Chemiluminescent Measurement of Total Urinary Nitrogen for Accurate Calculation of Nitrogen Balance

Kristen J. Skogerboe, Robert F. Labbé, Rebecca L. Rettmer, Joanne P. Sundquist, and Ann M. Gargett

This instrumental method for total urinary nitrogen (TUN) is based on the principle of gas-phase chemiluminescence. Results correlate well with measurements of TUN by the Kjeldahl method, which has long provided the means to calculate nitrogen balances for nutritional management. In recent years, because of speed and convenience of measurement, determination of urinary urea nitrogen (UUN) has been substituted for Kjeldahl TUN. However, in patients requiring aggressive nutritional support, the UUN may not be a valid indicator of total nitrogen excretion. We compared nitrogen balances calculated for patients, using both UUN and chemiluminescence TUN data. For both normal and hospitalized populations, nitrogen balance calculated from UUN data exceeded that calculated from TUN data. We show that use of UUN data in calculating nitrogen balance may result in an incorrect assessment of many patients as being in positive nitrogen balance. TUN determined by chemiluminescence evidently provides a simple means of calculating nitrogen balance more nearly accurately.

Additional Keyphrases: Kjeldahl analysis · assessment of nutritional status

Assessment of protein requirements for hospitalized patients is an important criterion of proper nutritional support. Nitrogen is a major constituent of protein, so measurement of nitrogenous metabolites in the urine reflects protein turnover in the body. Correspondingly, the calculation of "nitrogen balance" (the amount of nitrogen intake vs the nitrogen excreted) is a useful index of nutritional status and is widely used in assessing nutritional requirements of surgery and trauma patients (1, 2). A patient who is in negative nitrogen balance is utilizing muscle protein to meet the metabolic requirement of the body and is therefore in a catabolic state. Thus, the nitrogen balance indicates whether the metabolic requirements of the patient are being met.

Nitrogen balance is calculated from the amount of nitrogen fed and the amount of total urinary nitrogen (TUN) excreted.1 The classical technique for measuring TUN is the Kjeldahl method, which is of limited usefulness in the clinical laboratory, owing to technical difficulties associated with acid digestion. Given this limitation, simpler techniques for laboratory assessment of nitrogen status have been developed.

Because of its simplicity, the measurement of urinary urea nitrogen (UUN) has become a convenient substitute for TUN in the calculation of nitrogen balance. UUN can be used to estimate TUN because about 80% to 90% of total urinary nitrogen ordinarily is in the form of urea. If it is assumed that urea always constitutes a constant proportion of the total nitrogen excretion, the nitrogen balance calculation can be modified to correct for non-urea forms of nitrogen. However, nitrogen excretion may be underestimated in some patients when urea is used to calculate nitrogen balance (3, 4). This can occur when the fraction of total urine nitrogen contributed by urea is altered by stress, in which case the non-urea forms of nitrogen, such as ammonia, may be increased (5).

The limitation of the UUN measurement in nitrogen assessment indicates the need for a simple assay for urinary nitrogen that measures total nitrogen rather than just urea. It is possible that measurement of non-urea forms of nitrogen, combined with urea, might provide an adequate assessment of urine nitrogen excretion. One common clinical method for analyzing urea uses the coupled enzyme system of urease (EC 3.5.1.5) and glutamate dehydrogenase (EC 1.4.1.3). When performed as a fixed endpoint assay, the method detects both urea and ammonia. However, a potential disadvantage of this approach is that the absorbance signal produced by urea and ammonia are not equivalent because of the stoichiometry of the reaction (6): 1 mol of urea produces twice the absorbance signal of 1 mol of ammonia. Therefore, the reaction cannot be easily calibrated to yield the amount of total nitrogen in each specimen. The co-determination of these analytes might be advantageous for accurate nitrogen balance calculations, but this approach has yet to be evaluated.

