Ultra-long–term human salt balance studies reveal interrelations between sodium, potassium, and chloride intake and excretion[1,2]

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ABSTRACT

Background: The intake of sodium, chloride, and potassium is considered important to healthy nutrition and cardiovascular disease risk. Estimating the intake of these electrolytes is difficult and usually predicated on urine collections, commonly for 24 h, which are considered the gold standard. We reported on data earlier for sodium but not for potassium or chloride.

Objective: We were able to test the value of 24-h urine collections in a unique, ultra-long–term balance study conducted during a simulated trip to Mars.

Design: Four healthy men were observed while ingesting 12 g salt/d, 9 g salt/d, and 6 g salt/d, while their potassium intake was maintained at 4 g/d for 105 d. Six healthy men were studied while ingesting 12 g salt/d, 9 g salt/d, and 6 g salt/d, with a re-exposure of 12 g/d, while their potassium intake was maintained at 4 g/d for 205 d. Food intake and other constituents were recorded every day for each subject. All urine output was collected daily.

Results: Long-term urine recovery rates for all 3 electrolytes were very high. Rather than the expected constant daily excretion related to daily intake, we observed remarkable daily variation in excretion, with a 7-d infradian rhythm at a relatively constant intake. We monitored 24-h aldosterone excretion in these studies and found that aldosterone appeared to be the regulator for all 3 electrolytes. We report Bland–Altman analyses on the value of urine collections to estimate intake.

Conclusions: A single 24-h urine collection cannot predict sodium, potassium, or chloride intake; thus, multiple collections are necessary. This information is important when assessing electrolyte intake in individuals. *Am J Clin Nutr* 2016;104:49–57.

Keywords: sodium, salt, chloride, potassium, aldosterone, urine, diet, electrolyte intake

INTRODUCTION

We recently reported on an ultra-long–term salt balance study at 12 g/d, 9 g/d, and 6 g/d, with a re-exposure of 12 g/d, for months in healthy young men who were tested for the predicted constancy in urinary sodium excretion (UNaV)[11] and total-body sodium content (1). At constant salt intake, daily sodium excretion exhibited aldosterone-dependent, weekly (circaseptan) rhythms, resulting in periodic sodium storage. Changes in total-body sodium (±200–400 mmol) showed longer infradian rhythms about monthly and longer period lengths without parallel changes in body weight and extracellular water that were related directly to urinary aldosterone excretion (UAldoV) and inversely to urinary cortisol. The findings suggested rhythmic hormonal control. That such rhythms would interfere with the precision of 24-h UNaV as a predictor of salt intake was apparent. Sodium intake is associated with blood pressure increases in the population but only as the chloride salt, as shown in animals and man (2–6). Chloride could play a role in elevating blood pressure (7). Serum chloride concentrations were reported to affect mortality in hypertensive patients (8). Nonetheless, chloride has received little attention, even though chloride storage in skin was described in 1909 (9). In contrast, the interrelations between sodium and potassium excretion are well documented (10). Aldosterone results in urinary sodium retention and urinary potassium excretion (UKV). The dramatic rhythmic changes we

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[2] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[11] Abbreviations used: Mars105, simulated 105 d on Mars; Mars520, simulated 205 d on Mars; UAldoV, urinary aldosterone excretion; UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

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TABLE 1
Nutrient intake during the Mars105 study (descriptive statistics)[1]

	Salt phase			
	12 g/d, 35 d	9 g/d, 35 d	12 g/d, 5 d	6 g/d, 28 d
Anthropometric data				
Age, y	34.3 ± 5.2	34.3 ± 5.2	34.3 ± 5.2	34.8 ± 4.3
BMI, kg/m^2	24.3 ± 2.6	24.1 ± 2.0	24.0 ± 1.8	23.8 ± 1.6
Nutrients				
Energy, kcal	2775 ± 251	2818 ± 180	2857 ± 216	2712 ± 311
Carbohydrates, g	371.8 ± 46.4	387.4 ± 36.6	399.7 ± 41.7	384.3 ± 50.5
Fats, g	89.7 ± 12.2	87.6 ± 8.9	85.1 ± 6.8	80.3 ± 14.2
Proteins, g	103.5 ± 11.9	101.4 ± 10.9	103.7 ± 8.9	93.4 ± 16.0
Fiber, g	29.7 ± 7.0	27.5 ± 5.8	30.4 ± 7.1	27.7 ± 6.6
Minerals, mg				
Calcium	1196.0 ± 221.3	1318.9 ± 189.7	1043.6 ± 128.9	1239.3 ± 180.3
Potassium	3942.9 ± 923.6	3977.0 ± 784.0	4061.4 ± 855.2	3959.5 ± 644.2
Sodium	4782.1 ± 324.3	3622.8 ± 213.4	4835.0 ± 149.6	2453.6 ± 312.8
Chloride	7121.1 ± 565.7	5422.5 ± 411.2	7289.6 ± 214.4	3569.0 ± 602.9

[1]Values are means ± SDs for each salt phase. n = 4 subjects. Two subjects who did not meet the inclusion compliance criteria were excluded. Mars105, simulated 105 d on Mars.

uncovered in our study similarly should be true for potassium (1). The potassium intake in our subjects was generous at 4 g/d and fixed as closely as possible at each level of salt intake in our study. Chloride excretion, which seldom is measured directly in human studies nowadays, would be expected to track very closely with sodium excretion. Presumably, humans ingest sodium almost solely as sodium chloride (11). We now have analyzed daily chloride and potassium excretion in our study and compared the results with those for UNaV and UAldoV. Our findings shed new insights into the homeostasis of these electrolytes in the long term.

METHODS

We performed 2 long-term studies in the framework of a simulated flight to Mars, conducted in Moscow, Russia, from 2009 to 2011. We presented a detailed description of the experimental approach previously (1). The study was conducted at the Institute for Biomedical Problems in Moscow. Several ethical review boards approved the studies, including the internal review board–equivalent of the Russian Federation. Written informed consent was obtained from all participants, and all studies were done as outlined by the Declaration of Helsinki.

