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Correcting for Plasma Aldosterone Improves the Accuracy of Repeated Timed Urine Sampling for Estimation of Dietary Sodium Intake

Anne Myrthe C. van Vliet[a, b] Liffert Vogt[a, b] Bigina N.R. Ginos[a, b]
Petra Frings-Meuthen[c] Martina Heer[d] Rik H.G. Olde Engberink[a, b]

[a]Nephrology, Amsterdam UMC Location University of Amsterdam, Amsterdam, The Netherlands; [b]Microcirculation, Amsterdam Cardiovascular Sciences, Amsterdam, The Netherlands; [c]Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany; [d]Institute of Nutritional and Food Sciences, University of Bonn, Bonn, Germany

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24-h urine collection · Aldosterone · Repeated urine collection · Sodium · Urine

Abstract
Introduction: Long-term sodium balance studies show that sodium can be temporarily stored and released in tissues, mediated by circaseptan rhythms of aldosterone and cortisol. This complicates the reliability of a single 24-h urine collection to estimate individual sodium intake. We investigated whether repeated timed urine collection with and without correction for plasma aldosterone is a more accurate alternative for estimating daily sodium intake. ***Methods:*** We conducted a post hoc analysis of a metabolic ward study in which 16 healthy male adults consumed a diet with a fixed sodium content (50 or 200 mmol/day) for 7 days. Each day, urine was collected in 4 intervals (7:00–13:00 h, 13:00–19:00 h, 19:00–23:00 h, and 23:00–07:00 h). Plasma aldosterone was measured at 6:30 h, 12:30 h, and 18:30 h. Sodium intakes were estimated by various formulas using 3 timed urines of day 5–7. ***Results:*** During a 200-mmol daily sodium intake, sodium intake estimates based on three repeated timed urine samples and the Toft equation differed 10 [IQR: 3–14], 8 [6–19], 36 [16–49], and 20 [10–43] mmol from the actual intake for intervals 7:00–13:00 h, 13:00–19:00 h, 19:00–23:00 h, 23:00–7:00 h, respectively. These measurements did not significantly differ from a single 24-h urine (20 [12–55] mmol). During a 50-mmol daily sodium intake, repeated timed urine collection performed worse than a single 24-h urine collection. On both diets, correction for plasma aldosterone increased accuracy and sodium intake estimates were significantly more accurate than a single 24-h urine. ***Conclusion:*** In a controlled environment, repeated timed urine collection corrected for plasma aldosterone is more accurate than a single 24-h urine collection.

Introduction

Patients suffering from chronic kidney disease, hypertension, and cardiovascular disease are advised to lower their sodium intake to a maximum of 86 mmol (i.e., 5 g of salt) as this is associated with a lower blood pressure and lower risk of cardiovascular disease [1]. Clinical monitoring and personalized advice regarding sodium intake require accurate methods to estimate dietary sodium intake. Currently, in clinical practice and

karger@karger.com
www.karger.com/kbr

Correspondence to:
Rik H.G. Olde Engberink, r.h.oldeengberink@amsterdamumc.nl

research, a single 24-h urine collection is often used for estimation of sodium intake. This method is based upon the assumption that daily sodium intake equals daily sodium excretion. However, long-term sodium balance studies with a fixed sodium intake have shown that sodium is rhythmically retained and released under the influence of aldosterone and cortisol rhythms [2, 3]. Consequently, a single 24-h urine collection deviated more than 25 mmol (i.e., >1.5 g of salt) from the actual sodium intake in half of the cases. Sodium intake estimates based on just one 24-h collection could therefore lead to inadequate dietary advice [4–6].

More accurate estimation of sodium intake can be achieved by using multiple 24-h urine collections. In a controlled environment, collection of seven consecutive 24-h urine collections improved the accuracy of sodium intake estimates to 92% [4]. An observational study in an outpatient population demonstrated that, in a less controlled environment, the use of multiple 24-h urine collections versus a single collection also changed sodium intake estimates more than 34 mmol (i.e., >2 g of salt) in half of the subjects [7]. However, collection of seven consecutive 24-h urine collections is burdensome and infeasible in clinical practice and population studies.

