Journal of the American Association for Laboratory Animal Science
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Vol 54, No 4
July 2015
Pages 359–367

Hematology and Clinical Chemistry Measures During and After Pregnancy and Age- and Sex-Specific Reference Intervals in African Green Monkeys (*Chlorocebus aethiops sabaeus*)

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Clinical decisions and experimental analyses often involve the assessment of hematology and clinical chemistry. Using clinical pathology to assess the health status of NHP in breeding colonies or data from studies than involve pregnancy can often be complicated by pregnancy status. This study had 2 objectives regarding the hematology and clinical chemistry of African green monkeys (AGM, *Chlorocebus aethiops sabaeus*): 1) to compare pregnant or recently postpartum animals with nonpregnant, nonlactating animals and 2) to create age- and sex-specific reference intervals. Subjects in this study were 491 AGM from the Vervet Research Colony of the Wake Forest University Primate Center. Results indicated that changes in BUN, serum total protein, albumin, ALP, GGT, calcium, phosphorus, sodium, potassium, cholesterol, total CO_2, globulins, lipase, amylase, WBC, neutrophils, lymphocytes, platelets, RBC, Hgb, and Hct occur during pregnancy and the postpartum period. Age- and sex-specific reference intervals consistent with guidelines from the American Society for Veterinary Clinical Pathology were established and further expand the understanding of how to define health in AGM on the basis of clinical pathology. The combination of understanding the changes that occur in pregnancy and postpartum and expansive reference intervals will help guide clinical and experimental decisions.

Abbreviations: AGM, African green monkeys; tCO2, total carbon dioxide.

Appropriate interpretation of clinical chemistry and hematology measures requires understanding what values are representative of a 'healthy' animal and how those measures change during normal physiologic states. Data from several species have shown that clinical chemistry and hematologic measurements differ when animals are pregnant or postpartum.[4,10,19,22,23] These differences may be critical when interpreting clinical chemistry and hematology data that are part of clinical or experimental assessments.

The creation of reference intervals is based on the evaluation of samples from a well-characterized group of clinically healthy subjects and, by convention, is defined as the middle 95% of this healthy population.[5,8] The use of reference intervals is crucial to the identification and selection of subjects for biomedical research projects and the maintenance of the breeding colonies that provide those subjects.[12] The American Society for Veterinary Clinical Pathology provides guidelines for the determination of reverence intervals in veterinary species[7]

African green monkeys (AGM, *Chlorocebus aethiops sabaeus*), also known as vervets, have been used widely in biomedical research within diverse disciplines including arthrology, metabolism, immunology, gerontology, genetics, and behavior.[14-16,18,24,27] The Vervet Research Colony of the Wake Forest University Primate Center is an NIH-supported breeding colony and national biomedical research resource that provides animals, samples, and data from this species to numerous investigators. This breeding colony is ideally suited to provide samples and data on clinical chemistry and hematology to improve our understanding of the associated changes during pregnancy and lactation.

This study characterizes the difference between pregnant and nonpregnant female AGM and expands on the work of previous studies[21,25] by providing a more expansive set of reference intervals for the clinical chemistry and hematology for AGM. The aims of the study were 1) to describe how values for these parameters differ in adult female AGM according to reproductive status (that is, pregnant [1st, 2nd, or 3rd trimester], postpartum, or nonpregnant) and 2) to provide reference intervals for clinical chemistry and hematology according to age category and sex from a larger cohort than has been published previously.

Materials and Methods

Subjects. The subjects of this study were 491 United States-born, known-age AGM (*Chlorocebus aethiops sabaeus*). All animals were part of the Vervet Research Colony of the Wake Forest University Primate Center, which is a multigenerational, genotyped, and pedigreed biomedical research resource.[15] They represent the descendants of 57 founders imported from the islands of St Kitts and Nevis from 1975 to 1986 and which have maintained as a closed colony for the last 30 y. Animals were in the 2nd through 8th generation at the time of this study and ranged in age from 0.4 y to 26 y (mean, 11.1 y) at the time of sampling.

Animals were housed in 16 multigenerational social groups within large indoor–outdoor enclosures with climbing structures

Received: 30 Jun 2014. Revision requested: 29 Aug 2014. Accepted: 03 Dec 2014
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and perches. Social interaction, exercise, food, and water were available free choice. At the time of sampling, AGM were fed a commercial primate chow (Laboratory Diet 5038; LabDiet, St Louis, MO), with supplemental fresh fruits and vegetables provided 3 to 5 d each week.

All animal procedures were approved by the Wake Forest University Institutional Animal Care and Use Committee consistent with the recommendations in the *Guide for Care and Use of Laboratory Animals*[13] (8th edition, Institute for Laboratory Animal Research) and in compliance with the USDA Animal Welfare Act[1] and Animal Welfare Regulations[2] (Animal Welfare Act as Amended; Animal Welfare Regulations). Wake Forest is an AAALAC-accredited institution.

Sample collection and analysis. For both aims of this study, sample collection and analysis were identical. Animals were sampled as part of 15 triannual tuberculosis testing procedures that occurred over the course of 5 y. Individual animals had 1 to 5 samples (mean, 2.8) collected over this time period. Veterinary exams were performed once annually at 1 of these 3 time periods, along with blood collection for the assessment of clinical chemistry and hematology.

All animals were fasted overnight and anesthetized with intramuscular injection of ketamine (10 to 15 mg/kg). Blood samples (2 mL) were collected by venipuncture of the femoral vein into EDTA and serum separator blood tubes vacuum phlebotomy tubes (2 mL per tube; Becton Dickinson, Franklin Lakes, NJ).

The EDTA-treated blood samples were held on wet ice until shipment, and the serum-separator blood tubes were maintained at room temperature until processing. Serum-separator blood tubes were centrifuged at $1000 \times g$ for 25 min before aliquoting the serum. After processing, whole blood and serum samples were submitted to a commercial laboratory (Idexx Laboratories, Westbrook, ME) for analysis on the same day as collection.

