Inaccuracy of Osmometry for Measurement of Plasma Water in Acute Diabetes Mellitus

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Osmometry is reportedly a relatively imprecise technique for the measurement of plasma water. However, reconsideration of the theoretical background of the test reveals that it is also likely to be inaccurate and subject to both positive errors as well as viscosity-dependent negative errors simultaneously, when applied to samples from patients admitted in acute diabetes mellitus. This was confirmed by comparison with Waugh’s method of results for 59 specimens from 14 cases. Plasma water was shown to be relatively underestimated at high protein concentrations and overestimated at lower ones.

Indexing Term: analytical error

Serum water content reportedly exhibits relatively small biological variation in health (1) when measured osmotically by the dilution method of Rawles (2) as modified by Faye and Payne (3). This technique was originally described as a method to correct for the pseudohyponatremia that occurs in hyperlipemic diabetic ketosis (2). The resulting analytical goals for imprecision of measurements of serum water are particularly stringent (4); accordingly, the osmometry method has been criticized on the grounds of inadequate precision (5).

Theory

Calculation of sample water requires measurement of osmolality of the sample before and after dilution with a known volume of water. From the results obtained, water is calculated by application of the formula:

\[ f = \frac{D}{(U - D)} \times 0.5 \]  \hspace{1cm} (1)

where U and D are the measured osmolalities of the undiluted and diluted sample, respectively; 0.5 is the dilution factor (one volume of water:two volumes of sample); and f is the resulting plasma water fraction, which is virtually identical to the plasma water concentration when expressed in kg/L.

Apart from the effects of the inherent imprecision of osmolality and volume measurements (5), the general assumptions of this osmometry method are, first, that the sample is diluted accurately with an amount of water equal to one-half of the original sample volume, and, second, that the osmotic activity of crystalloid particles in the aqueous phase remains relatively unchanged. However, theoretical grounds exist to suggest that neither of these two assumptions is likely to be sufficiently valid, particularly in samples from patients admitted in acute diabetes mellitus.

Effect of sample viscosity. One source of potential error arises because of the requirement to measure volumes of sample and water with high accuracy, but with no account taken of differences in viscosity between the two. High viscosity of plasma can be associated with negative sample-volume errors (6–9). At constant temperature, plasma viscosity is largely determined by the plasma protein concentration (10). Ironically, patients admitted in acute diabetes often exhibit very high initial concentrations of plasma total protein (11, 12) and attendant high concentrations of plasma viscosity (13, 14), which decline during the next 24 h of treatment. Inspection of equation 1 reveals that viscosity-dependent differences in volume measurement between sample and subsequent water addition lead to a clinically significant underestimate in the value for plasma water by osmometry. For example, at a true sample water of 0.952 kg/L and U = 305 mmol/kg, 5% and 10% negative errors in the measurement of plasma sample volume would yield apparent values of 0.899 and 0.862 kg/L, respectively.

Effect of variation in osmotic coefficient. The measured osmolality of the undiluted sample (U) is described by the expression:

\[ U = \frac{(m \times n \times \phi_U)(v \times f)}{(v + f)} \]  \hspace{1cm} (2)

where m is the number of millimoles of osmotically active particles, n is the number of particles into which the solute dissociates at complete dissociation, \( \phi_U \) is the osmotic coefficient at osmolality U, v is the sample volume, and f is the fraction of water in the sample. If the sample is then diluted with a volume of water equal to a fractional increment of 0.5 (3), then the measured osmolality of the diluted sample (D) becomes:

\[ D = \frac{(m \times n \times \phi_U)(v \times f) + (v \times 0.5)}{(v + f)} \]  \hspace{1cm} (3)

where \( \phi_D \) is the osmotic coefficient now obtained at osmolality D. Combining equations 2 and 3 yields the expression:

\[ f = \frac{(0.5 \times D \times \phi_D)(U \times \phi_U) - (D \times \phi_U)}{(U \times \phi_U) - (D \times \phi_D)} \]  \hspace{1cm} (4)

Because the osmotic activity of plasma is largely due to sodium and chloride ions, one can determine the appropriate values for \( \phi_D \) and \( \phi_U \) from tables (15), which would allow correction of the original osmometry method. For example, if values of U = 305 mmol/kg and D = 200 mmol/kg are chosen, then f = 0.952 by equation 1 and 0.930 by equation 4. This overestimate of 0.022 repre-
sents a very large proportion of any likely reference interval (3).

Thus, one can hypothesize that estimation of plasma water by the original osmometry method is likely to be subject to (a) negative error influences at high viscosity values and (b) positive errors throughout a wide range of sample viscosities because of the failure to correct for variation in osmotic coefficient between the undiluted and diluted sample. The purpose of the present study was, therefore, to test these two hypotheses further by observing the results obtained in patients admitted as emergencies in acute diabetes mellitus.

