Original Article

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Development and reliability assessment of a liquid-phase measurement for skin taurine using skin blotting

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SUMMARY: Skin taurine is an indicator of dehydration and can be noninvasively collected through skin blotting. However, conventional taurine measurement using ninhydrin raises safety and specificity concerns, limiting its application in point-of-care testing. In this study, we propose a novel liquid-phase measurement of taurine collected *via* skin blotting. The aim of this study was to develop a method for liquid-phase measurement of skin taurine and to demonstrate its validity and reliability. This study consisted of (1) determining optimal recovery conditions, including the type and concentration of recovery solution and shaking method (mild, intense, or no shaking), (2) evaluating the specificity of taurine measurement, and (3) assessing the intra- and inter-rater reliability of the developed method for skin taurine measurement. Optimal recovery was achieved by intense shaking for 10 min with a 110 mM sodium chloride solution, and this method could measure taurine concentrations from 31.25 to 500 µM (r = 0.9983, p < 0.001), confirming its validity. Linear regression analysis showed that the addition of amino acids or skin lysates had little effect on taurine measurement. The developed method demonstrated high intra-rater reliability (intraclass correlation coefficient [ICC] (1,1) = 0.896, p < 0.001 for examiner A; ICC (1,1) = 0.755, p < 0.001 for examiner B), but inter-rater reliability was not significant (ICC (2,1) = 0.187, p = 0.15). The liquid-phase measurement of skin taurine demonstrated high sensitivity, specificity, and intra-rater reliability. Further studies are needed to improve inter-rater reliability for applying this method as a point-of-care tool for dehydration assessment.

Keywords: older adults, dehydration, taurine, skin blotting, liquid-phase measurement, taurine dioxygenase

1. Introduction

Hypertonic dehydration is defined as a loss of total body water (*1*) and leads to several disorders, such as headache, nausea, heat stroke, and in severe cases, impaired consciousness or death (*2*). In older adults, dehydration contributes to frailty (*3,4*), sarcopenia (*5,6*), and reduced activities of daily living, which further decrease fluid intake and exacerbate dehydration. Moreover, age-related changes in fluid distribution (*7*), reduced muscle mass (*4*), diminished thirst (*8,9*), and impaired osmotic regulation (*10,11*) make older adults particularly susceptible to dehydration. As dehydration worsens, the risks of hospitalization and mortality increase (*3,12*), along with healthcare costs (*12,13*). Its high prevalence, ranging from 19–28% among community-dwelling older adults (*6,14*) to 20–38% in nursing homes (*9,14,15*), underscores the urgent need for dehydration prevention in older adults.

Early identification of dehydration is important to prevent related disorders; however, older adults often show few or no symptoms (*16,17*). Regular monitoring using point-of-care testing could allow for early detection. Such testing should be noninvasive, quick, portable, safe for home use, and accurate. Several methods exist for identifying dehydration, with serum osmolality being the most reliable (values ≥ 300 mOsm/kg indicate hypertonic dehydration) (*1*). However, blood tests are invasive, time-consuming, and require laboratory analysis (*18*), making them unsuitable for point-of-care monitoring. Noninvasive methods have been proposed, but each has limitations. Physical assessment of dehydration based on symptoms shows poor validity (*19*). Body weight is not a reliable indicator due to fluctuations from diet and bowel movements (*20*). Urinalysis or saliva tests are affected by medications

(*21*) and diet (*22*). Bioimpedance analysis has limited accuracy (*23*). Echocardiographic evaluation of inferior vena cava diameter shows no clear correlation with dehydration (*24*). Therefore, no current method fully meets the criteria for practical point-of-care dehydration monitoring.

Recently, a novel, noninvasive, and highly accurate method for detecting dehydration has been proposed. This is based on the skin blotting technique, in which a specific membrane is applied to the skin to collect soluble molecules from interstitial fluid (*25*). Among the various molecules captured by the skin blotting technique, taurine has emerged as a promising biomarker for dehydration. Taurine plays a key role in regulating keratinocyte volume: under hypertonic conditions, it is taken up by cells to mitigate osmotic stress from excess electrolytes, whereas under hypotonic conditions, it is expelled to restore normal cell volume (*26,27*). This osmotic regulation explains the correlation between skin taurine levels and changes in osmolarity. Accordingly, Higashimura reported that skin blotting measurement of skin taurine using an anion-exchange membrane could detect current or impending hypertonic dehydration (serum osmolality > 291 mOsm/L) with 77.3% sensitivity, 81.8% specificity, and an area under the receiver operating characteristic curve of 0.789 (*16*). However, this method relies on ninhydrin staining (*16*), which requires lengthy staining and a plate reader, limiting speed and portability. Tsuchiya *et al.* improved this approach by using a ninhydrin spray to shorten the measurement time (*28*). Nevertheless, safety concerns remain because ninhydrin is a known skin irritant. Additionally, ninhydrin is nonspecific; it reacts with amino acids other than taurine, although with a different color tone. Thus, skin blotting for dehydration detection requires improvements in specificity, speed, and portability.