Blood chemistry values for BUN, blood glucose, total protein, albumin, total bilirubin, indirect bilirubin, direct bilirubin, creatinine, ALP activity, AST activity, ALT activity, GGT activity, creatinine kinase, total calcium, phosphorus, sodium, potassium, chloride, total plasma cholesterol, total carbon dioxide (tCO_2), anion gap, globulins, lipase, and amylase were measured by using an automated chemistry analyzer (Olympus AU5800, Beckman Coulter, Brea, CA). Hematology values for WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, RBC, Hct, MCV, MCH, and MCHC were measured by using an automated hematology analyzer (model XT-2000iV, Sysmex, Lincolnshire, IL).

Determination of pregnancy status. The following methods were used to determine pregnancy status at the time of sampling. All female AGM of breeding age (3 to 20 y old, $n = 311$) were assessed for pregnancy by using ultrasonography, with estimated gestational age determined via fetal measurements.[18] Pregnancy and postpartum status at the time of sampling was determined retrospectively through the evaluation of birthing records according to a 163-d gestational period.[18] Female AGM that had blood collected within 163 d prior to a birth were determined to be pregnant at the time of sampling.

Statistical analysis. Over the course of the 5-y period, clinical chemistry and hematology data were collected from a total of 491 animals, with 1 to 5 records per animal ($n = 871$ total records). This overall data set was used to generate 2 separate data sets: 1) a data set used to compare the effects of reproductive status on clinical chemistry and hematology parameters in adult female AGM and 2) a separate data set to establish reference intervals in male and nonpregnant female AGM of different ages. The inclusion criterion for both data sets was that the animal had to be clinically healthy by evaluation of a veterinarian at the time of sampling. Exclusion criteria were any clinical signs of disease or any medical therapy at the time of sampling.

A data set was created that consisted of breeding age female AGM only that were categorized as pregnant, postpartum, or nonpregnant. Pregnant and postpartum AGM were further divided into the following categories: 1st trimester (1 to 54 d gestation), 2nd trimester (55 to 108), 3rd trimester (109 to 163 d gestation), 1st month postpartum (days 1 to 30 postpartum), and 2nd month postpartum (days 31 to 60 postpartum). The term 'trimester' is used to divide the pregnancy into 3 equal time periods to allow comparisons with and translation to human pregnancy. If animals had multiple samples during the 1st trimester, 2nd trimester, 3rd trimester, 1st month postpartum, or 2nd month postpartum, only the most recent sample from each AGM was included in each category. The nonpregnant comparison group was selected from animals within the same age range as the pregnancy and postpartum animals but did not include any animals from those groups.

For the reference interval data set, all pregnant or postpartum female AGM were excluded. If animals had multiple samples while in an age category, only the most recent sample from each AGM was included to avoid overrepresentation of subjects. The resulting data set consisted of 561 samples. Animals then were separated into the following age categories: female AGM younger than 4 y old, male AGM younger than 4 y old, female AGM 4 to 12 y old, male AGM 4 to 12 y old, female AGM 12 to 20 y old, male AGM older than 12 y, and female AGM older than 20 y.

Data analysis. Statistical analyses of the pregnancy and postpartum data were performed by using Statistica version 12 (StatSoft, Tulsa, OK), and P values of less than 0.05 were considered significant. The ages of the pregnant and postpartum subjects were compared with those of the nonpregnant animals by using ANOVA. Clinical chemistry and hematologic measurements were summarized by using nonparametric methods, and categories were analyzed for differences by using Kruskal–Wallis and Dunn tests.

Statistical analysis for the reference intervals was performed by using Statistica version 12 (StatSoft). Mean, standard deviation, median, and interquartile range were determined for all groups. Reference intervals were determined nonparametrically as the central 95% of the reference population for categories with at least 40 samples.[7] For categories with fewer than 40 samples, the minimal and maximal values are given instead of reference intervals.

Results

Pregnancy. The groups did not differ by age ($P = 0.06$), and a summary of the groups is provided in Table 1. Median values and interquartile ranges during and after pregnancy and statistical differences as compared with data from nonpregnant AGM are presented in the Table 2. Most (but not all) differences in clinical chemistry and hematology values occurred in animals that were in the 3rd trimester. No statistically significant differences were seen in glucose, total bilirubin, indirect bilirubin, creatinine, AST, ALT, creatine kinase, chloride, triglycerides, monocyte count, eosinophil count, MCV, MCH, and MCHC.

Regarding clinical chemistry parameters, BUN showed significant ($P < 0.001$) differences as compared to nonpregnant AGM, with lower values in the 2nd and 3rd trimesters and

Table 1. Study groups for pregnancy-related analyses

Pregnancy or lactation stage	No. of animals	Age (y)		
		Mean (1 SD)	Minimum	Maximum
1st trimester (1 to 55 d of gestation)	33	10.2 (4.2)	3.9	19.0
2nd trimester (56 to 110 d of gestation)	43	9.8 (3.9)	3.7	17.8
3rd trimester (111 to 165 d of gestation)	38	10.5 (3.8)	4.8	19.0
1st month (1 to 30 d) after delivery	19	12.5 (4.0)	6.9	20.8
2nd month (31 to 60 d) after delivery	21	11.4 (4.3)	5.0	20.9
Nonpregnant or nonlactating	157	11.9 (5.5)	3.7	20.8
All animals	311	11.2 (4.9)	3.7	20.9

Age did not differ between groups. Gestational age was determined according to ultrasonography or birthing records.

