

Daily Water Intake by Common Marmosets (*Callithrix jacchus*) and Recommendations Regarding Fluid Regulation

Caroline B Winn,^{1,†} Elias B Issa,² Chiara P Curcillo,³ Catherine A Townes,⁴ Monika A Burns,¹ and Mary M Patterson^{1,*}

The typical daily water intake of common marmosets (*Callithrix jacchus*) in a research setting has not been well characterized. Because these New World primates are in demand as animal models for neurobehavioral experiments, which can include the potential use of fluid regulation for training, veterinary and research staff need to understand how marmosets keep hydrated under normal circumstances. In the current study, we measured the water consumption of older (age, 5 to 12 y; $n = 11$) and younger (age, 1 to 2 y; $n = 11$) marmosets every 3 h during the 12-h light phase in 2 different months (January and July). The overall daily water intake (mean \pm 1 SD) was 61.3 ± 20.4 mL/kg (range, 36.3 to 99.0 mL/kg); water intake by an individual marmoset or cohoused pair was fairly consistent from day to day. Water intake did not change across the four 3-h periods measured during the day, and minimal water was consumed overnight when the room lights were off. In addition, daily water intake did not differ between the 2 mo of measurements. Older animals drank significantly more than the younger group, and weight was directly correlated with water intake. Water intake was not affected by body condition score or housing status. The variation in water consumption among marmosets underscores the need for individualization of fluid regulation guidelines.

Abbreviation: BCS, body condition score

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Common marmosets (*Callithrix jacchus*) are New World primates that are increasingly popular in neurophysiologic and behavioral research because of their small size, cognitive abilities, and suitability for transgenic manipulation.^{4,6} A number of researchers working with marmosets train them to perform visual behavioral tasks as is done with Old World macaques; nevertheless, offering a liquid or food reward to assure consistent performance has limited efficacy in both primate groups. To improve task consistency in macaques, research staff often carry out some form of controlled water access; personnel working with marmosets may be interested in a similar training paradigm for their animals. However, although several reviews and studies are available to guide veterinarians, scientists, and IACUC regarding controlling water access and simultaneously ensuring an animal's health and welfare, these publications have focused principally on rodents and macaques.^{3,7,10,11} More recently, the Association of Primate Veterinarians produced a document in 2014 that focused on fluid regulation in macaques,¹ while a 2016 neuroscience article concluded that 2 different fluid control protocols had minimal effect on rhesus macaques.² In contrast, even the basics of daily water consumption by healthy, caged marmosets have not been evaluated in any detail previously, despite the fact that the majority of discussions regarding

controlled water access stress that baseline values for each species, if not for individual animals, are always warranted.

In this study, we determined the average daily water consumption of common marmosets in a research facility during 2 separate months. Differences in water intake related to the time of day and to an individual animal's age, weight, body condition score (BCS), and housing status were also investigated. Using the results obtained, we have implemented provisional recommendations for the fluid regulation of marmosets at our institution.

Materials and Methods

Animals. This study used 22 common marmosets; all were deemed clinically healthy, with a BCS of at least 2 on a scale of 1 to 5 (1, emaciated; 2, slightly thin; 3, excellent body condition; 4, somewhat heavy; 5, obese). The older group consisted of 4 male and 7 female marmosets, with an average age of 9.9 y (range, 5 to 12 y) and average weight of 402 g (range, 315 to 482 g); the younger group contained 8 male and 3 female marmosets, with an average age of 1.5 y (range, 1 to 2 y) and average weight of 371 g (range, 320 to 404 g). The animals were housed in the same holding room of an AAALAC-accredited facility as pairs ($n = 16$; 4 same-sex pairs, 4 male–female pairs) or individually ($n = 6$; 2 of these animals singly housed for only half of the study). All of the marmosets were on an animal use protocol approved by the Massachusetts Institute of Technology's IACUC. Custom-designed, stainless steel and polycarbonate cages (30 in. wide \times 32 in. deep \times 67 in. tall) were used for the majority of the cohort, except that the 4 singly housed animals were kept in a single cage divided into 4 quadrants (each 26 in. wide \times 21 in. deep \times 29 in. tall). The housing room was maintained at 74.0 ± 2.0 °F (23.3 ± 1.1 °C), with a relative humidity of 30% to 70% and a

