Endocrine and Fluid Metabolism in Males and Females of Different Ages After Bedrest, Acceleration, and Lower Body Negative Pressure

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Endocrine and Fluid Metabolism in Males and Females of Different Ages After Bedrest, Acceleration, and Lower Body Negative Pressure

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<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>2</td>
</tr>
<tr>
<td>Subjects</td>
<td>2</td>
</tr>
<tr>
<td>Treatment of Subjects</td>
<td>2</td>
</tr>
<tr>
<td>Analyses</td>
<td>3</td>
</tr>
<tr>
<td>Statistical Analysis of Results</td>
<td>4</td>
</tr>
<tr>
<td>RESULTS</td>
<td>4</td>
</tr>
<tr>
<td>Differences Between Males and Females</td>
<td>4</td>
</tr>
<tr>
<td>Hormones in Plasma</td>
<td>4</td>
</tr>
<tr>
<td>Hormones in Urine</td>
<td>4</td>
</tr>
<tr>
<td>Electrolytes and Other Parameters of Urine</td>
<td>5</td>
</tr>
<tr>
<td>Differences Between Age Groups</td>
<td>5</td>
</tr>
<tr>
<td>Hormones in Plasma</td>
<td>5</td>
</tr>
<tr>
<td>Hormones in Urine</td>
<td>6</td>
</tr>
<tr>
<td>Electrolytes and Other Parameters of Urine</td>
<td>6</td>
</tr>
<tr>
<td>Effect of Bedrest</td>
<td>7</td>
</tr>
<tr>
<td>Hormones in Plasma</td>
<td>7</td>
</tr>
<tr>
<td>Hormones in Urine</td>
<td>8</td>
</tr>
<tr>
<td>Electrolytes and Other Parameters of Urine</td>
<td>8</td>
</tr>
<tr>
<td>Effect of +Gz Acceleration</td>
<td>8</td>
</tr>
<tr>
<td>Hormones in Plasma</td>
<td>8</td>
</tr>
<tr>
<td>Hormones in Urine</td>
<td>9</td>
</tr>
<tr>
<td>Electrolytes and Other Parameters of Urine</td>
<td>9</td>
</tr>
</tbody>
</table>
FIGURES

Figure | Page
--- | ---
1 | Schedule of experiment phases showing days on which re-entry simulation tests and LBNP were performed. G1 = re-entry simulation, before bedrest; LBNP1 = LBNP, before bedrest; C (Control) = no tests, before bedrest; BR = bedrest; G2 = re-entry simulation, after bedrest; LBNP2 = LBNP, after bedrest; R (Recovery) = no tests, after bedrest ............... 27

2 | Urinary excretion of hormones in male and female subjects in four age groups. Each bar represents the mean for 7 to 10 subjects and for all days of the experiment phase; error bars represent standard error of the mean ............... 28

3 | Urinary excretion of electrolytes and other parameters of urine measured in male and female subjects in the age ranges 35 to 45 and 55 to 65 years. Each bar represents the mean for 7 to 10 subjects for all days of the experiment phase; error bars represent standard error of the mean ........ ..... 29

4 | Diurnal rhythm in the plasma cortisol concentration of healthy male and female subjects of various ages. Each point represents the mean for 7 to 10 subjects; error bars represent standard error of the mean. The broken line is the mean daily concentration .................. 30

5 | Plasma concentration of cortisol and ACTH in female and male subjects of different ages, before (-30 min), immediately after (15 min), and 10 min after centrifugation (+3 Gz) tests performed before and after bedrest. Each bar represents the mean for 8 to 10 subjects; error bars represent standard error of the mean ................. 31

6 | Plasma concentrations of norepinephrine and dopamine β-hydroxylase in female subjects of different ages, before (-30 min), immediately after (15 min), and 10 min after centrifugation (+3 Gz) tests performed before and after bedrest. The ratio of norepinephrine to DBH is also graphed. Each bar represents the mean for 8 to 10 subjects; error bars represent standard error of the mean............ 32

7 | Urinary excretion of hormones in male and female subjects in several age ranges. Each bar represents the mean for 7 to 10 subjects and for all days of the experiment phase; error bars represent standard error of the mean ............... 33

8 | Urinary excretion of electrolytes and other parameters of urine measured in male and female subjects in several age ranges. Each bar represents the mean for 7 to 10 subjects and for all days of the experiment phase; error bars represent standard error of the mean ............... 34
Urinary excretion of sodium and chloride in males and females of three age groups on the "non-stress" (no centrifugation or LBNP) days before, during, and after bedrest. Each point represents the mean for 7 to 10 subjects.
<table>
<thead>
<tr>
<th>ACRONYMS</th>
<th>MEANING</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>adrenocorticotrophic hormone</td>
</tr>
<tr>
<td>ADH</td>
<td>antidiuretic hormone</td>
</tr>
<tr>
<td>DPH</td>
<td>dopamine β-hydroxylase</td>
</tr>
<tr>
<td>EPI</td>
<td>epinephrine</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle stimulating hormone</td>
</tr>
<tr>
<td>LBNP</td>
<td>lower body negative pressure</td>
</tr>
<tr>
<td>LH</td>
<td>luteinizing hormone</td>
</tr>
<tr>
<td>NOR</td>
<td>norepinephrine</td>
</tr>
</tbody>
</table>
ABSTRACT

Space Shuttle flight simulations were conducted to determine the effects of weightlessness, lower body negative pressure (LBNP), and acceleration on fluid and electrolyte excretion and the hormones that control it. Measurements were made on male and female subjects of different ages before and after bedrest. After admission to a controlled environment, groups of 6 to 14 subjects in the age ranges 25 to 35, 35 to 45, 45 to 55, and 55 to 65 years were exposed to +3 Gz for 15 minutes (G1) and to LBNP (LBNP1) on different days. On 3 days during this pre-bedrest period, no tests were conducted. Six days of bedrest followed, and the Gz (G2) and LBNP (LBNP2) tests were run again. Hormones, electrolytes, and other parameters were measured in 24-hour urine pools throughout the experiment. During bedrest, cortisol and aldosterone excretion increased. Urine volume decreased, and specific gravity and osmolality increased. Urinary electrolytes were statistically unchanged from levels during the non-stress control period. During G2, cortisol increased significantly over its control and bedrest levels. Urine volume, sodium, and chloride were significantly lower; specific gravity and osmolality were higher during G2 than during the control period or bedrest. During LBNP2, volume was lower than during the non-stress control period, and specific gravity and osmolality were higher than during control or bedrest periods. The retention of fluids and electrolytes after +Gz may at least partially explain decreased urine volume and increased osmolality observed during bedrest in this study. There were some statistically significant differences between the sexes and age groups. Results of the study indicated that space flight would not affect the fluid and electrolyte metabolism of females or older males any more severely than it has affected that of male or female astronauts.

