**FINAL REPORT**

**NCC2-970**

# The Effect of Age in the Alteration in Fluid Balance of Rats in Response to Centrifugation

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## BACKGROUND & SIGNIFICANCE

With increasing age the ability of compensatory mechanisms of rats to respond to alterations in fluid and electrolyte balance maybe altered (14). These changes appear to be dependent on gender and strain, as well as, the age of the animals (9-11, 14, 26, 27). As rats age there is a slight reduction in the percentage of the total body mass that is water. This reduction in total body water (TBW) maybe accompanied by no change or an increase in plasma osmolality ( 12). The ability to sustain total body fluid and electrolyte balance occurs in the presence of a relative (adjusted for body weight) reduction in water balance (water intake minus urine production to) as urine production and water intake are altered in older animals compared to younger rats. In aged rats, greater than 10 months old, there is an increase in urine output (10, 14). The increase in urine production is associated with an increase in the excretion rate of solutes (5, 6, 10, 14, 23). In response to alterations in fluid balance, such as volume expansion, dehydration or hemorrhage, the ability of older rats to accommodate is attenuated due to a reduced thirst and delayed renal compensation (2, 7, 11, 23).

With onset of exposure to increased gravity levels by centrifugation there is an initial reduction in body mass (22, 25, 28). The decrease in body mass is sustained, compared to control rats, throughout the period of exposure. The reduction in body mass is, in part, due to an intial decrease in fluid intake and thus a negative water balance (3-5, 8, 16-18, 31). With chronic centrifugation, while body mass is decreased, the percentage of the mass that is water is maintained or slightly increased in rats (18, 21, 22). When an increase in percent total body water is reported it is associated with an increase in lean body mass. Following a period of hypohydration at the onset of centrifugation to rectify fluid balance there is a homeostatic adjustment, a transient increase in fluid intake and

reduction in urine output such that the net result is the maintenance of relative total body water (3-5, 16-18, 25, 31). The ability to make these changes, and maintain relative total body water, should be compromised in older rats exposed to centrifugation. Older animals have a larger body mass, and the response to centrifugation is mass dependent, and they have an attenuated ability to compensate alterations in water balance (11, 14 22, 24). To assess the effects of increased age on the response of fluid and electrolyte homeostasis to centrifugation at 2.0 G for 14 days we study young (growing) and mature male Sprague-Dawley rats.

## EXPERIMENTAL METHODOLOGIES

Before initiation of this study, approval was received from the Internal Animal Care and Use Committees (IACUC) at the University of California, Davis and at the National Aeronautics and Space Administration (NASA) Ames Research Center. The study conforms to NASA-Ames Research Center *Animal Users Guide* and the National Research Council guidelines for animal experimentation.

The study was conducted using 40 male Sprague-Dawley derived albino rats (Simonsen Laboratories, Gilroy, CA) and were either 1.5 months/150 g or retired breeders which were 8 months/450 g. Rats were received from the vendor at the University if California, Davis, Chronic Acceleration Research Facility (CARU). Each rat was weighed and housed (1 rat/cage) in standard vivarium cages for a three day acclimation period. The acclimation period was followed by surgery, a surgical recovery period, a baseline data collection period (days –7 to –1), and a test period of either centrifugation at 2.0 G or stationary control housing (days 1-14). Throughout the study the rats were maintained on a12:12-hour light dark cycle. The lights were turned on at 8:00 a.m. and off turned on at 8:00 p.m. Room temperature was maintained at 23 ± 1°C. Animals were fed a powdered diet (Purina Rat Chow no. 5012) and provided water *ad libitum*. Daily data collection and animal health checks occurred at 10:00 a.m. and lasted 45 minutes.

At the end of the acclimation period rats were surgically implanted with a telemeter (data not shown). Rats were allowed seven days to recover from the surgery. During the recovery period rats were housed in standard vivarium cages (1 rat/cage).

Body mass and food and water consumption were measured daily by weight (Ohaus, Florham Park, NJ). At the end of the recovery period rats were randomly selected and placed into one of four groups (n=8/group); 2.0-G mature, 1.0-G mature, 2.0-G young, or 1.0-G young.

