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ORIGINAL ARTICLE

Clinical chemistry and hematology values in a Caribbean population of African green monkeys

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Abstract

Background Hematology and clinical chemistry (HCC) reference values are critical in veterinary practice and *in vivo* pre-clinical research, enabling detection of health abnormalities, response to therapeutic intervention or adverse toxicological effects, as well as monitoring of clinical management.
Methods In this report, reference ranges for 46 HCC parameters were characterized in 331 wild-caught and colony-bred African green monkeys. Effects of sex, weight and duration of captivity were determined by one-way analysis of variance.
Results Significant sex differences were observed for several HCC parameters. Significant differences were also observed for select HCC variables between newly caught animals and those held in captivity for 1–12 months or longer.
Conclusions Comparison of this data with other non-human primate species and humans highlights similarities and disparities between species. Potential causes of interpopulation variability and relevance to the use of the African green monkey as a non-human primate model are discussed.

Introduction

The aim of this report is to summarize hematology and clinical chemistry (HCC) data for a colony of both colony-bred and wild-born African green monkeys (*Chlorocebus sabaeus*; herein referred to as SK-AGM) at the St. Kitts Biomedical Research Foundation (SKBRF) and to examine the impact of captivity on these measures. In addition to large populations in Africa, substantial wild populations of African green monkeys, also known as vervets, are present on several Caribbean islands, including St. Kitts and Nevis [17] where they have thrived and been considered an agricultural pest since introduction in the 17th century. The African green monkey shares significant anatomic, physiologic and genomic similarities with humans and is one of the most widely used non-human primate species in pre-clinical research [3]. Given the continuing need for non-human primates in pre-clinical research, particularly in efficacy and safety studies, creating a database that would facilitate intra- and interspecies comparisons of HCC data would be of considerable utility. Moreover, establishing normal ranges for HCC measures in the African green monkey would allow for more meaningful predictive value to be derived from safety endpoint measures in acute and chronic toxicity, and developmental and reproductive toxicology studies. Likewise, determination of means and ranges for each HCC test variable additionally facilitates monitoring monkey health during study interventions. It is therefore critical that HCCs be examined to determine both representative means and ranges for healthy African green monkeys in captivity.

Significant factors influencing reference HCC values for a population or species include the impact of genetic diversity, habitat and diet [1, 5, 9, 12, 19]. Age, sex and captivity duration, which reflects span of time

removed from a prior habitat, also play a key role in defining hematologic and clinical chemistry measures [11, 13, 18, 19]. Although some non-human primate colonies utilize populations with distant founder animals, many are supported or supplemented by wild-caught monkeys or those transferred from another facility and thus variability in HCC values as a result of differing captivity duration remains a significant issue. Although an active breeding colony exists at SKBRF, captive wild-born African green monkeys make up the majority of subjects used in biomedical studies at the facility. Such monkeys typically spend <1 year in captivity before being placed in a study or exported for research conducted in the United States. While a prior report described normal reference HCC values in the African green monkey [8], it was limited to a relatively small cohort of animals with no separation of data by sex and did not provide full coverage of standard HCC parameters commonly evaluated for research and veterinary purposes. Furthermore, the geographical origin of these animals is not described, and the values reported are not consistent with HCC values in this species measured in other investigations [1, 8, 18]. Demonstration of sex differences [1, 18], age-associated differences [18], effects of captivity duration [13] and influences of diet [5, 12] and stress [20] on select HCC parameters in prior studies highlights the critical need to consider such factors when evaluating these measures in the African green monkey or any other species.

Data that evaluate comprehensive HCC differences between colony-bred animals and animals born in the wild but held captive for different durations are lacking in the scientific literature. In this study, we extend the understanding of non-human primate HCC measures by characterizing the HCC profiles of a large subpopulation of captive wild-born, in addition to colony-bred, SK-AGM, examining differences associated with sex, weight and duration in captivity - variables of interest both with regard to veterinary care and study data interpretation. Larger sample sizes allow for the establishment of more representative normal values for a total population [1]. We also evaluate the relevance of SK-AGM, alongside chimpanzees and cynomolgus monkeys, as models of clinical chemistry and hematologic values in humans.

