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Effects of Weekly Blood Collection in Male and Female Cynomolgus Macaques (*Macaca fascicularis*)

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This study was designed to evaluate the maximal amount of blood that can be safely collected in healthy, adult male and female cynomolgus macaques for 4 consecutive weeks with minimal effect on animal wellbeing. General guidelines for blood collection volumes in laboratory animals are not species-specific, and currently there are few evaluations of blood collection in macaques. In this study, blood was removed at 7.5%, 10%, 12.5%, 15%, or 17.5% of total blood volume (TBV) for 4 consecutive weeks. Hematologic parameters and body weights were evaluated immediately prior to each blood collection time point and for an additional 4 consecutive weeks following the last collection. Male and female macaques tolerated removal of as much as 15% TBV with minor clinical effects, whereas macaques in the 17.5% TBV group exhibited an increased incidence of emesis and anorexia during the first 24 h after blood collection. According to these results, we recommend collecting no more than 15% TBV weekly for 4 consecutive weeks from healthy, adult male and female cynomolgus macaques.

Abbreviation: TBV, total blood volume.

Many experimental studies require blood sampling from laboratory animals to obtain data. The volume of blood collected is based on the specific needs of the study and the species that is being sampled. Some studies need small, repeated blood samples over a short period of time, whereas others require larger volumes over fewer time points. The challenge from a welfare and IACUC perspective arises when investigators propose studies that require a large volume of blood to be collected over a relatively short period of time. Current recommendations in the literature range from 10% of the total blood volume (TBV) collected weekly to 15% of TBV collected every other week.[6,19] These general recommendations are based on studies performed in rats and have been used as a guide for other species.

Previous studies evaluating maximal blood collection volumes in various species have demonstrated that more blood can be collected safely from healthy animals than is indicated in the currently accepted recommendations.[18,24,27] Serial blood collection of as much as 15% TBV weekly for 4 wk from laboratory Beagles was performed without any serious side effects.[24] Other colleagues demonstrated that 15% and 25% of TBV could be safely collected weekly for as long as 6 wk from male and female C57BL/6 mice, respectively.[27] One study removed 6%, 12%, 18%, or 24% of TBV weekly for 8 wk from male rhesus macaques and concluded that animals that had 12% or less of TBV removed weekly were able to maintain Hgb concentrations near baseline.[18] To our knowledge, there are no studies that evaluate maximal blood collection in cynomolgus macaques.

The goal of the current study was to evaluate and identify the maximal blood volume that can be collected for 4 consecutive weeks from male and female cynomolgus macaques without causing moderate to severe anemia or adverse clinical effects that could affect animal wellbeing. In light of previous studies[18,24,27] and our own experience with nonhuman primates, we hypothesized that at least 15% of TBV could be collected weekly for 4 wk from healthy cynomolgus macaques. The results of the current study likely will assist veterinarians and the IACUC in making safe recommendations for blood collection and will guide investigators in developing appropriate blood collection protocols for healthy cynomolgus macaques.

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Materials and Methods

Animals. Twelve adult male (age, 8.5 ± 2.9 y; weight, 7.0 ± 1.7 kg [mean ± SEM]) and 13 adult female (age, 7.4 ± 1.6 y; weight, 4.2 ± 0.9 kg) cynomolgus macaques (*Macaca fascicularis*) were used to complete this study. All macaques had a body condition score of 2.5 to 3.5 on a 5-point scoring system.[30] All procedures were performed under approval from the University of Illinois at Chicago Animal Care Committee. All macaques were housed in accordance with the *Guide for the Care and Use of Laboratory Animals*,[12] Public Health Service Policy,[23] and Animal Welfare Act[2] and Regulations[3] in an AAALAC-accredited facility. Animals were housed in visual and auditory contact with conspecifics at a minimum and were pair-housed whenever possible. Animals were provided commercial chow (15% Monkey Diet 8714, Harlan-Teklad, Madison, WI) once daily and municipal tap water ad libitum. Fresh produce or foraging items were provided once daily. Rooms were maintained at 22 ± 2 °C and 30% to 70% relative humidity with 100% conditioned air at 15 to 20 room air changes hourly. Fluorescent lighting was provided on a 12:12-h light:dark cycle (lights on, 0600 to 1800). Macaques had toys and manipulanda placed directly in the cage, and speakers in the animal rooms provided auditory enrichment. All animals tested negative for retroviruses and were tuberculosis-free as determined by semiannual skin testing. Prior to the initiation of the study, animals underwent a health assessment including a physical examination, baseline CBC counts, and chemistry analysis.

