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Species Differences in Urinary Specific Gravity of Various Nonhuman Primates

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Abstract

Specific gravity is a urinalysis parameter that measures the concentration of excreted molecules in urine. Clinically, specific gravity measures the kidney's ability to dilute or concentrate urine and reflects the concentration of urine during renal filtration. We explored species differences in the specific gravity of various primates' urine samples, including *Alouatta palliata*, *Alouatta caraya*, *Callithrix jacchus*, *Sapajus apella*, and *Saimiri sciureus.* A refractometer was used to measure the urine samples. The urine samples obtained from the wild howler monkeys, *Alouatta palliata,* had a higher specific gravity than the other primate species' samples, which were all obtained from captive animals and did not have differing values. These results held true even when controlling for individual differences between animals. Our results indicate that open access to water while in captivity affects specific gravity more than species differences in renal physiology, however specific gravity is a general parameter and might not detect other possible differences in renal physiology that exist between nonhuman primate species.

Introduction

Specific gravity is an urinalysis parameter that measures the concentration of excreted molecules in a subject's urine. More specifically, this parameter is the ratio of the density of urine relative to the density of water and reflects the concentration of urine during renal filtration (Chadha et al., 2001). Specific gravity measures the kidney's ability to dilute or concentrate urine, the higher the specific gravity, the more concentrated the urine and vice versa (Flasar, 2008). The specific gravity of an individual can vary over time and depends on the ability of the hypothalamic osmoreceptors to respond to varying plasma osmolarity, the ability of the body's baroreceptors to sense changes in blood volume or pressure, and the release of antidiuretic hormone (ADH) from the posterior pituitary in response to increased plasma osmolarity (Chew et al., 1989). Antidiuretic hormone regulates the reabsorption of water at the collecting ducts of the kidney. An increase in the secretion of ADH results in the loss of less water, causing the urine volume to decrease. When the secretion of ADH decreases, less water is reabsorbed by the collecting duct of the kidney, which increases the flow of water to the urinary tract and into the bladder, increasing urine volume (Reeves et al., 1992).

Used clinically, specific gravity measurements can evaluate kidney function, diagnose various renal diseases, and aid in the measurement of hormone concentration. Conditions such as diabetes insipidus, a disease in which the posterior pituitary halts its secretion of ADH, results in a low specific gravity. Renal tubular necrosis, kidney failure, and extreme kidney infection could also result in a decreased specific gravity. A high specific gravity could be the result of heart failure, dehydration, renal artery stenosis, and sugar or glucose in the urine (Dugdale, 2013).

Literature on this subject has shown that specific gravity can differ between individuals of the same species. In a study comparing the specific gravity between pet dogs of various ages, it was found that specific gravity values were lower in the evening than in the morning, unrelated to the dog's gender and lower in older dogs than in younger dogs (Vonderen et al., 1997). A different study comparing the specific gravity of humans, captive gorillas and woolly monkeys discovered that all three species had average urinary specific gravities that differed significantly between one another, but average specific gravity values did not differ between individuals within each species (White et al., 2010). In a study that conducted a urinalysis of three species of captive rhinoceros (*Rhinoceros unicornis*, *Dicerorhinus sumatrensis* and *Diceros bicornis*), it was found that all three species differed in average specific gravity values. The researchers concluded that the lower average specific gravity value of the *sumatran* rhinoceros was possibly due to the increased moisture content associated with the fresh browse diet of the rhinoceros species (Haffey et al., 2008).

Whether an animal lives in captivity or the wild can also have an impact on specific gravity values. Fasting brown rats (*Rattus norvegicus*) that are wild have been found to have a more sensitive adrenal cortex than fasting brown rats housed in captivity, causing the wild rats to ingest more water than their captive counterparts and have a lower specific gravity (Mosier and Richter, 1954).

There has been little research done into the comparison of renal physiology in primates. However, a previous study has shown that renal filtration rate may differ amongst nonhuman primate species (Fanelli et al., 1970), giving reason to believe that primate species may have different renal physiologies. Other observed differences in nonhuman primate renal physiology include varying degrees of urinary uric acid excretion between chimpanzees and rhesus, baboon, and guenon monkey. Primate species also differ in the presence or absence of the renal enzyme, uricase.

