**Accuracy of prediction equations for serum osmolarity in frail older people with and without diabetes**

Mario Siervo, Diane Bunn, Carla M Prado, Lee Hooper

**Affilitations:** Human Nutrition Research Centre, Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle on Tyne,UK (MS); Norwich Medical School, University of East Anglia, Norwich Research Park,Norwich, UK (DB, LH); Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, Canada (CP)

**Last name of each author**: Siervo, Bunn, Prado, Hooper

**Running title:** Accuracy of serum osmolarity in older people

**Keywords:** aged, osmolar concentration, prediction equations, dehydration, diabetes mellitus

**Corresponding author:** Lee Hooper (l.hooper@uea.ac.uk), Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, Norfolk NR4 7TJ, UK. Phone: +44 1603 591268

**Funding:** This report is independent research arising from a Career Development Fellowship to LH (NIHR-CDF-2011-04-025) supported by the National Institute for Health Research. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health.

**Conflict of interest statement:**  The authors have no conflict of interest to declare. The material presented in this manuscript is original and it has not been submitted for publication elsewhere while under consideration for AJCN.

**Clinical Trial Registry:** Research Register for Social Care Id 122273, <http://www.researchregister.org.uk/>

**Abbreviations:** BMI - Body Mass Index; CI - Confidence Interval; DRIE - Dehydration Recognition In our Elders; eGFR - Estimated Glomerular Filtration Rate; MMSE - Mini-Mental State Exam; RR - Relative Risk; UK - United Kingdom

**ABSTRACT**

**Background:** Serum osmolality is an accurate indicator of hydration status in older adults. Glucose, urea and electrolyte concentrations are used to calculate serum osmolarity, an indirect estimate of serum osmolality, but which serum osmolarity equations best predict serum osmolality in the elderly is unclear.

**Objective:** to assess agreement of measured serum osmolality with calculated serum osmolarity equations in older people.

**Design:** Serum osmolality was measured using freezing point depression in a cross-sectional study. Plasma glucose, urea and electrolytes were analysed and entered into 38 serum osmolarity prediction equations. The Bland-Altman method was used to evaluate agreement and differential bias between measured osmolality and calculated osmolarity. Sensitivity and specificity of the most promising equations were examined against serum osmolality (reference standard).

**Results:** 186 people living in UK residential care took part in the Dehydration Recognition In our Elders study (DRIE, 66% women, mean age 85.8±7.9 years, with a range of cognitive and physical impairments) and were included in analyses. 46% had impending or current dehydration (serum osmolality ≥295 mmol/kg). Those with diabetes (n=33, 18%) had higher glucose (p<0.001) and serum osmolality (p<0.01). Of 38 predictive equations used to calculate osmolarity, four showed reasonable agreement with measured osmolality. One (calculated osmolarity= 1.86×(Na++K+)+1.15×glucose+urea+14, all in mmol/L) was characterised by narrower limits of agreement and capacity to predict serum osmolality within 2% in >80% of participants, regardless of diabetes or hydration status. The equation’s sensitivity (79%) and specificity (89%) for impending dehydration (295+ mmol/kg) and current dehydration (>300 mmol/kg, 69% and 93% respectively) were reasonable.

**Conclusions:** Assessment of a panel of equations for prediction of serum osmolarity led to identification of one formula with greater diagnostic performance. This equation may be utilised to predict hydration status in frail older people (as a first stage screening) or to estimate hydration status in population studies.

**INTRODUCTION**

Water is a vital component of the human body, accounting for ~60% of its weight (1;2). The tight regulation of water balance and tonicity seen in humans involves several physiological functions including thirst, salt-seeking behaviour, neuro-endocrine and organ-specific responses. However, these functions tend to work less well in the elderly, so dehydration becomes more common. In the US National Health & Nutrition Examination Survey (NHANES) III cohort water-loss dehydration (serum tonicity of 300+ mOsm/L) was found in 16% of 20–29 year olds, increasing to 28% of 70–90 year olds (3), and in a study of Californian nursing homes 31% of residents were dehydrated at least once over 6 months (4). This high level of dehydration in older people has clinical and public health impact. Several prospective analyses of older people, carefully adjusted for concurrent risk factors, found that dehydration was associated with increased risk of mortality and disability (5-7). It is important to accurately identify older people with impending or current dehydration, to restore euhydration and improve disability-free life expectancy (8).

In young men and women plasma or serum osmolality is the only useful marker of static dehydration, with a “cut-off of 301 ± 5 mmol/kg” having the best diagnostic accuracy (9). While such rigorous analysis has not been carried out in older people, serum osmolality is likely to be the best indicator. Its advantages include 1) utilisation of standardised, objective analytical procedures, 2) determination of hydration status by a single measurement, and 3) no requirement for additional clinical and nutritional information. Serum osmolality is carefully controlled by the body. Increases in serum osmolality associated with dehydration stimulate cellular osmoreceptors that in turn stimulate thirst (leading to increased water intake) and vasopressin (or anti-diuretic hormone, ADH) secretion (reducing urinary water excretion) (10). The key physiological role of serum osmolality in maintenance of euhydration provides further support to its use as a reference standard for assessment of dehydration in older adults (11-13).

However, in some circumstances, the direct measurement of serum osmolality is not routinely undertaken due to cost implications (for example in UK hospitals measurement of serum osmolality is uncommon). If a valid equation for calculating serum osmolarity can be derived from osmotically-active determinants (serum Na, K, urea and glucose) generated from generic blood testing, this would improve the likelihood of detecting dehydration in older people. It would also be possible to assess hydration in existing research datasets, where these determinants are routinely available, but serum osmolality is not. Many equations have been used to calculate osmolarity, but it is not known which maps best onto measured osmolality in the elderly. Raised serum osmolality may be due to low fluid intake (general hemoconcentration) or poorly controlled diabetes (raised serum glucose) (14) so the accuracy of formulae should not be influenced by haematocrit level or diabetes status.