Methods based on gas-phase chemiluminescence have been reported for the measurement of total nitrogen (7-9), as is the present assay. Here, we demonstrate its advantage over UUN for calculation of nitrogen balance by comparing the nitrogen balance calculated from both UUN and TUN data for trauma and surgery patients as well as for normal, ostensibly healthy individuals.

Materials and Methods

Instrumentation

The chemiluminescence instrument (Antek Instruments Inc., Houston, TX 77070) consisted of a nitrogen detector (Model 720), pyroreactor (Model 771), and syringe drive (Model 735). The instrument converts all chemically bound nitrogen to nitric oxide at 1100 °C in the pyroreactor. This nitric oxide is mixed with oxygen in the nitrogen reaction chamber, where it forms excited nitrogen dioxide. As this molecule decays, it emits light that is specific for nitric oxide and is measured with infrared light (950 nm) by the photomultiplier tube. The instrument's gas-flow settings for Pyro O2, Inlet O2, and ozone were 350, 160, and 25 mL/min, respectively. The integrator attenuation was set at 5. The injection volume was constant at 5 μL of sample. For sample injection into the pyroreactor we used a syringe drive speed of 0.60 μL/s, corresponding to an instrument setting of 800.

We measured UUN with the urea chemistry module of
the Astra Automated Analyzer (Beckman Instruments Inc., Brea, CA 92621). This urease enzymatic method measures conductivity and is specific for urea, exhibiting no cross-reactivity with (e.g.) ammonia.

Specimens

We analyzed 76 urine specimens in correlating the Kjeldahl and chemiluminescence methods. This group included specimens obtained from both healthy and hospitalized subjects. Nineteen additional samples obtained from hospitalized patients were included in the UUN correlation study.

Standards and Controls

Aqueous standards, prepared from ammonium sulfate (Sigma Chemical Co., St. Louis, MO 63178), ranged in concentration from 3.0 to 22.5 g of nitrogen per liter. The standards were diluted further by adding 50 μL to 10 mL of de-ionized water.

Fisher Diagnostics’ (Orangeburg, NY 10962) “UriChem Urine Chemistry Control (Human) level II,” used as a control, was prepared according to the package insert directions except that 30 and 15 mL of water per vial were used to prepare controls I and II, respectively. Controls and urine samples were also diluted by adding 50 μL to 10 mL of de-ionized water.

Procedures

Sample injection and calculations. A linear calibration curve was prepared daily from data on triplicate injections of the aqueous standards. Injections were required to yield integrated areas that were within 3% of each other. Samples and controls were injected in duplicate and were accepted only if the CV for the injection was ≤3%. The nitrogen concentration in the samples and controls was calculated from the standard curve, and then the amount of nitrogen excreted in the urine per day was calculated.

Determination of total nitrogen by Kjeldahl. Samples were analyzed by the classic Kjeldahl method, with digestion of the sample in concentrated sulfuric acid. The digested samples were neutralized with sodium hydroxide, distilled into a boric acid solution containing brom cresol green, and titrated with standard 0.100 mol/L HCl reagent to a pH 4.8 endpoint.

Calculation of nitrogen balance. The nitrogen balance was calculated with standard formulas. When UUN data were used, the nitrogen balance (NB) equation was as follows:

\[ NB = \text{nitrorgen input} - (UUN + 4) \]  \hspace{1cm} (1)

where nitrogen input and UUN are given in g/24 h and the constant of 4 is a correction for perspiration and fecal losses and for non-urea forms of nitrogen in the urine. When TUN data are available, the equation for NB calculation becomes

\[ NB = \text{nitrorgen input} - (TUN + 2) \]  \hspace{1cm} (2)

where TUN is in g/24 h and the constant 2 corrects for fecal, perspiration, and other losses of nitrogen.

An alternative equation is sometimes used for calculating nitrogen balance from UUN data:

\[ NB = \text{nitrorgen input} - (1.2 \times UUN + 2) \]  \hspace{1cm} (3)

so that UUN is given in g/24 h, the 2 corrects for fecal and perspiration losses of nitrogen, and the factor 1.2 corrects for non-urea forms of nitrogen calculated as a percentage of the urea concentration (10).

