《Original article》

Estimation of salt intake based on Na/K ratio in overnight urine sample

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Abstract

Background: A method for accurate estimation of daily salt intake using spot urine sample has not yet been established.

Methods: We used data from 33 subjects who participated in our previous studies ingesting salt-adjusted formula diet. The subjects were healthy adults (9 males and 24 females; 21–58 years old). A new equation for estimating the previous day's salt intake by overnight urine sample was developed using a general linear model. Estimated values of salt intake by a newly developed equation were compared with Tanaka equation.

Results: A regression equation was calculated to estimate the previous day's salt intake using overnight urinary Na/K ratio and conductivity. The developed equation (best model equation) was "salt intake (g) = 0.43 × Na/K ratio + 0.07 × conductivity (mS/cm) – 1.04 × sex (male = 0, female = 1) + 7.52". The distribution of actual dietary salt intake was related more closely to estimated salt intake by our formula than that by Tanaka formula. The percentages of the error within ± 30% of the actual dietary salt intake were 74.5% by our equation and 62.7% by Tanaka equation, respectively.

Conclusion: A new equation developed in the present study is accurate satisfactorily to estimate for salt intake, compared with Tanaka equation. Our equation based on the Na/K ratio using overnight spot urine could be used to estimate individual daily salt intake.

Keywords: Na/K ratio, urinary sodium excretion, overnight urine, salt intake, estimation formula

Introduction

Sodium (Na) is one of the essential nutrients for the human body. However, excess sodium intake is closely associated with hypertension and the development of cardiovascular disease[1–4]. In addition, salt restriction has also been reported to be associated with decreased blood pressure and a reduced risk of cardiovascular disease[1,5,6]. Therefore, the World Health Organization (WHO) recommends that sodium intake be less than 2 g / day (equivalent to 5 g of salt / day) to reduce the risk of hypertension and cardiovascular diseases[7]. Nevertheless, sodium intake of most of people worldwide have reported to exceed WHO recommendations[8–12]. In addition, many patients are necessary to restrict salt to less than 6 g / day for treatments of hypertension, arteriosclerosis, and renal dysfunction[13–15].

Accurate estimation of a patient's salt intake is essential to provide appropriate guidance for salt restriction and improve the individual's adherence

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to appropriate dietary habit[16,17]. A number of methods for measuring dietary salt intake are currently available, including dietary surveys and urinary assessment of sodium. However, low correlations and differences were observed between 24-hour dietary recall method and 24-hour urine collection method[18]. Furthermore, these differences in the estimation results appear to depend on the characteristics of the subjects and reporting accuracy[19]. Although 24-hour urine collection method is likely to be the most accurate method for evaluating salt intake, collection of 24-hour urine involves considerable burden for participants. Also, there is no method ensuring complete urine collection[20]. On the other hand, there are several formulas that can be used to estimate the salt intake using spot urine, such as Tanaka, INTERSALT and Kawasaki equations[21]. However, these equations require determination of urinary creatinine (Cr) concentrations, the methods are cumbersome to calculate estimated values[21–27]. Furthermore, these estimation formulas are not suitable for evaluating individual salt intake, which were developed for use in populations in epidemiological studies.

Against this background, although a simple method that could be used to evaluate salt intake for individual patients would be extremely useful, a practical method has yet to be established. Therefore, in the present study, we aimed to develop a new equation to estimate the previous day's salt intake by analyzing overnight urine, and subsequently evaluated the estimation accuracy of this new equation, compared with Tanaka equation.

Methods

We conducted three studies investigating the association between salt intake and urinary Na and K excretion from 2018 to 2019. We developed a new equation using the data collected from these 3 experiments.

Subjects

Thirty-three adults of a healthy and standard physique (male 9, female 24, age 21–58 years old) participated in this study (**Table 1**). A detailed description of the study was provided by a researcher and the subjects consented to participate in the study. This study protocol was approved by the research ethics committee of Nagoya University of Arts and Sciences.

Salt formula diet

In all experimental periods, the salt intake of participants was managed precisely using a formula diet. Dietary salt was managed at a range of 5–15 g in formula diet, which was provided for 3 days or 10 days. When subjects ate additional foods other than formula diet or did not eat whole amount of formula diet, they were requested to record variety and quantity of ingested food. Nutrient intakes were calculated precisely by checking the nutritional labeling from the Standard Tables of Food Composition in Japan - 2015 - (Seventh Revised Version) (Ishiyaku Publishers, Inc., Tokyo)[28] or from nutritional labelling of food packaging. Beverage containing no salt was freely available regardless of kind or amount.