We conducted a validation study of equations for the calculation of serum osmolarity (mapping onto serum osmolality) in older people with and without diabetes. The primary objective was to identify a prediction equation not prone to differential bias associated with factors influencing body hydration, such as age, body size, or concentrations of particular effective solutes and characterised by good diagnostic accuracy.

**METHODS**

The DRIE (Dehydration Recognition In our Elders) study was a cohort study approved by the NRES Committee London – East Research Ethics committee (11/LO/1997, full ethical approval granted 25th January 2012), all study procedures were in accordance with the ethical standards of the Helsinki Declaration. The full study protocol, including measurement details, methods for assessment of capacity, and other study documentation are available in the Online Supplementary text files, Online Supplemental DRIE Letters and from the DRIE website (15).Baseline recruitment of 198 participants began in April 2012 and was completed in August 2013, and this publication utilizes the baseline (cross-sectional) data. Men and women aged 65+ living in residential care (residential care homes, nursing homes, specialist dementia care homes and mixed homes) in Norfolk and Suffolk, UK were recruited. Participants were excluded if they had been diagnosed with renal failure or heart failure, were in receipt of palliative care, had illnesses suggesting they were unlikely to survive for at least 3 months, or the care home manager reported that the resident did not wish to participate, or that they were too anxious or unwell for researchers to approach. Each participant signed informed written consent if they were willing to participate and able to answer several questions about the study. Participants who were willing to take part but unable to answer the questions (so unable to provide informed consent) were included where their designated consultee (a relative or close friend) provided a written declaration that they thought the participant would have chosen to take part if they still had capacity (described in full in the Online Supplementary text files).

*Data collection:* Study interviews were scheduled for times when participants were available and varied from 8am until 8pm. In summary, non-fasting venous blood samples were collected from an antecubital vein, or where necessary, from the back of the hand, after participants had rested for at least 5 minutes in a sitting (or occasionally lying) position. If a blood sample was not obtained after the second attempt, the procedure was abandoned and participant excluded. The interview continued with measurements of anthropometry, body composition, physical function, potential signs of dehydration (including skin turgor, capillary refill, mouth exam, sitting and standing blood pressure, urine testing), and standardised questionnaires assessing health status, and cognitive capability, including the Mini-Mental State Exam (MMSE). The MMSE scores from 0 to 30, with lower scores indicating greater cognitive impairment (16;17). Body weight was measured with participants wearing light clothes to the nearest 0.1kg using the care home scales. Height was obtained from care home records or estimated from ulnar length where necessary (18). Body Mass Index (BMI) was calculated (weight in kg divided by height in meters squared).

Data on age at interview, gender, co-morbidities (including diabetes) and current medication use were obtained from care home records. The Barthel Index is a measure of physical function (19;20), with potential scores from 0 to 100, 100 representing best functional status. The Barthel Index was completed for each participant, with questions answered by a senior member of care staff. Diabetes information was double checked – so that those identified as having diabetes were compared with participants found to have raised serum glucose, or using any diabetic medication. No additional potential diabetics were identified in this way.

Blood samples were collected using a needle and syringe, immediately inverted several times, then placed in a temperature controlled box (without heating or cooling, protected from outside temperature extremes) and driven to the Department of Laboratory Medicine, Norfolk and Norwich University Hospitals Trust (Norfolk, UK), delivered within four hours of collection, and samples were analysed immediately. The laboratory is fully accredited with Clinical Pathology Accreditation (UK) Ltd., has daily internal quality control run along with calibrators and is judged fortnightly against its peers (external quality control). Serum osmolality (measured by assessment of depression of freezing point, Advance Instruments Model 2020) was assessed in all samples. This model has a repeatability of ±3 mmol/kg (1 SD) in the 0 to 400 mmol region. The lab coefficient of variance for analysis of serum osmolality (at all levels) was 0.9%. Where sufficient blood was collected we also assessed serum urea (Abbott Architect using urease), serum creatinine (Abbott Architect using enzymatic method), serum sodium and potassium (Abbott Architect using Ion-selective electrode diluted), hemoglobin (Instrument Sysmex XN), and finally blood glucose (Abbott Architect using hexokinase/G-6-PDH). Estimated Glomerular Filtration Rate (eGFR) was calculated using the Cockcroft-Gault formula. Classification of hydration status was based on measured serum osmolality. Participants were categorised as being normally hydrated (serum osmolality 275 to <295 mmol/kg), having impending dehydration (serum osmolality 295 to 300 mmol/kg), or current dehydration (>300 mmol/kg) (9;12).

*Predictive Equations*: Fazekas and colleagues collected 36 different equations used to determine serum osmolarity (21). The equations involved summing multiples of serum sodium, potassium, glucose and urea, and occasionally ionized calcium, magnesium, lactate and bicarbonate. As sodium, potassium, glucose and urea are regularly measured in older people having blood tests our study has focussed on the 33 equations including only these factors [omitting 3 equations discussed by Fazekas that included ionized calcium or lactate as these test results are not routinely available (22-24)]. Fazekas and colleagues chose to multiply the results of several equations by 0.985 as they were reported in mOsm/L (25-27), however this was unlikely to have been the original authors intention so we ran the equations with and without this multiplication. In addition, we evaluated the predictive accuracy of widely used simple formulae for plasma osmolality (28), and tonicity (6), as well as using the aggregate method proposed by Wells and colleagues (29). This latter approach is based on the assumption that the osmolarity prediction equations are independent of one another and that these independent predictions can then be aggregated. Under these conditions, the error will not be correlated across the predictions, but will rather be randomly distributed across them and hence tend to cancel out, increasing the accuracy of the serum osmolarity aggregate prediction. All of the resulting 38 equations analysed in this study are provided in **Supplemental Table 1 (online supplementary material).**