During the baseline and test periods, rats were housed (1 rat/cage) in metabolic cages (58 x 36 x 33 cm). Food and water were provided on the side of the cage to prevent contamination in the urine and feces, which were collected below each cage. Water bottle lixits were modified to prevent dripping during the starting and stopping of the centrifuge. The 2.0-G and 1.0-G rats were housed in separate rooms, with the same temperature ( 23 ± 1.0 $^{O}$C) and lighting schedule (12L :12D). Body weights and food and water consumption were measured daily. Daily urine samples were collected from each rat. In each cage, urine was passed through a funnel, filtered by a urine and fecal separator, and collected into 30-ml conical tubes. To minimize evaporation, 1 ml of decahydronapthylene oil (Fisher Scientific) was added to each tube. At the end of the 24-hr collection period the tubes were brought to the lab, the samples were weighed (the scale was tared for the weight of the oil), the oil was removed, and the samples were centrifuged. The samples were frozen at –20 °C and shipped to NASA Ames Research Center for further analysis

Following the baseline period rats were exposed to either 14 days of centrifugation at 2.0G (27.75 RPM 5 ft radius) or kept in a 1.0 G stationary control environment. On day 1 of the test period each rat was given and intraperitoneal injection of doubly labeled

water O-18 [0.6 mg/kg], $D_2O$ [0.2 mg/kg] (Isotech, Inc., Miamisburg, OH). Daily data collection continued throughout the 14 day test period.

*Dissection.* A dissection was performed on each animal at day 14 of the test period. The rats were anesthetized with a minimal dose of Halothane and killed by decapitation. Trunk blood was collected from each animal, kept on ice, centrifuged, and frozen for further analysis. While collecting the trunk blood, careful attention was paid to prevent stomach acids from contaminating the samples.

*Analyses.* Plasma and urine electrolyte concentrations were measured by a Cobas Mirar (Roche Helios, Somerville, NJ). Plasma and urine osmolality were measured by freezing point depression (Fiske Associates, Norwood, MA ).

Glomerular filtration rate (GFR) was calculated on day 14 by the following formula:

$$\text{GFR (ml/day)} = (V \times U_{[\text{creatinine}]}) / P_{[\text{creatinine}]}$$

Where V is urine flow rate and P and U are the urine and plasma concentrations of creatinine.

The percent of the filtered load excreted (%FE) of electrolytes was calculated as:

$$\%FE = 100(V \times U_{[\text{electrolyte}]}) / (P_{[\text{electrolyte}]} \times \text{GFR})$$

Where V is urine flow rate, U and P are the urine and plasma concentrations of the electrolyte and GFR is the rate of glomerular filtration.

*Total Body Water.* Body composition analysis was performed at the University of California, Davis, Department of Nutrition. The analysis was performed on decapitated and eviscerated carcasses. Carcasses were analyzed for body water, fat, and

protein content. However, the protocol will refer only to the water analysis. Carcasses were placed in aluminum pans and frozen solid. Both the carcass and the pan were weighed before being placed in a freezer. The pan and frozen carcasses were placed in a freeze dryer for one week, then weighed and placed back in the freeze dryer for and additional 24-hours. This procedure was repeated until the weight had been secured within 1% of the weight from the previous day. This weight was referred to as the freeze-dried weight and was used to calculate total body water and % water. Total body water (TBW) and % water were determined from the following equations.

$$\text{Total Body Water (g)} = \text{carcass weight} - (\text{freeze dried weight} - \text{pan weight})$$

$$\% \text{ Water} = \text{TBW} / \text{ carcass weight}$$

*Statistics.* All statistics were performed using the Statistica software program (Statsoft, version 4.1, Tulsa, OK). Daily data were compared using a two-way ANOVA adjusted for repeated measure and Newman-Keuls pot hoc tests. The data obtained from the dissections were compared using a one-way ANOVA and Newman-Keuls post hoc tests. Differences with a $P \leq 0.05$ were considered significant.

