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Abstract

A simple and intuitive formula for the estimation of urine osmolality (U_{osm}) using the measured concentrations of major active urine osmolytes over a wide range of urine dilutions was proposed in healthy cats. Sixty-three urine samples were retrieved using ultrasound-guided cystocentesis from sixteen healthy cats under 5 years of age receiving intravenous infusion over a period of 24 hours. Samples were collected at baseline (T_0), T_2, T_6, T_{12}, and T_{24}. Urine osmolality was measured using a freezing-point osmometer, and the concentrations of osmolytes (urea, sodium, glucose, and potassium) were evaluated. A simple linear regression model for a clinical use was selected, and the agreement between the calculated and actual urine osmolalities was assessed. Urinary concentrations of urea, sodium and glucose were the three variables included in the model with the lowest AIC_C. Urine osmolality can be predicted accurately and precisely using urine urea, sodium and glucose with the following equation: U_{osm} = 1.25 × urea (mmol/l) or 20.87 × urea (g/l) + 1.1 × sodium (mmol/l) + 67 × glucose (mmol/l) or 3.72 × glucose (mg/dl). The concordance correlation coefficient for repeated measures between the actual and the calculated urine osmolality was extremely close to 1, which supported a high agreement : 0.996 (CI 95% : [0.993 ; 0.998]). In a population of healthy cats, urine osmolality can be predicted accurately and precisely using urinary urea, sodium and glucose concentrations. Similar formulae could potentially be established to help the clinician in pathological situations.

Key words

Feline, urine osmolality.

Introduction

Urinalysis is a routine in-clinic procedure that provides relevant information about the kidney function and systemic health in small animal practice. Urine osmolality (U_{osm}) depends on the amount of osmotically active molecules in the urine including nitrogenous waste compounds (urea, creatinine), uncharged molecules (glucose), and major ions (sodium and potassium mainly)[1–3]. In contrast to urine specific gravity (USG), U_{osm} is independent of the molecular weight of urine osmolytes (Cottam et al., 2002; Imran et al., 2010; Voinescu et al., 2002). In humans, U_{osm} might be more accurate than USG for the prediction of hydration status and renal tubular function in normal physiological situations but also in some pathologic states (Imran et al., 2010; Voinescu et al., 2002). A retrospective study in healthy cats reported that U_{osm} and USG correlated roughly over a narrow range of hypersthenuric urine samples (Di Bella et al., 2014). As U_{osm} evaluation requires a sophisticated device (freezing-point osmometer) and a skilled operator, its use in daily clinical practice remains considerably limited. In humans and cats, some studies propose additive formulas for the estimation of plasma osmolality in physiological and pathological states using the concentrations of the major components (Dugger et al., 2013; Khajuria and Krahn, 2005; Schermerhorn and Barr, 2006). To the authors' knowledge, no similar publications have proposed a comparable estimation of U_{osm} in feline species. We aimed at establishing a formula that estimate U_{osm} using the concentrations of osmotically active molecules in urine samples from healthy cats.

Material and methods

Inclusion criteria

Only healthy vaccinated and dewormed cats younger than 5-years-old and belonging to students of our Veterinary Teaching Hospital were enrolled. The included cats had normal physical findings and their history did not indicate any previous disease. They were receiving regular internal and external parasite control products. The included cats were never given any other medication. For every included cat, the protocol began at 8:00 am (corresponding to T_0). Baseline blood urea nitrogen, serum creatinine, urine dipstick analysis, urine specific gravity assessment and sediment examination were performed to rule out chronic nephropathy, pathologic glucosuria, proteinuria, pigmenturia, pyuria, bacteriuria and urinary tract inflammation. Systemic hypertension was ruled out by an ECVIM-CA resident [redacted] based on Doppler sphygmomanometry technique recommended by ACVIM consensus statement (Brown et al., 2007) using manual ultrasonic Doppler flow detector model (811-B, Parks Medical Electronics®; Aloha, USA).

