Urinary Excretion of LH and Testosterone from Male Rats During Exposure to Increased Gravity: Post-Spaceflight and Centrifugation (44557)

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Abstract. **A dissociation between plasma luteinizing hormone (LH) and testosterone (T) appears to exist during exposure to altered gravity. The pulsatile nature of LH release and the diurnal variability of T secretion may mask or bias the effects of altered gravity on the pituitary-gonadal axis when analyzing plasma concentrations. Therefore, we examined the relationship between the excretion of urinary LH and T in male Sprague-Dawley rats during exposure to increased gravity upon return to Earth following a 14-day spaceflight ($n = 6$) and by 12 days of centrifugation at 2g ($n = 8$). Excreted LH and T were elevated on the first 3 days postflight. Excreted T was elevated between Days 1 and 8 of centrifugation; however, excreted LH was reduced on Days 2 and 3 compared with control animals. Excreted LH and T were significantly correlated (R = 0.731 and 0.706, respectively) in postspaceflight and centrifuged animals. Correlation curves had similar slopes (0.0213 and 0.023, respectively), but different y-intercepts (–1.43 and 3.32, respectively). The sustained increase in excreted T during centrifugation suggests that the pituitary-gonadal axis in postspaceflight animals may adapt quicker to increased gravity. The upward shift in the correlation curve exhibited by the centrifuged animals suggests that the sensitivity of LH-induced T release is increased in these animals. The previous dissociation between plasma LH and T during altered gravity was not observed in the present study in which excreted LH and T were measured.**

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Testosterone (T) secretion in mammals is normally mediated by the gonadotropin, luteinizing hormone (LH). Exposure to altered gravity (i.e., microgravity and centrifugation) has been shown to affect the pituitary-gonadal axis in mammals (1–4). During spaceflight, astronauts exhibited a significant increase in plasma LH; however, plasma T was reduced (4, 5). This dissociation between circulating LH and T in spaceflight subjects was explained as the consequence of peripheral shunting resulting in a decrease in testicular microcirculation and therefore a reduction in testosterone's negative feedback on LH release (1, 4). Upon return to Earth, plasma LH was reported to be reduced on postspaceflight Day 8, whereas plasma T was elevated (4). During centrifugation at 2.3g, plasma LH in rats was not significantly reduced, although plasma T was reduced on Days 1 and 4 with concentrations returning to control levels on Day 15 of exposure (3). Data from these studies suggest that a dissociation between plasma LH and T during exposure to altered gravity may exist.

This dissociation between plasma LH and T may reflect the circadian periodicity of both LH (6) and T (7) secretion in rats. Blood sampling at various time points during the periodic changes in plasma concentrations may mask the actual effects of microgravity and centrifugation. For ex-

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ample, in rats, plasma T was reduced over the first 4 days of centrifugation at 2.3*g* (3); however, we have shown that excreted T was elevated over the first 8 days of centrifugation at 2*g* (8). The use of 24-hr urine excretion values is not biased by daily periodicity and may therefore provide a more accurate account of the effects induced by altered gravity on the pituitary-gonadal axis.

The present study analyzed excreted LH and T from 24-hr urine collections to compare the effects of $+1\Delta g$ induced by either return to Earth from space or centrifugation at 2*g* on the pituitary-gonadal axis in rats. Although both postspaceflight and centrifugation induce an increase of $+1\Delta g$, these conditions are similar but not identical. We hypothesized that if postspaceflight and centrifugation at 2*g* result in $+1\Delta g$, then both treatments would induce a similar response by the pituitary-gonadal axis during exposure.

Materials and Methods

Animal use protocols for centrifugation and postspaceflight procedures were reviewed and approved by the NASA Ames Research Center Animal Care and Use Committee. Protocols adhered to the NRC's *Guide for the Care and Use of Laboratory Animals*.