Table 1. Characteristics of study participants

	Overall (N=33)	Male (N=9)	Female (N=24)	
	Mean ± SD	Mean ± SD	Mean ± SD	p.value
Age. years	30 ± 12	37 ± 13	27 ± 10	0.026
Hight. cm	160.9 ± 6.0	167.6 ± 3.1	158.4 ± 4.8	<0.001
Body Weight. kg	55.2 ± 9.3	63.0 ± 13.2	52.3 ± 5.1	0.002
BMI. kg/m^2	21.3 ± 2.9	22.4 ± 4.7	20.8 ± 1.8	0.156

SD (Standard Deviation), BMI (Body mass index).
p.value : Male vs Female

Measurement of urine components

Over-night urine samples were collected each morning and the components excreted in the urine were measured daily. Concentrations of sodium, potassium, creatinine and sodium/potassium ratio (Na/K ratio) in the collected urine samples were measured at the Nagoya Medical Cooperative Association Nagoya Clinical Laboratory Center. Specific gravity was measured using a PAL-09S (ATAGO; Tokyo). Electrical conductivity (conductivity) were measured using a general purpose salt meter LAQUAtwin <EC-33B> (HORIBA; Kyoto). In addition, urine weight was also measured.

Statistical analysis

The variables used for analysis were the previous day's salt intake, Na/K ratio, concentrations of Na, K and Cr, conductivity, specific gravity, urine weight, height, body weight, body mass index (BMI), age, and sex. Data were expressed as mean ± standard deviation (SD), with statistical differences between the two groups determined using Student's t-test. The association between each variable was determined using Pearson's product-moment correlation coefficient. Using the previous day's salt intake as the target variable, an equation for estimating the salt intake was developed using a general linear model. Estimations of the previous day's salt intake were calculated using both our equation and Tanaka's equation[21]. The relationship between the actual measured value of the salt intake and the estimated value by the equation was determined using Pearson's product-moment correlation coefficient.

The significance level was $P < 0.05$, with EZR Ver.1.40[29] used for the analysis.

Results

Urine measurements

Table 2 shows the urine measurements for the samples. The Na/K ratio was 4.3 ± 2.6 overall, 3.4 ± 1.6 for males, and 4.5 ± 2.8 for females. The creatinine concentration was 131 ± 86 mg / dL overall, 173 ± 81 mg / dL for males, and 120 ± 84 mg / dL for females ($P < 0.001$). The average Na/K ratio and creatinine in urine in males was higher than those in females significantly ($P< 0.001$). Conductivity was 17.5 ± 6.5 mS / cm overall, 17.2 ± 5.9 mS / cm for males, and 17.6 ± 6.6 mS / cm for females.

Equation for estimating the previous day's salt intake based on overnight urine

Pearson's product-moment correlation coefficient was calculated for the previous day's salt intake and variables measured from overnight urine samples (Na/K ratio, conductivity, specific gravity, urine weight, height, body weight, and age). Results are shown as a correlation matrix between each of the variables (**Table 3**). The previous day's salt intake

Table 2. Urine measurements

	Overall (N=747)	Ma1519)	Female (N=596)	
	Mean ± SD	Mean ± SD	Mean ± SD	p.value
Urine measurements				
Na/K ratio. mmol/mmol	4.3 ± 2.6	3.4 ± 1.6	4.5 ± 2.8	<0.001
Na. mmol/L	102 ± 53	100 ± 44	103 ± 55	0.536
K. mmol/L	29 ± 16	33 ± 16	28 ± 16	0.002
Cr. mg/dL	131 ± 86	173 ± 81	120 ± 84	<0.001
Conductivity. mS/cm	17.5 ± 6.5	17.2 ± 5.9	17.6 ± 6.6	0.508
Specific gravity	1.020 ± 0.01	1.020 ± 0.01	1.020 ± 0.01	0.003
Urine weight. g	276 ± 138	313 ± 181	267 ± 124	<0.001

SD (Standard Deviation), BMI (Body mass index).
p.value : Male vs Female

Table 3. Correlation matrix between each variables.

