



Significance of Cholesterol Assays in the Investigation of Hepatic Lipidosis and Atherosclerosis in Psittacine Birds

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The aim of this study was to formulate normal ranges for cholesterol profiles in psittacine patients to aid in the diagnosis of hypercholesterolemia and associated conditions such as atherosclerosis or hepatic lipidosis (Figs 1-4). The effect of different husbandry conditions and species on blood cholesterol concentrations was also investigated. The blood cholesterol profile was used in birds with clinical evidence of liver disease in the author's practice to assess its diagnostic usefulness.



Fig 1. An enlarged pale liver is characteristic of hepatic lipidosis in parrots. Unfortunately, this is frequently a postmortem rather than an antemortem diagnosis.



Fig 2. Severe obesity in a medium sulphur-crested cockatoo. It is not unusual to see such large amounts of subcutaneous fat consistent with inactivity and a high fat diet. Subsequent blood tests indicated a total cholesterol concentration of 12.78 mmol/L.



Fig 3. Atherosclerotic plaques involved the cardiac vessels in this African grey parrot. This species is very susceptible to atherosclerosis and subsequent fibrosis of the vessels.



Fig 4. Radiograph of the heart and great vessels of an African grey parrot with atherosclerosis. Calcification of the blood vessels is easily identifiable.

Lipid Metabolism in Birds

Hypercholesterolemia can be associated with lipemia, liver disease, hypothyroidism, bile duct obstruction and high-fat diets. Dietary fatty acids, triglycerides and other lipids are insoluble in blood plasma and other tissue fluids. Lipids are transported throughout the body in cells as part of the cell membrane or in blood as lipoprotein complexes. Four types of lipoproteins have been identified in the bird: portomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Their density when measured by ultracentrifugation classifies them, but since this process is expensive, electrophoresis techniques have been developed to assay lipoproteins in man. Alternatively, formulas have been developed that will calculate LDL if HDL, cholesterol and triglyceride concentrations are known. These formulas are considered to be species-specific and have certainly not been validated for birds.

The liver synthesizes the majority of the body's cholesterol from VLDL. Cholesterol is found mainly as two lipoproteins in the body: high-density cholesterol (HDL) and low-density cholesterol (LDL). Following synthesis in the liver, HDL circulates through peripheral tissues, cleaning excess LDL and cholesterol from the walls of blood vessels. It then transports cholesterol back to the liver for subsequent conversion to bile acids, a safe form for excretion.

Abnormalities of Cholesterol Metabolism

Although LDL provides cells with their cholesterol requirements, excess amounts in man can lead to atherosclerosis. In general terms, a high concentration of LDL might also be considered abnormal in birds and could be expected to result in atherosclerosis. In a recent review of 107 avian necropsies, 36% of the birds had grossly identifiable cardiovascular lesions and 99% had cardiac lesions on histopathology, indicating that cardiac disease is presently a very common, yet undiagnosed, condition in avian medicine. The incidence of atherosclerosis in captive parrots is reported to be 5.0-12.6%. As in mammals, atherosclerosis in psittacine birds appears to have a genetic component; African grey parrots appear to be most susceptible followed by Amazon parrots, and cockatoos and macaws are less frequently represented. A high plasma LDL concentration is a risk factor for atherosclerosis in man, budgerigars and quail, and this would be expected to be the case in parrots.

Fatty liver disease, or hepatic lipidosis, is a common syndrome in psittacine birds, especially the South American species. Although high plasma concentrations of total cholesterol have been reported in parrots with hepatic lipidosis, little research has concentrated on HDL and LDL in clinical cases. Bavelaar and Beynen have demonstrated in African grey parrots that diets with very high saturated fat content signifi-

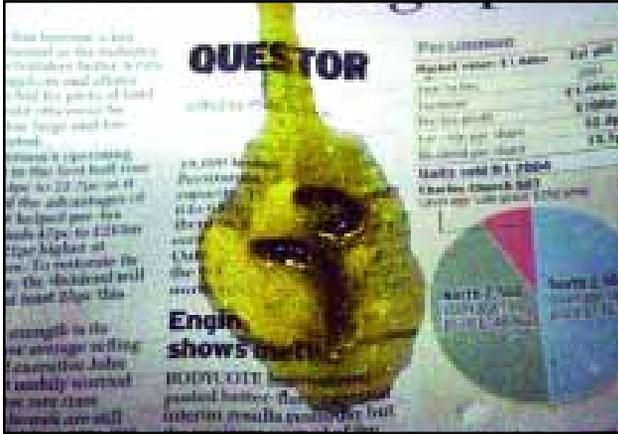


Fig 5. Classical appearance of green-stained urates indicating biliverdinuria in a parrot with hepatic disease.



