Urea and Osmotic Excretion in Rats Exposed to Chronic Centrifugation

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Background: A reduction in vasopressin was attributed to the initial diuresis reported in rats exposed to chronic centrifugation. However, it was suggested that urea may also contribute an osmotic component to this observed diuresis. ***Hypothesis:*** Increased urea excretion will contribute to osmotic excretion during chronic centrifugation, which may be partly responsible for the initial diuresis previously observed. ***Methods:*** Eight Sprague-Dawley rats were centrifuged (12 d at −2Gx) and eight were used as a control group. Daily urine samples were collected and an aliquot measured for excreted solutes and aldosterone. ***Results:*** Urine volume was elevated over the first 7 d of centrifugation with a peak on day 4. Urea and osmotic excretion were elevated over the first 5 d. Excreted Na^+ was elevated on days 1 and 2, which coincided with an increase in excreted aldosterone over the first 3 d of centrifugation. Urea excretion accounted for up to 54% of the increase in osmotic excretion during the initial portion of centrifugation suggesting that urea was, in part, responsible for the observed increase in urine output despite a reduction in water consumption. Following the first day of centrifugation, aldosterone appears to regulate Na^+ as suggested by the reduction in Na^+ excretion between days 2 and 3 when aldosterone excretion was elevated. ***Conclusions:*** It would appear that centrifugation induced an acute increase in protein catabolism as indicated by the increase in urea excretion which resulted in an increase in obligatory water loss. This increased diuresis may have acute consequences on the hydration state of centrifuged rats.

Keywords: aldosterone, GFR, hypergravity, osmotic diuresis, sodium excretion.

URINE IS COMPOSED of an osmotic and free water component. Osmotic clearance is the obligatory water loss associated with the excretion of solutes, whereby the composition of the solutes can consist of electrolytes, nitrogenous byproducts of protein catabolism, proteins, and steroids. Because each of these components, contributes to the osmotic pressure of the urine, the regulation of urinary osmolality relies on the specific regulation of each individual species. For example, aldosterone is the most potent mineralocorticoid that regulates plasma Na^+ and, indirectly K^+ (13). Therefore, alterations in urinary osmolality attributed to changes in Na^+ excretion may be reflected by changes in excreted aldosterone. Free water is the fraction of urinary water which is void of solutes and is usually regulated by arginine vasopressin (AVP) (4,22). Therefore, quantifying the contributing factors to urine production involves an examination of osmotic and/or free water clearance and the determination of the parameters responsible for osmotic and/or free water clearance.

Exposure of rats to centrifugation at both 1.7 and 3 G resulted in an increase in urine output which was attributed to a decrease in plasma AVP (4), thereby, increasing free water clearance, and not to an osmotic diuresis (5). This initial diuresis was observed despite a reduction in water consumption (3). However, the contribution of osmotic clearance and it's components to a potential osmotic diuresis during chronic centrifugation have not been examined. Mechanisms involved in the regulation of electrolyte homeostasis in mammals exposed to chronic centrifugation are also not well defined. Urea and other hormones, besides AVP, may be involved in the regulation of the renal handling of water and electrolytes as has been suggested (4,5).

Electrolyte homeostasis could potentially be affected by chronic centrifugation as indicated by initial decreases in food (3,11,16,23) and water intake (3,25). Following the first 24 h of centrifugation at both 1.7 G and 3.0 G, a decrease in excreted solutes and in the concentrations of urinary Na^+ and K^+ was exhibited in rats (5). This reduction in urinary Na^+ and K^+ was attributed to a simultaneous decrease in food intake since the excretion of electrolytes for pair-fed controls paralleled that for centrifuged rats (5). Although the authors suggest that osmotic excretion may have resulted from an increase in nitrogen compounds such as urea and creatinine, neither compound was quantified during exposure to chronic centrifugation. Therefore, the contributions of urea to solute excretion were examined in the present study.