Animals and Procedures. Details of the animals, their handling, and their care for both postspaceflight (9) and centrifugation (8) have previously been published. Characteristics of the animals and basic procedures for both study groups are summarized in Table I. Centrifuged animals were spun for 12 days at 2*g* (25.21 r.p.m.), and postspaceflight animals were flown aboard the Space Shuttle Columbia (STS-58) for 14 days. Upon landing of the shuttle, the animals underwent an examination by a veterinarian to assess the health of the animals and were turned over to the investigator within 6 hr of landing.

Urine Collection. For centrifugation, 24-hr urine samples were collected every morning (0730 PST) when the centrifuge was stopped for 1 hour to conduct routine maintenance of the animals. Urine was collected twice a day (0530 and 1530 PST) over the first 4 days postspaceflight (in accordance with other studies). Following Day 4, urine collections occurred at 1530 PST. Daily collections were taken on Days 5–7, and on Days 9, 11, and 13. Although urine was collected for 14 days postspaceflight, the sample volumes we received were not sufficient for analysis of LH and T on Day 14. Therefore, for the purpose of this paper, we set the duration of that experiment at 13 days. For the purpose of aliquoting samples for analysis, an aliquot of urine from the morning and afternoon collections during the first 4 days was pooled and used to represent the 24-hr sample. On Days 9, 11, and 13 urine was pooled over a 48-hr period, and volumes were divided by two to give an average daily urine volume for that particular collection period (i.e., urine volume on Day 9/2 = average urine volume for Days 8 and 9). Data are only shown for Days 9, 11, and 13 in these cases.

Hormone Analyses. Details of the T analyses and assay characteristics have been published previously(8). In short, an aliquot of urine was hydrolyzed with 12 *N* HCL and testosterone measured using a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). All samples were measured in duplicate, with interassay and intra-assay percentage coefficients of variation of 7.3 ± 1.0% and 6.2 ± 0.7%, respectively. A commercially available RIA kit (DPC, Los Angeles, CA) was also used for the determination of urinary LH concentrations. The metabolism of LH during filtration in the kidney has been well defined, and results in at least two subunits (α and β) (10). Since the kit antibody is specific for the

Table I. Summary of Animals and Procedures

	Post-spaceflight	Centrifugation
Rats		
Sample size		
Control	6	8
Experimental	6	8
Body mass (g)[a]		
Control	241 ± 3	224 ± 2
Experimental	246 ± 5	224 ± 2
Age (d)[a]	52	54
Sex	Males	Males
Strain	Sprague-Dawley	Sprague-Dawley
Vendor	Harlan Laboratories	Simonsen Laboratories
Procedures		
Study duration (days)	13	12
Diet	Food bars[b]	Rat chow[c]
Water	*ad lib*	*ad lib*
Housing	Metabolic cage	Metabolic cage
# Animals/cage	1	1
Light cycle	12L:12D	12L:12D

[a] Body mass and age of the animals at the beginning of the study.
[b] NASA Experimental Rodent Diet #TD 88179. (9)
[c] Powdered Purina Rat Chow Diet #5012. (8)

β-subunit as per the kit information, we recognize that our measured moiety is immunoreactive LH. However, for the contents of this paper, we will refer to it as LH. For the analysis of LH, a 250-μl aliquot of urine was diluted 1:2 with calibrating buffer (DPC, Los Angeles, CA), and 200-μl aliquots of diluted urine run in duplicate in the assay. Inter- and intra-assay percentage coefficients of variation were 5.2 ± 0.8% and 4.5 ± 0.4%, respectively. For both hormones, excreted values were determined by urine volume × urine [LH] or [T], and expressed as amount excreted per day.

Statistics. Two-way analysis of variance (ANOVA) corrected for repeated measures over time was used to compare the means of the experimental animals to their control group for each study (postspaceflight and centrifugation). A Fisher's PLSD test was applied *posthoc* if significance was determined for group × time interactions. Correlations between excreted LH and T were conducted using a simple regression of the daily mean values (11). A modified Student's *t* test was used to compare the regression slopes and intercepts for the experimental groups. ANOVAs and regressions were performed using StatView for the Macintosh software (12). Values are reported as means ± SE and were considered significantly different at $P < 0.05$.