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¹Division of Comparative Medicine and ²McGovern Institute for Brain Research, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts; ³University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania; and ⁴University of Illinois College of Veterinary Medicine, Urbana, Illinois

*Corresponding author. Email: mmpatt@mit.edu

[†]Current affiliation: Pfizer Worldwide Research and Development, Cambridge Massachusetts

12:12-h light:dark cycle. Enclosure enrichment was composed of perches, nest boxes, hammocks, manzanita wood branches, and hanging toys. Foraging trays and acacia gum treats were provided weekly.

The marmoset colony receives unrestricted access to water through an automatic watering system supplemented by 2 polycarbonate 500-mL water bottles per cage. Water bottles and cages are changed every 2 wk. The main diet is fed once daily in the late morning; it consists of biscuits (Teklad New World Primate Diet 8794, Envigo, Madison, WI), which are briefly soaked in water, and supplemented with fruits, vegetables, and additional protein sources (such as hard-boiled eggs, ZuPreem [Premium Nutritional Products, Mission, KS], and cottage cheese). The approximate moisture content of a typical meal is 22.3%. Marmoset colony health monitoring consists of semiannual sampling for potentially pathogenic bacteria (including *Salmonella* spp., *Shigella* spp., β -hemolytic *Escherichia coli*, *Klebsiella* spp., and *Campylobacter* spp.) and parasites (including *Enterobius* spp., *Entamoeba* spp., *Giardia* spp., and *Cryptosporidium* spp.). Animals are seronegative for squirrel monkey cytomegalovirus, *Saimiriine herpesvirus 1*, *Saimiriine herpesvirus 2*, and measles virus. Physical examinations are performed at least twice a year on all marmosets older than 6 mo, and CBC and serum chemistries are performed annually or more often as needed.

Study design. The automatic watering system was unhooked from all cages, and each cage was provided with a single water bottle, during a 1-wk acclimation prior to each study period. After acclimation, the water bottle on each cage was weighed on weekdays at 0700, 1000, 1300, 1600, and 1900 during the months of January and July (a total of 27 nonconsecutive days of measurements). To calculate water intake during each time period, 1 g of bottle weight was considered equivalent to 1 mL of water. Whenever the water in a bottle was close to being depleted halfway, the bottle was refilled and weighed again. Although no dripping was observed when water bottles were removed from the cages, limited dripping sometimes occurred when bottles were replaced after weighing. After determining that 10 drops comprised 1 mL of water, this loss was taken into account by counting all drops every time a bottle was put back on a cage and subtracting the lost water volume the next time the bottle was weighed. In addition, water consumption during the weekend (from 1900 Friday until 1900 Sunday) was measured on several occasions.

Data regarding water intake per cage was normalized by body weight of the animals in kilograms. When marmosets were pair-housed, the total water consumed by the pair was divided by the sum of their body weights to obtain relative water intake in milliliters per kilogram. Throughout the 2 mo of measurements, the animals were monitored to confirm that their eating and drinking habits were unaffected; weight and BCS remained stable also. Water measurements were excluded if an animal was out of the cage for more than 2 h (approximately 50 of 1750 observations), for example, when an animal was sedated for a procedure. The housing status of the animals was unchanged between January and July, with 2 exceptions: one animal singly housed in January was paired with another animal in July, and one animal was paired in January but singly housed in July (both situations are referred to as mixed-status cages).

Preliminary analysis with increased moisture content in diet. In a preliminary analysis of approximately 1 mo in duration, water intake by the marmosets was measured as described earlier for 16 nonconsecutive days. However, during this period, the moisture content in the diet was comparatively elevated because biscuits were soaked in water for about 20 min before

the water was decanted (estimated moisture content of a single meal equal to 61.5%). Subsequently, these data were used only to assess the effect of diet moisture content on the amount of water consumed from a water bottle.

