UC Davis

UC Davis Previously Published Works

Title

EFFECTS OF EXERCISE ON THE PLASMA LIPID PROFILE IN HISPANIOLAN AMAZON PARROTS (AMAZONA VENTRALIS) WITH NATURALLY OCCURRING HYPERCHOLESTEROLEMIA.

Permalink

https://escholarship.org/uc/item/0518488p

Journal

Journal of zoo and wildlife medicine : official publication of the American Association of Zoo Veterinarians, 47(3)

ISSN

1042-7260

Authors

Gustavsen, Kate A
Stanhope, Kimber L
Lin, Amy S
et al.

Publication Date

2016-09-01

DOI

10.1638/2015-0192.1

Peer reviewed

EFFECTS OF EXERCISE ON THE PLASMA LIPID PROFILE IN HISPANIOLAN AMAZON PARROTS (AMAZONA VENTRALIS) WITH NATURALLY OCCURRING HYPERCHOLESTEROLEMIA

Authors: Kate A. Gustavsen, Kimber L. Stanhope, Amy S. Lin, James L. Graham, Peter J. Havel, et. al.

Source: Journal of Zoo and Wildlife Medicine, 47(3) : 760-769

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/2015-0192.1

Journal of Zoo and Wildlife Medicine 47(3): 760–769, 2016

EFFECTS OF EXERCISE ON THE PLASMA LIPID PROFILE IN HISPANIOLAN AMAZON PARROTS (*AMAZONA VENTRALIS*) WITH NATURALLY OCCURRING HYPERCHOLESTEROLEMIA

Kate A. Gustavsen, Ph.D., D.V.M., Kimber L. Stanhope, Ph.D., Amy S. Lin, B.S., James L. Graham, B.S., Peter J. Havel, D.V.M., Ph.D., and Joanne R. Paul-Murphy, D.V.M., Dipl. A.C.Z.M., Dipl. A.C.A.W.

Abstract: Hypercholesterolemia is common in psittacines, and Amazon parrots (*Amazona* spp.) are particularly susceptible. Associations have been demonstrated between naturally occurring and experimentally induced hypercholesterolemia and atherosclerosis in psittacines. Daily exercise improves lipid metabolism in humans and other mammals, as well as pigeons and chickens, under varying experimental conditions. Hispaniolan Amazon parrots (*Amazona ventralis*) with naturally occurring hypercholesterolemia (343–576 mg/dl) were divided into two groups. An exercised group ($n = 8$) was housed as a flock and exercised daily with 30 min of aviary flight and 30 min walking on a rotating perch. A sedentary control group ($n = 4$) was housed in individual cages with no exercise regime. A plasma lipid panel, including total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, and triglycerides, was validated for this species. Body weight, chest girth, and the lipid panel were measured at 0, 61, and 105 days. Hematology and plasma biochemistry were measured at 0 and 105 days. Weight and girth were significantly lower in exercised than sedentary parrots at 61 and 105 days. HDL-C concentrations were significantly higher in exercised parrots at 61 days but returned to near baseline by 105 days. There were no significant changes in hematology, biochemistry, or other lipid panel parameters. Results were similar to studies in humans and animal models, in which increased HDL-C was the most consistent effect of exercise on circulating lipid and lipoprotein parameters. The return toward baseline HDL-C may have resulted from decreased participation in aviary flight. Additional investigation will be required to determine the amount of exercise and change in circulating lipid-related parameters necessary to improve long-term wellness in psittacine species predisposed to hypercholesterolemia.
Key words: Hispaniolan Amazon parrot, *Amazona ventralis*, exercise, cholesterol, HDL, LDL.

INTRODUCTION

Amazon parrots (*Amazona* spp.) are popular companion and zoologic collection birds, and are among the avian genera particularly susceptible to naturally occurring hypercholesterolemia and atherosclerosis.[6,7,40] The research colony of Hispaniolan Amazon parrots (*Amazona ventralis*) at the University of California, Davis, has a chronic history of hypercholesterolemia. The range of total cholesterol (TC) concentrations measured over 6 yr, using two independent clinical laboratories, was 350–550 mg/dl; this is well above published normal ranges of approximately 100–350 mg/dl for Amazon parrots.[7,20,28,41] None of the parrots was exhibiting signs of cardiovascular disease at the time of the study. Antemortem diagnosis of subclinical cardiovascular disease is challenging in avian species and has not been undertaken for these parrots. However, chronic hypercholesterolemia has been established as a risk factor for development of clinically significant disease.[7,40] The persistence of hypercholesterolemia, despite a species-appropriate formulated diet, motivated an investigation of other modifiable lifestyle factors.

Wild parrots may fly miles each day and spend 50% or more of their waking time foraging.[36,44] In contrast, captive parrots are typically restricted to cage or aviary housing and provisioned with food. Previous research has shown that orange-winged Amazon parrots (*Amazona amazonica*) housed alone in cages were inactive for >90% of their waking time.[44] The comparatively sedentary lifestyle of captive parrots is a potential point of intervention to manage hypercholesterolemia in these populations.

Physical activity is important for management of cardiovascular disease risk in humans. Exercise has been shown to increase high-density lipopro-

From the Department of Medicine and Epidemiology (Gustavsen, Lin, Paul-Murphy); the Department of Molecular Biosciences (Stanhope, Graham, Havel), School of Veterinary Medicine; and the Department of Nutrition (Stanhope, Graham, Havel), University of California Davis, 1 Shields Avenue, Davis, California 95616, USA. Present address (Gustavsen): Wildlife Conservation Society Zoological Health Program, 2300 Southern Boulevard, Bronx, New York 10460, USA. Correspondence should be directed to Dr. Paul-Murphy (paulmurphy@ucdavis.edu).

