Cell Metabolism
Short Article

Long-Term Space Flight Simulation Reveals Infradian Rhythmicity in Human Na^+ Balance

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SUMMARY

The steady-state concept of Na^+ homeostasis, based on short-term investigations of responses to high salt intake, maintains that dietary Na^+ is rapidly eliminated into urine, thereby achieving constant total-body Na^+ and water content. We introduced the reverse experimental approach by fixing salt intake of men participating in space flight simulations at 12 g, 9 g, and 6 g/day for months and tested for the predicted constancy in urinary excretion and total-body Na^+ content. At constant salt intake, daily Na^+ excretion exhibited aldosterone-dependent, weekly (circaseptan) rhythms, resulting in periodic Na^+ storage. Changes in total-body Na^+ (±200–400 mmol) exhibited longer infradian rhythm periods (about monthly and longer period lengths) without parallel changes in body weight and extracellular water and were directly related to urinary aldosterone excretion and inversely to urinary cortisol, suggesting rhythmic hormonal control. Our findings define rhythmic Na^+ excretory and retention patterns independent of blood pressure or body water, which occur independent of salt intake.

INTRODUCTION

The *milieu intérieur* (Bernard, 1864–1865) is maintained through extracellular electrolyte and water content (Cannon, 1932) and is relevant for blood pressure control (Guyton et al., 1974). Maintenance requires a steady state between daily Na^+ intake and Na^+ excretion (Bonventre and Leaf, 1982; Hollenberg, 1980). Most earlier human studies involved short-term shifts from very low to high salt intakes (Barber et al., 1956; Braunwald et al., 1965; Brown et al., 1971; Carey, 1978; Leaf and Couter, 1949; Ludwig, 1861). However, longer balance studies have not been consistent with the conventional idea that Na^+ intake and excretion are in balance within 1 day (Heer et al., 2000; Titze et al., 2002). Kirkendall investigated Na^+ balance and other variables and estimated a Na^+ recovery rate of about 70% (Kirkendall et al., 1976). Variable recovery has generally been attributed to lack of compliance. Animal data also cast doubt as to whether or not total-body Na^+ content is really maintained constant within narrow limits. We showed previously that Na^+ is stored without commensurate water retention in skin and in skeletal muscle of rodents (Titze et al., 2003, 2005; Ziomber et al., 2008). These observations caused us to reexamine Na^+ balance in humans. We asked ourselves three questions: Are salt intake and daily urinary Na^+ excretion (UNaV) related? Are total-body Na^+ and extracellular water related? Is total-body Na^+ necessarily a function of salt intake, as suggested by steady-state theory for salt and water homeostasis (Cannon, 1929, 1932; Pitts, 1974; Smith, 1959)?

We had a unique opportunity to study salt balance under metabolic ward conditions for 105 and 205 days within a controlled long-term simulated space flight program termed Mars500. During two simulations (Mars105 and Mars520) conducted in an enclosed habitat, we solely modified daily salt intake (12 g/day, 9 g/day, 6 g/day in Mars105 and back to 12 g/day NaCl in Mars520; see Table S3 and Figure S4 online) for 30–60 days. We collected 24 hr urine daily (105 days in

