COMPARISON OF SPECIFIC GRAVITY AND OSMOLALITY IN POST RACE URINE OF FUROSEMIDE-TREATED AND CONTROL THOROUGHBRED HORSES

J. M. Bosken, T. Tobin, G. D. Mundy*, M. Fisher* and R. O. Banks†

*Gluck Equine Research Center, Lexington, Kentucky 40546; *The Kentucky Racing Commission, Lexington, Kentucky 40511; †Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, Ohio 45267-0576, USA*

ABSTRACT

The specific gravities of urine samples from Thoroughbred horses are measured routinely following pari-mutuel races to monitor the potential misuse of diuretic agents such as furosemide. Specific gravity of a substance is the ratio of mass to volume expressed as g/ml. Osmolality is the number of osmoles of a solute per kg solvent (water). Specific gravity is the accepted procedure for estimating the osmolar concentration of urine samples and, generally, values less than 1.010 are targeted for further evaluation. However, a number of biological compounds, particularly proteins, have a greater effect on specific gravity than on osmolality determinations. Therefore, in the current study, values of specific gravity were compared to osmolality in urine samples (n=326) from horses in a mid-western racing jurisdiction. Specific gravity was measured at room temperature (24°C) with both a refractometer and a hydrometer, whereas osmolality was measured by freezing point depression (Precision Systems, Massachusetts, USA). Mean (± se) osmolality and specific gravities were significantly lower in furosemide-treated horses compared with control horses. Indeed, 24 horses had an osmolar concentration below 500 mOs/kg water, 19 of which had been treated with furosemide. There was an excellent correlation between specific gravities as estimated with a refractometer and those measured with a hydrometer. Based on linear regression analysis, the correlation coefficient (r) was 0.93 and the slope (m) was 0.71. In addition, there was a reasonable correlation (r = 0.71) between specific gravity and osmolality in the osmolality range of 500–1,000 mOs/kg water. Further, the slope of the linear regression in this range was 4×10^{-5}. By contrast, in urine samples below 500 and above 1,000 mOs/kg water, the correlation was less satisfactory (r=0.3 and 0.52, respectively), and the slopes of the regression analyses were significantly ($P<0.05$) reduced (7.3×10^{-6} and 2×10^{-5}, respectively). Thus, below 500 mOs/kg water, specific gravity does not correlate well with osmolality and is not an accurate measurement of solute concentration in that range. Therefore, it is concluded that the measurement of osmolar concentration is the better method to determine urine solute concentration.

INTRODUCTION

In most jurisdictions in North America, furosemide must be administered to Thoroughbred horses 4 h or more before they race.

Furosemide is used as a medication in horses thought to be susceptible to exercise induced pulmonary haemorrhage. There are concerns about its administration as it has been shown to affect the urinary and plasma concentrations of other co-administered drugs. Of particular concern is the fact that this effect is not predictable for yet untested drugs (Stevenson *et al.* 1990).

For this study, urine and blood samples were collected from each Thoroughbred horse that finished in the top 3 positions from each Thoroughbred race in the state of Kentucky.

Results from an earlier study (Uboh *et al.* 1992) led the authors to state that urine specific gravity values were not reliable for predicting the dose of furosemide administered to race horses. Measuring urine osmolar concentration may be a more accurate indication of the administration of furosemide.

Osmolality is not affected by the type of solute contained in a given sample. Specific gravity is only an approximation of the total solute concentration and can be affected by a number of physiological

variables including the amount of protein in the sample.

Urine samples from a Kentucky track were analysed to compare the reliability of osmolality with specific gravity in assessing the diuretic status of racehorses. The samples were divided into groups according to whether or not the subject was dosed with furosemide pre-race, whether the race was a 'sprint' (6.5 furlongs or less) or 'route' (one mile or more) and whether the horse was a winner or non-winner.

Materials and Methods

Collection of urine samples

The post race urine samples (n=326) were obtained from Thoroughbreds at a Kentucky racetrack. All samples were frozen at -70°C at the Department of Molecular and Cellular Physiology, University of Cincinnati, College of Medicine. All samples were collected as 'blind' samples and labelled only with a number. Specific gravity and osmolality determinations were performed on each sample.

Urine analysis

Specific gravity was determined with a National refractometer set to 1.000 with deionised water. Total osmolar concentration was determined by the freezing point depression method with a Precision Systems osmometer (Waltham, Massachusetts, USA).