Because of these differences, we believe that a difference between specific gravity will exist in various nonhuman primate species within the infraorder Platyrrhini (the animals in our study are much more closely related than those in the White et al. (2010) study mentioned previously.) We also believe that exploring this difference in specific gravity between primate taxa could lead to interesting insights into the differences in the primates' renal physiologies and lead to differences in veterinary treatments and procedures. Our central focus is to explore the species differences in the specific gravity of various primates' urine samples - specifically *Alouatta palliata*, *Alouatta caraya*, *Callithrix jacchus*, *Sapajus apella*, and *Saimiri sciureus*. *Alouatta palliata* is the only species of primate in this study that was wild, the rest of the primates were held in captivity. The specific gravity of primates within each species, and between individual animals, was also compared.

Methods

Urine samples were collected during previous research studies. Urine samples from *Alouatta palliata* were obtained from wild animals N= 10,74 (number of individuals, number of urine samples), located in Hacienda La Pacific, Costa Rica (10°28'N, 85°07'W). *Alouatta caraya* urine samples were obtained from captive animals N=3,15 housed at the Pittsburgh Zoo. *Callithrix jacchus* samples were collected from captive animals N=2,76 housed at Northeast Ohio Medical University's Comparative Medicine Unit. Urine samples from *Sapajus apella* were obtained from captive animals N=2,5 housed at Duke University. *Saimiri sciureus* samples originated from captive animals N=2,65 housed at the MD Anderson Cancer Center. Samples were collected by placing a plastic or aluminum foil sheet underneath animals, observing voiding, and pipetting urine from the sheet. Urine was stored frozen at -80°C until analysis. None of the primates sampled had any known health conditions that would potentially affect urinary concentration.

Urine collection was opportunistic, with most collections occurring in the morning. The measurement of specific gravity of the urine samples was conducted using a pocket refractometer (ATAGO PAL-10S.) There are numerous reasons why refractometers have historically been used in veterinary medicine to measure the specific gravity of urine, including its non-invasive nature, the ability to produce measurements with small amounts of urine, and its relative inexpensiveness (George, 2001). Urine samples were thawed to room temperature and a pasteur pipette was used to place a few drops of urine on the surface of the refractometer to measure the specific gravity. The resulting value was then recorded. To avoid cross-contamination, the surface of the refractometer was cleaned between samples. Refractometers work by measuring the angle of refraction between the air and the solution (in this study, the solution is urine) (DePalma, 2013).

Statistical analyses performed by the program SPSS Statistics were done to compare the resulting specific gravity values. The mean +/- SD of each species' specific gravity was calculated. An ANOVA was conducted to determine if the differences between species means were statistically significant. An ANCOVA was also performed, with individual identity as a covariate, to control for differences in specific gravity between individual animals. Bonferroni post-hoc tests were also conducted. Our level of significance was 0.05.

Results

The one-way analysis of variance (ANOVA) determined that there was a significant difference between the average specific gravity value of *Alouatta palliata* and the average specific gravity values of the other primate species (F=31.740, p<.001). The wild *Alouatta palliata*'s average specific gravity value was significantly higher (more concentrated) than all other species (*Alouatta caraya* p<0.001, *Sapajus apella* p=0.002, *Callithrix jacchus* p<0.001, *Saimiri sciureus* p<0.001). However, all other captive-housed species did not have significantly different specific gravity values (p>0.05). The mean of the specific gravity samples for the wild *Alouatta palliata* (N= 74, x̄ = 1.04466 ± 0.004453 kg/m^3) was significantly higher than that of the means of the rest of the captive primate samples (*Alouatta caraya* N= 15, x̄ = 1.02560 ± .017189 kg/m^3, *Sapajus* a*pella* N= 5, x̄ = 1.02120 ± .008408 kg/m^3, *Callithrix jacchus* N= 76, x̄ = 1.02637 ± .006697 kg/m^3, *Saimiri sciureus* N= 65, x̄ = 1.02137 ± .015307 kg/m^3) (F=31.740) (Figure 1 and Table 1).

The ANCOVA determined that individual differences in specific gravity between animals were not significant (F=2.368, p=0.126, η^2 = 0.012) (Table 1). Species still significantly differed in specific gravity after accounting for individual variation (F=10.986, p=<0.001, η^2 = 0.189).