*Terminology and units:* Measured osmolality is assessed in mOsm/kg or mmol/kg (molal units), while calculated osmolarity is in mOsm/L or mmol/L (molar units), which makes the terminology when comparing the two complex. Some authors of equations used herein have converted constituent mmol/L units into mmol/kg (dividing by 0.933) before carrying out regression so that inputting mmol/L units generates an output in mmol/kg (30). This means that some equations used in this study produced outputs in mOsm/L or mmol/L and some in mOsm/kg or mmol/kg, which would allow the osmolar gap to be expressed in mmol (31). For clarity within this paper, all equations were written using SI unit conversions and referred to as calculated osmolarity and expressed in mmol/L. Measured osmolality is reported herein as mmol/kg, although the units provided by our laboratory were mOsm/kg. As we were aiming for equivalence of osmolarity and osmolality where we have equations where measured osmolality and calculated osmolarity were added or subtracted units have been given as mmol.

*Statistical Analysis:* The cohort study was powered to allow development of a diagnostic decision tree to identify dehydration and so study size was not directly related to the current analysis. The t-test for independent samples was used to compare participants stratified by diabetes status, while the chi-square test was used to detect differences in the frequency of accurate predictive estimates in participants stratified by diabetes and hydration status. Analysis of variance was used to examine differences in predictive accuracy between subjects stratified by gender and diabetes status. The difference (∆, measured osmolality in mmol/kg minus calculated osmolarity in mmol/L) was expressed ±2SD and deemed accurate if the mean fell between -1 and +1mmol. The number of participants with calculated osmolarity values within ±2% of measured osmolality was also calculated. The paired t-test was used to determine the statistically significant differences between the measured osmolality and calculated osmolarity. The Bland-Altman method was used to evaluate the agreement of absolute (mmol) and relative (%) difference between measured osmolality and calculated osmolality (32). Pearson's correlation was used to assess the association of ∆ with age, BMI and biochemical parameters (serum hemoglobin, Na+, K+, glucose, urea and estimated glomerular filtration rate (eGFR)). Hydration status based on calculated osmolarity was plotted in 2x2 tables against measured osmolality. These tables were used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and other diagnostic criteria. All statistical analyses were carried out using PASW 19 for Windows (Polar Engineering and Consulting, formerly known as SPSS). Statistical significance was set at p< 0.05.

**RESULTS**

DRIE took place in 56 care homes, including 1816 residents, of whom 1077 were deemed ineligible by care home managers. Of the 739 potentially eligible residents approached by the researchers, 374 told us they were not interested while 365 wanted to take part, and 256 provided their own or consultee consent. We initiated research interviews with 232 (see **Figure 1** for further details), obtained serum osmolality for 198 individuals, plus serum sodium, potassium and urea data for 186, of whom 172 also had random serum glucose measurements. Of the 186 participants 33 (18%) had diabetes, and 35 (19%) had current dehydration (serum osmolality >300 mmol/kg), a further 50 (27%) had impending dehydration (serum osmolality 295-300 mmol/kg), 94 (51%) were normally hydrated (serum osmolality 275-<295 mmol/kg) and 7 (4%) had serum osmolality <275 mmol/kg. Of the 186 participants, 122 (66%) were women, mean age was 85.8 years (SD 7.9, range 65.7 - 105.5), and mean BMI 25.8 kg/m2 (SD 5.5, range 15.5 - 42.2). Mean MMSE score was 21.8 (SD 5.7, range 0 to 30), and mean Barthel Index was 66.6 (SD 26.4, range 0 to 100).

These characteristics did not differ between participants with and without diabetes (**Table 1**). Participants with diabetes did differ from those without diabetes in having higher serum osmolality, sodium, urea and glucose levels, and lower hemoglobin, but similar serum potassium, creatinine, and eGFR on average. Serum osmolality was significantly positively correlated with serum Na+ (r=0.73, p<0.001), urea (r=0.47, p<0.001), creatinine (r=0.30, p<0.001), and glucose (r=0.36, p<0.001), but not with serum potassium. (**Supplementary** **Table 2, online supplementary material**)

*Assessment of absolute bias (paired t-test)*: Analyses were conducted in the whole sample (of 186 for equations not including glucose, 172 for equations that involved serum glucose measures) and after stratification by diabetes status. The equations were characterised by wide range of predictive bias from 31 to -27mmol. Four equations (equations 24, 26, 32 & 33) had no significant differences between measured osmolality and calculated osmolarity, and the predictive bias was between -1 and 1mmol. Of these, only equation 32 showed no significant difference between measured osmolality and calculated osmolarity for the full sample, and for both subgroups (with and without diabetes), see **Table 2**.

*Bland-Altman Analysis*: The accuracy of the 4 selected equations was evaluated using Bland-Altman analysis (**Figure 2A-D**). Equation 32 was characterised by greatest agreement with measured osmolality. Specifically, equation 32 (**Figure 2C**) had narrower limits of agreement (±7.4) compared to the other 3 equations (**Figure 2A, 2B, and 2D**) and the bias was not associated with increasing values of osmolality.

*Predictive and diagnostic accuracy*: We assessed the number of individual predictions (calculated osmolarity) that fell within ±2% of measured osmolality for each of the four equations, stratified by diabetes status and hydration status. Again, equation 32 out-performed the other 3 equations by consistently predicting more than 80% of the osmolality values within the ±2% margin across diabetic (**Figure 3A**) and hydration subgroups (**Figure 3B).** Bland-Altman analysis of the percent distribution of the measurement bias confirmed the better agreement of equation 32 (**Supplementary Figure S1C**) compared to the other 3 equations (**Supplementary Figure S1A, S1B and S1D**). Further analyses showed that the absolute bias of equation 32 was not influenced by gender (as differences between measured osmolality and predicted osmolarity were not significantly different in men and women, **Figure 4**).