While research institutes that maintain large colonies of non-human primates have published normal values or maintain internal reference values for their respective species, data related to comparing colony-bred animals to animals born in the wild are not readily available. This report suggests important future lines of investigation related to environmental shifts in physiologic measures. A specific aim of this investigation was to determine the mean and range for HCC values for a captive population of African green monkeys at SKBRF, with analyses conducted to assess changes in HCC values associated with duration of captivity. This characterization of the mean and range of HCC values and physiologic changes that they may reveal, following various durations of captivity, will facilitate veterinary management and evaluation of safety and efficacy endpoints in biomedical studies.

Materials and methods

Subjects

Three hundred and thirty-one male and female African green monkeys ranging between 1.5 and 7.5 kg were employed in the study. Criteria for enrollment were that each monkey was found to be healthy on physical exam during screening for inclusion in a facility study. Sampling and analysis was conducted over a 3-year period in studies in which baseline blood samples were collected as a component of the study design. Because the baseline parameters analyzed were dictated by the specific aims of the studies into which the monkeys were enrolled, not all parameters were collected for all animals. All monkeys were from the colony at SKBRF, a non-profit primate research facility in St. Kitts, West Indies. Animals were either born at the facility or captured from the feral population on the island of St. Kitts and maintained at SKBRF. Monkey capture was conducted in a humane fashion by a network of professional trappers employed by the facility using methods similar to those described previously [7] with animals subsequently sedated with ketamine (8 mg/kg) during transportation to the facility and introduction to quarantine. Monkeys were housed singly in steel squeeze cages throughout the length of the study and provided water *ad libitum*. All monkeys were fed pelleted monkey chow biscuits (Harlan Teklad, Madison, Wisconsin, crude protein = 20%, crude fat = 5%, crude fiber = 10%). It is standard practice at the facility that all recently trapped animals are tested for tuberculosis, administered anti-helminthics and maintained in single cages in a designated quarantine structure for 1 month following introduction to the facility to minimize the risk of introducing pathogens to the colony. Only monkeys determined to be clinically healthy after a physical exam were utilized in the analysis, and no monkeys were subject to experimental procedures prior to the blood sampling to obtain HCC data. All protocols adhere to The

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Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and all studies were approved by the Institutional Animal Care and Use Committee.

Procedures

Clinical chemistry and hematology sample collection

HCC values were determined from blood and plasma samples obtained during routine physical examinations. Sedation was necessary for blood sampling. This was achieved using intramuscular injections of ketamine HCL (Ketaset, Fort Dodge, Iowa) at a dosage of 10 mg/kg. Food was withheld from all animals for a period of 12 hours prior to ketamine administration. Blood was collected from the femoral vein into 12 cc syringes using 22 gauge needles. This sample was then divided equally between (i) Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing EDTA as an anti-coagulant for hematology samples and (ii) polystyrene conical tubes (BD Falcon, Franklin Lakes, NJ, USA) containing heparin as an anti-coagulant for plasma isolation. After centrifugation at 2630 $\times$g for 7 minutes at 4°C, the plasma supernatant was transferred to 1.8 cc cryotubes (Nunc, Roskilde, Denmark). Both plasma and EDTA blood samples were shipped via express mail courier in cold storage to Antech Diagnostics, Inc. (Memphis, TN, USA).

Clinical chemistry and hematology analysis

All HCC procedures were conducted by Antech Diagnostics. Clinical chemistry values, as detailed in Table 1, were measured by Antech Diagnostics using an Hitachi 747 analysis system. Hematology assessments, as detailed in Table 1, were measured by Antech Diagnostics using optical and mechanical methodologies and automated cell counter.

Data analysis

The St. Kitts HCC values represent means, standard deviations and ranges calculated and evaluated according to captivity, sex and weight. Absolute value ranges are provided for whole population and sex comparisons. To establish the normal range of clinical chemistry and hematology in the SK-AGM population, a standard approach to remove outliers from the complete cohort analyzed was utilized by excluding values greater than two standard deviations from the mean. When the calculated lower range value was less than zero, the actual minimum value was reported. Absolute clinical values were analyzed using a one-way analysis of variance (ANOVA) to assess effects of weight, sex and captivity period. For assessment of the effect of duration in captivity, the dependent variable used was length of captivity prior to HCC measure following grouping of animals as colony-bred (born and raised at the facility), new caught (<1 month since capture), first year (1–12 months since capture) or >1 year (>12 months since capture). Significance was set at $P < 0.01$. Tukey–Kramer HSD *post hoc* analysis was performed where appropriate. Statistical analyses were performed using JMP v8.0.1 (SAS Institute, Cary, NC, USA).