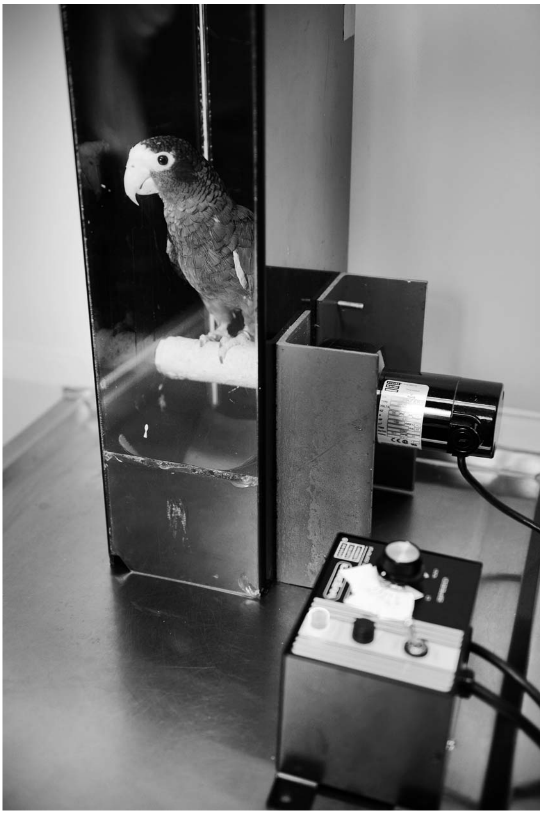

Figure 1. A rotating perch was used to provide walking exercise for Hispaniolan Amazon parrots. The apparatus consists of an opaque acrylic box with a clear front panel and a nonskid perch connected to a direct current motor, with a variable speed control box. The speed was adjusted to maintain a brisk but sustainable pace for the parrots, which was less than the maximum speed attainable for the apparatus.

tein cholesterol (HDL-C) concentration, decrease triglyceride concentration, and more variably, decrease TC and low-density lipoprotein cholesterol (LDL-C) concentrations, all of which have also been associated with lower risk of cardiovascular disease.[46] Similar effects of exercise have been demonstrated in mammalian and avian experimental models. In domestic chickens (*Gallus gallus*) fed a high-cholesterol diet, 1 hr per day of brisk walking resulted in significantly lower serum TC concentration and arterial cholesterol content within 8 wk.[47] In domestic pigeons (*Columba livia*) fed a high-fat, high-cholesterol diet, daily flights of 4 mi. (6.4 km) resulted in significantly lower plasma TC concentration and less extensive aortic atherosclerosis.[9]

The objective of this study was to determine whether a daily exercise regime, using activities that exploit natural behaviors, would affect plasma cholesterol and lipid concentrations in previously sedentary, hypercholesterolemic Hispaniolan Amazon parrots. Lipid metabolism was assessed by using plasma assays, including TC, HDL-C, LDL-C, and triglyceride. The hypothesis was that lipid profile changes would be similar to those seen in humans and avian experimental models, namely, increased HDL-C concentration and stable or decreased TC concentration.

MATERIALS AND METHODS

Animals and husbandry

Twelve adult Hispaniolan Amazon parrots, six males and six females, were used in the study. The parrots ranged in age from 9 to 25 yr, and with the exception of hypercholesterolemia, were healthy, as determined by physical examination, hematology, and plasma biochemistry. For at least 1 yr prior to the start of the study, all parrots were housed individually in powder-coated wire cages (61 × 58 × 66 cm) equipped with a water bottle, two wooden perches, and enrichment items that changed weekly. These cages allowed walking and climbing but did not permit flight of any appreciable distance. The housing room was kept at a constant temperature with a 12-hr light cycle and no natural light cues. Parrots were fed a pelleted diet ad libitum (ZuPreem Fruit Blend, Premium Nutritional Products Inc., Overland Park, Kansas 66214, USA). Eight parrots (four male and four female) were selected for the exercised group based on social compatibility and flight ability. These parrots were housed together in an aviary enclosure (2.75 × 3 × 2 m) with multiple perches, water sippers, and feed stations. Group housing was chosen to promote routine physical activity, in addition to the imposed exercise regime. Four parrots (two male and two female) were maintained in individual cages as sedentary controls. Both sedentary males had healed wing injuries and were unable to fly but navigated well in the individual cages. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of California, Davis.

Exercise program

Walking on a rotating perch: The rotating perch apparatus consisted of a black acrylic box (23.5 × 13.5 × 51 cm) with a transparent front panel. The perch had a radius of 1.83 cm and was connected to a direct current motor with a variable speed control (Fig. 1). This system was previously used to evaluate analgesic efficacy in Hispaniolan

Amazon parrots with experimentally induced arthritis.[13] The walking session was divided into three increments, with 1-min rest periods between increments. To acclimate the parrots, increment length was progressively increased from 3 to 10 min within the first week of the study, with a final walking time of 30 min per day. Rotational speed was progressively increased from 10 rev/min (slow walk) to 20 rev/min (moderate walk) in the first 2 wk of the study and then further increased to maintain a brisk but sustainable pace for each individual; six parrots achieved a speed of 30 rev/min (0.055 m/s, 0.123 mi/hr) within 4 wk, and the remaining two within 6 wk. All parrots sustained this pace without losing balance or showing signs of overexertion (e.g., panting or falling). Initially, manual restraint with a towel was used to move parrots from a transport carrier to the apparatus, but within 4 wk, all parrots climbed voluntarily into and out of the apparatus.