Therefore, in this study, we aimed to improve taurine measurement using skin blotting. First, to increase specificity and safety, we developed a method using the redox reaction of taurine dioxygenase (*29*), which is more specific for taurine and safer than the previous ninhydrin method. Next, to enable enzyme reaction, we designed a method to collect taurine from an anion-exchange membrane in the liquid phase. This approach builds on Ogai *et al.* (*30*), in which a recovery solution is applied to a membrane to collect proteins as a liquid phase. Because skin taurine is captured by the anion-exchange membrane, it is speculated that applying an ionic solution to the membrane can extract the trapped taurine into the liquid phase (*31*). The redox reaction of taurine dioxygenase offers high specificity and sensitivity (*32*), and the colorimetric measurement can be performed using a portable spectrophotometer.

Based on the above, the aim of this study was to develop a liquid-phase method for measuring skin taurine using skin blotting and to demonstrate the reliability of the method.

2. Materials and Methods

2.1. Study 1: Development of a liquid-phase measurement method of taurine *via* skin blotting

In Study 1, we first aimed to determine the optimal conditions for liquid-phase measurement of taurine blotted onto an anion-exchange membrane, focusing on the type and concentration of the recovery solution and the shaking method used during taurine collection. Once the optimal conditions for liquid-phase measurement of taurine were identified, we next assessed the validity of the method by evaluating the relationship between the amount of taurine applied and the measured results.

2.1.1. Preliminary experiments for determination of the recovery solution

To identify an appropriate recovery solution, we conducted a preliminary experiment in which several ionic solutions were tested based on previous studies (*33,34*), namely sodium chloride, sodium dihydrogen phosphate, and sodium sulfate solutions. Among the solutions tested, sodium chloride was the most suitable for taurine recovery. Sodium dihydrogen phosphate caused precipitation during taurine measurement using the Taurine Assay Kit (MET-5071; Cell Biolabs, Inc., San Diego, CA, USA), and sodium sulfate appeared to interfere with the measurement reaction (data not shown).

We then performed an experiment to estimate the optimal concentration of sodium chloride for liquid-phase taurine measurement. Sodium chloride solutions ranging from 1 M down to 31.25 mM were tested with mild shaking (see Section 2.5) to evaluate recovery rates. The 110 mM sodium chloride solution yielded the most stable measurements (data not shown) and was, therefore, selected as the starting condition for subsequent validation experiments.

2.1.2. Effect of sodium chloride on taurine measurement

Because sodium chloride solution is not the standard diluent for the Taurine Assay Kit, we evaluated its effect on taurine quantification. Taurine solutions at 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 0 μM were prepared with and without 110 mM sodium chloride in the assay buffer. The control group used distilled water (DW) instead of the sodium chloride solution. Taurine concentrations in both groups were measured using the Taurine Assay Kit. Absorbance was measured in triplicate for each sample, and the experiment was repeated three times.

2.1.3. Determination of the optimal sodium chloride concentration for liquid-phase taurine measurement

To determine the optimal sodium chloride concentration for taurine recovery, 0, 55, 110, and 220 mM sodium chloride solutions were used to collect taurine from an anion-exchange membrane preabsorbed with taurine *via* mild shaking (see Section 2.5). Taurine concentrations were then measured using the Taurine Assay Kit. Absorbance was measured in triplicate, and the experiment was repeated three times.

2.1.4. Determination of optimal shaking and time for liquid-phase taurine collection

To determine the optimal conditions for liquid-phase taurine collection, shaking intensity and duration were evaluated. An anion-exchange membrane prepared with a 149.8 μM taurine solution was used as a sample. This concentration was chosen based on the taurine threshold used to assess impending dehydration (serum osmolality > 291 mOsm/L) in a previous study (*16*). The recovery solution used in all conditions was 110 mM sodium chloride. The following shaking intensities and durations were tested: mild shaking for 60 min; intense shaking for 60, 10, or 1 min; and no shaking for 60, 10, or 1 min (see Section 2.5). The amount of taurine recovered under each condition was quantified using the Taurine Assay Kit, and the recovery rate was calculated.

2.1.5. Validation of the liquid-phase measurement of taurine

After determining the optimal conditions, we assessed the validity and measurable range of the method. Taurine solutions at concentrations of 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 0 μM were applied to anion-exchange membranes to allow taurine adsorption. Liquid-phase taurine measurement was then performed using the Taurine Assay Kit. To enable portable, point-of-care testing of skin taurine, a portable spectrophotometer (GDX-SVISPL; Vernier Science Education, Beaverton, OR, USA) was used for quantification. The correlation coefficient between readouts from the microplate reader (MPR-A100; AS ONE Corp., Osaka, Japan) and the portable spectrophotometer was 0.999, indicating high interchangeability of the two devices (supplementary Figure S1, *https://www.ddtjournal.com/action/getSupplementalData.php?ID=275*). Absorbance was measured in triplicate for each sample, and the experiment was repeated three times.