INTRODUCTION

One of the goals of the United States space program is to make it possible for more people of both sexes and most age groups to participate in space flight. Since the effects of space flight have so far been studied only in astronauts in excellent physical condition, it is desirable to consider the effects of flight on other types of individuals. Changes in water and electrolyte metabolism, probably mediated by the endocrine system and cardiovascular factors, are known to occur during and after weightless flight (Leach, 1981). These changes have also occurred in subjects on Earth in experiments using bedrest to simulate weightlessness (Vogt et al., 1967; Leach, 1976; Buznik and Kamforina, 1973; Natochin, 1977).

One objective of the bedrest study described here was determination of the probable effects of weightlessness on subjects of different ages and both sexes. The forces produced by acceleration at the beginning and end of a Space Shuttle flight are also a possible source of undesirable effects of space flight; re-entry stress after a period of weightlessness might be particularly likely to cause problems. For these reasons subjects were
exposed to centrifugation stress before and after bedrest. Because exposure to lower body negative pressure (LBNP) has been found to correct some of the cardiovascular changes produced by weightlessness (Lamb and Stevens, 1965; Gilbert et al., 1966; Stevens et al., 1966), the effects of LBNP were also examined in this study.

METHODS

Subjects

Groups of 6 to 14 male or female subjects (table 1) in the age ranges 25 to 35, 35 to 45, 45 to 55, and 55 to 65 years participated in the experiment. Each sex and age group was admitted at a different time. The study plan was reviewed by the Ames Research Center Human Research Review Committee, and informed written consent was obtained from subjects after they had been thoroughly informed about the experimental procedures. Subjects were selected following physical and psychological examinations to ensure that they were in good health. Smoking and beverages containing caffeine were not allowed during the experiment, medication was strictly controlled, and female subjects had not taken oral contraceptives for at least a month before the beginning of the experiment.

Treatment of Subjects

Subjects were housed in the Human Research Facility throughout the study and were maintained in a regulated photoperiod of 16L:8D (lights on at 0700). Diet was controlled to avoid foods containing substances that would interfere with biochemical analyses. Calorie intake range was 2200 to 3000 calories/day for males and 1900 to 2400 calories/day for females. Subjects were encouraged to remain ambulatory and active when they were not sleeping or undergoing bedrest.

On the second and seventh day after admission to the controlled environment, subjects were exposed to +2 or +3 GZ (head-to-foot) acceleration (the G1 phase of the experiment). The acceleration rate was 1.8 G/minute, and acceleration was held at 2.0 GZ for 15 minutes or at 3.0 GZ until loss of peripheral vision (advanced grayout) occurred or until 15 minutes had elapsed. A system of lights similar to that developed by Rogge (1968) was used to determine the onset of grayout. Protection against loss of consciousness was provided by use of temporal artery transcutaneous doppler velocity blood flow monitoring (Krutz et al., 1973). All subjects had undergone at least eight practice runs before the test day and were trained to ride without muscular contraction. Tolerance of +3 GZ was determined for most subjects as the mean duration (ramp plus plateau) of three consecutive runs with a 5-minute rest period between runs.

LBNP was used to test for cardiovascular deconditioning before (LBNP1) and after (LBNP2) bedrest. Heart rate, blood pressure, and cardiac output were monitored by noninvasive methods during the test. A negative pressure of 50 mm Hg was used and was terminated after 15 minutes or at the onset of
bradycardia and decrease in arterial blood pressure. Subjects had been familiarized with the LBNP test before the experiment began.

After the pre-bedrest period including G1 and LBNP1, subjects underwent 6 days of bedrest without any centrifugation or LBNP test (fig. 1). They were centrifuged again on the seventh, eighth, and ninth days of bedrest (the G2 phase of the experiment) and subjected to LBNP on the last (tenth) day of bedrest (LBNP2). A 5-day ambulatory recovery period followed the bedrest period. The group of 25- to 35-year-old females had a bedrest period of 14 days.

During bedrest, subjects maintained a strictly horizontal position (with one pillow) except that they were allowed to lean on one elbow during meals. All other activities were performed in the horizontal position. At the end of the bedrest period, subjects were transported to the centrifuge in a horizontal position.

Analyses

Throughout the studies, 24-hour urine collections were made for determination of hormones (including aldosterone, cortisol, epinephrine, norepinephrine, antidiuretic hormone, estradiol, estriol, and estrone), specific gravity, osmolality, electrolytes, uric acid, and creatinine.

On the fourth day after admission to the controlled environment, blood samples were collected from 35- to 65-year-old subjects every 4 hours by repeated venipuncture at 0700, 1100, 1500, 1900, 2300, 0300, and at 0700 the following day for the measurement of plasma cortisol concentration. Plasma gonadotrophins were measured in blood samples collected from 25- to 45-year-old female subjects during the pre-bedrest period on days when no other test was done (non-stress control days) and at the end of the bedrest period. Heparinized blood samples were drawn from some groups of subjects 30 minutes before the beginning of +3 Gz centrifuge runs and immediately after and 15 minutes after the completion of the run. In all cases, plasma was separated and stored frozen until used for determination of gonadotrophins, cortisol, adrenocorticotrophic hormone (ACTH), norepinephrine, and dopamine \(\beta\)-hydroxylase (D\(\beta\)H).

Cortisol (plasma and urine) was determined by the competitive binding method of Murphy (1967) or by radioimmunoassay (Foster and Dunn, 1974). ACTH (Vague and Oliver, 1972), luteinizing hormone (LH) (Midgley et al., 1966), follicle stimulating hormone (FSH) (Midgley et al., 1966), antidiuretic hormone (ADH) (Miller and Moses, 1969), and aldosterone (Ito et al., 1972) were determined by radioimmunoassay. Epinephrine and norepinephrine concentrations were estimated by the procedure of Von Euler and Lishajko (1961), estrogens were determined by gas chromatography (Roberts et al., 1968) and D\(\beta\)H was determined by enzymatic assay (Molinoff et al., 1971). Standard methods were used for determination of specific gravity of urine (Rubini and Wolf, 1957), osmolality (Johnson and Hoch, 1965), sodium (Henry, 1964), potassium (Henry, 1964), chloride (Cotlove et al., 1958), calcium (Willis, 1961), magnesium (Willis, 1961), inorganic phosphate (Fiske and SubbaRow, 1925), uric acid (Hawk et al., 1954), and creatinine (Hawk et al., 1954).
Statistical Analysis of Results

Analysis of variance with repeated measures was done with BMDP statistical computer programs (University of California, Los Angeles). To compare experiment phases and the sex and age groups by analysis of variance, the mean of each variable for each phase in each subject was used in the program. For analysis of age differences, only the control, bedrest, and recovery phases for each subject were used because the other phases were missing from one age group. When more than two means were compared, Tukey's *a posteriori* post-test for multiple comparisons was used after running the analysis of variance program. A different BMDP program that permits specification of contrasts in analysis of variance with repeated measures was used to compare other days of the experiment with the mean of control days. When a parameter was measured in only one or two groups of subjects, paired or unpaired t-tests were done to determine statistical significance of differences within or between groups. BMDP programs were also used to determine correlations and to perform least-squares regression analysis. The null hypothesis was rejected when $p < .05$.