Aviary flight: Two experimenters entered the aviary and walked slowly around the perimeter, gesturing with large plastic wands to encourage the parrots to fly. Several parrots chose to climb across the wire mesh aviary walls and ceiling rather than flying; these parrots were encouraged to maintain continuous motion around the aviary. The flight session was divided into three increments, with 3-min rest periods between increments. To acclimate the parrots, in the first 2 wk of the study, the length of each increment was progressively increased from 1 to 10 min so that the total daily flight time increased from 3 to 15 min during the first week and to 30 min by the end of the second week. Any parrot that showed signs of overexertion (e.g., panting, refusal to move when approached) was not actively pursued until it had recovered, typically within 1–2 min.

Schedule: Exercise sessions occurred 5 days/wk. The parrots participated in 30 min of flight exercise in the morning, followed by at least 30 min of rest, and were then caught with a hand net and separated into individual transport carriers. Each parrot spent 30 min walking on the rotating perch, in the same order each day. Parrots had no access to food while in the carriers. All parrots were released to the aviary together at the conclusion of the walking sessions. Parrots did not exercise on days when blood samples were taken.

Sedentary control group: Parrots in the sedentary group were maintained in individual cages, as described previously, in the same housing room as the aviary enclosure, for the duration of the study. Feeders were emptied while exercised parrots were participating in walking sessions and then refilled when the exercised parrots were returned to the aviary. Sedentary parrots were handled only three times during the study for data collection.

Data collection

Morphometric data and blood samples were collected at three time points during the study, days 0, 61 and 105, with day 0 being the day prior to starting the exercise program. Birds were fasted overnight (10–12 hr) prior to sample collection. Body weight was measured to the nearest gram using a digital scale. Chest girth was measured as body circumference around the axillae and the cranial end of the carina, where it joins the furcula, using a flexible tape measure. Measurement was taken at the fulcrum to minimize the effect of keel movement due to respiration. Blood was drawn from either jugular vein, with a maximum sample volume of 2 ml. On days 0 and 105, aliquots for hematology and plasma biochemistry were placed in ethylenediaminetetraacetic acid (EDTA) and lithium heparin microtubes, respectively, and submitted to the University of California–Davis Veterinary Medical Teaching Hospital Clinical Pathology Service for analysis. The remainder of the sample was placed in an EDTA tube and stored at 4°C for 6 hr or less before centrifugation to separate the plasma. Plasma samples were stored in cryovials at −80°C until analysis.

Plasma lipid profiles

Plasma cholesterol and lipid concentrations were determined by using an automated clinical chemistry analyzer (PolyChem 180, Polymedco Inc., Cortlandt Manor, New York 10567, USA) and commercially available reagents (MedTest DX, MedTest, Canton, Michigan 48188, USA). The profile included direct determinations of TC, HDL-C, LDL-C, and triglyceride concentrations. Preliminary validation of the lipid profile was performed on six pilot samples from the same group of parrots, prior to the start of the study. Measurements on undiluted plasma were compared with plasma that had been diluted 1 : 1 in isotonic phosphate buffered saline solution. Dilution resulted in a linear decrease in measured concentration, with $<10\%$ difference between the undiluted samples and the extrapolated results from diluted samples for TC and triglyceride and $<20\%$ difference for LDL-C. In addition, the TC concentrations measured in the experimental lipid panel and the plasma biochemistry panel

Table 1. Fasted plasma lipid profile of 10 Hispaniolan Amazon parrots (*Amazona ventralis*) consuming a commercial pellet diet, obtained prior to beginning an exercise program.[a]

Parameter[b]	Mean	SD	Median	Interquartile range	Range
TC (mg/dl)	389	36.5	396.5	355.75–410.25	339–461
HDL-C (mg/dl)	180	34.1	174	167–190.5	128–244
LDL-C (mg/dl)	140	53.8	142.5	98.25–161.25	75–268
Males ($n = 6$)	111	32.1	100.5	85.5–141	75–156
Females ($n = 4$)	183	51.0	166	155.25–193.75	132–268
TG (mg/dl)	150	24.4	140.5	135–155.75	119–204
HDL : TC ratio	0.463	0.072	0.477	0.426–0.505	0.311–0.584
Males ($n = 6$)	0.501	0.046	0.502	0.468–0.516	0.441–0.584
Females ($n = 4$)	0.406	0.067	0.407	0.373–0.441	0.311–0.499

[a] There was a significant effect of sex on LDL-C concentration and the HDL : TC ratio; summary statistics for these parameters were calculated for the entire group and for males and females separately.

[b] TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.

were well correlated ($r = 0.970$, $P < 0.001$; see "Data analysis" section). Concentrations of HDL-C in undiluted plasma were above the upper limit of the assay in all pilot samples, so comparison could not be made with diluted samples; for the study, samples were diluted 1 : 3 in isotonic phosphate buffered saline prior to HDL-C determination.

Data analysis

Data analysis was performed by using a statistical software package (Stata 13, StataCorp LP, College Station, Texas 77845, USA). Body weight, LDL-C, and triglyceride concentrations were not compatible with normal distributions based on the Shapiro–Wilk test. The baseline TC concentrations in the experimental lipid profile and in the standard plasma biochemistry panel were compared by using Pearson product moment correlation (r). Morphometric measurements and plasma lipid panel data were assessed using mixed-effects analysis of variance, which allowed assessment of multiple influences on the data in a single analysis. This model assumed that errors were approximately normally distributed; inspection of normal probability plots of the model residuals did not indicate any substantial departures from normality. An unstructured covariance matrix accounted for correlations among repeated measurements without imposing an assumption about the nature of those correlations. The model included the individual parrot as the random effect, categoric fixed effects of sex, exercise group, and time period, and the pairwise interaction between exercise group and time period. Other pairwise interactions, for example, to allow sex differences in the exercise effects, tended to produce overspecified models with multiple conflicting small magnitude effects and were not included in the final analysis. In all statistical tests, effects were considered significant at $P < 0.05$.