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Experimental design. The duration of the experimental period for each blood collection volume was 8 wk. Each macaque underwent blood collection of 7.5%, 10%, 12.5%, 15%, or 17.5% of TBV on the same day each week for 4 consecutive weeks. CBC and body weight were assessed before each blood collection (weeks 1 through 4) and for an additional 4 consecutive weeks after the last blood collection (weeks 5 through 8). Each of the 5 blood collection groups contained 4 male and 4 female cynomolgus macaques. Animals that were reused (7 male, 7 female) between groups were given a 2-wk rest period after the conclusion of the 8-wk experimental period or until the Hct percentage, Hgb concentration, and reticulocyte counts returned to within 10% of the original baseline measurement.

Blood collection and hematologic analysis. TBV was calculated as 6% of an animal's body weight, according to a study evaluating TBV in cynomolgus macaques.[1] Animals were weighed prior to each blood collection, and TBV was adjusted throughout the study to account for fluctuations in body weight.

Macaques were anesthetized with an intramuscular injection of ketamine (10 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA). Blood (3 mL) was collected from a femoral vein to evaluate hematologic parameters prior to blood collection; this volume was subtracted from the calculated blood volume to account for the blood removed for the CBC analysis. Either 7.5%, 10%, 12.5%, 15%, or 17.5% of TBV was removed weekly via a femoral vein during weeks 1 through 4. Hemostasis was achieved prior to returning macaques to their home cages. For weeks 5 through 8, macaques were anesthetized with ketamine as previously described and weighed, and blood (3 mL) was collected from a femoral vein for CBC analysis, which was performed on an automated hematology analyzer (Advia 120, Siemens Healthcare Diagnostics, Tarrytown, NY) using cynomolgus macaque-specific algorithms and parameters (Technicon H 1E MultiSpecies Software, version 3.0, Siemens Healthcare Diagnostics). Parameters evaluated were Hgb concentration, Hct percentage, RBC count, MCV, and absolute reticulocyte count.

Animal monitoring. All macaques were observed daily by husbandry staff for the duration of the study. Cageside observations were performed by study personnel twice daily on the day of blood collection and once daily during weeks 1 through 4. During weeks 5 through 8, macaques were monitored 3 times each week. Assessments evaluated alertness, posture, appetite, presence of vomit or salivation, and color of mucous membranes. To ensure animal wellbeing, the following criteria were used to remove an animal from study: Hgb concentration less than 7 g/dL, Hct percentage less than 25%, or weight loss greater than 10% of body weight for 2 consecutive weeks.

Baseline values and anemia characterization. Anemia is defined as a Hgb concentration, Hct percentage, or RBC count that is 2 standard deviations below the mean or the 2.5th percentile of the normal distribution of a healthy, iron-replete population.[7,13,16,25] For the purposes of the current study, Hgb concentration and Hct percentage were used to assess anemia, because Hgb concentration is used in human medicine and Hct percentage typically is used in veterinary medicine to define anemia.[34] Colony reference ranges for Hgb concentration and Hct percentage were based on samples obtained from healthy, adult cynomolgus macaques (male, n = 89; female, n = 53) during the past 3 y. These reference ranges were used to calculate the criteria for anemia. Macaques that fell below these criteria (that is, Hgb concentration or Hct percentage greater than 2 standard deviations below the mean) were considered anemic. For the purposes of this study, recovery was defined as a Hgb concentration that returned to within 10% of the original baseline value.

Although defining the lower limit of normal Hgb concentration and Hct percentage is helpful in identifying macaques that have a decreased erythrocyte mass, doing so does not indicate the severity to which they are affected. By using the World Health Organization's scale for categorizing anemia in humans[5] and using the colony reference ranges, the percentage change from the normal lower limit of Hgb concentration and Hct percentage for the various categories was calculated and applied to cynomolgus macaques.

Statistical analysis. All statistical analysis was performed by using MatLab software (MathWorks, Natick, MA). Each of the CBC parameters was analyzed by using a 3-way repeated-measures ANOVA to identify the effects due to animal sex, volume of blood collected, and time. Significance was determined as a P value of less than 0.05 for all statistical tests.