*Diagnostic accuracy:* The diagnostic accuracy of the four equations in identifying participants with current and impending dehydration, and euhydration as assessed by serum osmolality was assessed and is shown in **Supplementary Table 3.** The sensitivity of equation 32 in identifying participants with current dehydration (>300mmol/L) was modest (64%) whereas its specificity was high (93%). The positive and negative likelihood ratios were 8.85 and 0.39 respectively, with a diagnostic odds ratio of 22.6. The diagnostic accuracy of the equation improved for impending dehydration (295 to 300mmol/L) as sensitivity and specificity were 79% and 89% respectively, positive and negative likelihood ratios 7.53 and 0.23, with a diagnostic odds ratio of 32.4. If calculated serum osmolarity were to be used as a screening tool for current dehydration in this population it would be important not to miss cases of current dehydration, so high sensitivity would be crucial. As further assessment in those found to be at risk is simple (measuring serum osmolality), lower specificity would be acceptable. We examined the diagnostic accuracy of different calculated osmolarity cut-offs in screening for current dehydration (measured serum osmolality >300 mmol/kg) and suggest that a calculated osmolarity finding of >296 mmol/L has high sensitivity (97%) while retaining reasonable specificity (76%), with a diagnostic odds ratio of 99 (**Table 3**).

*Sensitivity Analyses*: We analysed the presence of differential bias by assessing the correlation of absolute bias (∆, measured-calculated) with factors associated with hydration including age, BMI, electrolytes (Na+, K+), glucose, urea, creatinine, eGFR, Urea/Cr, Hb, MMSE and Barthel Index. Equation 32 suggested least differential bias, as low-order correlations were only found with K+ (r=-0.28, p<0.001) and MMSE scores (r=0.21, p<0.01) (**Supplementary Table 4**).

**DISCUSSION**

The equation for calculated serum osmolarity developed by Khajuria and Krahn (1.86×(Na++K+)+1.15×glucose+urea+14, where all components were measured in mmol/L), was best able to predict measured serum osmolality in frail older people with and without diabetes (30). We did not detect evidence for differential bias related to the influence of factors associated with hydration such as age, BMI, sodium, urea and glucose. The equation’s sensitivity (79%) and specificity (89%) for impending dehydration (295+ mmol/kg) and current dehydration (>300 mmol/kg, 69% and 93% respectively) were reasonable.

Some limitations of our analyses need to be considered in the interpretation of these results. These results are specific to frail older people living in residential care, so should be extrapolated only with care. However, the high prevalence and prognostic value of dehydration in older participants (in this population 19% had current dehydration and a further 27% had impending dehydration) attribute a high clinical relevance to this analysis. Application of the equation in older people living in the community, those with heart or renal failure, and those at end of life may, or may not, be not appropriate, but needs to be tested. However, the population was heterogeneous for socio-demographic characteristics and health-related conditions which increased the variability of measured serum osmolality and allowed a more sensitive analysis of the diagnostic accuracy of the predictive equations. The cross-sectional study design is a minor limitation of the analysis as it did not attempt to establish causality of associations between hydration and health factors but we specifically focused on evaluation of agreement between measured serum osmolality and calculated serum osmolarity.

Scrutiny of the variables included in equation 32 reveals inclusion of the main solutes contributing to serum osmolality (Na+, K+, glucose, urea); other equations included the same variables in the equations and the main differences were the coefficients. External validation of predictive equations is important in establishing their accuracy, and is affected by the rigour of the study design, measurement protocols and representativeness of the population included in the validation sample. The validation of equation 32 was conducted in a sample of frail older people living in residential care, with a variety of chronic health problems and a wide range of cognitive and physical limitations. Our analysis framework included the presence of important variables such as age and BMI, but it is not clear whether the results will be generalisable to older people living independently.

Analytically we were unable to run duplicate assessments of serum osmolality, our reference standard, which may have reduced the accuracy of our hydration status assessment.

Our equation 32 was developed by Khajuria and Krahn to minimise the osmolar gap (the difference between serum osmolality and osmolarity) with a view to using any emerging osmolar gap to quantify alcohol intake (30). In our study we did not formally assess recent alcohol intake, but no participants were inebriated or smelled of alcohol at the study visit. Alcohol intake is low in UK care homes, many participants discussed drinking favourite alcoholic beverages when visiting family and friends, and some kept a bottle or two of alcoholic drinks to offer visitors, but only two men appeared to drink regularly, one drinking a pint of beer or cider daily, the other half a pint. Kahjuria and Krahn investigated the predictive capacity of coefficients for glucose, which may explain the good performance of this equation in our population and the maintenance of accuracy in both diabetic and non/diabetic patients.

There is evidence that dehydration is associated with increased risk of mortality and poorer functional status in older populations (5-7). In one study 561 non-disabled Americans aged at least 70 years were recruited. Having dehydration (tonicity of >300 mOsm/L) at baseline, compared to euhydration (normal tonicity, 285– 294 mOsm/L), was associated with a doubled risk of 4-year disability (RR 2.1, 95% CI: 1.2, 3.6) and a 40% increase in the risk of 8-year mortality (RR 1.4, 95% CI: 1.0, 1.9) (6). Since one of the reasons for increased tonicity and also for increased disability and mortality may be uncontrolled diabetes, the analyses were repeated omitting participants with raised glucose. These analyses also suggested an 80% increase in risk of 4-year disability and 50% increased risk of 8-year mortality in those with dehydration at baseline, however, the associations were no longer statistically significant (RR 1.8, 95% CI: 0.8, 3.9 for disability, RR 1.5, 95% CI: 0.9, 2.3 for mortality in normoglycaemics) due to the smaller sample size. The analyses were controlled for age, sex, race, weight, smoking, activity, plasma urea and creatinine, cognitive impairment, depression, and chronic disease (6). These data suggest, but do not prove conclusively, that it is important to identify older people with impending or current dehydration so that we can improve their hydration levels, and help to prevent long term functional and physical deficits (8). The equations for calculated tonicity (equation 35) used in this study (6) mapped quite well onto serum osmolality, with a difference between measured serum osmolality and calculated tonicity of 1.7 (2SD 9.6), but performed better in those without diabetes than in those with diabetes (see Table 2).

