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Original Study

Evaluation of Orally Administered Atorvastatin on Plasma Lipid and Biochemistry Profiles in Hypercholesterolemic Hispaniolan Amazon Parrots (*Amazona ventralis*)

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Abstract: Atorvastatin is a synthetic statin administered in its active form and used for the treatment of dyslipidemias. In the current study, the effects of atorvastatin were evaluated on plasma lipid profiles and the potential for adverse effects after once daily PO dosing of atorvastatin for 30 days in Hispaniolan Amazon parrots (*Amazona ventralis*). Sixteen adult parrots (10 female, 6 male) with hypercholesterolemia were used for this study. Birds were assigned to 2 groups (treatment and control) of 8 parrots each (3 male, 5 female) after balancing for age, sex, originating institution, and baseline plasma cholesterol values. Compounded atorvastatin oral suspension (10 mg/kg) was administered PO once daily via gavage into the crop. Equivalent volumes of placebo suspension were administered to the control group. Plasma biochemistry and plasma lipid profile analysis (total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides [TGs]) were analyzed on days 0, 14, and 30. Plasma samples and HDL-C fractions were evaluated for cholesterol and TG concentrations via enzymatic assays. Subtraction of HDL-C values from total cholesterol yielded the non-HDL-C concentration for each bird. Birds were routinely assessed for appetite, activity, and urofeces. Plasma atorvastatin concentrations were obtained from 7 of 8 birds in the treatment group from banked samples. Those samples were obtained on days 14 and 30, with drug administration 6 to 8 hours before collection. No significant differences were observed in total cholesterol, HDL-C, non-HDL-C, or TG between treatment and control groups at days 0, 14, and 30. Plasma atorvastatin concentrations were variable on day 14 (0.54–5.41 ng/mL for 6 of 7 samples, with 1 outlier of 307 ng/mL) and on day 30 (0.79–6.74 ng/mL). No adverse effects were noted in any of the birds during the study period. When dosed PO at 10 mg/kg once daily, atorvastatin did not result in significant changes to plasma lipid profiles (eg, lowering of plasma total or non-HDL-C concentrations) at any time point during this study. Future studies to investigate pharmacokinetic and pharmacodynamic properties of atorvastatin in parrots may require increased doses and/or frequency of administration.

Key words: atherosclerosis, dyslipidemia, hypercholesterolemia, lipid panel, atorvastatin, statin, psittacine, bird, avian, Amazon parrot, *Amazona ventralis*

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INTRODUCTION

Dyslipidemias are defined as abnormal concentrations of lipids and lipoproteins in the blood. These abnormalities in mammals may include elevated total cholesterol (TC), elevated low-density lipoprotein cholesterol (LDL-C), and decreased high-density lipoprotein cholesterol (HDL-C).^{1,2} In humans, several dyslipidemic disorders are established risk factors for atherosclerotic diseases.^{3–5} Dyslipidemic changes, in particular, increased TC, increased LDL-C, and TGs have been proposed to be risk factors for the development of atherosclerosis in psittacine birds.^{1,6–8} Dyslipidemia, including hypercholesterolemia are well-established targets for the prevention and treatment of atherosclerosis.^{1,9,10} In a study evaluating the ability to induce hypercholesterolemia in Quaker parrots (*Myiopsitta monachus*) via cholesterol feeding, it was found that the severity of atherosclerotic lesions and arterial cholesterol content were associated with plasma TC and LDL-C concentrations.¹¹ In that study, the authors found that the lesions induced by diet were similar to spontaneous lesions but should not be compared directly because the mechanism of lesion formation and progression may differ given the experimental diet and the accelerated time course of lesion formation.