2.2. Study 2: Evaluation of the specificity of taurine measurement

In skin blotting, various substances from the skin surface and underlying tissues are collected, including amino acids and proteins (*35-37*). Because taurine is an amino acid analog, the specificity of the taurine measurement used in this study was evaluated. To assess this, either amino acids or skin lysates (rich in skin-derived amino acids and proteins) were individually added as potential interfering substances to taurine solutions, and their effects on taurine measurement were examined. Taurine solutions were prepared at concentrations of 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 0 μM. To each, either 0, 0.1, or 1.0 μmol of an essential amino acid mixture (L4461; Promega Corp., Madison, WI, USA) or 0, 10, or 100 ng of human skin lysate (NB820-59254; Novus Biologicals, LLC, Centennial, CO, USA) was added. Taurine concentration in each sample was quantified using the Taurine Assay Kit. All samples were measured in triplicate, and the experiment was repeated three times.

2.3. Study 3: Reliability of liquid-phase taurine measurement of skin taurine

The intra- and inter-rater reliabilities of the developed method were assessed as follows. Two researchers (examiner A, with 2 years of experience in scientific research and primarily responsible for this study, and examiner B, with 17 years of research experience and only marginally involved in this study) first underwent training in skin taurine measurement. Twenty volunteers (mean ± standard deviation [SD]: 43.2 ± 8.7 years; 4 males and 16 females) were recruited, and four regions in total (1R, 2R on the right arm; 1L, 2L on the left arm) were designated on both arms of each participant, as shown in Figure 1. For each volunteer, examiner A randomly chose one site from each arm (1R or 2R, and 1L or 2L), and examiner B measured the region not selected by examiner A.

The skin blotting unit was prepared based on Tsuchiya *et al.* (*28*) with slight modifications. Specifically, while the structure consisting of a nitrocellulose membrane stacked with an anion-exchange membrane remained the same as in the previous study (*28*), a 2% agarose gel was incorporated to prewet the membrane (Figure 2). The skin blotting unit was applied to the skin in the same manner as the previous study (*28*). Briefly, the measurement region was first wiped with a nonwoven paper towel prewetted with distilled water, and the skin blotting unit was then attached for 20 min. After 20 min, the unit was removed and stored at 4°C until measurement. The sample size of 20 volunteers was determined by power analysis, assuming an intraclass correlation coefficient (ICC) (2,1) of 0.85 with a confidence interval (CI) of 0.2.

Each examiner performed liquid-phase collection of taurine from the anion-exchange membrane under the optimal conditions (10 min of intense shaking) determined in Study 1 and measured the collected taurine using the Taurine Assay Kit. A positive control (an anion-exchange membrane adsorbed with 7.35 nmol of taurine) was included in each measurement.

This experiment was approved by the Ethics

Committee of Ishikawa Prefectural Nursing University (reference number: 2024-12).

2.4. Taurine adsorption to the anion-exchange membrane

For Study 1, anion-exchange membranes adsorbed with different concentrations of taurine were prepared. Based on a previous study (*16*), a 10 × 10 mm filter paper was placed on a 12 × 12 mm anion-exchange membrane (A202-CE; Tokuyama Corp., Tokyo, Japan), and 30 µL of the designated concentration of taurine solution (32708-02; Nacalai Tesque, Inc., Kyoto, Japan) was applied to the filter paper. A 7.6-g weight was placed on top to ensure close contact between the filter paper and the membrane, and the assembly was left for 10 min to allow taurine adsorption. The anion-exchange membranes were then air-dried and used as samples in Study 1.

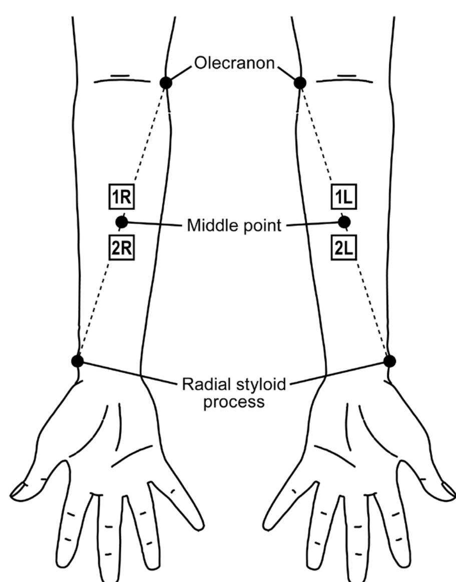

Figure 1. Position of skin blotting unit attachment. The middle point was determined as the midpoint between the olecranon and the radial styloid process of each arm.

