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ORIGINAL ARTICLE

Diurnal variation of urinary sodium-to-potassium ratio in free-living Japanese individuals

Toshiyuki Iwahori[1,2], Hirotsugu Ueshima[2,3], Sayuki Torii[2], Yoshino Saito[2,4], Keiko Kondo[2], Sachiko Tanaka-Mizuno[5], Hisatomi Arima[6] and Katsuyuki Miura[2,3]

High sodium-to-potassium ratios are associated with elevated blood pressure levels and an increased risk of cardiovascular diseases. We aimed to determine whether urinary sodium-to-potassium ratios fluctuate diurnally during the day to understand measured values of casual urinary sodium-to-potassium ratios. A total of 13,277 casual urine specimens were collected under free-living conditions from 122 Japanese normotensive and hypertensive individuals. Participants collected all casual urine samples in aliquot tubes, reported urine volumes and the time at each voiding for 10–22 days. Then, specimens were classified into hourly data. Diurnal patterns of urinary sodium-to-potassium ratios and urinary concentrations of sodium and potassium were evaluated. Overall mean values of hourly urinary sodium-to-potassium ratios were highest (4.1–5.0) in the early morning, lower (3.3–3.8) in the daytime and higher (4.0–4.4) toward evening hours. The mean urinary sodium and potassium concentrations were the lowest (90–110 and 24–32 mmol l^{-1}, respectively) during the early morning and higher (110–140 and 35–43 mmol l^{-1}, respectively) after mid-morning. Diurnal variability of potassium concentrations was larger than for sodium concentrations. Diurnal variations in urinary sodium-to-potassium ratios were comparable between normotensive and hypertensive individuals, between hypertensive individuals with and without antihypertensive medications, and among age and gender-specific subgroups. Overall mean hourly urinary sodium-to-potassium ratios fluctuated diurnally under free-living conditions and were higher during the morning and evening and lower during the daytime compared with 24-h urinary sodium-to-potassium ratios. Diurnal variation in urinary sodium-to-potassium ratios should be considered to understand actual daily dietary levels and avoid over- and under-estimation in clinical practice.
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Keywords: casual urine; circadian rhythm; diurnal variation; sodium potassium; sodium-to-potassium ratio

INTRODUCTION

Sodium-to-potassium (Na/K) ratios in 24-h urine are known to correlate well with blood pressure (BP) in epidemiological studies,[1–14] and it has been reported to be a superior metric to either sodium (Na) or potassium (K) alone in relation to BP outcomes and cardiovascular disease (CVD) risks.[1–4,12] Errors due to under- or over-collection of 24-h urine specimens are lower for Na/K ratios compared with Na or K alone because the ratio is independent of urine volume. Methods for estimating 24-h urine Na and K excretions from casual urine specimens are dependent on urinary creatinine measurements, which may degrade if specimens are not well temperature-controlled;[15–20] however, the ratio is independent of creatinine measurements. The measurement of urinary Na/K ratios could provide both onsite prompt feedback by portable handy devices[21] and good individual and population estimates of 24-h urinary Na/K ratios by using casual urine.[22–24] The accuracy of these estimates depends on the timing of casual urine collection.[23,24] Casual urinary Na/K ratios usually fluctuate[23–25] and are likely to reflect recent dietary habits, such as increases in the ratio after ingesting food with a high Na content and decreases in the ratio when such foods are avoided.[26] Urinary Na/K ratios are higher in the first void after arising and in the last void before bedtime but lower in a second void after arising compared with 24-h urine values.[23,24] However, the overall trend remains unknown. Previous studies have examined fluctuations in Na and K excretion by means of experimental trials in isolated unrealistic circumstances,[27–29] chronobiological assessments,[30,31] protocols requiring compulsory urine sampling at specific intervals,[29,31–36] or experiments requiring urine withdrawal.[37–39] However, the findings of such studies are not easy to translate into clinical values in terms of obtaining insight into daily dietary behavior. Therefore, the primary aim of this study was to determine the amplitude and timing of the fluctuations observed in the mean hourly values of urinary Na/K ratios to clarify whether diurnal variations in urinary Na/K ratios could be observed even