Fig 6. Black discoloration of the primary feathers in a green-winged macaw due to exposure of the basal feather pigments. This is thought to be due to hepatic disease and a failure of synthesis of endogenous carotenoids.



Fig 7. This budgerigar with chronic liver disease is thin, yet the abdomen is distended with ascitic fluid. Histopathologic analysis of a liver biopsy confirmed the diagnosis of yersinosis.



Fig 8. Dorsal-ventral radiograph of an Amazon parrot subsequently confirmed to be suffering from hepatic lipidosis. There is an obvious enlargement of the hepatic silhouette.

cantly affect plasma cholesterol concentrations. The study used artificially high concentrations of dietary fat not normally used in everyday feeding. Reducing dietary saturated fat or increasing the polyunsaturated fat levels in man can change plasma cholesterol concentrations, and Bavelaar and Beynen demonstrated similar results in African grey parrots. The use of soluble fiber, such as psyllium, has been shown to reduce plasma cholesterol concentrations in poultry, but a similar effect was not seen in African grey parrots.

Investigation of Liver Disease

Clinical signs of liver disease are nonspecific and

include lethargy, anorexia and biliverdinuria (Fig 5). In later stages, parrots can present with ascites, hepatomegaly and changes in plumage pigment (Figs 6, 7). Since liver disease in birds can be fatal if not diagnosed early, an accurate method of detecting early hepatic pathology is essential. Currently, the avian clinician needs to rely on a combination of investigative techniques to reach a specific diagnosis in the broad category of liver disease.

The majority of blood assays used in veterinary pathology laboratories for exotic pets are extrapolated from routine mammalian tests and are not validated for other species. Although biochemical evaluation of blood samples can increase the suspicion of liver disease, there appears to be a distinct lack of correla-



FIG 9. Positioning of ultrasound probe for investigating liver disease. Unfortunately, ultrasonography can be used in birds only to assess liver size or guide biopsy techniques despite its usefulness in mammalian medicine.



Fig 10. Typical positioning for diagnostic endoscopy. The author recommends endoscopy for any parrot with undiagnosed feather loss. This hyacinth macaw had chlamydiosis and after treatment made a full recovery. A ventral midline approach gives the endoscopist a full view of the liver, and endoscopic biopsies can be taken.



Fig 11. Endoscopic view of a normal parrot liver lobe from the caudal thoracic air sac.

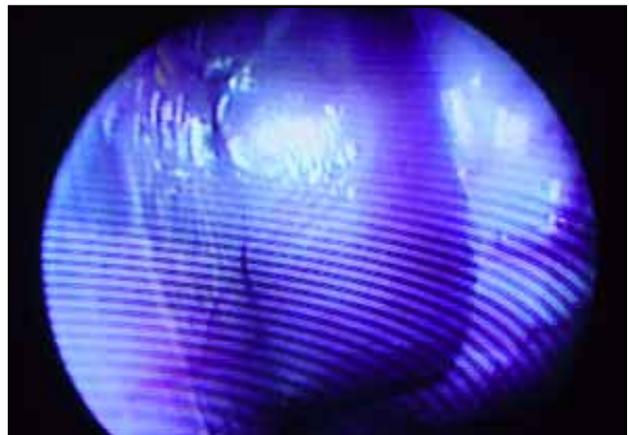


Fig 12. Endoscopic view of an abnormal liver lobe in a parrot. The liver is grossly enlarged and has swollen edges. The histopathologic diagnosis from a biopsy was hepatic lipidosis.

tion between changes in liver enzyme concentrations and histopathologic changes seen in the liver. Certain severe hepatopathies, such as aflatoxicosis, can result in dramatic loss of liver function without a significant elevation of liver enzymes. Although the hematology profile is not specific for liver problems, it can also give the veterinarian useful information. In particular, chlamydiosis and other infections can be suggested from elevated white blood cell counts; additional blood tests (e.g., serologic testing) are necessary in these cases. Bile acid assays have been validated in birds as a means of assessing liver function. In the author's opinion, bile acid concentrations >100 mmol/L are generally significant of hepatic disorders and correlate well with histopathologic changes in liver biopsies.

Diagnostic tests should also include survey radi-

ographs of the anesthetized bird, and positioning must be optimal to assess liver size. The liver silhouette should not extend beyond imaginary vertical lines drawn through the shoulder joints (Fig 8). However, radiography is likely to only suggest an enlarged liver and cannot establish a definitive diagnosis. Ultrasonography can also be used to assess liver size but, in the author's opinion, has the same limitations as radiography (Fig 9). Ultrasonography can be used to guide biopsy techniques, but this provides no apparent advantage over biopsies collected via endoscopy.