RESULTS

All parrots appeared to be healthy on physical examination prior to beginning the study. Mean body weight for all parrots on day 0 was 267 g (SD 26.1, median 259, and range 240–345). Two female birds, one each from the exercise and sedentary groups, had elevated calcium and phosphorus concentrations on the baseline plasma biochemistry panel and TC concentrations considerably higher than the other parrots. The birds did not display reproductive behavior; however, the pattern of plasma chemistry and lipid results strongly suggested folliculogenesis, and their data were excluded from analysis. With the exception of hypercholesterolemia, hematology and plasma biochemistry for the remaining 10 birds at days 0 and 105 were within published reference intervals for Amazon parrots,[20,28] as well as historic ranges for this colony.

Baseline plasma lipid profile data are presented in Table 1. The number of parrots was small, and LDL-C and triglyceride concentrations were not normally distributed; therefore, the summary statistics include median and interquartile range, as well as mean and standard deviation. There was a significant effect of sex on LDL-C concentration, regardless of exercise group or time period, with median LDL-C for female parrots being 65.5 mg/dl higher than males even after the two presumed reproductive females were excluded. TC concentrations were slightly higher in

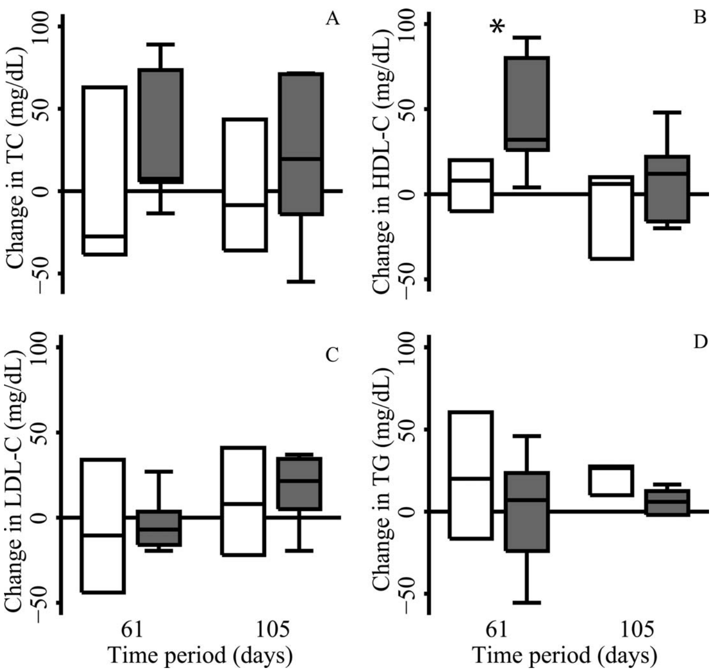

Figure 2. Changes of the plasma lipid profiles of Hispaniolan Amazon parrots during a controlled daily exercise program. For each plasma lipid profile parameter, the baseline (day 0) concentration for each parrot was subtracted from the concentrations at day 61 and day 105. In all panels, the horizontal axis is time (days), the vertical axis is change in plasma concentration (milligrams per deciliter), the gray boxes represent the median and interquartile range for the exercised group ($n = 7$), and the white boxes represent the median and range for the sedentary control group ($n = 3$). **A**. Total cholesterol (TC) concentration. **B**. High-density lipoprotein cholesterol (HDL-C) concentration; there was a significant interaction between exercise group and time at 61 days in a mixed-effects analysis of variance (asterisk). **C**. Low-density lipoprotein cholesterol (LDL-C) concentration. **D**. Triglyceride (TG) concentration.

females, and HDL-C concentrations were slightly higher in males, but neither difference was significant in the mixed effects model. The ratio of HDL-C to TC (HDL : TC ratio) was calculated. The mean value for both sexes combined was 0.46, but there was a significant effect of sex on this ratio, with a mean value of 0.40 for females and 0.50 for males.

Parrots in the exercised group quickly became accustomed to the rotating perch and maintained a brisk walking pace without signs of overexertion. The enclosing box provided sufficient room for birds to climb off the perch to avoid participating but none did. The flight exercise appeared to be more strenuous; most parrots exhibited increased respiratory effort that recovered to normal during the 3-min break periods. Several parrots climbed across the mesh walls and ceiling rather than flying, and overall participation in flight exercise declined throughout the study. Therefore, the intensity of exercise was variable within the group and decreased during the study.