To assess the difference between nitrogen balances as evaluated by chemiluminescence and those calculated from UUN, we calculated nitrogen balances for 67 of the 76 specimens divided into two groups: (a) specimens from 29 trauma, surgery, or burn patients, and (b) 38 specimens obtained from normal, healthy subjects who were participating in a controlled metabolic study. The patients in group a were all receiving all their nutrition parenterally. The normal subjects were eating food that had been carefully evaluated for its nitrogen content by use of food tables.

Results

Analytical Variables

Sensitivity. The minimum amount of nitrogen detectable by this method, 0.32 g/L, was established by calculating the concentration of a solution that would yield a signal three-fold above background at an attenuation factor of five. Corrections for sample dilution and attenuation yielded a minimum detectable concentration of 0.3 mg/L.

Linearity. The standard curve for the present method was linear from 48 to 1.5 g/L. Regression analysis of results for this experiment yielded slope = 1.0002, y-intercept = 0.75, and correlation coefficient = 0.9990.

Analytical recovery. Recovery was assessed by analyzing urine samples to which ammonium sulfate was added to give final concentrations of 10 and 20 g of nitrogen per liter. The mean recovery at each concentration was 97%.

Injection precision and carryover. Two urine samples with total nitrogen concentrations of 3.0 and 15.0 g/L were each injected 10 times. The CV for the signal obtained from this replicate determination was 3.0% at each concentration. The method did exhibit a carryover effect, caused by incomplete rinsing of the syringe between samples. Unfortunately, rinsing the syringe with water decreased the measured signal because residual water in the syringe slightly diluted the sample. Therefore, to minimize the carryover effect, we found it necessary to rinse the syringe 10 times with the solution to be injected.

Day-to-day precision. The precision of assaying the 5.9 and 12.1 g/L nitrogen controls over a 22-day period was 5.7% for both controls.

Correlation with UUN and Kjeldahl results. Correlation of chemiluminescence vs Kjeldahl method for TUN gave a slope = 1.028, y-intercept = 0.020, and correlation coefficient = 0.975 (n = 76). Similarly, correlation of chemiluminescence TUN vs UUN yielded a slope = 1.174, y-intercept = 0.799, and correlation coefficient = 0.975 (n = 95). Because the chemiluminescence and Kjeldahl methods correlated well, the correlation of Kjeldahl TUN vs UUN was similar: slope = 1.13, y-intercept = 0.837, and correlation coefficient = 0.971 (n = 76).

Discussion

The chemiluminescence-measured TUN correlated well with Kjeldahl TUN, where regression analysis yielded a slope very close to unity. The correlation results indicate that, within the analytical error of the two methods, TUN measured by chemiluminescence and Kjeldahl are equiva-
lent, and the more convenient chemiluminescence method can be readily substituted. Therefore, the comparison of nitrogen balances calculated with chemiluminescence TUN and UUN data would be equivalent to the comparison of balances calculated from Kjeldahl and UUN data. Although not expected, because UUN is not always a good indicator of TUN, the chemiluminescence TUN correlated equally well with UUN. This may be because of the number of specimens in this correlation set, as well as the diversity of the population from which the specimens were obtained. The slope of this correlation (1.17) indicates that the average total nitrogen composition of urine for the group studied was 83% urea.

For the abnormal group, we tested the hypothesis that the average nitrogen balance calculated from UUN data was greater than that calculated from chemiluminescence TUN results, an indication that UUN underestimates nitrogen excretion in the urine. Figure 1 shows the nitrogen balances for the hospitalized group and for the healthy population, with use of equations 1 and 2 to calculate the TUN and UUN nitrogen balances for the two groups. Table 1 summarizes the mean nitrogen balance determined for each group by use of equations 1–3.