Statin medications are routinely prescribed for humans for primary and secondary prevention of coronary artery disease, as well as for dyslipidemias and other lipid-related disorders.¹² Statin medications are hepatic 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, which reduce the amount of cholesterol produced by blocking primary enzymes of the mevalonate pathway needed for hepatic cholesterol synthesis.^{12,13} Statin drugs have also been shown to have antiatherosclerotic effects in humans independent of their lipid-lowering effects. Statins have been positively correlated with a decrease in the percentage of LDL-C, whereas angiographic studies have shown that patients receiving statin medications have reduced progression and may even be aided in the regression of atherosclerotic lesions.¹³ Several statin medications are commercially available, with atorvastatin and rosuvastatin characterized by long half-lives in humans of approximately 15 to 30 hours.^{2,14} Considering the increased metabolic rates of most avian species, using a medication with a longer half-life has the potential to reduce the required dosing interval in psittacine species.² In a pharmacokinetic study with psittacine birds, compounded rosuvastatin

was administered at 10 and 25 mg/kg and did not appear to reach plasma concentrations; however, no pharmacodynamic information was evaluated during that study to monitor the potential lipid-lowering effects.²

Atorvastatin is a synthetic statin administered in its active form, which is used for the treatment of dyslipidemia in humans.¹⁵ Described adverse reactions to atorvastatin have included nausea, diarrhea, and muscle and joint pain; more serious, but less frequently reported adverse effects in humans include renal and/or hepatic failure.^{15–17} In rats with dietary hypercholesterolemia, atorvastatin administered PO at 80 mg/kg once daily for 7 days, resulted in a reduction of the plasma cholesterol, TG, and LDL-C concentrations.¹⁸ When chickens were administered atorvastatin at a dose of 0.06% within the feed (approximately 24 mg/kg per day), plasma TC was reduced.¹⁹

The group of Hispaniolan Amazon parrots (*Amazona ventralis*) at the University of California, Davis, has been documented to have hypercholesterolemia and has participated in several previous studies, including the effects of exercise on the lipid profile.²⁰ The dosing for atorvastatin, based on anecdotal recommendations, is 3 mg/kg given PO once daily.²¹ To our knowledge, there are no published studies evaluating the effects of any statin drug (HMG-CoA reductase inhibitors) on plasma lipid profiles in any psittacine species. The purpose of this study was to evaluate the effects of atorvastatin administration in Hispaniolan Amazon parrots by monitoring plasma lipid and biochemistry profiles and monitoring for adverse effects of atorvastatin administration over a period of 30 days. We hypothesized that plasma TC would decrease in the Hispaniolan Amazon parrots during the 30-day PO administration of compounded atorvastatin, with no adverse effects.

MATERIALS AND METHODS

Animals and husbandry

Sixteen adult Hispaniolan Amazon parrots (10 females and 6 males) were enrolled in this study. Eleven (69%) of the animals (ranging in age from 12 to 28 years) were from a research flock at the University of California, Davis, School of Veterinary Medicine, Richard M. Schubot Parrot Wellness and Welfare Program. An additional 5 hypercholesterolemic animals (adults of undetermined age) were obtained from the Louisiana State University, School of Veterinary Medicine. The body weight range for all the parrots on day 0 was 251 to 391 g. All animals were housed individually

in wire cages ($61 \times 58 \times 66$ cm) with the University of California, Davis, birds in a single room and the Louisiana State University birds in a separate single room that allowed for visual and audible socialization within each group of birds, and which were temperature controlled at 25.5°C (78°F) and had a 12-hour daylight cycle. The animals were fed ad libitum pelleted diet (ZuPreem Fruit Blend, Premium Nutritional Products Inc, Overland Park, KS, USA) and had free access to water via sipper bottles. Before the study, all birds were deemed healthy via a complete physical examination and packed cell volume (PCV), total solids (TS), and plasma biochemistry. No significant abnormalities, other than mild to severe hypercholesterolemia, depending on the individual (reference interval, $87\text{--}364$ mg/dL)²² were noted on biochemistry and lipid panel analysis. A 30-day acclimation period was used before the start of the study. The birds were balanced for age, sex, originating institution, and baseline plasma cholesterol values before assignment to 2 groups (treatment and control) of 8 parrots each (3 males and 5 females). The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of California, Davis.