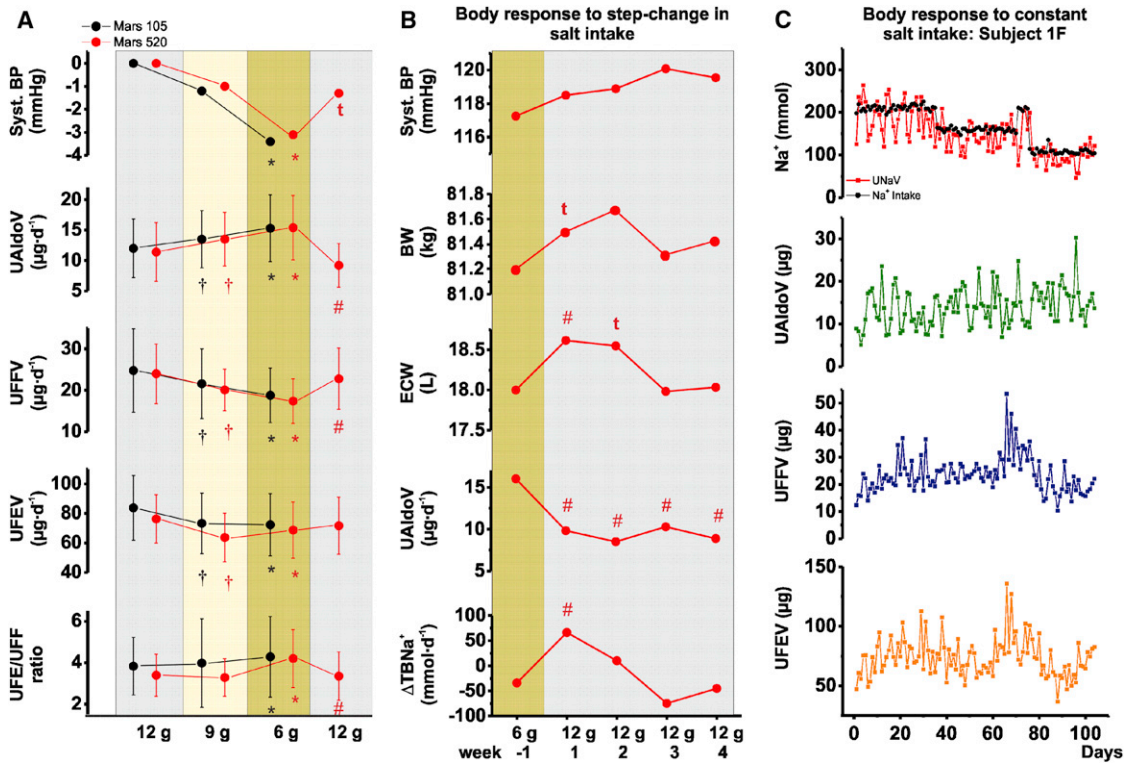

Figure 1. Response of the Body to Step Changes in Salt Intake during the Mars105 and the Mars520 Balance Studies

(A) Average systolic morning blood pressure (Syst. BP) and average ± SD for daily urinary aldosterone excretion (UAldoV), free cortisol excretion (UFFV), free cortisone excretion (UFEV), and UFE/UFF ratio (as a measure of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity) during the last 29 days of different fixed salt intakes of 12 g/day, 9 g/day, and 6 g/day.

(B) Weekly averages of blood pressure, body weight (BW), extracellular water (ECW), UAldoV, and change in total body Na^+ ($\Delta TBNa^+$) when 12 g/day salt was reintroduced in Mars520. The anticipated increase in BW, ECW, and total-body Na^+ ($TBNa^+$) occurred, but only in the first week after the dietary step change. Thereafter, BW, ECW, and $TBNa^+$ behaved independently, while blood pressure showed a gradual increase.

(C) Daily fluctuations in urinary Na^+ excretion (UNaV), UAldoV, UFFV, and UFEV in a single subject from Mars105. This daily variability occurred in all subjects.

$^*P_{(12\ g\ versus\ 6\ g)} < 0.05$; $\dagger P_{(9\ g\ versus\ 12\ g)} < 0.05$; $\#P_{(re\text{-}exposition\ to\ 12\ g)} < 0.05$; $^tP_{(re\text{-}exposition\ to\ 12\ g)} < 0.1$.

Mars105 and 205 days in Mars520). Our findings uncover rhythmic Na^+ excretory and retention patterns independent of blood pressure or body weight (BW) and give insights into aldosterone, cortisol, and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) as regulators.

RESULTS

Salt Intake, Na^+ Homeostasis, and Blood Pressure

We achieved 95% recovery of dietary Na^+ in UNaV over each dietary phase during both studies (Table S4), indicating steady-state Na^+ balance. Blood pressure decreased when daily salt intake was reduced from 12 to 6 g/day (Figure 1A and Table S1) and increased with reinstitution of the 12 g/day salt diet in Mars520. Daily aldosterone excretion (UAldoV) increased with stepwise dietary salt reduction in Mars105 and Mars520 and then decreased with reinstitution of the 12 g/day salt diet in Mars520. Daily urinary free cortisol (compound F) excretion (UFFV) behaved opposite to UAldoV in response to diets, as did urinary free cortisone (compound E) excretion (UFEV). Compared to a 12 g salt intake, the ratio between UFE and UFF was increased with 6 g salt.

In Mars520, long-term exposition of the 6 g/day salt diet was followed by a step-change re-exposition to 12 g/day. Within the first week, the body's adaptation to this environmental change was characterized by parallel increases in BW and extracellular water, an increase in total-body Na^+, and a decrease in UAldoV, as anticipated (Figure 1B). This increase in extracellular water was not paralleled by a significant increase in blood pressure. However, for the subsequent three weeks, blood pressure gradually increased, while BW and extracellular water decreased. Total-body Na^+ proceeded to decrease despite high-salt intake.