Table 2. Hematology and clinical chemistry data during and after pregnancy

Analyte	Median (interquartile range)					
	first trimester	second trimester	third trimester	1st month after delivery	2nd month after delivery	nonpregnant
BUN (mg/dL)	15 (4)	13 (4)[c]	12 (5)[c]	23 (8)	22 (5)[c]	15 (6)
Glucose (mg/dL)	65 (20)	62 (24)	61 (29)	66 (21)	68 (45)	60 (21)
Total protein (g/dL)	6.8 (0.4)	6.3 (0.6)[c]	5.9 (0.4)[c]	7.1 (0.9)	7.3 (0.5)	6.9 (0.5)
Albumin (g/dL)	3.9 (0.4)	3.0 (0.8)[c]	2.5 (0.2)[c]	3.6 (1.0)	4.0 (0.5)	4.0 (0.6)
Total bilirubin (mg/dL)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)
Indirect bilirubin (mg/dL)	0.1 (0.1)	0.1 (0.0)	0.1 (0.1)	0.1 (0.1)	0.1 (0.0)	0.1 (0.1)
Direct bilirubin (mg/dL)	0.2 (0.1)[a]	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)
Creatinine (mg/dL)	0.8 (0.2)	0.7 (0.2)	0.7 (0.3)	0.8 (0.2)	0.8 (0.2)	0.7 (0.3)
ALP (U/L)	96 (44)	78 (32)	70 (35)	135 (48)[b]	115 (37)	86 (48)
AST (U/L)	43 (15)	49 (14)	39 (13)	47 (21)	48 (20)	46 (18)
ALT (U/L)	84 (43)	104 (128)	85 (63)	77 (27)	66 (29)	83 (62)
GGT (U/L)	38 (19)	51 (30)[a]	43 (29)	53 (28)	43 (18)	37 (21)
Creatine kinase (U/L)	713 (554)	761 (948)	691 (356)	703 (657)	804 (881)	757 (652)
Calcium (mg/dL)	8.4 (0.6)	7.6 (0.5)[c]	7.5 (0.5)[c]	8.8 (0.8)	8.7 (0.5)	8.8 (0.7)
Phosphorus (mg/dL)	3.6 (1.4)[a]	3.4 (1.2)[c]	3.1 (0.9)[c]	4.9 (1.4)	5.4 (1.3)[a]	4.4 (1.4)
Sodium (mEq/L)	145 (2)	143 (3)[c]	142 (2)[c]	146 (2)	146 (4)	146 (3)
Potassium (mEq/L)	3.6 (0.6)	3.6 (0.5)[a]	3.7 (0.5)	4.1 (0.4)	3.9 (0.6)	3.7 (0.4)
Chloride (mEq/L)	109 (3)	110 (3)	110 (3)	109 (2)	109 (4)	109 (4)
Total protein cholesterol (mg/dL)	109 (47)[c]	99 (32)[c]	103 (22)[c]	155 (27)	129 (38)	145 (32)
Triglycerides (mg/dL)	65 (17)	65 (35)	63 (16)	71 (30)	67 (20)	63 (21)
tCO_2 (mEq/L)	18 (2)[c]	18 (5)[c]	18 (4)[c]	21 (4)	19 (5)	22 (4)
Anion gap	20 (4)	19 (5)	20 (4)	20 (3)	21 (4)[a]	19 (4)
Globulin (g/dL)	2.9 (0.3)	3.1 (0.7)[a]	3.4 (0.4)[c]	3.3 (0.6)[b]	3.2 (0.5)	2.9 (0.5)
Lipase (U/L)	85 (79)	70 (54)[a]	76 (61)	92 (47)	94 (34)	95 (72)
Amylase (U/L)	502 (194)[c]	442 (199)[c]	489 (129)[c]	568 (253)	553 (213)	647 (222)
WBC (K/uL)	6100 (3000)	7700 (3300)[b]	8700 (2300)[c]	6500 (4300)	6400 (2100)	6600 (3000)
Neutrophils (/uL)	3354 (2125)	4320 (2935)[b]	4524 (2004)[c]	3025 (2124)	3744 (1827)	3418 (2860)
Lymphocytes (/uL)	2160 (1784)	2613 (2052)	3300 (1134)[b]	2525 (1871)	2592 (1548)	2332 (1295)
Monocytes (/uL)	300 (203)	370 (242)	412 (256)	276 (536)	312 (128)	338 (294)
Eosinophils (/uL)	0 (61)	0 (62)	0 (104)	17 (81)	62 (73)	44 (89)
Basophils (/uL)	0 (0)[a]	0 (0)	0 (0)	0 (27)	0 (0)	0 (26)
Platelets (K/uL)	310 (134)	266 (160)[b]	242 (100)[c]	405 (118)	332 (171)	337 (113)
RBC (M/uL)	5.88 (0.60)	5.58 (0.48)	5.25 (0.63)[c]	5.31 (0.99)	5.48 (0.67)	5.80 (0.60)
Hgb (g/dL)	13.5 (1.4)	13.3 (1.1)	12.8 (1.1)[c]	13.1 (2.1)	13.2 (1.3)	13.6 (1.4)
Hct (%)	42.5 (3.8)	41.2 (3.5)	39.2 (3.7)[c]	40.6 (5.3)	41.0 (3.2)	42.7 (3.7)
MCV (fL)	73 (4)	74 (5)	75 (3)	77 (6)	75 (4)	74 (4)
MCH (pg)	23.4 (1.6)	23.7 (1.8)	24.2 (1.3)	24.5 (1.4)	23.8 (1.5)	23.6 (1.6)
MCHC (g/dL)	32.2 (1.3)	32.1 (1.4)	32.5 (1.4)	31.7 (1.9)	32.0 (1.0)	32.1 (1.5)

Value is significantly ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$; Dunn test) different from the nonpregnant age-matched study group.

Table 3. Clinical chemistry reference intervals for African green monkeys younger than 4 y

