博士論文（要約）

Development of the Measurement of Taurine Concentration by the Skin Blotting: A Non-Invasive Identifying Method for Chronic Dehydration of Vulnerable Older People in Home Care Setting

（スキンブロッティングによるタウリン濃度測定法の開発 ―在宅療養高齢者における慢性的な高張性脱水の非侵襲的な同定―）

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Dehydration is a highly prevalent health condition among older adults and can compromise independent daily activities and threaten their lives. It proceeds by water deficit or by both salt and water loss in the body, manifested by a reduced total body fluid. Ability to maintain water balance is attenuated due to physical changes in body composition, kidney function, anti-diuretic and sodium conserving hormones, and thirst sensation for age. Chronically developing dehydration is suggested in the elderly, however, prevalence and pathophysiology remain unclear. Finding early dehydration is essential to avoid severe consequences such as heat illness. To distinguish dehydration, serum osmolality is the most reliable indicator, but, is not useful at community settings because it requires a blood test. In this study, I aimed to develop a simple, portable and non-invasive measurement to identify impending and current dehydration of older adults by the new skin blotting method. The new measurement will contribute to find dehydration at home care setting, avoid advanced medical care needs, promote non-invasive treatment, and support health and daily lives of older adults.

I focused on an organic substance taurine (2-aminoethane sulfonic acid) as a biomarker, which is known as an osmolyte to respond to harmful stimuli such as hyperosmolality or ultraviolet in human skin. I hypothesized that the taurine concentration in skin changes corresponding to the intercellular osmolality, thus, planned to quantify the skin taurine concentration by a new non-invasive method for the purpose of estimating serum osmolality. The skin blotting was used in this study, which allows collection of soluble molecules contained within the intercellular space with electrostatic membranes. I used the method to quantify the skin taurine concentration using the colourimetric reaction with ninhydrin. The validity of the skin blotting has already been revealed in elsewhere, however, the procedure in this study was different from previous studies in that it employed an anion exchange membrane to collect taurine and ninhydrin colourimetric reaction for quantification. Therefore, I newly developed the skin blotting method for skin taurine measurement and verified its accuracy to find dehydration in older adults. Accordingly, this study consisted of two parts: in Study 1, I developed a new measurement by the skin blotting method and proved the validity to quantify the skin taurine concentration in animal; in Study 2, I investigated dehydration among the vulnerable community-dwelling older adults and verified the discriminative accuracy to

find impending and current dehydration by the new measurement.

Study 1 had four steps: *Step 1* demonstrated the taurine colourimetric reactivity with ninhydrin on anion exchange membranes; *Step 2* exhibited the specific taurine colour reaction with ninhydrin in comparison to glycine and fetal bovine serum; *Step 3* showed the dose-dependent taurine colourimetric reactivity with ninhydrin where taurine was within biotic level; and *Step 4* revealed validity of the skin blotting method to quantify the taurine concentration in rat skin by comparing with the taurine concentration measured by the high performance liquid chromatography (HPLC). In the animal study, anion exchange membranes applied on the lateral abdominal site of rats exhibited colourimetric reactions with ninhydrin, which absorbance had a linear correlation with the taurine concentration obtained by HPLC (R-squared value was 0.3647, $p = 0.038$). Accordingly, I concluded that the skin taurine can be specifically and quantitatively detected by the new skin blotting method.

In Study 2, dehydration was I investigated in 33 older adults at home care setting. The participants were using community nursing services and needed some medical examination, treatment, medication, monitoring and support for health as well as for daily activities, but their medical conditions were stable and chronic when participating in the investigation. Cross-sectional study revealed that two thirds of the participants had serum hyperosmolality over 291 mOsm/L. Further, serum osmolality in a third of the participants raised over 300 mOsm/L. They were suspected to be dehydrated, however, no one had acute causative episodes related to dehydration. Blood tests showed no hypernatremia even in the cases which serum osmolality was over 300 mOsm/L. In addition, BUN-creatinine ratio and hematocrit value inferred that there was a blood concentration. It was speculated that increased serum osmolality among the participants likely progressed gradually and chronically, accompanied by mild hypovolemia. No conventional dehydration symptoms and signs such as thirst or dry axilla were correlated to participant dehydrated status. Taurine absorbance obtained by the new skin blotting method was moderately correlated with serum osmolality (Pearson's correlation $r = 0.566$, $p = 0.001$). Logistic regression analyses demonstrated the largest impact of taurine absorbance to discriminate cases with serum osmolality over 291 mOsm/L. The odds ratio of taurine absorbance was 2.136 (95% CI: 1.145 - 3.986, $p = 0.017$), when taking into account of age and the Nursing Care Level as covariates. In another model, the odds ratio of taurine absorbance was 3.282 (95% CI: 1.064 - 10.126, $p = 0.039$) when age and HDS-R were covariates. The discriminative accuracy of taurine absorbance was confirmed by the receiver operating characteristics (ROC) curve analysis and the area under the curve (AUC) was 0.789 (95% CI: 0.606-0.972, $p = 0.008$). Sensitivity and specificity for identifying serum hypertonicity over 291 mOsm/L were 77.3% and 81.8% when the threshold of taurine absorbance was set at 0.272. As a result, taurine absorbance measured by the skin blotting method showed accurate discriminative power for impending and current dehydration in the community-dwelling older people. I concluded that the new skin blotting measurement will be a practical tool to identify

dehydration of vulnerable older people at home care setting.