Circaseptan Variability in Na^+ Excretion

We were struck by the high degree of variability in the data at each salt intake level (Figure 1A). We therefore inspected the data from each individual subject by daily time series analysis. Although salt intake was fixed, daily UNaV, UAldoV, UFFV, and UFEV also exhibited marked day-to-day fluctuations (Figure 1C). All subjects in both Mars105 and Mars520 exhibited these rhythmic excretory patterns (Figure S1). To investigate the body's rhythmic response to constant salt intake, we next normalized the data for salt intake (detrending of time

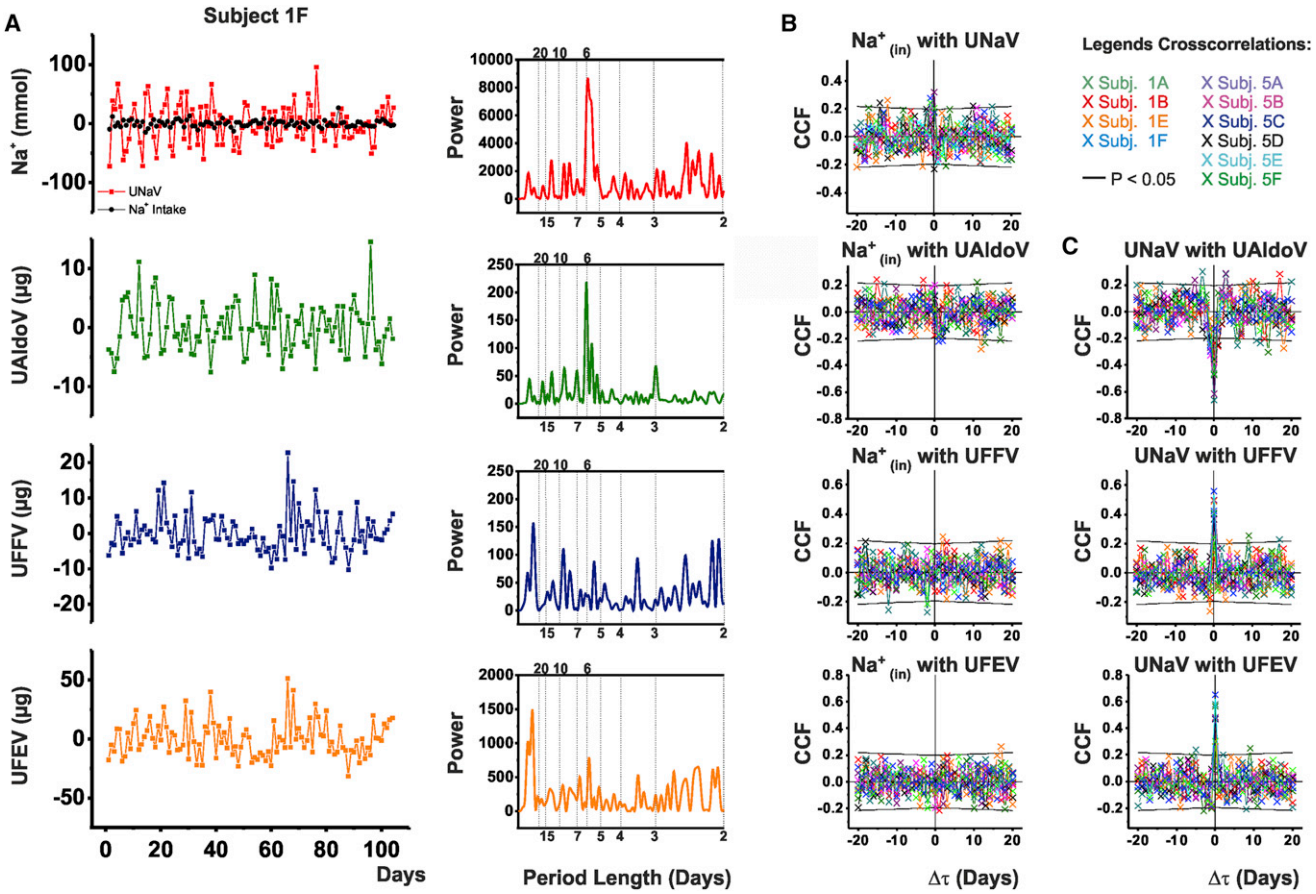

Figure 2. Response of the Body to Ultralong Constancy in Salt Intake

(A) Variability in UNaV, UAldoV, UFFV, and UFEV from one individual subject with salt intake set constant (detrended time series, left side) and Power Spectral Density (PSD) analysis of their rhythmical components (right side).