	Age	Body Weight	Previous day's salt intake	Height	Conductivity	Na/K ratio	Specific gravity	Urine Weight
Age		0.4460 ***	0.0293	0.2418 ***	–0.1356 ***	0.1133 **	–0.0855 *	0.0402
Body weight	0.4460 ***		0.0931 *	0.5171 ***	0.1248 **	–0.0040	0.1945 ***	–0.1518 ***
Previous day's salt intake	0.0293	0.0931 *		0.0115	0.2091 ***	0.3921 ***	–0.0066	0.063
Height	0.2418 ***	0.5171 ***	0.0115		–0.0350	–0.0212	0.0865 *	0.1131 **
Conductivity	–0.1356 ***	0.1248 **	0.2091 ***	–0.0350		0.0863 *	0.7854 ***	–0.5615 ***
Na/K ratio	0.1133 **	–0.0040	0.3921 ***	–0.0212	0.0863 *		–0.1735 ***	0.2224 ***
Specific gravity	–0.0855 *	0.1945 ***	–0.0066	0.0865 *	0.7854 ***	–0.1735 ***		–0.7117 ***
Urine Weight	0.0402	–0.1518 ***	0.0630	0.1131 **	–0.5615 ***	0.2224 ***	–0.7117 ***	

*** : P<0.001, ** : P<0.01, * : P<0.05 (Pearson's product-moment correlation coefficient)

exhibited a significantly positive correlation with Na/K ratio, conductivity and body weight (r=0.392, P < 0.001, and r=0.209, P < 0.001, and r=0.093, P < 0.05, respectively). Thus, analysis was performed using these three variables and sex. Utilizing a general linear model, we determined an equation for estimating the previous day's salt intake based on the overnight urine (**Table 4**). Results of the analysis showed that Model 4 using the Na/K ratio, conductivity, and sex as explanatory variables was the optimal model. The developed equation (Model 4 equation) is shown below:

The previous day's salt intake (g)
= 0.43 × Na/K ratio + 0.07×conductivity (mS/cm)
– 1.04 × sex (male = 0, female = 1) + 7.52.

Relationship between actual dietary salt intake and estimated value using our new equation

Table 5 presents actual salt intake and the estimated values using our equation developed in the present study (Model 4 equation) or Tanaka equation. The mean ± SD of the actual salt intake, our equation estimates, and Tanaka equation estimates were 9.85 ± 2.68 g, 9.75 ± 1.24 g, and 7.64 ± 1.63 g, respectively. The medians (Interquartile range) were 9.80 (8.10–11.90) g, 9.60 (8.86–10.49) g, and 7.50 (6.50–8.60) g, respectively. The Pearson's product-moment correlation coefficient between the actual and estimated salt intake was r = 0.456 (P < 0.001) for our equation estimates and r = 0.406 (P < 0.001) for Tanaka equation estimates. Both estimated values showed a significantly positive correlation with actual sodium intake. The percentages of the error within ± 30% of actual sodium intake were 74.5% for our

Table 4. Regression formula to predict Previous day's salt intake from Overnight urinary Na/K ratio.

	Model 1	Model 2	Model 3	Model 4
(Inrercept)	8.03 ***	6.19 ***	7.32 ***	7.52 ***
	(0.19)	(0.72)	(0.86)	(0.33)
Na/K ratio	0.42 ***	0.42 ***	0.43 ***	0.43 ***
	(0.04)	(0.04)	(0.04)	(0.04)
Body Weight		0.03 **	0.00	
		(0.01)	(0.01)	
Conductivity			0.07 ***	0.07 ***
			(0.01)	(0.01)
Sex			–1.01 ***	–1.04 ***
			(0.25)	(0.23)
AIC	3198	3193	3154	3152
Sample	686	686	685	685
R^2	0.20	0.16	0.20	0.21

The value was shown by the estimated regression coefficient (standard error).
*** : P<0.001, ** : P<0.01, * : P<0.05
Analyzed using general liner model.

Table 5. Actual measured value of salt intake and estimated value by regression equation.

