

Validation of equations used to predict plasma osmolality in a healthy adult cohort^{1–4}

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ABSTRACT

Background: Plasma osmometry and the osmol gap have long been used to provide clinicians with important diagnostic and prognostic patient information.

Objective: We compared different equations used for predicting plasma osmolality when its direct measurement was not practical or an osmol gap was of interest and identified the best performers.

Design: The osmolality of plasma was measured by using freezing point depression by microosmometer and osmolality calculated from biosensor measures of select analytes according to the dictates of each formula tested. After a rigid analytic prescreen of 36 originally published equations, a bootstrap regression analysis was used to compare shrinkage and model agreement.

Results: Sixty healthy volunteers provided 163 plasma samples for analysis. Of 36 equations considered, 11 equations met the pre-screen variables for the bootstrap regression analysis. Of the 11 equations, 8 equations met shrinkage and apparent model error thresholds, and 5 equations were deemed optimal with an original model osmol gap <5 mmol.

Conclusions: The use of bootstrap regression provides a unique insight for osmolality prediction equation performance from a very large theoretical population of healthy people. Of the original 36 equations evaluated, 5 equations appeared optimal for the prediction of osmolality when its direct measurement was not practical or an osmol gap was of interest. Note that 4 of 5 optimal equations were derived from a nonhealthy population. *Am J Clin Nutr* 2014;100:1252–6.

INTRODUCTION

Plasma osmometry has long been used to provide clinicians with diagnostic and prognostic information for a variety of disturbed states of body fluid imbalance (1–9). The automated calculation of plasma osmolality (mmol/L) is often favored as a more-expedient choice than directly measuring plasma osmolality (mmol/kg). The measured molal concentration of plasma is often compared with the molar concentration of plasma calculated from a few primary ionic elements (10). The impracticality of measuring the contribution of all osmotic substances, coupled to the colligative complexity and behavior of plasma ions produces a discrepancy between the measured molality and calculated molarity, even when accounting for the differences in the mass concentration of water (11, 12). The difference or bias is referred to as an osmol gap. The diagnostic or prognostic value of the osmol gap (13–15) depends heavily on the performance of the regression equation used to calculate osmolality.

The absence of a consensus over the most efficacious equations for use in predicting osmolality probably depends less on equation corrections for the water concentration (11, 12, 16, 17) [molal to molar (18) or molar to molal units (19)] and more on the fact that the fit of any regression equation to a new set of data will almost always produce an inferior prediction (20, 21). This effect is sometimes referred to as model “shrinkage” (20). The true usefulness of any regression equation depends on how well it predicts data other than those on which it was developed. Limitations of data splitting (22) and cross validation (23) make bootstrap regression an attractive alternative for prospectively estimating model shrinkage. Briefly, bootstrap regression is performed by computer simulation, whereby a sample test population is randomly resampled to draw statistical conclusions about the distribution of the parent population (24). Shrinkage (20) and model-agreement statistics (25) can be neatly applied to anticipate the future performance of any equation in a larger population. The adjunct diagnostic value of any prediction equation requires high test specificity in a population in which, in theory, no osmol gap should exist (26). Thus, a bootstrap regression analysis of equations for the prediction of osmolality in a healthy population is arguably the first step in identifying an equation that consistently produces the smallest osmol gap.

The purpose of this study was to systematically validate 36 equations used to predict plasma osmolality. To achieve this end, we performed a rigid but traditional 5-variable analytic assessment followed by the novel application of a bootstrap regression analysis. The equation performance was ultimately decided by using shrinkage and model-agreement calculations to better understand the potential equation performance in a healthy population where unidentified osmols do not contribute to an equation bias (19, 26, 27).

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SUBJECTS AND METHODS

Volunteers for this study were provided informational briefings and gave voluntary, informed written consent to participate. Volunteers in this investigation were studied under ordinary living conditions (nearly free living). Investigators adhered to Army Regulation 70–25 and US Army Medical Research and Materiel Command Regulation 70–25 on the use of volunteers in research. The US Army Research Institute of Environmental Medicine Institutional Review Board approved this study. Specific study instructions requested that volunteers continue their ordinary food and fluid intakes and physical activity patterns. Study restrictions were limited to abstinence from alcohol consumption for ≥ 24 h and food and fluid intakes for ≥ 90 min before each visit. The use of dietary supplements and any medication other than an oral contraceptive was also prohibited. Confirmation of adherence to study restrictions was obtained on arrival to the laboratory. Clothed body mass (kg) was measured (stationary scale, model WSI-600; Mettler Toledo) as well as standing height (cm) (portable stadiometer, model 217; Seca) with shoes removed. Subjects were seated, and after a 20-min stabilization period, blood was drawn from an antecubital vein without stasis.