Results

Baseline values and anemia categorization. The historical average Hgb concentrations (mean ± 1 SD) for male and female macaques were 12.3 ± 0.96 g/dL and 12.0 ± 1.1 g/dL, respectively. The average Hct percentages for male and female macaques were 40.9 ± 3.1% and 39.4 ± 3.0%, respectively. According to historical data, reticulocyte ranges were 13,306 to 103,300/µL and 10,600 to 107,800/µL for male and female macaques, respectively, whereas MCV ranges were 58.2 to 87.5 fL and 58.1 to 81.1 fL, respectively.

Applying the definition of anemia to these colony reference ranges for Hgb concentrations corresponds to lower limits of 10.4 g/dL and 9.7 g/dL for male and female macaques, respectively. Anemia based on Hct percentages corresponds to lower limits of 34.8% in male macaques and 33.4% in female macaques.

These data were used to construct a severity scale to qualify the level of anemia (Table 1) and gain a better understanding of the clinical significance of Hgb concentration and Hct percentage. Anemia was categorized as mild, moderate, or severe according to Hgb concentration or Hct percentage.

Hgb. Both sex and time had significant main effects on Hgb concentration ($P < 0.01$ for both comparisons), as did the interaction between blood collection volume and time ($P < 0.05$; Figures 1 A and 2 A). According to average Hgb concentrations, the male 17.5% TBV group and female 15% TBV group met criteria for anemia during weeks 3, 4, and 5. On an individual basis, one female macaque in the 10% TBV group, one macaque female in the 12.5% TBV group, 3 females in the 15% TBV group, and one female in the 17.5% and 3 male macaques in the 17.5% TBV group met criteria at one or more time points during the experimental period (Table 2). All animals that met criteria were considered to be mildly anemic according to the anemia categorization scale (Table 1). No animals were removed from the study due to decreased Hgb concentrations due to the blood collection procedures. One female macaque in the 17.5% TBV group was removed from study due to suspected endometriosis, which resulted in severe anemia during week 7.

According to average Hgb concentrations, the animals in the 7.5% TBV group had recovered by the first week after blood collection. The 10% and 17.5% TBV groups recovered after 1 wk of rest. Male macaques in the 12.5% TBV group and animals in the 15% TBV recovered after 2 wk, and female macaques in the 12.5% group recovered after 3 wk of rest.

Hct. Time had a significant main effect on Hct percentage ($P < 0.01$, Figures 1 B and 2 B). The average Hct percentage

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Table 1. Anemia categorization scale for humans and cynomolgus macaques according to Hgb concentrations and Hct percentages

	Humans		Cynomolgus macaques			
			Male		Female	
	Hgb (g/dL)	Hct (%)	Hgb (g/dL)	Hct (%)	Hgb (g/dL)	Hct (%)
Normal	> 13.0 (male); > 12.0 (female)	> 36.0	≥ 10.4	≥ 34.8	≥ 9.7	≥ 33.4
Mild	10.0–12.0	30.0–36.0	8.7–10.3	29.0–34.7	8.1–9.6	27.8–33.3
Moderate	7.0–10.0	21.0–30.0	6.1–8.6	20.3–28.9	5.7–8.0	19.5–27.7
Severe	< 7.0	< 21.0	< 6.1	< 20.3	< 5.7	< 19.5

Human data from references 3, 6, and 29.