2.5. Shaking method for liquid-phase collection of taurine

To collect taurine from each anion-exchange membrane, the following shaking conditions were used: (1) Mild shaking, as in the previous study (*30*): Membranes were placed in a 24-well plate (#144530; Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 500 µL of recovery solution and shaken on an orbital shaker (EM-36; Taitec Corp., Saitama, Japan) at 4.0 Hz = 240 revolutions per minute (rpm). (2) Intense shaking: The anion-exchange membrane and 125 µL of recovery solution were placed in a 1.5-mL microtube and set on a vortex mixer (Vortex-Genie 2; Scientific Industries, Inc., Bohemia, NY, USA) equipped with a tube support, with the tube lid facing down (Figure 3; the contact area of recovery solution and the membrane is 0.65 cm^2). Tubes were mixed at intensity level 1 (7.0 Hz = 420 rpm). (3) No shaking: Prepared using the same method as for intense shaking, except that tubes were left stationary without any shaking.

2.6. Analysis

Data are presented as individual measurements or as means ± SD. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 29; IBM Corp., Armonk, NY, USA) and Microsoft Excel (version 2506; Microsoft Corp., Redmond, WA, USA). A p-value < 0.05 was considered statistically significant.

2.6.1. Study 1

Linear regression analysis was used to evaluate the relationship between taurine concentration and

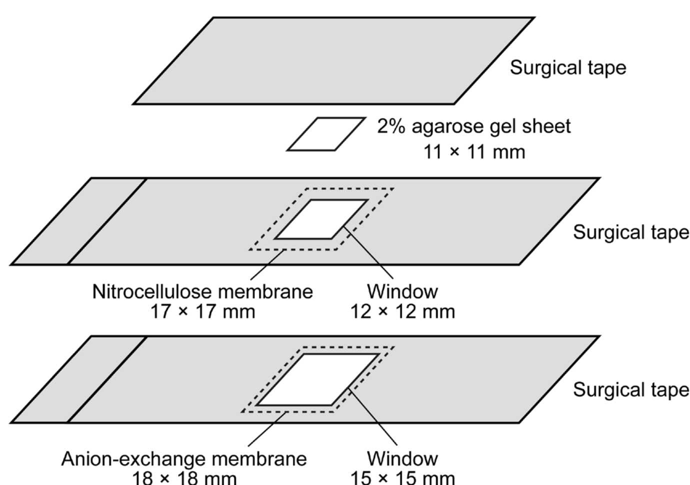

Figure 2. A skin blotting unit.

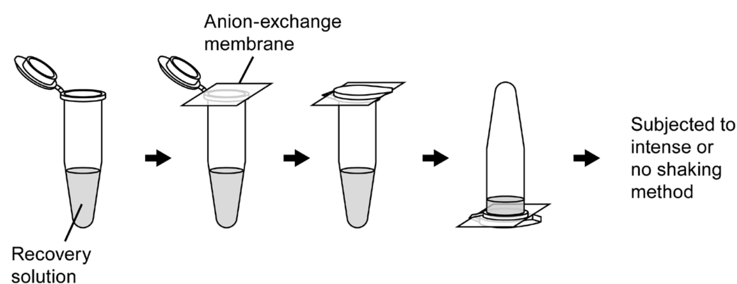

Figure 3. Modified method for liquid-phase collection of taurine.

background-adjusted absorbance, with the correlation coefficient (*r*) representing the strength of the linear association. The regression lines of the DW group and the 110 mM sodium chloride group were compared to determine whether the slope and intercept of the 110 mM sodium chloride group fell within the range of the mean ± 0.5 SD of the slope and intercept of the DW group. The recovery rate was calculated as the ratio of the recovered taurine amount to the applied taurine amount.

2.6.2. Study 2

The percent coefficient of variation (%CV) was calculated as the SD divided by the mean, multiplied by 100. Two-way analysis of variance (ANOVA) with Tukey's *post hoc* adjustment was performed to evaluate the interaction between taurine concentration and potential contaminants (amino acids or skin extracts).

2.6.3. Study 3

ICC (1,1) and ICC (2,1) were calculated to evaluate intra- and inter-rater reliability, respectively. Additional reliability analyses were performed using Bland–Altman analysis: fixed error was evaluated with the 95% CI of differences, proportional error was assessed with regression analysis of the plots, and random error was evaluated using limits of agreement (LOA), standard error of measurement (SEM), minimum detectable change (MDC), and the ratio of MDC to the measured value (MDC%) (*38*).

3. Results

3.1. Study 1: Development of a liquid-phase measurement method of taurine *via* skin blotting

3.1.1. Effect of sodium chloride on taurine measurement

The effect of sodium chloride on taurine measurement is shown in Figure 4A. The linear regression equation for the DW group was $y = 4.68 \times 10^{-4}x + 0.0143$ ($r = 0.991$, $p < 0.001$), and for the sodium chloride group, it was $y = 4.42 \times 10^{-4}x + 0.0107$ ($r = 0.980$, $p < 0.001$).

The mean ± 0.5 SD of the intercept in the DW group ranged from 0.0129 to 0.0157, and the mean intercept in the sodium chloride group (0.0108) fell below this range. Similarly, the mean ± 0.5 SD of the slope in the DW group ranged from 4.44×10^{-4} to 4.91×10^{-4}, and the mean slope in the sodium chloride group (4.42×10^{-4}) was also below the lower limit.

