A new method for determining plasma water content: application in pseudohyponatremia

Minhtri K. Nguyen,1 Vahram Ornekian,1 Anthony W. Butch,2 and Ira Kurtz1

Departments of 1Medicine and 2Pathology and Laboratory Medicine, University of California, Los Angeles, California

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Nguyen MK, Ornekian V, Butch AW, Kurtz I. A new method for determining plasma water content: application in pseudohyponatremia. Am J Physiol Renal Physiol 292: F1652–F1656, 2007. First published February 13, 2007; doi:10.1152/ajprenal.00493.2006.—Pseudohyponatremia is a clinical condition characterized by an increased fraction of protein or lipid in plasma, thereby resulting in an artificially low plasma sodium concentration ([Na+]P). Since the automated method of measuring [Na+]P in most laboratories involves the use of an indirect ion-selective electrode (I-ISE), this method does not correct for the degree of dilution based on the assumption that plasma is normally composed of 93% plasma water (1, 2, 8): In contrast, the plasma is not diluted in direct ISE (D-ISE), and this method directly measures Na+ activity in plasma and is therefore unaffected by the proportion of plasma occupied by water (6). In this study, we report a novel quantitative method for determining the PWC in pseudohyponatremia utilizing the difference in the [Na+]P as measured by I-ISE and D-ISE.

MATERIALS AND METHODS

Salt-free albumin was isolated using a mixed cation and anion exchange resin (MBD-10-LTOC; ResinTech, West Berlin, NJ) which exchanges Na+ for H+ and Cl− for OH−. Human albumin (126654; Calbiochem, EMD Biosciences, Darmstadt, Germany) was dissolved in ultrapure water (1 g/ml) and was subsequently loaded onto a column containing an equal volume of the exchange resin equilibrated with ultrapure water at a flow rate of 10 ml/h. The effluent solution was reloaded and run on the column five times. The salt-free albumin solution was lyophilized using a Savant Speed Vac concentrator (Savant Instruments, Holbrook, NY). Pseudohyponatremia was then induced by dissolving various amounts of salt-free albumin in human plasma to vary the plasma protein concentration and PWC. The PWC was then determined on 11 samples with varying plasma albumin concentration by three separate methods: 1) PWC determination based on differences in the [Na+]P as measured by I-ISE and D-ISE, 2) PWC determination based on measurements of the total protein concentration and total lipid concentration, and 3) gravimetrically determined PWC.

Determination of PWC based on direct and indirect ISE. Ion-selective electrode measures Na+ activity in plasma (3, 5, 6). An ISE adjustment factor (ISE-AF) is used to convert the ISE-measured activity of Na+ ([Na+]ISE) to the total Na+ concentration ([Na+]T) (3, 6):

\[
\text{ISE } [\text{Na}^+]_{\text{ISE}} \times \text{ISE-AF} = [\text{Na}^+]_{\text{ISE}} \\
\]

Let D-ISEa, and I-ISEa, represent the activity of Na+ as measured by direct ISE (D-ISE) and indirect ISE (I-ISE) respectively, and [Na+]D-ISE and [Na+]I-ISE represent the total Na+ concentration as measured by D-ISE and I-ISE, respectively. Therefore, in D-ISE:

\[
\begin{align} 
\text{D-ISE } [\text{Na}^+]_{\text{D-ISE}} \times \text{ISE-AF} &= [\text{Na}^+]_{\text{D-ISE}} \\
\end{align} \\
\]

In I-ISE, the plasma sample is diluted before the actual measurement is obtained, and the [Na+]I-ISE is then determined by correcting for the degree of dilution based on the assumption that plasma is normally composed of 93% plasma water (1, 2, 8):

\[
\begin{align} 
\text{I-ISE } [\text{Na}^+]_{\text{I-ISE}} \times (0.933 + \frac{\text{Vol}_{\text{diluent}}}{0.933 \times \text{ISE-AF}}) &= [\text{Na}^+]_{\text{I-ISE}} \\
\end{align} \\
\]

where the dilution correction factor is represented by the term (0.933 + Vol_{diluent}/0.933, and Vol_{diluent} is the volume of diluent per unit volume of plasma used in I-ISE.