The previously reported reduction in Na^+ excretion may suggest that the renin-angiotensin-aldosterone sys-

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tem (RAAS) was stimulated since decreasing Na^+ or increasing K^+ plasma levels results in an elevation of plasma aldosterone via RAAS (13,21). Therefore, changes in electrolyte excretion may be reflected by corresponding alterations in excreted aldosterone levels. However, the relationship between aldosterone and excreted electrolytes has yet to be examined in chronically centrifuged animals.

The objectives of this study were to determine the contribution of urea to osmotic excretion in rats exposed to chronic centrifugation, and examine the relationship between aldosterone and excreted Na^+ in rats exposed to chronic centrifugation. We hypothesized that increased urea excretion contributes significantly to the osmotic excretion during chronic centrifugation, which may be partly responsible for the initial diuresis previously observed.

METHODS

The animal use protocol was reviewed and approved by the NASA Ames Research Center Animal Care and Use Committee. This protocol adhered to the NRC's Guide for the Care and Use of Laboratory Animals. Vector and direction of the centrifugal force in the present study were $-2G_x$ as previously described (6,19,20), and force is referred to as 2 G throughout the paper. The present study used a gravitational force of 2 G for two reasons. First, the International Space Station will house a centrifuge facility which will be capable of producing an acceleration force of up to 2.0 G (10). Second, 2 G on Earth when compared with 1 G controls results in a 1 G delta which is similar to the 1 G delta experienced by spaceflight subjects between Earth and space (1 G to 0 G to 1 G). This 1 G delta allows for a comparison with spaceflight data.

Animals and Metabolic Cages

Control and centrifuged groups each consisted of eight male, specific pathogen-free Sprague-Dawley rats (50 d old) (Simonsen Laboratories, Gilroy, CA) weighing 223.9 ± 2.3 g and 223.9 ± 1.6 g, respectively, at the initiation of the study. Following their arrival at Ames Research Center, animals were housed two per vivarium cage for 4 d prior to being randomly assigned to one of the two study groups. While in their vivarium cages, animals were maintained on rat chow pellets (Diet #5012, Purina Mills, Brentwood, MO) and provided tap water ad libitum. As provided by the manufacturer, Na^+, K^+, Ca^{2+}, total phosphorous, protein, fat, and fiber contents of the diet were 0.28, 1.08, 1.01, 0.74, 22.5, 4.5, and 4.6%, respectively.

Once assigned to a treatment group, rats were switched to powdered Purina rat chow (Diet #5012) and housed individually in metabolic cages for the duration of the study. Design of the metabolic cage was similar to that previously published (9) with a slight modification in the height. Briefly, each metabolic cage was equipped with a grated bottom with a funnel underneath it. Connected to the funnel was a separator to which two 30 ml conical tubes were attached. Feces would collect in one tube and urine in the other. Metabolic cages for both groups of animals were placed in ventilated cabs large enough to hold two metabolic cages. Individual fluorescent lighting fixtures were attached to the cab's clear Plexiglas tops. Lighting was electronically controlled and set on a 12 L:12D light cycle. Cabs on the centrifuge were fastened to gimbaled platforms allowing the cages to swing out during centrifugation. Gimbaled platforms were mounted to the centrifuge's arms. Cabs housing the control animals were identical to those on the centrifuge and were placed on shelving units in the centrifuge room. Control animals were kept in the centrifuge room for the duration of the study so that all animals were exposed to the same noise, lighting, and temperature.

Prior to the study, filled water bottles in metabolic cages were tested for leaks by running the centrifuge for 10 min at the same G-load as that used in the study. Water bottles also had a lixit designed not to leak during centrifugation, therefore assuring the accuracy and reliability of the water consumption measurements and the collected urine volumes. Also, animals were adapted to obtaining water from water bottles with these lixits prior to centrifugation.