	Mean	SD	Percentile					Sample
			0%	25%	50%	75%	100%	
Actual measurement. g/day	9.85	2.68	4.19	8.10	9.80	11.90	18.62	708
Estimate (Model 4). g/day	9.75	1.24	7.01	8.86	9.60	10.49	15.86	698
Estimate (Tanaka). g/day	7.64	1.63	2.40	6.50	7.50	8.60	13.80	687

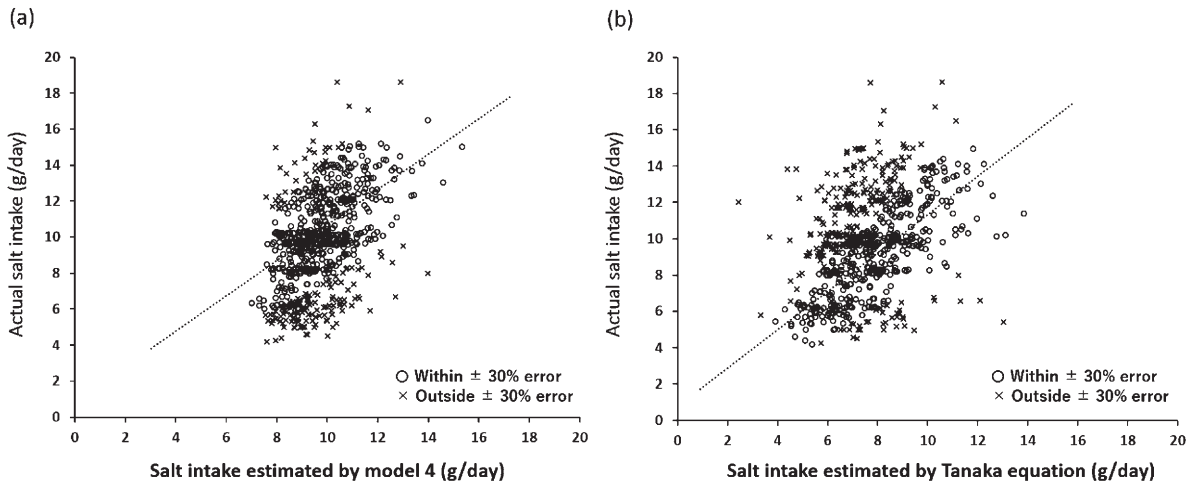

Figure 1. Association of actual salt intake and estimated previous day's salt intake.

a : Model 4
(Previous day's salt intake = 0.43 × Na/K ratio + 0.07 × Conductivity (mS/cm) – 1.04 × Sex (0, 1) + 7.52)
b : Tanaka equation
(Salt intake = (21.98 × Na concentration/Cr concentration/10 × Estimate 24-h urinary Cr excretion)$^{0.392}$/17)

equation estimates and 62.7% for Tanaka equation estimates (**Figure 1**). Furthermore, the percentages of error within ±1.0 g from actual sodium intake were 31.6% for our equation and 23.0% for Tanaka equation.

Discussion

Measurements of 24-hour urinary sodium excretion are useful for assessment of dietary salt intake, but 24-hour urine collection may be difficult and cost significant participant burden. As a result, several equations were developed to estimate 24-hour sodium excretion using spot urine samples. Nevertheless, most of spot urine equations, such as Tanaka, INTERSALT, and Kawasaki formulas, are in principle designed to estimate the average population intake. At the individual level, the precision and accuracy of these equations is inadequate. In the present study, we aimed to develop a simpler equation for estimation for individual intake. At first, we measured the concentrations of sodium, potassium and creatinine, Na/K ratio, electrical conductivity, specific gravity, and urine weight using overnight urine, in healthy adults who ingested a salt-adjusted diet. We calculated Pearson's product-moment correlation coefficient for the previous day's salt intake through salt-adjusted diet and variables measured from overnight urine samples. Then, we developed a best fitted model equation using only three factors (urinary Na/K ratio, conductivity and sex) to estimate the previous day's salt intake.

A new equation is accurate satisfactorily to estimate for daily dietary salt intake, compared with Tanaka formula[21]. Tanaka formula has been reported to be useful for estimation of the 24-hour urinary sodium excretion (mmol/day) from the sodium concentration in spot urine, and used in various studies as a method for estimating the salt intake in a population[25,30–32]. We compared the estimated salt intake obtained by our equation and actual dietary sodium intake values, showing a significantly positive correlation. The percentages of error by our formula within ± 30% and within ± 1 g from the actual value were 74.5% and 31.6%, respectively, which were better than those by Tanaka formula.

