Water balance in rats exposed to chronic centrifugation

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Ortiz, Rudy M., and Charles E. Wade. Water balance in rats exposed to chronic centrifugation. J Appl Physiol 89: 56–60, 2000.—Changes in gravitational load have been shown to alter renal function, which could potentially affect water balance. Therefore, the present study was conducted to determine the effects of chronic centrifugation on water balance. Eight Sprague-Dawley rats were centrifuged (12 days at 2 G), and eight rats were used as a control group. Water balance over the course of the study was determined by quantifying the percentage (%) of total body water (TBW; TBW/body mass) and water flux (water consumption – urine volume). TBW was estimated, by means of deuterium oxide dilution, before the study and after 3 days of centrifugation and by means of desiccation after 12 days of centrifugation. %TBW did not change in the centrifuged rats from initial levels or relative to controls over the course of the study. Differences between the sum of water consumption and sum of urine volume for the 12-day period were the same in both groups. Although an initial period of negative water balance was observed, the lack of a change in %TBW among the three measurement periods or in water flux over the 12 days of centrifugation suggests that water balance is not negatively affected as a result of centrifugation at 2 G.

deuterium oxide; hypergravity; lean body mass; total body water

INCREASED GRAVITATIONAL LOAD has been shown to affect renal function and total body water (TBW). Spaceflight subjects exposed to a + 1 G change on return to Earth are prone to postflight orthostatic intolerance (4) and increased diuresis (14, 23). Rats centrifuged over the range of 1.7–3.1 G have exhibited an immediate diuresis over the first 5–6 days of exposure (3, 15). However, whether increased diuresis induced by increased gravitational load results in a decrease in TBW and possible dehydration has not been established.

Because spaceflight data are limited and not readily attainable, Earth-based models have been developed to simulate microgravity (8, 10) and increased postflight gravity (5, 21) to address the effects of altered gravity on fluid balance. Centrifugation has been developed as a model to provide a better understanding of postflight effects. Alterations in renal function in rats after spaceflight (23) were similar to those reported for rats at the onset of chronic centrifugation (2, 15), providing an indication of the predictive value of centrifugation studies.

Fluid homeostasis could potentially be affected by chronic centrifugation. As indicated by the initial decrease in water (3, 15, 25) and food intake (3, 12, 15, 25) associated with centrifugation. In a variety of small mammals that were chronically centrifuged, percent body water was not altered (13, 18, 20) or increased (12), suggesting that hydration state was not ultimately compromised. Conversely, a decrease in water flux [water consumption (W) – urine volume (V)] in centrifuged rats (3) and mice (25) suggested that they were dehydrated as a result of centrifugation. Previous studies did not examine the effects of chronic centrifugation on TBW over the course of centrifugation within a group, which would provide insight to the changes in hydration state over time.

If changes in TBW are present during centrifugation, then alterations in lean body mass (LBM) and body fat may also result. LBM appears to increase in response to centrifugation (12, 18), suggesting an increase in TBW and/or a decrease in fat mass. Because previous studies have reported that body fat was reduced during (20) and after (12, 13, 18) centrifugation, changes in LBM may reflect alterations in fat metabolism.

Previous studies have addressed the problem of water balance during chronic centrifugation by examining the percentage (%) of TBW or water flux, but not both concurrently. Therefore, the present study quantified the effects of centrifugation on hydration state using two sets of independent measurements: TBW estimates and water flux rates. The objective was to examine the changes in %TBW (TBW/body mass) and water flux rates among the initial, early, and late periods of centrifugation at 2 G, and to determine whether hydration state was compromised during any of these periods. We hypothesized that water balance is compromised initially, as suggested by the reported diuresis (3, 15, 23, 25); however, the net effect is the maintenance of %TBW. In the present study, deuterium oxide (D2O) dilution was used to estimate TBW initially and on day 3 of exposure to chronic centrifugation.

METHODS

The animal use protocol was reviewed and approved by the NASA Ames Research Center Animal Care and Use Commit-
ter. This protocol adhered to the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Animals and metabolic cages. Control and centrifuged groups each consisted of eight male, specific pathogen-free Sprague-Dawley rats (50 days old) (Simonsen Laboratories, Gilroy, CA) weighing 223.9 ± 2.3 and 223.9 ± 1.6 g, respectively, at the initiation of the study. Animals were initially maintained on rat chow pellets (diet 5012, Purina Mills, Brentwood, MO) and provided tap water ad libitum. Once assigned to a treatment group, rats were switched to powder Purina rat chow (diet 5012) and individually housed in metabolic cages (9) for the duration of the study. Each metabolic cage was equipped with a grated bottom with a funnel underneath it. Connected to the funnel was a separator with two 30 ml-conical tubes attached. Feces would collect in one tube and urine in the other. Metabolic cages for both groups of animals were placed in ventilated cages large enough to hold two metabolic cages. Gimbaled platforms were mounted to the centrifuge’s arm, and cabs on the centrifuge were fastened to these platforms, allowing the cages to swing out during centrifugation. Cabs housing the control animals were identical to those on the centrifuge and were placed on shelving units in the centrifuge room. Individual fluorescent lighting fixtures were attached to the cabs’ clear Plexiglas tops. Lighting was electronically controlled and set on a 12:12-h light-dark cycle. 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, temperature, and airflow.