[1]Department of Research and Development, OMRON Healthcare Co., Ltd., Muko, Japan; [2]Department of Public Health, Shiga University of Medical Science, Otsu, Japan; [3]Center for Epidemiologic Research in Asia, Shiga University of Medical Science, Otsu, Japan; [4]Department of Nursing, Aino University, Ibaraki, Japan; [5]Department of Medical Statistics, Shiga University of Medical Science, Otsu, Japan and [6]Department of Preventive Medicine and Public Health, Fukuoka University, Fukuoka, Japan
Correspondence: Dr T Iwahori, Department of Public Health, Shiga University of Medical Science, Tsukinowa-cho Seta, Shiga, Otsu 520 2192, Japan.
E-mail: iwahori@belle.shiga-med.ac.jp
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under free-living conditions. The secondary aim was to clarify how the fluctuations of urinary Na/K ratios can be translated into daily clinical practice.

METHODS

Study design

This study was a cross-sectional study.

Participants and measurements

A total of 122 participants (age 25–69 years; male, $n=64$; female, $n=58$; normotensive, $n=45$; hypertensive with and without anti-hypertensive medication, $n=46$ and $n=31$, respectively) were recruited from the general population by advertisements. Participants were instructed to collect all urine samples under free-living conditions for 10–22 days, unless urine collection was unsuccessful or contaminated by feces. If participants declared that they failed to complete urine collection, we asked them to retry urine collection. In detail, participants were asked to record the volume (ml) of each casual urine sample and the time of collection. Urine samples were first collected in a standardized measuring cup for urine volume measurement, and then some of the urine was transferred into an aliquot tube (10 ml) at each voiding for the measurement of sodium and potassium concentrations. Each void was collected in a separate aliquot tube. The number of casual urine samples obtained during the study was dependent on the voiding frequency of each individual. The voiding samples before and after the longest sleep were defined as 'void before bedtime' and 'first void after arising', respectively. All casual urine samples were sent to the central laboratory at room temperature for measurement. Urinary Na and K concentrations (mmol l^{-1}) were measured using an ion-specific electrode method, and then the Na/K molar ratio (mmol/mmol) was calculated. The excretion of Na and K was calculated from reported urine volumes. Diurnal fluctuations in urinary Na/K ratios, urinary Na and K concentrations, and volumes and excretions were assessed in 13 277 specimens. Weight was measured twice using HBF-206IT (OMRON Healthcare Co., Ltd., Kyoto, Japan), and the height was measured using a stadiometer HP-M (Tsutsumi, Tokyo). The participants did not wear shoes during these measurements. Body mass index was calculated as weight divided by height squared (kg m^{-2}). Written informed consent was obtained from all participants. The ethics committee of the Shiga University of Medical Science and Omron Healthcare Company approved the study protocol.

Statistical analysis

Urinary Na/K ratios, Na and K concentrations and excretions, and urine volume were pooled and analyzed from 00:00–23:00 hours every hour on the hour. Midnight, before dawn, early morning, mid-morning, late morning, mid-afternoon, late afternoon and late evening were defined as 0:00, 1:00–4:00, 5:00–7:00, 8:00–10:00, 11:00, 14:00–15:00, 16:00–17:00 and 22:00–23:00 hours on the hour, respectively. Participants who changed their anti-hypertensive medication were excluded from the analysis. Adjusted overall mean values of hourly urinary variables were calculated by linear mixed models using the result of each urine sample as repeat measures for each participant to control intra-individual variation and inter-individual variation. Twenty-four time slots were classified into three time zones (overnight: 0:00–7:59 hours, daytime: 8:00–15:59 hours and evening: 16:00–23:59 hours). Statistical significance among time zones in the overall 122 participant and subgroup analyses, which evaluated the effects of time zones and subgroups, were conducted using a two-way analysis of variance with the GLM procedure in SAS statistical software version 9.4 (NC, USA).

