



Use of Hydrophobic Sand as a Refinement for Urine Collection in Mice and Rats

Introduction

Metabolic cages used to collect urine are a wire bottom, barren, non-enriched environment which does not allow for natural behaviors like nesting and digging and is known to increase stress in mice and rats.¹ Hydrophobic sand has been designed to allow urine collection in regular caging and provides rats and mice with an environment allowing more natural behaviors which should decrease stress. In addition, the space required and time to prepare the cages with hydrophobic sand is significantly lower than for metabolic cages.

This study aims to compare the volume and characteristics of urine samples collected in rats and mice after 6 or 24 hours in both caging systems as well as the stress levels in mice after 24 hours.



Cages with hydrophobic sand.



Metabolic Cages.

Methods

Thirty two male C57BL/6NCrI mice and sixteen male Sprague Dawley (CRL) rats were divided in 2 groups. Half the animals were placed in metabolic cages and the other half in static disposable caging filled with hydrophobic sand. The hydrophobic sand in each cage was approximately 5.5 grams per square inch of floor space. Animals were given access to water bottles and regular chow ad lib. Urine was collected using pipettes in the cages with hydrophobic sand and directly from the collection cup in metabolic cages and volumes were recorded at different time points (3, 6, and 24h for mice; 2, 4, and 6h for rats). After the last time point urine was combined from all time points for each animal and analyzed as one sample using ADVIA Chemistry XPT Systems.

Fecal samples were collected to measure cortisol levels from the mice at time of delivery to the facility (positive control), after a week of acclimation without being handled (negative control), and after 24 hours of urine collection in both set ups.

Prior starting this study, a pilot study was conducted which determined the best volume of hydrophobic sand to provide as well as confirming water bottles do not leak and contaminate the urine sample in the disposable cages used with hydrophobic sand.



Cage that needs more hydrophobic sand.



Collecting urine with a pipette.