## RESULTS

*Comparison of mature and young animals:* There were significant differences between the age groups prior to centrifugation (Table 1). The mature animals had a greater body mass and urine production rate with no difference between groups in water consumption. When adjusted for the differences in body weight the intake of water and production of urine of mature animals was significantly reduced. The rate of water turnover was therefore reduced in mature animals.

*Effect of centrifugation:* In 1.0 G control animals there was a significant increase in the body mass of both age groups over time (Fig. 1). In animals exposed centrifugation at 2.0 G there was a pronounced reduction of body mass, with the difference from control animals (11.6 % for mature and 9.4 % for young) sustained through out the study. The rate of change in body mass was similar in centrifuged and control animals after the second day of centrifugation for young rats and after the third day in mature animals. The initial decrease in body mass was associated with a reduction in absolute water intake (Fig. 2), which returned to levels similar to control animals by day 3 of centrifugation in young animals and by day 4 in mature rats. There were no significant changes in urine output during this period resulting in a reduction in water balance (Fig. 2). Water balance was stabilized within 3 days in the young animals and by 4 days of centrifugation in the mature animals.

Hydration status, assessed on day 14, was altered due to centrifugation (Table 2). Percent total body water was reduced in older animals and there was a significant increase

in both age groups due to centrifugation. Other indices of hydration status such as hematocrit, plasma protein concentration and plasma osmolality were not effected by centrifugation (Table 2). However, osmotic clearance rate in mature animals during centrifugation was reduced (Table 3). This was accompanied by a compensatory increase in free water clearance, such that there was no significant change in urine output.

Creatinine excretion rate was persistently reduced in centrifuged animals, but when adjusted for the reduction in body mass no difference was noted (Fig.3). There may have been a reduction in glomerular filtration rate (GFR), as estimated by the decrease in rate of excretion of creatinine during centrifugation. When calculated on the last day of centrifugation the glomerular filtration rate was not altered in young or mature animals (Table 3). To adjust for possible differences in glomerular filtration rates subsequent analysis of the excretion of solutes are expressed as function of creatinine excretion. Osmotic excretion rates were episodically altered in centrifuged animals, as were the excretion rates of sodium and potassium (Fig. 3). At the onset of centrifugation, in mature animals the changes in sodium, and potassium excretion rates when compared to controls of the same age tended to be greater than younger animals. In both young and mature rats, at the onset of centrifugation there was a reduction in the rate of excretion of calcium accompanied by an increase in the excretion rate of phosphorus (Fig. 3). In both groups the rate of phosphorus excretion was reduced after 6 days of centrifugation. The excretion of calcium in young animals returned to control levels by day 8 while the rate was significantly increased in older animals.

There was no change in the filtered load of solutes delivered to the kidney. Plasma concentrations of electrolytes were not significantly altered by centrifugation, though significant age effects were noted (Table 4). Calculated GFR was not altered by centrifugation (Table 3), therefore the filtered loads (plasma concentration x GFR) were not changed. On day 14 the percent of the filtered load excretion, indicative of the reabsorption by the kidney, was not changed for potassium and sodium in animals that were centrifuged. In centrifuged mature rats the percentage of the filtered load excreted for calcium was increased with phosphorus being reduced. Young animals showed only a significant reduction in phosphorus (Table 3).

## Conclusions

With an increase in gravity load induced by centrifugation or upon return to Earth following spaceflight, there is a period of adjustment in fluid balance in rats (3-5, 17, 18, 30). With centrifugation there is a reduced fluid intake with maintenance of the rate of urine excretion (3-5, 16-18, 31). Following spaceflight there is an increase in urine output and maintenance of fluid intake (30). The initial period of acclimation to hypergravity is associated with a net loss of fluids. In the present study in response to centrifugation at 2.0 G this period of acclimation is present in mature rats for a longer period of time, about 24 hours. Following this initial response a period of over compensation has previously been reported (3-5, 16-18). In the present study this was not observed. The net effect of these alterations in water intake and output in response to centrifugation for 14 days was slight increase in the percent total body water, with effective compensation seen in both young and mature rats.