Single spot urine sampling is an alternative that is easy to apply in large populations but is considered highly unreliable for estimation of individual sodium intake [5]. Previous studies showed that multiple overnight urine collections can be used to differentiate between sodium intakes of 10 and 200 or 65 and 110 mmol/day [8, 9]. However, this method lacks an actual estimation of sodium intake, which might be the reason why this method has not been adopted in clinical practice. The use of repeated spot urine collections for estimation of sodium intake has yielded inconsistent results, with one study reporting that additional spot urine collections increased the accuracy, whereas another study found no improvement [10, 11]. A major limitation of these studies is that 24-h urine sodium excretion was used as a reference, which as aforementioned has significant shortcomings. Therefore, it remains unknown how sodium intake estimates from repeated spot urine collections relate to the actual dietary intake.

This study aims to investigate whether repeated timed urine collections can more accurately estimate sodium intake than a single 24-h urine collection by comparing both estimates to a fixed known dietary sodium intake. Because of its influence on the rhythm of urinary sodium excretion, we will assess the effect of a correction for plasma aldosterone. We hypothesize that the use of multiple timed urine samples and/or the correction for plasma aldosterone will reduce the inaccuracy induced by circaseptan sodium excretion rhythms and overcome the known drawbacks of single spot urine sampling.

Materials and Methods

Study Design

We conducted a post hoc analysis on data from a 7-day metabolic ward study that aimed to evaluate the effects of dietary salt intake on body mass and body fluid compartments [12]. The Freiburg Ethics Committee and the Medical Board of the Deutsche Forschungsanstalt für Luft und Raumfahrt (DLR) approved the study protocol and written informed consent was obtained from all participants. All research activities were consistent with the World Medical Association Declaration of Helsinki. For the entirety of the study, locally recruited healthy male participants that were nonsmokers and nonathletes stayed in the metabolic ward (Institute of Aerospace Medicine, DLR, Cologne, Germany), which provided a constant environmental temperature of 24°C and humidity of 60%. During the study period, participants were not allowed to partake in physical exercise or use alcohol or drugs.

Study Diet

Complete details on food and water intake have been published previously [12]. Dietary sodium intake was fixed. Participants were randomized to consume 50 or 200 mmol sodium per day. Subjects did not fast during the study. Diets were isocaloric (11.300 MJ/day) and nutrient content corresponded with the German Recommended Dietary Allowances [13]. The weighed intake method was utilized to verify food intake [14]. PRODI 4.2 database and food manufacture information were used to calculate macronutrient, water, and sodium content of foods and beverages. High sodium foods (>20 mmol/100 g) were chemically analyzed with atomic absorption photometry to verify sodium content.

Urine Collections and Urinary Sodium Measurements

Throughout the study, urine was collected in four sampling periods per day: during morning (7:00–13:00 h), afternoon (13:00–19:00 h), evening (19:00–23:00 h), and night (23:00–7:00 h). We will refer to these collections as timed urine collections. Aliquots of urine were stored at −80°C until analysis. Weight and density of urine were used to calculate urine volume. Urinary sodium concentrations were measured with an ion-selective electrode (Hitachi 604) and urinary creatinine concentrations were determined according to the Jaffé method (Boehringer Mannheim).

Dietary Sodium Intake Estimation Methods

We used the population mean 24-h sodium excretion to determine when the study population reached steady-state sodium balance after initiation of the study diet. Consequently, we estimated dietary sodium intake from the timed urine samples collected during these three consecutive days: day 5, 6, and 7.