Procedures

A 23 Gauge intravenous (IV) catheter was placed on the right thoracic limb (cephalic vein). The cats received IV infusions of isotonic Lactated Ringer's solution (RL) at a flow rate of 4 mL/kg/h over 24 hours. Urine samples were collected by ultrasound-guided cystocentesis at baseline (T_0) and at several time-points subsequently (at 2 hours: T_2, only if the urinary bladder filled before cystocentesis, 6 hours: T_6, 12 hours: T_{12} and at 24 hours: T_{24}). Clinical and cardiopulmonary variables were assessed hourly during the 24-hour period by an undergraduate student using a dedicated examination sheet, an ECVIM-CA resident ([redacted]) and his supervisor

97 ([redacted]), ECVIM-CA Diplomate. The cats were provided regular dry feline diet and free water
98 access during the procedure. For ethical reasons, the cats were directly excluded from the
99 protocol if they showed any abnormal clinical finding or if they were reluctant to physical
100 restraint for the cystocentesis. A follow-up examination was systematically performed at 3, 6 and
101 12 months. During the visits, we recorded all information reported by the owners and we
102 performed a complete physical examination (including cardiopulmonary auscultation and
103 temperature assessment). The protocol was reviewed and approved by our institutional ethical
104 committee [redacted] and informed consents were obtained from owners.
105
106 Measurements
107 Urine samples were kept refrigerated in closed hermetic silicone tubes (Vacutainer®,
108 Coveto, La Guyonnière, France). The samples were processed within a delay of 1 to 12 hours to
109 avoid significant evaporation and measurement artifacts. Urine samples were initially assayed for
110 urea, sodium, glucose, potassium, creatinine, bicarbonates, chloride and lactates. Preliminary
111 statistical analyses were performed using urea, sodium, glucose, potassium and creatinine. Then,
112 considering the current knowledge relative to the calculation of plasma osmolality in humans
113 (Khajuria and Krahn, 2005) and small animals (Dugger et al., 2013; Schermerhorn and Barr,
114 2006), along with the findings extracted from an earlier report (Voinescu et al., 2002) relative to
115 the impact of urine osmolytes on osmolality, then to ensure the best clinical applicability on the
116 field, only urea, sodium, glucose, and potassium measurements were selected in the baseline
117 model. These variables were also selected considering their expected concentrations in feline
118 urines (Cottam et al., 2002). The samples were submitted for biochemical measurements of urea,
119 sodium and glucose using a dedicated analyser (Konelab 30i, Thermo Scientific®; Cergy
120 Pontoise, France). Potassium was assessed using the molybdate method, as reported by Zilva and

Nicholson (1973). Prior to osmolality assessment, the samples were acclimatized to ambient room temperature for 30 minutes. U_{osm} was measured using a freezing-point osmometer by 2 qualified technicians (Digital Micro-Osmometer, Roebling®; Giessen, Germany). For each individual urine sample, both operators evaluated U_{osm} once. The 2 technicians were blinded to the measurement of each other.

Statistical analysis

Statistical analysis was performed using statistical software (R® Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.). For baseline descriptive data (U_{osm} and urine osmolyte concentrations), numerical values were reported, as mean, median, minimum and maximum. Relationships between urinary osmolytes concentrations (urea, sodium, glucose, and potassium) and actual U_{Osm} were studied using a series of generalized linear mixed models (GLMM) with the variable 'Cat identification' used as an explanatory variable with a random effect in order to take into account the non-independence between measures made on a same cat. Furthermore, in order to take into account the non-independence between measures made on a same cat at the same time, we used an autocorrelation structure with a categorical time covariate, the variable "Time". Each of all possible combinations of one to four of the four different osmolytes selected in the study were tested as explanatory variables, with the actual osmolality as the response variable, using the glmulti package (Calcagno and de Mainzancourt, 2010). For model comparison, we considered the small number of samples. Therefore, we used the Akaike Information Criteria corrected for small sample sizes (AIC_C). We selected the model with the lowest AIC_C as the final model, and the differences of the AIC_C between models was interpreted according to Burnham (2002). For better

clinical applicability, we simplified the formula to obtain an easy-to-use additive formula without ordinate (we forced intercept to 0). For the same reason, the variable 'Time' was not included as an explanatory variable in this simplified model, as it would be impractical to use it for daily clinical activities. We additionally ran a similar model with an intercept and with 'Time' as a categorical factor (supplementary data).