Older rats have been shown to have a reduced relative thirst and compensatory renal function in response to hypohydration, hyperosmolality and pharmacological stimuli ( 2, 6, 7, 9-12, 14, 23). Responsiveness to these stimuli are delayed and/or attenuated in older animals. Similar findings were noted in the present study in the initial response to centrifugation. The older animal had a delayed return of fluid intake to control levels. The delay of one day did not appear to effect long-term fluid homeostasis, as there was difference in the response of percent total body water at the end of 14 days of centrifugation with both age groups having a slight but significant increase. This increase

has been attributed to the increase in lean body mass induced by centrifugation (13, 21, 22).

In previous studies of centrifugation an increase in urine output around the fourth or fifth day of centrifugation has been reported (3-5, 16-18, 31) This increase in urine output was the result of an increase in both free water and osmotic clearances (5, 17). In the present study we failed to observe this response. Urine output was not significantly altered during centrifugation in either young or mature animals. The absence of this response may be due to differences in the rate of rotation among the studies. While the previous studies were at a similar gravity level, 2.0 G, as the present study, the rotation rate was greater. It is possible that there may be a rotational component to the response of fluid intake and urine output to centrifugation.

There was a significant reduction of creatinine excretion rate during centrifugation irrespective of age. Creatinine excretion is often used as an index of glomerular filtration rate. When adjusted for the decrease in body mass, which persisted throughout centrifugation, there was no difference between groups in the rate of creatinine excretion suggesting GFR was not altered. Further when estimated on the last day, with correction for filtrate (plasma) concentration, no differences were noted due to centrifugation in either age group. It appears that GFR is not significantly altered by centrifugation.

In the absence of changes in the rate of filtration, and in plasma concentrations of electrolytes, changes in excretion rates would be the result of alteration of renal handling. The transient reduction in excretion rates of sodium and potassium during centrifugation, irrespective of age, suggest conservation of these electrolytes, possibly in response to the

period of hypophasia at the onset of centrifugation (9, 14). In addition Ortiz et al (17) reported an increase in urinary aldosterone concentrations at the onset of centrifugation, associated with a generalized "stress" response, which would facilitated the conservation of sodium. The increase in aldosterone persisted over the first four days of centrifugation. Though there were acute decreases in excretion of sodium and potassium, in response to centrifugation there were no significant changes in plasma concentrations suggesting maintenance of homeostasis of these electrolytes.

In contrast, while there were no significant changes in plasma levels of calcium or phosphorus, there were dynamic changes in excretion of these electrolytes during centrifugation. There was an inverse relationship in the excretion rates of these electrolytes. Exposure to hypergravity has been shown to significantly alter bone mineral content, with a no change or an increase in density reported (1, 20,32). However, this increased density is not associated with an increase in bone content of phosphorous or calcium. This would appear to be paradoxical in light of the increase in excretion of calcium and reabsorption of phosphorous at the end of centrifugation in the present study. Further, whole body calcium and phosphorous content are found by to be inconsistently altered following centrifugation (15, 20, 21). Keil et al (15) found total body content of calcium to be increased in young adult mice but not altered in mature animals. Further, the increase was only noted in male mice. In the present study alterations in excretion of calcium and phosphorus may reflect changes in absorption across the gut with no net change in body content. In the present study there is an

increased excretion of these electrolytes with centrifugation, which does not alter plasma concentration. The impact of these changes in total content is not known.

The absence of significant differences in fluid and electrolyte homeostasis of young and mature rats in response to centrifugation suggests age is not a confounding factor in the acclimation process. Though there were difference in the timing of compensation, with a delay in older animals, adequate compensation was observed as percent total body water was altered to a similar extent, and changes in urine output and water intake were similar between age groups. There were alterations in the excretion rates of a number of electrolytes, which were age dependent, suggesting compensation for exposure to centrifugation and establishment of a new homeostatic balance. Compensatory differences between ages should considered when studying systemic responses to hypergravity, as well as microgravity.