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Address for reprint requests and other correspondence: M. K. Nguyen, Div. of Nephrology, David Geffen School of Medicine at UCLA, 10853 Le Conte Ave., Rm. 7-155 Factor Bldg., Los Angeles, CA 90095 (e-mail: mtnguyen@mednet.ucla.edu).
Since D-ISE $a_{Na^+}$ is related to I-ISE $a_{Na^+}$ by the following equation:

$$D-ISE \, a_{Na^+} \times PWC/(PWC + V_{\text{diluent}}) = I-ISE \, a_{Na^+} \tag{3}$$

Substituting Eq. 3 for the term $I-ISE \, a_{Na^+}$ in Eq. 2:

$$D-ISE \, a_{Na^+} \times PWC/(PWC + V_{\text{diluent}}) \times (0.933 + V_{\text{diluent}}) = [Na^+]_{D-ISE} \tag{4}$$

Since $D-ISE \, a_{Na^+} \times I-ISE = [Na^+]_{I-ISE}$ (Eq. 1a), Eq. 4 can be rearranged as:

$$[Na^+]_{I-ISE} \times PWC/(PWC + V_{\text{diluent}}) \times (0.933 + V_{\text{diluent}})/0.933 = [Na^+]_{I-ISE} \tag{5}$$

Solving Eq. 5 for PWC:

$$PWC = (0.933 \times V_{\text{diluent}} \times [Na^+]_{I-ISE}/0.933)$$

$$- (0.933 \times [Na^+]_{I-ISE})$$

$$= (0.933 \times V_{\text{diluent}} - 0.933 \times [Na^+]_{I-ISE})$$

$$= PWC$$

where PWC is expressed as liter of plasma water per liter of plasma (or kg of plasma water per liter of plasma, assuming that the density of water is 1 kg/l).

Using Eq. 6, the PWC was determined on 11 samples with varying plasma protein concentration based on measurements of $[Na^+]_{D-ISE}$ and $[Na^+]_{I-ISE}$ in each sample. $[Na^+]_{D-ISE}$ was measured with a Nova 8 analyzer (Novo Biomedical, Waltham, MA), and $[Na^+]_{I-ISE}$ was measured with an Olympus AU5400 analyzer (Olympus America, Melville, NY), respectively.

**Determination of PWC based on total protein and lipid measurements.**

The PWC was also estimated based on measurements of total protein and lipid concentrations (9). Total protein and lipid concentrations were measured on each of the 11 samples using an Olympus AU5400 automated chemical analyzer. Total protein was measured by the biuret method, whereby proteins react with cupric ions in an alkaline solution to produce a violet-colored complex that absorbs light at 540 nm. The lipid concentration was determined as the sum of cholesterol and triglyceride concentrations. Cholesterol was measured using a coupled chemical reaction involving cholesterol esterase, cholesterol oxidase, and peroxidase. Hydrogen peroxide is produced and oxidatively couples 4-aminoantipyrine and phenol to form a red quinoneimine dye with an absorbance at 540 nm. Triglycerides were measured using a series of coupled reactions involving lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase. Hydrogen peroxide is produced and oxidatively couples p-chlorophenol and 4-aminoantipyrine to produce a red dye with an absorbance at 500 nm. The PWC was then calculated based on the following equation (9):

$$PWC = 0.991 - (0.73 \times [\text{protein}]) - (1.03 \times [\text{lipid}]) \tag{7}$$

where [protein] and [lipid] represent the total protein concentration and total lipid concentration (in kg/l), respectively.