The mean ± SD of the actual salt intake values, our equation and Tanaka equation estimate were 9.85 ± 2.68 g, 9.75 ± 1.24 g, and 7.64 ± 1.63 g, respectively. Also, medians (Interquartile range) were 9.80 (8.10–11.90) g, 9.60 (8.86–10.49) g, and 7.50 (6.50–8.60) g, respectively. Since the mean and distribution of the estimated values using our equa-

tion were close to the actual salt intake values, this suggests that the population mean can potentially be accurately estimated. However, the values using Tanaka equation underestimated the actual values. In previous studies, there have been some reports of errors when using Tanaka equation to estimate salt intake. Toft et al.[25] developed an equation (Danish prediction model) that could be used for estimating the 24-hour urinary Na excretion from spot urine in 473 Danish people, and then compared the actual values with the estimated values as determined by the equation. Their results showed that both Tanaka and Danish equations underestimated the values when there was an extremely high Na excretion of 300 mmol / day (17.6 g of salt) or more. Cogswell et al.[33] compared the estimated values of the 24-hour Na excretion as determined by multiple equations (INTERSALT, Tanaka, Kawasaki and Mage)[21–23,26,27] with the actual values that were obtained from a 24-hour urine collection, and found that all of these equations underestimated or overestimated both the low and high ranges of the actual values. In a further study that compared actual values with the estimated values of the 24-hour Na excretion, as determined by Tanaka and Kawasaki's equations, it was reported that the assessment of an individual's salt intake was only accurate when the actual value was close to the population mean[34]. Our regression estimates were very close to the actual values in the 25–75th percentile range. However, low salt intake (below the 25th percentile) were overestimated, while high salt intake (over the 75th percentile) was underestimated. These results indicate that while the regression estimates of the salt intake are accurate within the range that is close to the population mean, estimation errors can occur if the measured values are very small or large. Thus, when using these types of equations, the existence of such estimation errors should be carefully considered.

Both our equation estimates and Tanaka equation estimates showed significantly positive correlations with actual salt intake, and the correlation coefficients were comparable to those reported by Cogswell et al.[33]. For the estimations of the salt intake for individuals, the percentages of errors within ± 30% for estimates by our equation and Tanaka equation were 74.5% and 62.7%, respectively. In addition, the error rates within ± 1 g of the actual value by our equation and Tanaka equation were 31.6% and 23.0%, respectively. In a study by Zhou et al. of 141 Chinese subjects (aged 18–65 years old), the proportions of relative differences percentages, using the measured 24-h urinary sodium excretion as the reference, within ± 30% or ± 1 g were 57.4% and 17.7% for Kawasaki equation, 41.8% and 14.9% for INTERSALT equation, and 64.5% and 14.9% for Tanaka equation[35]. In a similar study by Dougher et al., who evaluated 129 patients with chronic kidney disease, the proportions of relative differences percentages within ± 30%, calculated using the INTERSALT, Tanaka, Nerbass, and Kawasaki equations, were 57%, 56%, 54%, and 50%, respectively[36]. Furthermore, our previous study, which estimated the salt intake using the overnight urine Na/K ratio, the proportions of relative differences percentages within ± 1 g from the actual values was 8.6%[37]. In other words, the present study indicates that a new equation we developed was more accurate than other commonly used equations, which is attributable to differences in the variables used in the equation and the suitability of the equation for the target population.

In the present study, we used the Na/K ratio and conductivity as the main indicators of the urinary Na. Electrical conductivity was determined by general purpose salt meter, so we can get the Na/K ratio and electric conductivity simultaneously. The Na/K ratio is an index that is independent of the amount of urine, the body weight of the subject, and the amount of creatinine excreted[38]. Therefore, this ratio is assumed to be less affected by these factors and fluctuations in Na excretion. Furthermore, since the conductivity reflects the electrolyte concentration of the sample, it can also be used as an index that reflects Na levels in the urine, similar to the Na/K ratio. Some of the estimation models, such as Kawasaki equation[22], were developed with sex differences taken into ac-

count[22–25]. Furthermore, previous studies have demonstrated that sex differences present in the urine measurements[18,39], so sex may affect urine measurements. In our present study, we used these variables to develop an equation that accounted for both the urine measurement values and the characteristics of the subjects, thereby helping to ensure a good estimation accuracy. In addition, we used the same population to develop the equation and to confirm the accuracy of the equation estimation. However, in other reports that have examined the accuracy of equation estimates, the characteristics of the equation development group and the accuracy confirmation group did not always match[35,36]. Toft et al.[25] reported that estimations of salt intake in the Caucasian population using a model that was uniquely developed from the Danish population were more accurate than those using Tanaka equation, which was developed from the Japanese population. In other words, these findings argue the importance of using a population that matches the characteristics of the target population when developing the equation.