**Patients and Methods**

A total of 59 plasma specimens were obtained from 14 patients (5 males and 9 females, ages 12–82 years), who were admitted to Croeshouse Hospital as emergencies in acute diabetic hyperglycemia. No specimen exhibited visible lipemia. Plasma water was determined osmotically, singly, in accordance with both equation 1 (3) and equation 4, and was also calculated from measurements of total protein, total lipid, and Waugh's formula (18), obtained by using methods and equipment described previously (12).

**Results and Discussion**

On admission, the plasma glucose results for the 14 patients ranged from 21.3 to 56.4 mmol/L. Figure 1 shows the changes in plasma total protein over the first 30 h after admission. The mean admission value was 78 g/L (range 67–97 g/L).

To investigate the effect of sample total protein on the difference between osmometry and Waugh's method, the results by each technique were grouped as shown in Figure 2. As predicted from hypothesis b, osmometry tends, on average, to yield higher results than does the method of Waugh (18), for sample protein concentrations <80 g/L. However, where sample protein ≥80 g/L, the situation is reversed, in accordance with hypothesis a above. Figure 2 also illustrates the much greater total variance in the osmometry method as evidenced by the relative sizes of the standard error bars.

The effect of viscosity on the methods was minimized by excluding from calculation all results with total protein ≥80 g/L. The results for plasma water by osmometry, either uncorrected for variation in osmotic coefficient as in equation 1, or corrected in accordance with equation 4, were each compared with Waugh's method by paired t-test. These results (Table 1) indicate that the uncorrected osmometry method does yield results that are, on average, significantly higher than those obtained with Waugh's method, by an amount that is very close to that predicted from hypothesis b. Subsequent correction of the osmometry method, by equation 4, gives results that are, on average, not significantly different (paired t-test) from those obtained by Waugh's method.

**Plasma water (kg/L), by Waugh's formula, is given by the expression:**

\[ f_{\text{Waugh}} = (991 - 1.03 L - 0.73 P)/1000 \]

where \( L \) (g/L) and \( P \) (g/L) represent plasma total lipids and protein, respectively (12). Therefore, the between-batch \( CV_{\text{Waugh}} \) is represented by the expression:

\[
[100(\text{mean} \times 1000)] \times [0.73SD(P)^2 + 1.03SD(L)^2]^{0.5}
\]

where SD(P) and SD(L) are the analytical, between-batch standard deviations for protein and lipid measuremen-

| Table 1. Comparison of Mean Values for Plasma Water, by Corrected and Uncorrected Osmometry, with Waugh's Method, for Samples with Total Protein < 80 g/L (n = 51) |
|----------------------------------|----------------|----------------|
|                                  | Uncorrected osmometry (equation 1) | Corrected osmometry (equation 4) | Waugh's method |
| Mean                            | 0.953* | 0.931 | 0.934 |
| Observed range                  | 0.863–1.109 | 0.843–1.064 | 0.917–0.947 |

* Significantly different \( P < 0.01 \) from results by other two methods.
ments, respectively (17). During the present study, the respective SD values for quality-assurance samples were 1.2 and 0.6 g/L. Hence, at a typical plasma water value of 0.933 kg/L (18), CV\textsubscript{Waugh} = (1/9.33) \times (0.767 + 0.382)^{0.5} = 0.1\%, which is of similar magnitude to that reported for Waugh's method by Faye and Payne (3).

The imprecision of the osmometry method, CV\textsubscript{osm}, is given by the expression (17): \(CV_{\text{osm}} = [CV(D)^2 + CV(U - D)]^{0.5}\). Respective values for CV(D) and CV(U - D) for quality-assurance samples were 1.1% and 5.2%, which yielded a CV\textsubscript{osm} of ~5.3%. Hence, the imprecision of the osmometry method, by single-tube analysis, is at least an order of magnitude greater than for Waugh's method, and this largely determined the resulting total variation (3-5). This difference is highlighted in Table 1, where the observed range of values by osmometry shows several that are >1.0, and hence clearly incorrect. Because of both the large imprecision difference between osmometry and Waugh's method and the narrow in vivo range for plasma water (as a proportion of the possible analytical range), it was not feasible to reliably compare the two methods by conventional linear-regression techniques (19).

Such wide disparity in analytical imprecision between the two methods also limits the usefulness of the method comparison technique of Bland and Altman (20). In that procedure, the difference between the results of the two estimations, for each individual sample, is plotted against the mean for the pair. Because the results obtained by Waugh's method remain relatively constant, by comparison with those by osmometry, then the magnitude of the differences, and of the means of each of the pairs, is largely determined by the relative variability of only the osmometry method. Hence, the difference appears as a linear function of the observed mean of the pairs. So far as I am aware, there is no existing statistical technique applicable in such situations.

In conclusion, despite the fact that there is no reference method for plasma water measurement (3), it is, nevertheless, probable that the osmometry method is an unreliable technique—in the absence of appropriate correction for variations in osmotic coefficient and in the presence of high total protein concentration or high viscosity. Comparability with Waugh's method should be possible if, in addition to replicate analysis (4), suitable corrections and exclusions are made as described above.

References