3.1.2. Liquid-phase collection of taurine at different sodium chloride concentrations

Figure 4B shows the recovery rates of taurine at different sodium chloride concentrations. The mean recovery rates (range) were 27.1% (14.4–41.3%) at 0 mM, 70.6% (56.4–79.5%) at 55 mM, 100.8% (97.7–106.4%) at 110 mM, and 124.6% (108.5–150.9%) at 220 mM.

3.1.3. Liquid-phase collection of taurine with different shaking methods and durations

Table 1 and Figure 4C present the results of the evaluation of shaking methods and durations on taurine recovery from the anion-exchange membrane. Mild shaking for 60 min yielded a mean recovery rate of 109.0 ± 16.3% with a %CV of 15.0%, confirming high and stable taurine recovery close to 100%.

The intense shaking method yielded the following mean recovery rates ± SD (%CV): 85.0 ± 8.8% (10.4%) at 1 min, 94.9 ± 4.4% (4.7%) at 10 min, and 92.7 ± 5.2% (5.6%) at 60 min. The no-shaking method yielded 27.5 ± 3.1% (11.2%) at 1 min, 58.6 ± 22.5% (38.3%) at 10 min, and 101.5 ± 9.9% (9.7%) at 60 min. Among the tested conditions, 10 min of intense shaking showed a recovery rate closest to 100% with the lowest %CV.

3.1.4. Measurable range and validation of the liquid-phase measurement of taurine

The mean values (range) of background-adjusted absorbance at taurine concentrations of 7.8 μM and 15.6 μM were 0.0146 (0.0102–0.0188) and 0.0175 (0.0125–0.0233), respectively, both falling within the range observed at a taurine concentration of 0 μM (0.0105–0.0173). Therefore, concentrations below or equal to

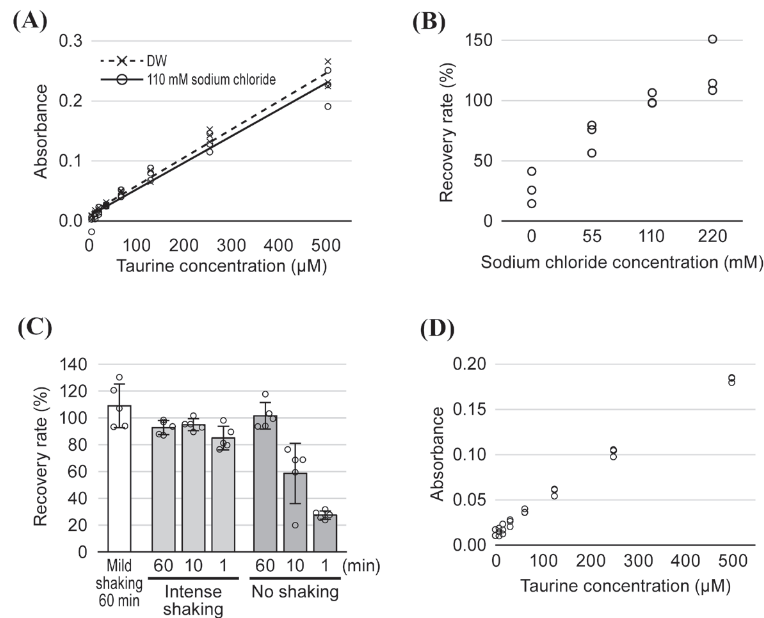

Figure 4. Evaluation of the liquid-phase measurement of taurine. (**A**) Effect of sodium chloride on taurine measurement. Absorbance values are background-adjusted. (**B**) Effect of sodium chloride concentration on taurine recovery in the liquid-phase measurement. Recovery rate is calculated as the ratio of taurine recovered to the amount applied to the anion-exchange membrane. (**C**) Effect of shaking method and time on taurine recovery. Data are expressed as mean ± standard deviation. (**D**) Correlation between taurine concentration and measurement results obtained using the developed liquid-phase measurement method. Absorbance values are background-adjusted. DW: distilled water.

Table 1. Effect of shaking method and time on taurine recovery in liquid-phase collection

Shaking method	Time (min)	Recovery rate* (%)	%CV
Mild Shaking†	60	109.0 ± 16.3	15.0
Intense shaking	1	85.0 ± 8.8	10.4
	10	94.9 ± 4.4	4.7
	60	92.7 ± 5.2	5.6
No shaking	1	27.5 ± 3.1	11.2
	10	58.6 ± 22.5	38.3
	60	101.5 ± 9.9	9.7

Mean ± standard deviation; CV: coefficient of variation. *Ratio of taurine recovered to the amount of taurine applied to the anion-exchange membrane. †Used in a previous study (*30*).

15.6 μM were considered unreliable and excluded.

The relationship between taurine concentrations and measurement results is shown in Figure 4D. A significant correlation was observed for concentrations between 31.2 and 500 μM, with the regression equation $y = 3.36 \times 10^{-4}x + 0.0165$ ($r = 0.998$, $p < 0.001$).

