Age- and sex-related changes in body weights and clinical pathology analytes in cynomolgus monkeys (*Macaca Fascicularis*) of Mauritius origin

Xiantang Li | Dingzhou Li | Kathleen E. Biddle | Susan S. Portugal | Mark R. Li | Rosemary Santos | John E. Burkhardt | Nasir K. Khan

Drug Safety Research & Development and Comparative Medicine. Pfizer, Inc., Groton, Connecticut, USA

Correspondence
Xiantang Li, Drug Safety Research & Development and Comparative Medicine, Pfizer, MS 8274-1259, Eastern Point Road, B274, Groton, CT 06340, USA.
Email: xiantang.li@pfizer.com

Abstract
Background: Clinical pathology and body weight information for the cynomolgus monkey in the literature is primarily derived from a small number of animals with limited age ranges, varying geographic origins, and mixed genders.

Objectives: This study aimed to summarize the age- and sex-related changes in clinical pathology analytes and body weights in cynomolgus monkeys of Mauritian origin.

Methods: Pre-study age and body weight data were reviewed in 1819 animals, and pre-study hematologic, coagulation, and serum biochemical analytes were reviewed in 1664 animals.

Results: Body weights were statistically higher (*P* <0.01) in males than females in all age groups (2–10 years). These measurements became prominent after 4 years of age and peaked at 7 to 8 years of age in both sexes. Sex-related differences were noted in reticulocyte (RETIC) counts, creatinine, cholesterol, and triglyceride concentrations, and alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) activities. Age-related differences were noted in RETIC and lymphocyte counts, creatinine, triglyceride, phosphorus, and globulin concentrations, and ALP and GGT activities. The youngest (2 to <3 year) age group had the fewest number of clinical pathologic analyte differences including ALP and GGT activity differences which occurred in all age groups from 2 to 10 years; they also had age-related lower globulin concentrations. There were no age- or sex-related differences in coagulation measurands.

Conclusions: Sexual dimorphism in body weight was apparent for all ages from 2 to 10 years of age. The only difference in clinical pathology analytes unique to the 2 to <3 years of age group were age-related lower globulin levels.

Keywords: body weight, coagulation, cynomolgus monkey, hematology, Mauritius, serum biochemistry

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. Veterinary Clinical Pathology published by Wiley Periodicals LLC on behalf of American Society for Veterinary Clinical Pathology.
1 | INTRODUCTION

Non-human primates (NHP) are the closest phylogenetic relatives of humans with similar physiologic traits; they especially resemble the anatomy and genetics of macaques and apes.\textsuperscript{1,2} Of the 22 macaque species, 21 species are distributed in Asia, including cynomolgus and rhesus monkeys.\textsuperscript{3,4} The cynomolgus monkey is recognized as having 10 subspecies based on their pelage colors and tail lengths. The cynomolgus monkey (\textit{Macaca fascicularis}, \textit{M. cynomolgus}, and \textit{M. irus}) is also known as the long-tailed macaque or crab-eating macaque.

The cynomolgus monkey is distributed primarily in Southeast Asia.\textsuperscript{2,3} Cynomolgus monkeys that inhabit the north of the Isthmus of Kra might have genetic introgression from the rhesus monkey in Cambodia, Laos, Myanmar, Thailand, and Vietnam, whereas those that inhabit the south of the Isthmus would have no genetic influences from the rhesus monkey.\textsuperscript{5,6} The cynomolgus monkey in Mauritius was introduced from Indonesia by shipping trades around the 16th century.\textsuperscript{7} The original founder population was estimated at approximately 10 to 15 monkeys.\textsuperscript{8} Thus, the genetic diversity of Mauritius monkeys is low.\textsuperscript{5} The cynomolgus monkey in China was introduced from Vietnam, Cambodia, Laos, and Myanmar in the late 1980s.\textsuperscript{4} Mauritius cynomolgus monkeys were shown to have differences in some hematologic and biochemical analytes and lower grades and incidences of background pathologic changes than Asian cynomolgus monkeys.\textsuperscript{9,10} Cynomolgus monkeys of Mauritian origin become sexually mature 1–2 years earlier than those of Asian origin.\textsuperscript{9} Despite geographic differences, there are no differences in physiologic and pharmacologic parameter values that impact the use of Asian or Mauritian cynomolgus monkeys for nonclinical safety assessments.\textsuperscript{10}

The cynomolgus monkey has become the most used NHP for biomedical research and nonclinical safety assessments since the Indian government banned the export of rhesus monkeys in 1978.\textsuperscript{11} Background information, including the body weight and clinical pathology reference intervals (RIs), has been reported in the literature and appears to vary with the geographic origins of animals and by different laboratory environments.\textsuperscript{12–17} These RIs were derived from a small number of animals, limited age ranges, and/or mixed genders.\textsuperscript{12,15,17–20} In the current study, we provide RIs for 2 to <3 and 3 to <4 years of age and summarize age- and sex-related changes in clinical pathology analytes (4 different age groups) and body weights (9 different age groups) in cynomolgus monkeys of Mauritian origin used in nonclinical safety assessment in Pfizer Drug Safety Research & Development laboratories over a period of 5 years (2016–2020).

2 | MATERIALS AND METHODS

2.1 | Animals and husbandry

During this 5-year period, we had a dataset of 1823 monkeys. All animals were of Mauritian origin with a specific-pathogen-free status to exclude cercopithecine herpesvirus 1, simian beta-retrovirus 1–5, simian T-lymphotropic virus, simian immunodeficiency virus, and \textit{Mycobacterium tuberculosis}. The animals were housed in the Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities in Mauritius and the United States. To ensure suitable health status, physical examinations were conducted, body weights measured, and blood samples collected for hematologic and serum biochemistry analyze screens prior to the pre-study group allocation. All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee. Clinically acceptable animals were allocated to study groups using a computer-assisted randomized procedure based on pre-study body weights.

2.2 | Body weight

Of the 1823 cynomolgus monkeys in this dataset, 1819 animals (960 males and 859 females) had pre-study age and body weight data. Animal body weights were taken during the acclimation periods right before the animals were assigned to study groups. If more than one body weight was measured, the last pre-study body weight immediately before the study started was used.

2.3 | Blood collection and analysis

Of the 1823 cynomolgus monkeys in this dataset, 1664 animals (860 males and 804 females) had both age and clinical pathology analyte data recorded. The clinical pathology data included hematologic, coagulation, and serum biochemical analytes. \textit{Table 1} lists analytes with the abbreviations, units, platforms, and/or methods. Also included in this table is an equivalency factor (%) of the expected biological variability, based on historical knowledge of the more common analytes (considered major and bolded in the table) for comparison purposes. All blood samples included were collected pre-study from animals that had been fasted overnight (approximately 12–18 hours) and before dosing started. If blood was collected more than once, the clinical pathology analyte values included were from the last blood collection before the study started. These blood samples were acquired by venipuncture of the femoral vein without anesthesia. Blood for hematologic was collected into a tube with K2EDTA anticoagulant, and a blood smear was made. The whole-blood sample was kept at room temperature (15–25°C) if it was analyzed within 8 hours of collection. If the samples were not analyzed at this time, they were refrigerated (2–8°C) for analysis on an ADVIA 2120i/120 analyzer (Siemens). Blood for coagulation was collected into a tube with 3.2% sodium citrate and centrifuged at approximately 2700 relative centrifugal force for 10 minutes. The plasma was kept at room temperature if it was to be analyzed within 4 hours of receipt or frozen at −80°C for processing on the STA-R Evolution coagulation analyzer (Diagnostica Stago). Blood for serum biochemical
TABLE 1  Hematologic, coagulation, and serum biochemical analytes, analytic methods, and equivalency values for the common (major) analytes used in the cynomolgus monkey

| Hematologic analytes | Abbreviation | Units | Platform              | Equivalency value (%) |
|----------------------|--------------|-------|-----------------------|------------------------|
| Red blood cells      | RBC          | ×10^6/μL | ADVIA 2120/120        | 10                     |
| Hemoglobin           | HGB          | g/dL   | ADVIA 2120/120        | 10                     |
| Hematocrit           | HCT          | %      | ADVIA 2120/120        | 10                     |
| Mean corpuscular volume | MCV   | fl     | ADVIA 2120/120        | 10                     |
| Mean corpuscular hemoglobin | MCH     | pg     | ADVIA 2120/120        | 10                     |
| Mean corpuscular hemoglobin concentration | MCHC | g/dL   | ADVIA 2120/120        | 10                     |
| Red cell distribution width | RDW | % | ADVIA 2120/120 | 25                     |
| Platelets            | PLT          | ×10^3/μL | ADVIA 2120/120        | 25                     |
| Mean platelet volume | MPV          | fl     | ADVIA 2120/120        | 25                     |
| White blood cells    | WBC          | ×10^3/μL | ADVIA 2120/120        | 25                     |
| Neutrophil, absolute | NEUT         | ×10^3/μL | ADVIA 2120/120 or Microscopy | 25                     |
| Lymphocyte, absolute | LYM          | ×10^3/μL | ADVIA 2120/120 or Microscopy | 25                     |
| Monocyte, absolute   | MONO         | ×10^3/μL | ADVIA 2120/120 or Microscopy | 50                     |
| Basophil, absolute   | BASO         | ×10^3/μL | ADVIA 2120/120 or Microscopy | 50                     |
| Eosinophil, absolute | EO           | ×10^3/μL | ADVIA 2120/120 or Microscopy | 50                     |
| Large unstained cell, absolute | LUC | ×10^3/μL | ADVIA 2120/120 | 50                     |
| Neutrophil percent   | NEUT_P       | %      | ADVIA 2120/120 or Microscopy | 25                     |
| Lymphocyte, percent  | LYM_P        | %      | ADVIA 2120/120 or Microscopy | 25                     |
| Monocyte, percent    | MONO_P       | %      | ADVIA 2120/120 or Microscopy | 50                     |
| Basophil, percent    | BASO_P       | %      | ADVIA 2120/120 or Microscopy | 50                     |
| Eosinophil, percent  | EO_P         | %      | ADVIA 2120/120 or Microscopy | 50                     |
| Large unstained cell, percent | LUC_P | % | ADVIA 2120/120 | 50                     |
| Reticulocytes, absolute | RETIC | ×10^3/μL | Calculation | 25                     |