*Clinical importance:* Further validation in other populations is needed, but a screening tool for dehydration based on an equation calculating serum osmolarity and involving routine clinical biochemical parameters could have a significant impact in the preliminary assessment and correction of current dehydration in older participants. If this formula is validated in further elderly populations, serum osmolarity, calculated according to our equation 32, could be automatically calculated on pathology lab reports for those aged 65 and over, providing an opportunistic method for the assessment of hydration status. A calculated serum osmolarity reading of >296 mmol/L could equate to a high suspicion of dehydration (as defined by measured serum osmolality >300mmol/kg (9;12)) and could usefully lead to serum osmolality testing, to confirm hydration status, unless clearly due to raised serum glucose (in which case diabetic control needs to be established).

This comprehensive analysis of equations for the calculation of serum osmolarity identified one equation with superior diagnostic accuracy in older participants. The equation, using routine biochemical parameters, needs to be confirmed in free living populations, but can be recommended as a valid substitute for the direct measurement of serum osmolality in existing data sets, and could usefully be used to screen for current dehydration in clinical situations.

**Acknowledgements**

We would like to thank all of the care homes and residents who generously participated in DRIE, and gave their time, energy and enthusiasm to this research. We are also grateful for the excellent support of Susan Kerry, Garry John and the Norfolk and Norwich University Hospital Pathology Laboratory who analysed our blood samples. The authors have no conflict of interest to declare.

**Author contributions**

LH is the guarantor of this work, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. LH and DB designed DRIE, recruited participants, and collected the data. MS and LH conceived the analysis discussed within this paper and MS, CP and LH carried out the analyses. MS and LH wrote the first draft of the manuscript and all authors edited the paper and agreed the final content.

**References**

 1. Olde Rikkert MGM, Melis RJF, Claassen JAHR. Heat waves and dehyration in the elderly: recognising the early warning signs can save lives. *BMJ* 2009;**339**:b2663.

 2. Olde Rikkert MGM, Hoefnagels WHL, Deurenberg P. Age-related changes in body fluid compartments and the assessment of dehydration in old age. In: *Hydration and Aging* (eds. Vellas B, Albarede JL, Garry PJ). Serdi: Paris, 1998; 9-32.

 3. Stookey JD. High prevalence of plasma hypertonicity among community-dwelling older adults: results from NHANES III. *Journal of the American Dietetic Association* 2005 August;**105**(8):1231-1239.

 4. Mentes J. Oral hydration in older adults: greater awareness is needed in preventing, recognizing, and treating dehydration. *American Journal of Nursing* 2006 June;**106**(6):40-49.

 5. Bhalla A, Sankaralingam S, Dundas R, Swaminathan R, Wolfe CD, Rudd AG. Influence of raised plasma osmolality on clinical outcome after acute stroke. *Stroke* 2000 September;**31**(9):2043-2048.

 6. Stookey JD, Purser JL, Pieper CF, Cohen HJ. Plasma hypertonicity: Another marker of frailty? *Journal of the American Geriatrics Society* 2004;**52**(8):1313-1320.

 7. Wachtel TJ, Tetu-Mouradjian LM, Goldman DL, Ellis SE, O'Sullivan PS. Hyperosmolarity and acidosis in diabetes mellitus: a three-year experience in Rhode Island. *Journal of General Internal Medicine* 1991 November;**6**(6):495-502.

 8. Hooper L, Bunn D, Jimoh FO, Fairweather-Tait SJ. Water-loss dehydration and aging. *Mechanisms of Ageing and Development* 2013;Published ahead of print 9 December 2013.

 9. Cheuvront SN, Ely BR, Kenefick RW, Sawka MN. Biological variation and diagnostic accuracy of dehydration assessment markers. *American Journal of Clinical Nutrition* 2010;**92**(3):565-573.

 10. Naitoh M, Burrell LM. Thirst in elderly subjects. In: *Hydration and aging* (eds. Vellas B, Albarede JL, Garry PJ). Serdi: Paris, 1998; 33-46.

 11. Cheuvront SN, Kenefick RW, Charkoudian N, Sawka MN. Physiologic basis for understanding quantitative dehydration assessment. *American Journal of Clinical Nutrition* 2013;**97**:455-462.

 12. Thomas DR, Cote TR, Lawhorne L, Levenson SA, Rubenstein LZ, Smith DA, Stefanacci RG, Tangalos EG, Morley JE, Dehydration Council. Understanding clinical dehydration and its treatment. *Journal of the American Medical Directors Association* 2008;**9**(5):292-301.

 13. Institute of Medicine. *Panel on Dietary Reference Intakes for Electrolytes and Water. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. National Academies Press, Washington DC, USA, 2004.

 14. Stookey JD, Pieper CF, Cohen HJ. Is the prevalence of dehydration among community-dwelling older adults really low? Informing current debate over the fluid recommendation for adults aged 70+years. *Public Health Nutrition* 2005;**8**(8):1275-1285.

 15. Hooper L, Bunn D. DRIE - Dehydration Recognition In our Elders. <http://driestudy> appspot com/ 2014 January 9;

 16. Young J, Meagher D, MacLullich A. Cognitive assessment of older people. *British Medical Journal* 2011;**343**:d5042.