Table 1 Chemistry and hematologic parameters

Parameter	Abbreviation	Units
Glucose		mg/dl
Urea nitrogen		mg/dl
Creatinine		mg/dl
Total protein		g/dl
Albumin		g/dl
Total bilirubin		mg/dl
Alkaline phosphatase	AP	U/l
Alanine aminotransferase	ALT	U/l
Aspartate aminotransferase	AST	U/l
Cholesterol		mg/dl
Calcium		mg/dl
Phosphorus		mg/dl
Sodium		mEq/l
Potassium		mEq/l
Chloride		mEq/l
Albumin/globulin ratio	Alb/Globulin	Ratio
Blood urea nitrogen/creatinine ratio	BUN/Creatinine	Ratio
Globulin		g/dl
Lipase		U/l
Amylase		U/l
Triglycerides		mg/dl
Creatine phosphokinase	CPK	U/l
Gamma glutamyl transpeptidase	GGTP	U/L
Magnesium		mEq/l
Calculated osmolarity	Osmolarity	mOsm/l
Hemoglobin	HGB	g/dl
Hematocrit	HCT	%
White blood cells	WBC	10^3/ml
Red blood cells	RBC	10^6/ml
Mean corpuscular volume	MCV	fl
Mean corpuscular hemoglobin	MCH	pg
Mean corpuscular hemoglobin concentration	MCHC	g/dl
Platelet count	PC	10^3/ml
Neutrophils		cells/ml
Lymphocytes		cells/ml
Monocytes		cells/ml
Eosinophils		cells/ml
Basophils		cells/ml
Neutrophils		cells/ml
Prothrombin time	PT	%
Partial thromboplastin time	PTT	%
Fibrinogen		SECS

Results

Effects of sex and weight on population clinical chemistry and hematologic values

Clinical chemistry and hematologic values were determined in a cohort of 331 African green monkeys. This population comprised juvenile and adult animals and a mixed sex population of wild-caught and colony-bred monkeys. Tables S1 and S2 illustrate overall population measures for standard clinical chemistry measures and hematology assessments, respectively. Abbreviations and units are described in Table 1. Significant overall sex differences ($P < 0.01$) were observed in a number of clinical chemistry parameters, including elevated creatinine ($P < 0.0001$), total bilirubin ($P < 0.0001$) and albumin/globulin ratio ($P = 0.0055$) in males and significantly higher alkaline phosphatase (AP), ($P < 0.0001$); aspartate aminotransferase (AST), ($P = 0.0007$); cholesterol ($P < 0.0001$); blood urea nitrogen (BUN)/creatinine ratio ($P < 0.0001$); triglycerides ($P = 0.0020$); and creatine phosphokinase (CPK), ($P < 0.0001$) levels in females. Significant effects of animal weight were observed on AP ($P < 0.0001$), AST ($P < 0.0001$), cholesterol ($P = 0.001$), phosphorus ($P < 0.0001$) and CPK ($P < 0.0001$) (Table S1). Significant sex differences were also observed with some hematology measures including significantly higher white blood cells (WBC) and neutrophils in females and elevated red blood cells (RBC) and mean corpuscular hemoglobin (MCH) levels in males (Table S2). RBC ($P = 0.0001$); mean corpuscular volume (MCV), ($P = 0.0004$); MCH ($P = 0.0006$); and lymphocyte measures ($P = 0.0027$) were also significantly affected by animal weight (Table S2). Significant differences in HCC variables by animal weight generally demonstrated a non-linear trend, as determined by locally weight scatter-plot smoothing (LOWESS) plots of individual parameters. AP and cholesterol measures did, however, demonstrate approximately linear decreases as animal weight increased while RBC counts demonstrated the opposite effect with elevated counts as animal weight increased (data not shown).

Effect of captivity on clinical chemistry and hematologic values

Data were further analyzed after grouping according to the specific captivity history of each animal. Effects of captivity were initially assessed across the entire cohort. No clinical chemistry or hematology assessments were made on colony-bred males, consequently sex-independent captivity effects could only be determined in the wild-caught males. Total protein, albumin, total bilirubin, AP, phosphorus and sodium levels were significantly higher in first year animals and those housed in captivity for >1 year, than animals that were newly caught. In contrast, calcium, amylase and triglyceride clinical chemistry variables (Table S3) and platelet counts from hematologic analyses (Table S4) were significantly lower in animals in their first year of captivity or housed for >1 year, than animals that were newly caught. Some measures differed significantly between groups defined by captivity history but did not show a consistent increase or decrease in levels associated with length of captivity, these included AST, albumin/globulin ratio, CPK and globulin (Table S3). Significant effects of captivity on RBCs and monocytes did not correspond directly to the duration of captivity as levels fluctuated between newly caught, first year animals and monkeys housed for longer than a year at the facility.