3.2. Study 2: Reaction specificity of taurine measurement

Figure 5A shows the results of adding amino acids to taurine solutions and their effect on taurine quantification. Two-way ANOVA revealed no significant interaction between taurine concentration and the amount of amino acids added ($p = 0.91$). However, both main effects were significant: taurine concentration ($p < 0.001$) and amino acid addition ($p = 0.031$). Multiple comparisons showed no significant differences in taurine quantification between the 0- and 0.1-μmol groups ($p = 0.318$) or between the 0.1- and 1.0-μmol groups ($p = 0.417$). In contrast, a significant difference was observed between the 0- and 1.0-μmol groups ($p = 0.023$).

Figure 5B shows the results of adding skin lysate to taurine solutions and its effect on absorbance. Two-way ANOVA indicated no significant interaction between taurine concentration and the amount of skin lysate added ($p = 0.977$). The main effect of taurine concentration was significant ($p < 0.001$), whereas the main effect of skin lysate amount was not significant ($p = 0.061$).

3.3. Study 3: Reliability of liquid-phase taurine measurement

The measured values for the positive control were 0.0597 for examiner A and 0.0587 for examiner B. Intra-rater reliability [ICC (1,1)] and its 95% CI were 0.896 (0.759–

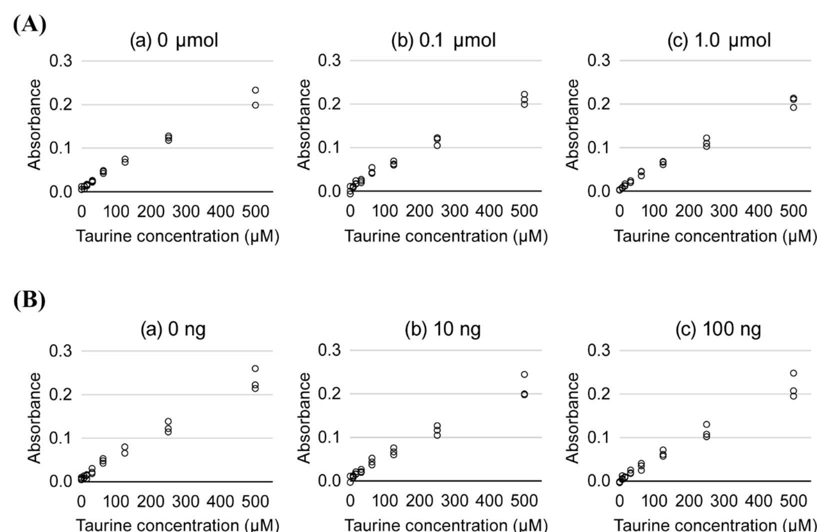

Figure 5. Specificity of taurine measurement. (**A**) Effect of amino acid addition on taurine measurement. Amounts added: (a) 0 μmol, (b) 0.1 μmol, (c) 1.0 μmol. (**B**) Effect of skin lysate addition on taurine measurement. Amounts added: (a) 0 ng, (b) 10 ng, (c) 100 ng. Absorbance values are background-adjusted.

0.957) for examiner A and 0.755 (0.485–0.894) for examiner B, both of which were statistically significant ($p < 0.001$for both). Bland–Altman plots for each examiner are shown in Figure 6A. All measurements were within the LOA for examiner A, while one measurement for examiner B fell below the lower LOA. Measurement errors for each examiner, calculated from the Bland–Altman plots, are shown in Table 2. The 95% CI for the mean difference was 0.0005 to 0.0017 for examiner A and −0.0017 to −0.0004 for examiner B, with both ranges not including zero. Regression analysis of the Bland–Altman plot for proportional error showed no significant coefficients for examiner A (−0.11, $p = 0.65$) or examiner B (−0.02, $p = 0.93$). Random error analysis showed that the MDC%, representing the degree of random error relative to the measurement, was 26.9% for examiner A and 35.4% for examiner B.

Inter-rater reliability [ICC (2,1)] and its 95% CI between the two examiners was 0.187 (−0.160-0.536), which was not statistically significant ($p = 0.15$). The Bland–Altman plot for inter-rater reliability is shown in Figure 6B, and the measurement error between examiners is shown in Table 3. The mean difference in skin taurine between examiners A and B was 0.00524, and the LOA was −0.0114 to 0.0219. All measurements fell within the LOA, and the 95% CI for the mean difference in fixed error was −0.0066 to 0.0039, including zero. The regression coefficient for proportional error was not significant (0.32, $p = 0.18$). The random error indicator MDC% was 68.7%.

4. Discussion

In this study, we developed and evaluated a new method for measuring skin taurine *via* skin blotting, employing liquid-phase collection of taurine from an anion-exchange membrane and redox reaction by taurine dioxygenase for faster, more specific, and safer quantification. The developed liquid-phase measurement method demonstrated higher specificity for taurine and greater measurement sensitivity than the conventional ninhydrin method. It also showed high intra-rater reliability, but inter-rater reliability was not significant, indicating the need to improve consistency between examiners. These results suggest that the developed method, once the remaining issues are addressed, has the potential to serve as a noninvasive point-of-care test for assessing skin taurine levels in clinical settings.