Statistics. Statistical analyses were performed by using Prism version 7.0d (GraphPad Software, La Jolla, CA). Sample distribution and variances were determined, and normally distributed data were tested at a 95% confidence level by using one-way ANOVA followed by the Tukey posthoc test for multiple comparisons, along with paired Student *t* tests, when applicable. Nonparametric and small sample-size data were tested by using the Kruskal-Wallis and Mann-Whitney tests, as warranted. Pearson correlation and linear regression analysis were used to evaluate the relationship between weight (in kilograms) and average daily water intake (in milliliters per day). Pair-housed data were used to compare water intake by age only when the paired animals were born within 6 mo of each other; similarly, only pairs in which members were within 40 g of each other in body weight and that had the same BCS were used to assess water intake relative to body weight and BCS, respectively. Outliers were removed from further consideration after an initial review of the entire dataset in a scatterplot and when determined to significantly skew the mean according to the 2-standard deviation rule. A *P* value of less than 0.05 was considered statistically significant.

Results

Average and characterization of daily water intake. Table 1 shows the study results according to older compared with younger animals, male compared with female marmosets, and singly compared with pair-housed marmosets. When 2 cages of animals (1 single-housed and 1 mixed housing) were removed as outliers due to significant skewing of the mean, the overall daily water intake was 61.3 ± 20.4 mL/kg (mean \pm 1 SD; median, 61.5 mL/kg; range, 36.3 to 99.0 mL/kg; $n = 19$; $m = 27$ d averaged over 2 mo of study). The distribution of average daily water intake values between singly, pair-housed, and mixed cages is presented in Figure 1. For measurements from individual cages of singly and pair-housed marmosets, water intake was fairly consistent from day to day (the SD was within 20% to 30% of mean values; Figure 1). Water intake did not differ across the 2 mo of the study (January, 60.2 ± 22.5 mL/kg daily; July, 62.0 ± 19.4 mL/kg daily; paired *t* test: $P = 0.47$, $t = 0.750$, $df = 10$). A significant main effect occurred between water measurement time periods (Figure 2) as determined by one-way ANOVA ($F_{4,50} = 6.591$, $P < 0.001$); Tukey posthoc testing subsequently revealed that the significant difference was between water consumption from 1900 to 0700 (4.5 ± 2.2 mL/kg) compared with every time period when room lights were on (0700 to 1000: 11.2 ± 5.6 mL/kg, $P < 0.05$; 1000 to 1300: 12.6 ± 5.2 mL/kg, $P < 0.01$; 1300 to 1600: 13.5 ± 5.2 mL/kg, $P < 0.01$; and 1600 to 1900: 14.2 ± 6.2 mL/kg, $P < 0.001$). No significant difference occurred in average water intake measurements during weekdays ($n = 27$) compared with the weekend ($n = 7$; paired *t* test, $P = 0.06$, $t = 2.085$, $df = 10$).

Water intake during preliminary analysis with increased dietary moisture content compared with water intake during 2 mo of study. Daily water intake during the preliminary set of water measurements, when the diet contained increased moisture (37.6 ± 17.7 mL/kg; median, 38.8 mL/kg, $n = 17$) differed significantly from intakes in January (60.2 ± 22.5 mL/kg) and July (62.0 ± 19.4 mL/kg; one-way ANOVA: $F_{2,437} = 13.39$, $P < 0.0001$). Tukey posthoc testing demonstrated that marmosets drank significantly less water during the preliminary period than during the 2 mo of study ($P < 0.0001$ for both months).

Table 1. Daily water intake (mL/kg; mean \pm 1 SD) of study marmosets according to age (older, 5–12 y; younger, 1–2 y), sex, and housing status

	No. of animals	Average water intake	Median	Range
Older male, singly housed ^a	1	143.1 \pm 23.6	140.3	103.9–206.2
Older females, singly housed	3	70.8 \pm 17.3	69.7	14.9–110.9
Older pairs, mixed sex	6	72.0 \pm 14.1	73.6	35.2–112.3
Younger pairs, both male	6	42.7 \pm 14.9	42.9	8.4–80.6
Younger pair, both female	2	66.2 \pm 19.8	75.3	20.5–96.1
Younger pair, mixed sex	2	50.1 \pm 16.1	48.4	15.6–85.8
Older (female)–younger (male) pair ^b	2	111.7 \pm 32.2	119.9	15.9–170.5
Overall	22	71.5 \pm 32.9	67.5	8.4–206.2
	19 ^c	61.3 \pm 20.4 ^c	61.5 ^c	8.4–112.3 ^c

The water intake of a pair is divided by their combined weight to determine individual daily intake. The range across days depicts minimum and maximum values of daily water consumption in each group.