| Coagulation analytes | Abbreviation | Units | Platform              |
|----------------------|--------------|-------|-----------------------|
| Prothrombin time     | PT           | Seconds | Diagnostica Stago      | 15                     |
| Activated partial thromboplastin time | APTT | Seconds | Diagnostica Stago      | 15                     |
| Fibrinogen           | FIB          | mg/dL  | Diagnostica Stago      | 20                     |

| Serum biochemistry analytes | Abbreviation | Units | Methodology            |
|-----------------------------|--------------|-------|------------------------|
| Creatinine                  | CREA         | mg/dL | Kinetic Alkaline Picrate | 20                     |
| Urea nitrogen               | BUN          | mg/dL | Urease                 | 20                     |
| Aspartate aminotransferase  | AST          | U/L   | Modified IFCC          | 30                     |
| Alanine aminotransferase    | ALT          | U/L   | Modified IFCC          | 30                     |
| Gamma glutamyltransferase   | GGT          | U/L   | Modified IFCC          | 20                     |
| Alkaline phosphatase        | ALP          | U/L   | Modified IFCC          | 30                     |
| Total bilirubin             | TBIL         | mg/dL | Vanadate Oxidation     | 25                     |
| Bile acids                  | BILE_AC      | μmol/L| Thio NAD               |                        |
| Total protein               | TP           | g/dl  | Biuret                 | 15                     |
| Albumin                     | ALB          | g/dL  | BCG Dye Binding        | 15                     |
| Globulin                    | GLOB         | g/dL  | Calculated             | 15                     |
| Albumin/globulin ratio      | AG           |       | Calculated             |                        |
| Glucose                     | GLUC         | mg/dL | Hexokinase             | 20                     |
| Cholesterol                 | CHOL         | mg/dL | Enzymatic              | 20                     |
analysis was collected into a serum separator tube without anti-
coagulant and left standing at room temperature for 15 minutes
prior to centrifugation at approximately 2700 relative centrifugal
force for 10 minutes. Serum was refrigerated (2 to −8°C) for analy-
sis on the day of collection or frozen at −80°C for processing on
the ADVIA 1800 (Siemens) for most analytes, except for insulin
(ADVIA Centaur XP; Siemens), TSH (Immunite 1000, Siemens), and
T3 and T4 (UPLC-MS/MS, Agilent 1290 UPLC coupled to an AB
Sciex 6500 triple quadrupole mass spectrometer).

2.4 | Study groups

For body weight comparisons, animals were grouped semi-annually from
2 to <3 years of age and yearly from 3 to 10 years of age. Age groups
and the number of animals per age group are presented in Figure 1.
For hematologic, coagulation, and serum biochemical analyses, animals
were grouped into the following 4 age categories: 2 to <3 years (111
males; 55 females), 3 to <4 years (435 males; 392 females), 4 to <5 years
(165 males; 182 females), and 5 to 10 years (149 males; 175 females) of
age for each sex. Body weight and clinical pathology comparisons were
made by age group and between male and female animals.

2.5 | Statistical analysis

The differences in body weights among age groups and between sex
were analyzed using the two-sided t test. Endpoints were assigned
to each animal as the value corresponding to the measurement taken
closest to the study start. Each value was then log-transformed to
normalize the data.

The differences in mean clinical pathology analyte values
among age groups and between sex groups were analyzed using
equivalence testing (ie, two one-sided t tests, or TOST). For equiva-
lence testing, a subjective equivalence factor or threshold was
assigned to common or major (as defined in the blood collection
and analysis section) analytes, based on the expected biological
equivalence or variation range of individual analytes in Mauritian
cynomolgus monkeys (Table 1). The thresholds were specified
in percentages predetermined for a set of relevant analyte val-
ues and used to specify upper and lower equivalence bounds.
Differences that did not fall inside the equivalence bounds sug-
gested biologically meaningful differences. For each analyte, an
equivalence test was run on every pair of the different age groups
using the R package TOST. Each test generated an accompa-
nying graph that contained a 90% confidence interval. In addition,
a Kolmogorov–Smirnov test was run to test the difference in the distribution, and an empirical cumulative distribution function graph was created. Values that were not equivalent had a P-value of <0.05 and appeared to be outside of normal biological variation were included and discussed in the results.

The other hematologic analytes (mean cell volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red blood cell distribution width [RDW], mean platelet volume [MPV], and white blood cell percentages) and serum biochemistry analytes (bile acid, amylase, lipase, insulin, TSH, T3, T4, high sensitivity C-reactive protein, and magnesium) were considered minor analytes. There were fewer numbers of historical controls or background data for some of these analytes in our facility and some of these analytes are not as relevant in nonclinical toxicity studies, so we were unable to create equivalency values. Thus, we did not include these analytes in the statistical analysis regarding age- or sex-related differences.

The RI calculations were conducted separately for each sex. Outliers were first identified for each analyte in each age group and removed.22 For parameters with sample sizes of n > 120 after the outlier removal, the nonparametric method was used for the RI by calculating the 2.5th and 97.5th percentiles as the lower and upper reference limits, respectively. For 20 ≤ n ≤ 120, when a parameter was normally distributed (ie, when the normality test P-value was >0.3 on either the raw or logarithmic scale), the parametric method was used; when normality did not hold (ie, when the normality test P-value was ≤0.3), a robust method was used.23 RIs were calculated for all (major and minor) parameters as long as all sample sizes were n > 40. When the final sample size was less than 20, only descriptive statistics were reported. For a few parameters, only one animal was available per sex, and thus no statistics were included. The RI calculations were conducted using the R package RIs. The 95% coverage criterion was used for all the methods, and 90% confidence intervals were calculated for the lower and upper RIs. All the RI calculations were conducted by age group, which were divided into 2 to <3 years and 3 to <4 years of age.

3 | RESULTS

3.1 | Age- and sex-related changes in body weights

Body weights per age group are presented in Figure 2. A total of 1819 animals ranged in age from 2.2 to 9.6 years for males (n = 960 or 52.8%) and 2.2 to 12.4 years for females (n = 859 or 47.2%). The mean age was 4.0 years for males and 4.3 years for females. Body weights were statistically higher (P < 0.01) in males than in females in all age groups from 2 to 10 years (Figure 2), suggesting that sexual dimorphism in body weights begins as early as 2 years of age. The sexual dimorphism in body weights became prominent after 4 years of age. Body weights reached peak values at 7 to 8 years of age in both males (7.9 kg) and females (5.5 kg), with a 2.4 kg difference in the peak mean body weights.

3.2 | Sex-related changes in hematologic, coagulation, and serum biochemistry analytes

Sex-related mean hematologic differences were limited to higher reticulocyte (RETIC) counts in females than males in some age groups. As shown in Figure 3, the RETIC index trended higher in females than males in the 3 to 10 years of age groups. In females, the group mean RETIC counts were statistically higher (#) in animals 5 to 10 years of age than in those 2 to <4 years of age. In males, the group mean RETIC counts were statistically higher (*) in animals 5 to 10 years of age than in those 2 to <4 years of age.
creatinine levels (from 2 to 10 years, 2 to 10 years, and 5 to 10 years of age, respectively) than females of the same age groups. Females had statistically higher triglyceride levels than males from 4 to 10 years of age, as shown in Figure 8. As shown in Figure 9, the cholesterol concentration trended higher in females than males, and females had statistically higher cholesterol levels than males from 5 to 10 years of age.

Sex-related changes occurred in mean hematologic and serum biochemistry analyte values. Higher ALP and GGT activities were seen in males compared with females from 2 to 10 years of age. Higher RETIC values were seen in females compared with males from 3 to 10 years of age, higher triglyceride concentrations were seen in females than males from 4 to 10 years of age, and higher creatinine concentrations were seen in males than females and higher cholesterol concentrations were seen in females compared with males from 5 to 10 years of age.

### 3.3 | Age-related changes in hematologic, coagulation, and serum biochemistry analytes

Age-related mean hematologic differences were observed in some age groups and included higher RETIC counts and lower lymphocyte counts in both sexes. As shown in Figure 3, RETIC values generally increased with age and were statistically higher in males 4 to 10 years of age and females 5 to 10 years of age (vs males and females 2 to <4 years of age). As shown in Figure 4, lymphocyte counts were comparable from 2 to 5 years of age but were statistically lower in males and females 5 to 10 years of age (vs males 4 to <5 years of age and females 2 to <3 years of age, respectively). There were no age-related changes in mean coagulation analytes.