Sex-specific effects of captivity on clinical chemistry and hematologic values

Data were further subgrouped by sex according to duration in captivity and further analyzed for captivity effects. Variables where there was a significant effect of captivity or sex on the whole population were examined to assess sex-specific effects of captivity. As the duration in captivity increased, the levels of total bilirubin, AP and sodium increased significantly in males (Tables S5 and S9). Moreover, males held in captivity for greater than 1 year showed significantly higher levels of total bilirubin and AP when compared to other captivity groups. By contrast, levels of CPK and AST were significantly lower in males housed in captivity for longer than a month. Platelet counts were significantly lower in males in year one and >1 year groups than in newly caught males, while RBC counts fluctuated over the course of captivity with significantly elevated levels over intermediate periods of captivity (between a month and 1 year) compared to newly caught and >1 year groups (Tables S6 and S9).

Levels of total bilirubin were significantly higher in females in captivity longer than a year when compared with newly caught females and those in their first year of captivity (Tables S7 and S9). AP measures demonstrated a similar but non-significant trend in captured animals where, in turn, levels in colony-bred females were significantly higher than the long-term captivity group. Calcium and amylase levels were significantly lower in long-term captivity females compared with those newly caught. AST and CPK levels were

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significantly higher in long-term captivity females compared with those newly caught and in their first year. Interestingly, in contrast to AP, levels of AST and CPK were significantly lower in colony-bred females than in the long-term captivity group, corresponding more closely with first year females. Platelet counts were significantly lower in first year and long-term captive females. RBC counts were elevated in first year females compared with those newly caught, but when comparing levels between newly caught and long-term captivity females there was no significant difference (Tables S8 and S9).

Comparison to other reference clinical chemistry and hematologic values

The primary goal of these studies was to establish the normal range of clinical chemistry and hematology values in the SK-AGM population (Tables 2 and 3). We broadly compared normal values and ranges for the SK-AGM population with prior reports of clinical chemistry or hematology values in a captive African green monkey population from the National Center for Primate Biology (University of California) [1], together with normal values for a captive cynomolgus monkey colony [19], a captive chimpanzee cohort [11] and reference human values from Massachusetts General Hospital [14] (Fig. 1). The greatest dichotomy between SK-AGM normal values and human reference values was observed for AST and AP levels. Conversely, normal values for glucose, calcium and total protein were very similar between all species examined. While normal gamma glutamyl transpeptidase (GGTP) levels were consistent among SK-AGM, chimpanzees [11] and humans [13], GGTP was quite divergent in cynomolgus monkeys [12]. Among HCC values evaluated, however, mean and range for normal total bilirubin in the chimpanzee cohort deviated the furthest from reference values in other species [11] (Fig. 1).

Discussion

The results of this investigation demonstrated that weight, sex and duration in captivity significantly affect