Atorvastatin was compounded as a PO suspension (10 mg/mL) by the William R. Pritchard Veterinary Medical Teaching Hospital Pharmaceutical Services using previously published methods and used for the duration of the study.²³ The placebo suspension was compounded with the same suspending agents (Ora-Blend, Perrigo Pharmaceuticals, Allegan, MI, USA) and flavoring (peanut butter, FlavoRx, Columbia, MD, USA). Compounded atorvastatin (10 mg/kg), was administered between 1800 and 2000 hours every 24 hours, for 30 treatments, followed by room lights being turned off. Equivalent volumes of placebo suspension were administered to the control group as described for the atorvastatin. The medications were mixed thoroughly for 1 minute before making individual doses. Medications were administered via metal gavage tube, followed by 1 mL of water to clear the tube, into each bird's crop.

Sample collection and plasma lipid profile analysis

Jugular venipuncture was performed on day 0 (morning before gavage), day 14, and day 30 with a 26-gauge, 1.59 cm (5/8-in) needle on a 3-mL syringe. Samples were collected 6 to 8 hours after the previous evening's drug administration. A maximum of 2.5 mL ($<1\%$ of the body weight) was collected from each bird. Microhematocrit

tubes were filled and centrifuged at $12\,000g$ for 5 minutes for PCV and TS. Blood was placed in ethylenediaminetetraacetic acid microtainers (Fisher Diagnostics, Middletown, VA, USA) for plasma lipid profiles and in lithium heparin tubes (Fisher Diagnostics) for plasma biochemistry panels. Samples were stored on ice for less than 3 hours, centrifuged at $1843g$ for 6 minutes, and plasma was harvested. Plasma for lipid profile analysis was stored in cryovials at -80°C .

Plasma biochemical analyses were performed at the William R. Pritchard Veterinary Medical Teaching Hospital, Clinical Diagnostic Laboratories, University of California, Davis.

Lipid panel analyses were performed in the Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis. Direct LDL-C, TG, TC, and HDL-C were analyzed with a PolyChem 180 clinical chemistry analyzer (Polymedco Inc, Cortlandt Manor, NY, USA) with reagents from Randox (Kearneysville, WV, USA). The profiles also included direct determinations of TC, HDL-C, LDL-C, and TG. One-part plasma and 1-part LDL/very low density lipoprotein precipitation buffer (abCam, Cambridge, MA, USA) were mixed together and incubated at room temperature for 10 minutes. The samples were then centrifuged for 10 minutes at $2000g$ and 4°C . After centrifugation, the supernatant HDL fraction was removed. The original plasma sample and HDL fraction were assayed for TC and TG with an enzymatic assay. The HDL values were then subtracted from the total values to calculate the non-HDL values for each respective assay. Validation of the lipid profile was performed during a previous study, from the same University of California, Davis, flock of parrots.²⁰

Banked plasma available from days 14 and 30 from 7 of the 8 treatment birds was evaluated for atorvastatin concentration. Plasma calibrators were prepared by dilution of the atorvastatin working standard solution (Toronto Research Chemicals, Toronto, ON, Canada) with drug-free plasma to concentrations ranging from 0.25 to 400 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality-control samples (plasma fortified with analyte at 2 concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Before analysis, 50 μL of plasma was diluted with 50 μL of methanol and 200 μL of ammonium acetate–1 M acetic acid (9:1, v:v) containing 0.0005 ng/ μL of d5-atorvastatin internal standard to pre-

Table 1. Mean $\pm$ standard deviation of plasma lipid profile (mg/dL) from centrifugation method from Hispaniolan Amazon parrots (control, $n = 8$; treatment, $n = 8$) at time point 0 and days 14 and 30 after once-daily PO dosing of atorvastatin (10 mg/kg).