**RESULTS**

The total protein concentration, total cholesterol, and triglyceride levels of each plasma sample are shown in Table 1. Salt-free albumin was dissolved in human plasma to vary the total protein concentration from 7.4 to 28.4 g/dl. Duplicate measurements of the $[Na^+]_p$ of each plasma sample were performed utilizing both D-ISE and I-ISE, and the mean of the two measurements of the $[Na^+]_{D-ISE}$ and $[Na^+]_{I-ISE}$ are shown in Table 1, respectively. Also shown are the ISE-determined PWC calculated based on Eq. 6.

Two separate aliquots of each sample were lyophilized to gravimetrically determine the mass of plasma water. The PWC was determined by dividing the mass of the plasma water of each aliquot by the volume of the plasma sample being lyophilized. In Table 1, the PWC of each sample was determined by calculating the mean of the gravimetrically determined PWC of the two aliquots.

The relationship between the ISE-determined PWC or protein/lipid-determined PWC and the gravimetrically determined PWC is analyzed by linear regression, as demonstrated in Figs. 1 and 2, respectively. In Fig. 1, linear regression analysis demonstrated that the gravimetrically determined PWC is related to the ISE-determined PWC by the following equation: gravimetrically determined PWC = 1.02 (ISE-determined PWC) - 0.0025 (Eq. 8: $r = 0.97$; 95% CI slope = 0.827–1.2128; 95% CI intercept = -0.1609 to 0.1558, where CI is confidence interval). In Fig. 2, the gravimetrically determined PWC is related to the protein/lipid-determined PWC by the following equation: gravimetrically determined PWC = 1.48

**Table 1. ISE and gravimetrically determined plasma water content**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$[Na^+]_{D-ISE}$, mmol/l</th>
<th>Average $[Na^+]_{D-ISE}$, mmol/l</th>
<th>$[Na^+]_{I-ISE}$, mmol/l</th>
<th>Average $[Na^+]_{I-ISE}$, mmol/l</th>
<th>Total Protein, g/dl</th>
<th>Total Cholesterol, mg/dl</th>
<th>Triglyceride, mg/dl</th>
<th>ISE-Determined PWC, kg/l</th>
<th>Mean Gravimetrically Determined PWC, kg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>143 144</td>
<td>143.5</td>
<td>142 142</td>
<td>142.0</td>
<td>7.4</td>
<td>166</td>
<td>68</td>
<td>0.923</td>
<td>0.946</td>
</tr>
<tr>
<td>2</td>
<td>144 144</td>
<td>144.0</td>
<td>140 140</td>
<td>140.0</td>
<td>8.8</td>
<td>172</td>
<td>69</td>
<td>0.906</td>
<td>0.919</td>
</tr>
<tr>
<td>3</td>
<td>144 145</td>
<td>144.5</td>
<td>137 138</td>
<td>137.5</td>
<td>10.9</td>
<td>180</td>
<td>72</td>
<td>0.887</td>
<td>0.892</td>
</tr>
<tr>
<td>4</td>
<td>145 145</td>
<td>144.0</td>
<td>133 133</td>
<td>133.0</td>
<td>13.7</td>
<td>181</td>
<td>70</td>
<td>0.860</td>
<td>0.867</td>
</tr>
<tr>
<td>5</td>
<td>146 143</td>
<td>144.5</td>
<td>132 133</td>
<td>132.5</td>
<td>13.7</td>
<td>181</td>
<td>72</td>
<td>0.853</td>
<td>0.857</td>
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<tr>
<td>6</td>
<td>145 143</td>
<td>144.0</td>
<td>128 128</td>
<td>128.0</td>
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<td>183</td>
<td>74</td>
<td>0.827</td>
<td>0.835</td>
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<tr>
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<td>0.784</td>
<td>0.821</td>
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<tr>
<td>8</td>
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<td>145.0</td>
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<td>119.0</td>
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<td>0.811</td>
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<tr>
<td>9</td>
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<td>145.5</td>
<td>120 120</td>
<td>120.0</td>
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<td>70</td>
<td>0.766</td>
<td>0.746</td>
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<tr>
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<td>113 112</td>
<td>112.5</td>
<td>24.8</td>
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<td>52</td>
<td>0.717</td>
<td>0.731</td>
</tr>
<tr>
<td>11</td>
<td>143 143</td>
<td>143.0</td>
<td>110 110</td>
<td>110.0</td>
<td>28.4</td>
<td>123</td>
<td>50</td>
<td>0.713</td>
<td>0.708</td>
</tr>
</tbody>
</table>

