#### Journal of the American Association for Laboratory Animal Science September 2012

stress in rodents, based on grooming characteristics and microstructure. This study aimed to evaluate the applicability of the grooming analysis algorithm to distinguish sleep-deprived and control rats in comparison with traditional grooming analysis. Forty-six animals were distributed into 3 groups: control (n = 22), paradoxical sleep-deprivation (96 h, n =10), and total sleep deprivation (6 h, n = 14). Immediately after the sleep deprivation protocol, grooming was evaluated using both the grooming analysis algorithm and traditional measures (grooming latency, frequency, and duration). Results showed that both sleep-deprived groups displayed grooming in a fragmented framework when compared to control animals. Variables from the grooming analysis algorithm were successful in distinguishing sleep-deprived and normal sleep animals regarding anxiety-like behavior. The grooming analysis algorithm and traditional measures were strongly correlated. In conclusion, the grooming analysis algorithm is a reliable methodology to assess the relationship between anxiety-like behavior and sleep deprivation in rodents.

## P198 Real-Time PCR Quantification of Metallothionein Gene Expression in Sprague–Dawley Rats Chronically Exposed to Cd

M Al-Ghanem<sup>2</sup>, G Al-Mahmoud<sup>2</sup>, M Shanmugakonar<sup>\*1</sup>, H Al-Naemi<sup>2</sup>

<sup>1</sup>Laboratory Animal Unit, <sup>2</sup>Biological and Environmental Sciences, Qatar University, Doha, Qatar

Toxicologic impact of Cd leads to multiple human pathologic conditions, and its effect on humans and animals has been extensively studied. Physiologic function of metallothionein (MT1) is not completely understood, but it is mainly associated with detoxification of Cd and Hg. Elevated synthesis of MT1 exposed to metals has been observed but data on quantitation in various tissues is limited. We measured MT1 levels in peripheral blood and tissue samples of rats exposed to CdCl<sub>2</sub>. The objective is to investigate the effect of chronic exposure of Cd on peripheral blood and tissue-specific expression of MT1. This will provide information of MT1 gene transcription regulation and its impact on the heavy metal detoxification process. Rats raised in our animal facility were assigned to 8 experimental groups. Daily dose of 15 mg/ kg body weight CdCl<sub>2</sub> in drinking water was administered for 8 wk. The control group received tap water free of Cd. Peripheral blood samples collected at 4 occasions (week 2, 4, 6, and 8) in EDTA tubes by retroorbital bleeding procedure. Liver and kidney tissue samples were collected and weighed. Total RNA/cDNA was prepared and quantified according to manufacturer's protocol. Premade MT1 gene expression assay was used while  $\beta$ -actin gene was the endogeneous control. Results from week 2 and 4 showed the trend of upregulation of MT1 gene (fold increase) while the sample from all the other occasions showed downregulated response of MT1. Week 4 sample showed the fold increase of 1.11 times compared to week 2 increase of 1.04. Though the recorded 1.1-fold difference in the gene expression is not high, it gives an indication that there was an induction of MT1 gene. The downregulated pattern of MT1 gene might be due to the overaccumulation of repressor apothionein protein which stops MT1 transcription. When the metal binds to the promoter region of the MT1 gene-repressor protein, it becomes inactive and increases the MT1 transcription, but at the same time accumulation of repressor protein downregulates MT1 gene. Our observations suggest that chronic Cd exposure elicits an elevated MT1 gene expression which in turn leads to detoxification. More elaborative study is warranted for further understanding of MT1 gene expression.