For the normal group the difference between the mean UUN and TUN NB was 0.56 g/24 h. This difference was significant at $P = 0.00029$ ($t = 3.76$; $t_{0.05} = 1.96$), so we accepted the hypothesis that the mean TUN NB was less than the mean UUN NB. The significant difference between the mean UUN and TUN NB indicates that equation 1 overestimates the NB by 0.56 g/24 h, as compared with equation 2. In this healthy population, a more nearly accurate equation for determining nitrogen balance from
Table 1. Comparison of Nitrogen Balances Calculated from UUN and TUN Data

<table>
<thead>
<tr>
<th>Nitrogen balance, g/24 h, mean ± SD</th>
<th>Normal pop. (n = 38)</th>
<th>Hospitalized pop. (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUN NB*</td>
<td>1.34 ± 1.4</td>
<td>-0.961 ± 7.25</td>
</tr>
<tr>
<td>TUN NBb</td>
<td>0.78 ± 1.7</td>
<td>-2.86 ± 8.17</td>
</tr>
<tr>
<td>Corrected UUN NBc</td>
<td>1.59 ± 1.6</td>
<td>-1.30 ± 7.78</td>
</tr>
</tbody>
</table>

* By equation 1: NB = nitrogen input - (UUN + 4).

b By equation 2: NB = nitrogen input - (TUN + 2).

* By equation 3: NB = nitrogen input - (1.2 x UUN + 2).

UUN data is thus:

\[ NB = \text{nitrogen input} - (UUN + 4.56) \]  

where 4.56 equals equation 1 with equation 2 for correction of other forms of nitrogen loss.

For the hospitalized group, the mean difference between TUN and the UUN nitrogen balances calculated from equations 1 and 2 was 1.9 g/24 h. This difference was statistically significant \((P = 0.00005)\), with a paired-sample \(t\)-test comparing the UUN and TUN nitrogen balances yielding \(t = 4.57\) \((t_{0.95} = 2.05)\), thus verifying the hypothesis that the mean TUN nitrogen balance was less than the mean UUN nitrogen balance. Six of the 29 patients within the surgery and trauma group (32%) were assessed as being in positive nitrogen balance by UUN but were actually in a negative nitrogen balance when calculated with TUN data. Given the mean and median difference between UUN and TUN nitrogen balances for the abnormal group of 1.9 g/24 h, there is more than a 50% probability that a positive UUN nitrogen balance of <1.9 g/24 h will actually reflect a negative TUN balance. The difference between the mean TUN and UUN NB is reduced to 1.34 g/24 h when the additional correction factor applied in equation 4 is used, but does not completely correct it.

Use of equation 3, also designed to correct for abnormal nitrogen excretion, to calculate nitrogen balances also gives incomplete correction. The mean difference between the equation 3-corrected UUN and TUN nitrogen balances for the hospitalized population was 1.55 g/24 h \((t = 4.90, t_{0.95} = 2.05; P = 0.000245)\). For the healthy population, the mean difference between the TUN and corrected UUN nitrogen balances, 0.8 g/24 h, was less than that observed for the hospitalized group and was also statistically different \((t = 3.94, t_{0.95} = 1.96; P = 0.00005)\).

To test for a significant difference between the nitrogen balance difference \((UUN NB – TUN NB)\) for the abnormal and normal groups, we used a simple two-sample \(t\)-test with unequal variances. We concluded that the difference between UUN and TUN nitrogen balances was greater for the hospitalized group than for the normal population \((P = 0.0032)\).

Results of the chemiluminescence method for TUN correlated well with those of both Kjeldahl and urea nitrogen assays. However, the nitrogen balance calculated from UUN data underestimated nitrogen excretion in both the healthy and hospitalized population when compared with the nitrogen balance calculated from TUN data. We conclude that correction factors do not provide an adequate adjustment of UUN data, and that non-urea forms of nitrogen should be measured carefully, not estimated, for accurate nitrogen balance calculations in a hospital population. The use of chemiluminescence TUN data to calculate nitrogen balance provides a convenient and accurate assessment of nutritional status.

References