Table 2 Normalized clinical chemistry values in St. Kitts African green monkeys

	Males					Females				
Parameters	N	Mean	SD	Low	High	N	Mean	SD	Low	High
Glucose	198	81.7	18.0	45.6	117.8	116	80.3	18.7	43.0	117.6
Urea nitrogen	206	19.8	4.4	10.9	28.7	114	20.1	5.1	9.9	30.3
Creatinine	206	0.9	0.2	0.5	1.4	116	0.7	0.2	0.3	1.1
Total protein	198	6.9	0.5	5.9	8.0	117	6.9	0.6	5.8	8.0
Albumin	170	4.1	0.4	3.3	4.9	94	4.0	0.5	3.1	4.9
Total bilirubin	204	0.8	0.6	0.1	1.9	121	0.4	0.5	0.1	1.3
AP	198	163.9	135.0	32.0	433.8	117	273.3	163.5	56.0	600.2
ALT	205	46.1	22.6	0.9	91.3	115	47.5	19.6	8.3	86.6
AST	170	48.0	23.5	0.9	95.1	96	62.2	26.5	9.2	115.2
Cholesterol	170	114.9	18.4	78.0	151.8	90	131.7	18.9	94.0	169.5
Calcium	205	9.1	0.6	7.8	10.3	112	9.1	0.7	7.7	10.4
Phosphorus	199	5.3	1.6	2.1	8.5	112	5.2	1.6	2.0	8.4
Sodium	168	151.7	5.0	141.8	161.7	92	150.8	4.7	141.3	160.3
Potassium	163	3.5	0.5	2.4	4.6	95	3.8	0.7	2.5	5.1
Chloride	174	104.6	6.8	91.1	118.2	100	106.5	6.5	93.4	119.5
Alb/globulin	92	1.6	0.4	0.9	2.4	95	1.4	0.4	0.5	2.3
BUN/creatinine	92	22.0	8.5	4.9	39.1	92	32.6	12.3	7.9	57.3
Globulin	171	2.9	0.6	1.6	4.1	98	2.9	0.7	1.6	4.3
Lipase	92	73.4	33.5	6.3	140.5	97	74.2	30.1	14.0	134.5
Amylase	93	473.2	132.5	208.1	738.2	95	430.3	139.4	151.4	709.1
Triglycerides	93	38.7	15.4	8.0	69.4	95	46.5	18.1	10.3	82.7
CPK	173	1509.7	1666.7	193.0	4843.1	90	2857.9	1872.4	140.0	6602.7
GGTP	92	41.8	16.3	9.2	74.3	96	40.3	16.7	6.9	73.7
Magnesium	94	1.6	0.2	1.1	2.1	97	1.5	0.3	0.9	2.1
Osmolarity	93	294.7	9.4	275.9	313.5	68	298.2	11.9	274.4	322.0

Range represents 2 × SD above and below the mean. Where this value fell below zero, the low value represents the actual lowest value observed during analysis. Male data represent only wild-caught males. Female data represent findings from both colony-bred and wild-caught females.

AP, alkaline phosphatase; CPK, creatine phosphokinase; GGTP, gamma glutamyl transpeptidase.

Table 3 Normalized hematology values in St. Kitts African green monkeys

	Males					Females				
Parameters	N	Mean	SD	Low	High	N	Mean	SD	Low	High
HGB	169	13.8	1.8	10.2	17.4	77	12.2	1.2	9.8	14.7
HCT	169	43.9	5.4	33.2	54.7	77	39.7	4.4	30.9	48.5
WBC	165	5.9	1.8	2.3	9.6	72	6.8	1.7	3.4	10.2
RBC	162	5.6	0.6	4.4	6.9	77	5.2	0.5	4.1	6.3
MCV	165	77.2	4.5	68.2	86.2	71	77.4	4.2	69.0	85.9
MCH	160	24.2	0.9	22.3	26.1	72	23.7	0.9	21.9	25.6
MCHC	169	31.3	1.9	27.5	35.2	77	30.9	1.7	27.4	34.3
PC	163	355.1	97.0	161.1	549.1	74	368.4	99.7	169.0	567.9
Neutrophils	164	2549.7	1152.8	244.0	4855.4	72	2995.7	1261.2	473.4	5518.0
Lymphocytes	163	2746.8	1132.6	481.7	5012.0	74	3146.2	1127.1	891.9	5400.4
Monocytes	165	226.7	153.3	0.0	533.2	68	172.2	164.4	0.0	501.1
Eosinophils	138	81.2	82.8	0.0	246.8	55	54.2	74.8	0.0	203.7
Basophils	104	40.3	43.6	0.0	127.6	52	33.9	41.5	0.0	116.9
% Neutrophils	159	45.0	12.8	19.4	70.5	72	45.0	12.8	19.4	70.7
% Lymphocytes	159	47.4	12.6	22.1	72.7	73	47.5	13.3	20.8	74.1
% Monocytes	163	3.7	2.5	0.0	8.6	73	2.8	2.6	0.0	8.0
% Eosinophils	135	1.3	1.3	0.0	3.9	55	0.8	1.0	0.0	2.8
% Basophils	106	0.7	0.7	0.0	2.1	54	0.5	0.6	0.0	1.7
PT	39	12.6	0.8	11.0	14.3	35	12.9	0.8	11.3	14.6
PTT	39	30.3	2.4	25.5	35.2	35	31.2	2.2	26.7	35.6
Fibrinogen	39	245.9	63.4	119.0	372.8	35	217.3	61.5	94.2	340.3

Range represents 2 × SD above and below the mean. Where this value fell below zero, the low value represents the actual lowest value observed during analysis. Male data represent only wild-caught males. Female data represent findings from both colony-bred and wild-caught females.