Statistical evaluation

Prior to statistical evaluation, the 326 samples were divided into 8 groups:

Group 1: Sprint winners pre-treated with furosemide;

Group 2: Sprint non-winners pre-treated with furosemide;

Group 3: Route winners pre-treated with furosemide;

Group 4: Route non-winners pre-treated with furosemide;

Group 5: Sprint winners not treated with furosemide;

Group 6: Sprint non-winners not treated with furosemide;

Group 7: Route winners not treated with furosemide;

Group 8: Route non-winners not treated with furosemide;

Differences between groups were evaluated using a one-way analysis of variance and the Student-Newman-Keuls *post hoc* test. Correlation between variables was evaluated using linear regression analysis and/or Sigmoidal (Boltzman) fit to the data.

Results

Urine specific gravity and osmolality data

Table 1 summarises the average and se values for specific gravity and osmolality. All furosemide groups had average specific gravity and average osmolality values less than the non-furosemide groups. The average osmolality of horses in route races groups was less than that for those in sprint races.

Tables 2 and 3 summarise the statistical evaluations of osmolality and specific gravity in the 8 groups of horses.

TABLE 1: A summary of the mean (± se) osmolality and specific gravity values for each group

	Pre-treated with furosemide				No furosemide treatment			
	Group 1 Sprint winner (n = 45)	Group 2 Sprint non-winner (n = 74)	Group 3 Route winner (n = 33)	Group 4 Route non-winner (n = 58)	Group 5 Sprint winner (n = 23)	Group 6 Sprint non-winner (n = 40)	Group 7 Route winner (n = 19)	Group 8 Route non-winner (n = 34)
Specific gravity (g/ml)	1.026	1.026	1.026	1.024	1.036	1.035	1.035	1.031
se	0.009	0.009	0.010	0.009	0.010	0.011	0.010	0.010
Osmolality (mOsm/kg H_2O)	796	757	754	716	1124	1181	1078	989
se	268	258	230	219	387	458	458	364

Sprint = ≤ 6.5 furlongs; Route = ≥ 1 mile

TABLE 2: Urine osmolality

	Pre-treated with furosemide				No furosemide treatment		
	Sprint winner	Sprint non-winner	Route winner	Route non-winner	Sprint winner	Sprint non-winner	Route winner
No furosemide route non-winner	Y	Y	Y	Y	N	Y	N
No furosemide route winner	Y	Y	Y	Y	N	N	
No furosemide sprint non-winner	Y	Y	Y	Y	N		
No furosemide sprint winner	Y	Y	Y	Y			
Furosemide route, non-winner	N	N	N				
Furosemide route, winner	N	N					
Furosemide sprint, non-winner	N						

Summarises statistical comparisons of urine osmolar concentrations among the various groups of horses. The data were analysed using a one-way analysis of variance and the Student-Newman-Keuls test. Values were accepted as significantly different (Y) when the probability of no difference was <5%

TABLE 3: Urine specific gravity

	Pre-treated with furosemide				No furosemide treatment		
	Sprint winner	Sprint non-winner	Route winner	Route non-winner	Sprint winner	Sprint non-winner	Route winner
No furosemide route non-winner	N	N	N	Y	N	N	N
No furosemide route winner	Y	Y	Y	Y	N	N	
No furosemide sprint non-winner	Y	Y	Y	Y	N		
No furosemide, sprint winner	Y	Y	Y	Y			
Furosemide, route non-winner	N	N	N				
Furosemide, route winner	N	N					
Furosemide, sprint non-winner	N						

Summarises statistical comparisons of urine specific gravity measurements among the various groups of horses. The data were analysed using a one-way analysis of variance and the Student-Newman-Keuls test. Values were accepted as significantly different (Y) when the probability of no difference was <5%.

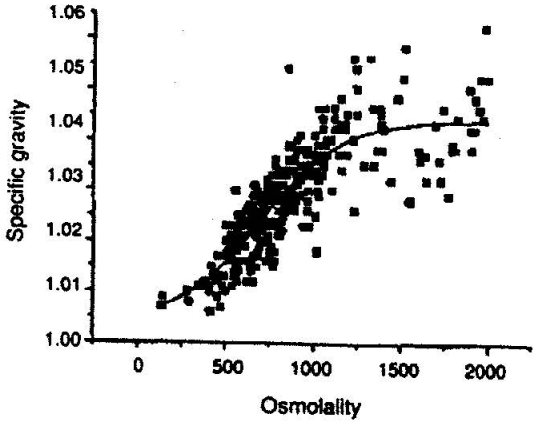

Fig 1: Comparison of specific gravity values to osmolality values for all urine samples in the study (n=326).

Table 2 shows that urine osmolalities in each of the furosemide groups were significantly different from the groups of horses that were not treated with furosemide. The only other groups which were significantly different from each other were the non-treated sprint non-winners vs. non-treated route non-winner groups.