Age-related differences in mean serum biochemistry analytes were observed in some age groups. These included lower ALP and GGT activities, and phosphorus and globulin concentrations in both sexes, as well as higher creatinine concentrations in males and triglyceride concentrations in females. As shown in Figures 5 and 6, ALP and GGT activities generally decreased with age; there were statistically significant decreases in ALP activity in males and females 3 to 10 years of age.
10 years of age (vs males and females 2 to <3 years of age) and statistically significant decreases in GGT activity in males 4 to 10 years of age and females 3 to 10 years of age (vs males 2 to <4 years of age and females 2 to <3 years of age, respectively). As shown in Figure 7, statistically significant age-related increases in creatinine concentrations were seen in males 4 to 10 years of age (vs males 2 to <4 years of age). Statistically significant increases in triglyceride concentrations were seen in females 5 to 10 years of age (vs females 2 to <3 years of age), as shown in Figure 8. There were statistically significant decreases in phosphorus concentrations in males 5 to 10 years of age and females 4 to 10 years of age (vs males and females 2 to <4 years of age), as shown in Figure 10. As shown in Figure 11, statistically significant lower globulin concentrations were found in males and females 2 to <3 years of age (vs. males and females 3 to 10 years of age).

Age-related changes were seen in mean hematologic and serum biochemical analytes. Globulin concentrations were lower in both sexes 2 to <3 years of age compared to older animals. ALP activity was lower in both sexes 3 to 10 years of age compared with younger animals. GGT activity was lower in males 4 to 10 years of age and females 3 to 10 years of age compared with younger males and females. Creatinine concentrations were higher in males 4 to 10 years of age compared with younger males. Phosphorus concentrations were lower in males 5 to 10 years of age and females 4 to 10 years of age compared with younger males and females. RETIC values were higher in males 4 to 10 years of age and females 5 to 10 years of age compared with younger males and females. Triglyceride values were higher in females 5 to 10 years of age compared with younger females. Finally, lymphocyte numbers were lower in both sexes at 5 to 10 years of age compared with younger animals.

3.4 | Hematologic, coagulation, and serum biochemical reference intervals.

RIs for clinical pathology analytes for the 2 to <3 years of age and 3 to <4 years of age groups were provided since these are the most typical age groups used in nonclinical safety studies (Tables 2 to 7).

**FIGURE 8** The sex- and age-related differences in serum triglyceride concentration. The group mean triglyceride concentration was statistically higher (#) in females than males in the 4 to 10 years of age groups. In females, the group mean triglyceride concentration was statistically higher (*) in animals 5 to 10 years of age than in those 2 to <3 years of age.

**FIGURE 9** The sex-related differences in serum cholesterol concentration. The group mean cholesterol concentration was statistically higher (#) in females than males in the 5 to 10 years of age group.

**FIGURE 10** The age-related differences in serum phosphorus concentration. In males, the group mean serum phosphorus concentration was statistically lower (*) in animals 5 to 10 years of age than in those 2 to <4 years of age. In females, the group mean serum phosphorus concentration was statistically lower (*) in animals 4 to 10 years of age than in those 2 to <4 years of age.

**FIGURE 11** The age-related differences in serum globulin concentration. The group mean globulin concentration was statistically lower (*) in males and females 2 to <3 years of age than in those 3 to 10 years of age.
### TABLE 2  Reference intervals of hematologic analytes for 2- to <3-year-old cynomolgus monkeys

| Analyte | Sex | Initial N | Final N | Mean | SD  | Median | Min  | Max  | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|---------|-----|-----------|---------|------|-----|--------|------|------|-----------------|-----------------|-------------|-------------|
| RBC     | M   | 111      | 110     | 6.82 | 0.43| 6.85   | 5.76 | 7.9  | (5.86, 6.09)    | (7.55, 7.78)    |             |             |
| RBC     | F   | 54       | 52      | 6.58 | 0.47| 6.63   | 5.52 | 7.53 | (5.47, 5.84)    | (7.31, 7.68)    |             |             |
| HGB     | M   | 111      | 109     | 13.44| 0.62| 13.4   | 11.9 | 14.9 | (12.06, 13.39)  | (14.49, 14.83)  |             |             |
| HGB     | F   | 54       | 53      | 13.00| 0.86| 13.0   | 11.4 | 14.7 | (10.98, 11.65)  | (14.35, 15.01)  |             |             |
| HCT     | M   | 111      | 109     | 46.29| 1.96| 46.4   | 41.6 | 50.1 | (41.91, 42.96)  | (49.78, 50.77)  |             |             |
| HCT     | F   | 54       | 52      | 45.37| 2.92| 45.05  | 39.5 | 51.6 | (38.51, 40.78)  | (49.95, 52.23)  |             |             |
| MCV     | M   | 111      | 108     | 67.85| 2.97| 67.9   | 60.7 | 74.3 | (61.22, 62.83)  | (72.87, 74.48)  |             |             |
| MCV     | F   | 54       | 52      | 68.18| 2.24| 68.3   | 63.7 | 72.9 | (62.90, 64.69)  | (71.67, 73.47)  |             |             |
| MCH     | M   | 111      | 107     | 19.73| 0.93| 19.8   | 17.5 | 22   | (17.66, 21.16)  | (21.30, 21.80)  |             |             |
| MCH     | F   | 54       | 52      | 19.49| 0.81| 19.5   | 18   | 21.4 | (17.60, 21.83)  | (20.76, 21.39)  |             |             |
| MCHC    | M   | 111      | 106     | 29.01| 0.86| 28.9   | 27.3 | 31.2 | (27.00, 27.45)  | (30.45, 30.96)  |             |             |
| MCHC    | F   | 54       | 52      | 28.56| 0.82| 28.65  | 26.8 | 30.3 | (26.62, 27.27)  | (29.86, 30.50)  |             |             |
| RDW     | M   | 111      | 110     | 14.28| 0.97| 14.1   | 12   | 16.5 | (12.01, 12.50)  | (15.96, 16.54)  |             |             |
| RDW     | F   | 54       | 54      | 14.18| 0.98| 14.25  | 12.5 | 16.4 | (11.88, 12.63)  | (15.73, 16.48)  |             |             |
| PLT     | M   | 111      | 104     | 368.54| 52.72| 364   | 235  | 501  | (250.68, 279.74)| (457.33, 486.40)|             |             |
| PLT     | F   | 54       | 50      | 393.84| 62.11| 391.5  | 274  | 548  | (247.41, 296.79)| (490.89, 540.27)|             |             |
| MPV     | M   | 111      | 109     | 8.73 | 1.09| 8.5    | 7    | 11.4 | (6.09, 6.64)    | (10.48, 11.20)  |             |             |
| MPV     | F   | 54       | 51      | 8.69 | 0.92| 8.6    | 7    | 10.7 | (6.35, 6.99)    | (10.02, 10.85)  |             |             |
| WBC     | M   | 111      | 105     | 11.48| 2.86| 11.5   | 6.4  | 19.2 | (4.91, 6.39)    | (16.29, 17.91)  |             |             |
| WBC     | F   | 54       | 49      | 11.05| 2.29| 10.8   | 6.1  | 15.9 | (5.64, 7.48)    | (14.61, 16.45)  |             |             |
| NEUT    | M   | 111      | 106     | 4.79 | 2.49| 4.32   | 1.99 | 11.29| (2.11, 3.03)    | (8.71, 9.63)    |             |             |
| NEUT    | F   | 54       | 49      | 4.43 | 1.51| 4.19   | 2.36 | 8.11 | (0.55, 1.79)    | (6.61, 8.13)    |             |             |
| LYM     | M   | 111      | 107     | 5.87 | 1.68| 5.82   | 2.21 | 10.14| (2.01, 3.25)    | (8.23, 9.47)    |             |             |
| LYM     | F   | 54       | 52      | 5.74 | 1.59| 5.59   | 2.81 | 9.28 | (0.09, 0.15)    | (0.55, 0.63)    |             |             |
| MONO    | M   | 111      | 106     | 0.37 | 0.12| 0.35   | 0.15 | 0.67 | (0.04, 0.14)    | (0.62, 0.75)    |             |             |
| MONO    | F   | 54       | 52      | 0.40 | 0.14| 0.39   | 0.16 | 0.74 | (0.00, 0.01)    | (0.06, 0.06)    |             |             |
| BASO    | M   | 111      | 106     | 0.03 | 0.01| 0.03   | 0.01 | 0.06 | (0.00, 0.01)    | (0.05, 0.06)    |             |             |
| BASO    | F   | 54       | 50      | 0.03 | 0.01| 0.03   | 0.01 | 0.06 | (0.00, 0.01)    | (0.05, 0.06)    |             |             |
| EO      | M   | 111      | 104     | 0.18 | 0.14| 0.15   | 0.01 | 0.56 | (0.39, 0.50)    | (BFA, BFA)      |             |             |