## APPENDIX A: TABLES

Table 1: The mean of three baseline days before exposure to 1G or 2G. The four groups are Young (Y), Young Centrifuged (YC), Mature (M) and Mature Centrifuged (MC).

| Group | Body Mass (g) | Water Intake (ml/day) | Urine Output (ml/day) |
|---|---|---|---|
| | | | |
| Y | 267 ±3.9 | 31 ± 1.0 | 9 ± 0.3 |
| YC | 266 ± 2.9 | 30 ± 0.2 | 9 ± 0.3 |
| | | | |
| M | 485 ± 0.6 | 29 ± 0.6 | 13 ± 0.6 |
| MC | 485 ± 1.1 | 31 ±0.8 | 13 ± 0.6 |
| | | | |

Table 2: Indices of hydration status in young (Y) and mature (M) rats who were controls or exposed to centrifugation (YC, MC) at 2G for 14 days.

| Group | Body Mass (g) | % Total Body Water (%) | Plasma Protein | Plasma Osmolality (mOsm/kg) | Hematocrit (%) |
|---|---|---|---|---|---|
| | | | | | |
| Y | 301 ± 4.0 | 69 ±0.2 | 5.9 ± 0.07 | 286 ± 3.1 | 44 ± 0.8 |
| YC | 268 ±4.0 | 70 ± 0.3 | 5.9 ± 0.08 | 288 ± 1.3 | 45 ± 0.7 |
| | | | | | |
| M | 495 ± 1.6 | 66 ± 0.5 | 6.2 ± 0.05 | 293 ± 1.4 | 46 ± 0.9 |
| MC | 439 ± 2.0 | 68 ± 0.3 | 5.9 ± 0.08 | 292 ± 1.5 | 46 ± 0.8 |
| | | | | | |

Table 3: Renal function on day 14.

| Group | GFR (l/day) | Urine Output (ml/day) | $C_{OSM}$ (ml/day) | $C_{H2O}$ (ml/day) | % FE Calcium | % FE Phosphor |
|---|---|---|---|---|---|---|
| | | | | | | |
| Y | 2.9 ± 0.11 | 9.8 ± 1.56 | 83 ± 4.1 | -72 ± 3 | 0.5 ± 0.06 | 6.5 ± 0.: |
| YC | 3.1 ± 0.18 | 10.6 ± 1.37 | 85 ± 3.0 | -75 ± 3 | 0.7 ± 0.09 | 5.1 ± 0.: |
| | | | | | | |
| M | 4.3 ± 0.27 | 14.4 ± 0.60 | 102 ± 4.1 * | -88 ± 4 * | 0.6 ± 0.07 | 7.1 ± 0.9 |
| MC | 3.9 ± 0.21 | 12.8 ±1.09 | 89 ± 3.3 | -76 ± 3 | 1.0 ± 0.08 | 3.9 ± 0.4 |
| | | | | | | |

Table 4: Plasma concentrations of young (Y) or mature (M) rats who were controls or exposed to centrifugation at 2G (YC, MC). There were significant ($p < 0.05$) age effects for creatinine and phosphorous concentrations.

| | Creatinine (mg/dl) | Sodium (mmol/L) | Potassium (mmol/L) | Calcium (mg/dl) | Phosphorus (mg/dl) |
|---|---|---|---|---|---|
| | | | | | |
| Y | 0.46 ± 0.18 | 141 ± 2.1 | 5.3 ± 0.25 | 10.1 ± 0.38 | 9.0 ± 0.22 |
| YC | 0.43 ± 0.16 | 138 ± 0.6 | 6.0 ± 0.32 | 9.9 ± 0.25 | 8.7 ± 0.38 |
| | | | | | |
| M | 0.50 ± 0.33 | 142 ± 0.7 | 5.5 ± 0.15 | 10.4 ±0.07 | 6.5 ± 0.52 |
| MC | 0.51 ± 0.23 | 141 ± 0.6 | 5.6 ± 0.22 | 10.2 ±0.17 | 6.3 ± 0.33 |
| | | | | | |