We initially examined the Toft equation as this equation is based on a European population [15]. Then we compared this equation to the Tanaka and Kawasaki equations, which are two commonly used Japanese equations [16, 17]. We adjusted all three equations to allow utilization of measured 24-h urinary creatinine

Table 1. Overview of adjusted spot urine-based equations

Type	Adjusted[a] equation
Toft et al. [15]	$33.56\times[uNa_{timed}/(uCr_{timed} \times 10) \times uCr_{24h}]^{0.345}$
Kawasaki et al. [16]	$16.3\times[uNa_{timed}/(uCr_{timed} \times 10) \times uCr_{24h}]^{0.5}$
Tanaka et al. [17]	$21.98\times[uNa_{timed}/(uCr_{timed} \times 10) \times uCr_{24h}]^{0.392}$

[a]Estimated 24-h urinary creatinine excretion was replaced by measured 24-h urinary creatinine excretion; timed urine sodium concentration (uNa_{timed}) in mmol/L; timed urine creatinine concentration (uCr_{timed}) in mg/dL; 24-h urinary creatinine excretion (uCr_{24h}) in mg.

excretion instead of estimated 24-h urinary creatinine excretion because the height of participants, which is required for these formulas, was not recorded during the study (Table 1).

For each single sampling period, we entered the measured sodium and creatinine concentrations in these three equations and averaged the resulting estimates of day 5, 6, and 7. We calculated mean sodium and creatinine concentrations in combined sampling periods: morning-afternoon (7:00–19:00 h), afternoon-evening (13:00–23:00 h), evening-night (19:00–7:00 h), and night-morning (23:00–13:00 h) to test whether combining sampling periods could improve sodium intake estimates.

Plasma Aldosterone Measurement

Blood was collected at 6:30, 12:30, and 18:30 h on day 5 and 7. During blood draw, subjects were in a supine body position. Collected blood was immediately separated by centrifugation and stored at −80°C until analysis. The plasma aldosterone concentration was measured using a commercially available radioimmunoassay kit (MAIA, Seronon Freiburg, Germany).

Statistical Analysis

The accuracy of the dietary sodium intake estimation was defined as the absolute difference between estimated and actual dietary sodium intake (|Δ sodium|). We deemed a discrepancy between actual and estimated sodium intake of >20 mmol sodium clinically relevant as this approximates a quarter of the maximum daily dietary sodium intake recommended by the World Health Organization [1].

Wilcoxon signed-rank tests were used to compare two groups. Comparison of more than two groups was performed using Friedman tests, followed by Dunn's pairwise post hoc tests with a Bonferroni correction. The association between urinary sodium excretion and plasma aldosterone was assessed via a repeated measures correlation using R package "rmcorr". Sodium excretion was coupled to the nearest plasma aldosterone measurement for nights and evenings and to the mean of the flanking plasma aldosterone measurements for mornings and afternoons.

We corrected dietary sodium estimates from repeated timed urine collections for plasma aldosterone in R. A linear mixed model using R package "nlme" was fitted with actual sodium intake as independent variable and estimated sodium intake, plasma aldosterone, and their interaction as both fixed and random effects. We used a leave-one-out cross-validation approach in which the aldosterone-corrected estimated dietary sodium intake was predicted for each participant using model coefficients that were calculated while that participant was excluded. Uncorrected estimates of dietary sodium intake that were used as comparison were predicted by identical models from which plasma aldosterone was omitted.

All data are expressed as median and interquartile range, unless otherwise specified. All statistical analyses were conducted with SPSS (version 28.0, SPSS Inc., Chicago, IL) and R studio (version 4.0.3). A p value below 0.05 was considered significant.

Results

Sixteen healthy adults were included in the study. All participants were male, nonsmokers, and nonathletes. Both sodium intake groups had comparable mean blood pressures at the start of the study, namely, 113 ± 3 mm Hg systolic and 67 ± 4 mm Hg diastolic pressure for the 50 mmol/day group and 109 ± 3 mm Hg systolic and 67 ± 3 mm Hg diastolic pressure for the 200 mmol/day group. The subjects who consumed 50 mmol sodium per day had a mean body weight of 74.6 ± 7.5 kg and the 200 mmol/day group had a mean body weight of 75.4 ± 4.8 kg. Below, we will first discuss the results of the 200-mmol sodium intervention followed by the 50 mmol/day results.

Normal Sodium Intake (200 mmol/day)

The population mean 24-h sodium excretion matched dietary sodium intake on day 5, indicating that steady-state sodium balance was achieved in the study population (Fig. 1a).