Study design and oversight

Twelve healthy male volunteers (**Supplemental Figure 1** and additional information) lived for a simulated 105 d on Mars (Mars105) and a simulated 205 d on Mars (Mars520) in an enclosed habitat consisting of hermetically sealed interconnecting modules. Microgravity and space radiation were not simulated. Selection criteria for the volunteers for the simulated mission to Mars were equal to that of real cosmonauts. No subject had any known medical condition and none ingested medications for any reasons. Environmental factors were maintained constant and enabled a "metabolic ward" setting for this experiment. Temperature and relative degree of humidity were maintained between 18°C

TABLE 2
Nutrient intake during the Mars520 study (descriptive statistics)[1]

	Salt phase			
	12 g/d, 61 d	9 g/d, 60 d	6 g/d, 48 d	12 g/d, 36 d
Anthropometric data				
Age, y	31.5 ± 5.0	32.0 ± 5.1	32.0 ± 5.1	32.2 ± 5.0
BMI, kg/m^2	27.1 ± 2.4	26.9 ± 2.3	26.3 ± 2.2	26.2 ± 2.3
Nutrients				
Energy, kcal	2649 ± 185	2572 ± 232	2472 ± 221	2472 ± 322
Carbohydrates, g	337.8 ± 37.0	314.8 ± 36.2	290.3 ± 36.5	315.4 ± 46.8
Fats, g	96.1 ± 14.3	100.8 ± 14.9	102.1 ± 15.1	89.7 ± 19.3
Proteins, g	93.8 ± 14.7	93.4 ± 13.5	91.3 ± 10.3	88.1 ± 16.7
Fiber, g	34.5 ± 8.0	37.0 ± 6.7	36.0 ± 7.4	32.6 ± 7.8
Minerals, mg				
Calcium	1018.3 ± 143.0	1039.4 ± 136.0	1036.2 ± 146.4	970.2 ± 181.0
Potassium	4300.7 ± 788.4	4233.4 ± 650.2	4173.7 ± 562.2	3969.1 ± 844.3
Sodium	4483.4 ± 523.5	3278.7 ± 510.5	2235.7 ± 488.3	4386.4 ± 635.3
Chloride	6639.4 ± 1058.7	5006.2 ± 907.0	3338.7 ± 862.2	6505.8 ± 1220.2

[1]Values are means ± SDs for each salt phase. n = 6 subjects. Mars520, simulated 205 d on Mars.

TABLE 3
Balance accuracies in the Mars105 and Mars520 studies (descriptive statistics)[1]

Study	Value
Mars105	
Sodium recovery by UNaV	95.0 ± 25.7
Potassium recovery by UKV	99.8 ± 23.0
Chloride recovery by UClV	105.2 ± 30.0
Mars520	
Sodium recovery by UNaV	93.4 ± 27.9
Potassium recovery by UKV	77.9 ± 24.2
Chloride recovery by UClV	98.8 ± 32.1

[1]Values are mean percentage recovery over time ± SDs. Mars105, simulated 105 d on Mars; Mars520, simulated 205 d on Mars; UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

and 25°C and between 30% and 85%, respectively. During the study, subjects had free access to water, although fruit juices were limited. The crews lived and worked like cosmonauts on the international space station. Daily calorie intake was between 2500 and 2800 kcal/d, satisfying the energy requirements for a light-activity lifestyle that characterized daily life conditions in industrial societies (12). Movement activity was measured continuously with wrist actigraphy in the 6 subjects participating in the Mars520 study (13).

The nutritional intervention took place during the complete Mars105 study and the first 205 d of the simulated flight to Mars during the Mars520 study. The dietary salt intervention during the Mars105 study was performed stepwise from 12 g salt/d to 9 g salt/d to 6 g salt/d. Because of the longer duration of the Mars520 study, we were able to re-expose the Mars520 subjects to 12 g/d, after salt depletion at 6 g/d, for a longer period. An oversight committee that felt the subjects should be exposed to salt reduction gradually determined the order of the diets. We maintained each salt intake level constant for ≥29 d. All other nutrients in the diet were maintained constant throughout the study (1).

We could not ask the subjects to eat the same breakfast, lunch, and dinner for weeks on end. Thus, we designed menus from