	Female (n = 88)			Male			
Analyte	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)	n	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)
BUN (mg/dL)	21 (5)	21 (7)	11–31	90	23 (6)	23 (10)	12–33
Glucose (mg/dL)	74 (19)	71 (19)	50–124	89	73 (18)	70 (23)	46–125
Total protein (g/dL)	6.9 (0.4)	6.9 (0.5)	6.2–7.6	89	6.8 (0.3)	6.8 (0.4)	6.1–7.3
Albumin (g/dL)	4.6 (0.3)	4.6 (0.3)	3.9–4.9	89	4.6 (0.2)	4.6 (0.2)	4.1–5.1
Total bilirubin (mg/dL)	0.3 (0.1)	0.2 (0.1)	0.1–0.4	89	0.2 (0.1)	0.2 (0.1)	0.1–0.4
Indirect bilirubin (mg/dL)	0.1 (0.1)	0.1 (0.0)	0.0–0.2	89	0.1 (0.1)	0.1 (0.0)	0.0–0.2
Direct bilirubin (mg/dL)	0.2 (0.1)	0.2 (0.1)	0.1–0.2	89	0.2 (0.1)	0.1 (0.1)	0.1–0.2
Creatinine (mg/dL)	0.7 (0.1)	0.7 (0.2)	0.5–1.1	90	0.7 (0.1)	0.7 (0.2)	0.5–1.0
ALP (U/L)	440 (170)	450 (235)	158–823	89	605 (151)	613 (188)	266–867
AST (U/L)	51 (12)	51 (16)	30–81	89	55 (19)	53 (17)	27–103
ALT (U/L)	64 (29)	58 (31)	33–136	90	52 (16)	50 (17)	31–101
GGT (U/L)	35 (13)	31 (14)	21–66	89	35 (12)	30 (10)	21–64
Creatine kinase (U/L)	602 (286)	499 (335)	256–1254	89	686 (648)	512 (333)	210–2687
Calcium (mg/dL)	9.2 (0.3)	9.2 (0.5)	8.6–9.7	89	9.0 (0.5)	9.0 (0.7)	8.3–10.0
Phosphorus (mg/dL)	6.2 (1.7)	6.0 (2.7)	3.2–9.5	90	7.3 (1.3)	7.35 (1.7)	4.7–9.6
Sodium (mEq/L)	145 (2)	145 (3)	142–149	89	146 (2)	146 (4)	142–151
Potassium (mEq/L)	3.8 (0.3)	3.8 (0.4)	3.4–4.4	90	3.8 (0.3)	3.8 (0.4)	3.4–4.5
Chloride (mEq/L)	108 (2)	107 (3)	104–113	89	107 (2)	107 (3)	103–112
Total protein cholesterol (mg/dL)	128 (22)	123 (26)	96–179	89	125 (22)	125 (34)	88–164
Triglycerides (mg/dL)	58 (14)	56 (17)	35–90	87	51 (15)	49 (15)	30–80
tCO_2 (mEq/L)	18 (4)	18 (4)	10–24	89	20 (4)	20 (4)	11–26
Anion gap	24 (5)	23 (7)	16–32	89	23 (4)	22 (5)	16–31
Globulin (g/dL)	2.4 (0.4)	2.3 (0.7)	1.7–3.2	89	2.2 (0.3)	2.1 (0.4)	1.7–2.7
Lipase (U/L)	196 (143)	129 (238)	40–545	89	155 (130)	98 (98)	45–531
Amylase (U/L)	620 (132)	594 (167)	409–941	89	602 (135)	592 (166)	371–894

higher values during the 2nd mo after delivery, without a corresponding difference in creatinine.

Liver enzyme activities showed a mixed pattern with no statistically significant differences during the 1st trimester, 3rd trimester, or 2nd mo postpartum. During the 2nd trimester, GGT activities were elevated ($P < 0.05$) compared with those in nonpregnant AGM. In the first month postpartum, ALP activity was increased ($P < 0.01$). Serum total protein levels and albumin were lower during the 2nd and 3rd trimesters ($P < 0.001$). Contrary to the lower serum total protein, globulins were higher ($P < 0.05$) during the 2nd and 3rd trimesters and continued to remain increased ($P < 0.05$) during the 1st mo postpartum. Serum total protein cholesterol levels were lower in AGM during the 1st, 2nd, and 3rd trimesters ($P < 0.001$).

Bicarbonate was lower in AGM during the 1st, 2nd, and 3rd trimesters ($P < 0.001$). Compared with those in nonpregnant AGM, serum calcium and phosphorus levels showed differences throughout pregnancy and after delivery. Calcium and phosphorus concentrations both were lower ($P < 0.001$) during the 2nd and 3rd trimesters. Phosphorus was decreased ($P < 0.05$) during the 1st trimester and increased ($P < 0.05$) in the 2nd mo postpartum.

During all trimesters of pregnancy, amylase was lower ($P < 0.001$) than that of nonpregnant AGM. Lipase was decreased ($P < 0.05$) during the 2nd trimester.

The WBC count was elevated ($P < 0.01$) during the 2nd and 3rd trimesters, with a corresponding elevation in neutrophil counts. Lymphocyte count was increased ($P < 0.01$) only during the 3rd trimester.

During the 3rd trimester, median RBC counts, Hgb concentrations, and Hct were lowest ($P < 0.001$), the only time that they differed from those in nonpregnant AGM. Platelet numbers were decreased ($P < 0.01$) in the 2nd and 3rd trimesters of pregnancy.

Reference intervals. Age- and sex-specific reference intervals for clinical chemistry values in AGM are provided in Tables 3 through 6; those for hematology are in Tables 7 through 10.

Discussion

To our knowledge, this report is the first to document the normal changes to blood chemistry and hematology encountered during pregnancy in AGM. Only a few publications examine these changes in any NHP.[4,9,12,26] Understanding these changes is critical to correctly interpreting these measures for clinical health management, breeding management, and research. Few measures remained stable during pregnancy and the postpartum period in AGM, and these changes need to be considered during data interpretation.

During the period of our study, the reproductive age range for female AGM was 3.9 to 20.9 y. Previous publications have described the reproductive value of AGM to peak between 5 to 7 y of age and then rapidly decline until 12 y of age.[6] Our examination of 35 y of colony records indicated that the reproductive age of AGM ranged from 2.6 to 23.9 y; 93.9% of births were in dams 3 to 15 y old.