RESULTS

Differences Between Males and Females

Hormones in Plasma

Sex differences in plasma cortisol and ACTH will be reported below with the description of age differences in these hormones.

Hormones in Urine

Sex differences in urinary excretion of hormones are shown in figure 2. In the age range 25 to 35 years, males had higher levels of aldosterone excretion during all phases of the experiment except G2 and the recovery period. In males of this age group, aldosterone excretion was highest during LBNP. In females it was lowest during LBNP. Cortisol excretion was more than twice as high in males as in females. Excretion of epinephrine was greater in females during most phases and excretion of norepinephrine was higher in males in all phases except LBNP1; the epinephrine/norepinephrine (EPI/NOR) ratio was considerably higher in females than in males except during LBNP1 and G2.

In the 35- to 45-year-old groups, the excretion of aldosterone, cortisol, and norepinephrine was higher in females than in males in all phases of the experiment, while epinephrine excretion and the ratio of epinephrine to norepinephrine was higher in males in all phases. In the 55- to 65-year-old groups the situation was almost reversed: higher excretion of epinephrine in females in four out of seven phases and higher EPI/NOR ratio in all phases, and higher excretion of aldosterone, cortisol, and norepinephrine in males in all phases except recovery. In this age group, the difference between the sexes was significant ($p < .05$) for excretion of norepinephrine. Results for the age group 45 to 55 years were a mixture of the results for the other
groups—greater excretion of aldosterone and epinephrine and higher EPI/NOR ratio in females, greater excretion of norepinephrine in males, and more cortisol in the urine of females during the control period and males during bedrest and recovery.

Differences between the sexes were not statistically significant, except for norepinephrine in 55- to 65-year-old subjects. Males showed a significant influence of age on excretion of norepinephrine, however.

**Electrolytes and Other Parameters of Urine**

In figure 3, non-endocrine parameters are graphed to compare urinary excretion levels in the two sexes for the youngest and oldest age groups in which all parameters were measured. When three age groups (35 to 65 years) were included in the analysis of variance, the sexes were significantly different with respect to specific gravity and osmolality (p < .01) but not with respect to volume. Although 35- to 45-year-old males excreted a greater volume of urine during all parts of the experiment than women in the same age group, the difference was not statistically significant, nor did the sexes differ in urine osmolality in this age group by itself. Males excreted significantly (p < .00001) more Na, K and Cl than females in all age groups. The excretion of phosphate (p < .001), uric acid (p < .001) and creatinine (p < .00001) was also greater in the males. The excretion of magnesium was about the same in the 35- to 45-year-old males and females. It decreased dramatically in the 55- to 65-year-old females (p < .001) whereas magnesium excretion was only slightly lower in the older males than in the younger group, so that there was a significant difference (p = .0001) between the sexes in this age group. Males in the oldest group also excreted significantly (p < .05) amounts of calcium than females of the same age. Before the LBNP2 phase, males in the 35- to 45-year-old group excreted more calcium than females, but the difference was not significant.

**Differences Between Age Groups**

**Hormones in Plasma**

The diurnal changes in circulating cortisol in male and female subjects of ages 35 to 65 years are compared in figure 4. Peak cortisol levels were reached at 0700 (lights on) in all groups, although after age 55 the peak levels decreased significantly (p < .05). The pattern of the diurnal rhythm between peaks varied with age and sex; for example, the trough was wider in the older groups (45 to 65 years) than in the 35- to 45-year-old subjects. The mean daily cortisol levels (dashed lines in fig. 4) were lowest in 55- to 65-year-old subjects.

The response of circulating cortisol and ACTH to the stress of centrifugation is shown in figure 5. Fifteen minutes after the onset of centrifugation, cortisol levels showed a significant increase (p < .05) in all age groups of both sexes except for 45- to 55-year-old males. ACTH levels increased significantly (p < .05) in all groups. The pre-stress level of cortisol, but not ACTH, decreased with age in males. The youngest females displayed a significantly greater plasma ACTH response than did males of the
same age. The oldest males had a pronounced plasma ACTH response that was approximately three times as great as that of the youngest males but not significantly different from that of the oldest females, whose response was similar to that of younger females. The correlation between plasma cortisol and ACTH was very low ($r = .08$).

Plasma norepinephrine and DBH were measured only in females, and there was little variation with age in the levels of these substances (fig. 6) except that the youngest group (25 to 35 years) had very high levels of norepinephrine and relatively low levels of DBH before and after centrifugation. Before centrifugation, the ratio of norepinephrine to DBH was significantly higher in the youngest group (25 to 35 years) than in women 35 to 55 years old. Plasma LH and FSH were measured only in females 25 to 35 and 35 to 45 years old, and the small differences between these groups were not significant (table 2).

**Hormones in Urine**

Urinary excretion of aldosterone, cortisol, epinephrine, and norepinephrine in both sexes and three or four age groups during three phases of the experiment are shown in figure 7.

Urinary excretion of cortisol and epinephrine was not significantly different between age groups (35 to 65 years). However, in females cortisol excretion decreased with age (35 to 65 years) and the decrease was significant ($p < .05$) for the control period. Excretion of cortisol and norepinephrine was lower in 25- to 35-year-old women than in the other age groups, and excretion of epinephrine was high in that group. Epinephrine excretion was also quite high in 35- to 45-year-old males, but the difference between age groups was not significant. Males and females 35 to 45 years old excreted significantly more aldosterone than either of the older groups, and in males aldosterone excretion was even higher in 25- to 35-year-olds. Cortisol excretion by 25- to 35-year-old males was much higher than that by any other group. Differences in urinary norepinephrine between the three oldest age groups were significant only in males. The youngest males (35 to 45 years) excreted significantly ($p < .01$) less norepinephrine than the older age groups.

The ratio of epinephrine to norepinephrine in 24-hour urine pools was highest in 25- to 35-year-old females and 35- to 45-year-old males. There was little difference between ratios for ages 45 to 55 and 55 to 65 years in men, but the difference between ratios for each of these age groups and those of men ages 35 to 45 was significant. The latter group had higher ratios than women of the same age, but for the older groups and 25- to 35-year-olds, women had higher ratios than men.

**Electrolytes and Other Parameters of Urine**

Urine volume, specific gravity, osmolality, and levels of electrolytes and other compounds in the different age groups are compared in figure 8.
The 45- to 55-year-old group had significantly less (p < .05) urine volume than the 35- to 45- or 55- to 65-year-old groups, and urine specific gravity (p < .01) and osmolality were greater in this group than in the 35- to 45-year-old subjects. Sodium and chloride did not show significant differences due to age for 35- to 65-year-olds, and potassium excretion was affected by age in males only: 45- to 55-year-olds had significantly lower levels of potassium excretion than the other groups (p < .05). The youngest males (25 to 35 years) had the highest level of sodium excretion.