RESULTS

Table 1 shows the characteristics and urinary findings of the study participants. The mean age was 50.9 years, and normotensive individuals were younger than hypertensive individuals (38.9 *vs.* 58.3 years). The mean 24- h urinary Na/K molar ratio was 3.77, and this value was higher among normotensive than hypertensive individuals (4.21 *vs.* 3.51) and higher in men compared with women (3.88 *vs.* 3.66; Table 1 and Supplementary Table 1). The mean 24- h urinary volume was 1799.9 ml, and values were similar among normotensive and hypertensive individuals and between men and women (Table 1 and Supplementary Table 1). The mean voiding frequency was 7.11 per day, and values were similar between men and women (7.06 *vs.* 7.16 voids/day). However, voiding frequency was lower among normotensive than hypertensive individuals (6.37 *vs.* 7.54 voids/day; Table 1 and Supplementary Table 1). The mean times of the first void after arising was in the early morning (6:52 hours); second void was during mid-morning (9:57 hours); and the void before bedtime was during late evening (10:43 hours). These times were similar between men and women, whereas urine was voided earlier by hypertensive than normotensive individuals (Table 1, Supplementary Table 1 and Supplementary Figure 1). The anti-hypertensive medications administered to 14 (30%), 9 (20%), 15 (33%), 1 (1%), 3 (7%) and 4 (9%) participants comprised calcium channel blockers (CCBs), angiotensin 2 receptor blockers (ARBs), both CCBs and ARBs, both CCBs and angiotensin converting enzyme (ACE) inhibitors, both CCBs and ARBs with other drugs, and other drugs, respectively.

The overall mean value of the hourly urinary Na/K molar ratio was highest (4.1–5.0; biased ~0.6 higher than the 24-h urine) between midnight and early morning (overnight time zone), lower (3.3–3.8; biased ~0.4 lower than the 24-h urine) between mid-morning and mid-afternoon (daytime time zone), and higher (4.0–4.4; biased ~0.4 higher than the 24- h urine) between late afternoon and late evening hours (evening time zone; Figure 1a). Statistical significance was observed among time zones (overnight, daytime and evening) in participants overall ($P<0.001$). The amplitude of the fluctuations, defined as the difference between the maximum and minimum values of the overall mean hourly urinary Na/K molar ratios (mmol $mmol^{-1}$), was ~1–1.5 (Figure 1a). The overall mean hourly urinary Na and K concentrations were the lowest (90–110 and 24–32 mmol l^{-1}, respectively) between midnight and early morning (around the time of the first void after arising) and increased to 110–140 and 35–43 mmol l^{-1}, respectively, after mid-morning and persisted until late evening (after the time of second void after arising; Table 1, Figures 1b and c). The diurnal variability of Na and K concentrations, defined as the s.d. divided by the mean value during a period of 24 h was 28.8% and 38.7%, respectively. The mean urine volume was the highest (320 ml per void) during the early morning (around the time of the first void after arising) and remained essentially constant at ~230 ml per void after the mid-morning (around the time of the second void; Figure 1d). The mean Na excretion was highest (32 mmol per void) during the early morning (around the time of the first void after arising), and it remained essentially constant at ~25 mmol per void after mid-morning (around the time of the second void after arising; Figure 1e). The mean K excretion was higher (8–9 mmol per void) between late morning and late afternoon, and it remained lower (6–7 mmol per void) from late evening until before dawn (Figure 1f).

Subgroup analysis showed that diurnal fluctuations in the mean urinary Na/K ratios were comparable between normotensive and hypertensive individuals (Table 1, Figures 2a–f) and between hypertensive individuals taking and not taking antihypertensive medications (Supplementary Figures 2A–F), hypertensive individuals taking and not taking Renin–Angiotensin System (RAS) antagonists (Supplementary Figures 5A–F), men and women (Supplementary Table 1, Supplementary Figures 3A-F), and age-specific subgroups (Supplementary Table 2, Supplementary Figures 4A–R). Statistical significance was observed in urinary Na/K ratios between both time zones and subgroups, among subgroups of gender-specific subgroups

Table 1 Characteristics and urinary findings of normotensive and hypertensive study participants, 64 men and 58 women aged 25–69 years, free-living Japanese volunteers, in 2012 and 2014.