Table 3 shows that the comparisons between specific gravity data were not characterised by as many significant differences among the groups. The furosemide treated, route non-winner group was the only group which was significantly different from all non-treated groups.

A Sigmoidal fit analysis was performed to display the relationship between specific gravity and osmolality. This analysis is illustrated in Figure 1, a graphical display of the Sigmoidal (Boltzman) fit to the specific gravity and osmolality data.The low chi squared value for the Boltzman fit of the data illustrated in Figure 1 is highly significant ($P<0.001$).

Table 4 diplays the samples which yielded specific gravity values of 1.010 and less along with their corresponding osmolality values. Seven of these 9 samples were from furosemide treated horses. No samples in either Group 5 or 7 had a specific gravity of 1.010 or lower.

Table 5 details all samples with osmolality values ≤ 500 mOsm/kg H_2O (n=24). Nineteen of these samples were from furosemide treated horses.

TABLE 4: Details of samples with specific gravity values ≤1.010 g/ml, with their corresponding osmolality values (n=9)

		Specific gravity (g/ml)	Osmolality (mOsm/kg H_2O)
Group 1	n = 2	1.010	500
		1.007	476
Group 2	n = 1	1.009	146
Group 3	n = 1	1.010	285
Group 4	n = 3	1.010	414
		1.008	296
		1.007	140
Group 6	n = 1	1.006	416
Group 8	n = 1	1.009	460

TABLE 5: Details of samples with osmolality values ≤ 500 mOsm/kg H_2O, with their corresponding specific gravity values (n=24)

		Specific gravity (g/ml)	Osmolality (mOsm/kg H_2O)
Group 1	n = 4	1.010	500
		1.015	423
		1.011	482
		1.007	476
Group 2	n = 5	1.017	499
		1.013	490
		1.013	457
		1.017	460
		1.011	381
Group 3	n = 5	1.013	428
		1.011	406
		1.010	285
		1.011	346
		1.013	427
Group 4	n = 5	1.010	414
		1.008	296
		1.012	452
		1.007	140
		1.011	345
Group 6	n = 2	1.006	416
		1.017	498
Group 7	n = 1	1.015	421
Group 8	n = 2	1.009	460
		1.012	379

Discussion

One method of describing mass, amount or quantity is osmoles or equivalents. This concept accounts for the number of particles released in solution. Mass per unit volume is the method used to describe concentration. Osmolality values for this study are expressed as mOsmoles/kg H_2O. Specific gravity is expressed as gram of solute/ml of solution. The greater the value of these 2 assays, the more solute is filtered from the blood by the kidneys to enter the urine. The lower the value, the less solute is filtered by the kidneys.

As expected, the diuretic state of the horse, post race, was significantly different according to the medication status of the animal. Horses pre-treated with furosemide showed significantly lower urinary solute concentrations than those not treated. This was seen in all cases for osmolality, but not for

specific gravity. The remainder of the comparisons within medication status groups provided only one significant difference, ie the osmolality results for the non-treated, sprint non-winner group and the non-treated, route, non-winner group.

The values in Table 4 indicate that samples whose specific gravities are equal to or below 1.010 can display osmolality values from 140 mOsm/kg H_2O to 500 mOsm/kg H_2O. Comparing the osmolality values of the sample in Group 1, whose specific gravity value is 1.010 and the sample in Group 2, whose specific gravity value is 1.009, the osmolality values are 500 mOsmoles/kg H_2O and 146 mOmoles/kg H_2O, respectively.

Based on the data and the data analysis, urine osmolality is a better method than specific gravity as an indicator of the diuretic state of the racehorse.

Acknowledgements

Published as No. 285 from the Equine Pharmacology and Experimental Therapeutics Program at the Maxwell H. Gluck Equine Research Center and the Department of Veterinary Science, University of Kentucky

Supported by grants funded by The Equine Drug Council and The Kentucky Racing Commission, Lexington, Kentucky and by research support from the National, Florida, and Nebraska Horsemen's Benevolent and Protective Associations and Mrs John Hay Whitney.

References

Stevenson, A.J., Weber, M.P., Todi, F., Mendonca, M., Fenwick, J.D., Kwong, E., Young, L., Leavitt, R., Nespolo, R. and Beaumier, P. (1990) The influence of furosemide on plasma elimination and urinary excretion of drugs in Standardbred horses. *J. vet Pharmacol. Ther.* **13**, 93-104.

Uboh, C.E., Soma, L.R., Rudy, J.A., Morgan, E., Mengeringhausen, K. and Sams, R. (1992) Plasma concentration of furosemide versus specific gravity of urine in predicting dose of administration in race horses. *Res. Commun. chem. Patbol. Pharmacol.* **77**, 201-219.