Whole blood samples were collected into 2.7-mL lithium-heparin tubes (Sarstedt-Monovette) and centrifuged at 1500 rpm for 15 min at 5°C to acquire plasma. A 20- μ L aliquot sample of plasma was immediately transferred to cuvettes for osmolality determination. The osmometry of plasma was performed in triplicate via freezing-point depression with a calibrated (Clin-itrol 290 Reference Solution; Advanced Instruments) micro-osmometer (Fiske Micro-osmometer, Model 210; Fiske Associates) dedicated for blood analysis. When the triplicate intrasample measures differed ≤ 3 mmol/kg ($\sim 1\%$), the median value was used. If the triplicate intrasample measures differed > 3 mmol/kg, 2 additional samples were measured, and the median value was used (28). Plasma (150 μ L) was also analyzed for sodium, potassium, calcium, magnesium, and blood urea nitrogen by using a direct ion selective electrode, and glucose was analyzed by using enzymatic determination. All direct ion selective electrode and enzymatic measures were made by using a Stat Profile Critical Care Xpress (Nova Biomedical). Plasma proteins were measured by using refractometry (1110400A TS Meter; AO Reichert Scientific Instruments) to estimate plasma water concentrations (29). All plasma constituents are reported in both standard international units and conventional units.

Plasma osmolality was calculated by using 36 different equations. To maintain fidelity with the intent of published equations, no adjustments were made. Although an $\sim 7\%$ numerical discrepancy may exist between osmolality (mmol/kg) and osmolality (mmol/L) because of a smaller molal water fraction, the rational suggestion to uniformly convert to molarity (18) or molality (19) units has not always produced consistent improvements in bias (16, 17). Conversions themselves require certain assumptions that may or may not hold true in theory (12) or reality when considering blood samples from which the original equations were developed. A mix of terms and units have been applied historically (13, 19), but no consensus exists for reporting. For consistency, all calculations are herein referred to as providing osmolality (mmol/L),

whereas direct measurements made on plasma by using freezing point depression are referred to as osmolality (mmol/kg). Therefore, the osmol gap or bias is given in standard international millimole units (mmol/kg minus mmol/L) (30). See Supplemental Table 1 under “Supplemental data” in the online issue for complete references and 36 equations evaluated in this study.

Each of the 36 calculated osmolality equations was compared with the measured osmolality and subjected to the same rigid, but conventional 5-variable assessment before being considered for bootstrap regression analysis. First, the osmol gap (bias or mean error) was required to be normally distributed (D’Agostino-Pearson omnibus test for skewness and kurtosis) and within ± 10 mmol (4, 10, 14, 26, 31, 32). Second, the mean absolute error was required to be < 10 mmol because a bias can underestimate the true error by virtue of extreme positive and negative values. Third, $\geq 95\%$ of individual osmol gaps were required to be within ± 10 mmol, thereby providing assurance that individual gaps would not be grossly underestimated by the mean (26). Any nonuniformity of the osmol gap across the range of a typical reference interval (33, 34) was ignored by using this framework. Fourth, the correlation coefficient (R value) between osmolality (x axis) and osmolality (y axis) was required to be ≥ 0.80 (between a very large and nearly perfect correlation) (35) with the homogeneity of residuals (35, 36). Fifth, 95% limits of agreement for the root mean squared error (RMSE_{95} ; $1.96 \times \sqrt{\text{mean squared error}}$) were required to be less than ± 5 mmol/L so that the model error did not exceed the day-to-day biological variation in plasma osmolality (34, 37). These criteria are consistent with the desire to keep any potential osmol gap to < 15 mmol (26, 31, 32).