(Continues)
| Analyte | Sex | Initial N | Final N | Mean  | SD     | Median | Min   | Max   |
|---------|-----|-----------|---------|-------|--------|--------|-------|-------|
| EO      | F   | 54        | 50      | 0.17  | 0.13   | 0.13   | 0.02  | 0.46  |
| LUC     | M   | 111       | 105     | 0.10  | 0.05   | 0.1    | 0.02  | 0.21  |
| LUC     | F   | 54        | 51      | 0.10  | 0.03   | 0.09   | 0.04  | 0.18  |
| NEUT_P  | M   | 111       | 111     | 41.12 | 15.77  | 40.85  | 13.97 | 77.83 |
| NEUT_P  | F   | 54        | 52      | 41.40 | 11.81  | 40.92  | 18.09 | 68.84 |
| LYM_P   | M   | 111       | 111     | 52.22 | 14.36  | 53.03  | 20.33 | 78.59 |
| LYM_P   | F   | 54        | 52      | 51.79 | 10.64  | 52.12  | 25.79 | 71.23 |
| MONO_P  | M   | 111       | 109     | 3.27  | 0.92   | 3.10   | 1.45  | 5.47  |
| MONO_P  | F   | 54        | 52      | 3.39  | 1.01   | 3.40   | 1.59  | 5.36  |
| BASO_P  | M   | 111       | 109     | 0.27  | 0.08   | 0.28   | 0.08  | 0.47  |
| BASO_P  | F   | 54        | 51      | 0.28  | 0.08   | 0.28   | 0.11  | 0.48  |
| EO_P    | M   | 111       | 104     | 1.62  | 1.31   | 1.21   | 0.07  | 5.41  |
| EO_P    | F   | 54        | 52      | 1.61  | 1.21   | 1.24   | 0.14  | 4.37  |
| LUC_P   | M   | 111       | 105     | 0.89  | 0.37   | 0.89   | 0.20  | 1.71  |
| LUC_P   | F   | 54        | 52      | 0.87  | 0.25   | 0.85   | 0.30  | 1.30  |
| RETIC   | M   | 111       | 105     | 31.07 | 12.64  | 29     | 12    | 67    |
| RETIC   | F   | 54        | 48      | 33.42 | 12.43  | 32     | 15    | 65    |

**Descriptive statistics**

**Reference interval (RI) and confidence interval (CI)**

| LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|------------------|------------------|---------------|---------------|
| 0.46             | 0.42             | 0.42          | 0.42          |
| 0.01             | 0.19             | 0.01          | 0.19          |
| 0.02             | 0.16             | (0.01, 0.03)  | (0.15, 0.18)  |
| 8.96             | 71.81            | (5.26, 12.34) | (7.47, 76.12) |
| 18.26            | 64.54            | (13.66, 22.86)| (59.94, 69.14)|
| 24.03            | 81.34            | (20.11, 27.75)| (78.02, 84.77)|
| 30.38            | 73.62            | (26.58, 35.20)| (69.26, 77.24)|
| 1.35             | 5.07             | (1.11, 1.55)  | (4.81, 5.39)  |
| 1.34             | 5.43             | (0.99, 1.68)  | (5.07, 5.78)  |
| 0.11             | 0.44             | (0.09, 0.14)  | (0.42, 0.47)  |
| 0.12             | 0.44             | (0.09, 0.15)  | (0.41, 0.47)  |
| 0.15             | 1.62             | (0.06, 0.24)  | (1.52, 1.72)  |
| 0.37             | 1.36             | (0.27, 0.47)  | (1.26, 1.46)  |
| 4.49             | 55.57            | (1.27, 7.79)  | (51.53, 60.05)|
| 6.53             | 57.46            | (1.96, 12.52) | (51.40, 63.45)|

**Note:** See Table 1 for abbreviations of individual analytes.

**Abbreviations:** BFA, beyond the feasibility of analysis; CI, confidence interval; F, female; LRI, lower reference limit; M, male; N, animal number/sample size; RI, reference interval; SD, standard deviation; URI, upper reference limit.
| Analyte | Sex | Initial N | Final N | Mean  | SD    | Median | Min   | Max   | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|---------|-----|-----------|---------|-------|-------|--------|-------|-------|-----------------|-----------------|--------------|--------------|
| RBC     | M   | 435       | 429     | 6.77  | 0.41  | 6.75   | 5.65  | 7.83  | (5.98, 6.10)    | (5.74, 7.75)    |              |              |
| RBC     | F   | 392       | 388     | 6.47  | 0.49  | 6.47   | 5.13  | 7.83  | (5.35, 5.64)    | (5.31, 7.55)    |              |              |
| HGB     | M   | 435       | 434     | 13.63 | 0.74  | 13.6   | 11.6  | 15.7  | (12.10, 12.40)  | (12.00, 15.20)  |              |              |
| HGB     | F   | 392       | 388     | 12.88 | 0.78  | 12.9   | 10.7  | 15.1  | (11.10, 14.40)  | (14.10, 14.40)  |              |              |
| HCT     | M   | 435       | 434     | 46.4  | 2.7   | 46.3   | 39.7  | 53.3  | (41.0, 41.9)    | (51.1, 52.5)    |              |              |
| HCT     | F   | 392       | 388     | 44.8  | 2.9   | 44.8   | 36.5  | 52.9  | (38.1, 39.8)    | (49.9, 51.2)    |              |              |
| MCV     | M   | 435       | 432     | 68.6  | 5.3   | 68.5   | 58.5  | 78.8  | (61.0, 62.4)    | (74.8, 76.7)    |              |              |
| MCV     | F   | 392       | 390     | 69.1  | 3.8   | 69.1   | 58.8  | 79.4  | (59.4, 62.5)    | (76.3, 78.1)    |              |              |
| MCH     | M   | 435       | 432     | 20.2  | 1.1   | 20.1   | 17.1  | 23    | (17.9, 18.2)    | (22.0, 22.6)    |              |              |
| MCH     | F   | 392       | 387     | 19.9  | 1.1   | 19.9   | 17.1  | 22.8  | (17.5, 18.1)    | (21.8, 22.4)    |              |              |
| MCHC    | M   | 435       | 433     | 29.4  | 1.2   | 29.4   | 26.2  | 32.7  | (26.8, 27.4)    | (31.5, 32.0)    |              |              |
| MCHC    | F   | 392       | 388     | 28.8  | 1.1   | 28.8   | 25.9  | 31.9  | (26.3, 26.8)    | (30.8, 31.4)    |              |              |
| RDW     | M   | 435       | 432     | 14.29 | 0.95  | 14.25  | 12    | 16.7  | (12.20, 12.50)  | (15.90, 16.30)  |              |              |
| RDW     | F   | 392       | 388     | 14.11 | 0.99  | 14.1   | 11.2  | 16.7  | (12.30, 12.70)  | (16.00, 16.40)  |              |              |
| PLT     | M   | 434       | 431     | 383   | 71    | 379    | 200   | 579   | (237, 258)      | (508, 534)      |              |              |
| PLT     | F   | 389       | 385     | 409   | 79    | 400    | 232   | 630   | (245, 269)      | (559, 601)      |              |              |
| MPV     | M   | 434       | 427     | 8.7   | 1.1   | 8.5    | 6     | 11.9  | (6.7, 7.0)      | (11.0, 11.5)    |              |              |
| MPV     | F   | 389       | 382     | 8.8   | 1.2   | 8.8    | 5.9   | 12.1  | (6.4, 7.2)      | (11.1, 11.9)    |              |              |
| WBC     | M   | 427       | 420     | 12.3  | 3.2   | 12.1   | 5.5   | 21.8  | (6.7, 7.4)      | (18.5, 21.1)    |              |              |
| WBC     | F   | 392       | 387     | 12.3  | 3.4   | 11.8   | 3.8   | 22    | (6.0, 7.3)      | (18.6, 20.7)    |              |              |
| NEUT    | M   | 435       | 419     | 5.1   | 2.5   | 4.5    | 0.9   | 12.6  | (1.3, 1.8)      | (10.2, 12.0)    |              |              |
| NEUT    | F   | 392       | 385     | 5.8   | 3     | 5.1    | 1.1   | 14.4  | (1.3, 2.0)      | (12.2, 13.5)    |              |              |
| LYM     | M   | 435       | 430     | 6.1   | 1.8   | 5.8    | 1.9   | 11.4  | (2.6, 3.1)      | (9.6, 10.7)     |              |              |
| LYM     | F   | 392       | 391     | 5.6   | 1.6   | 5.3    | 2.2   | 10    | (2.4, 3.2)      | (9.0, 9.5)      |              |              |
| MONO    | M   | 435       | 419     | 0.4   | 0.14  | 0.38   | 0.12  | 0.82  | (0.17, 0.20)    | (0.68, 0.78)    |              |              |
| MONO    | F   | 392       | 381     | 0.42  | 0.15  | 0.41   | 0.1   | 0.88  | (0.16, 0.20)    | (0.72, 0.79)    |              |              |
| BASO    | M   | 435       | 424     | 0.035 | 0.016 | 0.03   | 0     | 0.08  | (0.01, 0.01)    | (0.07, 0.08)    |              |              |
| BASO    | F   | 392       | 379     | 0.035 | 0.014 | 0.03   | 0.01  | 0.07  | (0.01, 0.01)    | (0.06, 0.07)    |              |              |
| EO      | M   | 435       | 409     | 0.22  | 0.18  | 0.17   | 0.01  | 0.81  | (0.02, 0.03)    | (0.62, 0.78)    |              |              |
| EO      | F   | 392       | 369     | 0.3   | 0.2   | 0.2    | 0     | 0.9   | (0.0, 0.0)      | (0.8, 0.9)      |              |              |
| Analyte | Sex | Initial N | Final N | Mean  | SD   | Median | Min  | Max  | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|---------|-----|-----------|---------|-------|------|--------|------|------|-----------------|-----------------|---------------|---------------|
| LUC     | M   | 435       | 424     | 0.105 | 0.043| 0.1    | 0.02 | 0.23 | 0.04            | 0.21            | (0.03, 0.04)   | (0.20, 0.22)   |
| LUC     | F   | 392       | 384     | 0.108 | 0.045| 0.1    | 0.02 | 0.23 | 0.04            | 0.21            | (0.04, 0.05)   | (0.19, 0.22)   |
| NEUT_P  | M   | 435       | 419     | 55    | 27   | 48     | 9    | 135  | 17              | 116             | (14, 19)       | (110, 129)     |
| NEUT_P  | F   | 392       | 385     | 62    | 32   | 55     | 12   | 155  | 19              | 138             | (14, 21)       | (131, 145)     |
| LYM_P   | M   | 435       | 430     | 65    | 20   | 63     | 20   | 123  | 31              | 109             | (28, 33)       | (103, 115)     |
| LYM_P   | F   | 392       | 391     | 60    | 17   | 57     | 23   | 107  | 32              | 100             | (26, 34)       | (97, 102)      |
| MONO_P  | M   | 435       | 419     | 4.4   | 1.5  | 4.1    | 1.3  | 8.8  | 1.9             | 8               | (1.8, 2.1)     | (7.3, 8.4)     |
| MONO_P  | F   | 392       | 381     | 4.5   | 1.6  | 4.4    | 1.1  | 9.5  | 1.9             | 8               | (1.7, 2.1)     | (7.7, 8.5)     |
| BASO_P  | M   | 435       | 424     | 0.38  | 0.17 | 0.32   | 0    | 0.86 | 0.11            | 0.75            | (0.11, 0.11)   | (0.75, 0.86)   |
| BASO_P  | F   | 392       | 379     | 0.37  | 0.15 | 0.32   | 0.11 | 0.75 | 0.11            | 0.75            | (0.11, 0.11)   | (0.65, 0.75)   |
| EO_P    | M   | 435       | 409     | 2.4   | 1.9  | 1.8    | 0.1  | 8.7  | 0.2             | 7.2             | (0.2, 0.3)     | (6.7, 8.4)     |
| EO_P    | F   | 392       | 369     | 2.9   | 2.2  | 2.3    | 0.2  | 9.8  | 0.5             | 8.7             | (0.3, 0.5)     | (8.2, 9.3)     |
| LUC_P   | M   | 435       | 424     | 1.13  | 0.46 | 1.08   | 0.22 | 2.47 | 0.43            | 2.26            | (0.32, 0.43)   | (2.15, 2.37)   |
| LUC_P   | F   | 392       | 384     | 1.16  | 0.49 | 1.08   | 0.22 | 2.47 | 0.43            | 2.26            | (0.43, 0.54)   | (2.04, 2.37)   |
| RETIC   | M   | 435       | 423     | 35    | 14   | 33     | 7    | 75   | 13              | 66              | (13, 13)       | (64, 73)       |
| RETIC   | F   | 392       | 380     | 44    | 18   | 42     | 12   | 98   | 14              | 86              | (13, 18)       | (79, 89)       |