 17. Psychological Assessment Resources (PAR). Mini-Mental State Exam (MMSE). 2011.

 18. Malnutrition Action Group SCoB. *The 'MUST' Explanatory Booklet: A Guide to the 'Malnutrition Universal Screening Tool' ('MUST') for Adults*. BAPEN, Redditch, UK, 2003.

 19. Mahoney FI, Barthel D. Functional evaluation: the Barthel Index. *Maryland State Medical Journal* 1965;**14**:56-61.

 20. Wilkinson A. TIME: toolkit of instruments to measure end-of-life care. <http://www> caringcommunity org/helpful-resources/models-research/time-toolkit-of-instruments-to-measure-end-of-life-care/ 2011;

 21. Fazekas AS, Funk G-C, Klobassa DS, Ruther H, Ziegler I, Zander R, Semmelrock H-J. Evaluation of 36 formulas for calculating plasma osmolality. *Intensive Care Medicine* 2013;**39**(2):302-308.

 22. Zander R. Optimale Berechnung der Osmolalita¨t. Physioklin, Mainz. <http://www> physioklin de/physiopoc/saeure-basen-sauerstoff-elektrolytstatus/optimale-berechnung-derosmolalitaet html 2012;

 23. Nelson VA, Scheidt RA. Personal communication to Fazekas et al 2013. 1969.

 24. Kopp JB. Osmolality study. 1973. Product literature for Wescor (Logan, UT) vapour pressure (''dew point'') osmometer Model 5100.

 25. Gerich JE, Martin MM, Recant L. Clinical and metabolic characteristics of hyperosmolar nonketotic coma. *Diabetes* 1971;**20**:228-238.

 26. Hoffman RS, Smilkstein MJ, Howland MA, Goldfrank LR. Osmol gaps revisited: normal values and limitations. *J Toxicol Clin Toxicol* 1993;**31**:81-93.

 27. Koga Y, Purssell RA, Lynd LD. The irrationality of the present use of the osmole gap: applicable physical chemistry principles and recommendations to improve the validity of current practices. *Toxicol Rev* 2004;**23**:203-211.

 28. Wikipedia the free encyclopedia. Plasma osmolality. <http://en> wikipedia org/wiki/Plasma\_osmolality 2014 January 28;

 29. Wells JC, Williams JE, Haroun D, Fewtrell MS, Colantuoni A, Siervo M. Aggregate predictions improve accuracy when calculating metabolic variables used to guide treatment. *Am J Clin Nutr* 2009;**89**:491-499.

 30. Khajuria A, Krahn J. Osmolality revisited-deriving and validating the best formula for calculated osmolality. *Clin Biochem* 2005;**38**:514-519.

 31. Cheuvront SN, Kenefick RW, Heavens KR, Spitz MG. A Comparison of Whole Blood and Plasma Osmolality and Osmolarity. *Journal of Clinical Laboratory Analysis* 2014 March 19.

 32. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res* 1999;**8**(2):135-160.

 33. Edelman IS, Leibman J, O'Meara MP, Birkenfeld LW. Interrelations between serum sodium concentration, serum osmolarity, and total exchangeable sodium, total exchangeable potassium, and total body water. *J Clin Invest* 1958;**37**:1236-1256.

 34. Holmes JH. *Measurement of osmolality in serum, urine and other biologic fluids by the freezing point determination*. American Society of Clinical Pathologists, Chicago, IL, 1962.

 35. Jackson WP, Forman R. Hyperosmolar nonketotic diabetic coma. *Diabetes* 1966;**15**:714-722.

 36. Winters RW. Disorders of electrolyte and acid-base metabolisms. In: *Pediatrics* 14th edn edition (ed. Barnett HL). Appleton-Century-Crofts: New York, 1968; 336-368.

 37. Mahon WA, Holland J, Urowitz MB. Hyperosmolar, non-ketotic diabetic coma. *Can Med Assoc J* 1968;**99**:1090-1092.

 38. Jetter WW. Clinical osmometry. *Pa Med* 1969;**72**:75-79.

 39. Ross EJ, Christie SB. Hypernatremia. *Medicine* 1969;**48**:441-473.

 40. Stevenson RE, Bowyer FP. Hyperglycemia with hyperosmolal dehydration in nondiabetic infants. *J Pediat* 1970;**77**:818-823.

 41. Hoffman WS. *The biochemistry of clinical medicine*. 4th edn ed. Chicago, IL, 1970.

 42. Sadler JH. Personal communication to Fazekas et al. 1970.

 43. Boyd DR, Baker RJ. Osmometry: a new bedside laboratory aid for the management of surgical patients. *Surg Clin North Am* 1971;**51**:241-250.

 44. Weisberg HF. *Osmolality*. Clinical Chemistry Check Sample CC-71 ed. American Society of Clinical Pathologists, Chicago, IL, 1971.

 45. Glasser L, Sternglanz PD, Combie J, Robinson A. A serum osmolality and its applicability to drug overdose. *Am J Clin Pathol* 1973;**60**:695-699.

 46. Wilson RF. *Fluids, electrolytes, and metabolism*. Charles C Thomas, Springfield, IL, 1973.

 47. Dorwart WV. Serum osmolality-methods of calculation from chemistry values and use of these values as a prognostic indicator (abstract 020). *Clin Chem* 1973;**19**:643.

 48. Dorwart WV, Chalmers L. Comparison of methods for calculating serum osmolality from chemical concentrations, and the prognostic value of such calculations. *Clin Chem* 1975;**21**:190-194.

 49. Jenkins PG, Larmore C. Letter: hyperglycemia-induced hyponatremia. *New Engl J Med* 1974;**290**:573.

 50. Bhagat CI, Garcia-Webb C, Fletcher E, Beilby JP. Calculated vs measured plasma osmolalities revisited. *Clin Chem* 1984;**30**:1703-1705.