^aSingle 1

^bMixed 1

^cData from outliers (Single 1 and Mixed 1) are removed.

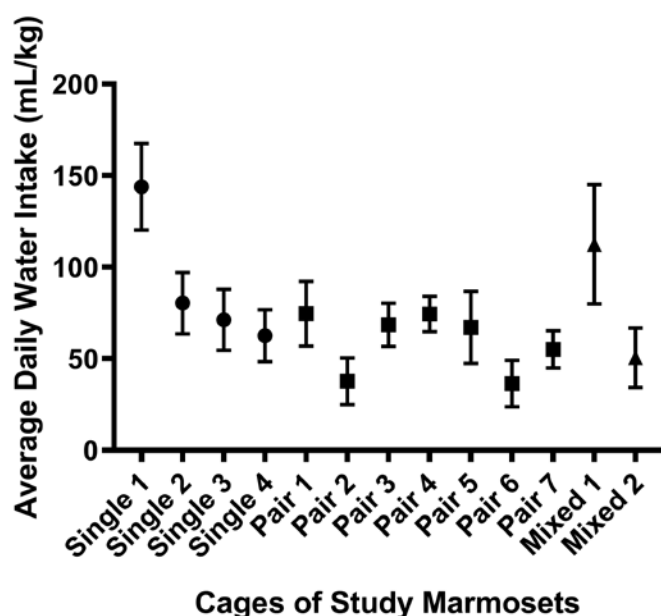


Figure 1. Daily water intake (mL/kg; mean \pm 1 SD) for singly housed, pair-housed, and mixed housing cages of marmosets (mean \pm 1 SD). The water intake of a pair is divided by their combined weight to determine individual daily intake. Mixed 1 refers to one animal housed alone in January and paired in July; Mixed 2 refers to 2 animals that were pair-housed in January, but only one animal was left in July. Single 1 and Mixed 1 are considered outliers and were removed from subsequent analyses.

Comparison of daily water intake according to age, weight, BCS, and housing status. Daily intake of drinking water was higher in older marmosets than younger animals (Mann–Whitney test; $P < 0.01$; Figure 3) and was significantly correlated with animal weight (Pearson correlation: $r = 0.76$, $df = 1, 13$, $P < 0.001$; regression line, $y = 145.4x - 31.81$, $R^2 = 0.58$; $P < 0.001$; Figure 4). Daily water intake was not significantly correlated with either BCS (Kruskal–Wallis test, $P = 0.84$; Figure 5) or housing status (Mann–Whitney test, $P = 0.48$; Figure 6).

Discussion

During the 2 mo of study, the marmosets ingested a daily average of 61.3 ± 20.4 mL/kg water in addition to any liquid consumed in the diet. This result is comparable to an earlier