This is the first study that investigated the prevalence of dehydration among vulnerable community-dwelling older people in Japan. A high prevalence of serum hyperosmolality was shown, however, there is a possibility that the detected hyperosmotic condition was not necessarily dehydration because new measurement was developed based on serum osmolality only. The taurine absorbance may reflect serum sodium concentration rather than serum osmolality and is possibly applicable to sodium concentration estimation including the case of hyponatremia. On the other hand, the relatively low sodium concentration in comparison to acute dehydration and the lack of any profound hypovolemia symptoms among the participants might be ascribed to the preferential adjustment for maintaining proper circulatory fluid volume and contribute to lower specificity observed in the conventional signs and symptoms. The mechanism of developing chronic hypertonicity and the underlying biological responses remain unclear and the hypothesis will require animal studies using chronic dehydration model and longitudinal examination to clarify the underlying physiology.

Physiological relationship between serum osmolality and the skin taurine concentration remains to be clarified. One possibility may be the 'skin sodium accumulation' hypothesis that there is a sodium pool in dermal glycosaminoglycan, with which the systemic sodium is regulated by a compensatory salt and fluid homeostasis mechanism. If there is an osmotic gradient due to skin sodium accumulation corresponding to the intercellular hyperosmolality, keratinocytes and dermal fibroblasts should be chronically exposed to high osmotic stimulus and need organic osmolytes like taurine to maintain cell functions. However, the actual sodium accumulation in skin or the osmotic stimulation related to skin sodium pool were not verified in this study. These hypotheses should be investigated in future studies with a chronically-developed dehydration model. There is another possibility that the ninhydrin colourimetric reaction observed in this study was originated from ninhydrin-reactive metabolic products other than taurine, which could have been generated by anti-oxidant mechanisms. To further evaluate the possible biological interactions, future experiments should implement metabolomic techniques.

There are some limitations about the new skin blotting method. The most important limitation is that the newly developed measurement for dehydration identification was derived from serum osmolality. The taurine absorbance detected by the skin blotting method was efficiently correlated with serum osmolality and taurine involvement in the state of body fluid osmolality and/or sodium concentration in the skin is valid. Therefore, the measurement is not a diagnostic method but a discriminative tool for identifying body fluid hyperosmolality including dehydration. In that sense, the specificity of the skin blotting method could be overestimated to some extent. Next, the cutoff point of serum osmolality for identifying dehydration in the community-dwelling older people was set at 291 mOsm/L in this study. The influence of hyperosmolality and chronically-developing

dehydration on elderly health conditions are left to future studies. Therefore, the threshold of serum osmolality needs to be reconsidered based on pathophysiology and healthcare strategies. Third, in consideration of various taurine properties *in vivo*, it is likely that taurine has different functions throughout life and the concentration in skin can be altered for age. A larger population study will be needed to clarify the generalizability of the method and how it is influenced by possible health conditions of older adults such as glycaemia, chronic inflammatory diseases, malfunctioning of skin lymphatic system, deficit of glycosaminoglycan metabolism, dietary sodium intake, and acute diseases. At the last, to implement the measure to clinical practice in home care settings, further adjustment for portable apparatus is essential. Better wavelengths and chemical reaction conditions for taurine absorbance measurement may be identified when attaining miniaturization.

Medical intervention provided by home-visit nurses is expected to change by detecting dehydration with the new skin blotting method. The dehydration identified by the method may not always require emergent medical treatments, but rather need precautionary care to avoid advanced dehydration because chronically-developed dehydration will progress and revert into acute life-threatening conditions in older people. The amount of water intake in the participants differed between the Nursing Care Levels in this study and that indicates hydration status was unevenly different between the care levels. The amount of the fluid and its administration frequency should by modified according to individual body composition and activity level. Further, treatment of chronic dehydration should be performed intermittent use of isotonic fluid containing optimal dose of glucose and ex-post monitoring. Based on the skin sodium hypothesis, the sodium storage is likely to be quite temporary, therefore, the new skin blotting method may be a possible monitoring tool for dehydration care. From a long-term perspective, treatment should include an individual fluid intake regimen and physical exercise to maintain muscle mass, which may expand the potential for the body fluid maintenance against irregular and dysregulated water loss.

In conclusion, this is the first study to elucidate the hydration states in community-dwelling vulnerable elderly individuals in Japan. There was a high prevalence rate of dehydration, reaching 66.7 % when impending dehydration was included. However, the bulk of the blood data and physical signs which are conventionally used to detect dehydration were not correlated with the dehydrated status. Therefore, dehydration in this population could have been developing insidiously and chronically. The new skin blotting method was developed in this study, and its reliability, validity and discriminative accuracy were verified. This method is useful to find chronically presented, impending or current dehydration in community-dwelling elderly individuals in a non-invasive manner. This newly developed measurement will contribute to the early detection and intervention of dehydration and enhance the autonomous daily living of the older people.