Note: See Table 1 for abbreviations of individual analytes.

Abbreviations: CI, confidence interval; F, female; LRI, lower reference limit; M, male; N, animal number/sample size; RI, reference interval; SD, standard deviation; URI, upper reference limit.
### TABLE 4  Reference intervals of coagulation analytes for 2- to <3-year-old cynomolgus monkeys

| Analyte | Sex | Initial N | Final N | Mean | SD | Median | Minimal | Maximal | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|---------|-----|-----------|---------|------|----|--------|---------|---------|----------------|----------------|-------------|-------------|
| PT      | M   | 110       | 109     | 12.71| 0.57| 12.7   | 11.4    | 14      | 11.6           | 13.83           | (11.44, 11.75) | (13.68, 13.99) |
| PT      | F   | 53        | 52      | 12.39| 0.51| 12.4   | 11.4    | 13.5    | 11.39          | 13.39           | (11.19, 11.59) | (13.19, 13.59) |
| APTT    | M   | 110       | 108     | 22.29| 1.23| 22.2   | 19.3    | 24.9    | 19.76          | 24.67           | (19.45, 20.07) | (24.32, 25.06) |
| APTT    | F   | 53        | 52      | 21.73| 1.05| 21.75  | 19.4    | 23.8    | 19.67          | 23.78           | (19.26, 20.08) | (23.37, 24.19) |
| FIB     | M   | 110       | 104     | 189.14| 23.64| 186    | 130     | 245     | 142.81         | 235.48          | (136.29, 149.32) | (228.97, 242.00) |
| FIB     | F   | 53        | 51      | 176.08| 17.95| 174    | 140     | 214     | 140.9          | 211.25          | (133.84, 147.97) | (204.19, 218.32) |

Note: See Table 1 for abbreviations of individual analytes.

Abbreviations: CI, confidence interval; F, female; LRI, lower reference limit; M, male; N, animal number/sample size; RI, reference interval; SD, standard deviation; URI, upper reference limit.

### TABLE 5  Reference intervals of coagulation analytes for 3- to <4-year-old cynomolgus monkeys

| Analyte | Sex | Initial N | Final N | Mean | SD | Median | Minimal | Maximal | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|---------|-----|-----------|---------|------|----|--------|---------|---------|----------------|----------------|-------------|-------------|
| PT      | M   | 389       | 385     | 12.93| 0.59| 13     | 11.3    | 14.6    | 11.77          | 14.2           | (11.6, 11.9) | (13.9, 14.4) |
| PT      | F   | 377       | 369     | 12.71| 0.54| 12.7   | 11.3    | 14.2    | 11.7           | 13.9           | (11.6, 11.8) | (13.70, 14.0) |
| APTT    | M   | 390       | 383     | 22.4 | 1.4 | 22.2   | 19      | 26.2    | 20.2           | 25.5           | (19.7, 20.4) | (25.2, 26.3) |
| APTT    | F   | 377       | 366     | 22.2 | 1.3 | 22.2   | 19.2    | 25.8    | 19.9           | 25.1           | (19.7, 20.1) | (24.6, 25.3) |
| FIB     | M   | 390       | 383     | 195  | 26  | 193    | 129     | 265     | 151            | 247            | (136, 154)  | (241, 256)  |
| FIB     | F   | 377       | 367     | 182  | 26  | 181    | 115     | 249     | 129            | 236            | (125, 130)  | (232, 241)  |

Note: See Table 1 for abbreviations of individual analytes.