Results

Figure 1: Total Urine Collected in Mice after 24 Hours

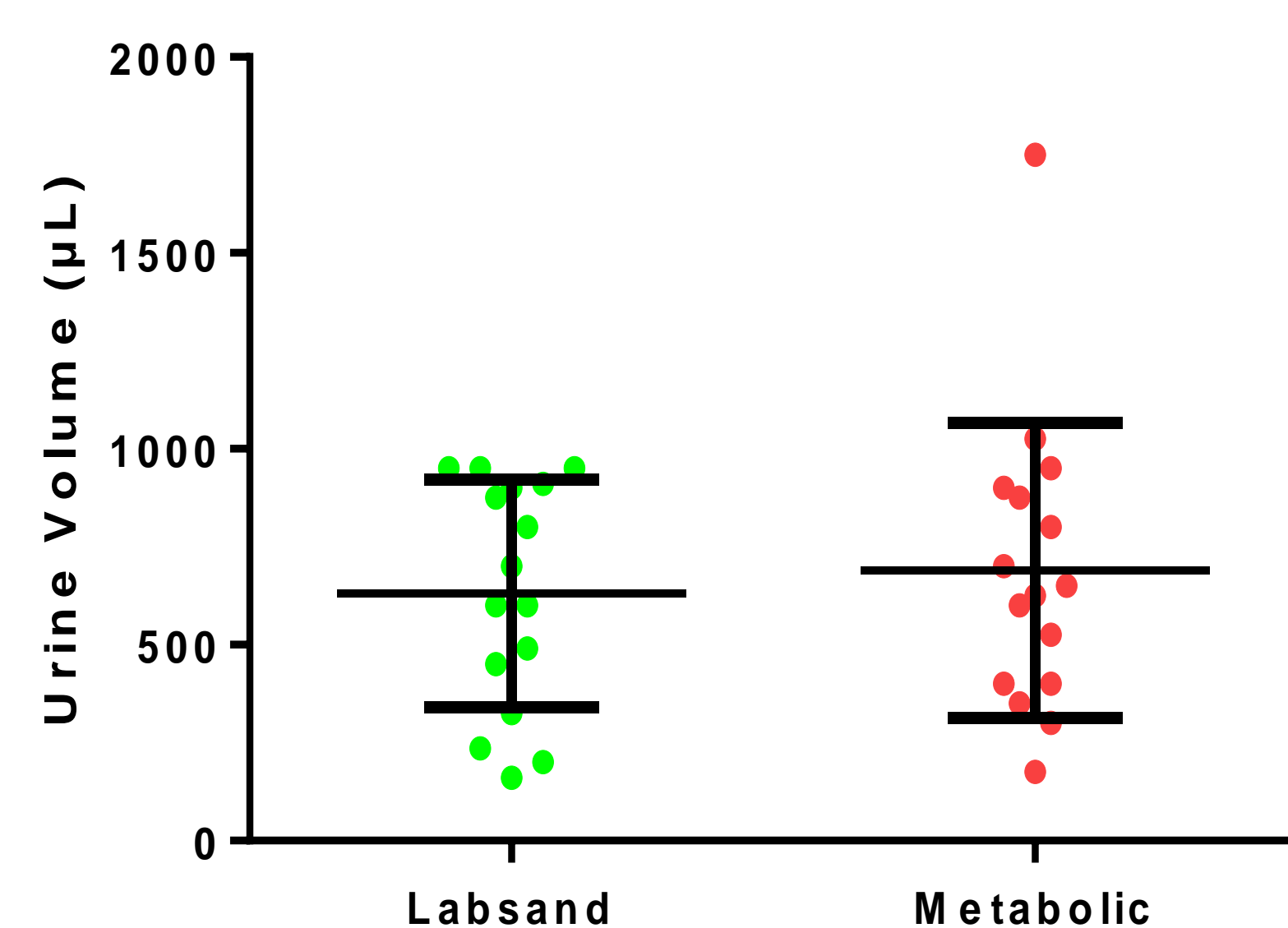