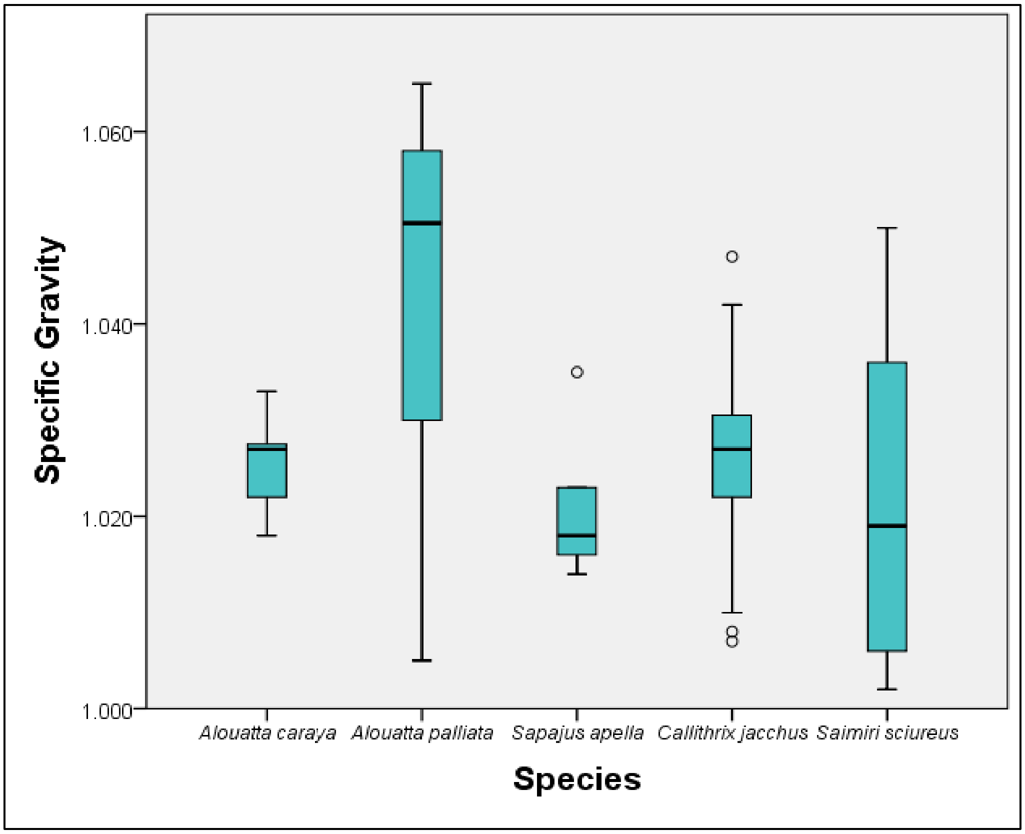

Figure 1. Comparisons of urinary specific gravity values of various nonhuman primate species. Circles on graph represent outliers in the data (outliers exceed 1.5 interquartile ranges above/below the upper/lower quartile of data).

Individual	Aguacate	Blue Diamond	Juniper	Mimosa	Orange Keychain	Orange Triangle	Orf	Silver Triangle	Teaford	White Keychain
Specific Gravity ($\bar{x}$, s)	1.041 ± 0.015	1.057 ± 0.003	1.041 ± 0.018	1.041 ± 0.021	1.052 ± 0.009	1.043 ± 0.012	1.041 ± 0.016	1.056 ± 0	1.023 ± 0	1.055 ± 0.007
Sample Size	11	2	9	3	4	2	4	1	1	2

Table 1. Comparison of average urinary specific gravity value between known ID individuals of the same species (*Alouatta palliata*).

Discussion

The primary goal of this study was to compare the average urinary specific gravity values between various species of nonhuman primates. The only species of primate that had an average specific gravity value that differed from the other species was *Alouatta palliata*, which was the only primate species in our study that was not housed in captivity. The fact that the wild primate urine samples had a higher average specific gravity value suggests that primates living in the wild may not have a constant water source, which could lead to less water intake, and thus more concentrated urine. In this study, primates with a steady source of water while in captivity lead to lower average urinary specific gravity values, and thus more dilute urine.