Plasma lipid profiles were measured at day 0 prior to starting the exercise program, at day 61 after approximately 9 wk of exercise, and at day 105 after 15 wk of exercise. Mixed-effects analysis of variance was performed by using the raw data at each time point; changes due to exercise were reflected as a significant interaction between exercise group and time point in the model. There was a wide range in baseline lipid concentrations and morphometric measurements among the parrots, so for visual clarity, the data from days 61 and 105 are presented as changes relative to day 0 for each parrot. TC concentrations were higher in the exercised than the sedentary group at days 61 and 105 (Fig. 2A), though the effect was not statistically significant. The difference in TC was primarily due to an increase in plasma HDL-C in the exercised group, for which there was a significant exercise–time interaction at day 61 (Fig. 2B). There was also a significant exercise–

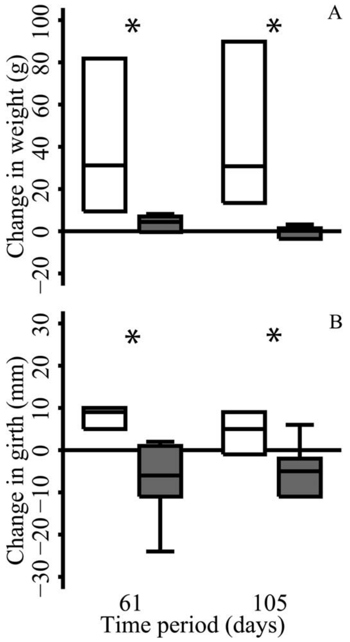

Figure 3. Changes of body weight and chest girth of Hispaniolan Amazon parrots during a controlled daily exercise program. For both parameters, the baseline (day 0) measurement for each parrot was subtracted from the measurements at days 61 and 105. In all panels, the horizontal axis is time (days), the vertical axis is the change in measurement, the gray boxes represent the median and interquartile range for the exercised group ($n = 7$), and the white boxes represent the median and range for the sedentary control group ($n = 3$). **A**. Body weight (grams). There was a significant effect of time, as well as significant interactions between exercise group and time at 61 and 105 days, in a mixed-effects analysis of variance (asterisks). **B**. Chest girth (millimeters). There was a significant interaction between exercise group and time at 61 and 105 days in a mixed-effects analysis of variance (asterisks).

time interaction for the HDL : TC ratio at this time point. Interestingly, this increase was not maintained throughout the exercise program; HDL-C returned to near the baseline value by day 105. There were no significant effects of exercise or time on either plasma LDL-C or triglycerides (Fig. 2C, D).

Body weight and chest girth data are presented similarly to the lipid profile (Fig. 3). Parrots in the exercised group maintained weight during the study, while the sedentary group gained weight (Fig. 3A). This resulted in a significant exercise–time interaction at days 61 and 105, as well as a significant effect of time period. There was a significant exercise–time interaction for chest girth at days 61 and 105 (Fig. 3B), due to a moderate decrease in girth for the exercised group and a moderate increase for the sedentary group.

DISCUSSION

Daily exercise had a potentially beneficial effect on the plasma lipid profile in Hispaniolan Amazon parrots in this study. The lipid panel included plasma assays for TC, HDL-C, LDL-C, and triglyceride. Automated assays for HDL-C and LDL-C rely on reagents that selectively solubilize these lipoprotein fractions (MedTest DX Direct HDL-Cholesterol and Direct LDL-Cholesterol product inserts, MedTest). Avian HDL and LDL particles are biochemically similar to their mammalian counterparts,[5,12] and an underlying assumption of this study was that the assays accurately detected and differentiated these particles. Automated biochemical assays for TC, HDL-C, and triglycerides have been used extensively in avian species, including Amazon parrots.[4,7,15,16,38,41] It is common to estimate LDL-C concentration by using the Friedewald equation, which relies on an assumed 5 : 1 ratio of triglyceride to cholesterol in very-low-density lipoprotein (VLDL) particles in human plasma.[19] However, this assumption is known to be inaccurate in humans with triglyceride concentrations >150 mg/dl,[37] and 8 of 10 parrots in this study had triglyceride concentrations above that value. Therefore, a direct enzymatic assay for LDL-C was used. The same significant effects were identified when the mixed-effects analysis was repeated by using LDL-C concentrations estimated via the Friedewald equation (data not shown).

The median HDL : TC ratio in this study was 0.477, which is similar to other Amazon parrots and indicates that just under half of the plasma cholesterol is contained in HDL particles.[7,38,41] This is low compared with many other avian species, including other psittacines, for which HDL : TC ratios are commonly 0.6–0.8.[1–3,7,17,25,29–32] Low HDL-C and low HDL : TC ratio are associated with increased cardiovascular disease risk in humans.[23] In several prior studies, feeding excess cholesterol to granivorous birds resulted in a disproportionate increase in LDL-C and VLDL-C concentrations and increased the prevalence of atherosclerosis.[2,8,27] Surprisingly, median HDL-C concentration was positively correlated with the prevalence of atherosclerosis across psittacine genera in one retrospective study.[7] However, the genera with the highest atherosclerosis prevalence also had the lowest HDL : TC ratios, as calculated from the reported data. Therefore, it remains possible that proportionally low HDL-C contributes to increased susceptibility to cardiovascular disease in Amazon parrots.

In this study, there were significant sex differences in the plasma lipid profile, with LDL-C being higher and the HDL : TC ratio being lower in females, even after suspected reproductively active birds were excluded. In a previous study of mixed-species Amazon parrots, females had significantly lower HDL-C than males,[41] which is compatible with this proportional difference. The prevalence of atherosclerosis is higher in female parrots,[6,21] and it is possible that proportionally higher LDL-C or lower HDL-C contributes to this difference. However, a recent retrospective study of laboratory databases did not identify an effect of sex on any plasma lipids,[7] so it would be premature to make a strong conclusion based on existing data.

Increased HDL-C is the most consistently reported effect of exercise on the lipid profile in humans and animal models,[18,34,46,49] even in the absence of a change in TC, and is associated with reduced risk of cardiovascular disease.[46] There was a significant effect of exercise on HDL-C concentration in this study, with a mean increase of 45.7 mg/dl (26%) at day 61. To the authors' knowledge, no prior studies of exercise in avian models have measured HDL-C separately from TC concentration. Although the parrots remained hypercholesterolemic, the significant increase of HDL-C concentration and HDL : TC ratio may be beneficial changes in the plasma lipid profile.