Centrifugation. Animals were given a 72-h period to adapt to their metabolic cages and the powered chow before initiation of centrifugation. Centrifuged animals were then spun for 12 days on a 7.32-m (24 ft)-diameter, 10-powered centrifuge at 2 G (25.21 rpm). Animals were allowed to maintain their natural tetrapod position during centrifugation. The centrifuge was stopped for 1 h every morning (7:30 AM) to record measurements on all animals. Body mass (BM), food consumption, W, and V were determined daily (15).

Isotope infusion and analysis. Before initiation of centrifugation, animals were dosed intraperitoneally with 1 g D₂O (99.9 atom%, Isotec, Miamisburg, OH). After day 3 of centrifugation, animals were redosed with 0.6 g D₂O (99.9 atom%, Isotec, Miamisburg, OH). After day 3 of centrifugation, animals were redosed with 0.6 g D₂O. Animals were redosed to quantify any changes in TBW during the initial 3-day period because previous data had shown that the greatest variability in water flux occurred during this time (3, 15, 25). From each animal, a 1-ml aliquot of the 24-h urine sample was collected daily and frozen for isotope analysis. Analyses for all samples were conducted at the end of the study. Deuterium was measured by 2H-to-1H isotope ratios of the hydrogen gas with a Delta-E gas/isotope mass spectrometer (Finnigan MAT, San Jose, CA). Details of the analytical procedures for isotope measurement and sample handling have been previously described (24).

Calculations. Isotope dilution was used to estimate %TBW and %LBM for control and centrifuged animals initially and after 3 days of centrifugation. In this study, isotopic dilution space (IDS) was used to estimate TBW. Instantaneous dilution of the isotope [D₂O at time zero (T₀)] in the body was calculated by standard regression of the natural log of the isotope concentration vs. time, assuming the density of D₂O was 1.105 g/ml at room temperature

\[
\text{IDS (l) = dose/1.105 \times [D}_2\text{O] at } T_0 - \text{background [D}_2\text{O]}
\]  

where dose was the dose (mg) of the isotope administered and [D₂O] is D₂O concentration. The redose IDS was calculated by using the measured concentration on day 2 as background

Redose IDS (l) = redose/1.105\times[D₂O] at T₀

where [D₂O] at T₀ is the instantaneous dilution of the isotope on day 3. %TBW was calculated as a function of BM

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\%\text{TBW} = (\text{IDS}/\text{BM}) \times 100
\]

%LBM was estimated using 73.3% hydration (determined in the present study)

\[
\%\text{LBM} = \%\text{TBW}/0.733
\]

Cumulative water flux difference was calculated as Σ from 1 to 3 (W₋V). Mean daily water flux was the average difference between W and V, each of which were previously reported (see Fig. 2 of Ref. 15). Total relative water flux was determined as Σ(W₋V)/100 g BM for the 12-day centrifugation period. Water flux calculations did not account for preformed water in the diet, insensible water losses, or metabolic water production.

Carcass analysis. After day 12 of measurements, animals were anesthetized with methoxyflurane (Metafane, Pitman-Moore, Mundelein, IL) and prepared for carcass analysis. Animals were shaved, the gastrointestinal tract was emptied, and carcasses were weighed to the nearest 0.1 g. The gastrointestinal tract was emptied by cutting open the stomach and removing its contents and by squeezing the remaining digesta out of the intestines. Once weighed, carcasses were freeze dried under a vacuum to constant mass, and TBW was determined as the change in mass. Body fat was then extracted from the desiccated carcass with petroleum ether. After the extraction of body fat, the fat-free carcass was ground, and the protein content of the powdered carcass was determined by standard Kjeldahl procedure (2). Values for each body component were then used to determine %TBW and %LBM. Percent hydration of LBM (%TBW/LBM) was determined from the direct measurement of LBM after 12 days of centrifugation. Direct carcass analysis was used to determine TBW and LBM immediately after the 12 days of centrifugation as opposed to isotopic estimation, because additional days after centrifugation would have been required to obtain urine samples for the calculations of TBW and LBM. The effects of centrifugation on TBW and LBM may have been masked by the acclimatization of the centrifuged animals to this postcentrifugation period, as previously indicated (22).