Parameters	Day 0		Day 14		Day 30	
	Control	Treatment	Control	Treatment	Control	Treatment
Total cholesterol	384.1 $\pm$ 63.0	376.9 $\pm$ 77.5	395.9 $\pm$ 42.5	327.9 $\pm$ 77.5	405.6 $\pm$ 38.0	359.4 $\pm$ 69.8
Non-HDL cholesterol	170.4 $\pm$ 59.0	173.8 $\pm$ 101	162.6 $\pm$ 43.2	139.9 $\pm$ 48.7	181.9 $\pm$ 33.5	140.7 $\pm$ 42.2
HDL cholesterol	213.7 $\pm$ 63.9	203.2 $\pm$ 51.2	233.3 $\pm$ 40.0	188.0 $\pm$ 69.0	223.6 $\pm$ 40.2	218.7 $\pm$ 56.7
Triglycerides	84.5 $\pm$ 25.6	68.0 $\pm$ 18.8	76.7 $\pm$ 15.2	97.0 $\pm$ 32.3	82.8 $\pm$ 22.4	84.4 $\pm$ 12.9
Non-HDL triglycerides	29.9 $\pm$ 15.8	21.9 $\pm$ 11.2	24.2 $\pm$ 14.8	49.3 $\pm$ 34.9	40.7 $\pm$ 20.2	42.3 $\pm$ 16.6
HDL triglycerides	54.5 $\pm$ 13.3	46.1 $\pm$ 10.3	52.5 $\pm$ 13.9	47.7 $\pm$ 8.7	42.0 $\pm$ 4.4	42.1 $\pm$ 4.4

Abbreviation: HDL, high-density lipoprotein.

cipitate proteins. The samples were vortexed for 1 minute to mix, refrigerated for 20 minutes, vortexed for an additional 1 minute, centrifuged at 3830g for 10 minutes at 4°C, and 30 μ L was injected into the liquid chromatography tandem mass spectrometry system. Quantitative analysis of atorvastatin was performed on a TSQ Vantage triple-quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) coupled with a turbulent flow chromatography system (TFC TLX2, Thermo Scientific) having LC-10ADvp liquid chromatography systems (Shimadzu, Kyoto, Japan) and operated in laminar-flow mode. Detection and quantification were conducted with selective reaction monitoring of the initial precursor ion for atorvastatin (559 mass-to-charge ratio). The response for the product ions for atorvastatin (250, 276, 292, and 440 mass-to-charge ratios) was plotted, and peaks at the proper retention time were integrated with Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and to quantitate analyte in all samples by linear-regression analysis. A weighting factor of $1 \times$ was used for all calibration curves.

The response for atorvastatin was linear and provided correlation coefficients of 0.99 or better. Accuracy was reported as the percentage of nominal concentration, and precision was reported as the percentage of the relative standard deviation. For atorvastatin, accuracy was 103% for 0.3 ng/mL and 89% for 5 ng/mL. Precision was 8% for 0.3 ng/mL and 6% for 5 ng/mL. The technique was optimized to provide a limit of quantitation of 0.25 ng/mL and a limit of detection of approximately 0.1 ng/mL.

Monitoring

Before daily administration of the drug, birds were subjectively monitored for signs of food

consumption (decreased food in dish, food particles on bottom of cage), water consumption via water bottle and urofeces. The animal activity before daily manual restraint was also monitored. All monitoring was performed by the individuals administering the medications (J.A.R. and J.M.D.).

Statistical analysis

Data were analyzed with R statistical software (version 3.0.1, R Foundation for Statistical Computing, Vienna, Austria). Longitudinal data analysis was performed with linear mixed modeling on the TC, LDL-C, HDL-C, TG, HDL-TG, and non-HDL-TG and plasma biochemistry values as the outcome variables with time, treatments (placebo or atorvastatin), and interaction as fixed effects, and birds (nested within treatments because this was not a crossover study) as a random effect. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Quantile plots were also performed on the residuals by treatment groups for normality assessment. Residuals resulting from the fitted model were verified to be normally distributed and had no evidence of heteroskedasticity. A type III analysis of variance was performed on the fixed effects and post hoc comparisons were performed using a Tukey's adjustment. Values of $P < .05$ were considered significant.