ISE, ion-selective electrode. D-ISE and I-ISE, direct and indirect ion-selective electrode, respectively; $[Na^+]$, Na+ concentration; PWC, plasma water content.
Since the automated method of measuring \([\text{Na}^+]_p\) in most laboratories involves the use of I-ISE, Eq. 5 is especially helpful in evaluating clinical disorders characterized by hyperproteinemia and hyperlipidemia. The more widespread use of I-ISE in most clinical laboratories is due to the fact that the plasma is diluted to reduce the volume of blood needed for analysis of the \([\text{Na}^+]_p\). Therefore, in hyperproteinemia and hyperlipidemia, the true \([\text{Na}^+]_{\text{D-ISE}}\) can be extrapolated from the \([\text{Na}^+]_{\text{I-ISE}}\) by rearranging Eq. 5 as follows:

\[
[\text{Na}^+]_{\text{D-ISE}} = \frac{[\text{Na}^+]_{\text{I-ISE}}}{\text{PWC} / (\text{PWC} + \text{Vol}_{\text{diluent}}) 	imes (0.933 + \text{Vol}_{\text{diluent}})/0.933}.
\]

Of course, one has to first calculate the PWC with Eq. 6 before one can determine \([\text{Na}^+]_{\text{D-ISE}}\) using the measured \([\text{Na}^+]_{\text{I-ISE}}\) and Eq. 5a. Therefore, the measurement of \([\text{Na}^+]_{\text{D-ISE}}\) must be performed initially at least once to calculate the PWC. However, once the PWC is known and is assumed to be stable, subsequent \([\text{Na}^+]_{\text{D-ISE}}\) can be calculated from the measured \([\text{Na}^+]_{\text{I-ISE}}\) with the use of Eq. 5a. Equation 5a can therefore be helpful in minimizing the volume of blood needed for the serial analysis of \([\text{Na}^+]_p\) in patients with hyperproteinemia or hyperlipidemia and superimposed true hyponatremia who are undergoing treatment of the hyponatremia. For instance, in a patient with pseudohyponatremia due to multiple myeloma who develops superimposed true hyponatremia in the setting of myeloma kidney, it is vitally important to differentiate the component of hyponatremia that is due to true hyponatremia from that of pseudohyponatremia. Since inappropriate treatment of pseudohyponatremia can lead to significant patient morbidity, \(Eq. 5a\) can be used to extrapolate the true \([\text{Na}^+]_p\) to help guide therapy and to avoid overcorrection of the hyponatremia due to inappropriate treatment of the pseudohyponatremia. Similarly, it has been recently reported that intravenous immunoglobulin can cause both pseudohyponatremia due to postinfusional hyperproteinemia and true hyponatremia resulting from sucrose-induced translocation of water from the intracellular

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**DISCUSSION**

Plasma is normally composed of 93% plasma water and 7% proteins and lipids (1). Therefore, clinical conditions characterized by hyperproteinemia and hyperlipidemia will result in a relative decrease in the aqueous phase of plasma. Since \(\text{Na}^+\) is only present in plasma water, each volume of plasma measured will contain less \(\text{Na}^+\) and will be reported by most automated laboratory methods employing I-ISE as a low \([\text{Na}^+]_p \) (2, 8). In I-ISE, the plasma sample is diluted before the actual measurement is obtained. The \([\text{Na}^+]_{\text{I-ISE}}\) is then determined by correcting for the degree of dilution based on the assumption that plasma is normally composed of 93% plasma water (1, 2, 8). Therefore, in clinical conditions characterized by an expanded nonaqueous phase of plasma, the degree of dilution will be underestimated, thereby resulting in an artificially low \([\text{Na}^+]_{\text{I-ISE}}\). In contrast, the plasma is not diluted in D-ISE, and this method directly measures \(\text{Na}^+\) activity in plasma and is therefore unaffected by the proportion of plasma occupied by water (6).