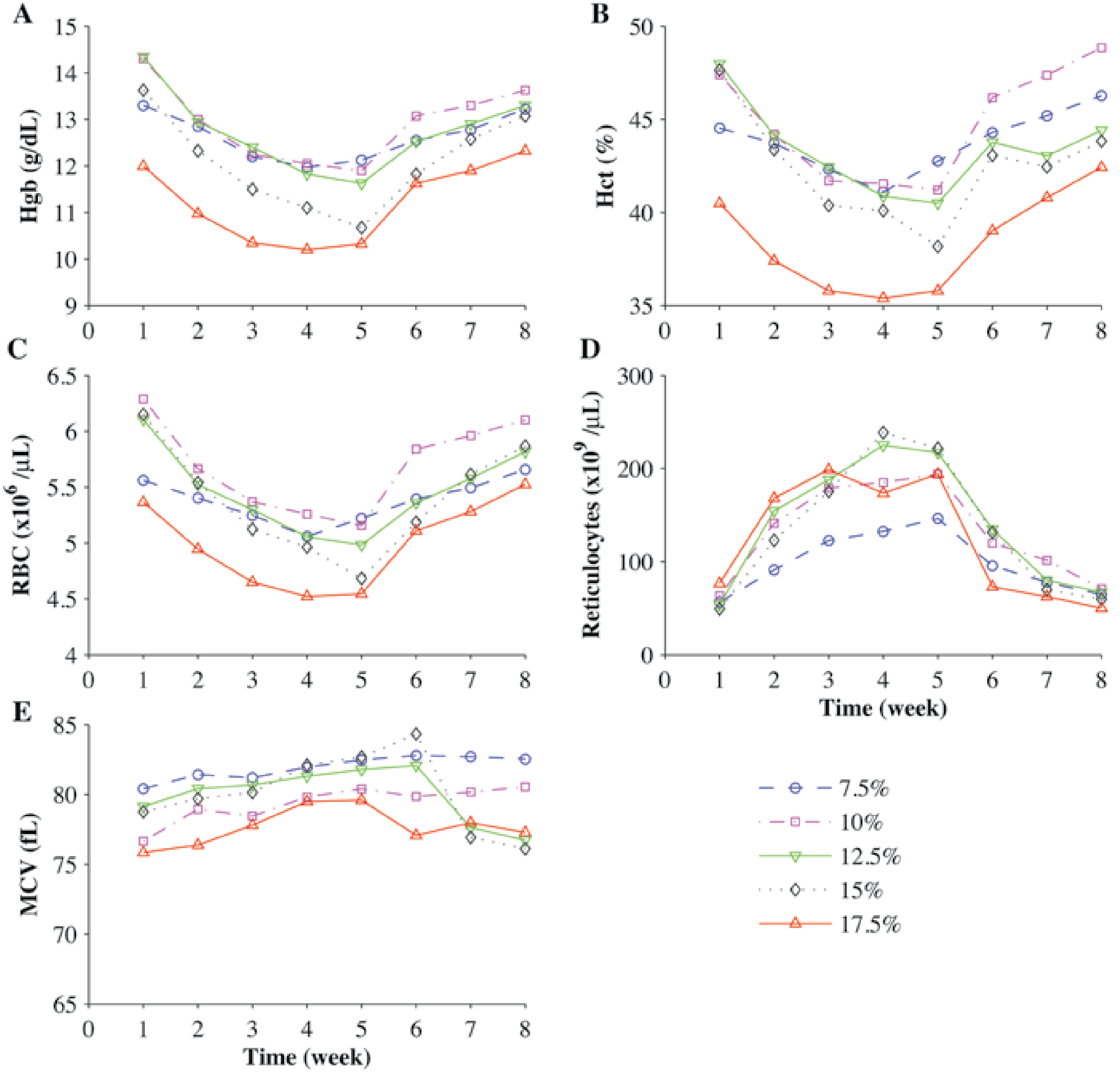

Figure 1. Average hematologic parameters in male cynomolgus macaques. Blood was collected for hematologic analysis prior to removal of 7.5%, 10%, 12.5%, 15%, or 17.5% TBV during weeks 1 through 4. During weeks 5 through 8, blood was collected to monitor hematologic parameters. (A) Average Hgb concentration. (B) Average Hct percentage. (C) Average RBC count. (D) Average absolute reticulocyte count. (E) Average MCV.