 51. Snyder H, Williams D, Zink B, Reilly K. Accuracy of blood ethanol determination using serum osmolality. *J Emerg Med* 1992;**10**:129-133.

 52. Wojtysiak B, Duma D, Solski J. The new equation for calculated osmolality. *Ann Univ Mariae Curie Sklodowska* 1999;**7**:59-64.

 53. Rasouli M, Kalantari KR. Comparison of methods for calculating serum osmolality: multivariate linear regression analysis. *Clin Chem Lab Med* 2005;**43**:635-640.

 54. Varley H, Gowenlock AH, Bell M. *Practical clinical biochemistry*. 5th edn ed. Heinemann, London, 1980.

 55. Bianchi V, Bidone P, Arfini C. Siero ed urine: osmolalita calcolata o osmolalita misurata? *RIMeL/IJLaM* 2009;**5**:206-211.

|  |
| --- |
| **Table 1: Descriptive characteristics of participants stratified by diabetes status** |
|  | **All** | **Without Diabetes** | **With Diabetes**  | **p-value**  |
| N | 186 | 153 | 33 |  |
| Age, *years* | 85.8±7.9 | 85.8±8.0 | 85.5±7.5 | 0.85 |
| Gender*Male**Female* | 64122 (66%) | 49104 (68%) | 1518 (55%) | 0.16 |
| Weight, *kg* | 69.0±17.2 | 67.4±16.7 | 76.3±17.6 | **0.007** |
| Height, *cm* | 163.1±10.4 | 162.0±10.2 | 168.1±9.7 | **0.002** |
| BMI, *kg/m2* | 25.8±5.5 | 25.5±5.4 | 27.0±6.0 | 0.17 |
| MMSE\* | 21.8±5.7 | 21.6±5.9 | 22.5±4.8 | 0.43 |
| Barthel Index | 66.6±26.4 | 66.9±26.9 | 65.3±3.9 | 0.74 |
| Serum Osmolality, *mmol/kg* | 292.1±9.3 | 291.3±9.1 | 295.9±9.5 | **0.01** |
| Sodium, *mmol/L* | 137.5±3.7 | 137.7±3.7 | 136.2±3.6 | **0.03** |
| Potassium, *mmol/L* | 4.2±0.4 | 4.2±0.4 | 4.2±0.3 | 0.36 |
| Urea, *mmol/L* | 6.9±2.6 | 6.7±2.4 | 8.2±3.1 | **0.003** |
| Creatinine, *µmol/L* | 89.4±35.2 | 87.4±34.3 | 98.7±38.2 | 0.09 |
| Glucose \*\*, *mmol/L* | 6.9±3.1 | 5.9±1.5 | 11.0±4.8 | **<0.001** |
| eGFR, mL/min/1.73 m2 | 63.8±18.8 | 64.5±18.4 | 60.5±20.4 | 0.26 |
| Hb, g/dL | 12.4±1.4 | 12.5±1.4 | 11.9±1.5 | **0.02** |

All values given were mean±SD, except for N and gender, which were numbers of participants.BMI=body mass index; MMSE=Mini Mental State Examination; eGFR= estimated glomerular filtration rate; Hb=hemoglobin. **\***MMSE scores available in 179 participants. **\*\***Glucose measurements were available in 172 participants. The p-value refers to a t-test for independent samples (continuous variables) and chi square test (categorical variables) used to compare subjects categorised according to diabetes status.