Subjects in the 45- to 55-year-old group excreted amounts of calcium significantly (p < .05) greater than those of the 25- to 35-year-old and 55- to 65-year-old groups. Magnesium excretion was significantly (p < .01) greater in 35- to 45-year-old subjects than in 45- to 55-year-old subjects, and the latter subjects excreted higher (p < .05) levels than the oldest group. Phosphate excretion was significantly (p < .01) lower in the 45- to 55-year-old group than in the oldest subjects.

The 35- to 45-year-old subjects excreted significantly (p < .05) more uric acid than either of the older groups. Creatinine excretion was significantly lower (p < .01) in the 45- to 55-year-old group than in the youngest or oldest subjects.

**Effect of Bedrest**

**Hormones in Plasma**

In most age groups, bedrest led to a greater increase in the amount of plasma cortisol, immediately after and 15 minutes after the end of the exposure to +3 Gz (fig. 5). In 25- to 35-year-old females, however, subjects had a lower level of plasma cortisol immediately after exposure to +3 Gz after bedrest than they did before bedrest. (The bedrest period was twice as long for this group as it was for other subjects.)

With regard to plasma ACTH, data for only two age groups (45 to 55 and 55 to 65 years old) of males were available (fig. 5). After bedrest these showed an increase in the plasma ACTH response to +3 Gz exposure, especially in 55- to 65-year-olds. In most groups of females, plasma ACTH increased or remained about the same with bedrest, but in 45- to 55-year-old females it was lower after bedrest and centrifugation than it was after centrifugation without bedrest (fig. 5).

Bedrest had a considerable effect on plasma norepinephrine in females (no data were available for males) only in the 25- to 35-year-old group, in which the hormone level was lower after bedrest (fig. 6).

Plasma DBH was higher with bedrest before and immediately after centrifugation in females 25 to 35 and 35 to 45 years old (fig. 6), but it was lower after bedrest in 45- to 55-year-olds at the same times. The norepinephrine/DBH ratio (fig. 6) was lower after bedrest in the youngest group of female subjects before and after centrifugation, but this difference was of borderline significance (p < .1). In 45- to 55-year-old women, the ratio was significantly higher after bedrest before centrifugation (p < .05).
Before a third centrifugation (data not shown) of 25- to 35-year-old females after 2 days of recovery, the NOR/DβH ratio was quite low, 0.089. The ratio increased after centrifugation, reaching 0.241 by 15 minutes after centrifugation ended.

Hormones in Urine

Bedrest affected subjects of different ages and sexes differently, and it affected urinary excretion of some hormones more than that of others (fig. 7). The change most consistent across groups was a reduction in nor-epinephrine in all age groups of females and males in which it was measured. This reduction was significant (p < .01); it was the only effect of bedrest on excretion of the four hormones measured that was statistically significant when all subject groups were included in the analysis. In males and the two older groups of females, epinephrine decreased also. Aldosterone was significantly decreased on the first day of bedrest in 55- to 65-year-old men and women. The EPI/NOR ratio was higher (but not significantly higher) in 25- to 35- and 35- to 45-year-old females and 35- to 45-year-old males after bedrest; in other groups the ratio remained about the same. Excretion of ADH decreased slightly in 25- to 35-year-old women after bedrest (table 3), and excretion of estrogen (estrone, estriol, and estradiol) increased in 35- to 45-year-old females during bedrest (table 4).

Electrolytes and Other Parameters of Urine

Urine volume was increased on the first day of bedrest in all groups except 55- to 65-year-old males, and the difference was significant when all groups were included in the analysis. On the other days of bedrest, however, urine volume was significantly lower than it was during the control period (fig. 8). Specific gravity and osmolality were highest during bedrest, and the difference between control and bedrest was significant (p < .05) except in the group of 45- to 55-year-old females (fig. 8).

For several parameters there were significant differences between the first and last halves of the non-stress bedrest period. Urinary excretion of sodium (fig. 9), chloride (fig. 9), calcium, and uric acid was significantly (p < .05) lower at the end of bedrest than at the beginning. Excretion of sodium and chloride followed the same pattern (fig. 9), which was slightly different in the two sexes. There was a sharp increase in excretion of these electrolytes on the first day of bedrest and a decrease on the first day of the recovery period. The decrease was sharp in males and 35- to 45-year-old females but more gradual in the other females. Most groups had a second small peak of excretion of sodium and chloride after several days of bedrest.

Effect of +Gz Acceleration

Hormones in Plasma

The effects of bedrest and centrifugation on plasma hormones have been described above. Plasma cortisol and ACTH increased after acceleration stress in both sexes, all age groups, and before and after bedrest. Effects on norepinephrine and DβH were variable.
Hormones in Urine

Specimens from days on which subjects were centrifuged or exposed to LBNP were not collected for the 45- to 55-year-old groups. Urinary excretion of aldosterone, cortisol, epinephrine, and norepinephrine by the other groups of subjects is shown in figure 2.

Excretion of aldosterone after centrifugation was not significantly different from excretion levels during the control or bedrest periods except in 25- to 35-year-old females, in whom excretion of aldosterone increased by 127 percent after initial centrifugation (G1). Excretion of cortisol increased after G1 in 35- to 45-year-olds and 55- to 65-year-olds, but the increase was not statistically significant. After the post-bedrest centrifugation (G2), excretion of the hormone was increased significantly (p < .05), not only over control levels but also over levels during bedrest, in 35- to 45- and 55- to 65-year-olds. In 25- to 35-year-old males, who had the highest level of cortisol excretion, the level decreased (not significantly) during both centrifugations.

Excretion of epinephrine after centrifugation did not differ significantly from excretion during the control period or bedrest. Changes in the EPI/NOR ratio also were not significant.

Excretion of norepinephrine decreased (but not significantly) after G1 in all groups except 25- to 35-year-old women and men. After G2, excretion of this hormone decreased significantly (p < .01) with respect to the control period. It also decreased, but not significantly, with respect to bedrest and G1 in males and with respect to G1 in females.

Data on ADH and estrogen excretion were available for only the younger age groups. Excretion of ADH increased by 90 percent after G1 in women 25 to 35 years old (table 3). Levels for G2 were greater than those for the control and bedrest periods for women in this age group but less than those for G1. In males, ADH excretion increased by only 41 percent after G1, and excretion of the hormone was greatest during the G2 period.

Excretion of total estrogens increased (compared to the control period) after G2 in 35- to 45-year-old women, the only group in which estrogen was measured (table 4). There was considerable variation among the three types of estrogen examined (estrone, estriol, and estradiol).