Low Individual Accuracy of 24-h Urine Collections

Despite the match on study population level, the individual difference between estimated and actual sodium intake ranged from an underestimation of 69 mmol to an overestimation of 60 mmol, with a median difference of −11 mmol (IQR: −34 to 15 mmol; online suppl. Table S1; for all online suppl. material, see https://doi.org/10.1159/000540658). This wide range was reduced to a maximum underestimation and overestimation of 46 and

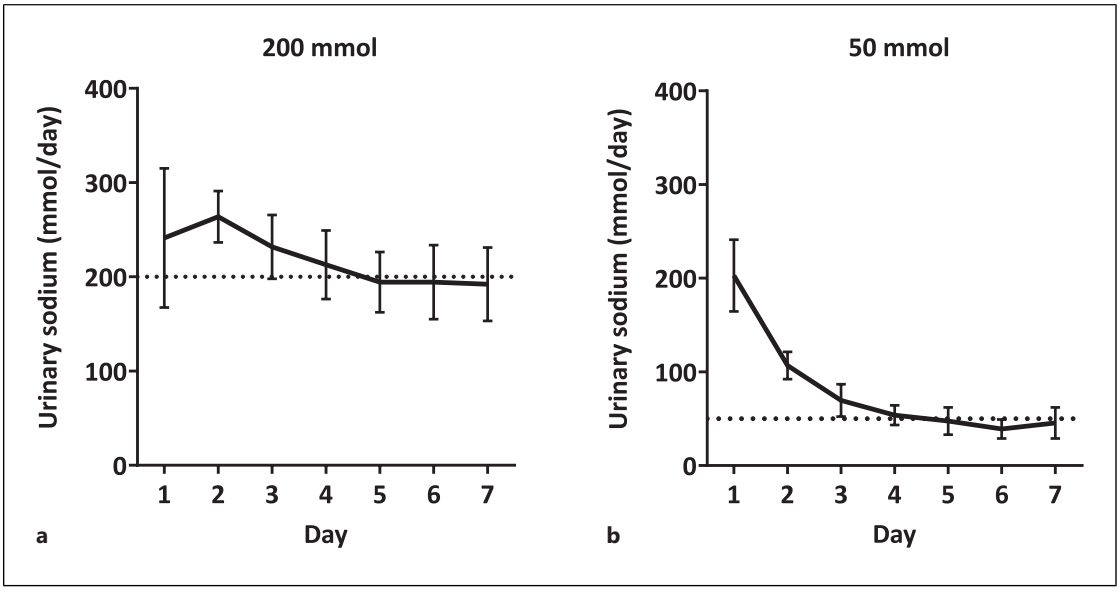

Fig. 1. Urinary sodium excretion in the study population. Mean daily urinary sodium excretion of the study population during a sodium intake of 200 mmol/day (**a**) or 50 mmol/day (**b**).

29 mmol, respectively, by calculating the mean sodium excretion in three consecutive 24-h urine collections on day 5–7 (median −13 mmol, IQR: −24 to 20 mmol). However, in 5 out of 8 of participants these estimates still differed more than 20 mmol from the actual intake.

Repeated Timed Urine Estimation Using the Toft Equation Is Not Superior to 24-h Urine

Calculations with the Toft equation showed that the accuracy of repeated timed urine-based estimates did not significantly differ from the accuracy of a single 24-h urine collection. However, all estimates derived from morning urine samples fell within the predetermined clinically acceptable error margin of 20 mmol (Fig. 2a). As accuracy may have been improved by using the measured 24-h urinary creatinine, we performed a sensitivity analysis with a 24-h creatinine excretion that was measured on day 4. This yielded similar results (online suppl. Fig. S1).

The Tanaka and Kawasaki Equations Do Not Outperform the Toft Equation

When comparing the Tanaka and Kawasaki equations to the Toft equation, the Toft equation had the highest accuracy and was significantly more accurate than the Tanaka equation for morning samples (Fig. 2a), whereas the equations performed similarly when afternoon samples were used. When sodium intake was estimated from evening samples, both the Tanaka and Toft equation were more accurate than the Kawasaki equation. Lastly, estimations based on night urine samples resulted in a significantly higher accuracy for the Toft equation compared to the Tanaka equation.