(B) Crosscorrelations to test for relationships between the urinary rhythms and Na^+ intake ($Na^+_{(in)}$) in all subjects. Urinary variability occurred independent of salt intake.

(C) Crosscorrelations between UNaV with UAldoV, UNaV with UFFV, and UNaV with UFEV. Rhythms in UNaV were inversely correlated with UAldoV and directly correlated with UFFV and UFEV. CCF, crosscorrelation coefficient.

series) and analyzed the period lengths of these rhythmic change patterns by power spectral analysis (Figure 2A and Figure S1). UNaV, UAldoV, UFFV, and UFEV all showed peaks at about 6 days period length (circaseptan). UFFV and UFEV in addition showed prominent peaks with monthly period lengths.

Interrelation between Rhythmical Na^+, Aldosterone, and Cortisol Excretion

To inspect the rhythmic relationship between salt intake and the excretory parameters further, we performed a crosscorrelation time series analysis (Figures 2B and 2C). Crosscorrelation is a measure of waveform similarities as a function of a time lag. $\Delta\tau$ represents the phase shift or time lag in days (Figure S2). With fixed salt intake, there was no day in which any excretory parameter was uniformly dependent on Na^+ intake in any subject (Figure 2B). In contrast, UNaV and UAldoV were inversely correlated at $\Delta\tau = 0$ in the subjects, while UNaV and UFFV, or UFEV, were directly correlated at $\Delta\tau = 0$ (Figure 2C). Thus, the relationship between UNaV and UAldoV confirmed the antinatriuretic action of aldosterone. In contrast, urinary free cortisol and cortisone excretion unexpectedly were associated with enhanced urinary Na^+ excretion.

A serendipitous feature of Mars105 allowed us to test the influence of sleep deprivation on Na^+ homeostasis. In Mars105, the subjects were required to serve a nightshift duty, every sixth night, which was not the case in Mars520. Blood pressure on the morning after nightshift was higher compared to nonnightshift (Figure 3A). Besides the expected UAldoV response to changes in salt intake, we found increased urinary aldosterone excretion during days with nightshift. In contrast, UFFV and UFEV were not increased with nightshift. Additional individual time series crosscorrelation analysis revealed a strong inverse correlation between nightshift and UNaV in three of four subjects, which recurred every 6 days (Figure 3B). The time course of this antinatriuretic response was paralleled by increased UAldoV in the same subjects. One subject did not increase UAldoV in response to nightshift (subject 1A) but instead showed increased UFFV and UFEV, which then were coupled with a slight increase in UNaV during nightshift.