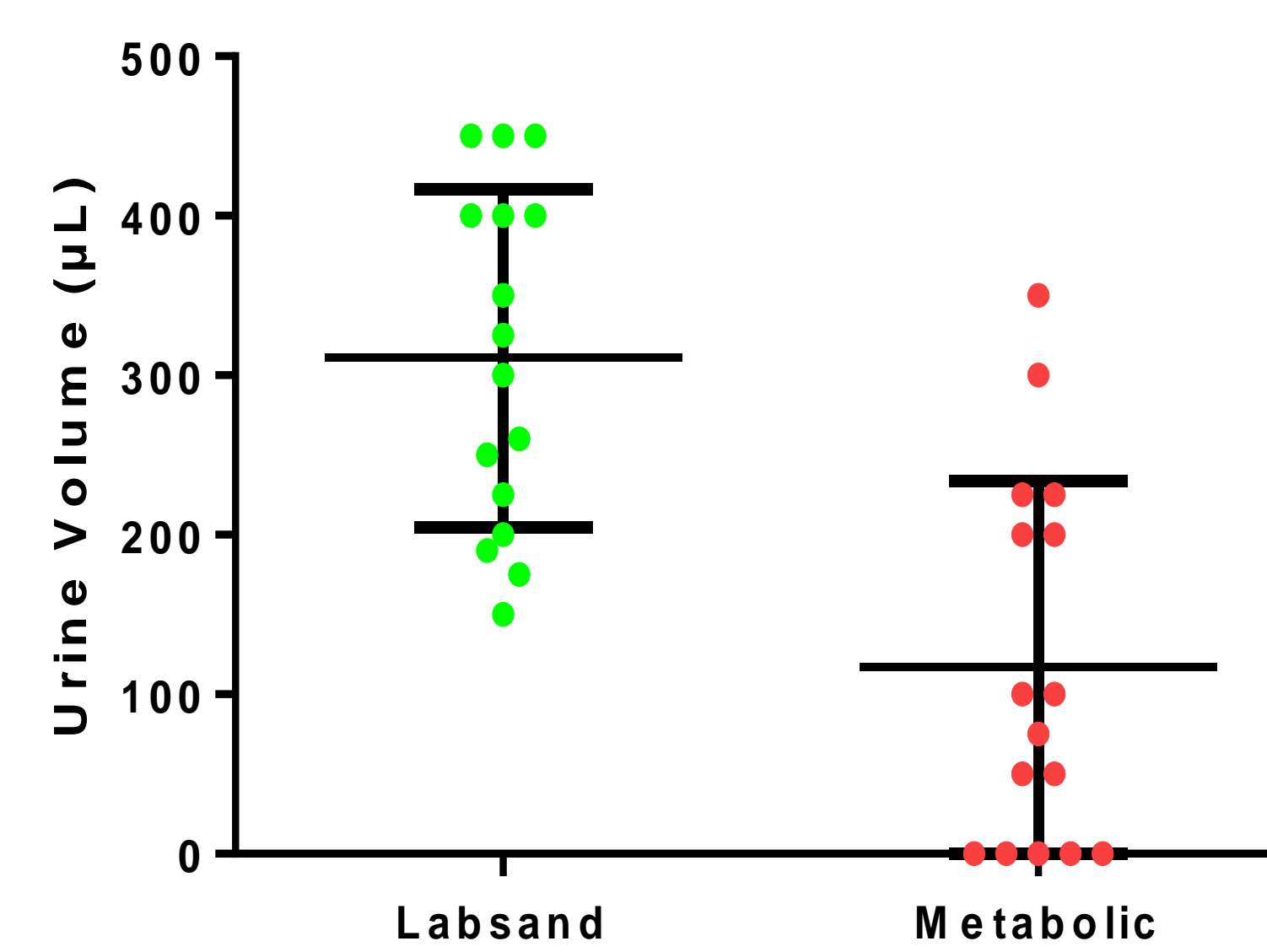
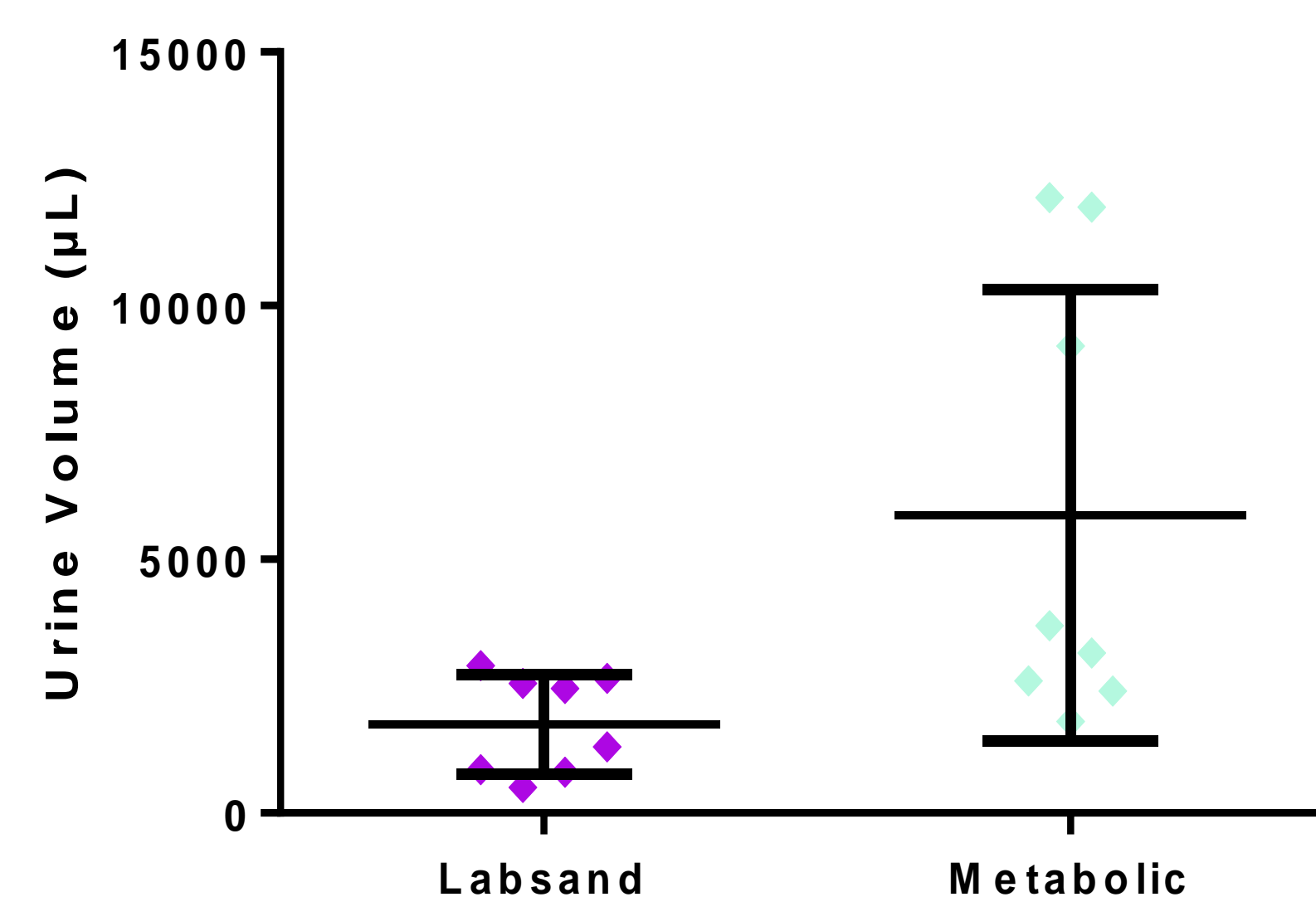