## APPENDIX B: FIGURES

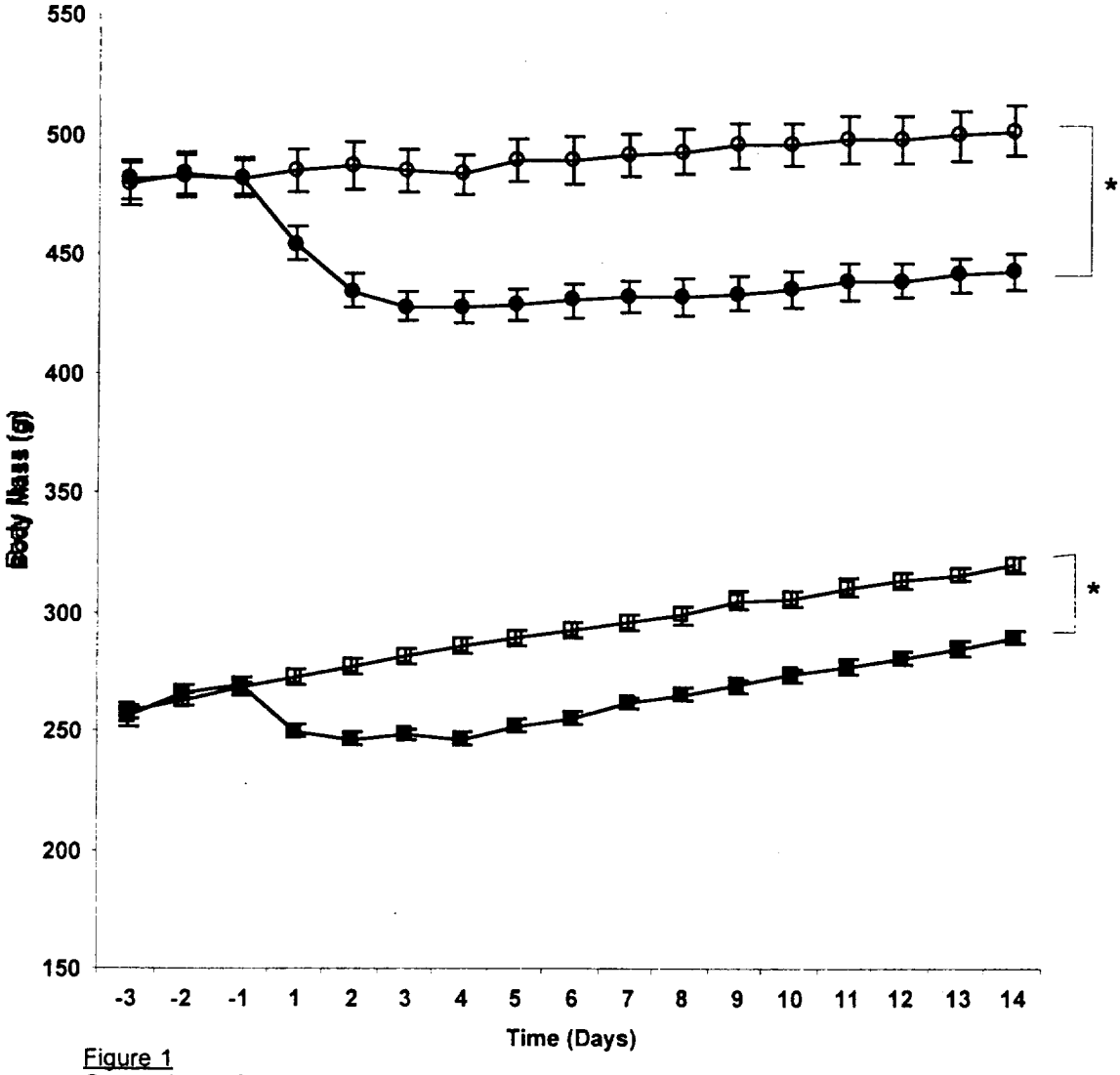



Figure 1
Comparison of body mass between the mature controls (open circles), mature 2.0 G (closed circles), young controls (open squares), and young 2.0 G (closed squares). Values are group means ±SE. * denotes a significant differeces ($p \leq 0.05$) between the young groups, and ** between the mature groups.

2

Figure 1.

Comparison of water consumption, urine volume, and water balance between mature controls (open circles), mature 2.0 G (closed circles), young controls (open squares), and young 2.0 G (closed squares). Values are expressed as absolute group means + se, and corrected for body weights + se. * denotes significant difference ($p \leq 0.05$) between young groups, ** between mature groups.