Procedures

Animals were given a 72-h period to adapt to their metabolic cages and powered chow prior to the initiation of centrifugation. Animals were provided with tap water ad libitum for the duration of the study. Centrifuged animals were then spun for 12 d on a 7.32-m (24-ft) diameter, 10 armed centrifuge at 2 G (25.21 rpm). Animals were allowed to maintain their natural tetrapod position during centrifugation. Every morning at 0730 hours the centrifuge was stopped for 1 h to record measurements on all animals. Body mass (BM), food and water consumption (W), and urine output (V) were determined daily. Following the 12th d of measurements, animals were anesthetized with methoxyflurane (Metafane, Pitman-Moore, Inc., Mundelein, IL) and approximately 5 ml of blood from each animal were drawn into a 30 ml syringe from the descending aorta and transferred into one heparinized and one EDTA treated vacutainer.

Analyses

Blood samples were centrifuged for 15 min (1400 G at 4°C) and plasma was collected and frozen at −20°C for analyses of aldosterone, creatinine, Na^+, K^+, osmolality, and urea. Daily urine samples were analyzed for the same parameters as plasma with the addition of total protein. Excreted values (urine concentration of the substance multiplied by urine volume) were determined. Values were then adjusted by dividing by the amount of excreted creatinine and expressed as mmol substance · mmol creatinine^{-1} (except aldosterone: pmol · mmol creatinine^{-1}, and total protein: g · mmol creatinine^{-1}). Except for "excreted creatinine," reference to "excreted" values refers to values adjusted for excreted creatinine.

Aldosterone was extracted from urine with ethyl acetate, and samples were measured in duplicate with a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). Inter-assay and intra-assay coefficients of variation were 8.0% and 2.7%, respectively. To illustrate that excreted aldosterone levels accurately reflected circulating levels, excreted levels on the last day were correlated with plasma concentrations measured from samples taken on the last day. Creatinine, electrolytes, total protein and urea were measured on a clinical auto-analyzer (Roche Diagnostics, Somerville, NJ). Osmolality was determined using a freezing point osmometer (Fiske, Norwood, MA).

Statistics

Means for the centrifuged group were compared with the control group by two way analysis of variance corrected for repeated measures over time. Fisher's PLSD test was administered post-hoc if significance was found. Student's *t*-test was used to analyze plasma constituents taken on the last day of centrifugation. A simple regression was conducted to determine the correlation between plasma and excreted aldosterone. Significance of the correlation was determined by a Fisher's r to z test. All statistical tests were performed on StatView for the Macintosh (1). Means ± SE are reported and were considered significantly different at $p < 0.05$.

RESULTS

Food and Water Consumption, and Urine Volume

Analysis of variance comparisons between control and centrifuged groups for body mass, relative food consumption (g · 100 g BM ^{1}), water consumption and urine volume revealed significant group × time interactions. Centrifugation chronically reduced body mass while relative food consumption was reduced for the first 9 d of centrifugation (**Fig. 1**). Centrifuged rats significantly decreased water consumption during the first 2 d of centrifugation, returning to control level on day 3. Water consumption was elevated in centrifuged rats from day 4–6 (**Fig. 2**). Urine output was significantly higher in centrifuged rats between days 1 and 6 with a peak on day 4 (Fig. 2).