AGM have a decrease in BUN during pregnancy, a change that occurs in baboons and rabbits also.[4,9,23] However, contrary to these other species, AGM do not have a concurrent decrease in creatinine.[4,9,23] A decrease in BUN and creatinine during

Table 4. Clinical chemistry reference intervals for African green monkeys 4 to 12 years old

	Female (n = 147)[a]			Male (n = 46)[a]		
Analyte	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)
BUN (mg/dL)	16 (4)	16 (5)	10–25	16 (4)	16 (5)	10–26
Glucose (mg/dL)	61 (18)	60 (21)	31–102	61 (16)	62 (17)	31–94
Total protein (g/dL)	7.0 (0.4)	7.1 (0.5)	6.2–7.6	6.9 (0.4)	6.9 (0.7)	6.2–7.7
Albumin (g/dL)	4.1 (0.4)	4.2 (0.5)	3.3–4.7	4.2 (0.5)	4.4 (0.6)	2.8–4.8
Total bilirubin (mg/dL)	0.2 (0.1)	0.2 (0.1)	0.1–0.4	0.3 (0.1)	0.3 (0.2)	0.1–0.5
Indirect bilirubin (mg/dL)	0.1 (0.1)	0.1 (0.0)	0.0–0.2	0.2 (0.1)	0.1 (0.1)	0.0–0.3
Direct bilirubin (mg/dL)	0.1 (0.1)	0.1 (0.1)	0.0–0.2	0.2 (0.1)	0.1 (0.1)	0.1–0.3
Creatinine (mg/dL)	0.8 (0.1)	0.8 (0.2)	0.5–1	0.8 (0.2)	0.8 (0.2)	0.5–1.1
ALP (U/L)	97 (40)	86 (47)	46–191	105 (56)	90 (67)	40–263
AST (U/L)	49 (21)	48 (17)	26–92	60 (22)	56 (20)	31–105
ALT (U/L)	105 (83)	79 (69)	34–381	93 (63)	66 (73)	33–254
GGT (U/L)	41 (14)	37 (21)	19–71	40 (12)	38 (13)	23–68
Creatine kinase (U/L)	920 (517)	772 (584)	305–2400	1175 (1157)	674 (1187)	220–4848
Calcium (mg/dL)	8.9 (0.5)	8.9 (0.7)	8.0–9.7	9.2 (0.5)	9.2 (0.8)	8.3–10.1
Phosphorus (mg/dL)	3.9 (1.0)	3.9 (1.2)	2.0–6.5	4.9 (1.1)	4.8 (1.6)	3.1–6.9
Sodium (mEq/L)	146 (2)	146 (3)	141–150	147 (2)	147 (3)	142–150
Potassium (mEq/L)	3.7 (0.4)	3.7 (0.4)	3.1–4.5	4.1 (0.5)	4.0 (0.7)	3.3–5.0
Chloride (mEq/L)	109 (3)	109 (4)	105–114	107 (3)	107 (4)	102–111
Total protein cholesterol (mg/dL)	142 (23)	138 (29)	102–203	133 (27)	128 (33)	not done[b]
Triglycerides (mg/dL)	68 (26)	64 (20)	40–149	48 (14)	46 (18)	not done[b]
tCO_2 (mEq/L)	21 (3)	21 (5)	14–28	24 (4)	24 (6)	19–32
Anion gap	20 (3)	19 (5)	13–27	20 (4)	19 (6)	13–28
Globulin (g/dL)	2.9 (0.4)	2.8 (0.5)	2.2–3.7	2.6 (0.7)	2.6 (0.7)	1.7–4.4
Lipase (U/L)	172 (147)	99 (222)	42–566	123 (101)	97 (35)	34–458
Amylase (U/L)	660 (351)	587 (205)	411–1122	667 (151)	652 (183)	437–987

[a]Sample size as indicated except for total protein cholesterol (male, 37), triglycerides (female, 96; male 21), lipase (male, 45), and amylase (male, 45).
[b]Sample size insufficient to calculate reference interval.

pregnancy is commonly attributed to an increase in glomerular filtration rate due to expanded circulatory volume. An explanation for the lack of decrease in creatinine in AGM was not determined in the current study.

The observed differences in the liver enzyme activities (ALP, GGT) of AGM were not of sufficient magnitude to be considered clinically or physiologically relevant. In humans, rabbits, squirrel monkeys, rhesus, and baboons, statistically significant changes occur in liver enzyme activity during pregnancy but may not be clinically relevant.[4,9,19,23,26] The patterns of changes in liver enzyme activity are dissimilar among AGM and other species.

The lower serum total protein levels in the 2nd and 3rd trimesters of AGM are similar to what has been reported in humans, squirrel monkeys, rhesus, baboons, and rabbits.[4,9,19,23,26] In humans but not AGM, this decrease in total protein continues into the 1st mo postpartum.[19] The lower serum total protein levels in AGM are driven by albumin, which is decreased during the 2nd and 3rd trimesters.

Serum total protein cholesterol is decreased during the 2nd and 3rd trimesters in AGM, squirrel monkeys, baboons, and rabbits.[9,23,26] In AGM, this parameter is also decreased during the 1st trimester. The current study and the studies in squirrel monkeys, baboons, and rabbits all controlled for diet by maintaining the same diet throughout all stages; therefore these values likely reflect true changes in cholesterol metabolism. The decreases in serum total protein, albumin, and cholesterol may be a result of plasma volume increasing faster than albumin and cholesterol production during pregnancy.[11,19] This 'hemodilution' effect typically causes a corresponding decrease in globulin concentration, but that change did not occur in the current study in AGM. The increase in globulins that peaks in the 3rd trimester likely is the result of a marked increase in production that is not reported in other species.

The decrease in tCO_2 during pregnancy indicates a shift in acid–base balance and is likely the result of respiratory compensation for an unmeasured serum anion or change in strong ion difference from renal alterations. The timing of this change supports it as being the result of increasing demands of the fetus. In horses, bicarbonate is decreased during the 1st trimester and postpartum.[10]

AGM, similar to baboons, show a decrease in calcium during pregnancy and an increase in the calcium:phosphorus ratio.[9] Rabbits show a different pattern, with decreased calcium during the 3rd trimester and increased phosphorus during the 2nd and 3rd trimesters.[23] These differences likely are related to the changing demands associated with fetal development and lactation. The patterns of serum sodium and potassium concentrations in AGM differ from those in rats, baboons, and horses.[9,10,20]

The decrease in amylase during pregnancy only in AGM is an unexpected finding that is not reported in any other species. In humans, lipase is reported to be decreased during the 1st trimester, but no change is seen in amylase.[17] The mechanism for this decreased amylase in AGM is unclear but may be related to

Table 5. Clinical chemistry reference intervals for African green monkeys 12 to 20 y old