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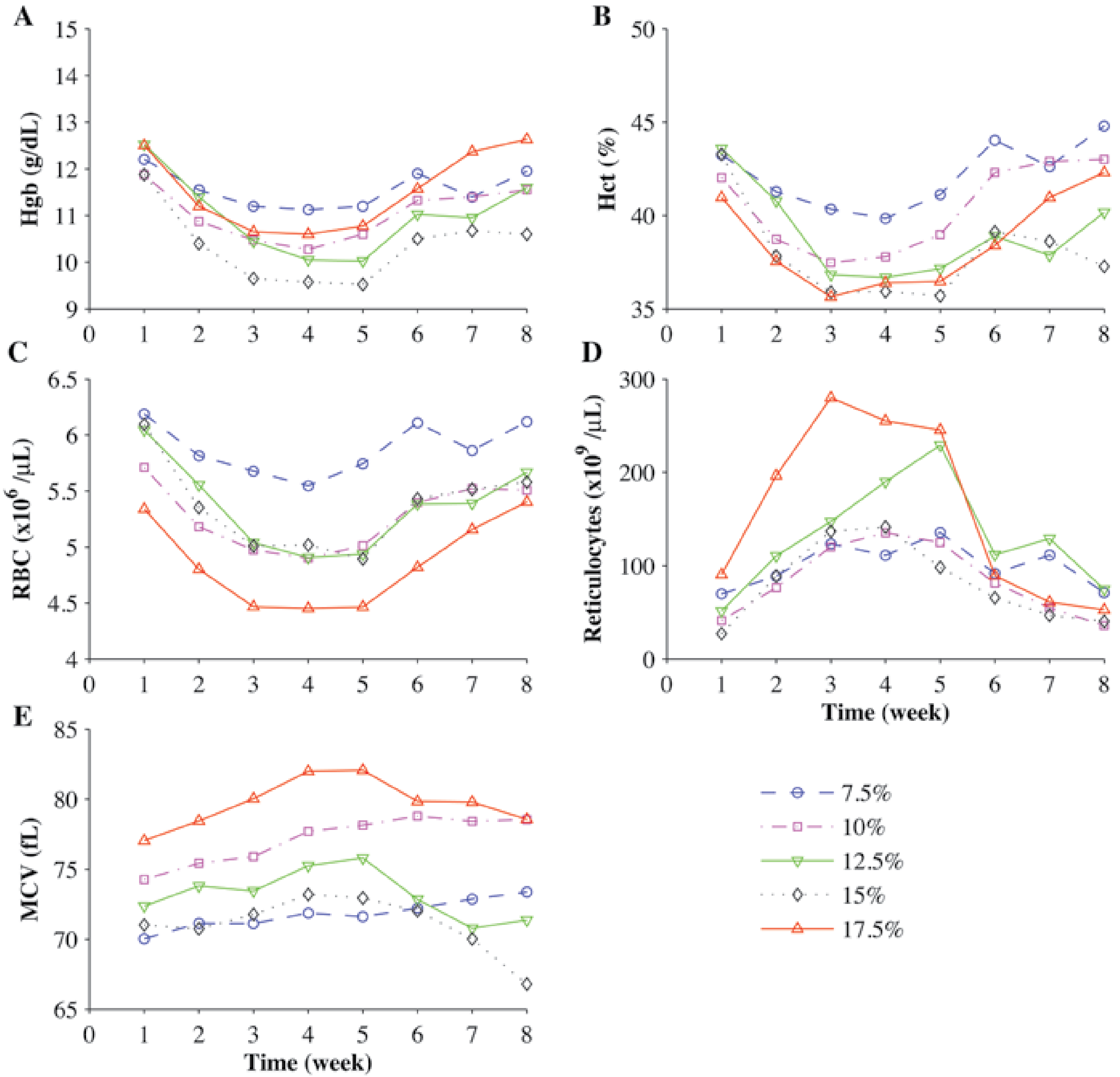

Figure 2. Average hematologic parameters in female cynomolgus macaques. Blood was collected for hematologic analysis prior to removal of 7.5%, 10%, 12.5%, 15%, or 17.5% TBV during weeks 1 through 4. During weeks 5 through 8, blood was collected to monitor hematologic parameters. (A) Average Hgb concentration. (B) Average Hct percentage. (C) Average RBC count. (D) Average absolute reticulocyte count. (E) Average MCV.

did not drop below 34.8% in males or 33.4% in females in any of the blood collection groups. One female in the 12.5% TBV group, one female in the 17.5% TBV group, and 2 males in the 17.5% TBV group met anemia criteria at a minimum of one time point or more during the experimental period (Table 3). All were considered to be mildly anemic based on the anemia categorization scale (Table 1). No animals were removed from study based on decreased Hct percentages due to blood collection procedures. One female in the 17.5% TBV group was removed from study due to suspected endometriosis, which resulted in severe anemia in week 7.

Other hematologic parameters. Time had a significant main effect on RBC count, reticulocyte count, and MCV ($P < 0.01$ for all comparisons, Figures 1 C through E and 2 C through E). There was also a significant interaction between collection volume and time on RBC count and MCV ($P < 0.05$ for both comparisons, Figures 1 C through E, and 2 C through E). All macaques demonstrated a regenerative response according to absolute reticulocyte count (43,500 to 424,100/µL) and MCV (62.3 to 97.3 fL in male and 63.9 to 89.9 fL in female macaques).