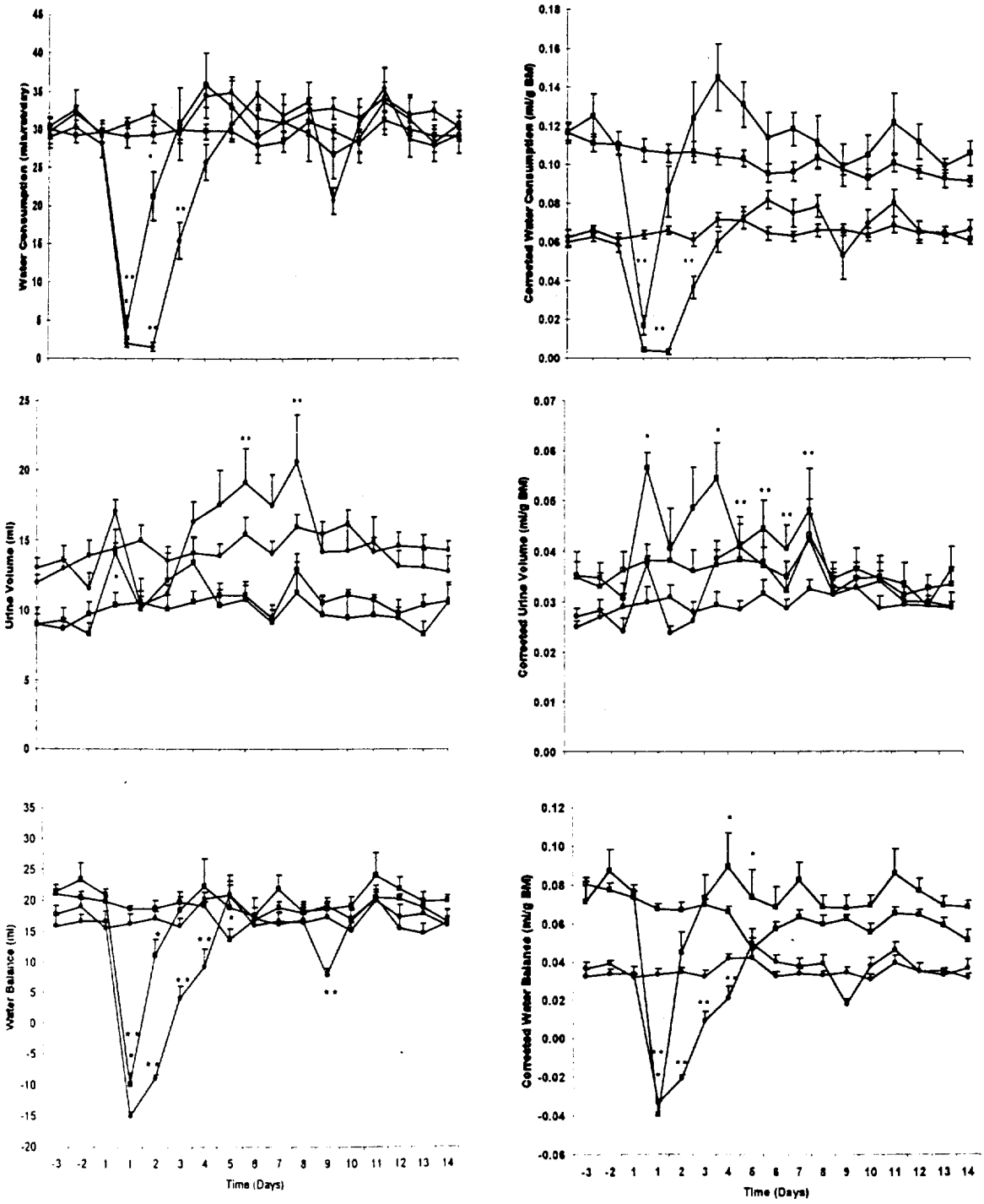
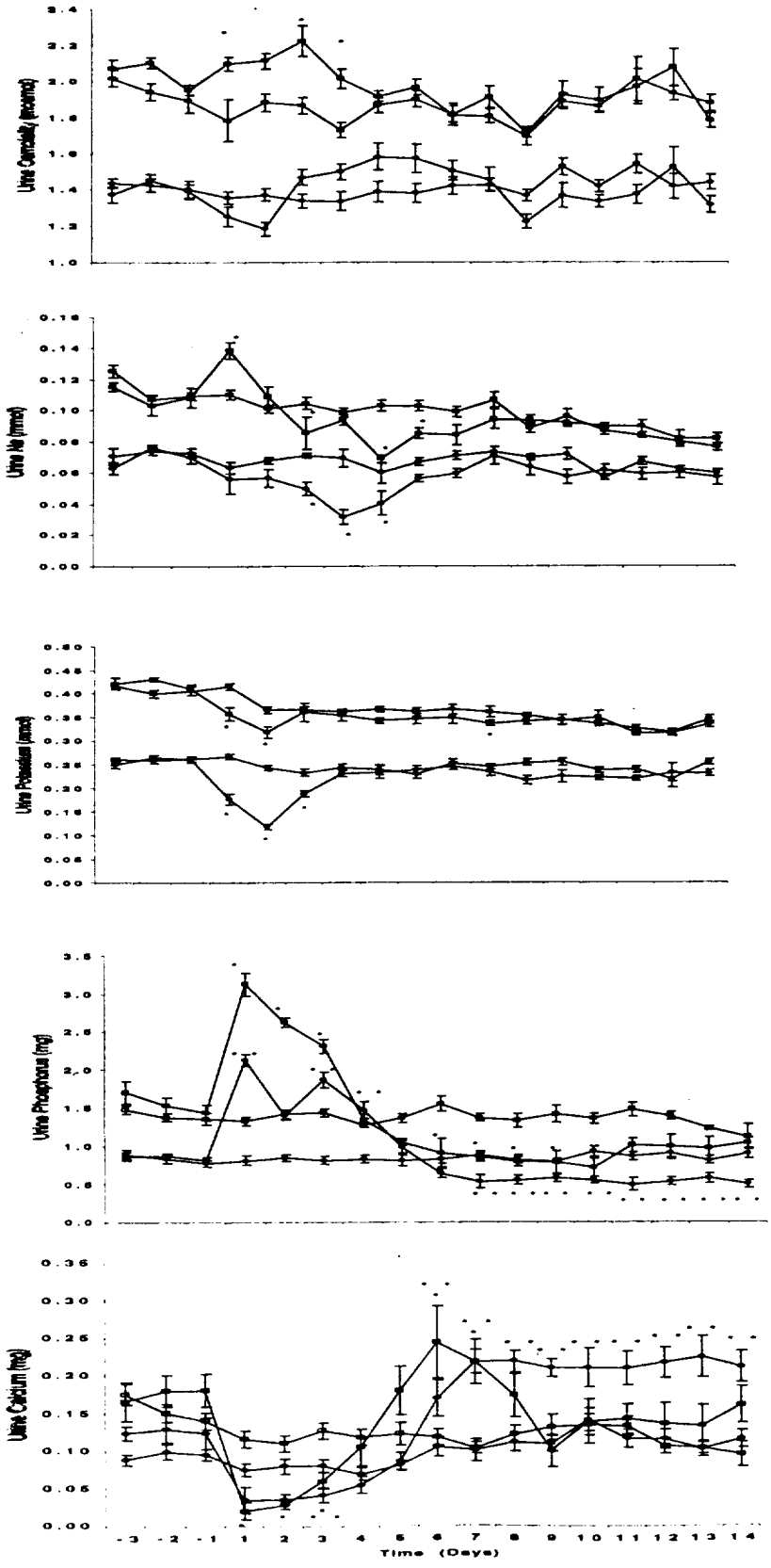


Figure 2. Comparison of urine electrolytes between mature controls (open circles), mature 2.0 G (closed circles), young controls (open squares), and young 2.0 G (closed squares). Values are group means + SE. * denotes a significance ($p \leq 0.05$) between young, ** between mature rats.



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