	Female (n = 122)[a]			Male (n = 9)		
Analyte	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)	Mean (1 SD)	Median (interquartile range)	Minimum–maximum[b]
BUN (mg/dL)	18 (5)	17 (8)	10–27	18 (6)	16 (5)	12–30
Glucose (mg/dL)	66 (23)	64 (20)	31–130	73 (20)	72 (26)	42–103
Total protein (g/dL)	6.8 (0.5)	6.9 (0.5)	5.8–7.7	6.8 (0.3)	6.8 (0.3)	6.3–7.3
Albumin (g/dL)	3.8 (0.4)	3.8 (0.5)	2.9–4.4	4.1 (0.4)	4.2 (0.4)	3.1–4.5
Total bilirubin (mg/dL)	0.2 (0.1)	0.2 (0.1)	0.1–0.4	0.3 (0.1)	0.3 (0.1)	0.2–0.4
Indirect bilirubin (mg/dL)	0.1 (0.1)	0.1 (0.1)	0.0–0.3	0.2 (0.1)	0.2 (0.1)	0.1–0.3
Direct bilirubin (mg/dL)	0.1 (0.1)	0.1 (0)	0.0–0.2	0.1 (0.0)	0.1 (0.0)	0.0–0.1
Creatinine (mg/dL)	0.8 (0.2)	0.8 (0.2)	0.5–1.2	0.9 (0.1)	0.8 (0.1)	0.8–1.2
ALP (U/L)	85 (32)	79 (35)	49–148	84 (25)	78 (24)	60–143
AST (U/L)	48 (16)	45 (15)	27–86	47 (7)	46 (9)	37–60
ALT (U/L)	89 (62)	71 (51)	33–308	87 (75)	63 (31)	41–280
GGT (U/L)	43 (19)	38 (19)	23–85	38 (8)	36 (6)	23–53
Creatine kinase (U/L)	897 (504)	780 (691)	300–2115	704 (301)	636 (243)	319–1360
Calcium (mg/dL)	8.6 (0.5)	8.6 (0.5)	7.7–9.7	8.9 (0.4)	9.0 (0.2)	8.4–9.5
Phosphorus (mg/dL)	4.4 (1.1)	4.3 (1.5)	2.6–6.8	5.6 (1.2)	6.0 (1.2)	3.2–7.1
Sodium (mEq/L)	145 (3)	146 (3)	133–149	146 (2)	147 (2)	144–148
Potassium (mEq/L)	3.7 (0.4)	3.7 (0.5)	3–4.7	3.9 (0.3)	4.0 (0.4)	3.2–4.2
Chloride (mEq/L)	108 (3)	108 (4)	98–113	106 (2)	107 (2)	101–109
Total protein cholesterol (mg/dL)	151 (25)	152 (33)	102–206	143 (34)	131 (47)	103–205
Triglycerides (mg/dL)	71 (20)	65 (22)	40–127	not done	not done	not done
tCO_2 (mEq/L)	22 (3)	22 (3)	16–28	25 (2)	25 (2)	20–28
Anion gap	19 (4)	19 (4)	13–27	19 (3)	19 (3)	14–23
Globulin (g/dL)	3.1 (0.5)	3.0 (0.5)	2.5–4.2	2.7 (0.5)	2.5 (0.2)	2.1–3.7
Lipase (U/L)	106 (77)	91 (44)	49–351	95 (41)	85 (39)	48–171
Amylase (U/L)	701(190)	670 (248)	427–1213	671 (221)	612 (322)	468–1138

[a]Sample size as indicated except for total protein cholesterol (female, 118) and triglycerides (female, 118; male, 0).
[b]Sample size insufficient (n < 40) for calculation of reference interval.

changes in glomerular filtration rate, given that renal filtration is the primary route for the excretion of amylase.

WBC and neutrophil counts are increased in the 2nd and 3rd trimesters in AGM and humans.[3,19] Rhesus macaques show similar increases in WBC and neutrophil counts but only during the 1st trimester of pregnancy.[11] This increase contrasts with the situation in baboons, in which there is no change,[9] and rabbits, in which the WBC count is decreased.[23] In addition, AGM had a unique increase in lymphocytes during the 3rd trimester, but the increase in WBC was still predominantly due to the elevated neutrophil count. In humans, the pregnancy-associated increase in WBC count can limit its diagnostic value clinically in the context of concerns regarding infection,[19] and clinicians working with AGM should carefully consider these changes when interpreting hematologic values during pregnancy.

The RBC count, Hgb, and Hct were trended lower over the course of the pregnancy and became statistically different from nonpregnant animals in the 3rd trimester. Similar changes in these measures are reported in humans, rats, rabbits, baboons, rhesus macaques, and squirrel monkeys.[4,9,19,20,23,26] These changes may reflect the aforementioned hemodilution effect seen with albumin concentration, during which the total body erythroid mass remains stable or increases but not as rapidly as does the plasma volume. During the first month postpartum, the median RBC count was similar to that during the 3rd trimester, but the range was much wider. This finding may be a result of individual variance in blood loss during parturition. Baboons show a decrease of platelet counts in the 3rd trimester, as do AGM, but not the in the 2nd trimester.[9] Humans show no change in platelet counts, whereas rabbits show increases in late pregnancy.[19,23] These changes are not likely clinically important in any of these species, given that levels are still adequate for normal clotting.

The reference intervals presented in the current report are the most extensive that have been reported for AGM and likely are representative of all Caribbean-origin AGM, according to previous genetic studies.[14] Analysis of the effects of sex and age were not pursued in this study since they have been previously described.[21,25] We followed the reference interval guidelines of the American Society for Veterinary Clinical Pathology, except that reference intervals were not calculated for any group with fewer than 40 samples.[7] The groups that had fewer than 40 samples were the geriatric populations, male AGM 12 and 20 y old, and female AGM older than 20 y old. None of these populations is commonly used in research, but we felt that providing these data was important to expand the knowledge on the species as a whole.