In this study, pseudohyponatremia was induced by dissolving salt-free albumin in human plasma to vary the plasma protein concentration. As the plasma protein concentration increased from 7.4 to 28.4 g/dl, there was a corresponding decrement in the PWC as measured gravimetrically (Table 1). As expected, there was a progressive artificial decrease in the \([\text{Na}^+]_{\text{I-ISE}}\) as the aqueous phase of plasma was reduced (Table 1). In contrast, the \([\text{Na}^+]_{\text{D-ISE}}\) was unaffected by the increased plasma protein concentration (Table 1). Utilizing the differences in the \([\text{Na}^+]_p\), as measured by I-ISE and D-ISE, the PWC was calculated based on \(Eq. 6\). As demonstrated in Fig. 1, there is excellent agreement between the ISE-determined PWC and the gravimetrically determined PWC. Indeed, our results demonstrated that the ISE-determined PWC was not significantly different from the gravimetrically determined PWC \((P = 0.68)\).
compartment to the extracellular compartment as well as the infusion of large volume of dilute fluids in patients with an underlying defect in urinary free water excretion (7). In this setting, extrapolation of the true $[\text{Na}^+]_{D-ISE}$ from the $[\text{Na}^+]_{I-ISE}$ can be especially helpful in the serial analysis of $[\text{Na}^+]_p$ during treatment of the true hyponatremia. Importantly, determination of the PWC by Eq. 6 can also be utilized to correct for any other plasma solute concentration that is factitiously low due to the reduced PWC. For example, there is recent evidence that the plasma K$^+$ concentration ([K$^+]_p$) and plasma Cl$^-$ concentration ([Cl$^-$]$_p$) as measured by I-ISE in clinical laboratories are also falsely decreased in clinical conditions where the PWC is $<93\%$ (4). In these clinical conditions, the PWC as calculated by Eq. 6 can be used to correct for the factitiously low $[\text{Na}^+]_p$ and $[\text{Cl}^-]_p$:

$$\frac{[\text{Ion}]_{D-ISE}}{[\text{Ion}]_{I-ISE}} = \frac{\text{PWC}/(\text{PWC} + \text{Vol}_{\text{diluent}}) \times (0.933 + \text{Vol}_{\text{diluent}})/0.933}{(5b)}$$

where $[\text{Ion}]_{D-ISE}$ and $[\text{Ion}]_{I-ISE}$ represent the plasma concentration of the ion of interest as measured by D-ISE and I-ISE, respectively.

Since inappropriate treatment of pseudohypokalemia can lead to iatrogenically induced hyperkalemia, determination of PWC to correct for the factitiously low $[\text{K}^+]_p$ is therefore important in avoiding further unnecessary diagnostic testing and potentially detrimental treatment.

Given that the protein/lipid concentration typically does not change significantly in the short term in patients with hyperproteinemia/multiple myeloma or hyperlipidemia, the PWC will also not vary significantly in the short term, and therefore multiple repeated D-ISE/I-ISE determinations will not be clinically necessary. Consequently, to determine a subsequent $[\text{Na}^+]_{D-ISE}$ value, one only needs a repeat measurement of $[\text{Na}^+]_{I-ISE}$ and the initial calculated PWC value. Moreover, in the short term, the PWC can also be used to mathematically correct for the factitiously low serum $[\text{K}^+]$ and $[\text{Cl}^-]$ in hyperproteinemia and hyperlipidemia. It is important to appreciate, however, that a new D-ISE/I-ISE measurement will be needed to determine the new PWC if there is a significant change in the serum protein/lipid concentration.