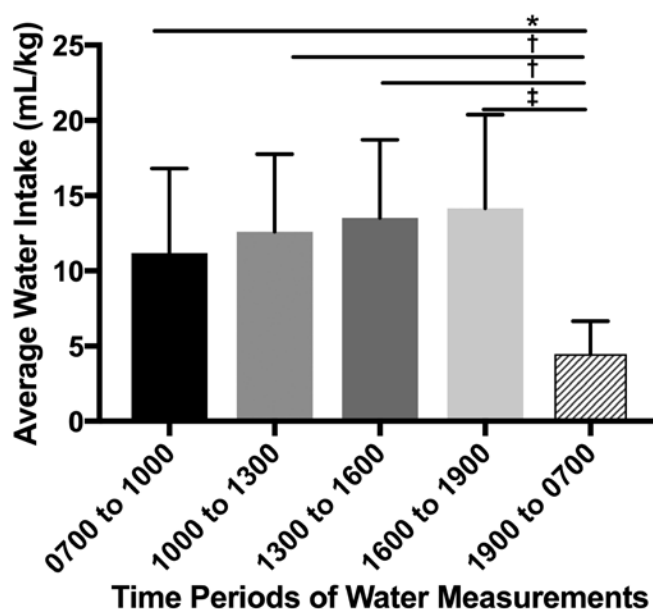


Figure 2. Water intake (mL/kg; mean \pm 1 SD) of study marmosets for each time period during the day (0700 to 1000, 1000 to 1300, 1300 to 1600, and 1600 to 1900), and night (1900 to 0700). One-way ANOVA ($F_{4,50} = 6.591$, $P < 0.001$) followed by Tukey posthoc testing (*, $P < 0.05$; †, $P < 0.01$, and ‡ $P < 0.001$).

study that estimated the daily water intake of common marmosets, housed individually inside metabolic cages, as 37.0 mL/kg.⁵ In that study, notably, the animals received no food in the metabolic cages for 24 h, or diet was given but not described. In addition, we found that water consumed per cage of marmosets (on a mL/kg/d basis) varied between cages, but intracage variation was limited; this outcome demonstrates the necessity of getting baselines of daily water intake for each animal prior to any fluid regulation. In the preliminary period of measurements when biscuit moisture was increased (61.5% moisture content per meal) relative to the 2 mo of the study (22.3% moisture content per meal), the marmosets drank significantly less, which highlights that auxiliary sources of water should be well defined. Because they are a diurnal species, we expected that the marmosets would drink minimally at night, and such was the case; healthy marmosets likely do not need access to water during this time. Importantly, overall water intake did not

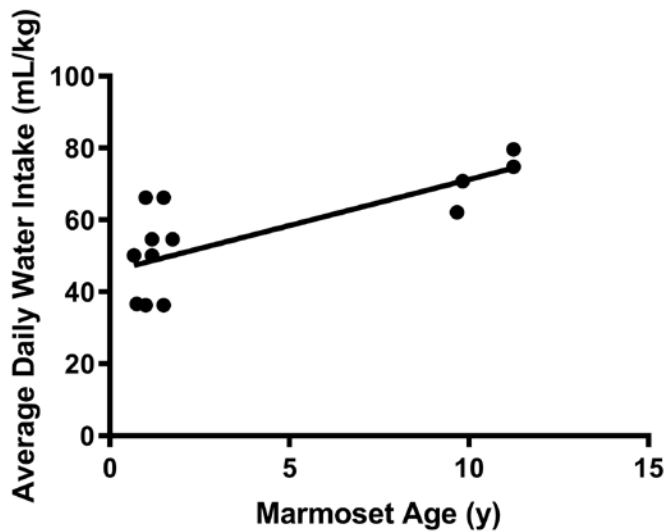


Figure 3. Average daily water intake (mL/kg) of younger (1–2 y, $n = 10$) and older marmosets (5–12 y, $n = 5$) represented in a box-and-whisker plot. Whiskers extend down to the 10th percentile and up to the 90th percentile. Mann–Whitney test, $P < 0.01$.

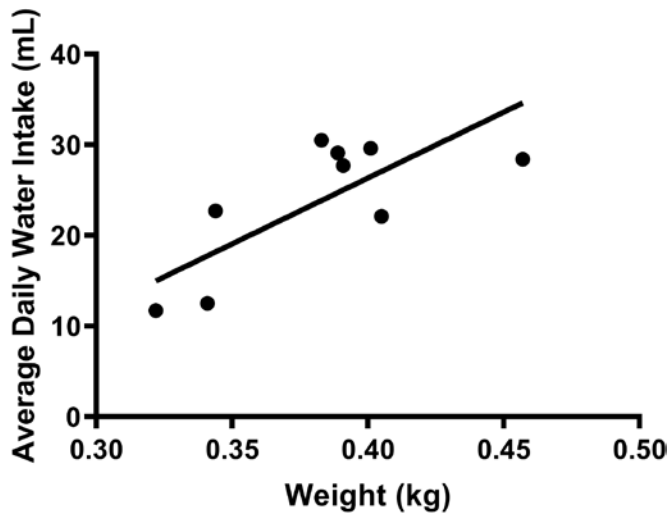


Figure 4. Average daily water intake (mL) of study marmosets ($n = 15$) according to weight (kg); members of a pair were included only when their weights were within 40 g of each other. Pearson correlation ($r = 0.76$, $P < 0.001$) with regression line ($y = 145.4x - 31.81$, $R^2 = 0.58$; $P < 0.001$).

differ between weekday and weekend measurements, providing support that bottle manipulations during the week did not influence the animals' drinking patterns.