	Normotensive individuals (N = *45*)		*Hypertensive individuals with anti-hypertensive medication* (N = *43*)		*Hypertensive individuals without anti-hypertensive medication* (N = *34*)		*Overall* (N = *122*)	
Variables	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Age	38.9	10.1	61.2	7.7	54.3	8.5	50.9	13.1
Height (cm)	165.0	10.3	161.2	7.2	163.7	7.5	163.5	8.7
Weight (kg)	62.4	13.0	63.5	11.3	64.8	13.1	63.7	12.6
Body mass index (kg m^{-2})	22.8	3.4	24.3	3.5	24.0	3.7	23.7	3.5
	N (%)		*N* (%)		*N* (%)		*N* (%)	
Women (overall)	23 (51.1)		19 (44.2)		16 (47.1)		58 (47.5)	
	Mean (s.d.)	*Median*	*Mean (s.d.)*	*Median*	*Mean (s.d.)*	*Median*	*Mean (s.d.)*	*Median*
24-Hour urine volume (ml)	1797.6 (996.0)	1540.0	1746.8 (582.4)	1700.0	1870.0 (595.4)	1780.0	1799.9 (765.1)	1690.0
24-Hour Na excretion (mmol per 24 h)	183.7 (90.1)	157.7	187.7 (72.8)	177.9	209.7 (75.7)	202.9	192.4 (81.0)	182.2
24-Hour K excretion (mmol per 24 h)	45.4 (16.9)	43.3	59.3 (18.7)	59.1	58.6 (18.2)	55.0	54.0 (19.1)	52.8
Urine voiding frequency (No. of voids per day)	6.37 (2.37)	6	7.87 (2.25)	8	7.12 (1.70)	7	7.11 (2.24)	7
Time frame of urine voiding								
First void after arising	7:37 hours (1:26)	7:30 hours	6:42 hours (1:18)	6:40 hours	6:27 hours (1:20)	6:25 hours	6:52 hours (1:26)	6:50 hours
Second void after arising	11:13 hours (2:32)	10:50 hours	9:15 hours (1:56)	9:05 hours	9:24 hours (1:58)	9:30 hours	9:57 hours (2:21)	9:43 hours
Void before bedtime	23:13 hours (1:53)	23:07 hours	22:27 hours (1:06)	22:31 hours	22:35 hours (1:19)	22:50 hours	22:43 hours (1:28)	22:50 hours
Na concentration (mmol l^{-1})								
24-h urine	112.2 (44.6)	106.4	112.8 (41.0)	112.0	117.5 (42.8)	112.1	113.9 (42.9)	110.4
First void after arising	100.8 (47.8)	91.3	104.3 (46.7)	100.0	113.7 (46.7)	108.7	106.4 (47.2)	100.0
Second void after arising	121.5 (61.3)	117.4	116.0 (50.1)	113.1	123.1 (47.7)	126.1	119.7 (52.7)	117.4
Void before bedtime	124.2 (62.3)	117.4	117.4 (60.7)	113.1	133.0 (59.7)	134.8	124.1 (61.1)	121.8
K concentration (mmol l^{-1})								
24-h urine	28.9 (11.6)	26.8	36.3 (13.1)	33.8	33.6 (12.0)	31.6	32.8 (12.7)	31.1
First void after arising	26.0 (13.0)	24.2	33.4 (18.7)	30.7	27.8 (14.1)	25.6	29.7 (16.2)	25.6
Second void after arising	37.0 (20.7)	33.2	47.0 (23.7)	46.0	46.1 (24.4)	43.5	44.0 (23.5)	40.9
Void before bedtime	29.5 (16.2)	28.1	40.3 (26.5)	35.8	37.0 (19.9)	35.8	36.8 (22.7)	33.2
Na/K ratio								
24-h urine	4.21 (1.71)	3.93	3.35 (1.37)	3.09	3.72 (1.31)	3.56	3.77 (1.53)	3.52
First void after arising	4.50 (2.55)	3.86	3.92 (2.54)	3.21	4.65 (2.16)	4.20	4.30 (2.45)	3.74
Second void after arising	3.88 (2.21)	3.40	3.21 (2.54)	2.49	3.23 (1.69)	2.88	3.40 (2.23)	2.83
Void before bedtime	4.99 (2.76)	4.42	3.79 (2.37)	3.27	4.23 (2.09)	3.76	4.21 (2.42)	3.68

Abbreviations: K, potassium; Na, sodium.
Urinary findings are the means of the 7 days.