The Accuracy of Sodium Intake Estimates Depends on Sampling Period

The Toft equation underestimated dietary sodium intake with night samples (median −20, IQR: −43 to −1), whereas it overestimated sodium intake using evening samples (median 36, IQR: 16 to 49; online suppl. Fig. S2). The Tanaka and Kawasaki equations showed a similar trend. Of note, the Tanaka equation demonstrated highest accuracy using the evening sampling period but tended to underestimate sodium intake during all other sampling periods. When sampling periods were compared within each equation, all three equations showed a significant difference between estimates based on evening and night sampling periods.

Combined Sampling Periods Are Not Superior to the Single Sampling Periods

As evening samples generally overestimated and night samples underestimated sodium intake, we investigated whether combining sampling periods could improve the accuracy of the sodium intake estimates. For the Toft equation, the combined morning-afternoon sampling period was significantly more accurate than the afternoon sampling period (p = 0.016), the afternoon-evening and evening-night sampling periods were more accurate than

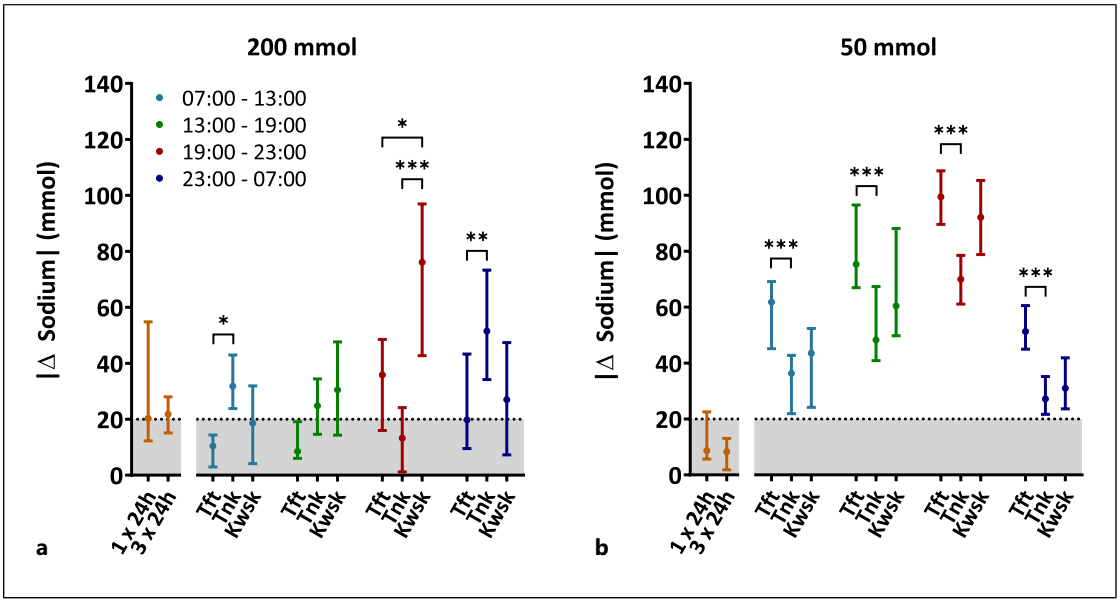

Fig. 2. Accuracy of sodium intake estimation stratified by time and equation. Overview of absolute difference between estimated and measured sodium intake (|Δ sodium|) for several methods during a 200-mmol (**a**) or 50-mmol sodium intake (**b**). Tft, Toft equation; Tnk, Tanaka equation; Kwsk, Kawasaki equation. *$p < 0.05$; **$p < 0.01$; ***$p \leq 0.001$. The dashed gray line depicts the clinically acceptable error margin of 20 mmol sodium.

the evening sampling period ($p = 0.008$ and $p = 0.023$, respectively), and the night-morning sampling period significantly improved the night sampling period ($p = 0.016$; online suppl. Fig. S3). However, none of the combined sampling periods outperformed both separate sampling periods. Similar to the Toft equation, no combined sampling period outperformed the two separate sampling periods for the Tanaka and Kawasaki equations.