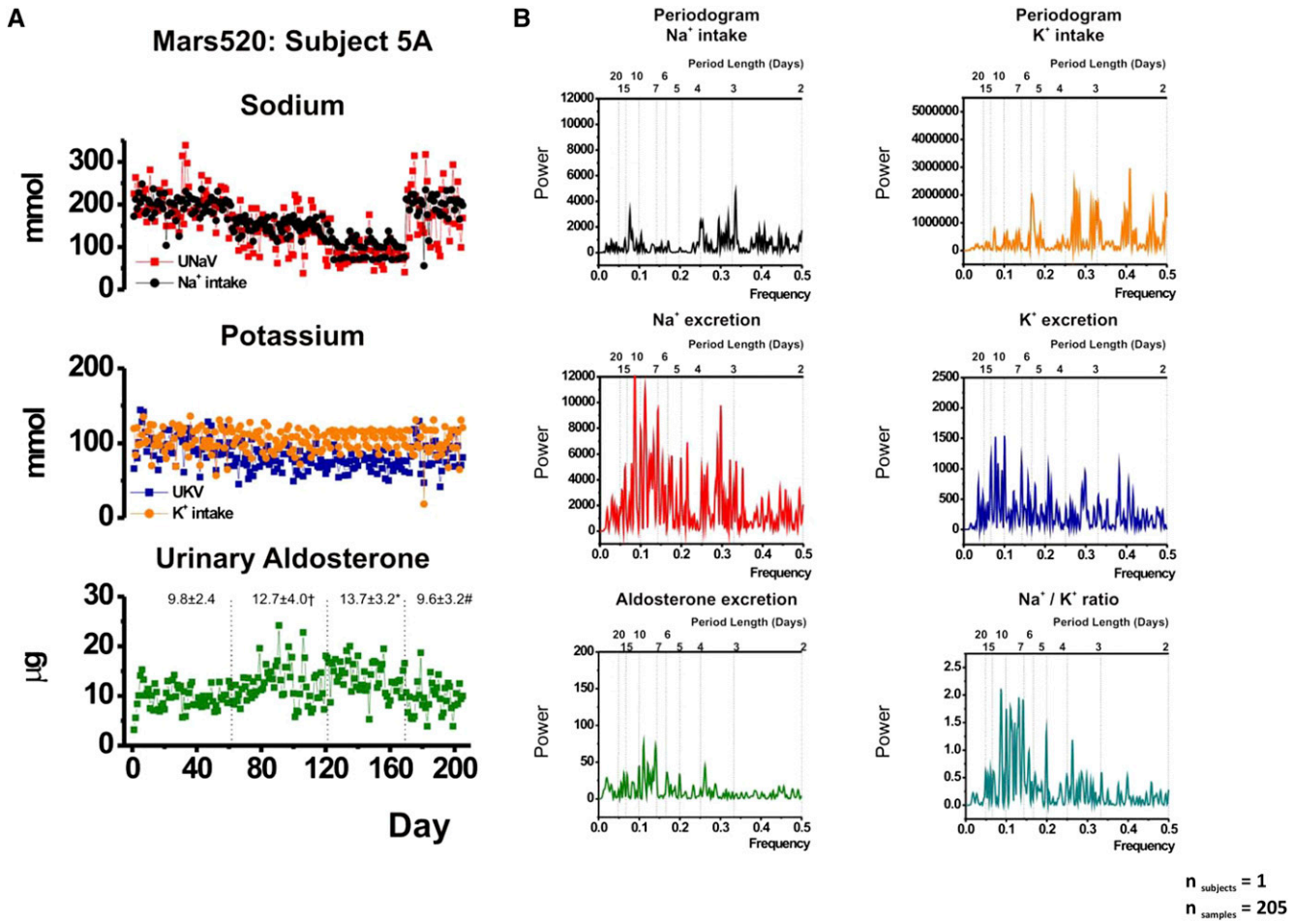

FIGURE 1 Sodium, potassium, and aldosterone excretion from a single representative subject studied daily for 205 d (descriptive statistics) in the Mars520 study (A). The panel shows 205 d of relatively stable sodium intake, with the highly variable 24-h UNaV as reported previously (1). The less-stable potassium intake, the highly variable UKV, and the highly variable 24-h UAldoV in the same representative subject are given. Power spectral analysis of the rhythmic change patterns for UNaV, UAldoV, UKV, and urinary Na^+:K^+ ratio in the same subject (B). UNaV had a dominant 6–8 d rhythmic change pattern, as did potassium intake, UAldoV, UKV, and the urinary Na^+:K^+ ratio. Day-to-day sodium intake was maintained more constantly during the Mars105 study than during the Mars520 study, whereas potassium intake was more variable during the Mars105 study than during the Mars520 study (data not shown). The data were analyzed with Fourier Transform. $^{\dagger,*,\#}$Significantly different from low-salt diet [ANOVA statistic all $P < 0.0001$, 12 g re-exposure significantly different from 6 g salt diet (4th and 3rd salt phases, respectively)]. Mars105, simulated 105 d on Mars; Mars520, simulated 205 d on Mars; UAldoV, urinary aldosterone excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion. Reproduced from reference 1 with permission.

varied processed foods that maintained all constituents as constant as possible, modifying only the salt intake. The ionic content of the foods was determined by chemical analysis as required by regulatory food agencies (14). Each subject had an individual daily menu plan that listed all food products he was to consume on a particular day. Each subject was asked to adhere to the menu plan as strictly as possible and consume every listed item. Each subject also was asked to document directly onto the menu plan (daily diary), as a percentage, the contents of any food items he failed to eat completely. The nutritionist afterward made the necessary adjustments to the amount of ingested nutrients for the subject on that day, resulting in precise information on the long-term prescribed and day-to-day recorded electrolyte intake. In total, the day-to-day recorded food products contained 14.8 kg salt, of which we found a total 13.7 kg in the subjects' urine (14). Subjects were allowed to drink water ad libitum, measured fluid intake gravimetrically, and recorded the intake amounts directly onto the menu plans. The subjects collected all of their urine for a 24-h period every day and determined the urine volume gravimetrically. Urinary creatinine excretion was constant at all salt intake levels. Less than 0.1% of our 1646 urine samples showed creatinine excretion <0.8 g/d or >2.4 g/d. We therefore used all samples for analysis (14).

Biochemical analyses

The food industry that supplied the food chemically analyzed the electrolyte content in their products by ashing and atomic absorption spectrometry. We analyzed urine sodium and potassium content with a clinical flame photometer. Urinary chloride concentration was measured with silver-nitrate titration. Creatinine was measured by an automated technique. Daily UNaV, UKV, and urinary chloride excretion (UClV) was calculated by the multiplication of urine sodium, potassium, and chloride concentrations and urine volume. Methods to determine urinary aldosterone have been outlined earlier (1).