Electrolytes and Other Parameters of Urine

Urine volume, sodium, and chloride were significantly (p < .01) lower during G2 than they were during G1, bedrest, or the non-stress control period (fig. 3). Urine volume was also lower (p < .01) during G1 than it was during the control period. Potassium excretion was significantly lower (p < .05) during G2 than during the non-stress bedrest period. Specific gravity and osmolality were higher during G2 than during the control period or bedrest; these two parameters were also significantly higher during G2 than during G1.
Effect of LBNP

Hormones in Urine

Only norepinephrine changed significantly after LBNP, but some of the changes in other parameters were consistent across groups.

In females, urinary excretion of aldosterone decreased after LBNP in all age groups measured (fig. 2). In males it remained about the same as it had been during the control period. Excretion of the hormone after LBNP2 was usually less than during the control period or LBNP1.

Excretion of cortisol (fig. 2) was greater after LBNP1 than during the control period for 35- to 45-year-olds of both sexes but less than controls for 55- to 65-year-olds of both sexes. For LBNP2, excretion of cortisol was the same as or greater than during the control period and LBNP1 for 35- to 45-year-old females and 55- to 65-year-old males, and it was less than those values for the other groups, including 25- to 35-year-old males.

After LBNP1, excretion of epinephrine (fig. 2) increased in all groups except the youngest females. However, in most groups it was lower after LBNP2 than during the control period and/or after LBNP1. Norepinephrine excretion (fig. 2) generally decreased slightly after LBNP1 and decreased significantly after LBNP2 as compared to either the control period or LBNP1. The EPI/NOR ratio (fig. 2) was not significantly different from the ratio during the control period.

Excretion of ADH (table 3) increased after LBNP1 and LBNP2 in 25- to 35-year-old females but decreased in males in that age group. Excretion of estrogens (table 4) was increased after both LBNP tests, the second increase being greater than the first for all three types of estrogen.

Electrolytes and Other Parameters of Urine

Urine volume (fig. 3) was significantly (p < .01) lower during LBNP2 than it was during LBNP1 or the control period. Specific gravity and osmolality (fig. 3) were significantly (p < .01) higher during LBNP2 than during the control period, the bedrest period or LBNP1.

Recovery

When phases of the experiment were compared, there was no significant difference between the control and recovery periods for any urine constituent or other measurement. However, on the first day of recovery, urinary levels of several parameters were significantly different from levels during the control period. Urine volume, sodium, potassium, and chloride decreased and specific gravity, osmolality, and calcium increased. Aldosterone was significantly increased when all groups were included in the analysis but was not different in any one group.
Correlation, Cluster, and Regression Analyses

The r values at different times during the experiment are shown in table 5 for many parameter pairs. The highest correlation, between specific gravity and osmolality, remained constant except for a very small decrease during GI. The second highest correlation, between sodium and chloride, decreased slightly during GI and LBNP1 and increased slightly during LBNP2 and recovery. Potassium levels were highly correlated with sodium, chloride, and creatinine. Specific gravity and osmolality had high negative correlations with volume. The highest correlations of hormones with each other and with other parameters were those of cortisol with aldosterone and of epinephrine with volume. Epinephrine and norepinephrine were negatively correlated throughout the experiment.

During most phases of the experiment, correlation clusters were formed by volume, specific gravity, and osmolality; epinephrine and norepinephrine; and calcium and magnesium. The cluster pattern was not the same for any two phases. The number of clusters increased during bedrest, at which time sodium and chloride constituted one cluster, and potassium, creatinine, phosphate, and uric acid another.

When data from all phases of the experiment were used in a linear regression program, sodium was a significant predictor of volume, and aldosterone and cortisol were significant independent predictors of volume and potassium. Aldosterone was a significant predictor of chloride, and cortisol was a significant predictor of magnesium and uric acid.

DISCUSSION

Differences Between Males and Females

Regarding levels of aldosterone, cortisol, epinephrine, or norepinephrine in urine, the only statistically significant difference due to sex was in the excretion of norepinephrine in 55- to 65-year-old subjects, where males showed consistently higher norepinephrine levels than females. This and other differences dependent on age group will be discussed below.

Males had significantly higher values for urine specific gravity and osmolality and for urinary sodium, potassium, chloride, phosphate, uric acid, and creatinine. The greater body mass of males and their greater intake of food may account for the greater excretion rate of these compounds in males. No attempt was made to coordinate experiment phases with the menstrual cycles of female subjects but this does not appear to have caused a problem for interpretation. Variability in fluid and electrolyte excretion of females was no greater than that in males and was usually smaller.

Differences Between Age Groups

Magnesium was the only compound that increased or decreased in the urine through at least three age groups; it decreased with age. The difference between age groups was more pronounced in females than in males.
The oldest groups of subjects (55 to 65 years) of both sexes had the lowest excretion of calcium and magnesium, although for calcium the difference between these groups and the others was not statistically significant.

The 45- to 55-year-old group was significantly different from the other groups with respect to several parameters. In both sexes, this age group had the lowest urine volume and level of creatinine excretion and the highest level of calcium excretion. Phosphate excretion in this group was significantly lower than that of the oldest subjects. In males, 45- to 55-year-olds had the lowest levels of potassium excretion.

Uric acid excretion was highest in 35- to 45-year-old subjects of both sexes. In males only, norepinephrine excretion was significantly lower in this age group than in older subjects, and in females, cortisol excretion was significantly higher than in older subjects. The ratio of plasma norepinephrine to DPH, measured only in women, was highest in subjects 25 to 35 years old.

Palmer et al. (1978) found that plasma norepinephrine was lower in subjects (of both sexes) 10 to 20 years old than in subjects (mostly females) older than 40 years. Christensen (1973), Lake et al. (1976), Ziegler et al. (1976) and Shimada et al. (1985) have also documented increasing levels of plasma norepinephrine with age. In the studies reported here there was no such clear-cut relationship of plasma norepinephrine with age in females. The reasons for the discrepancy are unclear.

Carlson et al. (1970) measured urinary excretion of catecholamines in patients 64 to 98 years old and found it to be similar to that in younger people. The subjects in these studies were not segregated by sex. In the present study, females 35 to 65 years old excreted about the same amount of norepinephrine, but in males 35 to 45 years old, excretion of the hormone was significantly lower than in the two older groups, in which excretion levels were similar. The youngest males (25 to 35 years) excreted more norepinephrine than the females did, but in the next group (35 to 45 years) the females excreted more norepinephrine during all phases of the experiment. These results are not inconsistent with those of Cuche et al. (1975) for recumbent subjects, all but one of whom were 21 to 41 years old: females excreted more norepinephrine in the urine than males did. Excretion of norepinephrine appears to depend on sex and on age.

Urinary aldosterone and cortisol had the highest correlation coefficient of hormone pairs in this experiment. Plasma and urinary cortisol were not highly correlated.