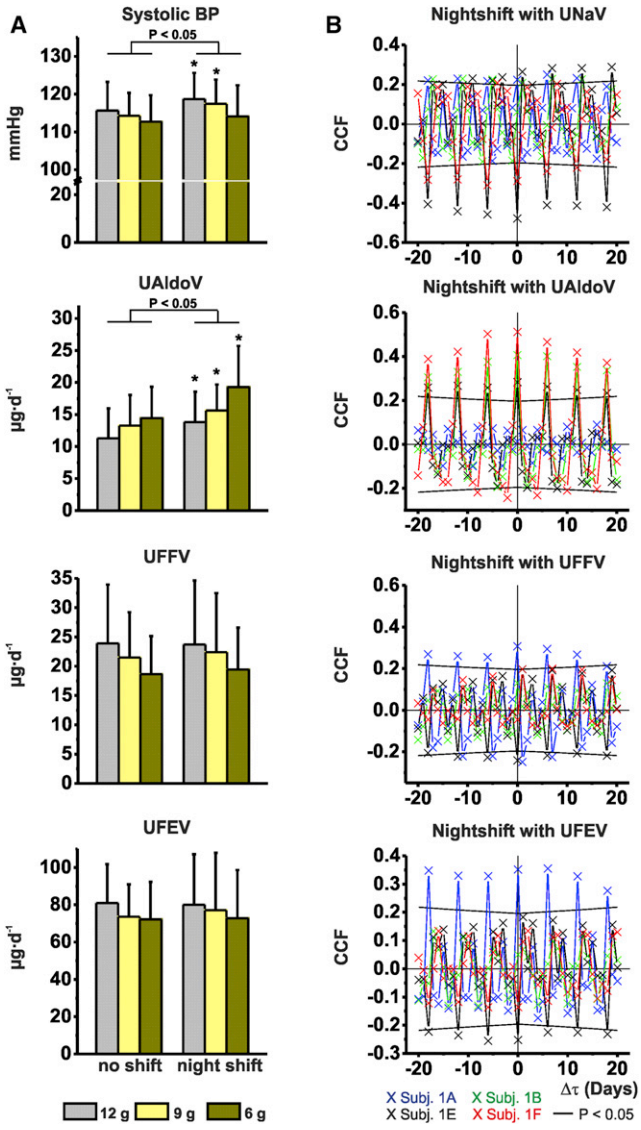

Figure 3. Influence of Night-Shift Work during Mars105 on Na^+ Metabolism

(A) Comparison of average ± SD of morning systolic blood pressure, UAldoV, UFFV, and UFEV on days without and days with nightshift at the three levels of salt intake. Nightshift increased average blood pressure and UAldoV in the subjects, but not UFFV and UFEV.

(B) Individual time series examination of the rhythmical interrelation between the parameters with crosscorrelation analysis. In three out of four subjects, nightshift repetitively increased UAldoV and decreased UNaV. In one subject, nightshift repetitively increased UFFV and UFEV, which then increased UNaV. The findings suggest that nightshift work triggered or synchronized the pronounced circaseptan rhythmicity in Mars105. * $P_{(nightshift)} < 0.05$.

Long-Term Changes in Total-Body Na^+

The ultralong nature of the Mars520 study allowed us to inspect day-to-day changes in total-body Na^+ based on Na^+ intake and UNaV. Individual time series analysis revealed an infradian (longer than circadian) rhythm in total-body Na^+ with phases that extended over weeks (Figure 4A), irrespective of salt intake, in all subjects (Figure S3). We crosscorrelated total-body Na^+ with UAldoV, UFFV, UFEV, and UFE/UFF ratio to detect potential regulatory hormones involved in generation of these long-term rhythmical change patterns. Rhythmic variability between total-body Na^+ and UAldoV was parallel and phase shifted by 1–2 weeks (Figure 4B and Figure S3). Rhythmic variability between total-body Na^+ and UFFV or UFEV was in counterphase and exhibited similar phase shifts (Figure 4B and Figure S3). Rhythmic variability between total-body Na^+ and UFE/UFF ratio was in phase, phase shifted, or in counterphase in the subjects. We found no significant association between variability in total-body Na^+ and blood pressure in the same subjects (Figure 4C and Figure S3).

While crosscorrelation analysis between total-body Na^+ and urinary hormone excretion delivered variable results, time series analysis of interrelation between the urinary hormones revealed underlying similarity. We found that secretion patterns in UAldoV and UFFV or UFEV were in counterphase around $\Delta\tau = 0$. UAldoV and UFE/UFF ratio were directly correlated in most of the subjects. These findings suggest that increases in total-body Na^+, which appeared independent of salt intake, were associated with increased UAldoV and decreased UFFV, while decreases in total-body Na^+ were coupled with low UAldoV and high UFFV levels. The inverse relationship between UAldoV and UFFV may in part be coordinated by suppression or activation of 11β-HSD2 activity.

DISCUSSION

We grasped an opportunity during a simulated space flight program (Mars500) to test whether salt intake and daily UNaV are related, whether total-body Na^+ and extracellular water are related, and whether total-body Na^+ is necessarily a function of salt intake. The Mars500 studies on salt intake and Na^+ balance are distinct from earlier studies that investigated Na^+ homeostasis at extremes of salt intake or in response to provocative maneuvers (Barber et al., 1956; Leaf and Couter, 1949; Ludwig, 1861; Strauß et al., 1958). Earlier studies were brief, and the investigators assumed that once steady state was achieved, daily UNaV would reliably reflect salt intake.

We aimed to test the reverse experimental approach, namely the response of the body to constant salt intake. Thus, the Mars500 balance studies were not only designed to examine Na^+ balance in response to abrupt increases in dietary salt (traditional step change approach) but also to investigate Na^+ metabolism in response to ultra-long-term constant salt intake (ultra-long-term constancy). Such experiments cannot be performed under daily life conditions, because precisely controlled food intake and specimen collection by each test subject over months would be necessary. The design of the Mars500 Na^+ balance studies allowed us to address the question.