Statistical analyses

To analyze the predictive value of a single 24-h urine sample in order to accurately estimate real electrolyte intake, we compared true sodium, potassium, and chloride intake with measured 24-h sodium, potassium, and chloride excretion in the urine. Because current computerized models often calculate the projected effect of a 3-g reduction in salt intake on cardiovascular health outcomes, we tested the accuracy of UNaV and UClV to correctly estimate real salt intake within a 3-g (50-mmol) range. The accuracy of each individual UNaV and UClV for correct assessment of daily salt intake was performed by the definition of true positives of salt intake. We investigated the difference between recorded sodium, potassium, and chloride intake and UNaV, UKV, and UClV with Bland–Altman plots. We considered a $\pm$25-mmol (corresponding to $\pm$ 1.5 g salt) deviation of the mean difference between the recorded sodium, chloride, and potassium intake and UNaV, UClV, and UKV to be a true positive urine sample (correct prediction of salt or potassium intake). UNaV, UKV, and UClV samples that

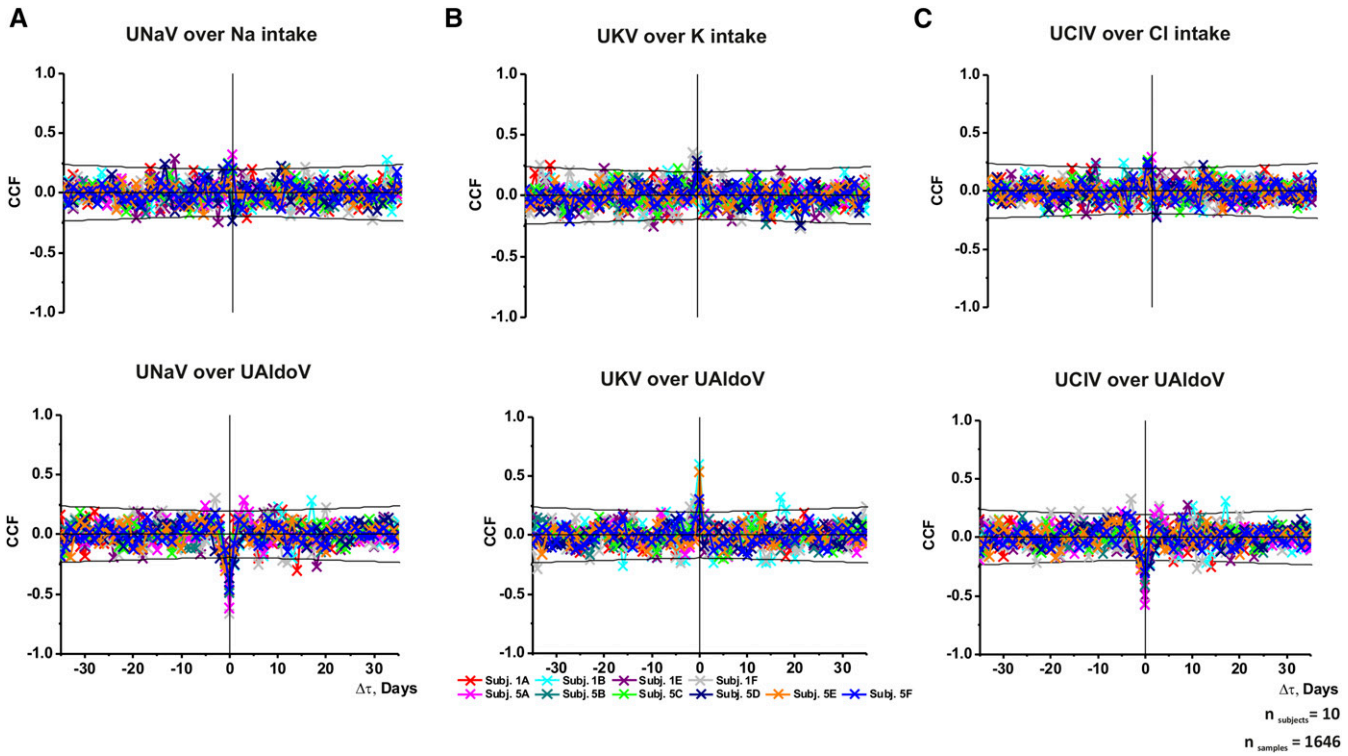

FIGURE 2 Analysis for the detection of rhythmic relations between salt intake, UNaV (A), UKV (B), UClV (C), and UAldoV (A–C) for both the Mars105 and Mars520 studies. $\Delta\tau$ represents the phase shift in days. There was no day on which UNaV, UKV, and UClV reflected sodium, potassium, or chloride intake. In contrast, $\Delta\tau$ shows that UNaV and UAldoV were inversely correlated as reported previously (A). Cross-correlation relations between UKV, UClV, and UAldoV for both the Mars105 and Mars520 studies are shown (B and C); the relation was direct for UKV and inverse for UClV, very similar to the relation for UNaV. CCF, cross-correlation coefficient; Mars105, simulated 105 d on Mars; Mars520, simulated 205 d on Mars; Subj., subject; UAldoV, urinary aldosterone excretion; UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

were outside this range were considered to be true negatives (misclassification of salt or potassium intake). A P value <0.05 was considered to be statistically significant. Data analysis was performed with IBM/SPSS software, version 20.0. Time series analysis to test for rhythmic changing patterns was performed by power spectral density estimation with the use of a periodogram with a rectangular window function. For analysis of the interrelation between the various markers, we analyzed our data by crosscorrelation to detect time-shifted interrelations between rhythmic components within the time series. These methods were described in more detail previously (1).

RESULTS

Our subjects were aged 38 ± 4.3 y. They were in excellent health and had ingested no medications. Energy, carbohydrate, fat, protein, and fiber intake were fixed throughout both the Mars105 and Mars520 studies (**Tables 1** and **2**) (1, 14). Accuracy in daily salt intake, urine sample collection, and sodium, potassium, and chloride balance throughout the time course of ultra-long continuous investigation was substantiated by ∼95% recovery of all 3 dietary electrolytes during the Mars105 study (**Table 3**). In the Mars520 study, the potassium recovery was 78% (Table 3). In the Mars105 study, subjects 1C and 1D opted out of the study for personal reasons and were not included in the analysis further. Sodium intake was acceptably controlled in the Mars520 study, whereas 24-h UNaV fluctuated greatly around the intake at all 3 levels (**Figure 1**A). Potassium intake was maintained fairly constant at all levels of salt intake; however, daily potassium excretion was more variable than intake (Figure 1A). Daily UAldoV also varied markedly, although an increase in excretion can be observed when salt intake was reduced to 9 g/d and then to 6 g/d, with an expected decrease as the high-salt intake was resumed (Figure 1A). The data were detrended and subjected to power spectral analysis that revealed a rhythmic approximate circaseptan 7-d infradian rhythm (Figure 1B), as reported earlier (1).