Linear regression bootstrapping was performed with SPSS Statistics Premium software (version 20, 2012; IBM Corp) to assess the potential of the top-performing equations in a much larger healthy population (20, 24, 25). A split-file structure was imposed on data so that each equation was analyzed separately. For each equation, 163 cases were resampled (with replacement), and an ordinary least squares regression was performed. This method was repeated 1000 times for each equation to produce a theoretical universe of 163,000 different sample pairs for analysis. A specific syntax was written to obtain model variables of interest including the R value and the mean squared error. The shrinkage and model agreement were determined by simplifying approaches of Copas (20) and Mielke et al (25), respectively, so that changes in absolute model variables could be more easily interpreted. Shrinkage, or the drop in prediction ability, was assessed by using a minimum threshold bootstrap $R = 0.7$. The basis for this threshold was 1) it is the lower bound of a very large correlation (35); 2) it implies that x should explain, at minimum, 50% of the variance in y because the causal relation between x and y is already known; and 3) the small typical range for measured osmolality in a healthy cohort was expected to suppress R (36). The model agreement, or an increase in apparent model error, was considered acceptable so long as the maximum threshold bootstrap RMSE_{95} did not inflate the original model $\text{RMSE}_{95} > 1$ mmol/L beyond the day-to-day biological variation (37). Any model that met these criteria was considered acceptable for the purposes of predicting plasma osmolality. Acceptable bootstrap models with the smallest original bias (< 5 mmol) were further considered optimal (19).



Parametric data are presented as means ± SDs (ranges). Non-parametric data are presented as medians (ranges).

RESULTS

A total of 60 volunteers (42 men and 18 women) provided between 1 and 3 blood samples each, which resulted in an analysis of 163 independent blood samples. The median time interval between intraindividual samples was 2 d (1–349 d). The age of volunteers was 26 ± 7 y (19–46 y) with height of 172 ± 9.2 cm (149–193 cm) and weight of 77.7 ± 15.4 kg (48.8–112 kg). More-descriptive data for the 9 individual plasma analytes considered as well as the measured plasma osmolality and plasma proteins are shown in **Table 1**. All measured values were within typical population reference intervals (27, 38, 39). Plasma water was 93 ± 0.3% (92–94%). The analytic CV for plasma osmolality was calculated from 10 triplicate measures as

$$(SD \div \text{mean}) \times 100 \tag{1}$$

and was 0.5%. All other CVs <3% were calculated from duplicate measures as follows (40):

$$SD = (\sum \text{differences}^2 \div 2n)^{1/2} \tag{2}$$

No outliers were identified when we applied robust linear regression to 36 plasma osmolality (x) and plasma osmolarity (y) data pairs. Of 36 equations considered for analysis, 11 equations passed the initial variable evaluations. Variable details for the 11 equations selected for evaluation using bootstrap regression are shown in **Table 2**. Results of the bootstrap regression analysis are shown in **Table 3**. There were 8 equations that met the shrinkage and apparent model error thresholds for acceptance. Of 8 acceptable equations, 5 equations (27, 41–44) were identified as optimal by having an original mean bias <5 mmol (19). Slope and intercept terms ranged narrowly for the 5 optimal equations from 0.616 to 0.685 and 96.03 to 113.1, respectively. The osmol gap range for the 5 optimal equations (Tables 2 and 3; see Supplemental Table 1 under “Supplemental data” in the online issue) was from 0.7 to –4.5 mmol (Table 2).

DISCUSSION

The purpose of this study was to systematically validate 36 equations used to predict plasma osmolality. Our goal was to identify the most efficacious equations for use in a healthy population where unidentified osmols would not contribute to an equation bias (19, 26, 27). The primary finding of this study was that, of 36 equations used for predicting plasma osmolality, only 11 equations were considered acceptable for the bootstrap analysis. We showed that 8 equations met the required bootstrap thresholds, and we ultimately recommend 5 equations as optimal for the prediction of plasma osmolality (Tables 2 and 3). An acceptable model shrinkage and model agreement provided evidence that the 5 best performing equations should perform well consistently in a healthy cohort (high test specificity), thereby making osmol gap detection more likely when present in a nonhealthy cohort (high test sensitivity) (26).