RESULTS

Plasma lipid profiles

Oral administration of atorvastatin for 30 days to Hispaniolan Amazon parrots did not result in statistically significant changes in mean values for TC, HDL-C, LDL-C, or TG at either day 14 or day 30 by the centrifugation method (Table 1) or the clinical chemistry analyzer (Table 2).

Table 2. Mean $\pm$ standard deviation of plasma lipid profile (mg/dL) from automatic chemistry analyzer from Hispaniolan Amazon parrots (control, $n = 8$; treatment, $n = 8$) at time point 0 and days 14 and 30 after once-daily PO dosing of atorvastatin (10 mg/kg).

Parameters	Day 0		Day 14		Day 30	
	Control	Treatment	Control	Treatment	Control	Treatment
Total cholesterol	447.1 $\pm$ 63.0	443.3 $\pm$ 2.1	432.2 $\pm$ 41.8	362.5 $\pm$ 85.1	460.5 $\pm$ 40.4	402.4 $\pm$ 66.3
LDL cholesterol	209.7 $\pm$ 118	220.6 $\pm$ 131	197.6 $\pm$ 70.9	154.8 $\pm$ 74.8	215.1 $\pm$ 82.2	162.3 $\pm$ 71.9
HDL cholesterol	186.9 $\pm$ 54.2	172.2 $\pm$ 34.3	191 $\pm$ 49.8	153.4 $\pm$ 49.8	193.2 $\pm$ 28.8	178.6 $\pm$ 34.3
Triglycerides	175 $\pm$ 55.2	157.8 $\pm$ 39.8	142 $\pm$ 30.7	177.6 $\pm$ 52.0	178.7 $\pm$ 56.1	185.5 $\pm$ 28.6

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein.

The administration of the placebo suspension resulted in similar statistical outcomes for mean TC, HDL-C, LDL-C, and TG value comparisons on days 14 and 30. The mean HDL-TG was significantly lower at 30 days than at prior times, regardless of treatment ($P = .01$). This corresponded to a higher mean non-HDL-TG at 14 days compared with day 0 ($P = .01$).

PCVs, TS, and plasma biochemistry panels

Time or treatment did not have any significant effect on PCV or TS. Neither time nor treatment had any clinically significant effect on biochemical analyte.

Atorvastatin plasma concentration

Plasma atorvastatin concentrations were variable on day 14 (0.54–5.41 ng/mL for 6 of 7 samples, with 1 outlier of 307.5 ng/mL) and on day 30 (0.79–6.74 ng/mL) (Table 3).

Adverse effects

No changes or abnormalities were noted for appetite, water consumption, or uofeces within either the control or treatment group of birds.

Weight loss was noted over the 30-day period; however, neither time nor treatment had a significant effect on weight, and weight loss was less than 10% for all birds over the 30-day period.

DISCUSSION

To our knowledge, this is the first study to evaluate the effects of statins on plasma lipid profiles and biochemistry profiles in a psittacine species. Atorvastatin at the dose and frequency administered in this study did not significantly lower TCs in this group of Hispaniolan Amazon parrots. These results were in contrast to a previous study with a chicken experimental model of hyperlipidemia, in which chickens were provided a daily feed containing 0.06% atorvastatin (estimated to deliver 60 mg/hen or approximately 24 mg/kg) for 20 days¹⁹ and resulted in lowered plasma concentrations of TC and TG and that also reduced liver steatosis, inflammation, and hepatocellular damage.¹⁹ Studies with other statin medications (pravastatin) in both chickens and pigeons have shown TC-lowering effects, along with reductions in the severity of atherosclerotic plaques.^{24–27} The lack of detectable effect from atorvastatin on the plasma lipid profiles in the

Table 3. Total cholesterol (TC, mg/dL) and atorvastatin plasma concentration (ng/mL) from Hispaniolan Amazon parrots (treatment, $n = 8$) at time point 0 and days 14 and 30 after once oral dosing of atorvastatin (10 mg/kg).