Plasma and Urine Analyses

Plasma parameters did not differ significantly between the two groups following 12 d of centrifugation (**Table I**). Significant group × time interactions were determined for excreted creatinine, aldosterone, Na^+, total protein, urea, and osmotic excretion. Excreted K^+ did not demonstrate a group × time interaction. Excreted creatinine (mmol · d^{-1}) was significantly reduced on days 2, 7, and 9–12 of centrifugation (**Fig. 3**). Excreted total protein was significantly elevated on day 1, returned to control level on day 3, and remained significantly reduced from days 6–12 of centrifugation (Fig. 3). Excreted urea and osmotic excretion were significantly elevated over the first 5 d of centrifugation (**Fig. 4**). Excreted Na^+ was elevated on the first 2 d of centrifugation, returning to control levels on day 3 (**Fig. 5**). Excreted K^+ for control and centrifuged groups was not significantly different over the 12 d of centrifugation (0.370 ± 0.009 and 0.369 ± 0.012 mmol · mmol creatinine^{-1}, respectively). Following the first 24 h of centrifugation a prominent spike in excreted aldosterone was observed. Levels remained elevated on days 2 and 3 of centrifugation, decreasing below control levels on days 5 and 6, and returning to control levels for the remainder of the centrifugation period (Fig. 5). Excreted and plasma aldosterone were positively correlated ($R = 0.705$; $p = 0.0024$; $df = 14$) on the last day of centrifugation.

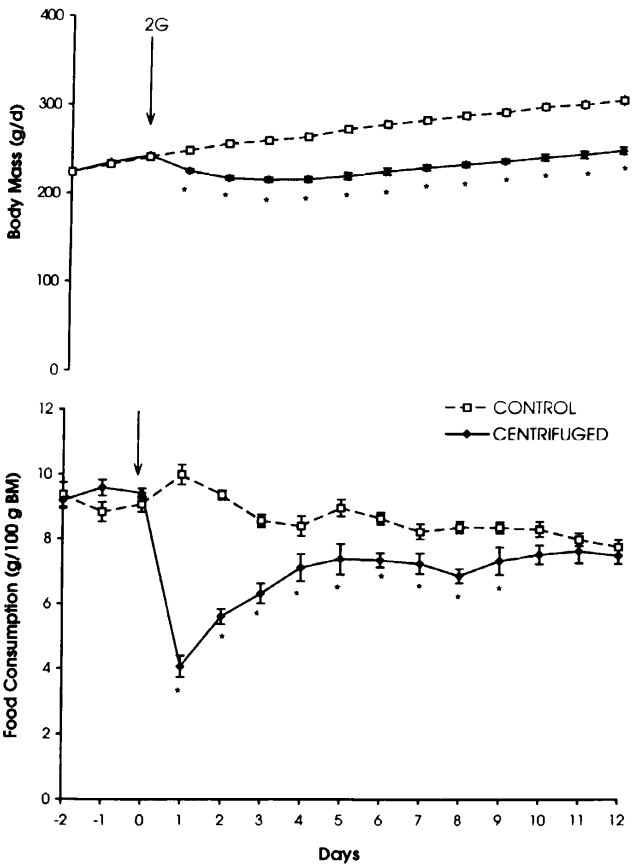

Fig. 1. Daily body mass (BM) and relative food consumption (g · 100 g BM^{-1}) for control and centrifuged rats. 2G refers to the day when centrifugation was initiated. Means (±SE); * ($p < 0.05$).

DISCUSSION

As predicted by the results of previous centrifugation studies (11,16,25), a reduction in body mass associated with an initial reduction in food consumption was observed in rats in the present study. Also, the immediate decrease in water consumption and diuresis is similar to that reported for rats exposed to chronic centrifugation at both 1.7 G and 3.0 G (3). However, the present study provides evidence that an increase in urea excre-