A limitation of our study design was that water consumed by a pair of marmosets was determined by weighing a single water bottle, and we cannot assume that dividing the total water volume by the combined body weight of both animals reflects the water intake of either animal by itself. In addition, individual food consumption, activity, and fecal and urinary output were not quantified for the study marmosets; these parameters, among others, can influence water intake.^{2,7,10} Our results show the 5- to 12-y-old marmosets drank more than the younger marmosets (1 to 2 y) on an mL/kg/d basis. Although a study involving rhesus macaques found that the oldest group of animals (20 to 36 y) drank less than their middle-aged (13 to 17 y) and young adult (7 to 9 y) conspecifics,⁹ water intake by the middle-aged cohort did not statistically differ from that

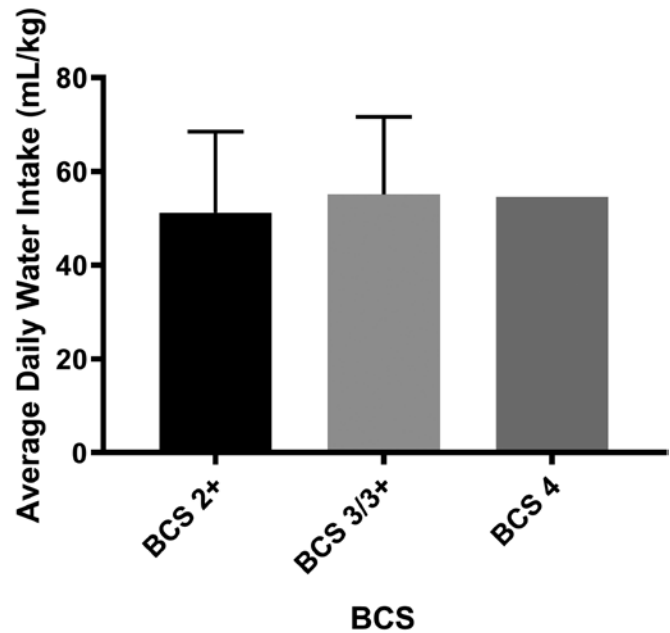


Figure 5. Daily water intake (mL/kg; mean \pm 1 SD) of study marmosets according to BCS (BCS 2+, $n = 4$; BCS 3 or 3+, $n = 7$; BCS 4, $n = 2$ [no SD]). Members of a pair were included only when both had the same BCS. Kruskal–Wallis test, $P = 0.84$.