Diagnostic endoscopy is perhaps the most useful tool for the investigation of hepatic disease. The author normally prefers the standard left lateral endoscopic approach. Entering the cranial thoracic air



Fig 13. The author favors a right lateral approach for hepatic biopsy in parrots. This allows easy identification of the liver. The liver is grasped in micro hemostats and the biopsy is removed. These biopsy samples are larger than those obtained by endoscopy and therefore provide more diagnostic information. The small piece of tissue is fixed to filter paper to prevent artifactual change. Histopathology of the biopsy is the preferred method of obtaining an accurate diagnosis of chronic liver disease.



Fig 14. Bronze-wing pious parrot. The 29 parrots used in the second study were a group of mixed *Pionus* spp. Despite eating a diet with a very low fat content, the birds had significantly higher levels of LDL than the African grey parrots.

sac, the liver lobe can be visualized and assessed for gross changes. To view the entire liver, a ventral midline approach is recommended, and the endoscope is introduced under the sternal arch. Using this approach, biopsies can also be taken (Figs 10-12). If a liver biopsy is definitely required at the outset, the author favors a right-sided approach entering behind the last rib.

To collect a liver biopsy, a small incision is made to access the liver immediately below the muscle. A thin capsule surrounds the liver, which may need to be incised prior to biopsy. The liver is grasped with hemostats for the biopsy procedure. The author favors the LigaSure™ blood vessel sealing generator for liver biopsy, which ensures hemostasis and causes minimal damage to hepatic tissue. Using experienced avian pathologists, histopathologic analysis of small liver biopsies can be a very useful diagnostic tool and is often the only method of obtaining a definitive diagnosis (Fig 13).

Hemorrhage or release of ascitic fluid into the caudal air sacs is a potential complication of liver biopsy in parrots, although this is normally a routine procedure. A working diagnosis of atherosclerosis may be reached if fibrosis or calcification of the great vessels is evident radiographically. Histopathology reveals fibrosis of the vessels.

Methodology

The main population of birds from which the study

subjects were drawn consisted of 100 healthy adult African grey parrots housed indoors as 50 pairs. All birds had been purchased from a single source and were wild-caught imports from Guyana. Each pair was housed in a separate brick and wire aviary measuring 2 m x 2 m containing a wooden nest box measuring 40 cm x 30 cm. All aviaries were positioned in a farm building of brick and slate roof construction with no exposure to natural ultraviolet light. The birds were fed a seed-based diet (Tidymix) without additional vitamin or mineral supplementation. The birds had been kept under these husbandry conditions for 24 months prior to the start of the study.

Each bird had been examined prior to purchase in 1998 using a standard format. Fecal samples were collected for parasitologic, microbiologic and Gram's stain examination. Blood samples were submitted for routine hematologic and biochemical analysis as well as PCR tests for circovirus, polyomavirus and chlamydia. Each bird was examined by laparoscopy to confirm its gender and stage of sexual maturity. As requested by the owner, the birds were examined and blood samples analyzed annually.

Forty birds were randomly selected from the main population to form the study group; these were then randomly divided into 2 groups of 10 female/male pairs. The birds were kept in the same building under the same conditions as the main population. One group was fed a pellet diet (Harrison's High Potency Coarse bird food), and the second group was main-



Fig 15. Many captive reptiles are reported to suffer from hepatic lipidosis normally associated with overfeeding. This water dragon presented with anorexia. Endoscopy indicated an enlarged pale liver consistent with lipidosis. The animal was euthanized on humane grounds. Plasma cholesterol levels in reptiles have not been researched to date.

tained on the seed diet. During the annual health examination, additional blood was collected from the study group for measurement of cholesterol levels. Samples were assayed for HDL, LDL, triglyceride and total cholesterol concentrations using an Alfa Wassermann wet chemistry analyzer. All birds were anesthetized with isoflurane for blood collection.

In a separate study, blood was collected from a group of 29 sexually mature healthy pionus parrots fed a pulse-based diet, and the cholesterol parameters described above were analyzed (Fig 14). (A pulse-based diet in the UK consists of a mixture of mung beans, chick peas, black-eyed peas and harricot beans soaked for 48 hours, to which apple/carrot and a multivitamin are added.) Blood samples were collected in duplicate from each bird and the mean used for statistical purposes.

All clinical cases presenting at the author's practice and presumed to have hepatic lipidosis were blood sampled routinely for the cholesterol profile described above; other diagnostic tests were also performed. Cholesterol levels from these birds were then compared with the normal ranges derived from the main study and with the histopathologic diagnosis from liver biopsies to determine if there was a correlation between cholesterol concentrations and histopathologic changes.