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

a number of clinical chemistry and hematology measures in African green monkeys. Many of the clinical chemistry and hematology parameters demonstrating significant sex differences were consistent with previously published observations. Similar to our findings, Sato et al. [18] reported significantly higher levels of creatinine, total bilirubin, MCH and RBCs in male African green monkeys, while neutrophil counts were significantly higher in females. The demonstration of higher red blood cell parameters, such as RBC and MCH measures, in males, is consistent with previous measures in African green monkeys [13, 18] and cynomolgus monkeys [16, 19] and established differences in humans, reflecting hormonal environment and the proportionately larger muscular mass of males [15]. In contrast to prior reports in the African green monkey [1, 18], however, we also observed a significantly higher albumin/globulin ratio in males, similar to recent observations in cynomolgus monkeys [19], while females showed significantly higher levels of AP. Differences with the reported values of Sato et al. may reflect the larger cohort examined in our study or environmental or dietary differences. The fact that the African green monkey cohort Sato evaluated derived from a St. Kitts origin founder population would suggest that genetic contributions were less significant [18]. Similar sex differences have been observed for creatinine, triglycerides and RBC counts in cynomolgus monkeys [19], although in these same cynomolgus studies sex differences in total bilirubin and neutrophil measures contradicted our observations illustrating the challenges and limitations of evaluating interspecies differences.

As reported previously in African green monkeys [13], males were significantly heavier in the SKBRF cohort than in females, and importantly, several HCC variables demonstrated a significant effect of weight in addition to, or separately from sex. In particular, AP activity was significantly lower in males but assessing the distribution of data by weight we also observed a linear trend of distribution negatively associated with bodyweight. It is possible that this effect corresponds to age-related differences in AP activity, as has previously been described in African green monkeys and capuchin monkeys [18, 21], whereby low bodyweight and high AP activity reflect younger animals still undergoing skeletal development. The majority of HCC parameters demonstrating significant effect of weight also demonstrated a significant gender difference, with MCV, lymphocyte and phosphorus values

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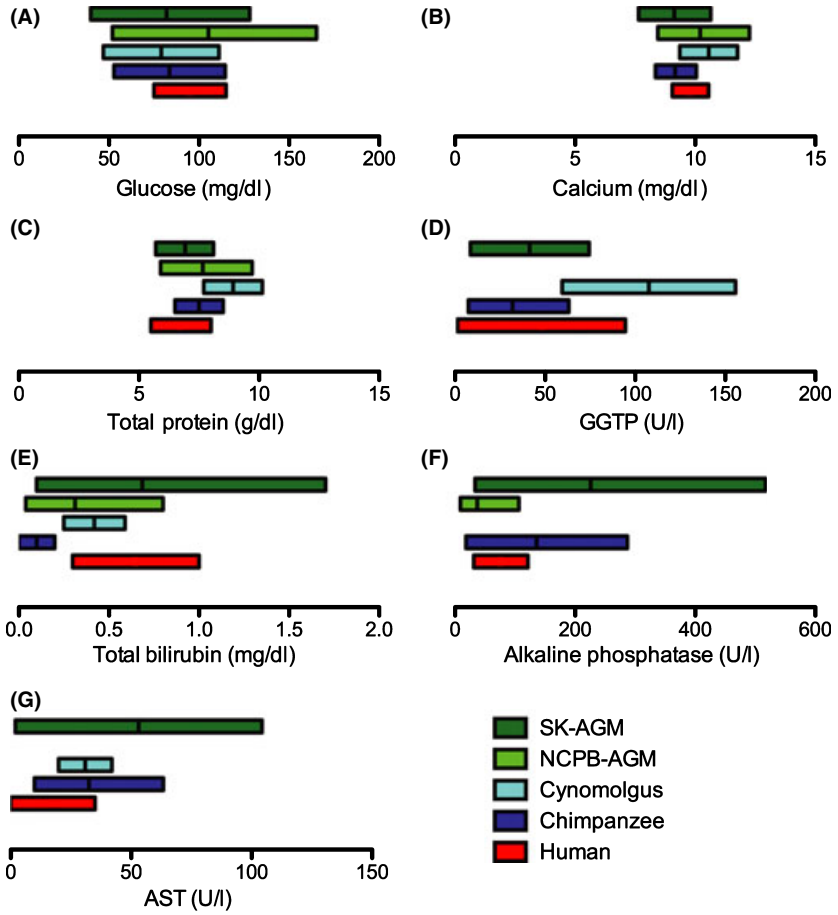