Abbreviations: CI, confidence interval; F, female; LRI, lower reference limit; M, male; N, animal number/sample size; RI, reference interval; SD, standard deviation; URI, upper reference limit.
| Analyte | Sex | Initial N | Final N | Mean  | SD   | Median | Min  | Max  |
|---------|-----|-----------|---------|-------|------|--------|------|------|
| CREA    | M   | 100       | 100     | 0.68  | 0.10 | 0.7    | 0.5  | 0.9  |
| CREA    | F   | 53        | 51      | 0.66  | 0.09 | 0.7    | 0.5  | 0.8  |
| BUN     | M   | 100       | 94      | 18.17 | 2.63 | 18     | 12   | 25   |
| BUN     | F   | 53        | 51      | 17.24 | 2.31 | 17     | 12   | 22   |
| AST     | M   | 100       | 98      | 44.49 | 8.54 | 44     | 26   | 64   |
| AST     | F   | 53        | 49      | 40.27 | 4.85 | 40     | 31   | 52   |
| ALT     | M   | 100       | 97      | 48.24 | 13.02| 47     | 24   | 78   |
| ALT     | F   | 53        | 50      | 46.70 | 12.68| 45     | 29   | 73   |
| GGT     | M   | 110       | 109     | 193.67| 39.70| 193    | 97   | 284  |
| GGT     | F   | 53        | 53      | 127.64| 26.16| 130    | 82   | 174  |
| ALP     | M   | 100       | 98      | 892.66| 226.77| 851.5  | 493  | 1436 |
| ALP     | F   | 53        | 53      | 677.09| 152.88| 662    | 352  | 987  |
| TBIL    | M   | 100       | 96      | 0.20  | 0.07 | 0.2    | 0.1  | 0.3  |
| TBIL    | F   | 53        | 50      | 0.23  | 0.09 | 0.2    | 0.1  | 0.4  |
| BILE_AC | M   | 13        | 12      | 1.77  | 0.81 | 1.7    | 0.7  | 3.1  |
| BILE_AC | F   | 5         | 4       | 2.70  | 2.40 | 1.9    | 0.8  | 6.2  |
| TP      | M   | 100       | 99      | 7.57  | 0.42 | 7.5    | 6.7  | 8.4  |
| TP      | F   | 53        | 53      | 7.42  | 0.48 | 7.4    | 6.6  | 8.5  |
| ALB     | M   | 100       | 98      | 4.66  | 0.21 | 4.7    | 4.2  | 5.1  |
| ALB     | F   | 53        | 52      | 4.55  | 0.19 | 4.5    | 4.2  | 4.9  |
| GLOB    | M   | 100       | 100     | 2.91  | 0.44 | 3      | 2    | 3.9  |
| GLOB    | F   | 53        | 52      | 2.84  | 0.40 | 2.85   | 2.2  | 3.7  |
| AG      | M   | 100       | 98      | 1.62  | 0.28 | 1.6    | 1.2  | 2.3  |
| AG      | F   | 53        | 51      | 1.61  | 0.23 | 1.6    | 1.1  | 2.1  |
| GLUC    | M   | 100       | 93      | 68.67 | 8.17 | 69     | 51   | 91   |
| GLUC    | F   | 53        | 51      | 77.59 | 12.09| 78     | 54   | 102  |
| Analyte | Sex | Initial N | Final N | Mean  | SD    | Median | Min  | Max  |
|---------|-----|-----------|---------|-------|-------|--------|------|------|
| CHOL    | M   | 100       | 99      | 102.67| 19.80 | 99     | 57   | 151  |
|         | F   | 53        | 50      | 113.02| 13.01 | 112.5  | 84   | 141  |
| TRIG    | M   | 100       | 93      | 45.04 | 9.47  | 44     | 26   | 69   |
|         | F   | 53        | 51      | 47.82 | 11.32 | 48     | 26   | 71   |
| PHOS    | M   | 100       | 97      | 6.46  | 0.86  | 6.5    | 4.4  | 8.6  |
|         | F   | 53        | 51      | 6.23  | 0.82  | 6.1    | 4.5  | 7.8  |
| CA      | M   | 100       | 96      | 10.16 | 0.29  | 10.2   | 9.5  | 10.8 |
|         | F   | 53        | 53      | 10.04 | 0.37  | 10     | 9.3  | 10.7 |
| CL      | M   | 110       | 106     | 107.53| 1.64  | 107    | 104  | 111  |
|         | F   | 53        | 52      | 108.40| 1.75  | 108    | 105  | 112  |
| NA      | M   | 110       | 108     | 148.14| 2.06  | 148    | 143  | 153  |
|         | F   | 53        | 52      | 146.81| 1.77  | 146    | 143  | 150  |
| K       | M   | 110       | 103     | 4.23  | 0.32  | 4.2    | 3.5  | 5    |
|         | F   | 53        | 48      | 4.26  | 0.29  | 4.25   | 3.7  | 4.9  |
| AMYL    | M   | 14        | 13      | 325.15| 99.00 | 301    | 206  | 520  |
|         | F   | 1         | 1       | NA    | NA    | NA     | NA   | NA   |
| LIPASE  | M   | 14        | 14      | 17.21 | 4.10  | 15     | 13   | 24   |
|         | F   | 1         | 1       | NA    | NA    | NA     | NA   | NA   |
| CRPHS   | M   | 15        | 14      | 1.20  | 0.52  | 1.15   | 0.3  | 1.9  |
|         | F   | 10        | 9       | 1.07  | 0.77  | 0.8    | 0.2  | 2.6  |

| Reference interval (RI) and confidence interval (CI) | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|-----------------------------------------------------|------------------|------------------|---------------|---------------|
| CHOL                                                | 141.77           | 141.77           | (56.02, 66.25) | (135.70, 149.02) |
| TRIG                                                | 63.32            | 63.32            | (22.57, 27.59) | (60.36, 66.53) |
| PHOS                                                | 7.94             | 7.94             | (4.23, 4.89)   | (7.65, 8.26)   |
| CA                                                  | 10.75            | 10.75            | (9.47, 9.67)   | (10.68, 10.83) |
| CL                                                  | 110.9            | 110.9            | (103.52, 104.36) | (110.52, 111.47) |
| NA                                                  | 112.07           | 112.07           | (104.03, 105.37) | (111.46, 112.86) |
| K                                                   | 4.86             | 4.86             | (3.48, 3.66)   | (4.76, 4.95)   |
| AMYL                                                | NA               | NA               | (NA, NA)       | (NA, NA)       |
| LIPASE                                              | NA               | NA               | (NA, NA)       | (NA, NA)       |
| CRPHS                                               | NA               | NA               | (NA, NA)       | (NA, NA)       |

Note: See Table 1 for abbreviations of individual analytes.
Abbreviations: CI, confidence interval; F, female; LRI, lower reference limit; M, male; N, animal number/sample size; NA, not applicable; RI, reference interval; SD, standard deviation; URI, upper reference limit.
TABLE 7 Reference intervals of serum biochemistry and other serum analytes for 3- to <4-year-old cynomolgus monkeys

| Analyte | Sex | Initial N | Final N | Mean | SD | Median | Min | Max | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|---------|-----|-----------|---------|------|----|--------|-----|-----|-----------------|-----------------|--------------|--------------|
| CREA | M  | 322 | 319 | 0.79 | 0.13 | 0.8 | 0.5 | 1.1 | 0.6 | 1 | (0.50, 0.60) | (1.00, 1.10) |
| CREA | F  | 303 | 300 | 0.75 | 0.11 | 0.8 | 0.5 | 1 | 0.6 | 1 | (0.50, 0.60) | (0.90, 1.00) |
| BUN | M  | 322 | 317 | 18.4 | 2.5 | 18 | 12 | 25 | 14 | 23.1 | (13.0, 14.0) | (23.0, 25.0) |
| BUN | F  | 303 | 291 | 18.3 | 2.5 | 18 | 11 | 25 | 14 | 23.7 | (13.0, 15.0) | (23.0, 24.0) |
| AST | M  | 322 | 321 | 39.7 | 8 | 39 | 20 | 61 | 26 | 57 | (25.0, 28.0) | (55.0, 59.0) |
| AST | F  | 303 | 295 | 35.6 | 6.7 | 35 | 20 | 54 | 23.4 | 49.6 | (21.0, 25.0) | (48.0, 54.0) |
| ALT | M  | 322 | 319 | 7.8 | 0.46 | 7.8 | 6.6 | 8.9 | 21 | 68 | (19.22) | (64.75) |
| ALT | F  | 303 | 301 | 7.8 | 0.46 | 7.8 | 6.6 | 8.9 | 22 | 86 | (19.25) | (80.97) |
| GGT | M  | 403 | 396 | 93 | 17 | 91 | 53 | 143 | 64 | 130 | (61, 67) | (128, 134) |
| GGT | F  | 379 | 373 | 93 | 17 | 91 | 53 | 143 | 467 | 1310 | (420, 490) | (1267, 1375) |
| ALP | M  | 322 | 319 | 824 | 215 | 797 | 396 | 1393 | 106 | 263 | (100, 117) | (253, 273) |
| ALP | F  | 303 | 301 | 426 | 113 | 797 | 396 | 1393 | 64 | 130 | (61, 67) | (128, 134) |
| TBIL | M  | 322 | 319 | 3.12 | 0.41 | 3.2 | 2.1 | 4 | 1.0 | 3.9 | (2.10, 2.60) | (3.80, 3.90) |
| TBIL | F  | 303 | 301 | 3.37 | 0.41 | 3.4 | 2.3 | 4.5 | 2.4 | 4.1 | (2.40, 2.60) | (4.00, 4.30) |
| BILE_Ac | M  | 93 | 84 | 2.5 | 1.5 | 2.4 | 1.2 | 3.9 | 5.4 | BFA | (4.9, 5.9) |
| BILE_Ac | F  | 87 | 79 | 2.8 | 1.5 | 2.5 | 1.8 | 4.9 | 5.7 | BFA | (5.2, 6.3) |
| TP | M  | 322 | 319 | 7.8 | 0.46 | 7.8 | 6.6 | 8.9 | 6.8 | 8.6 | (6.70, 7.00) | (8.60, 8.70) |
| TP | F  | 303 | 301 | 7.8 | 0.46 | 7.8 | 6.6 | 8.9 | 6.9 | 8.6 | (6.70, 7.00) | (8.50, 8.80) |
| ALB | M  | 322 | 314 | 4.65 | 0.21 | 4.65 | 4.1 | 5.2 | 4.2 | 5.1 | (4.20, 4.30) | (5.00, 5.20) |
| ALB | F  | 303 | 302 | 4.47 | 0.22 | 4.5 | 4 | 5 | 4 | 4.9 | (4.00, 4.10) | (4.90, 5.00) |
| GLOB | M  | 322 | 322 | 3.12 | 0.41 | 3.2 | 2.1 | 4 | 2.3 | 3.9 | (2.10, 2.30) | (3.80, 3.90) |
| GLOB | F  | 303 | 303 | 3.37 | 0.41 | 3.4 | 2.3 | 4.5 | 2.4 | 4.1 | (2.40, 2.60) | (4.00, 4.30) |
| AG | M  | 309 | 303 | 1.5 | 0.21 | 1.5 | 1 | 2 | 1.2 | 2 | (1.20, 1.20) | (2.00, 2.00) |
| AG | F  | 297 | 293 | 1.34 | 0.19 | 1.3 | 1 | 1.8 | 1.04 | 1.8 | (1.00, 1.10) | (1.70, 1.80) |
| GLUC | M  | 322 | 315 | 76 | 14 | 73 | 40 | 114 | 53 | 109 | (51, 56) | (106, 112) |
| GLUC | F  | 303 | 298 | 82 | 19 | 80 | 43 | 130 | 52 | 122 | (48, 55) | (118, 128) |
| CHOL | M  | 322 | 316 | 98 | 16 | 97 | 55 | 141 | 65 | 130 | (61, 71) | (127, 132) |
| CHOL | F  | 303 | 302 | 107 | 17 | 108 | 71 | 154 | 74 | 142 | (72, 79) | (137, 146) |
| TRIG | M  | 322 | 315 | 46 | 11 | 45 | 23 | 74 | 28 | 71 | (25, 30) | (68, 72) |
| TRIG | F  | 303 | 296 | 52 | 14 | 50 | 21 | 91 | 29 | 82 | (24, 31) | (78, 88) |
### TABLE 7 (Continued)