The findings of this investigation depended on accepting performance variables applied to the initial screen of the 36 equations evaluated, bootstrap model thresholds, and intuitive rules of osmometry. To this end, we applied well-established criteria and analytic methods appropriate for the purposes of this validation study, and we referenced a wealth of published literature in support of our choices. It is also worth repeating that the correct identification of an osmol gap requires the absence of any such gap in a healthy cohort (19, 26, 27). All measured osmolality values in this study were within typical population reference intervals (27, 38, 39) and well below values frequently reported for severe dehydration, (37) poor clinical outcomes (6), or ethanol poisoning (27, 45). Because only a healthy cohort was examined in this study, note that the 5 optimal equations identified (27, 41–44) appeared to also work well in the smaller, nonhealthy cohorts used in their development. Indeed, the comprehensive and seminal study of Edelman et al (10) corroborates that the calculated osmotic contribution of a few primary ions (sodium, glucose, and nonprotein nitrogen) should compare well with the measured osmolality even when there are pathologic elevations in those ions as a result of a variety of diseases. Close agreement between measured and calculated osmolality using a few primary ions also makes osmol gap identification, which is often critical in emergency

TABLE 1
Descriptive statistics for the concentrations of select substances in plasma¹

	Standard international units		Conventional units	
	Mean ± SD	Range	Mean ± SD	Range
Sodium	138.6 ± 1.4 mmol/L	135.7–145.0 mmol/L	138.6 ± 1.4 mEq/L	135.7–145.0 mEq/L
Potassium	4.1 ± 0.3 mmol/L	3.5–5.7 mmol/L	4.1 ± 0.3 mEq/L	3.5–5.7 mEq/L
Chloride	106.6 ± 1.7 mmol/L	102.1–110.6 mmol/L	106.6 ± 1.7 mEq/L	102.1–110.6 mEq/L
Calcium ²	1.15 ± 0.04 mmol/L	1.00–1.28 mmol/L	4.62 ± 0.15 mg/dL	4.00–5.10 mg/dL
Magnesium ²	0.61 ± 0.05 mmol/L	0.52–0.75 mmol/L	1.48 ± 0.11 mg/dL	1.27–1.83 mg/dL
Glucose ³	5 ± 1 mmol/L	4–7 mmol/L	99 ± 11 mg/dL	69–134 mg/dL
BUN ⁴	6 ± 1 mmol/L	3–11 mmol/L	16 ± 4 mg/dL	9–30 mg/dL
Plasma proteins	0.8 ± 0 g/L	0.7–0.9 g/L	7.5 ± 0.4 g/dL	6.6–8.6 g/dL
Plasma osmolality	295 ± 4 mmol/kg	285–307 mmol/kg	295 ± 4 mOsm/kg	285–307 mOsm/kg

¹ Data are for 163 blood samples.
² Ionized.
³ Measured enzymatically.
⁴ BUN, blood urea nitrogen.

TABLE 2Five-variable analysis of equations meeting established criteria¹

Equation no.	Equation	<i>R</i>	RMSE ₉₅	Bias	MAE	Percentage within ± 10 mmol
6	$2 \times \text{Na}^+ + \text{BUN} \div 2.8 + \text{glucose} \div 18$	0.81	3.7	-4.5	4.6	99.3
9	$2 \times (\text{Na}^+ + \text{K}^+) + \text{BUN} \div 2.8 + \text{glucose} \div 18$	0.82	3.7	3.8	3.9	100
11	$(1.86 \times \text{Na}^+ + \text{BUN} \div 2.8 + \text{glucose} \div 18) \div 0.93$	0.81	3.8	-3.7	3.8	99.3
13	$1.86 \times (\text{Na}^+ + \text{K}^+) + \text{urea} + \text{glucose} \div 10$	0.82	3.6	-6.2	6.2	97.5
14	$1.89 \times \text{Na}^+ + 1.38 \times \text{K}^+ + 1.03 \times \text{urea} + 1.08 \times \text{glucose} + 7.45$	0.82	3.6	-6.0	6.0	98.2
16	$2 \times \text{Na}^+ + \text{BUN} \div 3 + \text{glucose} \div 20 + 8$	0.81	3.7	2.5	2.9	100
26	$2 \times (\text{Na}^+ + \text{K}^+) + \text{glucose} \div 18 + 0.93 \times 0.5 \times \text{BUN} \div 2.8$	0.81	3.5	0.7	1.9	100
30	$1.897 \times \text{Na}^+ + \text{glucose} + \text{BUN} + 13.5$	0.81	3.6	5.3	5.3	100
31	$1.9 \times (\text{Na}^+ + \text{K}^+) + \text{glucose} + \text{BUN} + 5$	0.82	3.6	5.5	5.5	100
34	$1.86 \times (\text{Na}^+ + \text{K}^+) + 1.15 \times \text{glucose} + \text{urea} + 14$	0.82	3.6	-1.4	2.2	100
35	$1.09 \times (1.86 \times \text{Na}^+ + \text{glucose} + \text{urea})$	0.81	3.9	-0.3	1.9	100

