-dehydrocholesterol (provitamin D) onto featherless areas of skin. Conversion of provitamin D to cholecalciferol (vitamin D_3) occurs by an ultraviolet light-dependent isomerization reaction. Cholecalciferol is a sterol prohormone, which is subsequently activated by a 2-stage hydroxylation process. It is initially metabolized to 25-hydroxycholecalciferol in the liver, and this

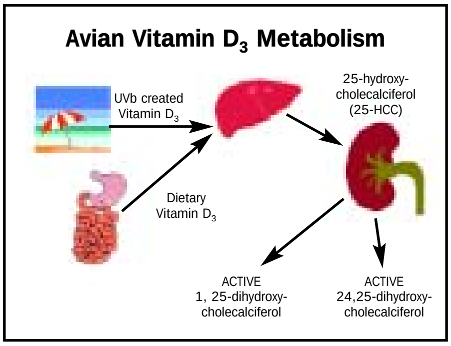


Fig 15. Vitamin D metabolism

metabolite is transported to the kidney via carrier proteins where it is converted to either 1, 25-dihydroxycholecalciferol or 24, 25-dihydroxycholecalciferol, the active metabolites of cholecalciferol in domestic fowl. In egg-laying hens, 30-40% of the calcium required for eggshell formation is acquired from medullary bone. The control of this highly labile calcium pool involves both 1, 25-dihydroxycholecalciferol and estrogen.

Vitamin D_3 metabolism has not been well researched in psittacine birds, but significant differences in vitamin D_3 levels under different lighting and dietary regimens, similar to those studied with domestic poultry, are predicted in African grey parrots. The use of artificial ultraviolet lights and improved diets should significantly reduce the common disorders of calcium metabolism seen in this species. Psittacine birds kept indoors would be expected to receive inadequate UV-B light exposure for cutaneous synthesis of vitamin D. Vitamin D is usually supplied in avian diets in the form of ergol (vitamin D_2), which requires conversion to active metabolites in order to prevent vitamin D_3 toxicity. However, only 10% of dietary vitamin D_2 is bioavailable in domestic fowl.[5]

Vitamin D Assays

Serum concentration of 25-hydroxycholecalciferol is considered the most reliable measure of the vitamin D status of an individual due to its long half-life compared with other vitamin D metabolites. Traditionally, radioimmunoassays have been used to assay 25-hydroxycholecalciferol, but the more recently available enzyme immunoassays have the advantages of convenience and economy. Enzyme immunoassays correlate well with recognized radioimmunoassays. The IDS OCTEIA 25-hydroxycholecalciferol assay was used in this study for the quantitative determination of 25-hydroxycholecalciferol. Each sample was submitted for both enzyme immunoassay and radioimmunoassay. The enzyme immunoassay was easy to perform, and the cost was $15 per test compared with $110 for each radioimmunoassay. The enzyme immunoassay methodology would also be of use in reptile medicine for the diagnosis of metabolic bone disease.

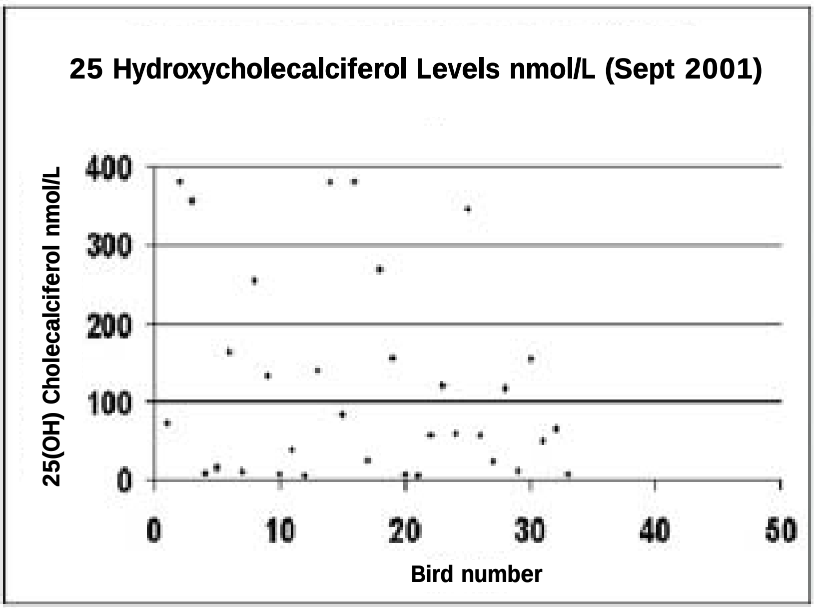


Table 3. Initial vitamin D results graph

Initial Vitamin D Results

Table 3 indicates the values obtained by enzyme immunoassay for 25-hydroxycholecalciferol in 40 seed-fed African grey parrots. The study demonstrated that 25-hydroxycholecalciferol could be consistently recovered from psittacine blood using an enzyme immunoassay. The 25-hydroxycholecalciferol level in these 40 seed-fed birds was 7.2-380.0 nmol/L with a mean of 116.52 nmol/L and a standard deviation of 126.7 nmol/L. In man, the normal range for 25-hydroxycholecalciferol is 47.7-144.0 nmol/l.[2] In the laying hen, 25-hydroxycholecalciferol would not be expected to fall below 26 nmol/L and would normally be greater than 50 nmol/L.[12]

Conclusions

★ Analysis of ionized calcium levels is vital if correct diagnosis of calcium disorders is to be achieved in exotic animals. The measurement of total calcium should be avoided now that there are reliable and affordable tests for measuring ionized calcium levels.

★ The measurement of 25-hydroxycholecalciferol levels in exotic animals is now practical and economical using an enzyme immunoassay. This should lead to advances in the treatment and prevention of calcium disorders in both reptiles and birds as well as allow for dietary vitamin D requirements to be devised under different lighting regimens.

★ One would expect the routine measurement of ionized calcium and 25-hydroxycholecalciferol to be useful in other species, particularly many species of reptiles that routinely present with abnormalities of calcium metabolism. Normal 25-hydroxycholecalciferol ranges need to be developed for most exotic species.

References and Further Reading

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