Fig. 1 Select hematology and clinical chemistry (HCC) reference value ranges for African green monkey (AGM), cynomolgus monkey, chimpanzee and humans. Reference HCC values shown for SKBRF (SK-AGM), and National Center for Primate Biology, U.C. Davis African green monkeys (NCPB-AGM) [3], cynomolgus monkeys (cynomolgus) [12], chimpanzees [11] and humans [13]. Range values represent mean and 2 × standard deviation (SD) for high- and low-range measures across males and females for SK-AGM and cynomolgus monkey HCC parameters shown. Range determination method was not detailed for human or NCPB-AGM datasets, which also include both male and female HCC values. No mean values were detailed for human reference values and thus no middle bar is included for human measures. Chimpanzee data comprise mean and range values for adult males only from Howell et al., (2003) [13]. This comparison was made as adult males comprised the majority of the 331 animals examined in the current study, and no combined sex data were provided for the chimpanzee analysis.

the exceptions to this. Phosphorous was the only measure of these three to show a clear weight-associated trend, whereby levels decreased as body weight increased. In contrast, similar studies in cynomolgus monkeys demonstrated phosphorus levels increased significantly with weight [19]. Serum phosphorus concentrations were lower in obese perimenopausal women compared to normal weight control groups [10], more closely resembling our observations in SK-AGM but more extensive evaluations of the effect of weight in humans are limited by the complex influence of dietary intake, exercise, gastrointestinal absorption and urinary excretion on circulating phosphorus levels.

Impact of captivity on hematologic values in African green monkeys has been reported previously [13], and the impact on HCC measures have been reported in rhesus monkeys [4, 9]. This is the first report examining the effect of captivity on clinical chemistry measures in African green monkeys. Analyzing HCC measures in the St. Kitts colony revealed a number of variables significantly impacted by the duration in captivity. Several measures, including total protein, albumin, AP, phosphorus and sodium, increased significantly over the course of captivity from the first month to >1 year in captivity. In contrast, other variables such as calcium and triglyceride levels were significantly lower in the >1 year in captivity group. CPK activity, an indicator of muscle inflammation or injury, was significantly lower in long-term captivity males yet significantly higher in comparable females

when compared to recently captured animals. The reason for these reverse sex-specific captivity trends is unclear and worthy of further investigation.

Kagira et al. [13] investigated the impact of captivity on hematologic values in an East African cohort of African green monkeys over an 8-month period, observing significant effects of captivity on erythrocyte parameters, total white blood cell (WBC) counts and platelet counts in adults. As a result of these findings, the authors highlighted the need for a long adaptation period and individual baseline measures before experimental use [13]. Our findings demonstrated some similarities with this prior report, such as platelet counts, which were significantly lower in both males and females housed in captivity >1 month when compared to newly caught animals. In addition, RBC counts were significantly elevated after intermediate periods of captivity in males, while MCH levels were consistently higher, although not significantly, in intermediate and long-term captivity males and females compared to newly caught animals. By contrast, we found no effects on mean corpuscular hemoglobin concentration (MCHC). Important methodological differences exist between our studies and this previous report. In addition to differing analytical approaches and genetic differences associated with the distinct Kenyan and Caribbean subpopulations, animal housing and dietary differences must also be considered as potential sources of variability.

Appreciating the different methodological approaches employed between much of the previously published literature on reference HCC values in Old World monkeys, particularly the African green monkey, we have been cautious to minimize comparison of absolute values with those previously reported and instead evaluated overall sex and captivity differences. Nonetheless, in evaluating the potential benefits or limitations of working with African green monkeys as a pre-clinical model for evaluating therapeutic efficacy or safety, it is important to consider the reference range in the SKBRF colony in relation to reference human values along with those of other Old World monkeys widely used in biomedical research. Such comparisons confirm similar ranges for a number of clinical chemistry and hematologic parameters with cynomolgus monkeys [19], rhesus monkeys [2], chimpanzees [11] as well as prior reports in the African green monkey [1, 18]. Parameters exhibiting similar ranges included total protein, creatinine, glucose, calcium, sodium, chloride and cholesterol (select examples illustrated in Fig. 1). Erythrocyte measures (HGB, HCT, MCH and RBC) were also broadly consistent with previous reports in cynomolgus monkeys [19].