In line with accepted notions, abrupt increases in salt intake induced short-term increases in total-body Na^+ and extracellular water, rapid suppression of aldosterone excretion, and expected adjustment in UNaV to the next salt intake level. Unexpected was the observation that high-salt diet increases cortisol excretion in humans, an effect that we attribute in part to diminished action of 11β-HSD2. Our results also showed a modest, variable salt effect on blood pressure; however, the effect was delayed and required several weeks to achieve plateau. These findings support the assumption that endocrine and/or blood pressure

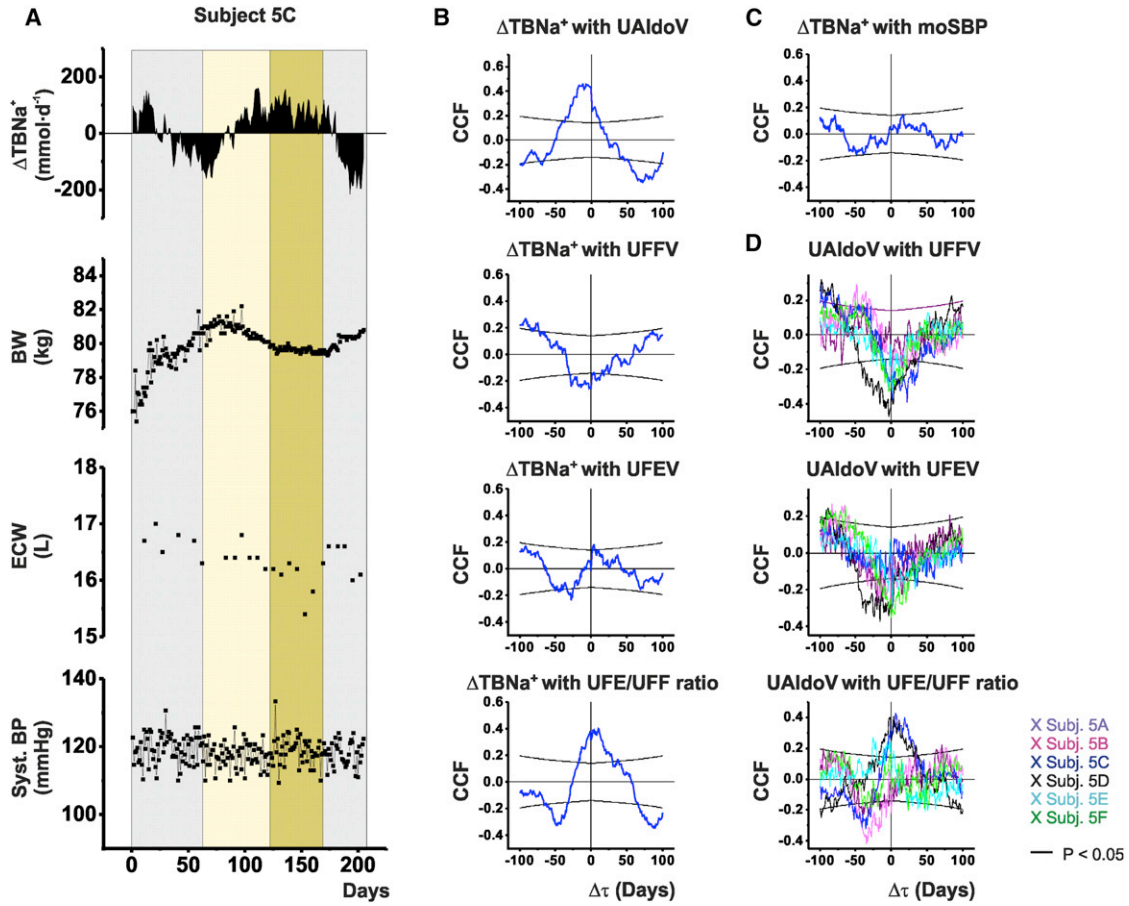

Figure 4. Ultralong Periodicity of Changes in Total-Body Na^+ ($\Delta TBNa^+$) and Its Association with Urinary Aldosterone and Glucocorticoids

(A) Individual time series of $\Delta TBNa^+$, body weight, ECW, and blood pressure in a single Mars520 subject across the salt intakes. An infradian rhythm of $\Delta TBNa^+$ is apparent, which is uncoupled from changes in body weight, ECW, and blood pressure.