Bird ID	Day 0	Day 14		Day 30	
	TC concentration	TC concentration	Atorvastatin plasma concentration	TC concentration	Atorvastatin plasma concentration
1	416.2	331.0	0.62	417.9	0.79
2	387.8	410.2	4.3	445.5	1.5
3	348.2	232.0	1.18	256.1	1.2
4	396.4	245.8	307.5	372.3	6.74
5	520.4	374.9	ns	ns	ns
6	255.2	242.3	5.41	292.3	1.34
7	375.8	417.9	3.02	404.2	2.11
8	315.5	368.9	1	327.5	6.14

Abbreviation: ID, identification; ns, insufficient sample.

Hispaniolan Amazon parrots was unexpected. Poor efficacy of atorvastatin in the parrots may have resulted from low oral bioavailability (<30%),^{2,17} the short half-life of statin medications, species-specific differences in atorvastatin metabolism influencing the drug's mechanism of action, or the relatively short duration of the treatment.

Plasma concentrations of atorvastatin were evaluated after the preliminary pharmacodynamic data were available. There was variability in the plasma concentration of atorvastatin during the treatment within this study on day 14 and day 30, which could be explained in part by the variability of time points of collection because that was not in the initial protocol, and blood samples were obtained anywhere from 6 to 8 hours after drug administration for day 14 and day 30. Although the parrots showed presence of drug in the plasma, those concentrations were relatively low and difficult to interpret and correlate to plasma concentrations in other species without knowing the pharmacokinetic profile (eg, maximum plasma concentration after a single dose [C_{max}], the amount of time the drug was present in the plasma at maximal concentration [T_{max}], or the half-life) of this drug in Hispaniolan Amazon parrots. In humans, atorvastatin at a dosage of 2.5 to 80 mg/d is readily absorbed with a C_{max} of 4.34 to 187 ng/mL within 1 to 2 hours, and plasma concentrations of atorvastatin increase based on dose administered; however, plasma drug concentration increases are nonlinear.^{28,29} In humans, the half-life of atorvastatin is approximately 14 hours,^{28,30} is rapidly absorbed with peak plasma concentrations noted at 1 to 2 hours, and has a C_{max} of 4.34 to 187 ng/mL after administration of the standard starting doses (5, 20, or 80 mg/d).²⁹ In contrast to our study, a previous study of the pharmacokinetics of the statin drug rosuvastatin reported non-detectable plasma concentrations following a single PO dose of 10 mg/kg administered to Hispaniolan Amazon parrots.²

Plasma atorvastatin metabolites were not directly measured in this study. In humans, statin medications have overall poor bioavailability orally because of the first-pass effect by the liver and a high level of plasma protein binding. At least 70% of the HMG-CoA reductase inhibitory effects are due to active metabolites, which are thought to extend the half-life to 20 to 30 hours.^{2,17,28,30} The total body clearance of statin medication is also high because of first-pass removal and extensive microsomal metabolism by cytochrome P450.¹⁷ It is possible that no active metabolites or low

concentrations of atorvastatin metabolites contributed to the lack of significant effects on plasma lipid profiles. At this time, in this parrot species, the metabolism and plasma concentrations of atorvastatin metabolites are unknown, and there is a poor understanding of the role of metabolites in this drug's mechanism of action. A pharmacokinetic study of atorvastatin in Hispaniolan Amazon parrots after PO administration of 10 mg/kg dose would be needed to help understand the relationship between the plasma concentrations of atorvastatin, its metabolites, and its pharmacodynamic studies compared with plasma concentrations known to be effective in other avian species.

Another consideration for the minimal effects on plasma lipid parameters, is the masking effect of stress associated with daily administration of medication. In our study, the birds may have developed a negative association with the investigators and study procedure over time, as indicated by increased occurrence of behaviors associated with stress and decreased cooperation with net capture for manual restraint. In humans, it has been shown that mental, physical, and emotional stress lead to increases in blood TC levels.^{31–33} Psychologic stress has been shown in humans and nonhuman primates to increase circulating glucocorticoids, which can lead to plasma lipid profile changes consistent with atherogenic dyslipidemia, including elevated TC and LDL-C and decreased HDL-C.^{20,34} Although there are no studies, to our knowledge, evaluating the effect of stress on TC in avian species and although stress associated with the daily restraint and gavage may not exclusively explain the increase in plasma TC after day 14, it does warrant future investigation.