Figure 2: Total Urine Volume Collected in Mice after the 6 Hour Timepoint



For mice after 24 hours, the total volume was not significantly different ($p=0.6289$) between the two set ups (Fig 1). However after 6 hours, there was significantly more urine collected from mice in hydrophobic sand than from metabolic cages ($p < 0.0001$ – Fig. 2). In rats, significantly more urine ($p = 0.0350$) was collected in rats in metabolic cages versus hydrophobic sand after 6 hours (Fig 3). Urine analysis was run on commonly

Figure 3: Total Urine Volume Collected in Rats



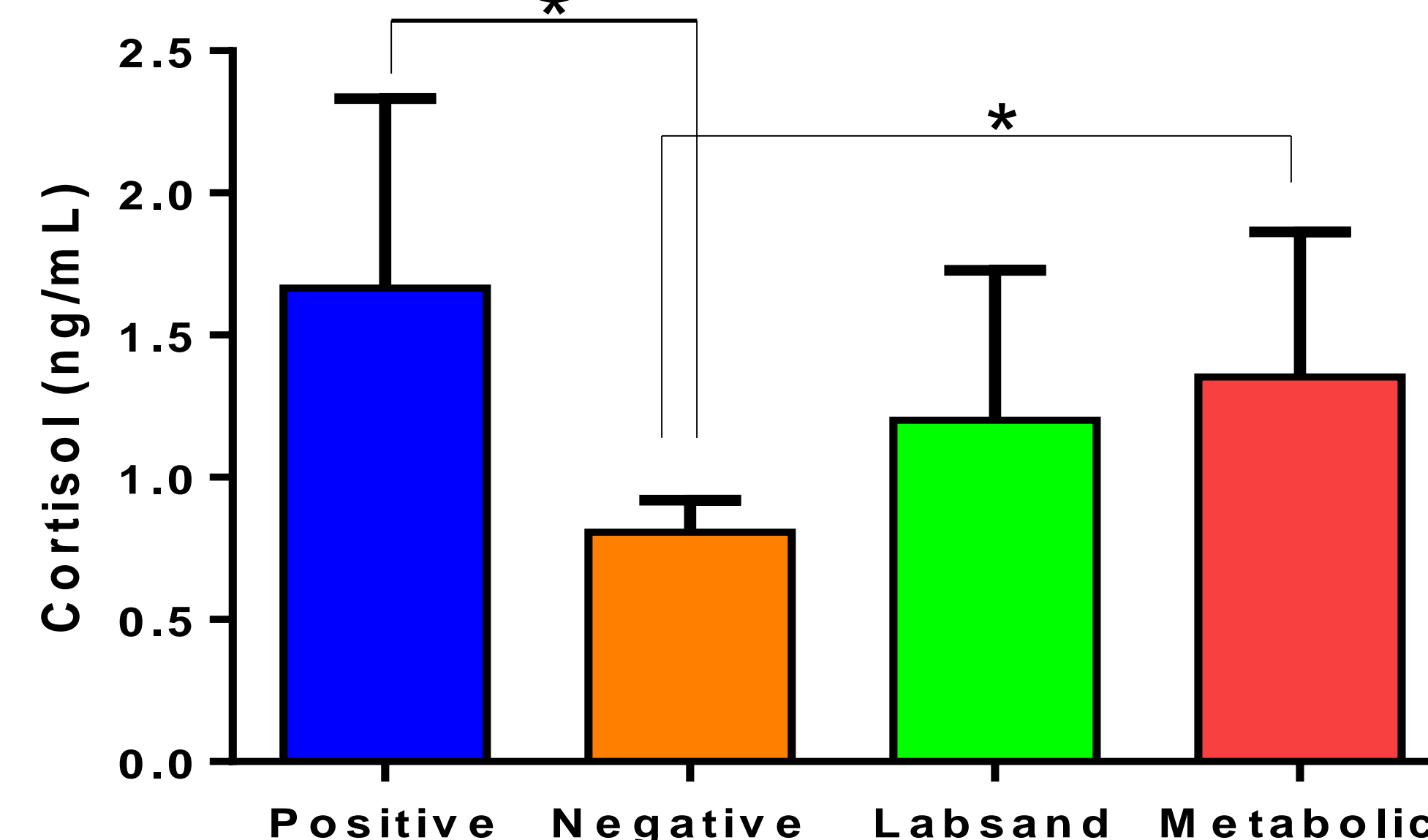
looked at electrolytes like Ca, Na, Cl, K, Phosphorus, and also creatinine. The data was inconclusive with differences which were varying depending of the species and parameters.