Historically, Waugh (9) reported that the PWC could be estimated based on measurements of the total protein concentration and the total lipid concentration according to Eq. 7. However, this protein/lipid-determined PWC has not yet been validated experimentally. In this study, we demonstrated that the protein/lipid-determined PWC does not correlate as well with the gravimetrically determined PWC (slope $= 1.48$, y-intercept $= -0.448$) compared with the ISE-determined PWC (slope $= 1.02$, y-intercept $= -0.0025$). This finding is demonstrated in Fig. 2. Interestingly, the total cholesterol concentration progressively increased from 166 to 189 mg/dl with addition of the salt-free albumin as the total protein concentration increased from 7.4 to 17.5 g/dl (Table 1). This increase in the total cholesterol concentration was attributed to the addition of cholesterol contained in the salt-free albumin preparation to the sample. However, the total cholesterol concentration surprisingly decreased from 189 to 123 mg/dl with further addition of the salt-free albumin to the sample as the total protein concentration increased from 17.5 to 28.4 g/dl. Similarly, there was a similar trend of initial increase and subsequent decrease in the triglyceride level as the total protein concentration increased from 7.4 to 28.4 g/dl. The subsequent decrement in the total cholesterol and triglyceride levels with further increment in the total protein concentration was likely due the displacement of the lipid phase by the excess albumin. The displacement of lipids by the excess albumin can be verified by demonstrating an increase in the total cholesterol and triglyceride levels with reduction of the plasma total protein concentration. The sample consisting of 28.4 g/dl of total protein was therefore diluted with ultrapure water to lower the plasma total protein concentration. As predicted, the cholesterol and triglyceride levels actually increased (cholesterol: from 117 to 152 mg/dl; triglyceride: from 36 to 47 mg/dl) despite dilution with ultrapure water when the total protein concentration decreased from 28.4 to 24 g/dl. This was due to less displacement of lipids by the excess albumin. Additional dilution of the sample to lower the total protein concentration from 24 to 17.9 g/dl, however, resulted in a decrease in the cholesterol and triglyceride levels (cholesterol: from 152 to 108 mg/dl; triglyceride: from 47 to 36 mg/dl). This finding simply reflected the fact that the dilutional effect was now greater than the displacement effect of albumin.

Since the measurement of cholesterol and triglyceride levels is affected by the aqueous phase of plasma, the measured cholesterol and triglyceride levels may be inaccurate in clinical conditions characterized by hyperproteinemia and hyperlipidemia. As discussed earlier, the protein/lipid-determined PWC is less accurate than the ISE-determined PWC compared with the gravimetrically determined PWC. Therefore, the inaccuracy in the protein/lipid-determined PWC is likely due to the fact that the measured cholesterol and triglyceride levels may be inaccurate in hyperproteinemia and hyperlipidemia.

In conclusion, clinical conditions characterized by hyperproteinemia and hyperlipidemia will result in a relative decrease in the aqueous phase of plasma. In such clinical disorders, pseudohyponatremia results since the $[\text{Na}^+]_p$ measured by most automated laboratory methods using I-ISE will be reported as a low $[\text{Na}^+]_p$. In contrast, the measurement of the $[\text{Na}^+]_p$ by D-ISE is unaffected by the proportion of plasma occupied by water. In this study, we demonstrated that the PWC can be accurately determined based on differences in the $[\text{Na}^+]_p$ as measured by I-ISE and D-ISE. Our results also indicated that the ISE-determined PWC correlates more closely to the gravimetrically determined PWC than the protein/lipid-determined PWC. Therefore, this new quantitative method can be especially helpful in evaluating the PWC in clinical conditions characterized by hyperproteinemia and hyperlipidemia.

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