Clinical assessments. There were no clinically significant effects on body weight for any of the macaques on study. Emesis and anorexia were the only 2 adverse effects that were observed during cageside assessments. Of the 160 total blood collections, there were 10 episodes of emesis and 11 occasions of anorexia observed within the 24 h after collection (Table 4). One female macaque in the 17.5% TBV group developed unilateral mydriasis after the 4th blood collection. There were no other

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Table 2. Macaques meeting anemia criteria according to Hgb concentration

		Hgb (g/dL)							
Group	Macaque sex and no.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
10.0%	Female 7834	11.4	10.3	**9.5**	**9.3**	9.7	10.6	11.2	10.0
12.5%	Female 7809	11.9	11.4	**8.9**	**8.9**	**9.1**	10.4	**9.5**	11.1
15.0%	Female 7837	11.7	10.1	**9.6**	10.1	9.8	10.8	10.7	10.6
15.0%	Female 7834	10.4	**9.4**	**8.6**	**8.7**	**8.3**	9.7	10.3	10.3
15.0%	Female 8175	12.5	11.1	10.0	**9.5**	**9.6**	10.8	10.9	10.2
17.5%	Female 8343	**9.5**	**9.2**	**9.3**	**9.4**	9.8	10.2	11.2	11.2
17.5%	Female 7826	12.3	11.7	10.7	11.2	11.0	11.9	**5.0**[a]	not included
17.5%	Male 8107	11.8	10.7	**10.2**	**9.7**	10.4	11.4	12.2	12.9
17.5%	Male 8345	11.0	10.4	**9.6**	**9.8**	**9.5**	11.6	11.1	11.3
17.5%	Male 7850	10.4	**10.0**	**10.0**	**9.9**	**10.2**	10.6	11.8	11.5

Values in bold indicate that that the macaque met the criteria for anemia (male, < 10.4 g/dL; female, < 9.7 g/dL).
[a]Macaque removed from study during week 7 due to suspected endometriosis.

Table 3. Macaques meeting anemia criteria according to Hct percentages

		Hct (%)							
Group	Macaque sex and no.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
12.5%	Female 7809	43.7	42.3	**31.8**	33.5	35.2	36.7	34.6	40.4
17.5%	Female 8343	**31.2**	**30.9**	**32.1**	**32.8**	34.3	**33.1**	36.2	38.2
17.5%	Female 7826	39.6	39.8	34.9	38.8	37.6	39.5	**17.8**[a]	not included
17.5%	Male 8107	38.2	**34.6**	**34.3**	**32.7**	35.2	37.4	40.7	42.9
17.5%	Male 8345	36.3	35.2	**32.7**	**34.0**	**32.1**	38.2	37.3	38.0

Values in bold indicate that the macaque met the criteria for anemia (male, < 34.8%; female, < 33.4%).
[a]Macaque removed from study during week 7 due to suspected endometriosis.

Table 4. Adverse effects of emesis and anorexia within 24 h after blood collection

		Emesis				Anorexia (≤ 24 h)			
Group	Macaque sex and no.	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
7.5%	Female 8176	+	—	—	—	—	—	—	—
15%	Female 8172	—	+	—	—	—	—	—	—
15%	Female 8175	—	—	+	—	—	—	—	—
17.5%	Female 8172	+	—	—	—	+	—	—	+
17.5%	Female 7826	—	—	—	—	—	—	+	+
17.5%	Female 8343	+	+	+	+	+	+	+	+
17.5%	Male 7850	+	—	+	—	+	—	+	+

abnormalities observed in this animal, and the anisocoria persisted through the end of the study. One female macaque in the 17.5% TBV group was observed to be pale, quiet, and mensing heavily during week 7. CBC results indicated severe anemia in week 7; however, Hgb concentration and Hct percentage were both within normal limits during the 6 wk of monitoring prior to this finding (Tables 2 and 3). Endometriosis was considered a differential diagnosis for this animal, given her age, clinical presentation, and hematologic abnormalities. Data from weeks 7 and 8 were not included in the study because the hematologic abnormalities were considered due to an underlying and unrelated disease process.

Discussion

The results of this study support the hypothesis that as much as 15% of the TBV can be safely collected weekly over a 4-wk period in healthy, adult male and female cynomolgus macaques. Removal of 15% of the TBV for 4 consecutive weeks resulted in mild anemia at one or more time points in 3 of the 8 animals, according to the Hgb concentration. There was no evidence of anorexia and only single episodes of emesis, which occurred in 2 of the 8 animals. According to Hgb concentration, macaques that had 17.5% TBV removed demonstrated mild anemia at one or more time points in 5 of 8 animals. Furthermore, there was an increase in adverse effects in both male and female macaques.