|  |
| --- |
| **Table 2:** Difference (*∆*) between measured plasma osmolality and calculated osmolarity (measured serum osmolality minus calculated osmolarity) in all participants and stratified by diabetes status |
|  | **All** **(N=186)** | **No Diabetes****(N=153)** | **Diabetes****(N=33)**  |
| Equation number (references for equations 1 to 33 taken from Fazekas (21)) | *∆* (measured serum osmolality in mmol/kg minus calculated osmolarity in mmol/L), mmol |
| Equation 1\* (33) | 30.9±8.6 c | 30.6±8.8 c | 32.3±8.8 c |
| Equation 2 (33) | -4.0±14.0 c | -5.6±11.6 c | 3.0±15.6 a |
| Equation 3\* (34) | 25.9±8.6 c | 25.5±8.6 c | 27.5±8.2 c |
| Equation 4\* (25;35) | -1.7±8.2 c | -2.0±8.2 c | -0.2±7.4 |
| Equation 5 (36) | 17.1±12.6 c | 15.7±10.4 c | 23.4±14.4 c |
| Equation 6\* (37) | 6.7±8.8 c | 6.3±8.8 c | 8.4±8.2 c |
| Equation 7 (38) | 10.1±12.6 c | 8.7±10.4 c | 16.4±14.4 c |
| Equation 8 (39) | 7.1±12.6 c | 5.7±10.4 c | 13.4±14.4 c |
| Equation 9\* (40) | 10.2±10.0 c | 9.6±9.8 c | 12.6±10.0 c |
| Equation 10 (41) | 3.3±12.8 c | 2.0±10.4 c | 9.8±14.4 c |
| Equation 11\* (42) | 6.9±8.8 c | 6.5±8.8 c | 8.7±8.4 c |
| Equation 12\* (25) | -2.6±8.2 c | -2.3±8.2 c | -4.3±7.4 c |
| Equation 13\* (43) | 20.9±8.6 c | 20.5±8.6 c | 22.5±8.2 c |
| Equation 14\* (44) | 7.6±9.0 c | 7.1±8.8 c | 9.8±8.4 c |
| Equation 15 (44) | 13.6±11.2 c | 12.4±9.4 c | 19.3±12.6 c |
| Equation 16\* (45) | 5.9±8.6 c | 5.6±8.6 c | 7.3±8.2 c |
| Equation 17\* (46) | 12.4±8.0 c | 12.0±8.2 c | 13.9±7.4 c |
| Equation 18\* (47) | 18.7±8.6 c | 18.4±8.6 c | 20.3±8.0 c |
| Equation 19\* (48) | 16.9±8.6 c | 16.5±8.6 c | 18.5±8.2 c |
| Equation 20\* (48) | 13.4±8.0 c | 13.2±8.0 c | 14.3±7.2 c |
| Equation 21\* (49) | -1.4±8.2 c | -1.8±8.2 c | 0.1±7.6 |
| Equation 22\* (50) | 4.2±7.6 c | 4.1±7.6 c | 4.7±6.8 c |
| Equation 23\* (50) | 4.5±7.4 c | 4.4±7.6 c | 5.3±6.6 c |
| **Equation 24\* (51)** | **-0.4±9.0** | **-0.8±8.8 a** | **1.8±8.4 a** |
| Equation 25\* (26) | 24.7±8.4 c | 24.5±8.6 c | 26.0±7.8 c |
| Equation 25a\* (26) | 28.7±8.4 c | 28.4±8.4 c | 30.0±7.9 c |
| **Equation 26\* (52)** | **-0.9±10.0** | **-0.5±9.8** | **-2.5±10.6 a** |
| Equation 27\* (27) | -32.0±8.2 c | -32.2±8.2 c | -31.0±7.4 c |
| Equation 27a\* (27) | -27.1±8.0 c | -27.3±8.2 c | -26.1±7.2 c |
| Equation 28\* (53) | 7.3±8.6 c | 6.9±8.6 c | 8.9±8.2 c |
| Equation 29\* (53) | 7.4±8.0 c | 7.0±8.2 c | 8.9±7.4 c |
| Equation 30\* (54) | 14.5±7.4 c | 14.4±7.6 c | 15.3±6.6 c |
| Equation 31\* (30) | 2.1±8.0 c | 2.0±8.2 c | 2.6±7.6 c |
| **Equation 32\* (30)** | **-0.4±7.4** | **-0.4±7.6** | **-0.3±7.0** |
| **Equation 33\* (55)** | **-0.5±8.2** | **-0.8±8.2 a** | **0.5±7.4** |
| Equation 34\* (28) | 5.2±7.4 c | 5.4±7.6 c | 4.3±6.6 c |
| Equation 35\* (tonicity) (6) | 1.7±9.6 c | -1.2±9.7 c | 4.0±9.4 c |
| Equation 36 (29) | 7.4±8.6 c | -6.9±8.4 c | 9.6±8.0 c |

N= number of participants; data are presented as means±2SD. ap<0.05; cp<0.001. \*Equations include glucose concentrations and therefore calculations are based on a final sample of 172 participants (all other calculations are based on 186 participants). The equations with the best performance are highlighted in bold. The paired t-test was used to determine the statistical significance of differences between measured osmolality and calculated osmolarity.

|  |
| --- |
| **Table 3:** Diagnostic characteristics of different serum osmolarity cut-offs, using equation 32, to use in screening for current dehydration (measured serum osmolality >300 mmol/kg). |
| Serum osmolarity cut-offs for equation 32 (mmol/L)  | Sensitivity | Specificity | PV+ | PV- | LR+ | LR- | DOR  | Pre-test probability | Post-test probability given T+ | Post-test probability given T- |
| >300  | 0.64 | 0.93 | 0.68 | 0.91 | 8.85 | 0.39 | 22.58 | 0.19 | 0.68 | 0.09 |
| >299 | 0.79 | 0.91 | 0.68 | 0.95 | 9.13 | 0.23 | 39.31 | 0.19 | 0.68 | 0.05 |
| >298  | 0.82 | 0.89 | 0.64 | 0.95 | 7.58 | 0.20 | 37.2 | 0.19 | 0.64 | 0.05 |
| >297  | 0.88 | 0.81 | 0.53 | 0.97 | 4.70 | 0.15 | 31.51 | 0.19 | 0.53 | 0.03 |
| >296 | 0.97 | 0.76 | 0.48 | 0.99 | 3.96 | 0.04 | 98.82 | 0.19 | 0.48 | 0.01 |
| >295 | 0.97 | 0.73 | 0.46 | 0.99 | 3.55 | 0.04 | 85.05 | 0.19 | 0.46 | 0.01 |

PV+: positive predictive value; PV-: negative predictive value; LR+ positive likelihood ratio; LR- negative likelihood ratio; DOR diagnostic odds ratio; prob probability; T+ positive test; T- negative test; NC not calculable

Figure legends

**Figure 1**: Flow chart for inclusion of care home residents into DRIE.

**Figure 2:** Bland-Altman plots describing the agreement between measured osmolality and predicted osmolarity using four different equations [Equation 24 (Figure 2A), Equation 26 (Figure 2B), Equation 32 (Figure 2C), Equation 33 (Figure 2D)],) characterised by the lowest ∆ values (see Table 1). Scatter plots have been stratified by diabetes status. A regression line has been fitted to identify the presence of differential bias with increasing osmolality. Grey solid lines are limits of agreement (±2SD). Dotted lines are average differences between measured and predicted values.

**Figure 3:** Predictive accuracy of four equations, evaluated by calculating the percentage of predicted osmolarity values within ±2% of measured osmolality in participants stratified by diabetes status (Figure 3A) and degree of dehydration (Figure 3B). The chi Square test was used to evaluate differences between those with and without diabetes (Figure 3A) and between those who were hydrated, had impending dehydration or current dehydration (Figure 3B) in percentage of accurate predictions for equations 24, 26, 32 and 33).

**Figure 4:** Accuracy of equation 32 in participants stratified by gender and diabetes status. A factorial analysis of variance was used to evaluate whether gender (G) and diabetes (D) status had an interactive effect on the accuracy of equation 32. G\*D= interaction term. Number of participants=172. Data showed as mean±1SEM.