Despite the significant increase at day 61, HDL-C concentrations in the exercised group returned to near baseline by day 105. This may be attributable to declining participation in aviary flight sessions in the latter part of the study. It was hoped the parrots would participate readily in flight exercise. They did not, and instead, the motivation to fly was largely negative. In contrast, the parrots moved voluntarily from a transport carrier into the rotating perch apparatus and continued to participate throughout the study period, suggesting that this form of exercise was not threatening. Frequency, duration, and intensity of exercise all impact the magnitude of changes to the lipid profile in humans and animal models.[22,24,33,46] Flight requires two to three times the energy expenditure of running in avian species.[11] Thus, when parrots did not participate in the flight sessions, both the duration and intensity of daily exercise were substantially reduced. Studies of detraining effects in humans and animal models have found that exercise-induced changes in plasma or tissue lipid concentrations regressed within 4 to 6 wk of discontinuing an exercise program.[24,35,45] Continued participation in walking exercise alone may not have been sufficient to sustain the early increase of HDL-C concentrations in the present study. Extending the duration of walking sessions or increasing the rotational speed of the perch may have increased effectiveness; however, increasing the duration or difficulty of the task could also compromise voluntary participation.

There was no significant effect of exercise on LDL-C or TC concentration in the present study. Previous studies of exercise in domestic chickens and pigeons induced severe hypercholesterolemia by supplementing the diet with fat or cholesterol or both in excess of normal intake.[9,47] TC concentration was still elevated in exercised animals but significantly lower than sedentary controls. In pigeons that were fed an atherogenic diet and subjected to flight exercise, switching to a species-appropriate commercial diet resulted in a much greater decrease in TC than exercise alone.[9] In the present study, parrots were receiving a formulated diet, and effects of diet modification in combination with exercise were not tested. Note that decreased TC concentration may not be necessary to improve cardiovascular health. In domestic rabbits (*Oryctolagus cuniculus*) and cynomolgus macaques (*Macaca fascicularis*) fed atherogenic diets, TC concentration did not change in exercised animals, but they had significantly less extensive atherosclerosis at necropsy compared with sedentary controls.[33,34] Assessing the direct effect of exercise on severity of atherosclerosis in parrots would require either improved antemortem diagnostic capability or a terminal study design.

Exercise is a physiologic stressor, triggering an adrenomedullary and adrenocortical response to mobilize energy and maintain homeostasis. Plasma catecholamine and corticosteroid concentrations are transiently increased during exercise in mammals, as well as domestic ducks (*Anas platyrhynchos*), chickens, and pigeons.[26,42,43] In the present study, there may also have been an adverse psychological impact of compulsory flight and daily capture from the aviary. In humans and nonhuman primates, psychological stress has been associated with sustained corticosteroid elevation and an atherogenic lipid profile, including increased TC and LDL-C and lower HDL-C and HDL : TC ratio.[10] Thus, any psychological stress associated with the procedures in the present study would be expected to diminish, rather than mimic, the effects of exercise on plasma lipids.

Group housing prevented quantification of caloric intake for parrots in the exercised group, but the stable weights indicate that they increased consumption as necessary to meet their increased energy expenditure. The substantial weight gain among sedentary parrots was unexpected. Because all parrots were housed individually prior to this study, there should have been no change in daily energy expenditure for the sedentary group. The exercised and sedentary groups had access to food for the same time each day. However, the exercised group was removed from the aviary for walking sessions, whereas the sedentary group remained in their cages with the feeders emptied. Presentation of food-related cues can induce additional food consumption in humans and laboratory rats that have eaten to satiation.[14,48] In one prior study, young domestic chickens consumed more food and gained more weight when feeders were removed and immediately replaced several times a day than when they were left in place.[39] It is possible that refilling feeders in the presence of the sedentary parrots stimulated them to overeat. Effects on the plasma lipid profile were limited, with TC, HDL-C, and LDL-C concentrations remaining near baseline throughout the study for the sedentary group. Thus, differences attributed to exercise are unlikely to be artifacts of weight gain in the sedentary parrots.

Cardiovascular disease is a common postmortem finding in psittacines, and Amazon parrots are among the genera that are predisposed. However, antemortem diagnosis is challenging in birds, and death may occur without premonitory signs. Hypercholesterolemia is a predisposing factor to cardiovascular disease in birds as it is in humans, and appropriate diet alone may not prevent hypercholesterolemia in sedentary parrots. This study investigated the effect of daily exercise on lipid metabolism in previously sedentary, hypercholesterolemic Hispaniolan Amazon parrots and found effects of exercise that paralleled protective changes in other species. Although it was an unintended consequence and a notable limitation of the study design, the suspected detraining effect observed in the latter half of the study highlighted the importance of increased physical activity as a permanent lifestyle change. Creating positively reinforcing opportunities for high intensity flight or flapping exercise may be challenging in captive birds, and strategies will necessarily vary with temperament and husbandry. The findings of the present study suggest that increasing daily physical activity may be an important consideration for increasing plasma HDL-C and in the prevention or management of adverse lipid profiles in captive psittacines.

Acknowledgments: Support for this study was provided by a Mazuri Grant from the American Association of Zoo Veterinarians Wild Animal Health Fund and by the Center for Companion Animal Health and the Richard M. Schubot Parrot Wellness and Welfare Program at the University of California–Davis School of Veterinary Medicine. Dr. Havel's laboratory receives support from the National Institute of Health (grants HL091333, HL107256, and DK-095960) and a multicampus grant from the University of California Office of the President (award 142691). The authors thank Dr. Philip Kass for consultation on statistical analysis and Ms. Huai-Chen Jessica Chang and Ms. Marinelle Nunez for technical assistance.