Afterward, the agreement between the calculated and actual U_{osm} was assessed thanks to the concordance correlation coefficient (CCC) for repeated measures in the cccrm:ccclon package (Carasco et al., 2009; Carasco et al., 2013). This method relies on a linear mixed modeling approach, the assumptions of the linear model (linearity, homoscedasticity and normality of the residuals) were thus verified graphically prior to the analysis. To account for the lack of independance of the observations and due to the design of the study, an autoregressive correlation structure was chosen for the mixed model. The results of the CCC were interpreted using the scale proposed by McBride (2005). The limits of agreement represented by the bias and their 95% confidence intervals were estimated using the Bland Altman method (Bland and Altman, 2007). The plot was generated to reinforce the interpretation of the data.

Results

Population included

Twenty-one cats were initially enrolled. However, 5 cats were excluded. The cystocentesis was not possible for 3 cats and 2 other cats had dyspnea after 0 and 2 hours of intravenous infusion, respectively. Sixteen domestic short haired cats completed the protocol without any complication. All five urine samples were available for 6/16 cats, while 1/5, 2/5 and 3/5 samples were unavailable for 5/16, 3/16 and 2/16 cats, respectively. Sixty-three samples were

finally processed. No complication was reported during the procedure. Fifteen out of the 16 included cats had normal 3, 6 and 12-months rechecks. The 16th died from a hit-by-car accident at 2 month.

Urine osmolality and osmolytes evaluation

The paired measurements of urine osmolality with the freezing-point osmometer were completely identical between the 2 operators. Urine osmolality varied over a wide range. Mean, median, minimum and maximum values for urinary urea, sodium, glucose, and potassium are indicated within Table 1. The relationship between the actual and the calculated U_{osm} can be appreciated on Figure 1.

Derived formula

Of all possible subsets and combinations of urea, sodium, glucose, and potassium the AIC indicated that the model with urea, sodium, and glucose preferable (Table 2 and supplementary data 1 and 2). The GLMM including urea, sodium and glucose (all in mmol/l) and no intercept resulted in the ensuing formula: $U_{osm} = 1.25252 \times \text{urea} + 1.05320 \times \text{sodium} + 66.72193 \times \text{glucose}$ ($AIC_C = 843.551$). The difference between the AIC_C indices of the first and the second model was greater than 2. This supports our choice of the first model (Burnham, 2002).

The following simplified model would be acceptable for better practical use: $U_{osm} = 1.25 \times \text{urea (mmol/l)}$ or $20.87 \times \text{urea (g/l)} + 1.1 \times \text{sodium (mmol/l)} + 67 \times \text{glucose (mmol/l)}$ or $3.72 \times \text{glucose (mg/dl)}$. The concordance correlation coefficient for repeated measures was 0.996 (CI 95% : [0.993 ; 0.998]), which can be considered, as 'almost perfect' according to McBride (2005). The visual inspection of the limits of agreement plots suggested the closeness of agreement between the 2 variables (Figure 2). The fixed bias, defined as the means of the

differences between the calculated and atual U_{osm} was -7.30 mosm/kg, meaning that, on average, the calculated U_{osm} was 7.30 units lower than the actual value. The range of the differences (defined by calculated minus actual osmolality) was -480,85 : +612,25 mosm/kg. Bland-Altman 95% limits of agreement between the actual and calculated U_{osm} were -370.32 : +334.21 mosm/kg. The differences were not homogeneous across the range of means of the calculated and actual U_{osm} but were more negative at low means and more positive at high means.