The response of plasma ACTH to acceleration stress varied with sex and age, much more than the response of plasma cortisol did. In females, the greatest responses occurred in subjects 25 to 35 and 45 to 55 years old, whereas in males, the greatest response occurred in subjects 55 to 65 years old. Aging appears to affect the pituitary‐adrenal and adrenergic systems differently in males and females.
An "age-related diminution in the ability to conserve solute" was observed by Rowe et al. (1976). No clear-cut relationship between age and conservation of any solute except magnesium was found in the present study, and excretion of magnesium decreased with age. Among male subjects, specific gravity and osmolality of the urine were generally lowest in the youngest group (35 to 45 years), but the two older groups had about the same specific gravities and osmolalities. In 55- to 65-year-old male subjects, during the recovery period osmolality of the urine remained at the increased level reached during bedrest, indicating that solute conservation after bedrest might have been diminished in this group. There was no evidence for such a change with age in females.

Effect of Bedrest

Plasma cortisol increased during bedrest in most groups. Plasma norepinephrine decreased significantly only in the youngest group of female subjects, but urinary norepinephrine decreased significantly when all groups were analyzed. The response of plasma DBH was about the opposite of that for plasma norepinephrine: DBH increased in females 25 to 45 years old and decreased in 45- to 55-year-olds. The NOR/DBH ratio decreased in the youngest group and increased in the 45- to 55-year-olds. Diminished norepinephrine in young females may have induced an increase in DBH, which converts dopamine to norepinephrine.

Urine volume decreased during bedrest, except for the first day, and specific gravity and osmolality of urine increased. Sodium, chloride, calcium, and uric acid excretion decreased significantly between the first and last halves of the bedrest period. Sodium and chloride had very similar patterns of excretion, with a sharp increase on the first day of bedrest and a decrease on the first day of recovery.

These results are similar in some respects but not in others to results of other bedrest studies. A decrease in norepinephrine excretion is a common finding (Leach et al., 1973; Leach, 1976; Sandler and Winter, 1978). A decrease in epinephrine excretion with bedrest has been observed by Leach (1976) and by Wegmann et al. (1980). The present study resulted in a decrease in epinephrine excretion in all groups of males and in the two older groups of females, but in the youngest females excretion of epinephrine increased during bedrest. Sudoh (1971) found that a decrease in norepinephrine output occurred with "monotonous light work," and Carlson et al. (1970) found a positive correlation between catecholamine excretion and habitual motor activity in elderly people. The decrease in catecholamine excretion with bedrest in most subjects may be due to physical and mental inactivity.

Leach (1976) found that cortisol and aldosterone excretion decreased during bedrest. In the present study, urinary levels of both hormones increased in most groups during bedrest, but the changes were not statistically significant. Excretion of both hormones is increased during (Leach and Rambaut, 1977) and immediately after (Leach et al., 1975; Leach, 1977; Leach and Rambaut, 1977) space flight also.

13
In most bedrest studies, urine volume increased (Vogt et al., 1967; Leach, 1976). However, in at least one study (Sandler and Winter, 1978) besides the one reported here, a decrease in urine volume occurred during bedrest. The higher specific gravity and osmolality of urine during bedrest in the present study also indicate a lack of diuresis.

Increased excretion of electrolytes and nitrogenous compounds is a common finding in bedrest studies (Deitrick et al., 1948; Biryukov et al., 1967; Vogt et al., 1967; Buznik and Kamforina, 1973; Leach, 1976; Natochin, 1977). In the present study, early losses of sodium, chloride, calcium, uric acid, and creatinine diminished during the 6 days of bedrest so that the average levels of these compounds during bedrest were not significantly different from average levels during the control period. Calcium and phosphate, which are of particular interest due to the possibility of loss of bone substance, did not show a significant increase in excretion level during bedrest as they have in some other studies (Deitrick et al., 1948; Birkhead et al., 1963; Parin et al., 1970; Leach, 1976; Natochin, 1977), probably because of the short duration of bedrest. According to several studies (Deitrick et al., 1948; Birkhead et al., 1963; Natochin, 1977), the peak in calcium excretion is reached after several weeks of bedrest.

The earliest, largest, and most consistent changes measured after bedrest began were loss of sodium and chloride through the urine. About the same time, plasma and urinary cortisol (which promotes sodium retention) increased in most groups of subjects. Measurement of plasma levels of aldosterone would probably be useful in studying the relationship between this hormone and electrolytes. In urine, neither aldosterone nor cortisol was very highly correlated with any electrolyte in the present study.

The increase in urine specific gravity and osmolality during bedrest was probably caused by the decrease in volume rather than an increase in solutes. Fluids, electrolytes, and nitrogenous compounds were conserved during most of the bedrest period in this experiment. The correlation between specific gravity and osmolality remained constant throughout the experiment, indicating that there was little change in the proportion of solutes that were not osmotically active.

**Effect of +Gz Acceleration**

In spite of the briefness of +3 Gz exposure, centrifugation affected many of the parameters measured in 24-hour urine pools, especially after bedrest (G2). Urinary cortisol (except in males 25 to 35 years old), ADH (in subjects 25 to 35 years old), estrogen (in females 25 to 45 years old), specific gravity, and osmolality were higher after G2 than during the control period and in most cases were significantly higher than during the bedrest period. Urinary norepinephrine, volume, sodium, and chloride were significantly lower after G2 than during the control period or (except for norepinephrine) the bedrest period, and potassium was lower than during the non-stress bedrest period for most age groups. All of these parameters except ADH (which was measured in only two groups of subjects) were affected in the same way by bedrest and by centrifugation, and when the two conditions were combined a particularly large response was obtained.
The effects of acceleration in the present study are consistent with results obtained by several other investigators. Epstein et al. (1974) found a decrease in sodium but not potassium excretion after centrifugation and proposed that the change was mediated by renal hemodynamic factors. Greenleaf et al. (1977) noted an increase in plasma ADH after exposure of young men to acceleration. An increase in excretion of cortisol after centrifugation of young women was reported by Sandler and Winter (1978).

In 25- to 35-year-old females, there was a good correlation between mean daily amplitude of plasma cortisol and acceleration tolerance. The mean amplitude decreased during bedrest as did the tolerance of subjects to centrifugation (Vernikos-Danellis et al., 1978). In the present study, acceleration tolerance was decreased after bedrest.1 Plasma and urinary cortisol, however, were greater after G2 than G1. Correlation analysis showed an inverse relationship \( r = -0.21 \) between urinary cortisol and acceleration tolerance, which had its highest positive correlations with sodium, potassium, chloride, and urine volume.

**Effect of LBNP**

Although a significant difference between LBNP and other phases was seen only for norepinephrine, some trends in excretion of fluid and electrolytes were apparent.

Urinary estrogen, specific gravity, and osmolality were higher after LBNP at the end of bedrest (LBNP2) than they were during the control period or after LBNP1. During LBNP2, specific gravity and osmolality were higher than during bedrest, and both were most highly correlated (negatively) with urine volume then. Excretion of norepinephrine and urine volume were lower during LBNP2 than they were during the control period or LBNP1. All of these parameters were affected in the same way by bedrest, centrifugation and LBNP, and bedrest appeared to exacerbate the response to LBNP.