In addition, the results in this group of Hispaniolan Amazon parrots may have been confounded by a genetic predisposition of hypercholesterolemia.³⁵ In humans, familial hypercholesterolemia is an autosomal codominant inherited disorder (usually the result of mutations in the gene encoding the LDL receptor), which affects lipoprotein metabolism and leads to high plasma levels of LDL-C.³⁶ The penetrance of familial hypercholesterolemia in human patients is 100%, which means that half the children from an affected parent will have increased plasma cholesterol levels.³⁶ Statin medications alone tend not to lower cholesterol values to appropriate ranges in humans with familial hypercholesterolemia.³⁷ Given the relatedness of most of this particular group of Hispaniolan Amazon parrots (the University of California, Davis, flock), true familial hypercholesterolemia cannot be ruled out. Additionally,

pharmacogenetic explanations for our findings cannot be ruled out, which include possible variants in the cholesterol transport or metabolic pathways. Genetic variants in the cholesterol transport pathway of birds have only been reported in abstract form, but similar mechanisms are well understood in humans.³⁵ A genetic polymorphism resulting in an overall high clearance rate of the drug may also exist given this flock's previously reported enhanced metabolism of other drugs.³⁸ To our knowledge, there are no studies that have positively identified familial hypercholesterolemia in any avian species.

Persistent elevations in hepatic transaminases can occur in humans administered statin medications, most specifically within the first 3 months of administration, and routine monitoring of biochemistry parameters (eg, liver and myocellular enzymes) is recommended before starting and when taking atorvastatin.^{15–17} Myopathies, characterized by muscle weakness and pain, along with elevated creatine kinase levels, have been observed in human patients administered statin medications.¹⁷ Plasma biochemistry panels evaluated on days 14 and 30 did not have any significant changes in birds receiving atorvastatin. Mild weight loss occurred in all birds; however, the loss was noted to be less than 10% over the 30 days. Routine daily handling for gavage and venipuncture on days 0, 14, and 30 were thought to be the main contributing factor for the weight loss. Subjectively, none of the birds had any evidence of lethargy or anorexia, clinical signs that could be associated with nausea and/or muscle pain in the birds, which has been reported in humans.^{15,17} Subjectively, no changes in fecal characteristics were noted in the birds throughout the 30 day study period.

As a part of a separate study, concentrations of the formulated atorvastatin suspension were evaluated before administration to the birds in this study and remained within 87% to 93% of its nominal concentration of 10 mg/mL (unpublished data; stability study of compounded atorvastatin). Changes in the dose of atorvastatin the birds received over time were considered minimal; therefore, we do not believe that these changes affected the pharmacodynamic effects of the drug during the 30-day study period.

Limitations of the study included its small sample size. A larger sample size may have improved significance of some data points. Stress and daily handling of the birds may have a confounding effect on study outcomes. To counteract this potential, it would be desirable to

develop a training protocol to allow for animal compliance, thereby decreasing possible influence of stress on plasma lipid profiles. Lastly, additional studies that use animals with dietary-induced hypercholesterolemia may provide different results when evaluating the effects of atorvastatin on plasma lipid profiles in psittacine species.

In conclusion, there were no significant changes observed in the TC, LDL-C, HDL-C, or TGs in Hispaniolan Amazon parrots treated once daily with 10 mg/kg atorvastatin at any time point during this study. No adverse physical or behavioral effects were noted in any of the birds administered atorvastatin. It is possible that administration of higher doses or twice daily administration would result in significant changes to TC, HDL-C, LDL-C, or TG. Future studies to investigate dose and dosing intervals on plasma pharmacokinetics of atorvastatin in parrots are warranted before additional efficacy studies are performed.

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