(men and women), age-specific subgroups, hypertensive and normotensive individuals, hypertensive individuals with and without medication, and individuals with and without RAS inhibitors (all $P<0.001$).

DISCUSSION

The main finding of the present study was that the mean hourly values of urinary Na/K ratios varied diurnally in individuals under free-living conditions. The amplitude of the fluctuation, defined as the difference between the maximum and minimum values of the overall mean of the hourly urinary Na/K molar ratio, was relatively large (~1–1.5). The mean hourly values of urinary Na/K ratios and 24-h urinary Na/K ratios differed systematically and depended on the time of day; that is, casual urine sampling only during one specific time frame will provide values that are biased toward being either lower (during the daytime) or higher (during the morning and evening) compared with the Na/K ratio of the 24-h urine. Thus, appropriate bias correction for time should improve the accuracy and precision of the 24-h urinary Na/K ratio derived from the casual urine Na/K ratio, for example, subtracting values of 0.4–0.6 from morning and evening casual urine Na/K ratios and adding the value 0.4 to the daytime casual urine Na/K ratio to estimate the true 24-h urine Na/K ratio. Furthermore, repeated casual urine sampling from diverse time frames

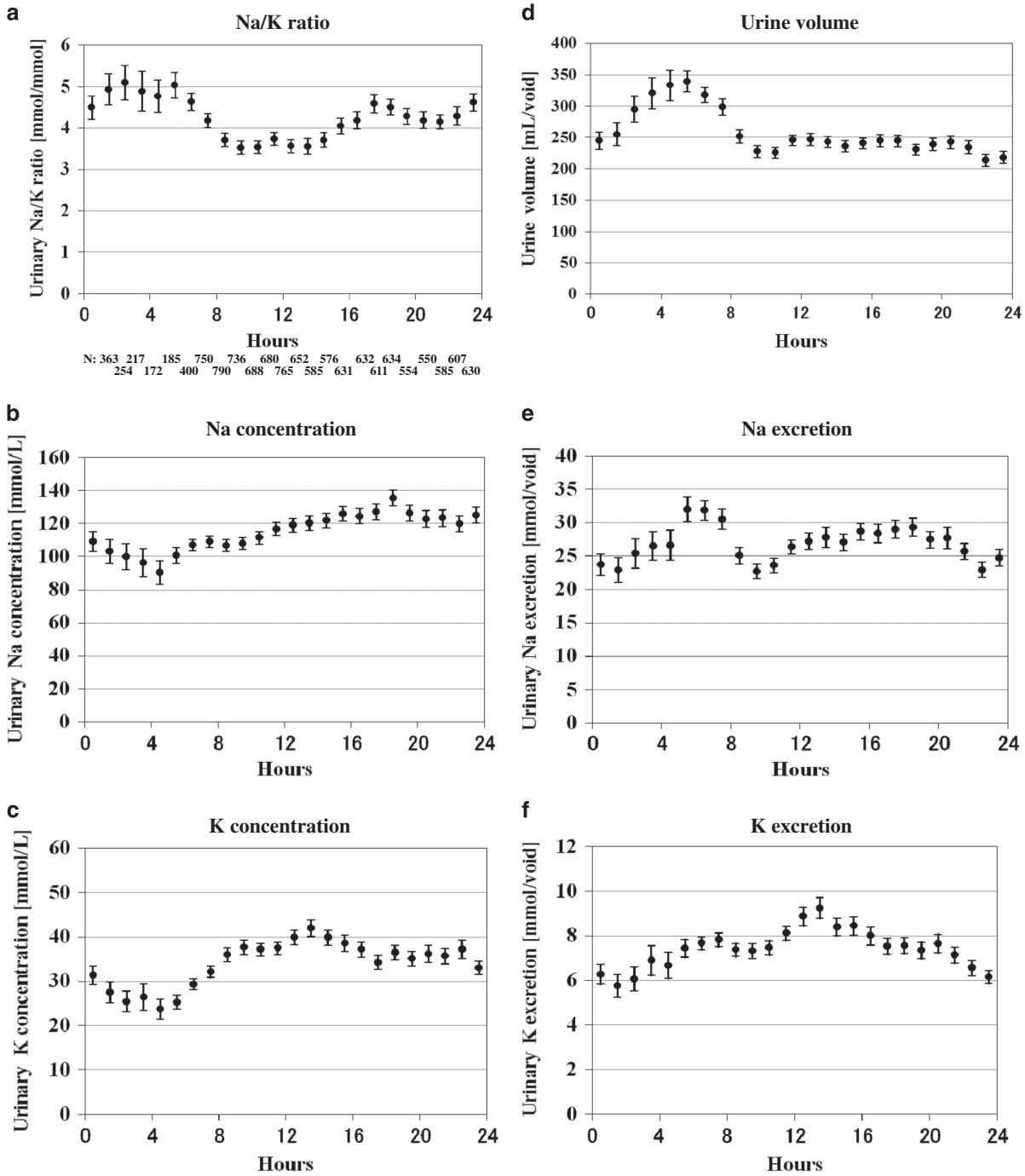