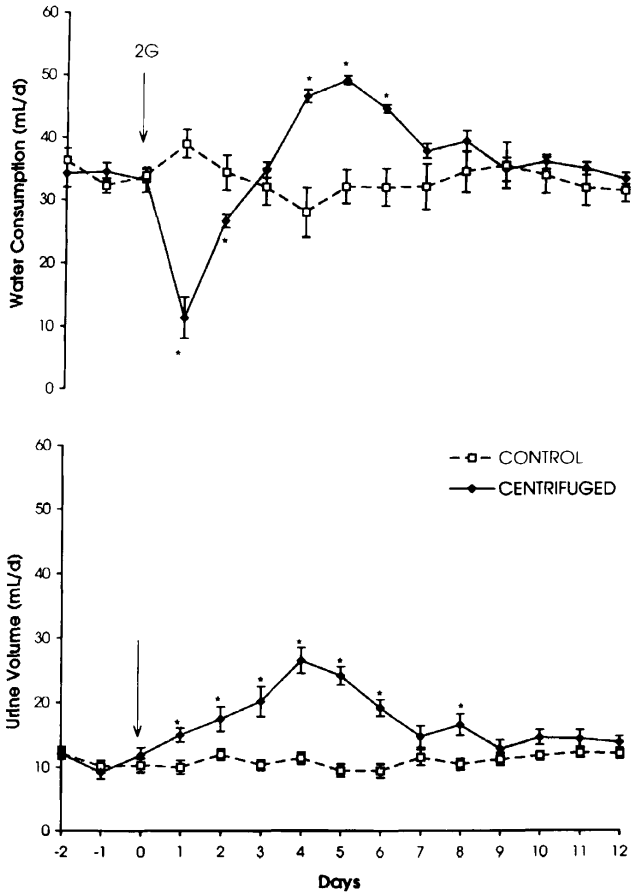

Fig. 2. Daily water consumption and urine volume for control and centrifuged rats. 2G refers to the day when centrifugation was initiated. Means (±SE); * ($p < 0.05$).

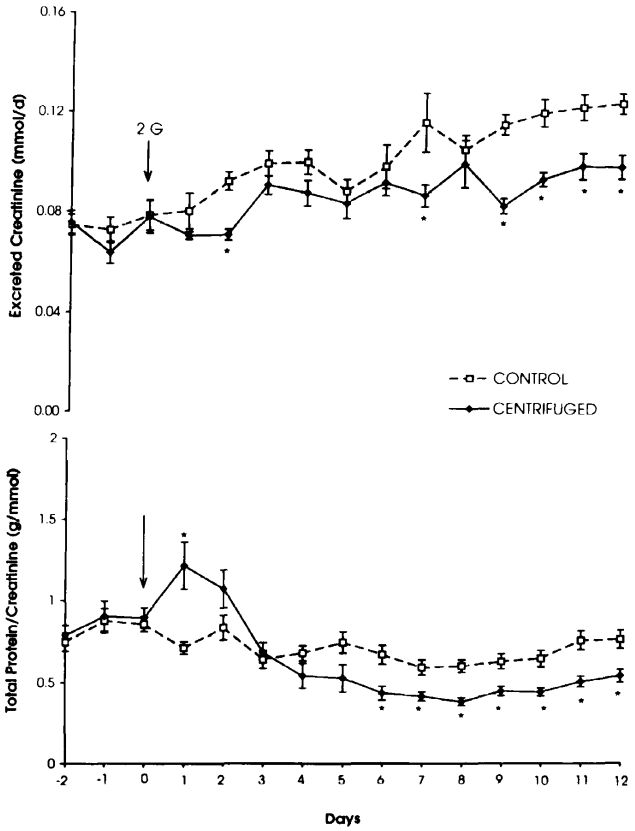

Fig. 3. Excreted creatinine, and excreted total protein adjusted for excreted creatinine for control and centrifuged rats. 2G refers to the day when centrifugation was initiated. Means (±SE); * ($p < 0.05$).

tion most likely contributed to the observed increase in osmotic excretion, and thus, to the immediate diuresis. The acute natriuresis associated with the elevation in aldosterone excretion, is reported for the first time for any chronically centrifuged animal.

A general consequence of chronic centrifugation is an initial reduction in food consumption resulting in a decrease in body mass (16,19,23) and electrolyte excretion (5). A reduction in food consumption induced by centrifugation may have led to an initial increase in protein catabolism as indicated by the elevated excretion of total protein and urea at the onset of exposure to hypergravity. Reduced food intake has been shown to acutely induce protein catabolism in rats resulting in an increase in urea excretion (7). Excreted urea remained elevated for the first 5 d of centrifugation which suggests that protein catabolism was prominent during this period. Increased protein catabolism leads to an increase in circulating nitrogen (7,12), which ultimately results in an increase in obligatory water loss to void the excess nitrogen load (12). This cause and effect association between increased protein catabolism and increased obligatory water loss appears to be the sequence of events responsible for initiating the observed diuresis.