In contrast, other HCC parameters were quite divergent. CPK in the SKBRF cohort was detected at far higher levels than previous reports in cynomolgus monkeys [19] and chimpanzees [11] as well as prior reports in African green monkeys [18]. While muscle injury as a result of ketamine administration has been reported to elicit increases in serum CPK levels [2], this is unlikely to explain the difference between species and between different subpopulations of African green monkey as all these studies utilized ketamine to sedate animals prior to blood collection. ALT and AST ranges were also notably higher than reported levels in cynomolgus monkeys [19] and chimpanzees [11]. Similar variability in liver transaminases within other vertebrate orders would be consistent with species rather than environmental differences [6].

Normal ranges for the SK-AGM cohort showed many similarities with published reference ranges in humans [14]. Common to other Old world monkey HCC normal values, glucose, creatinine, total protein, calcium, sodium, potassium and erythrocyte parameters in SK-AGM were very similar to reference human values. Normal GGTP mean and ranges for cynomolgus monkeys were substantially higher than levels observed in SK-AGM, chimpanzees [11] and humans [13]. Other measures, including ALT, AST, phosphorus, triglycerides and CPK, were more similar between chimpanzees and humans than African green monkeys. These findings suggest that some measures are influenced by greater interspecies genetic homology. Because normal mean values for total bilirubin were particularly divergent between humans and chimpanzees but very similar between humans and SK-AGM, it is evident that genetic variance will not suffice as the source of all observed HCC discrepancies. It is well established, however, that different reference laboratories generate different HCC values for the same species, thus a component of the apparent differences may reflect the assay stringencies of the laboratories employed. A more accurate assessment of interspecies and interpopulation biological differences would be achieved by utilizing the same laboratory for all analyses.

An unavoidable consequence of utilizing wild-caught animals is the inability to accurately determine the age of study animals although dental examination is an effective means of estimation in non-human primates [13]. Consequently, effects of age on HCC values cannot be accurately determined on wild-caught animals within the SKBRF cohort. Expansion of the present dataset to include more data from the colony-bred colony will permit reevaluation of normal SK-AGM HCC values across age groups.

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Non-human primate research is critical to the translational development of basic biological findings into validated therapeutic interventions for improving human health. Non-human primates are appropriately reserved for experimentation where no other animal model will provide the necessary anatomic, physiologic or genetic homology with humans, most often in the final stages of pre-clinical research, prior to clinical trials. Animal care costs additionally constrain the use of non-human primates. For these reasons, academic and commercial non-human primate research needs are supplied by a relatively small number of captive populations, which are augmented by wild-caught monkeys at certain facilities. Our findings, along with prior examinations of HCC values in African green monkeys [1, 8, 18], highlight the impact of time in captivity and sex, in addition to age, on normal HCC measures, and in so doing, the importance of establishing baseline HCC values in individuals prior to research use and utilizing these as the primary reference for determining therapeutic or adverse effects. Our findings additionally provide further support to prior reports demonstrating the considerable physiologic similarities that the African green monkey shares with humans and other closely related non-human primates. Benefits of being able to reference normal HCC values for a species, and now more accurately for the African green monkey, include the facilitation of veterinary management, evaluation of study outcomes and characterization of phenotypic and genotypic subpopulations, although all factors influencing individual and subpopulation HCC measures must be appreciated.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Effect of Sex and Weight on Clinical Chemistry Values in St. Kitts African Green Monkeys.

Table S2. Effect of Sex and Weight on Hematologic Measures in St. Kitts African Green Monkeys.

Table S3. Effect of Captivity and Sex on Clinical Chemistry Values in St. Kitts African Green Monkeys.

Table S4. Effect of Captivity and Sex on Hematologic Measures in St. Kitts African Green Monkeys.

Table S5. Effect of Captivity on Clinical Chemistry Values in Male St. Kitts African Green Monkeys.

Table S6. Effect of Captivity on Hematologic Measures in Male St. Kitts African Green Monkeys.

Table S7. Effect of Captivity on Clinical Chemistry Values in Female St. Kitts African Green Monkeys.

Table S8. Effect of Captivity on Hematology Values in Female St. Kitts African Green Monkeys.

Table S9. *P*-Values for Post-hoc Analysis of Sex-Specific Captivity Effects on HCC Measurements.

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