Discussion

Urine osmolality can be predicted accurately and precisely using urine urea, sodium and glucose in healthy cats with the following equation: $U_{osm} = 1.25 \times \text{urea} + 1.1 \times \text{sodium} + 67 \times \text{glucose (mmol/l)}$ or $3.72 \times \text{glucose (mg/dl)}$.

One important finding is that the mean osmolyte gap (or fixed bias) estimated with the preceding formula was very small (-7.30 mosm/kg) when compared to the huge variation of urine osmolality of the cats included (ie. 302 to 2940 mosm/kg). This small gap strongly suggests that the formula is accurate and reinforces its applicability in clinical practice. Moreover, the Bland-Altman 95% limits of agreement between calculated and actual U_{osm} were -370.32: +334.21 mosm/kg. This finding means that one would expect 95% of samples to have estimates of urine osmolality between 370.32 mosm/kg less than and 334.21 mosm/kg greater than the actual value. Considering the large physiological variation in feline urine osmolality (Table 1 and Figure 1), this is an acceptable difference (around 5%) for healthy cats in physiological condition and highly concentrated urines. However, this gap is not negligible for the cats with diluted urines (i.e. those receiving IV infusion during hospitalisation). Moreover, the method used treated each sample as if it was independent of each other sample. In this setting, one must know the generated 95% confidence intervals in the Bland Altman method results are probably unduly

narrow. Interestingly, the differences observed between the 2 methods were not homogeneous across the range of means of the calculated and actual osmolalities. Indeed, calculated values tended to be lower than actual at high means and higher than actual at high means. This proportional bias might probably occur subsequently to the fact that we selected a model without intercept. Actually, the presence of an intercept for this type of formula would have improved fit. Indeed, considering the low concentration of glucose within urines with comparison to urea and sodium, the implication of this osmolyte is more important in diluted urines (with a low osmolality) than in highly concentrated urines (higher osmolality). This effect could have been reduced and even corrected if we had allowed an intercept in the GLMM (rather than forcing the intercept to be 0). However, we had the objective to develop a formula that would the easiest to use for the practitioner in daily clinical practice. In this setting, we considered the model proposed by Dugger et al (2013) for the estimation of plasma osmolality in cats. This model consisted in a formula that involved only urea, sodium and glucose and that had no intercept. Its clinical applicability and relevance justified the choice that led to the final formula in the present study.

Currently, the use of U_{osm} in small animals remains limited and its role needs to be evaluated. Some studies reported the physiologic variations of U_{osm} in dogs and cats, while several reports documented some degree of correlation or agreement with USG (Ayoub et al., 2013; Di Bella et al., 2014; Imran et al., 2010; Van Vonderen et al., 1997; Wardrop, 2008). These studies suggested that USG could be an acceptable marker of renal tubular function in daily clinical practice but is still imperfect. Unfortunately, the relationship between USG and U_{osm} has mostly been evaluated and validated in canine species (Ayoub et al., 2013). Indeed, the few series available in cats are subjected to some major analytical pitfalls (Di Bella et al., 2014: Ross and Finco, 1981). An earlier study evaluated some pathologic urine samples retrieved from azotemic