We next investigated the rhythmic interrelation between 2 harmonically oscillating signals by cross-correlation analysis. There were no significant cross-correlations between intake of these electrolytes and 24-h excretions of urine (**Figure 2**A–C). We detected an inverse relation between UNaV and UAldoV when the phase shift was equal to zero (Figure 2A) and a direct relation between UKV and UAldoV at that time point (Figure 2B). We found an inverse cross-correlation between UClV and aldosterone, no different from that for sodium (Figure 2A and C).

In our study, salt intake was varied at 12 g/d, 9 g/d, and 6 g/d (Mars105 study), with a re-exposure of 12 g/d in the Mars520 study, while potassium intake was maintained constant at 4 g/d. We found an expected but nonrobust correlation between sodium intake and UNaV across intake levels (**Figure 3**A). Because potassium intake was maintained constant, the relation between potassium intake and UKV was flat (Figure 3B). As expected, the relation between chloride intake and UClV was very similar to that observed for sodium (Figure 3C). As predicted, UNaV was correlated highly with UClV (**Figure 4**A), but not robustly with UKV (Figure 4B). In contrast, UNaV was correlated robustly with UNaV and UKV (Figure 4C), whereas UClV and UKV were not robustly correlated (Figure 4D).

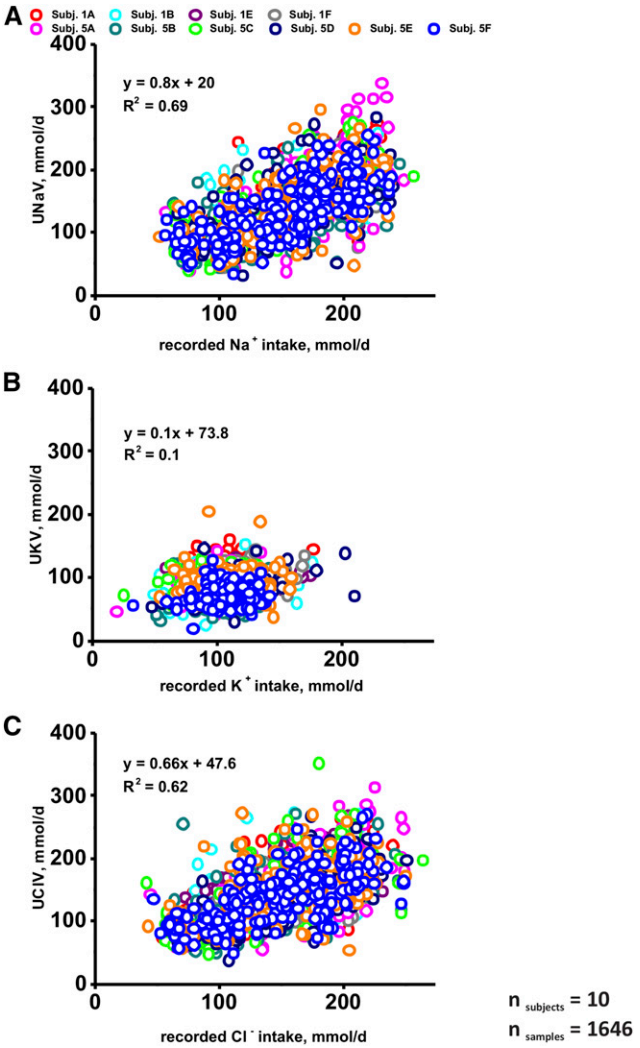

FIGURE 3 Although there was a correlation between daily recorded sodium intake and 24-h UNaV, the variability in the latter makes this correlation less robust than expected (A). The potassium intake was flat across the various salt intake levels (B). The correlation between recorded chloride intake and UClV was similar to that observed for sodium (statistic by linear regression) (C). Subj., subject; UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

The Bland–Altman plot is a method of data plotting used in analyzing the agreement between 2 different measurements. We used this method to assess differences in measured electrolyte intake and 24-h excretion. With a single 24-h urine collection, sodium intake levels of 6 g/d, 9 g/d, or 12 g/d could not be separated from one another, because misclassifications (**Figure 5**A) occurred one-half of the time (14). Although potassium intake was fixed, the daily variability in excretion led to misclassifications one-third of the time (Figure 5B). Estimating chloride intake from UClV showed no better results than for sodium (Figure 5C), as would be predicted from their tight correlation (Figure 4A). Increasing the number of collections improves the precision of predicting intake of these electrolytes. Any 3 collections to 75% accuracy for sodium (**Figure 6**A), 81% accuracy for potassium (Figure 6B), and 72% accuracy for chloride (Figure 6C). Collecting urine for 1 wk reduces the number of misclassifications further (**Figure 7**A–C), although an error rate of ≤13% remains.