We sought to follow the guidelines of the American Society for Veterinary Clinical Pathology to create reference intervals most consistent with the standards of veterinary medicine and to mimic the standards in human medicine set forth by the Clinical and Laboratory Standards Institute.[7] The individual animals in the Vervet Research Colony have well-documented health statuses, standardized husbandry practices, and well-defined genetic backgrounds and are divided into distinct

Table 6. Clinical chemistry reference data for female African green monkeys older than 20 y (n = 37)

Analyte	Mean (1 SD)	Median (interquartile range)	Minimum–maximum
BUN (mg/dL)	19 (6)	18 (7)	7–34
Glucose (mg/dL)	72 (26)	63 (35)	42–135
Total protein (g/dL)	6.8 (0.5)	6.9 (0.6)	5.1–7.7
Albumin (g/dL)	3.6 (0.3)	3.7 (0.4)	2.8–4.2
Total bilirubin (mg/dL)	0.2 (0.1)	0.2 (0.1)	0.1–0.3
Indirect bilirubin (mg/dL)	0.1 (0.1)	0.1 (0.1)	0–0.3
Direct bilirubin (mg/dL)	0.1 (0.1)	0.1 (0.1)	0–0.2
Creatinine (mg/dL)	0.8 (0.2)	0.8 (0.1)	0.4–1.1
ALP (U/L)	96 (41)	89 (58)	44–224
AST (U/L)	42 (14)	43 (17)	20–83
ALT (U/L)	84 (72)	54 (42)	20–300
GGT (U/L)	39 (17)	34 (19)	17–84
Creatine kinase (U/L)	802 (528)	657 (560)	187–2539
Calcium (mg/dL)	8.7 (0.5)	8.7 (0.6)	7.7–10.1
Phosphorus (mg/dL)	4.8 (1.2)	4.7 (1.5)	2.1–8.6
Sodium (mEq/L)	145 (3)	146 (3)	137–151
Potassium (mEq/L)	3.8 (0.5)	3.8 (0.6)	2.9–5.3
Chloride (mEq/L)	106 (3)	106 (3)	100–113
Total protein cholesterol (mg/dL)	154 (36)	146 (54)	91–240
Triglycerides (mg/dL)	71 (12)	68 (19)	54–85
tCO_2 (mEq/L)	25 (4)	24 (4)	15–34
Anion gap	18 (3)	18 (3)	12–29
Globulin (g/dL)	3.2 (0.4)	3.1 (0.4)	2.1–4.0
Lipase (U/L)	106 (85)	89 (44)	39–575
Amylase (U/L)	812 (221)	780 (329)	413–1490

Table 7. Hematology reference intervals for African green monkeys younger than 4 years

	Female (n - 96)			Male (n = 99)		
Analyte	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)
WBC ($\times 10^3$/μL)	5340 (2584)	4600 (3050)	2600–12500	4890 (1856)	4700 (2400)	2500–9300
Neutrophils (/μL)	2821 (1958)	2166 (2144)	806–9240	2295 (1285)	1960 (1917)	756–5767
Lymphocytes (/μL)	2188 (1273)	1926 (1369)	720–5040	2304 (1294)	1920 (1011)	931–5550
Monocytes (/μL)	276 (216)	210 (253)	0–924	248 (243)	224 (182)	0–574
Eosinophils (/μL)	39 (116)	0 (13)	0–427	37 (61)	0 (57)	0–246
Basophils (/μL)	11 (30)	0 (0)	0–90	6 (20)	0 (0)	0–66
Platelets ($\times 10^3$/μL)	318 (111)	323 (132)	106–491	319 (137)	307 (119)	147–695
RBC ($\times 10^6$/μL)	5.69 (0.36)	5.75 (0.32)	4.80–6.27	5.82 (0.44)	5.79 (0.50)	4.90–6.76
Hgb (g/dL)	13.1 (0.9)	13.2 (0.9)	11.2–15.1	13.3 (1.2)	13.4 (1.4)	10.6–15.4
Hct (%)	41.8 (2.5)	42.2 (2.5)	35.4–46.0	42.1 (3.1)	42.1 (3.8)	34.0–47.9
MCV (fL)	73.45 (2.95)	73.71 (3.85)	67.62–79.61	72.41 (3.60)	72.24 (3.08)	66.00–79.79
MCH (pg)	23.09 (0.92)	23.13 (1.00)	21.36–24.73	22.83 (1.40)	22.77 (1.41)	20.40–25.17
MCHC (g/dL)	31.45 (1.05)	31.33 (1.50)	29.98–33.74	31.52 (1.02)	31.36 (1.43)	29.63–33.26

biologic groups. The preanalytical factors, analytical methods, and data analysis were consistent and are well-documented for the presented reference intervals. We believe that these details increase the value and applicability of these reference intervals.

The interpretation of hematology and clinical chemistry measures is often the basis of clinical decisions and is best guided by well-defined reference intervals. Without reference intervals, correct interpretation during pregnancy may be limited, because even significant differences between groups may not exceed the reference interval or may not be of a magnitude to be physiologically significant. However, these statistically significant differences may be important in study designs that use animals that may be pregnant or lactating. Overall, the combination of understanding how pregnancy changes hematology and clinical chemistry parameters and reference intervals for AGM helps to better characterize their biology and increase their value as an animal model.

Limitations of the current study include variations in physiology due to social rank in the social housing, but this variation

Table 8. Hematology reference intervals for African green monkeys 4 to 12 y old

	Female (n = 150)			Male (n = 46)		
Analyte	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)
WBC ($\times 10^3/\mu L$)	6984 (2438)	6750 (3000)	3400–13400	5928 (2026)	5450 (2700)	2800–9400
Neutrophils (/μL)	3847 (2174)	3366 (2680)	1064–9322	2726 (1525)	2313 (1160)	825–7013
Lymphocytes (/μL)	2645 (1041)	2338 (1268)	1132–4872	2727 (1260)	2614.5 (1758)	828–5166
Monocytes (/μL)	421 (257)	384 (278)	67–1072	376 (242)	332 (238)	86–1025
Eosinophils (/μL)	50 (79)	10 (78)	0–306	74 (93)	47 (130)	0–300
Basophils (/μL)	14 (38)	0 (0)	0–134	12 (31)	0 (0)	0–119
Platelets ($\times 10^3/\mu L$)	320 (103)	311 (90)	135–571	305 (94)	286 (94)	142–508
RBC ($\times 10^6/\mu L$)	5.81 (0.47)	5.80 (0.55)	4.83–6.85	6.86 (0.82)	7.07 (0.77)	5.68–8.41
Hgb (g/dL)	13.7 (1.1)	13.7 (1.2)	11.4–15.9	16.6 (2.0)	17.0 (2.2)	13.9–20.3
Hct (%)	43.0 (3.0)	43.1 (3.1)	36.4–49.7	52.2 (6.0)	52.6 (6.9)	44.6–63.4
MCV (fL)	74.16 (2.93)	74.33 (3.95)	68.36–81.58	76.22 (3.37)	76.42 (3.98)	68.76–81.97
MCH (pg)	23.63 (1.03)	23.57 (1.49)	21.71–25.87	24.25 (1.18)	24.35 (1.28)	21.29–26.06
MCHC (g/dL)	31.87 (0.98)	31.73 (1.42)	30.2–33.75	31.83 (1.22)	31.72 (2.08)	29.77–33.96