241 cats following renal mass reduction via vascular ligation or nephrectomy (Ross and Finco, 1981).
242 A more recent study evaluated the relationship between USG and U_{osm} in healthy cats, for which
243 almost all urine samples were highly concentrated (Di Bella et al., 2014). As a general principle,
244 predicting with high precision is more difficult when the outcome variable has a narrow range. In
245 that study, the narrow range of urine density might actually have affected the accuracy of the
246 predicted linear correlation. Moreover, the operators used a refractometer, which was not
247 designed to estimate USG over the value of 1.050, and no urine dilution was performed for
248 severely hypersthenuric samples. Thus, for a subset of highly concentrated urine samples, USG
249 could not be actually estimated. Instead, a random approximate of USG value was assigned to
250 meet the prerequisites of the statistical analysis. In this background, the relationship between
251 USG and U_{osm} could hardly be evaluated with these results. It is thus valuable to evaluate the
252 agreement between USG and U_{osm} and build the formula through the use of wider range of urine
253 dilution in healthy cats, as it has recently been proposed in dogs[11]. In our study, the proposed
254 formula was derived using a wider range of urine osmolality (from 302 to 2940 mosm/kg).
255 We aimed at developing the easiest additive model and the final formula fulfilled our
256 requirements. The final model only consisted of 3 osmolytes, which were found to collectively
257 result in the model that was most likely to predict future values accurately (Table 2). The
258 proposed models did not consider the impact of pathologic osmolytes accumulation (e.g., ketone
259 bodies or toxins). The reliability of the final formula is thus only proven in normal physiological
260 conditions. Similar formulas could thus be tested at some point for clinical evaluation of
261 pathologic cases. Indeed, it would be interesting in the future to ascertain the reliability of
262 supplementary models on urine samples retrieved from sick cats suffering from different
263 urological conditions (chronic kidney disease or acute kidney injury), metabolic (diabetes
264 mellitus or primary hyperaldosteronism) or inflammatory/infectious diseases.

Our study includes several limitations. Urine ammonia, calcium and phosphate concentration were not selected during the statistical analysis, though some studies report that their concentrations are not negligible (Cottam et al., 2002; Di Bella et al., 2014; Imran et al., 2010; Voinescu et al., 2002; Wardrop, 2008). We did not perform urine bacterial culture to rule out subclinical bacteriuria. However, this condition occurs rarely in younger healthy cats showing no clinical or cytological finding consistent with urinary tract inflammation (Lister et al., 2011). Moreover, it would have been better to rule out any subclinical chronic nephropathy (IRIS stage I CKD) to completely ascertain the healthy status of the cats. This would have required an ultrasonographic examination and the measurement of symetrical dimethylarginine (SDMA).

The basic concepts of objective standardization in clinical laboratory require specific anticipation and preparation that are documented in a dedicated review (Jensen et al., 2006). The present protocol does not appear to be completely standardized according to these recommendations. However, the design of our study still meets the conditions proposed in the preceding review. Among these 9 points, the investigators need to estimate the random error for both methods. The estimation should be done before the analysis. Our statistical model suggests that the formula underestimates urine osmolality by a mean of 7.30 mosm/kg. Unfortunately, this fixed bias was obtained only after the completion of the study. Thus it could only be applied in future research. Additionally, according to the aforementioned review, the investigators needed to predict an acceptable difference for the measurement of U_{osm} with the osmometer (i.e. the analytical error). We initially projected this analytical error around less than 10% of the physiologic feline U_{osm} (i.e. 200–300 mosm/kg). This means that if the paired measurements for every given sample would have differed by less than 200 to 300 mosm/kg between the 2 operators, then the final model could be considered acceptable. Our projection was finally far much greater than the actual analytical error. Indeed, the paired measurements of urine osmolality

were completely identical between the 2 operators. For these reasons, this final point is no longer an issue. Moreover, using some specific tools, the projected number of samples to be included could roughly be predicted around 30 to 50. For this reason, we included the largest number of samples possible during the study period, and this number (63 samples) is above the minimal recommended threshold (40 samples).

In this setting, our methodology to built the equation appears suitable to constitute a theoretic basis and a preliminary pilot study that would help for future research.

Cystocentesis was prefered over manual urinary bladder compression for urine sampling because the latter can cause traumatic rupture and ureteral reflux, especially in male cats (Osborne et al., 1996). Furthermore, repeated urinary catheterization can predispose to iatrogenic urinary tract infections (Lees et al., 2006). None of the cats developed complications after sampling and every cat that was reluctant to the procedure at first attempt was excluded.

Conclusion

The proposed formula is easy to use and can help for a rapid estimation of urine osmolality in healthy cats. At this step, our findings are only relevant to healthy cats and the proposed model needs to be re-evaluated and validated on a larger population using a prospective design. It would be interesting in the future to find similar equations suitable for pathological conditions, especially in diabetic or glucosuric patients.