LITERATURE CITED

1. Averbeck C. Haematology and blood chemistry of healthy and clinically abnormal great black-backed gulls (*Larus marinus*) and herring gulls (*Larus argentatus*). Avian Pathol. 1992;21:215–223.

2. Barakat HA, St Clair RW. Characterization of plasma lipoproteins of grain- and cholesterol-fed White Carneau and Show Racer pigeons. J Lipid Res. 1985;26:1252–1268.

3. Bavelaar FJ, Beynen AC. The relation between diet, plasma cholesterol and atherosclerosis in pigeons, quails and chickens. Int J Poultry Sci. 2004;3:671–689.

4. Bavelaar FJ, van der Kuilen J, Kuilen J, Hovenier R, Lemmens AG, Beynen AC. Plasma lipids and fatty acid composition in parrots in relation to the intake of α-linolenic acid from two feed mixtures. J Anim Physiol Anim Nutr. 2005;89:359–366.

5. Beaufrere H. Atherosclerosis: comparative pathogenesis, lipoprotein metabolism, and avian and exotic companion mammal models. J Exot Pet Med. 2013;22: 320–335.

6. Beaufrere H, Ammersbach M, Reavill DR, Garner MM, Heatley JJ, Wakamatsu N, Nevarez JG, Tully TN Jr. Prevalence of and risk factors associated with atherosclerosis in psittacine birds. J Am Vet Med Assoc. 2013;242:1696–1704.

7. Beaufrere H, Cray C, Ammersbach M, Tully TN Jr. Association of plasma lipid levels with atherosclerosis prevalence in psittaciformes. J Avian Med Surg. 2014;28:225–231.

8. Beaufrere H, Nevarez JG, Wakamatsu N, Clubb S, Cray C, Tully TN Jr. Experimental diet-induced atherosclerosis in Quaker parrots (*Myiopsitta monachus*). Vet Pathol. 2013;50:1116–1126.

9. Bhattacharya SP, Ordor OE, Banks MD, Adkins JS. Reversal of experimental atherosclerosis in pigeons: role of flying-exercise and non-atherogenic diet. Nutr Res. 1984;4:1003–1012.

10. Brindley DN, McCann BS, Niaura R, Stoney CM, Suarez EC. Stress and lipoprotein metabolism: modulators and mechanisms. Metabolism. 1993;42(9 Suppl. 1):3–15.

11. Butler PJ. Exercise in birds. J Exp Biol. 1991; 160:233–262.

12. Chapman MJ. Animal lipoproteins: chemistry, structure, and comparative aspects. J Lipid Res. 1980; 21:789–853.

13. Cole GA, Paul-Murphy JR, Krugner-Higby LA, Klauer JM, Medlin SE, Keuler NS, Sladky KK. Analgesic effects of intramuscular administration of meloxicam in Hispaniolan parrots (*Amazona ventralis*) with experimentally induced arthritis. Am J Vet Res. 2009;70:1471–1476.

14. Cornell CE, Rodin J, Weingarten H. Stimulus-induced eating when satiated. Physiol Behav. 1989;45: 695–704.

15. Cray C, Rodriguez M. Analysis of psittacine lipoproteins. In: Proc Assoc Avian Vet; 2007. p. 257–259.

16. Deem SL, Noss AJ, Cuéllar RL, Karesh WB. Health evaluation of free-ranging and captive blue-fronted amazon parrots (*Amazona aestiva*) in the Gran Chaco, Bolivia. J Zoo Wildl Med. 2005;36:598–605.

17. Facon C, Beaufrere H, Gaborit C, Albaric O, Plassiart G, Ammersbach M, Liegeois JL. Cluster of atherosclerosis in a captive population of black kites (*Milvus migrans* subsp.) in France and effect of nutrition on the plasma lipid profile. Avian Dis. 2014;58:176–182.

18. Forsythe WA, Miller ER, Curry B, Bennink MR. Aerobic exercise effects on lipoproteins and tissue lipids in young pigs. Atherosclerosis. 1981;38:327–337.

19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.

20. Fudge AM. Laboratory medicine: avian and exotic pets. Philadelphia (PA): W. B. Saunders Co.; 2000. p. 380.

21. Garner MM, Raymond JT. A retrospective study of atherosclerosis in birds. In: Proc Assoc Avian Vet; 2003. p. 59–66.

22. Gass GC, Romack FE, Lohman TG. The effect of exercise on atherosclerosis in the coronary artery and abdominal aorta of mature female swine. Eur J Appl Physiol O. 1979;42:235–246.

23. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB, on behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics: 2013 update: a report from the American Heart Association. Circulation. 2013;127:e6–e245.

24. Gollnick PD, Simmons SW. Physical activity and liver cholesterol. Int Z Angew Physiol. 1967;23:322–330.

25. Graczyk TK, Cranfield MR, Bicknese EJ. Evaluation of serum chemistry values associated with avian malaria infections in African black-footed penguins (*Spheniscus demersus*). Parasitol Res. 1995;81:316–319.

26. Haase E, Rees A, Harvey S. Flight stimulates adrenocortical activity in pigeons (*Columba livia*). Gen Comp Endocrinol. 1986;61:424–427.

27. Hammad SM, Siegel HS, Marks HL. Total cholesterol, total triglycerides, and cholesterol distribution among lipoproteins as predictors of atherosclerosis in selected lines of Japanese quail. Comp Biochem Physiol A Mol Integr Physiol. 1998;119: 485–492.