The results of the present study suggest that our equation may have a higher estimation accuracy compared to currently used methods to estimate salt intake. However, there were some limitations in the present study. First, most of the participants in the present study were young adults. Thus, in order to accurately estimate salt intake in Japanese adults, data from middle-age and elderly people, who have hypertension, cardiovascular diseases and chronic kidney disease (CKD). Further study is necessary. In addition, although the current equation was developed from a total of 685 data samples, there were several consecutive samples that were collected from the same participants in part. Therefore, the estimation accuracy in external groups remains unclear. Uechi et al.[40] divided 470 participants into a "development group" and a "confirmation group" in consideration of both age and sex, and developed an equation for estimating salt intake from spot urine in order to evaluate estimation accuracy. Thus, further testing of our current equation will be needed to evaluate its estimation accuracy in an external population. Furthermore, the distribution range of the estimated values for our equation was narrower than that of Tanaka equation. Therefore, it will be necessary to extend the estimation range by incorporating into this equation the urine measurement value data for extreme low or high salt intake, such as 3 g / day or 20 g / day.

Previous studies have suggested that daily salt intake can be estimated with a high accuracy using the Na/K ratio of spot urine. Moreover, evaluations performed by monitoring the urinary Na/K ratio are less of a burden on subjects, and additionally would be useful when evaluating individual salt intake and the effect of salt restriction[41]. Furthermore, the advantage of using the Na/K ratio instead of urinary Na and creatinine is that our measurements are quicker and simpler to perform. Recently, it has become possible to easily measure the concentrations of urinary Na and K due to the development and availability of smaller instruments capable of performing rapid measurements[42]. When combined with our new equation, use of the Na/K ratio should make it possible to perform continuous assessments of an individual's daily salt intake.

Conclusion

A new equation was newly developed for estimating salt intake based on the Na/K ratio, conductivity and sex using overnight urine, and the accuracy of this equation was subsequently evaluated. Our developed equation was able to estimate salt intake with a high accuracy at both a population and an individual level, and measurement procedure is simple, using general purpose salt meter. Therefore, these findings suggest that daily salt intake can potentially be individually evaluated using our equation. Further studies are necessary to examine the estimation accuracy of our equation in different populations or individuals.

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contend with undergoing a long-term strict dietary intake and daily urine collection.

Conflicts of interest

This study was conducted in collaboration with LIXIL Corporation. HA, NI, and TS are employees of LIXIL Corporation. All other authors declare that they have no conflicts of interest.

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《原著》

第1尿ナトリウム / カリウム比を用いた食塩摂取量の推定

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要旨

背景：個人の日々の食塩摂取量を正確かつ簡便に推定できる方法は、未だ確立されていない。
方法：食塩摂取量と尿中ナトリウム（Na）/ カリウム（K）比の関連をみた先行研究に参加した33名の被験者から共通して得られたデータを用いて、食塩摂取量を推定する回帰式を一般線形モデルで作成した。対象は成人男性 9 名、成人女性24名で、年齢は30 ± 12歳であった。作成した推定式および既存の推定式（Tanaka の式）を用いて前日食塩摂取量の推定値をそれぞれ算出し、比較した。
結果：第 1 尿から前日食塩摂取量を推定する回帰式を作成した。その回帰式は、前日食塩摂取量 = 0.43 × Na/K 比 + 0.07 × 導電率（mS/cm）− 1.04 × 性別（男 = 0 , 女 = 1 ）+ 7.52となった。前日食塩摂取量の分布は Tanaka 式で算出した推定値の分布よりも本研究で作成した式で算出した推定値の分布の方が似ていた。作成した推定式による推定値と Tanaka 式による推定値の実測値30％以内の誤差の割合は、それぞれ74.5％および62.7％であった。
結論：本研究で作成した推定式は、既存の回帰式より高い精度で推定できた。第 1 尿 Na/K 比を用いて個人の食塩摂取量を簡便に推定できる可能性が示唆された。

キーワード：Na/K 比、尿中ナトリウム排泄量、夜間尿、食塩摂取量、推定式

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