Urinary Sodium Excretion Varies among Timed Urine Sampling Periods

We found a circadian rhythm of sodium excretion: sodium excretion significantly increased from morning to evening and then significantly declined from evening to night (Fig. 3a; online suppl. Fig. S4). This variation in urinary sodium excretion was associated with plasma aldosterone levels. Plasma aldosterone was significantly higher at 6:30 than at 18:30 h (Fig. 3b), and urinary sodium excretion was negatively correlated with plasma aldosterone levels ($r = -0.89$; $p < 0.001$; Fig. 3c).

Repeated Timed Urine Sampling with Aldosterone Correction Is Superior to 24-h Urine

Since we revealed a strong correlation between sodium excretion and plasma aldosterone, we examined whether a correction of urine-based sodium intake estimates for plasma aldosterone could improve accuracy. Correction for aldosterone significantly improved the accuracy of the Toft equation for all but the morning sampling period (Fig. 4a). The median improvement of estimates was largest for sampling periods during the evening (19 mmol, IQR: 7 to 27 mmol) and night (21 mmol, IQR: 4 to 43 mmol). The morning and afternoon sampling periods showed improvements of 5 mmol (IQR: −1 to 16 mmol) and 8 mmol (IQR: 7 to 11 mmol), respectively. Moreover, all aldosterone-corrected estimates were significantly more accurate than both a single and three consecutive 24-h urine collections.

Low Sodium Intake (50 mmol/day)

We assessed whether these results were consistent in participants who consumed 50 mmol sodium per day. In this group, the mean 24-h sodium excretion matched dietary sodium intake on day 4 (Fig. 1b).

In contrast to the 200-mmol sodium diet, repeated timed urine collections combined with the Toft equation performed significantly worse than a single 24-h urine collection during a 50-mmol sodium diet ($p = 0.008$; Fig. 2b). The Tanaka equation significantly outperformed the Toft equation during all sampling periods ($p \leq 0.001$), whereas the Kawasaki equation performed

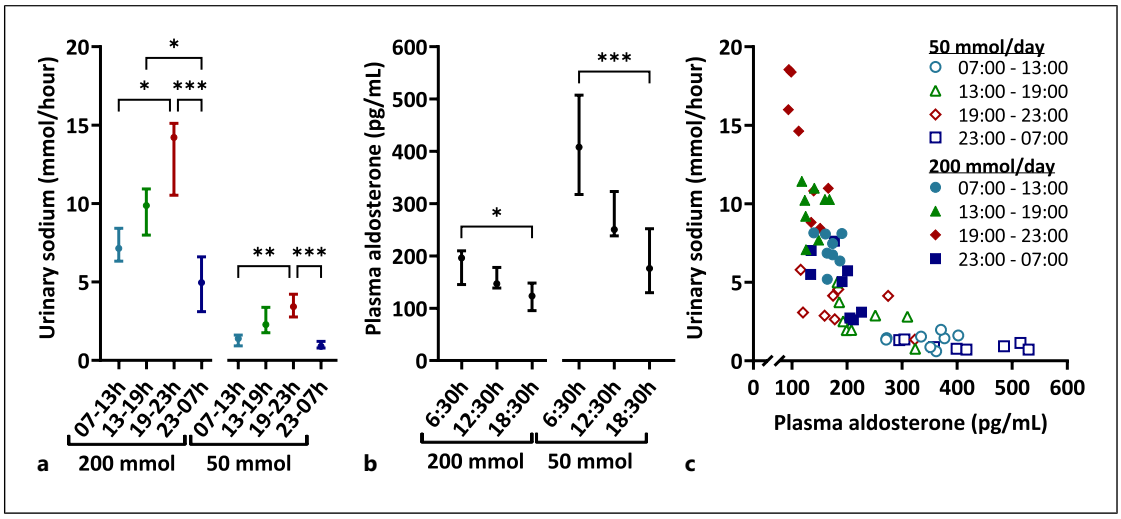

Fig. 3. Plasma aldosterone and urinary sodium excretion. **a** Urinary sodium excretion per sampling period during steady state, corrected for sampling period duration. **b** Plasma aldosterone at different time points on day 5 and 7. **c** Correlation between urinary sodium and plasma aldosterone. *$p < 0.05$; **$p < 0.01$; ***$p \leq 0.001$.