Plasma analysis. While the animals were anesthetized, ~5 ml of blood from each animal was drawn from the descending aorta into a 30-ml syringe and transferred into a heparinized vacuum tube. Two aliquots were taken into capillary tubes for hematocrit (Hct) measurements. Capillary tubes were spun on a microcentrifuge (10,000 rpm for 5 min), and Hct was read on a microcapillary reader (Damon/IEC, Needham Heights, MA). Blood samples were then centrifuged for 15 min (1,400 g at 4°C), and the plasma was collected and frozen at −20°C for analyses of total protein concentration, measured on a clinical auto-analyzer (Roche Diagnostics, Somerville, NJ). Plasma osmolality was determined using a freezing-point osmometer (Fiske, Norwood, MA).

Statistics. Means for BM, TBW, %TBW, LBM, %LBM, cumulative water flux difference, and daily water flux were compared between control and centrifuged groups by two-way ANOVA corrected for repeated measures over time. Fisher’s paired least significant difference test was administered post hoc if significance was determined. Student’s t-test was used to compare ΣW, ΣV, Σ(W₋V), plasma constituents, Hct, and %TBW/LBM. All statistical tests were performed.
using StatView software for the Macintosh (1). Means ± SE are reported and were considered significantly different at $P < 0.05$.

**RESULTS**

Due to poor dilution of isotope in one of the control animals, isotopic estimations for the control group were calculated from seven animals. BM was the same between groups initially but was reduced in the centrifuged group on day 3 of the study (Table 1). After 12 days of centrifugation, both groups exhibited a significant increase in BM, but the centrifuged group was ~19% lower than controls (Table 1). TBW and LBM were reduced in centrifuged animals after days 3 and 12 (Table 1); however, %TBW and %LBM were similar between groups over the course of centrifugation (Fig. 1). Means for %TBW/LBM (73.6 ± 2.6 and 72.9 ± 0.2%) and crude protein percentage (18.9 ± 0.3 and 22.2 ± 0.3%) were similar between the control and centrifuged groups, respectively, after 12 days of centrifugation. Plasma total protein (5.5 ± 0.10 and 5.6 ± 0.10 g/dl), plasma osmolality (300 ± 3.2 and 303 ± 1.6 mosmol/kgH$_2$O), and Hct (42 ± 1.0 and 43 ± 1.0%) measurements did not differ significantly between control and centrifuge groups, respectively, after 12 days of centrifugation.

Mean cumulative water flux difference did not result in a significant group × time interaction (Fig. 2). The slopes, or rate of gain, for the two mean cumulative water flux difference curves were similar (22.3 ± 0.2 and 22.0 ± 0.5 ml/d, for control and centrifuge, respectively). In the centrifuged animals, negative water flux was observed on the first day of centrifugation, and flux was significantly lower on days 2 and 3 (Fig. 3). Although $\Sigma W$ and $\Sigma V$, on a relative basis (ml/100 g BM), were significantly greater in centrifuged rats, the difference between $\Sigma W$ and $\Sigma V$ for both groups was similar (Fig. 3).

**DISCUSSION**

During the initial days of centrifugation, food and water consumption were reduced, resulting in a decrease in BM (15). This decrease appears to be a gen-

**Table 1. Body mass, total body water, and lean body mass of rats initially and after 3 and 12 days of centrifugation**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Centrifuged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_0$</td>
<td>241.7 ± 2.2</td>
<td>243.3 ± 2.0</td>
</tr>
<tr>
<td>$T_3$</td>
<td>259.3 ± 2.3†</td>
<td>216.6 ± 2.8§</td>
</tr>
<tr>
<td>$T_{12}$</td>
<td>307.0 ± 4.1†</td>
<td>256.7 ± 3.1*</td>
</tr>
<tr>
<td>TBW, ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_0$</td>
<td>168 ± 8</td>
<td>175 ± 3</td>
</tr>
<tr>
<td>$T_3$</td>
<td>177 ± 7</td>
<td>149 ± 8*</td>
</tr>
<tr>
<td>$T_{12}$</td>
<td>188 ± 6§</td>
<td>163 ± 2*</td>
</tr>
<tr>
<td>LBM, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_0$</td>
<td>217 ± 9</td>
<td>236 ± 3</td>
</tr>
<tr>
<td>$T_3$</td>
<td>253 ± 10</td>
<td>214 ± 11*†</td>
</tr>
<tr>
<td>$T_{12}$</td>
<td>256 ± 5‡</td>
<td>223 ± 3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. $T_0$, before study; $T_3$, after day 3 of centrifugation; $T_{12}$, after day 12 of centrifugation; TBW, total body water; LBM, lean body mass. *Significant difference from control; †significant difference from preceding value in a group; ‡significant difference from $T_0$; $P < 0.05$ for all values with significant differences.
eral consequence of centrifugation (3, 5, 12, 13, 21, 22, 25). These changes in BM and consumption could, therefore, result in changes in TBW and water flux and, ultimately, hydration state. Previous studies have suggested that rodents were dehydrated (3) or that water balance was negatively influenced (25) as a result of chronic centrifugation because water flux between the control and centrifuged animals was different. In the present study, the reduction in BM, food consumption, and W (15) did not appear to affect hydration state over the course of the study. Also, the mean value determined in the present study for the hydration of LBM (73.3%) is similar to that originally reported for rats (17).