Rat on hydrophobic sand.

Mice fecal cortisol levels were not significantly different between hydrophobic sand versus metabolic cages ($p=0.5631$). There was a significant difference between the positive and negative control ($p=0.0081$) and also between the negative control and the mice in metabolic cages ($p=0.0190$) (Fig 4). *significant difference

Figure 4: Cortisol Levels in Mice After a 24 Hour Urine Collection Compared with a Positive and Negative Control



Discussion

In mice, the absence of a significant difference in urine volume after 24 hours demonstrates that hydrophobic sand is a reliable alternative to metabolic cages for urine collection even when maximum volume is required. The increase of urine volume collected from the hydrophobic sand after 6 hours is likely because mice were housed in a more familiar environment with hydrophobic sand while they might require more time to acclimate to a wire bare flooring of the metabolic cages before urinating.

In rats, because the total urine volume was significantly lower when collected from the hydrophobic sand after their final time point at 6 hours, hydrophobic sand would only be a reliable alternative to metabolic cages if the study does not require maximum urine volume. The urine collection was not extended past 6 hours because the volume of urine would be too great for the cage size overnight. Rats were observed playing with the sand and many came out of the cages covered in urine and sand. Increasing the sand volume did not resolve the issue as it also increased the play behaviors and amount of sand mixed with urine accumulating in the rats' fur. Hydrophobic sand would still be an option to consider if a urine sample of no more than 500mL was needed after 6 hours.

Additional studies would be required to confirm the absence of impact of hydrophobic sand on electrolytes, creatinine or drug excretion in the urine.

Cortisol levels were not significantly different between both caging systems and were between the positive and negative control. However, there was a significant difference between the negative control and metabolic cages. Both environments seem to increase stress level but the cortisol levels in the hydrophobic sand cage may also reflect an opportunity for the mice to have an increased activity by digging and exploring this new environment.

Urine collected on hydrophobic sand took slightly longer to collect compared to metabolic cages but that is offset by the decreased time needed to set up and take down cages, also less space is needed. Metabolic cages took much longer to set up and also required hours of work to take down and run through cage wash.

Conclusions

Hydrophobic sand offers an efficient alternative to metabolic cages for mice in most studies as it does not impact the total urine collected and offers a more natural environment for the animals.

In rats, hydrophobic sand can be used to collect a urine sample but only when urine volume is not a parameter assessed.

References

- Kurien, B.T., Everds, N.E., & Scofield, R.H., (2004). Experimental animal urine collection: a review. *Laboratory Animals*, 38(4), 333-361.