Results

An independent sample t2 test with 95% confidence limits was used to analyze all data. The units for all values listed are mmol/L.

Table 1. Results from African Grey Parrots Fed a Seed Diet with a Fat Content of 192.2 g/kg Dry Weight Total Diet (n=19)

Parameter	Mean	Standard deviation	Reference range
LDL-Cholesterol	1.86	1.02	0.84-2.88
HDL-Cholesterol	4.51	0.23	4.28-4.74
Cholesterol	7.0	1.27	5.73-8.27
Triglycerides	1.56	0.66	0.9-2.22

Table 2. Results from African Grey Parrots Fed a Pellet Diet with a Fat Content of 158 g/kg (n=20)

Parameter	Mean	Standard deviation	Reference range
LDL-Cholesterol	1.60	0.98	0.62-2.58
HDL-Cholesterol	4.36	0.19	4.17-4.55
Cholesterol	6.72	0.96	5.76-7.68
Triglycerides	1.66	0.49	1.17- 2.15

No significant difference was seen between the cholesterol concentrations in each dietary group.

Table 3. Results from Pionus Parrots Fed a Pulse Diet with a Fat Content of 70.2 g/kg (n=29)

Parameter	Mean	Standard deviation	Reference range
LDL-Cholesterol	2.33	0.88	1.95-2.67
HDL-Cholesterol	4.52	0.14	4.45-4.58
Cholesterol	7.4	0.97	6.99-7.8
Triglycerides	1.22	0.22	1.13-1.31

The LDL concentration was found to be significantly greater in the pionus group compared with either African grey parrot group.

Table 4. Mean Cholesterol Concentrations in Clinical Cases of Confirmed Hepatic Lipidosis (n=9)

Parameter	Mean
LDL-Cholesterol	4.86
HDL-Cholesterol	6.72
Cholesterol	11.08
Triglycerides	2.05

All 9 birds with histopathologic evidence of hepatic lipidosis had significantly higher LDL concentrations compared with any of the clinically normal pionus or African grey parrots.

Discussion

The study produced normal ranges for triglycerides, total cholesterol, HDL and LDL in African grey parrots fed both seed and pellet diets. Cholesterol parameters of the 2 dietary groups were not significantly different in spite of the different concentrations of dietary fat. Although the study previously mentioned demonstrated that dietary fat could influence cholesterol concentrations, the diets in that study contained very high saturated fat concentrations that would only be used experimentally and not in captive parrots kept under normal conditions.

However, a significant difference was seen between the values obtained for the pionus group and the corresponding values in the African grey parrot; this occurred in spite of the pionus group being fed a diet with a lower saturated fat content than either of the diets used for the African grey parrots. This suggests that there is a genetic difference in cholesterol metabolism in parrots as in man, and it might explain why some species are susceptible to disorders of cholesterol metabolism. Traditionally, South American species eat diets very low in fat in the wild, essentially being consumers of fruit. The variation also demonstrates that different psittacine species should have specific diets developed for individual species requirements.

In all cases of hepatic lipidosis seen at the clinic, LDL values were significantly higher than those from any of the clinically normal parrot groups. A high plasma LDL concentration also correlated well with histopathologic evidence of hepatic lipidosis in liver biopsies.

Using cholesterol profiles either in clinical cases or in health profiles might be helpful in diagnosing hepatic lipidosis or even in predicting the development of hepatic lipidosis before clinical signs are

evident. Although a controlled study must be undertaken to further examine normal and abnormal ranges of the parameters in the cholesterol profile among avian species, we can presently say that an LDL concentration >3 mmol/L should be considered abnormal in psittacine birds. Treatment could involve dietary correction or hypocholesterolemic drugs. The author is presently evaluating the use of LDL assays in monitoring the response to treatment for hypercholesterolemia.

It was not possible in this study to correlate LDL concentrations with the incidence of atherosclerosis due to the difficulty of diagnosing atherosclerosis in the early stages of the disease.

Acknowledgements

The author is extremely grateful for financial assistance for this work from Harrison's Bird Foods. Thank you to Nigel Harcourt-Brown and Beck's Bird Barn for providing the African grey parrot and pionus blood samples.

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Resources at a Glance

- * Blood vessel sealing generator - LigaSure™, Tyco Health Products, www.ligasure.com
- * Seed-based food for birds - Tidymix™, John Heath Hull

- * Formulated food for birds - High Potency Coarse™, Harrison's Bird Foods, www.harrisonsbirdfoods.com