The lack of a change in %TBW among the three measurements in the centrifuged group indicates that hydration state was not altered over the 12-day period, despite a negative water flux value on the first day. The decrease in TBW pool size in the centrifuged animals between the initiation of the study and day 3 is consistent with the observed negative water flux; however, this decrease in pool size did not result in a change in %TBW. In monkeys, %TBW also did not change at 2 G, despite an 8.3% decrease in TBW pool size (16). The observed maintenance of %TBW is consistent with the previously reported lack of a change in %TBW in a variety of small mammals (hamster, rat, guinea pig, and rabbit) after 6 wk of centrifugation at 2 G (18). Greater gravitational loads (2.76 and 3.18 G) for longer periods (30 days) also did not significantly alter TBW content in Sprague-Dawley rats (20). Previous studies with small mammals have only measured TBW at the end of the centrifugation period; therefore, data from the present study provide a better understanding of the effects of hypergravity on hydration state during the early period of centrifugation.

The similarity in the difference between \( \Sigma W \) and \( \Sigma V \) for both groups in the present study indicates that centrifuged animals ultimately maintained water balance. Water flux data showed a negative value on the first day of centrifugation and reduced levels on days 2 and 3, which can be attributed to elevated osmotic excretion in the form of urea resulting from increased protein catabolism (15). This initial alteration in water flux suggests that an imbalance in fluid homeostasis is an acute episode in centrifuged animals since water flux rates for centrifuged animals reach control levels by day 4 and are maintained for the remainder of the study. Therefore, the difference in cumulative water flux does not accurately indicate a state of dehydration in centrifuged animals as previously suggested (3, 25). The fact that the slopes of the two curves were similar suggests that overall water turnover between the two groups was similar. Also, plasma total protein, osmolality, and Hct, which provide other indexes of an animal’s state of hydration, were similar after 12 days of centrifugation. These findings further substantiate our contention that animals are not ultimately dehydrated following centrifugation at 2 G. Therefore, water flux data in conjunction with TBW and plasma indexes of hydration provide a more accurate analysis of an animal’s state of hydration than water flux data alone.

The observed reduction in LBM after day 3 of centrifugation suggests that acute intermittent changes in body composition of centrifuged animals occurred. This decrease in LBM, along with a previously reported increase in excreted total protein (15), indicates an initial increase in protein catabolism (7, 15). Acute elevations in the concentrations of plasma triglycerides and free fatty acids (6) have also been reported for centrifuged rats, suggesting that fat oxidation was increased initially as well. Therefore, reduction in body mass (5, 13, 15, 20, 21) during the initial portion of centrifugation appears to be attributed to a decrease in both lean tissue and body fat (19). However, these reductions were probably in proportion to one another, thereby not affecting %TBW or %LBM, as observed in the present study. The ability of these animals to maintain %LBM is important because onboard centrifugation as been proposed as a countermeasure to the deleterious effects, such as muscular atrophy (5, 11), of prolonged exposure to microgravity. Therefore, centrifugation at 2 G appears to be synonymous to regular exercise, whereby %LBM is maintained, if not increased (12).
In conclusion, the present study provides data from two sets of independent measurements, TBW and water flux, to confirm that water balance is maintained in chronically centrifuged rats. The lack of a change in %TBW among the three measurement periods suggests that water balance is not negatively affected beyond the initial 3 days of exposure to centrifugation at 2 G. Although negative water flux was observed on the first day of centrifugation, centrifuged animals remained in positive water balance for the remainder of the study, suggesting a lack of dehydration. The similarities in plasma total protein, osmolality, and Hct at the end of the study also support the contention that centrifuged animals were not ultimately dehydrated. The absence of a change in %TBW and %LBM suggests that body composition as a percentage of BM was not significantly altered over the course of the centrifugation period. However, acute decreases in absolute amounts of lean tissue and fat mass likely occurred, resulting in the observed decrease in BM at the onset of centrifugation. Because centrifugation was previously thought to induce dehydration, these results may have significant implications on the use of onboard centrifuges during spaceflight as a countermeasure to some of the adverse effects of microgravity and may further assist our exploration of space.

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