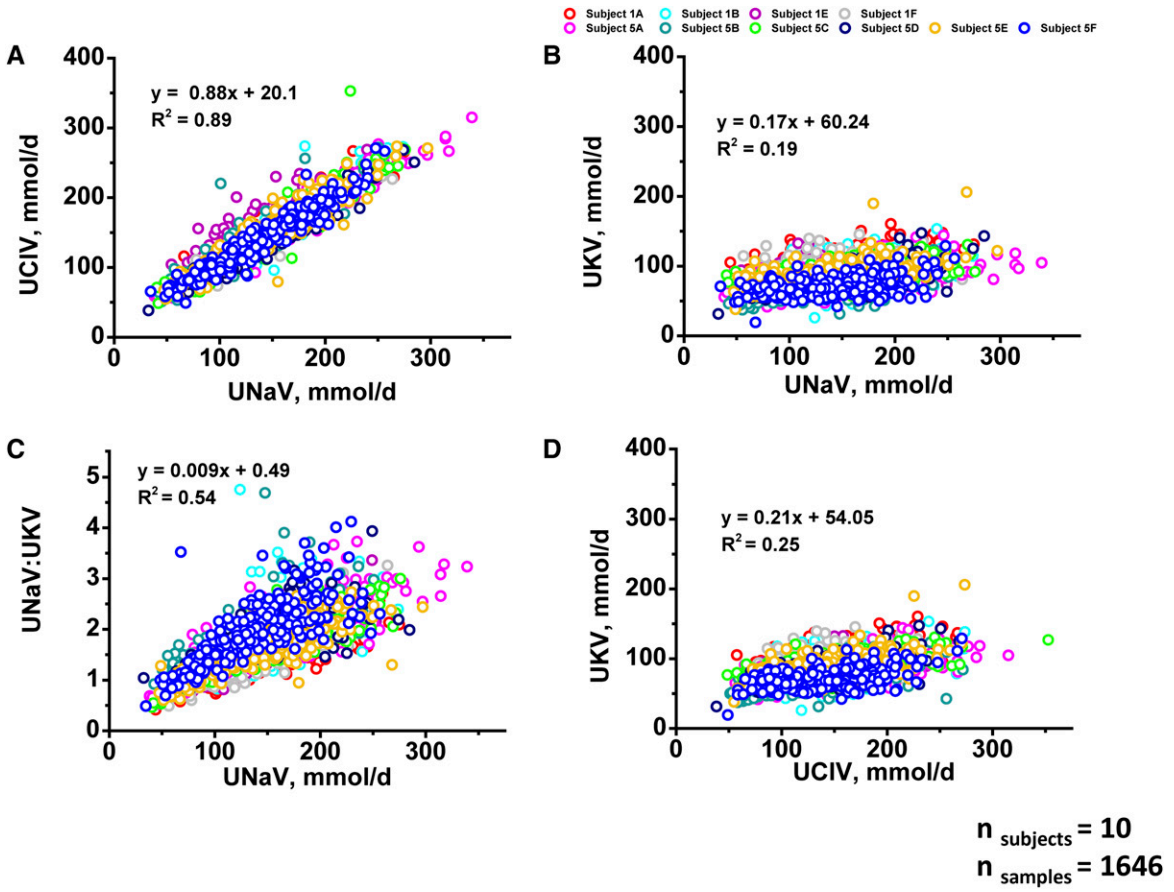

FIGURE 4 UNaV was tightly correlated with 24-h UClV at all sodium intake levels (A). Increasing UNaV was associated with a modest rise in UKV (B). UNaV was correlated with UNaV:UKV (C). The correlation between UClV and UKV was similar to that observed for sodium (statistic by linear regression) (D). UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

DISCUSSION

The important findings in our study are that potassium and chloride excretion exhibit similar infradian rhythms in excretion patterns, as does sodium. In the case of potassium, the correlation between the cations is inversely related. In contrast, for chloride and sodium, the relation is direct. Similarly, cross-correlations for sodium and chloride excretion were inverse with aldosterone, whereas that for potassium was direct. According to the WHO, because of extrarenal potassium loss, potassium excretion should be multiplied by 1.30 to estimate potassium intake, as specified in their potassium intake guidelines (http://www.who.int/nutrition/publications/guidelines/potassium_intake/en/). This conclusion is based on the findings of the International Population Study on Macronutrients and Blood Pressure (15). The potassium recoveries in the Mars105 study were almost 100%, whereas those in the Mars520 study were closer to those predicted by the International Population Study on Macronutrients and Blood Pressure. We have no immediate explanation for the discrepancies in our 2 studies; the same methodology was used in both.

Dahl et al. (16) found that the blood pressure–reducing effect of a low-salt diet in hypertensive patients was uncoupled from a reduction in exchangeable body sodium and featured an initial transient increase in exchangeable body potassium in some patients. Similarly, an earlier short-term study of extremes of salt intake showed that parallel increases in blood pressure were obviated when the associated renal potassium losses were replaced (17). We also showed the close relation between sodium and chloride excretion when salt intake was altered. We admit that this finding would be expected. However, to our knowledge, the rhythmic behavior in chloride excretion has not been reported previously.

Our study was necessarily a clinical observational investigation of subjects in a unique environment, and many mechanistic issues could not be addressed. We are aware that electrolyte homeostasis is complex, involving the central nervous system and the effector organs, including the heart, kidneys, and other regulatory systems. In the brain, the role of salt-and-water drives and subsequent downstream effects has given insights into regulatory mechanisms. We present data regarding aldosterone. A novel role for that hormone has been identified in the brain (18) that also involves the enzyme responsible for the cortisol–cortisone conversion, 11β-hydroxysteroid dehydrogenase type 2 (19). The ramifications of these findings has been discussed (20).

Our Bland–Altman plots extend the observation that UNaV at a fixed intake is variable but exhibits a circaseptal rhythm to chloride and potassium (14). Ostensibly, if 24-h excretion is used to determine population means for a huge number of samples, this variability is less of a confounder (21). Spot urine collections have been advocated to substitute for 24-h collections, under the assumption that 24-h UNaV or UKV is a gold standard (22). The authors of a relatively small population study in which actual intake was unknown concluded that the spot