These results indicate that LBNP caused subjects to retain fluids and electrolytes. Another study (Gilbert et al., 1966) has also indicated that LBNP stimulates the retention of electrolytes and water, and Grigoriev (1983) has used a combination of water and salt supplements and LBNP to prevent fluid and electrolyte loss during bedrest and during space flight of cosmonauts.

At least one research group (Suvorov and Beleda, 1972) has found that in subjects with high LBNP tolerance, LBNP was followed by increased excretion of epinephrine and norepinephrine; in subjects with low tolerance, excretion of the compounds decreased after LBNP. Since our subjects were not grouped by tolerance level, the results presented here probably represent an average effect of LBNP on these catecholamines.

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1D. Goldwater, unpublished observations
Recovery

Changes in several urine parameters occurred on the first day of ambulation after bedrest. Fluid and electrolytes were retained, a phenomenon that has also been observed in astronauts immediately after their return to Earth (Leach, 1983). An increase in urinary aldosterone has also been observed in returning astronauts. These findings are thought to result from the redistribution of body fluids so that stretch receptors in the left atrium detect a decrease in blood volume and set in motion the homeostatic mechanisms necessary to increase volume. Within 4 days after cessation of bedrest, all measured variables in all age groups returned to levels not significantly different from control values. This recovery was more rapid than metabolic recovery of astronauts from space flight of equal or shorter duration (Leach, 1983).

Implications of Results for Space Flight

The main bedrest finding that is not commonly seen during space flight was a decrease in norepinephrine excretion, which was probably due to the inactivity inherent in bedrest. The effects of return to Earth's gravity and reambulation were quite similar to each other: increases in urine osmolality and specific gravity and decrease in urine volume, sodium, chloride, and potassium.

It appears likely that the exposure of subjects to centrifugation and/or LBNP affected the metabolic response of subjects to bedrest (Lamb and Stevens, 1965; Stevens et al., 1966; Grigoriev, 1983). In the study of Sandler and Winter (1978), in which urine volume decreased during bedrest as it did in the present study, centrifugation and LBNP were also done before bedrest. The effects of $G_Z$ and LBNP on physiological changes during bedrest should be studied separately at intervals throughout a bedrest period.

CONCLUSIONS

1. Males excreted up to twice as much sodium, potassium, chloride, magnesium, phosphate, uric acid, and creatinine in the urine as females, but no statistically significant differences due to sex were found in urinary levels of aldosterone, cortisol, epinephrine, or norepinephrine.

2. Magnesium was the only compound measured that increased or decreased in the urine through at least three age groups; it decreased with age. However, there were significant differences between age groups in the excretion of several hormones and other compounds.

3. Urinary excretion of norepinephrine depends on sex and age. Females 25 to 35 years old excreted less than two-thirds as much norepinephrine as those in older groups. Males had higher excretion rates than females of the same ages, except in the 35- to 45-year-old subjects. Aging appears to affect the pituitary-adrenal and adrenergic systems differently in males and females.
4. Bedrest produced some of the same effects on fluid and electrolyte metabolism as are seen in astronauts during space flight. Increased urine volume, sodium, and chloride occurred only on the first day of bedrest; urinary sodium, potassium, and chloride were increased throughout flight in Skylab astronauts. Significant increases in urinary aldosterone and cortisol that occurred during the Skylab flights were not observed during bedrest. The main bedrest finding that is not commonly seen during space flight was a decrease in norepinephrine excretion, which was probably due to the inactivity inherent in bedrest. The effects of return to Earth's gravity and reambulation were quite similar to each other: increases in urine osmolality and specific gravity and decreases in urine volume, sodium, chloride, and potassium.

5. Centrifugation and lower body negative pressure generally had the same metabolic effects as bedrest in this study; but since some of the effects of bedrest were different from those found in most other studies, centrifugation or LBNP tests may have prevented the loss of fluid and electrolytes usually reported during bedrest. Re-entry acceleration may play a role in initiation of fluid and electrolyte conservation in astronauts returning from space flight.

6. Results of this study indicate that space flight would not affect the fluid and electrolyte metabolism of non-astronaut females or older males any more seriously than it has affected that of male or female astronauts. Effects on astronauts are not clinically important and last for no longer than a few weeks.

ACKNOWLEDGMENTS

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REFERENCES


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<th>Height, cm</th>
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### TABLE 3. - URINARY EXCRETION OF ANTIDIURETIC HORMONE IN 25- TO 35-YEAR-OLD SUBJECTS

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<th>BR</th>
<th>G2</th>
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<tr>
<td>Female</td>
<td>8</td>
<td>28.6 ± 2.7</td>
<td>54.3 ± 7.5</td>
<td>46.6 ± 20.1</td>
<td>27.9 ± 1.7</td>
<td>47.5 ± 14.2</td>
<td>53.5 ± 12.2</td>
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<tr>
<td>Male</td>
<td>4</td>
<td>57.1 ± 22.1</td>
<td>80.3 ± 25.6</td>
<td>54.2 ± 5.7</td>
<td>21.4 ± 10.5</td>
<td>112.8 ± 59.0</td>
<td>47.1 ± 29.1</td>
<td>52.8 ± 10.8</td>
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### TABLE 4. - URINARY EXCRETION OF ESTROGENS IN 35- TO 45-YEAR-OLD FEMALE SUBJECTS

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<th>μg/24h, mean ± SE</th>
<th>Change from control period, mean ± SE</th>
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<td>3.48 ± 1.31</td>
<td>-0.19 ± .31 1.13 ± 1.36 1.35 ± .68 -0.23 ± .22 2.93 ± 1.68 1.08 ± .59</td>
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<td>Estradiol</td>
<td>28.52 ± 5.32</td>
<td>-3.50 ± 3.08 1.89 ± 4.23 8.95 ± 4.04 2.42 ± 5.94 18.95 ± 18.03 4.66 ± 1.76</td>
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<td>Estriol</td>
<td>11.68 ± 2.28</td>
<td>3.48 ± 2.74 0.17 ± 3.36 1.77 ± 2.15 0.49 ± 2.88 53.47 ± 56.17 1.16 ± 3.84</td>
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<td>Total estrogens</td>
<td>43.1 ± 4.62</td>
<td>0.47 ± 4.67 3.0 ± 6.5 11.64 ± 3.3 2.76 ± 8.32 76.0 ± 59.51 8.40 ± 3.57</td>
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FIGURE 1. - Schedule of experiment phases showing days on which re-entry simulation tests and LBNP were performed. G1 = re-entry simulation, before bedrest; LBNP1 = LBNP, before bedrest; C (Control) = no tests, before bedrest; BR = bedrest; G2 = re-entry simulation, after bedrest; LBNP2 = LBNP, after bedrest; R (Recovery) = no tests, after bedrest.
FIGURE 2. - Urinary excretion of hormones in male and female subjects in four age groups. Each bar represents the mean for 7 to 10 subjects and for all days of the experiment phase; error bars represent standard error of the mean.
FIGURE 3. - Urinary excretion of electrolytes and other parameters of urine measured in male and female subjects in the age ranges 35 to 45 and 55 to 65 years. Each bar represents the mean for 7 to 10 subjects for all days of the experiment phase; error bars represent standard error of the mean.
FIGURE 4.- Diurnal rhythm in the plasma cortisol concentration of healthy male and female subjects of various ages. Each point represents the mean for 7 to 10 subjects; error bars represent standard error of the mean. The broken line is the mean daily concentration.
PLASMA CORTISOL AND ACTH