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During the first 24 h after 17.5% TBV removal, 3 of 8 macaques had an episode of emesis and 4 of 8 animals demonstrated anorexia at one or more time points.

Hgb concentration is considered the most sensitive and direct hematologic parameter to identify anemia.[13,20,26,28] Measurement of Hgb concentration is performed by spectrophotometry, which directly determines the Hgb per unit volume of blood.[32] In contrast, the Hct percentage is an indirect measurement, given that it is calculated as the product of the MCV and the RBC count.[32] This indirect measurement makes the Hct percentage inherently less sensitive than Hgb concentration. However, Hct percentages continue to be used in veterinary medicine. In addition, findings of the current study suggest that Hgb concentration is a more sensitive indicator of anemia than Hct percentage. Nine individual macaques met anemia criteria according to Hgb concentration, whereas only 4 met criteria based on the Hct percentage.

The interaction between blood volume and time was statistically significant for Hgb concentration, RBC count, reticulocyte count, and MCV; however time was the only variable that was statistically significant after evaluation of Hct percentages. As expected for serial collection of blood over several weeks, values for Hgb concentration, Hct percentage, and RBC count all decreased during the blood-collection period (Figures 1 and 2). A general recommendation in the literature on blood removal indicates that 1 to 3 wk of rest may be required for hematologic parameters to return to normal after 7.5% to 20% TBV is removed, respectively, over a 24-h period.[6] The results of the current study were consistent with this recommendation. The mean Hgb concentrations returned to within 10% of baseline values in 1 to 4 wk, depending on the proportion of TBV removed. Individual recovery times were variable, but on average, macaques that had 10% TBV or less removed weekly required 1 wk of rest. Animals in the 12.5% and 15% TBV groups required an average recovery period of 2 to 3 wk after 4 wk of consecutive blood collections. The average recovery time of the 17.5% TBV group was 1 wk after the last blood collection. This group demonstrated an unexpectedly short recovery period; however, recovery time increased as blood volume increased, as expected, for the the 7.5%, 10%, 12.5%, and 15% TBV groups.

Other important hematologic parameters to evaluate when anemia is suspected are the absolute reticulocyte count and MCV. The presence of reticulocytes in the blood indicates that the bone marrow is functional and that the body is responding appropriately to the blood loss.[32] The increased reticulocyte counts observed during weeks 2 through 5 indicated that the macaques in this study were able to produce a regenerative response to the ongoing blood collections.

MCV, a measure of the average RBC size, is an indicator of both the presence of a regenerative response as well as of adequate iron stores.[32] In response to blood loss, healthy animals develop a macrocytic anemia due to the larger size of immature RBC that are released from the bone marrow. Animals undergoing repeated blood collections have the potential to develop iron deficiency over time and develop a microcytic anemia due to low iron stores. The increased MCV in the current study indicated that the macaques were able to mount an adequate regenerative response to blood loss and that iron deficiency was not a concern.

Several studies have evaluated the effects of ketamine on hematologic parameters.[15,35] These studies have conflicting results on the effect of ketamine in relation to specific hematologic parameters in both male and female cynomolgus macaques.[15,32] Because the colony reference ranges used to define anemia were established by using animals under ketamine restraint and because they were in agreement with other published reference ranges,[4] we considered that the effect of ketamine on the hematologic results of this study were minimal.

Animals have the ability to compensate for a decrease in RBC mass up to a point, at which time they begin to demonstrate clinical signs associated with anemia.[22] The ability to compensate is based on several factors, including the underlying cause of the anemia, the speed and severity of onset, and the health status of the animal.[22,32] In general, healthy animals are able to compensate for mild anemia without demonstrating clinical signs, which can include weakness, exercise intolerance, lethargy, increased respiratory rate, increased heart rate and pallor.[8,32,34] When an animal is able to compensate and there are no clinical signs associated with mild anemia, treatment or removal from study is not warranted. As an animal loses its ability to compensate and becomes moderately to severely anemic, clinical signs become evident, resulting in the need to address the underlying condition and administer appropriate treatment. It has been reported that a Hgb concentration of 7 g/dL is sufficient to maintain oxygenation for most human patients.[10] In general, blood transfusions to treat anemia in human patients are rarely indicated when the Hgb concentration is above 10 g/dL but always indicated when it is below 6 g/dL.[31] In the current study, all macaques that met anemia criteria were categorized as having mild anemia, except for one female macaque in the 17.5% TBV group which was categorized as having severe anemia and was removed from the study during the recovery period because of suspected endometriosis. Screening of middle-aged female macaques for endometriosis may be warranted prior to removal of large volumes of blood.