TABLE I. PLASMA VALUES FOLLOWING 12 D OF CENTRIFUGATION.

	Control	Centrifuged
Osmolality (mOsm · L^{-1})	299.6 ± 3.2	303.0 ± 1.6
Na^+ (mmol · L^{-1})	142.9 ± 0.7	143.9 ± 0.4
K^+ (mmol · L^{-1})	5.0 ± 0.1	5.2 ± 0.2
BUN (mmol · L^{-1})	3.64 ± 0.13	3.54 ± 0.20
Creatinine (mmol · L^{-1})	0.050 ± 0.002	0.046 ± 0.001
Aldosterone (pmol · L^{-1})	769.0 ± 158.5*	530.4 ± 78.2

* Calculated from n = 7 due to a lack of sample.
No significant differences ($p < 0.05$) were determined for any of the means (±SE) reported.

The tubular movement of urea is also associated with the movement of water (17). Therefore, the increase in urea excretion over the first 5 d of centrifugation, which coincides with the increase in the urine output over the same period, suggests that urea excretion was partly responsible for the observed diuresis. Centrifugation has been shown to reduce AVP activity and the concentration of urine osmolality, resulting in free water diuresis (4). Free water clearance is increased when AVP concentrations are reduced, resulting in an increase in urine volume (22). Therefore, the observed increase in urine volume in the present study was likely the result of both an osmotic diuresis induced by increased urea

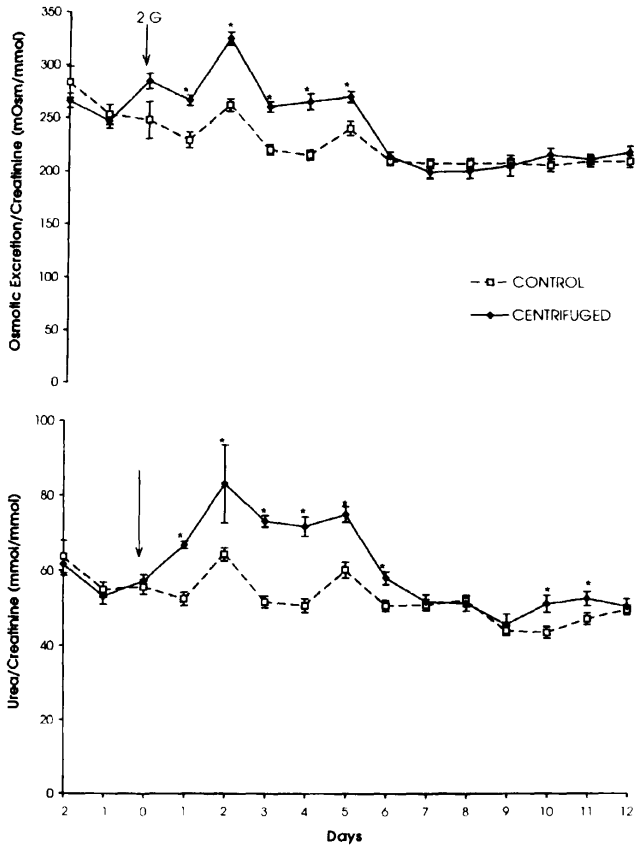

Fig. 4. Excreted urea and total solutes adjusted for excreted creatinine for control and centrifuged rats. 2G refers to the day when centrifugation was initiated. Means (±SE); * ($p < 0.05$).

excretion and free water diuresis induced by a reduction in AVP as previously reported (4). Although attenuated, a similar diuresis and osmotic excretion were observed in post-spaceflight rats on returning to Earth (24) suggesting that the renal effects of approximately + 1ΔG associated with centrifugation at 2 G and with return to Earth following spaceflight may be parallel.