¹Data are for 163 pairs of measured osmolality (x axis) and calculated osmolality (y axis). Analysis includes (ordinary least squares regression) the correlation coefficient *R* and RMSE₉₅. Descriptive statistics include the mean difference (bias), the MAE, and percentage of values within a ± 10 -mmol difference. Equation numbers align with the equation numbering system provided (see Supplemental Table 1 under "Supplemental data" in the online issue). BUN, blood urea nitrogen; MAE, mean absolute error; RMSE₉₅, 95% limits of agreement for the root mean squared error.

situations (31, 32), more likely when unidentified osmols are present. Therefore, it is plausible that equation performances observed in this study should also be applicable to nonhealthy populations.

Recommendations of this study provide the clinical nutrition community with a much-needed systematic validation (27) study of the many equations used to predict plasma osmolality (see Supplemental Table 1 under "Supplemental data" in the online issue). In practice, a number of original equations considered (most commonly equation 6) are already in use for the automated calculation of osmolality in many bench-top analyzers. However, some analyzers, including the instrument used in this study (Stat Profile Critical Care Xpress; Nova Biomedical), incorporate an equation for the automatic calculation of osmolality (46) that did not meet bootstrapping regression analysis thresholds. The choice of an equation used by manufacturers or clinicians is largely based on experience or the many equation comparisons published that used single population cohorts over the past 30 y. There remains no consensus over which equation is the best, most probably because the application of existing equations to novel data or cohorts invariably produces an inferior agreement (20, 21). To our knowledge, our study is the first to comprehensively validate the performance of multiple equations beyond a large, single dataset. Our use of bootstrap regression afforded a glimpse at equation performances from a theoretical universe of healthy people (24) obtained by performing 1000 regression analyses on each equation by using 163 newly resampled data pairs each time.

All 5 formulas considered optimal in this study (Tables 2 and 3) contained only 3 or 4 ions in their calculations. This fact underscores the experimental conclusions of Edelman et al (10) and supports the contention that the colligative complexity of plasma (12) does not lend itself easily or accurately to the quantitative summation of too many ionic substances (11). Of the 5 most optimal equations identified in this study, one equation was published 46 y ago (41) and previously identified by Worthley et al (47) as the "best" and "simplest" formula for the calculation of

osmolality. When expressed in the International System of Units, it can be easily calculated mentally as follows (41):

$$2 \times \text{sodium} + \text{glucose} + \text{blood urea nitrogen} \quad (3)$$

All of the top-performing equations produced osmol gaps that ranged from 0.7 (27) to 4.5 mmol (41).

In conclusion, 5 (27, 41–44) of 36 equations evaluated appear optimal for predicting osmolality. The acceptable performance of the 5 optimal equations in healthy people with no unidentified osmols suggests that their use in other populations should reliably predict osmolality and aid in identifying meaningful osmol gaps (27, 41–44), but only prospective tests of this conclusion will provide greater certainty.

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The authors' responsibilities were as follows—SNC and KRH: data analysis and interpretation of data; and all authors: study design and writing and editing of the manuscript. None of the authors had a conflict of interest.

TABLE 3Bootstrapping regression analysis of the top-performing equations¹

Equation no.	Bootstrap range	
	<i>R</i>	RMSE ₉₅ ²
6 ³	0.71–0.89	3.2–4.3
9 ³	0.72–0.90	3.2–4.3
13	0.72–0.90	2.9–4.0
14	0.71–0.90	3.0–4.2
16 ³	0.71–0.89	3.0–4.2
26 ³	0.71–0.89	2.9–4.1
31	0.72–0.90	3.0–4.2
34 ³	0.71–0.89	3.0–4.2

¹Data are for 163 resampled (with replacement) pairs of measured osmolality (x axis) and calculated osmolality (y axis). An ordinary least squares regression was performed 1000 times for each 163 newly resampled pairs to produce a range of possible *R* and RMSE₉₅ outputs from a theoretical universe of 163,000 pairs. Equation numbers align with the equation numbering system provided (see Supplemental Table 1 under "Supplemental data" in the online issue).

²RMSE₉₅, 95% limits of agreement for the root mean squared error.

³Optimal on the basis of an original osmol gap <5 mmol.



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