Figure 1 Overall hourly mean values of casual urinary variables (urine specimens, $N = 13\,277$); Na/K ratios were highest from midnight to early morning, lower from mid-morning to mid-afternoon and higher toward evening, and statistical significance was observed between time zones (overnight, daytime, and evening) in overall participants ($P < 0.001$) (**a**). Na and K concentrations were lowest from midnight to early morning, and higher from mid-morning to late evening (**b**, **c**). Urine volume was highest in the early morning and remained constant thereafter (**d**). Na excretion was highest in the early morning (around the time of first void after arising), and remained constant thereafter (**e**). K excretion increased between late morning and late afternoon, and decreased between late evening and before dawn (**f**). Bars indicate the 95% confidence interval of the mean.

is also known to improve the accuracy of identifying the 24-h urinary Na/K ratio,[23,24] and the present findings could explain this result by washing out values with lower and higher bias by averaging them to converge with the 24-h urinary Na/K ratio. Therefore, it can be inferred that estimation by repeated sampling of casual urinary Na/K ratios could improve its accuracy for identifying the 24-h urinary Na/K ratio with appropriate bias correction based on when they were measured.

The diurnal variations in casual urinary Na/K ratios could reflect postprandial surges, hormonal activities, or other factors. Previous studies have suggested that food intake is not an important contributor to the circadian rhythm.[33,40,41] In the present study, the variation observed in the overall mean values of hourly urinary Na/K ratios were not likely explained by postprandial surges. Thus, it can be inferred that food intake was irregularly reflected in urinary