| Analyte | Sex | Initial N | Final N | Mean   | SD     | Median | Min    | Max   | LRL of RI (2.5%) | URL of RI (2.5%) | CI 90% of LRL | CI 90% of URL |
|---------|-----|-----------|---------|--------|--------|--------|--------|-------|-----------------|-----------------|---------------|--------------|
| PHOS    | M   | 322       | 318     | 6.49   | 0.82   | 6.4    | 4.4    | 8.7   | (4.80, 5.00)    | (8.00, 8.20)    |               |              |
| PHOS    | F   | 303       | 299     | 5.65   | 0.85   | 5.6    | 3.6    | 7.8   | (3.70, 4.00)    | (7.10, 7.70)    |               |              |
| CA      | M   | 322       | 319     | 10.21  | 0.38   | 10.2   | 9.3    | 11.2  | (9.40, 9.60)    | (10.90, 11.10)  |               |              |
| CA      | F   | 303       | 301     | 10.08  | 0.38   | 10.1   | 9.2    | 11.1  | (9.20, 9.50)    | (10.80, 11.00)  |               |              |
| CL      | M   | 403       | 394     | 107.4  | 1.9    | 107    | 103    | 112   | (103.0, 104.0)  | (111.0, 112.0)  |               |              |
| CL      | F   | 379       | 374     | 108.7  | 2.1    | 109    | 103    | 114   | (105.0, 106.0)  | (113.0, 113.0)  |               |              |
| NA      | M   | 403       | 398     | 148.2  | 2.1    | 148    | 143    | 154   | (144.0, 145.0)  | (153.0, 154.0)  |               |              |
| NA      | F   | 379       | 373     | 147.4  | 2      | 147    | 143    | 153   | (143.0, 144.0)  | (151.0, 152.0)  |               |              |
| K       | M   | 403       | 391     | 4.28   | 0.33   | 4.3    | 3.4    | 5.2   | (3.6, 3.7)      | (5.2, 5.3)      |               |              |
| K       | F   | 379       | 377     | 4.4    | 0.4    | 4.3    | 3.2    | 5.5   | (3.6, 3.7)      | (5.2, 5.3)      |               |              |
| AMYL    | M   | 83        | 79      | 355    | 100    | 330    | 204    | 608   | (89, 152)       | (492, 577)      |               |              |
| AMYL    | F   | 84        | 82      | 349    | 97     | 338    | 206    | 589   | (113, 168)      | (498, 568)      |               |              |
| LIP     | M   | 83        | 80      | 16     | 2.9    | 15.5   | 11     | 22    | (9.2, 11.0)     | (20.9, 23.1)    |               |              |
| LIP     | F   | 84        | 76      | 15.7   | 2.3    | 15.5   | 10     | 21    | (10.2, 11.7)    | (19.3, 21.1)    |               |              |
| INS     | M   | 11        | 8       | 14.5   | 2      | 14.9   | 10.8   | 17.1  | (NA, NA)        | (NA, NA)        | (NA, NA)      | (NA, NA)     |
| INS     | F   | 17        | 14      | 12.3   | 3.1    | 11.1   | 9      | 16.8  | (NA, NA)        | (NA, NA)        | (NA, NA)      | (NA, NA)     |
| MG      | M   | 45        | 44      | 2      | 0.13   | 2      | 1.8    | 2.2   | (1.68, 1.78)    | (2.21, 2.32)    |               |              |
| MG      | F   | 46        | 43      | 1.99   | 0.11   | 2      | 1.8    | 2.2   | (1.72, 1.81)    | (2.17, 2.25)    |               |              |
| TSH     | M   | 82        | 80      | 0.6    | 0.3    | 0.6    | 0.1    | 1.2   | 0.03           | 2.46           | BFA           | (2.19, 2.72) |
| TSH     | F   | 79        | 76      | 0.78   | 0.43   | 0.67   | 0.1    | 1.77  | 1.59           | BFA (1.42, 1.80)|               |              |
| T3      | M   | 82        | 78      | 221    | 33     | 222    | 144    | 304   | (146, 167)      | (274, 295)      |               |              |
| T3      | F   | 79        | 72      | 213    | 33     | 216    | 122    | 297   | (138, 159)      | (267, 289)      |               |              |
| T4      | M   | 82        | 81      | 4      | 1.1    | 3.7    | 2.1    | 6.6   | 1.6            | 6.2            | (5.8, 6.6)    |              |
| T4      | F   | 79        | 77      | 4.2    | 1.1    | 4      | 2.2    | 6.9   | 1.8            | 6.4            | (6.0, 6.8)    |              |
| CRPHS   | M   | 81        | 79      | 1.3    | 0.66   | 1.3    | 0.2    | 2.8   | BFA            | BFA            |               |              |
| CRPHS   | F   | 46        | 42      | 1.24   | 0.62   | 1.2    | 0.2    | 2.6   | 0.03           | 2.46           | BFA           | (2.19, 2.72) |

Note: See Table 1 for abbreviations of individual analytes.

Abbreviations: BFA, beyond the feasibility of analysis; CI, confidence interval; F, female; LRI, lower reference limit; M, male; N, animal number/sample size; RI, reference interval; SD, standard deviation; URI, upper reference limit.
Cynomolgus monkeys are often the most pharmacologically and metabolically relevant animal species for nonclinical safety assessment, especially for biologics.\textsuperscript{24-26} Target organ development and age correlations between the monkey and the intended patient population are critical factors for study design. The juvenile toxicity study is typically conducted in postweaning monkeys starting at 10 to 12 months of age, whereas safety assessments of biologics require using monkeys of sexual maturity. For general toxicity studies, the starting ages of monkeys vary from 2 to 5 years. In the current study, therefore, we reviewed the age- or sex-related differences in body weights and hematologic, coagulation, and serum biochemical analyte values and saw sexual dimorphism in body weights at all ages from 2 to 10 years. Sex-related changes in mean hematologic and serum biochemical analytes also occurred. Age-related changes in mean hematologic and serum biochemical analytes were also seen. The only difference in clinical pathology analytes unique to the 2 to <3-year age group were age-related lower globulin concentrations compared with older animals. No age- or sex-related differences were seen with the coagulation analytes. Hematologic, coagulation, and serum biochemical RIs and body weights have been previously reported for cynomolgus monkeys with a small number of animals, limited age ranges, mixed genders, and/or different geographic origins.\textsuperscript{16,17} In the current study, we report similar indices in a large cohort of cynomolgus monkeys of Mauritian origin, which will provide useful RIs worldwide, especially for laboratories using similar testing methods, animal age ranges, and animal origins.

The age- and sex-related changes in serum biochemical analyte values are related to physiology or organ system development.\textsuperscript{18,19} Age-related decreases in ALP activity and phosphorus concentrations reflect decreased bone formation in older animals, and higher ALP and GGT activities in male than female monkeys have also been previously reported in the literature.\textsuperscript{18,27} Sex differences in ALP activity have been reported in a similar species, the Japanese macaque, which has higher ALP activities in males likely associated with growth in males; during which time females have stopped growing. It could also be associated with the expression of secondary sex characteristics induced by sex hormones.\textsuperscript{28} Similarly, higher GGT activity has been reported in human males than females, which is also thought to be due to sex hormones.\textsuperscript{29} Age-related increases in creatinine concentrations and higher creatinine levels in male than female monkeys are likely due to increased muscle mass with age and in males, respectively.\textsuperscript{27} There was a trend for higher serum lipids in older female monkeys compared with younger female monkeys and similarly-aged male monkeys, which was due to higher triglyceride concentrations. Cholesterol was also higher in older female monkeys compared to similarly-aged male monkeys. It was recently published that, as in humans, female cynomolgus monkeys tend to have higher serum lipids than males.\textsuperscript{30} Sex-specific differences in lipid and glucose metabolism are known to occur in both humans and nonhuman primates and may be due at least in part to the unique requirements of females for gestation and/or lactation.\textsuperscript{31} The slightly lower globulin concentrations in male and female monkeys 2 to <3 years of age compared with older monkeys is consistent with other reports showing increasing globulin concentrations with age, which could be due, at least in part, to increasing environmental antigen exposure and adaptive immunity.\textsuperscript{27}