However, this diuresis may not be a consistent response to centrifugation in all subjects. Chronic centrifugation at forces between 2 and 6 G has been shown to reduce urine output in mice (25) and in rats (18,20). However, the responses observed in mice may be a species specific phenomenon. The previous studies used Wistar rats which suggests that renal responses exhibited by rats may be strain specific since Sprague-Dawley rats were used in the present study. A decrease in GFR has been reported as a possible mechanism for the antidiuresis exhibited in positively accelerated animals (6), which may be responsible for the previously observed reductions in urine output in response to the initiation of centrifugation.

Increased urea excretion contributed to the increase in osmotic excretion in the present study. During centrifugation at 1.7 G, osmotic excretion was greater than that of pair-fed controls despite a decrease in excreted electrolytes which led the authors to suggest that the increase in osmotic excretion may have been the result of an increase in a nitrogen source such as urea (5). The elevation in urea excretion in the present study contributed between 30 and 54% to the observed increase in osmotic excretion, while the natriuresis observed during the first 2 d of centrifugation contributed 12 and 7%, respectively, to the increase in osmotic excretion.

Renal reabsorption of Na^+ induced by aldosterone and the stimulation of aldosterone release by angiotensin II in response to reduced circulating levels of Na^+ have been well documented in mammals (13,21). In the present study, centrifuged animals apparently maintained elevated excretion of aldosterone during days two and three in response to the reduction in Na^+ reabsorption, or increased natriuresis. Although this increase in Na^+ excretion despite elevated excretion of aldosterone may appear to be paradoxical, this response is identical to that observed in post-spaceflight rats on the first day on return to Earth (24). As in the post-spaceflight rats (24), excreted aldosterone in the present study returned to control levels one day after excreted Na^+ levels returned to control values. Because the plasma and excreted aldosterone levels measured on the last day of the study were significantly correlated, the excreted aldosterone levels provided an accurate representation of circulating concentrations. Therefore, increased Na^+ excretion may have lead to a

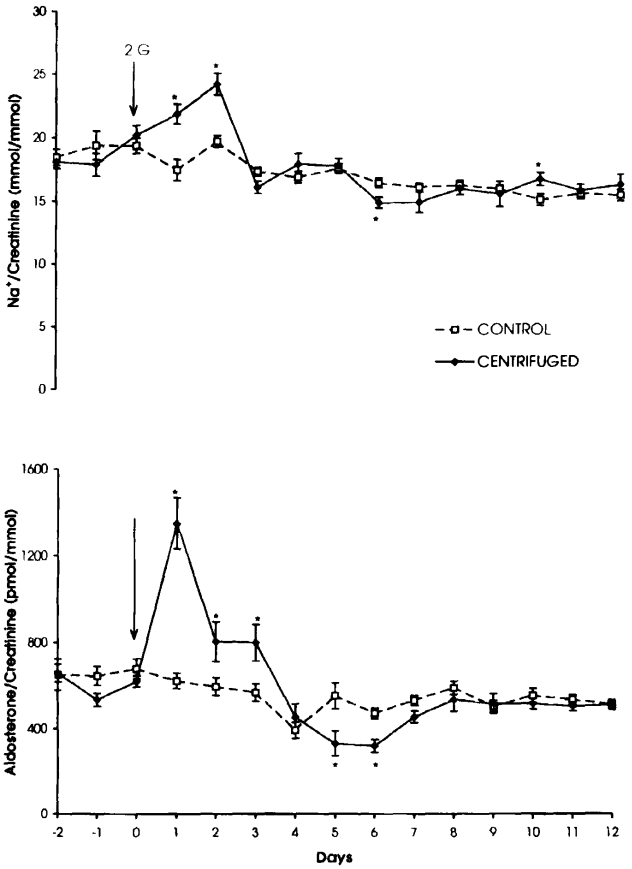

Fig. 5. Excreted sodium and aldosterone adjusted for excreted creatinine for control and centrifuged rats. 2G refers to the day when centrifugation was initiated. Means (±SE); * ($p < 0.05$).

reduction in circulating levels and, thus, stimulating aldosterone release during days two and three of centrifugation. Also, the natriuretic actions of ANP (8) may have contributed to the observed natriuresis in the present study, however the effects of centrifugation on ANP have yet to be examined.