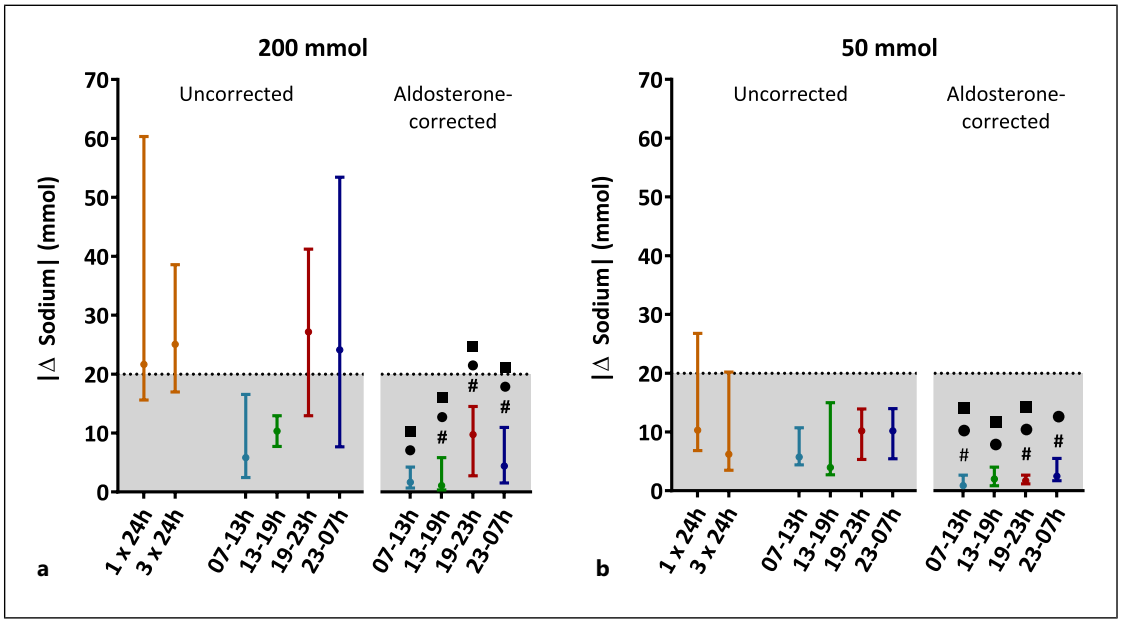

Fig. 4. Accuracy of sodium intake estimations corrected for plasma aldosterone. Overview of absolute difference between estimated and measured sodium intake (|Δ sodium|) for predicted aldosterone-corrected and uncorrected estimates during a 200-mmol (**a**) and 50-mmol sodium intake (**b**). The Toft equation was used for estimation of dietary sodium intake. #$p < 0.05$ versus repeated timed urine collections; •$p < 0.05$ versus a single 24-h urine collection; ▪$p < 0.05$ versus 3 consecutive 24-h urine collections. The dashed gray line depicts the clinically acceptable error margin of 20 mmol sodium.

similar to the Toft equation. Nevertheless, the Tanaka equation was significantly inferior to a single 24-h urine collection ($p < 0.05$). Although all equations overestimated sodium intake considerably, morning and overnight collections were significantly less inaccurate than evening collections (online suppl. Fig. S2d–f).

The circadian rhythms in sodium excretion and plasma aldosterone levels were similar to the patterns

during a 200-mmol sodium intake (Fig. 3a, b). Urinary sodium excretion remained negatively correlated with plasma aldosterone levels ($r = -0.78$; $p < 0.001$; Fig. 3c). A correction for plasma aldosterone also improved sodium intake estimates on the 50-mmol sodium diet (Fig. 4b). All aldosterone-corrected estimates were significantly more accurate than a single 24-h urine collection ($p < 0.05$).