BEFORE BEDREST

AFTER BEDREST

FEMALES

25-35YR 35-45YR 45-55YR 55-65YR

MALES

25-35YR 35-45YR 45-55YR 55-65YR

CORTISOL (µg/100ml)

ACTH (pg/100ml)

TIME (MIN) FROM BEGINNING OF ACCELERATION TEST

FIGURE 5. - Plasma concentration of cortisol and ACTH in female and male subjects of different ages, before (-30 min), immediately after (15 min), and 10 min after centrifugation (+3 Gz) tests performed before and after bedrest. Each bar represents the mean for 8 to 10 subjects; error bars represent standard error of the mean.
FIGURE 6. - Plasma concentrations of norepinephrine and dopamine β-hydroxylase in female subjects of different ages, before (-30 min), immediately after (15 min), and 10 min after centrifugation (+3 Gz) tests performed before and after bedrest. The ratio of norepinephrine to DBH is also graphed. Each bar represents the mean for 8 to 10 subjects; error bars represent standard error of the mean.
FIGURE 7. - Urinary excretion of hormones in male and female subjects in several age ranges. Each bar represents the mean for 7 to 10 subjects and for all days of the experiment phase; error bars represent standard error of the mean.
FIGURE 8. - Urinary excretion of electrolytes and other parameters of urine measured in male and female subjects in several age ranges. Each bar represents the mean for 7 to 10 subjects and for all days of the experiment phase; error bars represent standard error of the mean.
FIGURE 9. - Urinary excretion of sodium and chloride in males and females of three age groups on the "non-stress" (no centrifugation or LBNP) days before, during, and after bedrest. Each point represents the mean for 7 to 10 subjects.
### APPENDIX A - PLASMA MEASUREMENTS

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#### Cortisol circadian rhythm, μg/100 ml

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APPENDIX A (Concluded)

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| Acceleration before bedrest |
| -30 min | 12.6 | 4.7 | 7.4 | 3.1 | 11.6 | 3.1 | 6.7 | 1.5 | 11.7 | 4.6 | 10.9 | 2.4 | 8.7 | 3.5 |
| 15 min | 18.0 | 3.0 | 12.4 | 2.3 | 16.2 | 4.5 | 13.3 | 2.9 | 14.6 | 7.9 | 15.0 | 2.7 | 10.7 | 4.8 |
| 25 min | 19.7 | 3.1 | 12.7 | 4.8 | 13.1 | 3.3 | 10.5 | 4.2 | 14.3 | 5.9 | 12.4 | 2.0 | 14.2 | 6.5 |
| Acceleration after bedrest |
| -30 min | 10.3 | 2.1 | 11.0 | 4.6 | 9.8 | 3.2 | 7.9 | 3.2 | 12.3 | 2.8 | 5.9 | 3.6 | 6.4 | 2.0 |
| 15 min | 14.9 | 4.0 | 16.2 | 6.3 | 15.4 | 2.2 | 14.3 | 2.1 | 16.6 | 2.9 | 12.5 | 4.5 | 14.4 | 4.2 |
| 25 min | 21.3 | 7.0 | 16.0 | 3.4 | 16.0 | 5.8 | 15.3 | 5.1 | 15.6 | 2.3 | 14.3 | 2.9 | 15.4 | 7.1 |
| ACTH, pg/ml |
| Acceleration before bedrest |
| -30 min | 39.0 | 13.8 | 14.5 | 7.8 | 31.5 | 18.8 | 36.7 | 12.5 | 42.6 | 22.7 | 17.7 | 13.7 | 37.3 | 23.5 |
| 15 min | 131.0 | 49.8 | 31.5 | 25.7 | 132.0 | 53.4 | 100.0 | 60.3 | 71.2 | 36.9 | 73.0 | 20.3 | 141.0 | 47.2 |
| 25 min | 122.0 | 68.6 | 22.4 | 12.9 | 71.1 | 37.0 | 67.0 | 27.1 | 46.1 | 21.6 | 46.5 | 31.1 | 102.1 | 30.0 |
| Acceleration after bedrest |
| -30 min | 45.5 | 16.3 | 14.2 | 7.7 | 29.4 | 15.7 | 43.6 | 18.9 | 21.0 | 14.9 | 41.3 | 23.3 |
| 15 min | 176.0 | 98.1 | 28.3 | 20.2 | 81.3 | 29.9 | 103.7 | 74.3 | 78.1 | 68.0 | 196.5 | 158 |
| 25 min | 116.0 | 54.4 | 25.0 | 23.2 | 45.1 | 13.5 | 84.3 | 34.4 | 52.0 | 53.3 | 98.1 | 71.0 |
### APPENDIX B - URINE MEASUREMENTS

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Space Shuttle flight simulations were conducted to determine the effects of weightlessness, lower body negative pressure (LBNP), and acceleration on fluid and electrolyte excretion and the hormones that control it. Measurements were made on male and female subjects of different ages before and after bedrest. After admission to a controlled environment, groups of 6 to 14 subjects in the age ranges 25 to 35, 35 to 45, 45 to 55, and 55 to 65 years were exposed to +3 Gz for 15 minutes (G1) and to LBNP (LBNP1) on different days. On 3 days during this pre-bedrest period, no tests were conducted. Six days of bedrest followed, and the Gz (G2) and LBNP (LBNP2) tests were run again. Hormones, electrolytes, and other parameters were measured in 24-hour urine pools throughout the experiment. During bedrest, cortisol and aldosterone excretion increased. Urine volume decreased, and specific gravity and osmolarity increased. Urinary electrolytes were statistically unchanged from levels during the non-stress control period. During G2, cortisol increased significantly over its control and bedrest levels. Urine volume, sodium, and chloride were significantly lower; specific gravity and osmolarity were higher during G2 than during the control period or bedrest. During LBNP2, volume was lower than during the non-stress control period, and specific gravity and osmolarity were higher than during control or bedrest periods. The retention of fluids and electrolytes after +Gz may at least partially explain decreased urine volume and increased osmolarity observed during bedrest in this study. There were some statistically significant differences between the sexes and age groups. Results of the study indicated that space flight would not affect the fluid and electrolyte metabolism of females or older males any more severely than it has affected that of male or female astronauts.