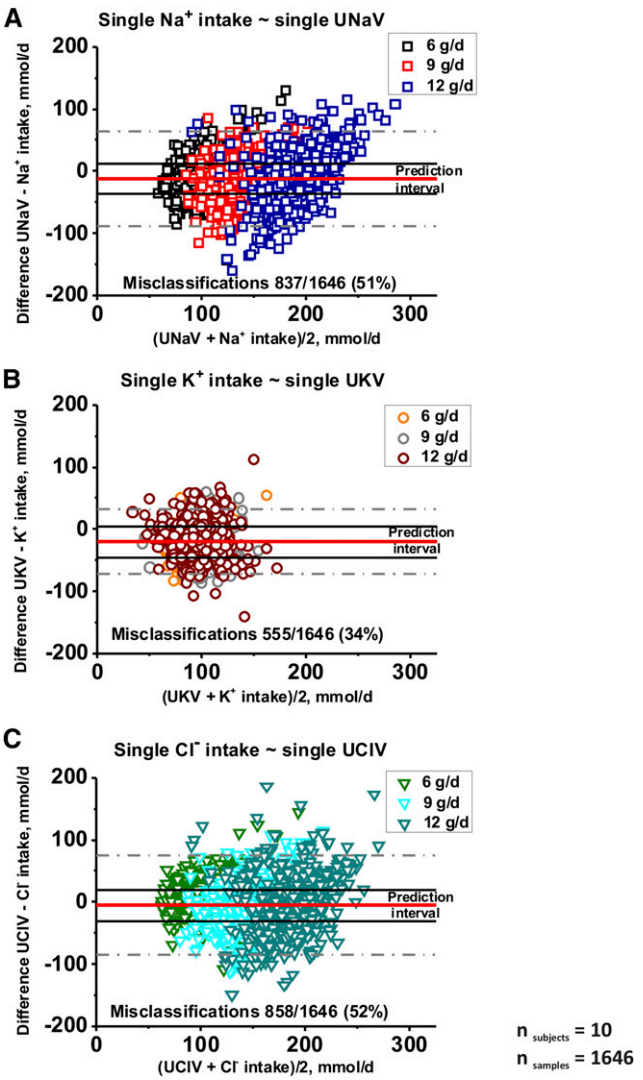

FIGURE 5 Bland–Altman plot to test the agreement between single recorded 24-h electrolyte intake levels and single 24-h urine collection for UNaV (A), UKV (B), and UClV (C). The prediction interval to accurately predict intake by excretion is defined as ±25 mmol/d of the mean difference between intake and excretion. With a single collection, the misclassification for sodium is 51%; for potassium, the value is 34%; and for chloride, the value is 52%, similar to sodium. The red solid line indicates the regression line; the gray dotted lines are the upper and lower confidence levels (statistic by Bland–Altman). UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

urine method is acceptable for estimating 24-h UNaV and UKV to assess sodium and potassium intake in a black population (22). However, the CI for the mean difference, which is too large, makes the sodium results inadmissible at the clinical level for individual assessments. To date, to our knowledge, not a single reliable conversion equation for timed-spot specimens to 24-h urine samples exists for widespread international use. It has been shown that spot urine collections are completely inadequate in estimating the individual mean electrolyte intake (23, 24). The validity for estimating population electrolyte intake with spot urine collections is still under investigation, and remains highly controversial in analytic epidemiologic research (25, 26). To our knowledge, prospective studies validating the extrapolation equations are still lacking.

Our subjects were given ~4 g (100 mmol) dietary potassium/d. Our UKV results generally corroborated the intake and recovery. Far different is potassium intake and excretion in African Americans. Unhealthy diets that are often low in potassium likely contribute to racial disparities in blood pressure (27). Investigators recently tested the effectiveness of providing weekly dietary advice, assistance with selection of higher potassium grocery items, and a $30/wk food allowance on blood pressure and other outcomes in African American adults with hypertension (28). The control group was estimated to have a potassium intake of 2.6 g/d. It is likely that the intake was even lower, because urinary excretion (only a single specimen at baseline) was 34.3 mmol/d. Despite the counseling and financial subsidy, blood pressure did not decrease and the increase in potassium intake and excretion was modest. Other populations

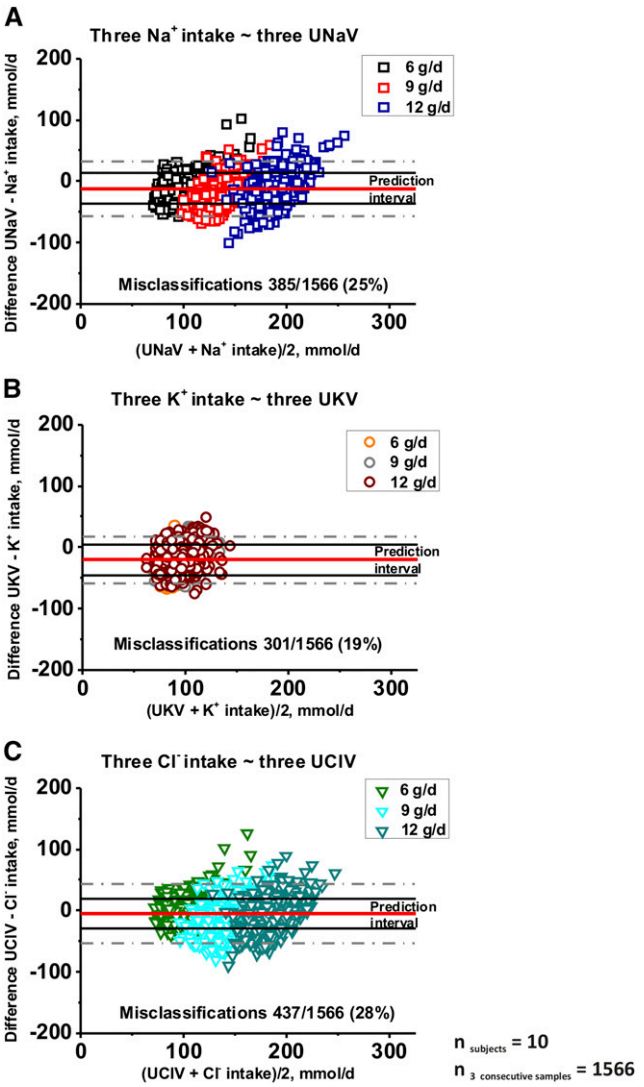

FIGURE 6 Analysis of agreement between 3 consecutively recorded sodium, potassium, and chloride intake levels and 3 consecutive UNaV, UKV, and UClV collections. Multiple collections reduce the variability and thereby improve the predictive value of UNaV, UKV, and UClV. The red solid line indicates the regression line; the gray dotted lines are the upper and lower confidence levels (statistic by Bland–Altman). UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