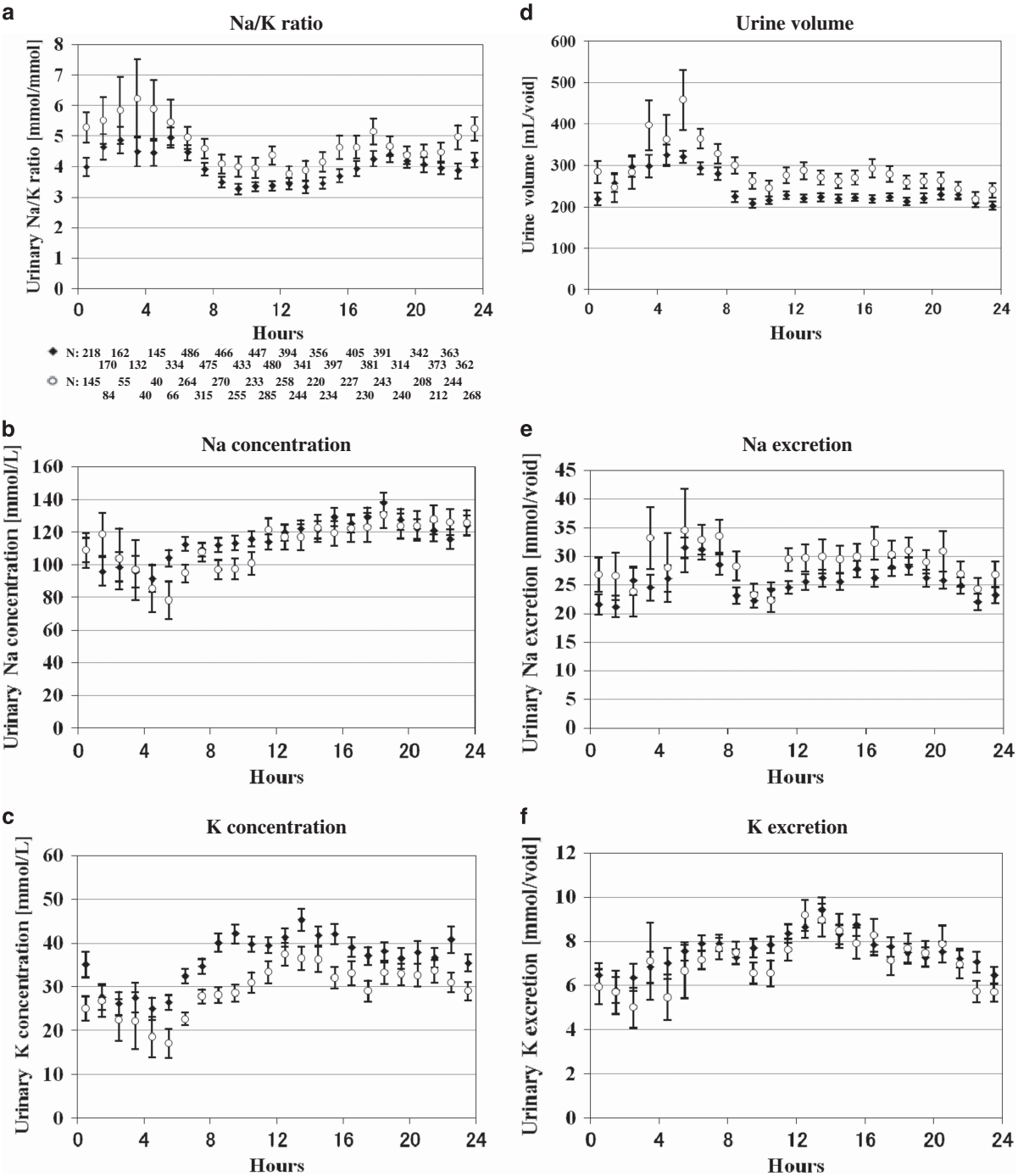

Figure 2 Overall hourly mean values of casual urinary variables in subgroups of normotensive and hypertensive individuals (urine specimens, $N=13\,277$); Characteristics of the Na/K ratio, Na and K concentrations, urine volume, Na and K excretions were similar among subgroups of normotensive (◆; $N=45$) and hypertensive (○; $N=77$) (**a–f**). Bars indicate 95% confidence interval of the mean.

Na and K excretions, and the fluctuation in overall mean hourly urinary Na/K ratios is mainly related to something other than food intake, for example, hormonal factors. One of the hormonal factors underlying this diurnal variation is renal Na and K excretion.[42–44] Renal Na and K excretion is regulated in the distal tubule under the control of hormones such as aldosterone.[42–44] High aldosterone secretion causes Na reabsorption, which induces an increase in renal K excretion and a decrease in Na excretion.[42–44] Therefore, aldosterone functions in increasing Na absorption from the urine into serum, and in K release from serum to urine.[42–44] Plasma aldosterone is subject to circadian rhythms, as it is inactive during sleeping hours and active after arising in humans and other animals.[27,45,46] Thus, the diurnal variation of Na may be explained mainly by that of aldosterone. The bladder collects urine before casual urinary excretion and induces a time lag between diurnal variations in serum and urinary Na/K ratios. Therefore, the first urine void after arising would mainly reflect inactive Na reabsorption while asleep, causing relatively increased renal Na excretion that results in the highest urinary Na/K