List of abbreviation

ACVIM: American College of Veterinary Internal Medicine

AIC_C: Akaike Information Criteria for small samples

CKD: Chronic Kidney Disease

ECVIM: European College of Veterinary Internal Medicine

IRIS: International Renal Interest Society

IV: Inravenous

RL: Ringer Lactate

U_{osm}: Urine osmolality

USG: Urine Specific Gravity

Ethical approval

Ethical committee (cometh 2015_1527, VAS, n°18) – Data provided

Consent for publication

All authors gave approval and consent for publication.

Availability of data and material

The data generated or analysed are included as supplementary material or within additional files that are available.

Competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Authors contribution

TB, JMB, and JLC substantially contributed to the conception of the protocol while BR, JC, JMB, and JLC contributed to the design of the study. TB and JLC were involved in the acquisition of data, JLC, BR and JMB in the interpretation of data and JC in the analysis of data. TB and JLC drafted the manuscript while BR, JMB, JC and JLC critically revised and prepared the manuscript. All authors gave final approval and approved the final manuscript.

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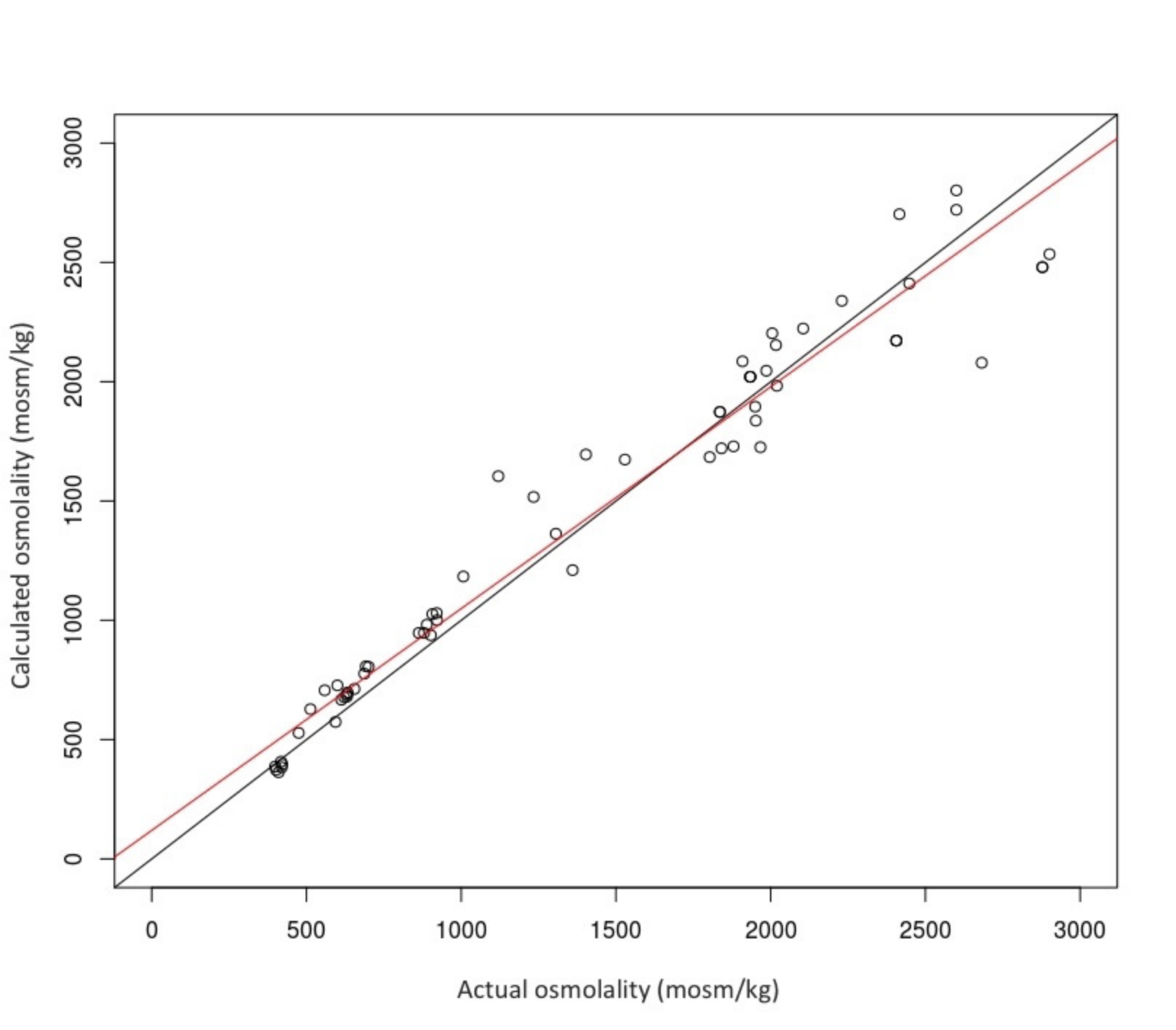
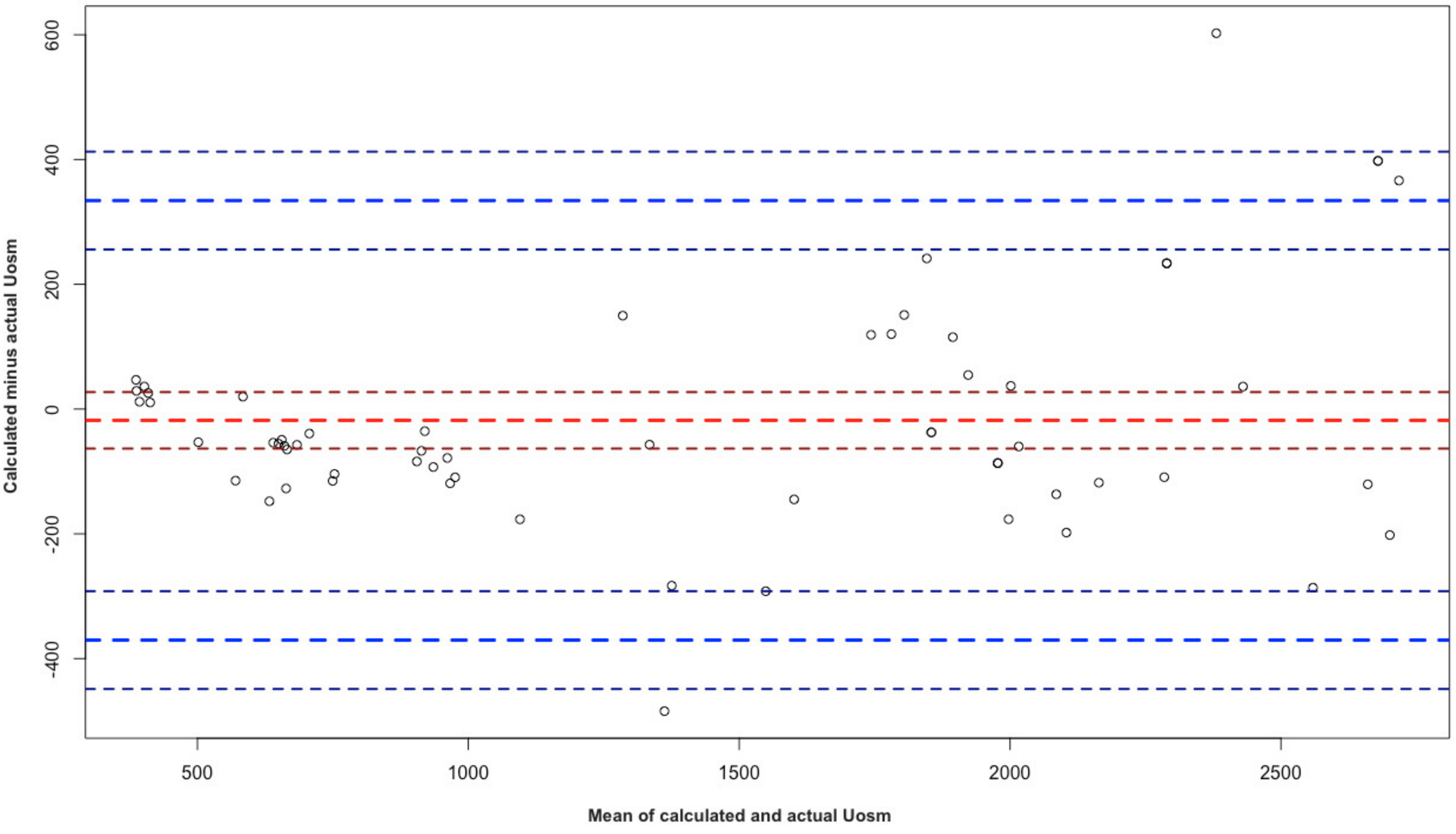
Figure 1. Agreement between calculated and actual urine osmolalities in healthy feline patients. The line of best fit (red) is the line that best crosses the scatter plot to express the relationship between the 2 variables. This line was obtained by applying the reduced major axis method. The black line represents the line of perfect concordance (ie the line of perfect agreement between the 2 variables).