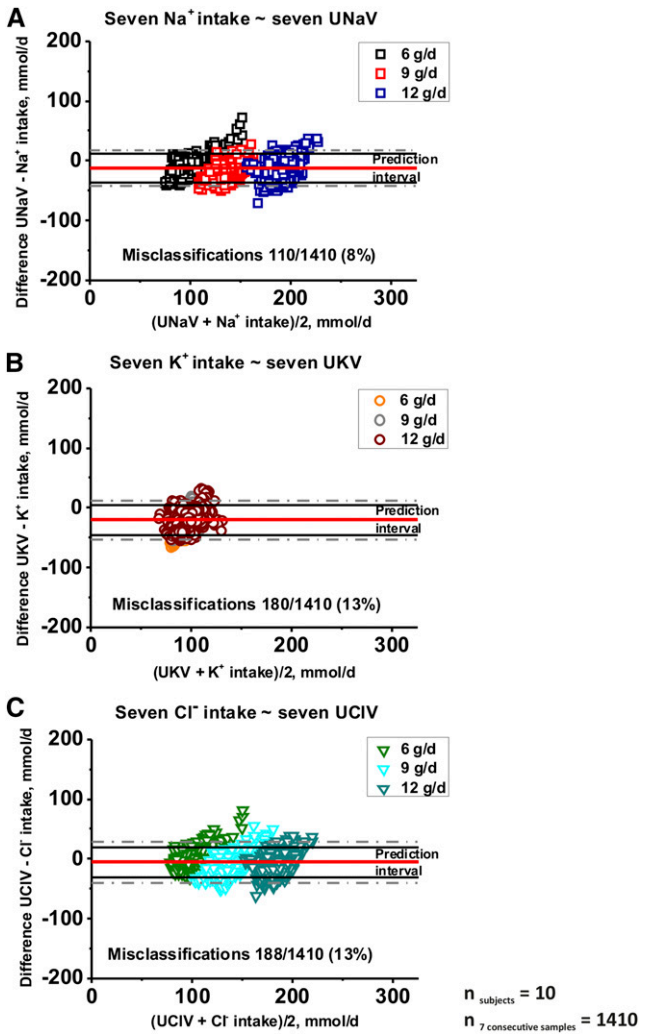

FIGURE 7 Seven consecutive collections reduce the number of misclassifications of 24-h UNaV (A), UKV (B), and UClV (C) to ≤13%. The red solid line indicates the regression line; the gray dotted lines are the upper and lower confidence levels (statistic by Bland–Altman). UClV, urinary chloride excretion; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

are hardly better off. In a recent Chinese study, whereas sodium excretion in men and women exceeded 220 mmol/d, potassium excretion was a paltry 40 mmol/d in both sexes (29).

We have not uncovered the mechanisms of our infradian rhythms, the effects of which are mediated by aldosterone. Although the function of the suprachiasmatic nucleus in circadian rhythm regulation has been studied extensively, we have limited understanding of how infradian clocks might function. Peripheral tissue clock function contributes to the regulation of physiologic processes. The adrenal gland plays a special role in this context, with adrenal hormones showing strong circadian secretion rhythms that affect downstream physiologic processes (30). We have shown that the release of sodium and ostensibly chloride from glycosaminoglycan stores in the skin and elsewhere is regulated by immune cells (31, 32). Interestingly, infradian and ultradian rhythms have been described in oscillating immune function of birds (33). Similarly, investigators have carried out prolonged investigations of thymic cells, melatonin, and corticosterone biorhythms in male Wistar rats (34). Conceivably, the infradian rhythms we observed could be modeled experimentally in animals.

Our study necessarily has limitations. The number of subjects was relatively small. We studied only men, and the responses in women could be different. It is possible that volunteers for a Mars flight simulation are not representative of the general population, although we do not believe that state of affairs to be a confounder in this study. The diets were not presented in random order for the reasons we stated. However, perhaps the long duration of the study might decrease any effects of order.

Our findings have practical implications. Because the Mars flight simulation menus were composed of readily available industrial processed foods, the results were not dependent on special diet kitchens and would be available for well-controlled dietary intervention in population studies (35). As pointed out in a recent report, processed foods can be healthy (36). However, the high degree of variability in UNaV, UClV, and UKV underscores the difficulty of assessing salt and potassium intake with brief urine collections. Continuous monitoring would be necessary to correctly separate subject groups. Epidemiologic studies relying on a single urine sample to classify dietary behavior over years yielded contradictory findings (37, 38). It is tempting to speculate that misclassifications in dietary salt or potassium intake represent a relevant confounder; however, population means are not the same as individual data. A recent meta-analysis that included >50,000 persons indicated a daily sodium intake based on a UNaV of ∼160 mmol/d, close to the intake level of 9 g salt/d of our subjects (39). Intervention trials will require designs in terms of compliance monitoring based on individual data or population means.

Novel magnetic resonance techniques for measurements of sodium, potassium, or even chloride storage in tissues may provide more reliable information to evaluate the relation between salt intake, electrolyte disposition, and blood pressure in humans (40). We speculate that the third-space storage compartment largely residing in skin participates in the rhythmic regulation of these electrolytes (41, 42). Additional studies in animals and humans will be necessary to test that hypothesis.

The authors' responsibilities were as follows—JT: conceived of and designed the study; AB and BJ: performed the biostatistical analysis; AB and RHGOE: analyzed the data; AB: prepared the figures; NR: conducted the clinical study and measured the electrolytes in all samples; KL: composed and designed the diets; PW and UM: performed the periodic rhythm analyses; MR: measured the aldosterone; FCL: wrote the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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