### P199 Rodent and Swine Spectrophotometric Urinalysis

NA Forbes-McBean\*, CF Brayton

Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD

Urinalysis is a common noninvasive diagnostic test for humans and animals. Urine chemical strips (dipsticks) are sometimes used in research settings, but these are designed for human use or for dogs and cats, and their utility or validity for laboratory species is not well documented. Compared with chemstrip results based on qualitative assessments of a color change on the test strip, automated spectrophotometric clinical

chemistry analyzers offer many more test options and quantitative results. Urinary uric acid, amylase, alkaline phosphatase (ALP), glucose, albumin, blood urea nitrogen (BUN), total protein, and lactate dehydrogenase (LDH) are potentially relevant biomarkers for kidney, prostate, pancreatic, and other disease, and were the focus of this study. The objective of this study was to establish the reliability (coefficient of variance) of a spectrophotometric analyzer and to assess ranges of some urine analytes from healthy pigs and rodents. Based on at least 10 determinations of the same sample, the coefficient of variance was less than 5.0%. Urine from 10 nonfasted healthy adult laboratory pigs, and 10 nonfasted healthy adult laboratory mice (various strains) were analyzed on a chemistry analyzer. In pigs, the range values were: UA = 2.4 to 7.4 U/L, amylase = 29 to 655 U/L, glucose = 1.0 to 21.0 mg/ dL, BUN = 143 to 320 mg/dL, ALP = 2.0 to 15.0 U/L, albumin = 0.1 g/ dL, total protein = 0 to 1.8 g/dL, and LDH = 3.0 to 30.0 U/L. In mice, the range values were: UA = 0 to 1.6 U/L, amylase = 143 to 798 U/L, glucose = 13.0 to 51.0 mg/dL, ALP = 9.0 to 27.0 U/L, albumin = 0.1 to 3.5 g/dL, total protein = 0 to 2.0 g/dL, and LDH = 1.0 to 33.0 U/L. Creatinine exceeded measurable ranges of this instrument. In summary, some relatively inexpensive urine chemistry tests may offer presumptive testing for suspected pathologic dysfunctions, or for monitoring progression of an expected or induced disease condition.

# P200 Mouse Urine Specific Gravity: Chemical Strip Method Compared with Veterinary Refractometer

### NA Forbes-McBean\*, CF Brayton

Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD

Urine is approximately 95% water and 5% solute. The specific gravity (SG) of water is 1.000. Urine SG represents its solute concentration and osmolarity, whether solutes are electrolytes, metabolites, or 'lost' protein or glucose. More concentrated specimens have higher SG. Determination of urine SG is used primarily to assess kidney function or concentrating ability. SG can also help to assess hydration (elevated in ill dehydrated patients, or in intentionally dehydrated athletes), or to assess for urine dilution, when urine SG is lower than blood plasma SG (about 1.010), as in polydypsic polyuric patients without renal failure. Expected urine SG of nonfasted laboratory mice is about 1.030, and mice have exceptional concentrating ability, such that their urine SG can range considerably higher. The upper limit of urine chemical strips (also called chemstrips, multistix, or dipsticks), and of some refractometers is 1.030 or 1.040. The objective of this study was to compare the chemical strip method with the handheld veterinary refractometer (upper limit 1.080). Urine was collected from 10 nonfasted, healthy adult mice, and tested with the chemical strip and the refractometer. Results showed a significant difference (P < 0.05) between the chemical strip and the refractometer on the same urine samples. Average SG using the chemical strip was 1.017 with a range of 1.010 to 1.030. Average SG using the refractometer was 1.026 with a range of 1.013 to 1.049. The chemical strip method required 30 µL of urine. The refractometer required 60 µL of urine. In conclusion, the chemical strip SG determination was significantly lower than that of the refractometer, and the chemical strip upper limit probably is too low for reliable determination of mouse urine SG. The handheld veterinary refractometer is a practical method for determination of mouse urine SG, requiring about 60 µL of urine specimen.

#### P201 Analysis of the hr Gene in a Hairless Mouse Strain (HR)

O Suzuki\*, M Koura, Y Noguchi, K Uchio-Yamada, J Matsuda

Laboratory of Animal Models for Human Diseases, National Institute of Biomedical Innovation, Ibaraki, Japan

We analyzed the *hr* gene of the hairless mouse strain HR to determine whether the strain shares the same mutation with other hairless strains such as HRS/J and Hos:HR-1. We also developed a method for genotyping hairless mice using PCR to determine the genotypes of pups before the phenotype (hair coat loss) appears (approximately 2 wk of age). Genomic DNA was obtained from the livers of HR and wildtype