(B and C) (B) Investigation of the rhythmical interrelation between these changes in $\Delta TBNa^+$ with UAldoV excretion, with UFFV excretion, with UFEV excretion, and with UFE/UFF ratio in the same subject. Ultra-long-term variability in $\Delta TBNa^+$ was associated with parallel changes in UAldoV and with inverse changes in UFFV. In contrast, we found no significant correlation between changes in $\Delta TBNa^+$ and systolic morning blood pressure (C) in the same subject.

(D) Individual time series analysis of the interrelation between UAldoV, UFFV, UFEV, and UFE/UFF ratio. UAldoV and urinary glucocorticoid excretion were inversely correlated in all Mars520 subjects. UAldoV was associated with parallel direct increases in UFE/UFF ratio in most of the subjects, suggesting enhanced 11β-HSD2 activity with increased UAldoV. For each subjects' individual data, see also Figure S3.

homeostatic mechanisms are primarily operative to prevent variability in extracellular Na^+ and volume content, despite variable salt intake (Cannon, 1929, 1932; Pitts, 1974; Smith, 1959).

However, our experiments featured an additional, hithertofore unappreciated component of Na^+ metabolism in humans, namely endocrine-driven generation of variability in urinary Na^+ excretion and total-body Na^+ content in response to a constant salt intake. We found that UNaV in humans was characterized by weekly (circaseptan) rhythmic change patterns, which were not related to salt intake but instead were paralleled by inversely changing patterns in UAldoV and directly changing patterns in UFFV.

Because our subjects received time information from the outside world, we cannot conclusively prove the existence of endogenous free-running weekly cycles in our subjects. However, the almost complete loss of all other variability in UNaV and UAldoV except for sharp circasemiseptan and circaseptan frequencies that were precisely synchronized with days on which the subjects had to serve a nightshift, resemble endogenous oscillatory entrainment to light-dark *Zeitgebers* (Pittendrigh, 1981). Halberg and colleagues have demonstrated that endogenous circaseptan and circasemiseptan rhythms exist in algae (Schweiger et al., 1986). Separating isolated human subjects from all possible factors that could act as *Zeitgebers* (Aschoff, 1965) for months or years to prove free-running circaseptan periodicity would probably require a real trip to Mars.

The periodicities we observed are in line with earlier observations on ketosteroid excretion in a human subject under daily life conditions (Halberg et al., 1965). Sealey et al. observed similar day-to-day oscillations in UAldoV at a fixed high-salt intake (Sealey et al., 1972). In line with previous studies under daily life conditions (Luft et al., 1982), our findings suggest that repetitive daily urine samples are preferable for estimating dietary salt consumption. A particularly interesting animal study is germane to our findings (Uezono et al., 1987). The investigators fed Dahl salt-sensitive and salt-resistant rat strains 8% salt diets and

collected urine at 4 hr intervals for 30 days and uncovered a circaseptan variation in Na^+ excretion at high-salt intake. We have not solved the molecular basis for the weekly rhythmic UNaV, UAldoV, and UFFV excretion, let alone the interrelation analysis of the infradian rhythmically changing patterns we described. Transfer of our observation in humans to bench research in animal models with circadian clock-gene disruption may help unraveling, whether or not these clocks are also instrumental in the generation of aldosterone-driven infradian rhythms in electrolyte and water excretion (Doi et al., 2010).

Rhythmic retention and excretion of Na^+ resulted in additional long-term variability of total-body Na^+ that was not related to BW, blood pressure changes, or salt intake, suggesting that Na^+ was rhythmically stored and released from the body without parallel changes in water content. We have addressed molecular mechanisms of Na^+ storage in earlier animal experiments and are necessarily pursuing these studies further (Machnik et al., 2009, 2010). To the best of our knowledge, the infradian rhythm we observed in total-body Na^+ has not been reported previously. Our crosscorrelation time series analyses suggest that when the total-body Na^+ infradian rhythm peaks, UAldoV is at zenith, UFFV is at nadir, and 11β-HSD2 activity is high. We are not aware of earlier reports regarding a significant inverse crosscorrelation between aldosterone and cortisol excretion. The enzymes responsible for aldosterone synthesis (CYP11B2) and cortisol synthesis (CYP11B1) are highly homologous, and investigators have considered the possibility that aldosterone synthesis blockade could also suppress cortisol release (Andersen et al., 2012). Our findings suggest that both aldosterone and cortisol are regulators of Na^+ metabolism in man and exert functional antagonism for the body's electrolyte mass balance. When UNaV was increased or reduced in response to dietary changes in salt intake in our step change experiments, aldosterone was uniformly associated with urinary Na^+ retention, while cortisol was associated with enhanced Na^+ excretion. Similarly, the long-term changes in total-body Na^+, which occurred independent of salt intake, were associated with parallel changes in UAldoV and inverse changes in UFFV. This finding is not in line with the idea that both aldosterone and cortisol regulate body Na^+ balance synergistically by sharing the mineralocorticoid receptor. However, in agreement with our observations is a recent study suggesting that high-salt intake downregulates 11β-HSD2 (Lienhard et al., 2012). Our study supports the idea that high cortisol levels are operative in steady-state regulation of salt excretion. The mechanisms by which elevated cortisol levels may enhance urinary Na^+ excretion in man remain unclear.