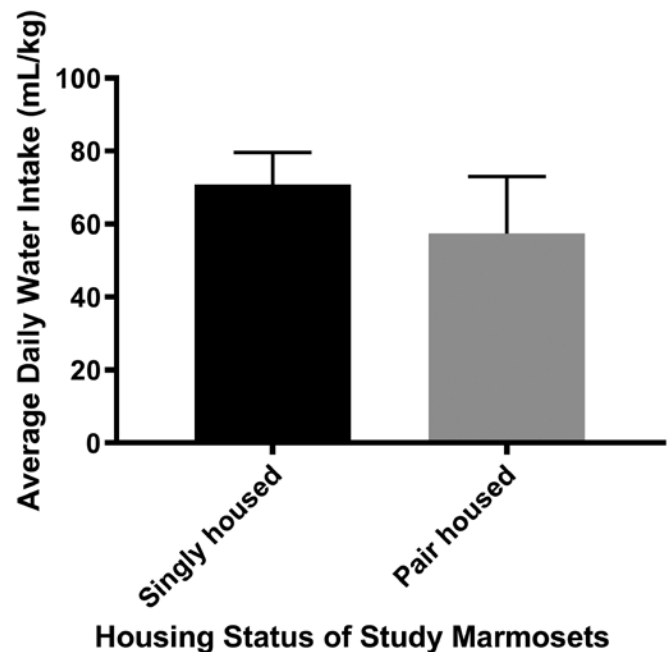


Figure 6. Daily water intake (mL/kg; mean \pm 1 SD) of study marmosets according to housing status: singly housed, $n = 3$; pair-housed, $n = 16$. The water intake of a pair is divided by their combined weight to determine individual daily intake. Mann–Whitney test, $P = 0.28$.

of the young adults. Because our 5- to 12-y-old marmosets are comparable to the geriatric and middle-aged groups of macaques in the earlier study, and our group of marmosets aged 1 to 2 y is best described as including both subadults and young adults,⁸ it is difficult to compare age-related water intake between marmosets and macaques. Water intake in the study marmosets was positively correlated with weight but not with BCS. In water regulation protocols for other species, animal weight is sometimes used to set a minimum for daily

water requirements,³ however, we consider it premature to do so for marmosets because of the possibility of outlier animals, as discussed later. No difference in water intake according to sex was noted, but the numbers were too low to evaluate this association by using statistics.

Two outlier cages (Single 1 and Mixed 1) were removed from further data analyses because of their excessively high water intake compared with other marmosets in the room. One of these marmosets (Single 1) was a 12-y-old male with an average daily water intake of 143.1 ± 23.6 mL/kg, about twice that of age-equivalent older male–female pairs (72.0 ± 14.1 mL/kg/d). Behavioral observations through video monitoring revealed that Single 1 was hyperactive, with intermittent locomotor stereotypies (for example, vertical flipping and circling), in addition to having longer and more frequent drinking bouts. Although repeated CBC and serum chemistries were unremarkable, Single 1's urine specific gravity was low (1.007 ± 0.002 , $n = 2$) in comparison to an average of 1.024 ± 0.013 obtained from the urine samples of 11 other animals in the room. Differential diagnoses for polydipsia and hyposthenuria in Single 1 include renal disease, diabetes insipidus, psychogenic polydipsia, and excessive thirst due to hyperactivity; the clinical evaluation of Single 1 is ongoing. The second outlying group (Mixed 1) had a high water intake in the first month of study when an adult female lived alone; this animal was not observed to exhibit any unusual behavior and had normal laboratory results. Both of these cases again reinforce that each marmoset needs to be evaluated individually.

Given these findings, we have developed some early recommendations for establishing a fluid regulation protocol applicable to our marmoset colony. Current physical examinations and bloodwork evaluations that establish overall health are required before placing an animal on study. Next, given the variability in water intake between individual marmosets, baseline water intake of each on-study animal and any cage-mate must be evaluated during unrestricted water access over a minimum of 10 to 14 d; this requirement means that paired animals have to be separated for part of the day. At the outset of training, the average fluid intake from the baseline measurements is used as each animal's daily minimum; once training has become routine and pending discussions between researchers and the veterinary staff, an animal's daily minimum may be modified depending on individual motivation and other factors. On training days, on-study, paired animals are kept apart for 2 to 3 h in the morning and afternoon while each is trained; when one animal of a pair is off-study, it receives free access to water during the 2 daily training sessions of its cage-mate. While these recommendations undergo refinement, daily water intake is logged every day of regulation for on-study animals as well as for any cage-mates; in addition, both are weighed daily whenever fluid access is controlled. Water bottles do not have to be provided overnight, and animals are given water without restriction on the weekends. Because the amount of water in the food greatly affects how much water is consumed through drinking, no appreciable dietary changes can be made during water regulation unless the research staff is notified and approves the proposed modification; water content from meals should be discussed in any water regulation protocol. Another consideration is that experimental animals should not be transferred to new rooms or different cage setups unless a baseline water intake is reestablished after the move. Following a standard operating procedure based on these guidelines, researchers at our institution have undertaken water regulation with several animals; to date, test performances have been

markedly improved in the absence of any deleterious effect on animal health or behavior.

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