In mammals, ACTH-induced gluco- and mineralocorticoid release in response to emotional disturbance and stress has been well documented (2). Plasma corticosterone concentration in rats was increased within 0.5 h of centrifugation (15) indicating a neuroendocrine stress response. Therefore, the 2.5- and 3-fold increases in excreted aldosterone and corticosterone (14), respectively, on the first day of centrifugation can be attributed to a similar neuroendocrine stress response as previously indicated. However, this stress response is short-lived and is abated by the third day of centrifugation as indicated by similarities between centrifuged and control animals in the rate of gain in body mass and of food consumption following day 3 in the present study. Normal rates of gain in body mass and in food consumption have been suggested as indices of "non-stress" conditions (23).

Rats chronically centrifuged at 1.7 G and their pair-fed controls exhibited a reduction in Na^+ and K^+ excretion for at least the first 5 d (5). This reduction in electrolyte excretion was attributed to the decrease in dietary electrolyte intake. In the present study, the excretion of Na^+ was elevated over the first 2 d despite a decrease in food consumption, which suggests that this natriuresis was the result of an increase in GFR or a decrease in tubular reabsorption or both. Excreted creatinine, used as an indication of GFR, was reduced on day 2, which would typically result in a decrease in excretion, however Na^+ excretion was elevated during this period suggesting that tubular reabsorption of Na^+ was reduced. Estimating GFR by fractional excretion of creatinine, GFR in the centrifuged animals (2.1 ± 0.1 vs. 2.5 ± 0.1 $L \cdot d^{-1}$, respectively) was significantly lower at the end of the centrifugation period. Since the calculated GFR on the last day of centrifugation was lower in this group, the observed decrease in creatinine excretion during the last 4 d of centrifugation may reflect a decrease in GFR. If so, this reduction over the last 4 d may have been an important mechanism for maintaining ionic and osmotic homeostasis during this period since plasma values were similar after 12 d of centrifugation.

In conclusion, centrifuged animals exhibited a neuroendocrine stress response on the first day of exposure as indicated by 2.5- and 3-fold increases in excreted aldosterone and corticosterone levels (14), respectively. The natriuresis exhibited on the first 2 d of centrifugation was likely the result of a decrease in tubular Na^+ reabsorption which lead to the sustained elevation in aldosterone excretion on days 2 and 3 of centrifugation. Elevated excretion of total protein and urea at the onset of centrifugation indicated an acute increase in protein catabolism (7), which is most likely since food consumption was reduced. The increase in excreted urea probably contributed to a reported (5) and to the observed elevation in osmotic excretion, resulting in the increase in urine output. Therefore, increased mass loading such as the re-entry of spaceflight subjects to Earth may acutely induce protein catabolism which will result in an increased obligatory loss of water to remove the excess nitrogen. This increased diuresis could potentially affect the hydration state of these subjects, especially if the catabolism of lean tissue is not attenuated within a short period. The recently reported increase in urine output in post-spaceflight rats immediately following re-entry to Earth (24) is similar to the immediate diuresis on exposure to 2 G in the present study suggesting a synonymous response of Sprague-Dawley rats to $+ 1\Delta G$. Although the magnitude of the diuretic response to an increase of 1 G was different between the two studies, this difference in magnitude should help elucidate the renal response of rats to various gravitational loads, which is important if we continue to use this species as a model for gravitational studies.

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