Our results differ from Mosier and Richter's (1954) results which found that wild brown rats had lower specific gravity values than domesticated brown rats. This can most likely be explained by the fact that Richter and Mosier's brown rats were studied under fasting conditions, whereas the environment of the wild *Alouatta palliata* animals was not altered in any way, and the captive primates were given a steady supply of food. Our data indicate that the environmental difference in available water had more of an effect on the differing specific gravity values between *Alouatta palliata* and the rest of the primate species, than differences in these species' renal physiologies.

Our findings differed from both White's (2010) study and Haffrey's (2008) study, since only one of the species we were studying (*Alouatta palliata*) had an average specific gravity that differed significantly from the other species. These differences can most likely be attributed to the fact that the species in our study were much more closely related than the species in those studies mentioned previously, and all belonged to the infraorder Platyrrhini. Our results indicated that species do not differ in average specific gravity values, but rather environmental factors caused *Alouatta palliata*'s values to differ.

When comparing average specific gravity values between individuals of the same species, our results differed from Vonderen et al. (1997) in that we found urinary specific gravity values to differ between primate species even once individual animals were controlled for. Vonderen found urinary specific gravity values for domestic dog breeds ranging from 1.006-1.050. In a different urinalysis study of wild chimpanzees, urinary specific gravity values ranged from 1.000- 1.050 (Leendertz et al., 2010). In comparison, our overall specific gravity values ranged from 1.002-1.065. Our values are similar in range to the average specific gravity values found in both Vonderen and Leendertz studies, however our values for *Alouatta palliata* are somewhat higher (more concentrated) than the maximum values of both of the studies. This means that the urine samples from our *Alouatta palliata* animals might have been abnormally more concentrated than what is typically found in nature, which might explain why the species had an average specific gravity value higher than the averages of the other primate species in our study.

Past research has shown that different primate species may have varying rates of renal filtration (Fanelli, 1970). Humans also have varying rates of renal filtration that can change based on age, blood creatine measurement, ethnicity, gender, height, weight and water intake (Dugdale, 2013). To compare this with our results, it can be concluded that the wild *Alouatta palliata* has a different renal filtration rate than the rest of the species in our study, which appear to have equal renal filtration rates between each another. However, we can attribute these differences in renal filtration rates to varying environmental conditions, not differing renal physiologies between primate species. This could affect the world of veterinary medicine when conducting urinalysis on primates to detect various renal and renal-related diseases. For example, when attempting to detect renal failure in wild primates, a higher specific gravity baseline should be used for detection since their normal average urinary specific gravity values are higher than their domestic counterparts.

Possible sources of error that could have occurred in our study include our low number of individual animals per species, (besides *Alouatta palliata*) and our low number of *Sapajus apella* urine samples. Most of the species sampled only had 2-3 individuals from which urine was collected. In comparison, the primate studies referenced previously included no fewer than 8 individual animals from each species group. This might have given an inaccurate representation of the average specific gravity values from those species, since so few individuals were involved in the analysis. Also, the *Sapajus apella* primates were represented by a total of only 5 urine samples (collected from 2 individuals), which might not have accurately represented this species average specific gravity value. Although our samples were repeatedly frozen and thawed, this should not have affected our results (however, these freeze-thaw cycles are known to affect urinary hormone concentrations) (Anestis et al., 2009).

Future studies to be conducted in this area could include the comparison of specific gravity values of urine between more species of primates with larger numbers of individual animals being studied. The differences in urinary specific gravity values between wild and captive primates of the same species should also be studied to determine if wild animals would still have higher average specific gravity values than their domestic counterparts. Studying different species of primates and manipulating their access to water to observe how the average specific gravity value changes over time is also a suggested future study. If two or more groups of different primate species had a higher specific gravity without a constant water source than with, this would support our conclusion that specific gravity is based off of environmental factors and not differences in renal physiologies. Overall, our study suggests that a difference in average urinary specific gravity values exists between wild and captive primates. The wild species of primate *Alouatta palliata* had a higher average specific gravity value than the captive *Alouatta caraya*, *Callithrix jacchus*, *Sapajus apella*, and *Saimiri sciureus.* It was concluded that differences in the availability of water was what led to the differing specific gravity values, and not differences in renal physiologies between primate species. Our data indicates that there are no major differences in renal physiology, related to the concentration of urine, between various nonhuman primate species. Specific gravity is a general measure, so studying this one parameter itself might not detect other possible differences in renal physiology that exist between nonhuman primate species.

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