There is concern that removing more than 10% TBV over a short period of time can result in substantial physiologic changes. Blood donation in humans can cause temporary hypotension, which can lead to adverse reactions such as nausea, weakness, and lightheadedness.[14] In humans, blood loss greater than 10% TBV decreases cardiac output and oxygen delivery to tissues, whereas blood loss over 20% TBV results in decreased arterial pressure.[17] In animals, cardiac output and blood pressure decreases with removal of 15% to 20% TBV.[21] To put this information into perspective, approximately 18% TBV (similar to the highest TBV removed in the current study) is removed from human blood donors who meet the minimal weight requirement of 50 kg,[14]. Reports of human blood donors demonstrate that nausea or emesis occurs in 8.6 per 1000 first-time male donors and 45.4 per 1000 first-time female donors.[9] This observation is consistent with the current study, in which only female macaques had episodes of emesis when 15% or less of TBV was removed. However, both male and female macaques in the 17.5% TBV group demonstrated multiple episodes of emesis. Although physiologic parameters were not evaluated during this study, it is likely that alterations in physiology accounted for the increase in adverse effects in the 17.5% TBV group. Because emesis only occurred on the day of blood collection, the effects likely were due to temporary hypotension rather than anemia. To reduce adverse reactions and prevent potential decreases in cardiac output associated with blood removal, one should consider administering intravenous fluids when collecting more than 15% TBV. When administering replacement fluids, normal saline equivalent to the volume of blood removed is recommended.[21]

In addition to blood removal, the episodes of emesis and anorexia observed in our macaques may be due to the use of ketamine for chemical restraint. According to the clinical practice

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guidelines for use of ketamine in humans, emesis during recovery occurs in 5.9% of patients.[11] In addition, decreased food consumption has been observed to occur for as long as 48 h after ketamine anesthesia in male rhesus macaques and for as long as 120 h postanesthesia in female rhesus macaques.[29] The administration of ketamine may have exacerbated the adverse clinical signs seen in the 17.5% TBV group.

After the last blood collection, anisocoria was observed in one female macaque in the 17.5% TBV group. Reasons for anisocoria include trauma, increased intracranial pressure, aneurysm, glaucoma, meningitis, and seizures. The animal was otherwise healthy, according to physical exam and behavioral observation. One of the most commonly reported adverse reactions associated with blood donation in humans is feeling faint or weak.[9] Blood donors have experienced delayed episodes of fainting and subsequent head trauma that occurred after the 15-min recommended postdonation resting period.[14] If our macaque was hypotensive from blood collection and unsteady due to recovery from ketamine, she may have sustained head trauma during a fall in the recovery phase and subsequently developed anisocoria.

Limitations of the current study include a small sample size and a limited blood collection period of 4 wk. It should not be concluded that these blood volumes can be collected on a weekly basis for an undefined period of time. According to the trends observed in Hgb concentration and Hct percentage, the values would likely continue to decline if blood collection was performed for additional weeks.

In light of our current results, we consider that 15% TBV can be safely collected weekly for 4 consecutive weeks from healthy, adult male and female cynomolgus macaques. With the removal of 17.5% TBV, macaques demonstrated additional adverse effects that were likely due to temporary hypotension, indicating that the animals should receive supplemental intravenous fluids to minimize these effects. For a 4-wk blood collection protocol, we recommend evaluation of hematologic parameters at baseline to determine an animal's suitability for high-volume blood removal and reassessment prior to blood removal at week 3, because this was the point at which most macaques met criteria for anemia. If blood is to be removed beyond 4 wk, we recommend evaluating hematologic parameters every 1 to 2 wk. In general, a 1-wk recovery period is sufficient after weekly removal of 10% TBV or less for 4 consecutive weeks, whereas a 2- to 3-wk recovery period is recommended when more than 10% TBV is removed weekly for 4 wk. It is important to recognize that individual animals may not be able to compensate for repeated blood loss. Therefore, evaluation of hematologic parameters is important for any animal suspected to be anemic or when there is concern about the capacity of the bone marrow to mount an adequate regenerative response.

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