The age- and sex-related changes in hematologic analytes seem to be mostly related to physiology and/or environmental exposure.\textsuperscript{10,16-17} Lower lymphocyte counts in older monkeys of both sexes are consistent with the literature.\textsuperscript{32} RETIC counts increased with age in both sexes and were higher in older female monkeys compared to similarly aged male monkeys. While we did not find anything in the literature regarding age- or sex-related differences in RETIC values, sex-related differences in RETIC values have been seen in our internal historical RIs where females had a higher upper RI than males. In this study, despite the increased RETIC values, no significant differences in RBC mass measurands (RBC, hemoglobin [HGB], and hematocrit [HCT]) were observed. Increased RETIC values without concurrent decreases in the RBC mass measurands can indicate that the bone marrow is responding to a need for increased RBC production. Because RETICs only circulate for a few days and RBCs circulate for significantly longer (~100 days in cynomolgus monkey), a significant lag in decreased RBC, HGB, and HCT values could occur compared with increased RETIC counts.\textsuperscript{35} In this study, higher RETIC counts in older females may be related to menstruation-related blood loss which was also suspected to be the cause of lower RBC measurands in older females compared to similarly-aged males in a large cohort of Asian cynomolgus monkeys.\textsuperscript{27}

The hematologic, coagulation, and serum biochemical intervals for cynomolgus monkeys are variable in the literature.\textsuperscript{27,32,24} The geographic origin might have impacted the hematologic and clinical biochemical analytes.\textsuperscript{9,19,35} It has been reported that Mauritian monkeys have higher RBC counts but lower RETIC and WBC counts, and MCV and MCH values than Asian monkeys.\textsuperscript{9} Mauritian monkeys have higher serum calcium and lower serum phosphorus than Asian monkeys.\textsuperscript{7} Cynomolgus monkeys indigenous to the Philippines have lower creatine phosphokinase and alanine aminotransferase values than the Mauritian or Vietnamese monkeys,\textsuperscript{9} and Vietnamese monkeys had lower ALP and aspartate aminotransferase than Philippine and Mauritian monkeys.\textsuperscript{9} Among Mauritian monkeys, many hematologic and biochemical analytes measurements differ between purpose-bred and captured animals.\textsuperscript{19}

In cynomolgus monkeys, sexual dimorphism in body sizes or weights has been reported to become significant after 3 to 4 years of age.\textsuperscript{37} In a large cohort of Asian cynomolgus monkeys (588 males and 1930 females), there was no sex-related difference in body weights before 4 years of age.\textsuperscript{17} In males, body weights increased dramatically after 4 to 5 years, peaked at 14 to 15 years (~8.40 kg), and declined after 17 years of age. In females, body weights increased gradually after birth, peaked at 12 to 13 (~5.0 kg) years, and declined after 15 years of age.\textsuperscript{17} In the current study, body weights were significantly higher in males than
females of all age groups from 2 to 10 years of age, suggesting the sexual dimorphism in body sizes as early as at least 2 years of age in Mauritian cynomolgus monkeys. It has been reported that the cynomolgus monkey of Mauritian origin is generally larger in body weight/size and becomes sexually mature 1 to 2 years earlier than Asia cynomolgus monkeys. 9,36 In the current study, body weights of Mauritian monkeys peaked at 7 to 8 years of age in both males (7.9 kg) and females (5.5 kg). Apparently, body weight reaches its peak in Mauritian monkeys (7 to 8 years of age) earlier than in Asian monkeys (12 to 15 years of age). Once the plateau is reached, body weights are comparable between Mauritian and Asian cynomolgus monkeys. Age and body weight have been proposed as a predictor of puberty and clinical pathology analyte values in cynomolgus monkeys. 36-42 Correlations between body weight and clinical pathology analytes have been observed in female cynomolgus monkeys. 42 Body weight has been shown to correlate positively with HGB, HCT, MCV, glucose, triglycerides, leptin, and insulin levels. 42 In our study, triglyceride levels were higher in older females as compared to younger females and in females as compared to similarly-aged males. Body weights alone are not a reliable predictor of clinical pathology analyte values in cynomolgus monkeys.

Clinical pathology RIs play important roles in clinical laboratory diagnoses and nonclinical safety assessments, especially in large animal studies where animal numbers are often limited and for those labs where appropriate internal references have not been established. Guidelines on determining, establishing, and verifying controls or RIs have been established for both clinical and veterinary laboratories. 14,36,43 However, many other factors can influence the results and interpretation of both internal and published clinical pathology RIs, such as variations associated with individual animals, laboratory settings, study-related procedures, and environmental conditions. 23,27,35,44,45 Animal factors might include animal species, origin, numbers, sex, age, health status, inter- and intra-animal variability, and animal husbandry and care practices. The laboratory or facility variations can include species- or parameter-specific assay methods, instruments and reagents, sample frequency and preparations, and technologic variation. The study-related procedures include study design variables, such as handling frequency and intensity, dosing methods and devices, sample collection frequency, volume, invasiveness, fasting states, anesthesia restraints, instrumentation, such as telemetry or catheterization, and environmental stress. Thus, it is prudent to adopt a weight of evidence approaches to use and interpret RI ranges or values, especially when using external reference datasets.

There were some limitations in the current study. The animals were restricted to cynomolgus monkey of Mauritian origin only so caution should be taken when using the RIs for cynomolgus monkey of other origins. Most animals included in this data set were 3 to 5 years of age and while at these ages both sexes were generally equally represented there were lesser numbers of females than males in the 2 to <3 years of age group. So RIs provided for the 3 to <4 years of age group were more robust than the RIs for the 2 to <3 years of age group and the RI for males 2 to <3 years of age were more robust than the RI for similarly-aged females. This dataset was generated using Pfizer animal use and care conditions, analytic instruments, and analytical methods so there may be variations from other animal care facilities and laboratory settings.

In summary, we saw sexual dimorphism for body weights from 2 to 10 years of age in cynomolgus monkeys of Mauritian origin. Sex-related differences were noted in RETIC counts, creatinine, cholesterol, and triglyceride concentrations, and ALP and GGT activities. Age-related differences were noted in RETIC and lymphocyte counts, creatinine, triglyceride, phosphorus and globulin concentrations, and ALP and GGT activities. The youngest (2 to <3 year) age group had the fewest number of clinical pathologic analyte differences including ALP and GGT activity differences which occurred in all age groups from 2 to 10 years and age-related lower globulin concentrations.

ACKNOWLEDGMENTS

The authors would like to thank Dr. William Reagan, Steven Bailey, Donald Fish, Deborah Burt, and Kimberly Navetta as well as other Pfizer colleagues for statistical review, manuscript preparation, or other technical assistance.

DISCLOSURE

The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

ORCID

Xiantang Li https://orcid.org/0000-0001-5061-8886
Kathleen E. Biddle https://orcid.org/0000-0001-7503-0640

REFERENCES

1. Magden ER, Mansfield KG, Simmons JH, Abee CR. Nonhuman primates. Laboratory Animal Medicine. 3rd ed. Elsevier Inc; 2015:771-930. doi:10.1016/B978-0-12-409527-4.00017-1
2. Roos C, Zinner D. Diversity and evolutionary history of macaques with special focus on Macaca mulatta and Macaca fascicularis. In: Bluemel J, Korte S, Schenck E, Weinbauer GF, eds. The Nonhuman Primate in Nonclinical Drug Development and Safety Assessment. Academic Press; 2015:3-16.
3. Liedigk R, Kollecke J, Böker KO, et al. Mitogenomic phylogeny of the common long-tailed macaque (Macaca fascicularis fascicularis). BMC Genomics. 2015;16(1):222. doi:10.1186/s12864-015-1437-0
4. Fan Z, Song Y. Chinese primate status and primate captive breeding for biomedical research in China. The Future of Nonhuman Primate Resources. (China) National Academies Press; 2003:36-48.
5. Kanthaswamy S, Ng J, Satkoski Trask J, et al. The genetic composition of populations of cynomolgus macaques (Macaca fascicularis) used in biomedical research. J Med Primatol. 2013;42(3):120-131. doi:10.1111/jmp.12043
6. Yan G, Zhang G, Fang X, et al. Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaques. Nat Biotechnol. 2011;29(11):1019-1023. doi:10.1038/nbt.1992
44. Yang H, Richman L, Hall R. Implications and limitations of reference values and historical data for the interpretation of nonhuman primate toxicology studies. In: Bluemel J, Korte S, Schenck E, Weinbauer GF, eds. The Nonhuman Primate in Nonclinical Drug Development and Safety Assessment. Academic Press; 2015: 173-183.

45. Perretta G, Violante A, Scarpulla M, Beciani M, Monaco V. Normal serum biochemical and hematological parameters in *Macaca fascicularis*. J Med Primatol. 1991;20(7):345-351.

How to cite this article: Li X, Li D, Biddle KE, et al. Age- and sex-related changes in body weights and clinical pathology analytes in cynomolgus monkeys (*Macaca Fascicularis*) of Mauritius origin. Vet Clin Pathol. 2022;51:356-375. doi: [10.1111/vcp.13094](https://doi.org/10.1111/vcp.13094)