Discussion

This is the first study to compare the accuracy of repeated timed urine collections to a single 24-h urine collection with measured sodium intake as a reference. Using the Toft equation, the accuracy of three repeated timed urine collections did not significantly differ from a single 24-h urine collection during a sodium intake of 200 mmol/day. However, when sodium intake was 50 mmol/day, repeated timed urine collections performed significantly worse than a single 24-h urine collection. With additional correction for plasma aldosterone, the sodium intake estimations from the repeated timed urine collections outperformed a single 24-h urine collection during both diets.

Considering the reported inaccuracy of a single 24-h urine collection for estimation of sodium intake, it is crucial that new methods are validated against measured sodium intake. In our study, we were able to compare multiple strategies for estimation of sodium intake to the measured intake. We found that the accuracy of three consecutive timed urine samples, which greatly reduces patient burden, was similar to a single 24-h urine during a sodium intake of 200 mmol/day. In our analysis, the Toft equation yielded better estimations than the Kawasaki and Tanaka equations, which can be explained by the Danish population that was included in the Toft study, which may be more similar to ours [15–17]. Although the Toft equation does not require a specifically timed spot urine sample, we found that morning urine samples were most accurate in estimating measured sodium intake. Considering the substantial differences in sodium excretion during the day, the Toft equation may be further improved by using a fixed time period.

During low sodium intake, all spot urine equations overestimated intake substantially. This could be attributed to the fact that the subjects in whom the equations were developed excreted around 200 mmol sodium per day. These data support the advice of the International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) not to use single spot urine sodium measurements for estimation of individual dietary intake and demonstrate that this is particularly problematic when sodium intake is low.

We found a close association between the diurnal variation in sodium excretion and plasma aldosterone, which has been previously reported [18]. When correcting for the aldosterone-induced variations in sodium excretion, sodium intake estimates were more accurate than a single and repeated 24-h urine collections. A drawback of this method is the necessity of a plasma aldosterone measurement during the sampling period. Previous research suggests that plasma aldosterone measurements can be replaced by more convenient timed urinary aldosterone measurements as a strong correlation was found between morning plasma aldosterone measurements and overnight urinary aldosterone excretion ($r = 0.90$) [18].

Our current analysis is limited to 16 healthy men. Further research in a larger sample should be undertaken to confirm our findings and assess whether similar results can be found in women and patients with a clinical indication for estimation of sodium intake. Another limitation is that due to the unavailability of the height and age of participants, we had to replace estimated 24-h urine creatinine excretion with measured 24-h urinary creatinine excretion. This may have improved the accuracy of the repeated timed urine collections. However, we showed that using a 24-h urine creatinine excretion from a different day yields comparable results. This approach could be easily reproduced in daily clinical practice with collection of a single 24-h urine to determine 24-h creatinine excretion and subsequent use of timed urine sampling with this creatinine value, as long as kidney function remains stable.

Notwithstanding these limitations, our findings shed new light on the use of repeated timed urine collections. Future studies should assess whether our data can be generalized to patients with hypertension, cardiovascular disease, and kidney disease, the actual populations of interest, as these patients often use medication that influences sodium excretion, such as diuretics and renin-angiotensin-aldosterone system inhibitors, and are advised to reduce their sodium intake. Considering the contrasting findings of the 50- and 200-mmol diets, other levels of sodium intake are also a topic for future research.

In conclusion, estimation of sodium intake in healthy subjects using repeated timed urine measurements is complicated because of its poor accuracy during low sodium intake. Additional correction of these estimates from timed urine collections for plasma aldosterone significantly improves the accuracy.

Acknowledgment

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Statement of Ethics

All research activities were consistent with the World Medical Association Declaration of Helsinki. The Freiburg Ethics Committee and the Medical Board of the Deutsche Forschungsanstalt für Luft und Raumfahrt (DLR) approved the study protocol. Written informed consent was obtained from all participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.M.C.V. and B.N.R.G.: analysis, investigation, methodology, visualization, and writing article. L.V.: conceptualization, supervision, and critical review article. P.F.-M. and M.H.: data curation, investigation, methodology, resources, and critical review article. R.H.G.O.E.: conceptualization, funding acquisition, methodology, resources, supervision, and critical review article.

Data Availability Statement

The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon reasonable request.

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