Preliminary experiments confirmed the feasibility of using sodium chloride solution for liquid-phase taurine collection. To evaluate its applicability, we examined the effect of sodium chloride on taurine quantification. Regression slopes and intercepts from the DW group were used as standards (mean ± 0.5 SD) for comparison. The slope and intercept values for the sodium chloride group were slightly below the lower limits of the DW group, suggesting that 110 mM sodium chloride may mildly inhibit taurine quantification, possibly by reducing the substrate reactivity of taurine dioxygenase in the sodium chloride solution (*39*). Nevertheless, significantly high correlations between taurine concentration and measured values were observed in both groups, indicating that measurement accuracy is maintained using the sodium chloride-based liquid-phase collection method.

To determine the optimal sodium chloride concentration, taurine recovery was compared across different concentrations of sodium chloride. Recovery rates exceeded 100% under both the 110 mM and 220 mM conditions, with the 220 mM condition reaching

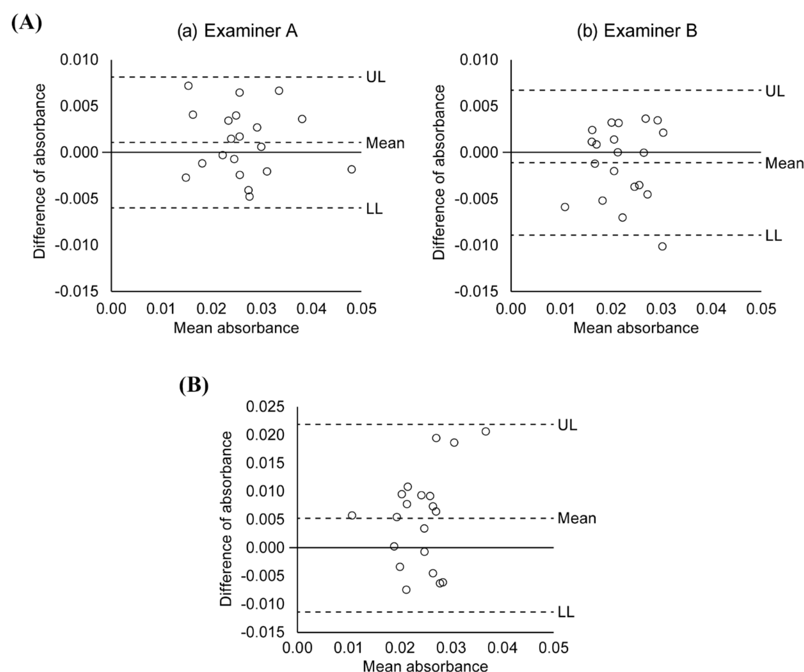

Figure 6. Bland–Altman plots of measurement errors. (**A**) Intra-rater error for each examiner. (**B**) Inter-rater error between the two examiners. UL: upper limit of agreement; LL: lower limit of agreement.

Table 2. Measurement error of each examiner in the Bland–Altman analysis

Examiner	LOA	Fixed error	Proportional error		Random error		
		95% CI	Regression coefficient	*p*-value	SEM	MDC	MDC%
A	−0.0060 – 0.0081	0.0005 – 0.0017	−0.11	0.65	0.0025	0.0071	26.9
B	−0.0089 – 0.0067	−0.0017 – −0.0004	−0.02	0.93	0.0028	0.0078	35.4

LOA: limits of agreement; CI: confidence interval; SEM: standard error of measurement; MDC: minimal detectable change.

Table 3. Measurement error between the two examiners in the Bland–Altman analysis

LOA	Fixed error	Proportional error		Random error		
	95% CI	Regression coefficient	*p*-value	SEM	MDC	MDC%
−0.0114 – 0.0219	−0.0066 – 0.0039	0.32	0.18	0.0060	0.0166	68.7

LOA: limits of agreement; CI: confidence interval; SEM: standard error of measurement; MDC: minimal detectable change.

150.9%, considerably exceeding the theoretical maximum. This suggests that excessive sodium chloride may cause measurement instability (*39*). Therefore, 110 mM sodium chloride was selected as the optimal solution for stable taurine recovery.

Next, we evaluated the shaking methods and durations required for liquid-phase taurine collection from anion-exchange membranes using 110 mM sodium chloride. We considered nearly 100% recovery and low variability as essential conditions for effective collection, with desirable conditions including shorter shaking times and minimal use of equipment. Mild shaking for 60 min, as used in the previous study (*30*), served as a reference and achieved approximately 100% recovery. The no-shaking method, the simplest collection approach, showed increasing recovery over time: 27.5% at 1 min, 58.6% at 10 min, and 101.5% at 60 min. In contrast, intense shaking achieved higher recovery in less time: 85.0% at 1 min and 94.5% at 10 min. To assess variability, we compared the %CV across conditions. The lowest %CV was 9.73% for the no-shaking method at 60 min and 4.67% for the intense shaking method at 10 min, indicating that 10 min intense shaking provided the best balance of recovery and reproducibility.