We return to our initial questions: are salt intake and daily UNaV related; are total-body Na^+ and extracellular water related; and is total-body Na^+ necessarily a function of salt intake, as suggested by steady-state theory for salt and water homeostasis? Had we terminated the study 1 week after the step change in salt intake, we would have answered each question with "yes" and would have confirmed preconceived notions. However, our long-term time series analysis leads us to answer the same questions with "no." While the results of our long-term balance experiment support the steady-state concept of Na^+ balance, our findings also suggest that current physiological concepts on Na^+ metabolism and their clinical implications may underestimate the time frame and variability by which such steady state is achieved. The astounding degree of variability in UNaV that we observed in our studies questions the widely accepted notion that a single 24 hr UNaV sample is a valid measure of daily salt intake in humans, and that body Na^+ content is steadily maintained within very narrow limits via rapid urinary excretion of dietary salt. In addition, we uncovered complex rhythms and suggest unappreciated functions for corticoid hormones. We believe these findings could lead to further hypotheses concerning salt intake, Na^+ regulation, and blood pressure that can be addressed under daily life conditions, in part with magnetic resonance imaging techniques that we introduced earlier (Kopp et al., 2012).

EXPERIMENTAL PROCEDURES

Subjects and Environmental Conditions

In the Mars105 and Mars520 space flight simulation studies, 12 young male healthy volunteers provided written informed consent after due approval to spend 105 and 520 days in an enclosed habitat consisting of hermetically sealed interconnecting modules. The crews lived and worked like cosmonauts on the international space station. Environmental factors were maintained constant. Microgravity was not simulated. Anthropometric data of each participant were determined (Table S2). The isolation study was conducted at the Institute for Biomedical Problems in Moscow and approved by several ethical boards of the Russian Federation and European Space Association authorities. Written informed consent was obtained and all studies were done as outlined in the Declaration of Helsinki.

Diets and Ultralong Balance Approach

Nutritional intervention took place during the complete Mars105 study and the first 205 days of the simulated flight to Mars during the Mars520 study. Dietary salt reduction during Mars105 was performed stepwise from 12 g to 9 g to 6 g salt per day (Supplemental Experimental Procedures, Table S3, and Figure S4). Due to the longer duration of the Mars520 study, we could re-expose the subjects to 12 g salt per day after salt depletion to 6 g salt per day. We maintained each salt intake level constant for at least 29 days. All other nutrients in the diet were maintained constant throughout the study. The subjects collected all their urine for a 24 hr period and every day transferred four aliquots of 10 mL, which then were frozen for later analysis. We defined inadequate caloric intake and lack of accuracy in daily Na^+ balance as dropout criteria. Accuracy was evaluated by individual average daily urinary Na^+ excretion (UNaV) as percentage of actual daily Na^+ intake. We excluded subjects from analysis when their average UNaV was repeatedly less than 80% of Na^+ intake or when the subjects did not adhere to our daily menu plans. Only this strict focus on experimental accuracy allowed us to implement a long-term balance approach. Because two subjects did not comply with these criteria (Supplemental Experimental Procedures and Table S4), we had to exclude them from further analysis. Analytical methods, blood pressure, and body impedance techniques are given in the Supplemental Experimental Procedures.

Statistical Analysis

Comparison of means was performed either by paired t test or by multivariate analysis (general linear model) to test for the interaction of multiple effectors on various parameters. Data are given as averages ± SD. Time series analysis to test for rhythmical changing patterns was performed by power spectral density estimation using a periodogram with a rectangular window function. For analysis of interrelation between the various parameters, we analyzed our data by crosscorrelation to detect time-shifted interrelations between rhythmical components within the time series. Except for power spectral analysis, data analysis was performed with SPSS software (Version 20.0).

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article at http://dx.doi.org/10.1016/j.cmet.2012.11.013.

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