Figure 2. Bland-Altman plot for all samples illustrating the difference between calculated and actual urine osmolalities. The thick red dashed reference line represents the osmolyte gap value, defined as the mean of the differences between calculated and actual urine osmolalities (-7.30 mosm/kg). The thinner red dotted lines correspond to its lower and upper 95% confidence intervals (– 63.32 ; + 27.21 mosm/kg). The thick blue dashed lines represent the lower and upper bounds of 95% limits of agreement for the difference between calculated and actual osmolality (– 370.32 : + 334.21 mosm/kg). The thinner blue dotted lines correspond to the lower and upper limits of their 95% confidence intervals..


Calculated osmolality (mosm/kg)
0
500
1000
1500
2000
2500
3000
Actual osmolality (mosm/kg)
0
500
1000
1500
2000
2500
3000


Calculated minus actual Uosm
600
400
200
0
-200
-400
500
1000
1500
2000
2500
Mean of calculated and actual Uosm

Variables / Units	Mean	Median	Minimum	Maximum
Urea (mmol/L)	808.6	931.4	71.9	1736
Sodium (mmol/L)	149.8	146.0	87.0	202.0
Glucose (mmol/L)	4.20	4.20	1.30	9.70
Potassium (mmol/L)	19.2	21.1	8.9	29.2
Actual osmolality (mosm/kg)	1429.8	1360	399	2901
Predicted osmolality (mosm/kg)	1422.5	1302	302	2940

Table 1. Summary statistics for urine concentration of relevant osmolytes evaluated using Konelab 30i, Thermo Scientific®; Cergy Pontoise, France (Urea, Sodium, Glucose) and the molybdate method (Potassium).

Model	Sodium (mmol/l) [95% CI]	Urea (mmol/l) [95% CI]	Glucose (mmol/l) [95% CI]	AIC_C value
1	**1.05320 [0.22819 – 1.87821]**	**1.25252 [1.07350 – 1.43155]**	**66.72193 [21.09324 – 112.35062]**	**843.551**
2	Not fitted	1.22839 [1.04243 – 1.41435]	101.78429 [63.74247 – 139.82611]	847.524
3	1.781978 [1.08258 – 2.48138]	1.46643 [1.35722 – 1.57564]	Not fitted	856.3768
4	Not fitted	1.701341 [1.64298 – 1.77171]	Not fitted	876.9481
5	Not fitted	Not fitted	339.9172 [319.361 – 360.473]	926.2244

Table 2. Summaries of the 5 best models. The difference between the AICC values of the first and the second model was greater than 2, which is basis for selecting the first model over the second model (Burnham, 2002). 95% CI: 95% confidence interval.