ratio during the early morning. Thereafter, the period of active Na reabsorption would be reflected in the urine void after mid-morning (from around the time of the second void after arising until daytime casual urine voids), causing relatively less renal Na excretion and lower Na/K ratios. Plasma aldosterone activity decreases toward nighttime and induces another increase in the urinary Na/K ratio. The reported diurnal fluctuation of urinary aldosterone is symmetrical and that of serum aldosterone is similar to fluctuations in the mean hourly urinary Na/K ratios;[34,45] however, further study is needed to clarify this issue.

Hypertensive individuals taking RAS inhibitors ($N=28$) also showed diurnal variation of Na/K ratios. This finding was reasonable because RAS inhibitors do not fully block the pathway of aldosterone production,[47] and aldosterone is also regulated by other factors such as serum K and adrenocorticotropic hormones.[47]

The limitations of this study were that we did not evaluate the diurnal variation in hormonal factors, and the sample size was limited to $N=122$. It is not known whether these findings are also applicable to individuals taking blood pressure drugs that are not predominantly used in the Japanese general population because we could not include the complete variety of blood pressure drugs that patients may use. However, it is reasonable to infer that diuretics may not affect the diurnal variation because casual urine collected mainly during the daytime showed lower Na/K ratios compared with the 24-h urinary Na/K ratios in the INTERSALT study, which was carried out in the 1980 s when diuretics were mainly used for anti-hypertensive medication.[22] In addition, blood flow into the kidneys might be variable under free-living conditions and may affect the secretion rates of urinary and hormonal factors during casual urine sample collection. It is unknown whether the findings are also applicable to hydroelectrolytic disorder patients, patients suffering acute illness, those who did not have recent medication, individuals with renal dysfunction, and children or the elderly. However, the strength of this study was that the overall mean hourly urinary variables were determined from more than 13 000 specimens collected from a general population, which might have washed out the noise and retained signals. In addition, some participants awoke on their own to urinate after sleep onset, which is a natural occurrence under free-living conditions. Thus, diverse and complex lifestyle factors such as dietary intake, hydration status, hemodynamic status, posture and acid–base balance might have been similar to the average value of the general population within each time frame. Epidemiological findings have shown consistencies in higher casual urinary Na/K ratios observed during early morning and night and lower urinary Na/K ratios during the daytime compared with 24-h urine values in worldwide diverse populations.[23,24,48–53] Reverse causality is known to affect the studies with diet as an exposure because sick individuals are likely to modify their diet toward healthier food either because of medical advice or an illness-related reduction in food consumption.[54,55] The difference observed in the level of urinary Na/K ratios between hypertensive individuals and normotensive individuals is likely explained by reverse causality; however, the diurnal fluctuations in overall mean hourly urinary Na/K ratios in subgroups of normotensive and hypertensive participants, hypertensive individuals with and without medication, and hypertensive individuals with and without RAS inhibitors were comparable in the present study. Similarly, findings observed among subgroups in gender- and age-specific individuals were comparable. Thus, the present findings might be applicable to diverse demographic backgrounds and would be useful in daily clinical practice and epidemiological studies.

In conclusion, we identified diurnal variations in the overall mean hourly urinary Na/K ratios under free-living conditions. The ratios were higher in the morning and evening but lower in the daytime compared with 24-h urine values. Diurnal variations in urinary Na/K ratios should be considered when measuring casual urine Na/K ratios under free-living conditions to avoid over- and under-estimating actual daily dietary values in clinical practice.

CONFLICT OF INTEREST

TI is an employee of OMRON Healthcare Co., Ltd. HU served as a consultant for this project. KM received a research grant from OMRON Healthcare Co., Ltd.

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Author contributions: All authors contributed to the design of the study. TI, ST and YS participated in data collection. TI participated in data analysis. TI participated in writing the report. HU, ST, KK, HA and KM participated in critical revision of the manuscript. All authors approved the final version of the report for submission.

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Supplementary Information accompanies the paper on Hypertension Research website (http://www.nature.com/hr)