Using the optimized protocol (10-min intense shaking), we confirmed the validity and measurable range of liquid-phase taurine measurement. A strong, significant correlation was observed between taurine

concentration and measured values within the range of 31.25 to 500 μM ($r = 0.9983$, $p < 0.001$). This range is lower than that reported in previous studies (51.4–6,400 μM) (*16,28*), indicating improved sensitivity for detecting the dehydration threshold (149.8 μM) estimated in earlier work (*16*).

Because skin blotting can collect various contaminants, such as amino acids other than taurine, skin proteins, sebum, and cells, we next evaluated the specificity of the liquid-phase taurine measurement. Adding a high concentration of the amino acid mixture (1.0 μmol) had a slight effect on quantification, but no significant effects were observed at the lower concentration (0.1 μmol) or with any of the skin lysate additions. Sylvestre *et al.* reported that the total amount of amino acids collected by iontophoresis from the stratum corneum ranged from 0.3 to 0.6 $\mu mol/cm^2$ (*35*). In the present study, taurine was measured from an anion-exchange membrane with a surface area of 0.65 cm^2, corresponding to an estimated amino acid contamination of 0.2–0.4 μmol. This value falls within the 0.1–1.0 μmol range of amino acids tested in this study. Because no statistically significant difference in taurine measurement was observed between the 0.1 μmol and 1.0 μmol groups, the influence of physiological amino acid contamination on skin taurine measurement appears negligible. Eichhorn *et al*. reported that taurine dioxygenase can react with several sulfonic acids other than taurine, but their reactivity is much lower than that of taurine (*32*). Moreover, these sulfonic acids are not naturally found in the human body (*40*). These findings further support the high specificity of the present method for measuring skin taurine.

Taurine is an amino acid analog, and ninhydrin, which has been used in previous studies, reacts with amino acids and proteins other than taurine to produce color, although the color is distinct from that of taurine (*16*). Therefore, taurine measurement by ninhydrin staining could be affected by skin contaminants. In contrast, the high specificity of the newly developed liquid-phase taurine measurement provides a significant advantage for more accurate quantification of skin taurine.

Intra-rater reliability [ICC (1,1)] was 0.896 for examiner A and 0.755 for examiner B, both of which meet the "good" criteria defined by Koo and Li (*41*), indicating high intra-rater reliability for both examiners. Bland–Altman plots showed significant fixed and random errors for both examiners, but these were considered within practical limits. In contrast, inter-rater reliability [ICC (2,1)] was not significant ($p = 0.15$), indicating that inter-rater reliability could not be confirmed. This suggests that several factors may have contributed to the lower inter-rater reliability. Bland–Altman analysis revealed a large random error between examiners. The high degree of agreement between the positive control measurements performed by the two examiners suggests that both could properly perform liquid-phase taurine collection and the subsequent measurement of taurine. This suggests that the random errors may arise from the procedure between the application and removal of the skin blotting unit.

In taurine measurement using skin blotting, the skin surface is wiped with a nonwoven cloth moistened with DW to remove contaminants before attaching the blotting unit (*16,28*). Differences between examiners in the amount of DW applied, the force used during wiping, or wiping speed may have contributed to higher random error. Indeed, previous studies report variation in the forces applied to the skin among skilled caregivers during bed baths (*42*), suggesting considerable variability in wiping parameters. Therefore, further standardization of techniques, along with prior education and training, is necessary for consistent results. Despite this, the higher intra-rater reliability indicates that the developed method can be considered reliable when performed by a single operator.

Currently, the entire process takes approximately 60 min (20 min for skin blotting, 10 min for liquid-phase collection, and 30 min for enzymatic reaction). While this study demonstrates the validity of the developed method, further work is needed to improve speed and confirm its clinical reliability.

A limitation of this study is that sampling sites on the inner sides of the forearms were assumed to be homogeneous, and reliability was evaluated based on this assumption. However, differences between sites may exist. To verify this, skin taurine concentration could be measured using invasive methods such as tissue biopsy or microdialysis, but these methods are not suitable because of their invasiveness. Future studies should clarify potential differences in skin taurine concentration depending on the sampling site.

5. Conclusion

In this study, we developed a novel method for measuring skin taurine using skin blotting by combining liquid-phase taurine collection with redox reaction by taurine dioxygenase. The developed method showed improved specificity and sensitivity, as well as high intra-rater reliability, although inter-rater reliability was not significant. By improving inter-rater reliability through measures such as operator training, this method has the potential to serve as a practical tool for skin taurine measurement and point-of-care dehydration testing, contributing to dehydration prevention and the maintenance of daily living activities and cognitive function in older adults.

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