Table 9. Hematology reference intervals for African green monkeys 12 to 20 y old

	Female (n = 122)			Male (n = 9)		
Analyte	Mean (1 SD)	Median (interquartile range)	Reference interval (central 95%)	Mean (1 SD)	Median (interquartile range)	Minimum–maximum
WBC ($\times 10^3/\mu L$)	6962 (2405)	6650 (3300)	3300–12500	5322 (1522)	5700 (1800)	3100–8300
Neutrophils (/μL)	4140 (2383)	3944.0 (3496)	1037–9025	2757 (1197)	2324 (1359)	1132–4826
Lymphocytes (/μL)	2364 (897)	2162 (1445)	975–4456	2208 (966)	2646 (1315)	719–3420
Monocytes (/μL)	318 (185)	279 (204)	78–783	166 (70)	171 (130)	86–257
Eosinophils (/μL)	72 (82)	55 (76)	0–277	63 (60)	49 (46)	9–165
Basophils (/μL)	27 (39)	15 (39)	0–125	33 (27)	29 (33)	0–87
Platelets ($\times 10^3/\mu L$)	355 (125)	358 (138)	113–592	319 (67)	303 (72)	235–451
RBC ($\times 10^6/\mu L$)	5.85 (0.59)	5.81 (0.83)	4.85–7.08	6.80 (0.23)	6.76 (0.21)	6.47–7.25
Hgb (g/dL)	14.1 (1.6)	14.0 (1.7)	11.4–17.1	17.0 (1.2)	16.5 (1.9)	15.4–19.0
Hct (%)	43.3 (4.6)	43.0 (5.7)	36.0–52.3	51.4 (3.6)	50.4 (6.9)	46.9–56.2
MCV (fL)	74.07 (4.43)	74.12 (4.45)	66.43–81.43	75.60 (4.35)	76.66 (5.60)	69.38–81.34
MCH (pg)	24.06 (1.67)	24.16 (1.91)	21.20–26.67	24.99 (1.38)	25.10 (1.37)	22.78–27.38
MCHC (g/dL)	32.47 (1.04)	32.52 (1.41)	30.41–34.17	33.06 (0.52)	32.85 (0.80)	32.44–33.81

Table 10. Hematology reference intervals for female African green monkeys older than 20 y (n = 37)

Analyte	Mean (1 SD)	Median (interquartile range)	Minimum–maximum
WBC ($\times 10^3/\mu L$)	7046 (2565)	6200 (4100)	2600–12500
Neutrophils (/μL)	4092 (2555)	3264 (4134)	699–9492
Lymphocytes (/μL)	2452 (720)	2294 (932)	1115–4538
Monocytes (/μL)	330 (194)	287 (260)	55–800
Eosinophils (/μL)	96 (100)	76 (117)	0–375
Basophils (/μL)	33 (52)	14 (38)	0–220
Platelets ($\times 10^3/\mu L$)	382 (121)	422 (159)	98–723
RBC ($\times 10^6/\mu L$)	5.83 (0.56)	5.87 (0.79)	4.59–6.70
Hgb (g/dL)	13.7 (1.7)	13.9 (2.6)	8.9–16.2
Hct (%)	42.3 (4.8)	42.0 (7.3)	30.4–50.9
MCV (fL)	72.57 (4.67)	73.69 (4.05)	51.19–78.72
MCH (pg)	23.44 (1.84)	23.88 (1.16)	14.15–25.42
MCHC (g/dL)	32.27 (1.19)	32.55 (1.24)	27.64–34.52

should be considered a strength because it is representative of the spectrum of health. An additional limitation is subclinical systemic disease, which likely is limited in nature do to vigilant observation and veterinary care. The number of pregnant AGM included in this study is relatively limited, ranging from 19 to 43 depending on the study group, although this population is

well defined by virtue of being a closed, genotyped, pedigreed colony, and this report is the largest evaluation of the effect of pregnancy on clinical pathology in any NHP. A final limitation is that the time point at which sample collection occurred is not controlled according a specific pregnancy or postpartum day, given that pregnancy state was determined retrospectively, but the sample is representative of the pregnancy or lactation state.

In conclusion, we have provided the extensive reference intervals for hematology and clinical chemistry measures in AGM and have established that similar patterns in these values occur in AGM and other species. The Vervet Research Colony population we studied is likely similar to the St Kitts population, on the basis of genetic studies showing similar genetic variation.[14] Not all parameters with significant differences during pregnancy are considered to be clinically relevant, but changes in BUN, albumin, globulins, lipase, amylase, WBC, neutrophils, RBC, Hgb, and Hct have been proven important in the diagnosis and treatment of spontaneous disease. Other parameters may be important for specific research endpoints, depending on the needs of the study for which the animal is enrolled. These normative data are crucial when analyzing samples obtained during both nonpregnant and pregnant states, given that diagnoses and therapy may be initiated on the basis of the results of hematology and clinical chemistry measures. The understanding of these effects of pregnancy may provide the basis to study reproduction, pregnancy, and the postpartum period in AGM.

Acknowledgments

We thank Jeff Cranfill, Valerie Allen, Gordon McDole, Charles Boyd, Kelsey Finnie, and Tara Chavanne for their technical expertise.

Support for this research was provided by NIH grant OD010965 (principal investigator: Jay R. Kaplan).

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