28. Harrison GJ, Lightfoot TL. Clinical avian medicine. Palm Beach (FL): Spix Publishing; 2006. p. 1005–1008.

29. Heinze CR, Hawkins MG, Gillies LA, Wu X, Walzem RL, German JB, Klasing KC. Effect of dietary omega-3 fatty acids on red blood cell lipid composition and plasma metabolites in the cockatiel, *Nymphicus hollandicus*. J Anim Sci. 2012;90:3068–3079.

30. Hermier D, Forgez P, Chapman MJ. A density gradient study of the lipoprotein and apolipoprotein distribution in the chicken, *Gallus domesticus*. Biochim Biophys Acta. 1985;836:105–118.

31. Hermier D, Forgez P, Laplaud PM, Chapman MJ. Density distribution and physicochemical properties of plasma lipoproteins and apolipoproteins in the goose, *Anser anser*, a potential model of liver steatosis. J Lipid Res. 1988;2:893–907.

32. Kelley JL, Alaupovic P. Lipid transport in the avian species: part 1: isolation and characterization of apolipoproteins and major lipoprotein density classes of male turkey serum. Atherosclerosis. 1976;24:155–175.

33. Kobernick SD, Niwayama G. Physical activity in experimental cholesterol atherosclerosis of rabbits. Am J Pathol. 1960;36:393–409.

34. Kramsch DM, Aspen AJ, Abramowitz BM, Kreimendahl T, Hood WB Jr. Reduction of coronary atherosclerosis by moderate conditioning exercise in monkeys on an atherogenic diet. N Engl J Med. 1981; 305:1483–1489.

35. LeMura LM, Duvillard von SP, Andreacci J, Klebez JM, Chelland SA, Russo J. Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and diet during and after resistance, aerobic and combination training in young women. Euro J Appl Physiol. 2000;82:451–458.

36. Lightfoot TL, Nacewicz CL. Psittacine behavior. In: Bays TB, Lightfoot TL, Mayer J (eds.). Exotic pet behavior: birds, reptiles, and small mammals. St. Louis (MO): Saunders Elsevier; 2006. p. 51–101.

37. Martin SS, Blaha MJ, Elshazly MB, Brinton EA, Toth PP, McEvoy JW, Joshi PH, Kulkarni KR, Mize PD, Kwiterovich PO, DeFilippis AP, Blumenthal RS, Jones SR. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. J Am Coll Cardiol. 2013;62:732–739.

38. Otten B, Quesenberry KE, Jones MP. Reference ranges for serum lipid levels in Amazon parrots. In: Proc Assoc Avian Vet; 2001. p. 95–97.

39. Petherick JC, Waddington D. Can domestic fowl (*Gallus gallus domesticus*) anticipate a period of food deprivation? Appl Anim Behav Sci. 1991;32:219–226.

40. Pilny AA, Quesenberry KE, Bartick-Sedrish TE, Latimer KS, Berghaus RD. Evaluation of *Chlamydophila psittaci* infection and other risk factors for atherosclerosis in pet psittacine birds. J Am Vet Med Assoc. 2012;240:1474–1480.

41. Ravich M, Cray C, Hess L, Arheart KL. Lipid panel reference intervals for Amazon parrots (*Amazona* species). J Avian Med Surg. 2014;28:209–215.

42. Rees A, Hall TR, Harvey S. Adrenocortical and adrenomedullary responses of fowl to treadmill exercise. Gen Comp Endocrinol. 1984;55:488–492.

43. Rees A, Harvey S, Phillips JG. Transitory corticosterone responses of ducks (*Anas platyrhynchos*) to exercise. Gen Comp Endocrinol. 1985;59:100–104.

44. Rozek JC, Danner LM, Stucky PA, Millam JR. Over-sized pellets naturalize foraging time of captive orange-winged Amazon parrots (*Amazona amazonica*). Appl Anim Behav Sci. 2010;125:80–87.

45. Tsai AC, Bach J, Borer KT. Somatic, endocrine, and serum lipid changes during detraining in adult hamsters. Am J Clin Nutr. 1981;34:373–376.

46. Vanhees L, Geladas N, Hansen D, Kouidi E, Niebauer J, Reiner Z, Cornelissen V, Adamopoulos S, Prescott E, Borjesson M, Bjarnason-Wehrens B, Björnstad HH, Cohen-Solal A, Conraads V, Corrado D, De Sutter J, Doherty P, Doyle F, Dugmore D, Ellingsen Ø, Fagard R, Giada F, Gielen S, Hager A, Halle M, Heidbüchel H, Jegier A, Mazic S, McGee H, Mellwig KP, Mendes M, Mezzani A, Pattyn N, Pelliccia A, Piepoli M, Rauch B, Schmidt-Trucksäss A, Takken T, van Buuren F, Vanuzzo D. Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the EACPR. Part II. Eur J Prev Cardiol. 2012;19:1005–1033.

47. Warnock NH, Clarkson TB, Clarkson TB, Stevenson R, Stevenson R. Effect of exercise on blood coagulation time and atherosclerosis of cholesterol-fed cockerels. Circ Res. 1957;5:478–480.

48. Weingarten HP. Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. Science. 1983;220:431–433.

49. Yashiro M, Kimura S. Effect of voluntary exercise and dietary protein levels on serum lipoprotein distributions and lecithin: cholesterol acyltransferase (LCAT) activity of mice. J Nutr Sci Vitaminol. 1980;26:59–69.

Received for publication 1 September 2015