Results

A significant group effect between postspaceflight animals and their controls was determined for excreted T. A significant group × time interaction was determined for both excreted LH and T during the postspaceflight period. Both excreted LH and T were significantly elevated on the first 3 days postspaceflight (Fig. 1). A significant group effect and a significant group × time interaction were observed for both excreted LH and T during centrifugation. Excreted LH was reduced on Days 2, 3, and 10–12 of centrifugation compared with controls (Fig. 2, top panel). Conversely, excreted T was elevated significantly from Days 1–6 and 8 with a peak at Day 5 of centrifugation compared with controls (Fig. 2, bottom panel). Excreted LH and T were correlated significantly in both postspaceflight and centrifuged animals (Fig. 3). Intercepts for the two curves were significantly different; however, the two slopes were not.

Discussion

Testicular testosterone secretion in mammals is normally mediated by LH. In the present study, changes in LH and T were correlated during exposure to increased gravity induced postspaceflight or by centrifugation. However, previous studies of the effects of altered gravity on the pituitary-gonadal axis have reported a dissociation between LH and T (3–5).

Rats centrifuged at 2.3*g* exhibited reduced plasma T and unchanged plasma LH measured on the fourth day (3). Gray *et al.* (3) suggested that the paradox between suppressed T levels and unaffected LH levels may have been the consequence of (i) T not being entirely mediated by LH

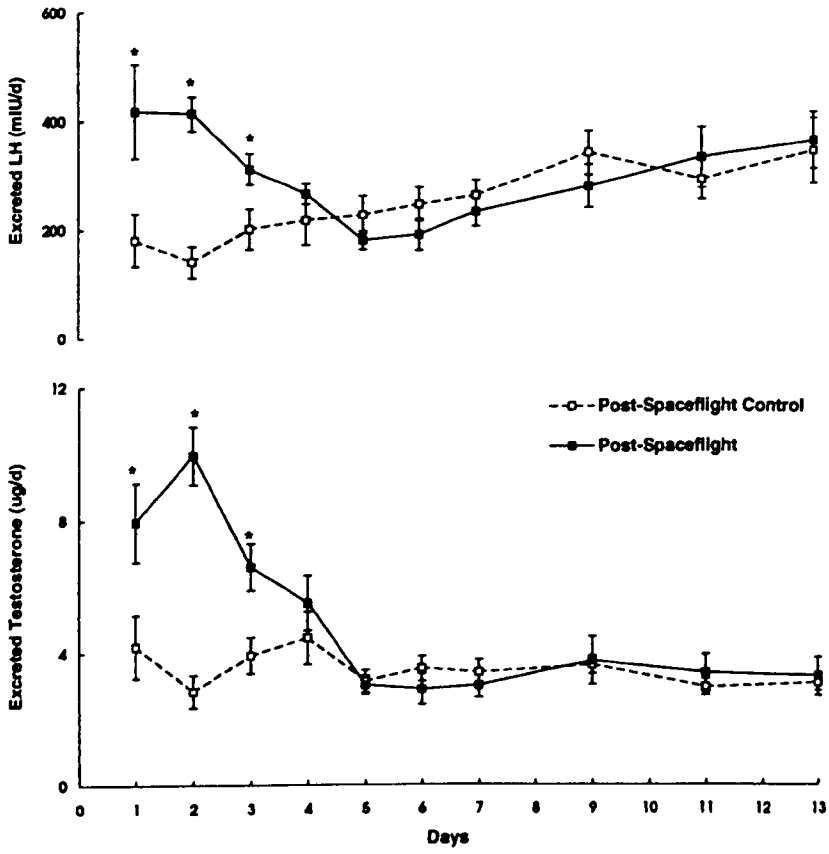

Figure 1. Effects of +1Δ*g* induced by postspaceflight on excreted LH and T for postspaceflight animals and their controls. Significant ($P < 0.05$) differences are designated by an asterisk (*). Means (± SE) are reported.

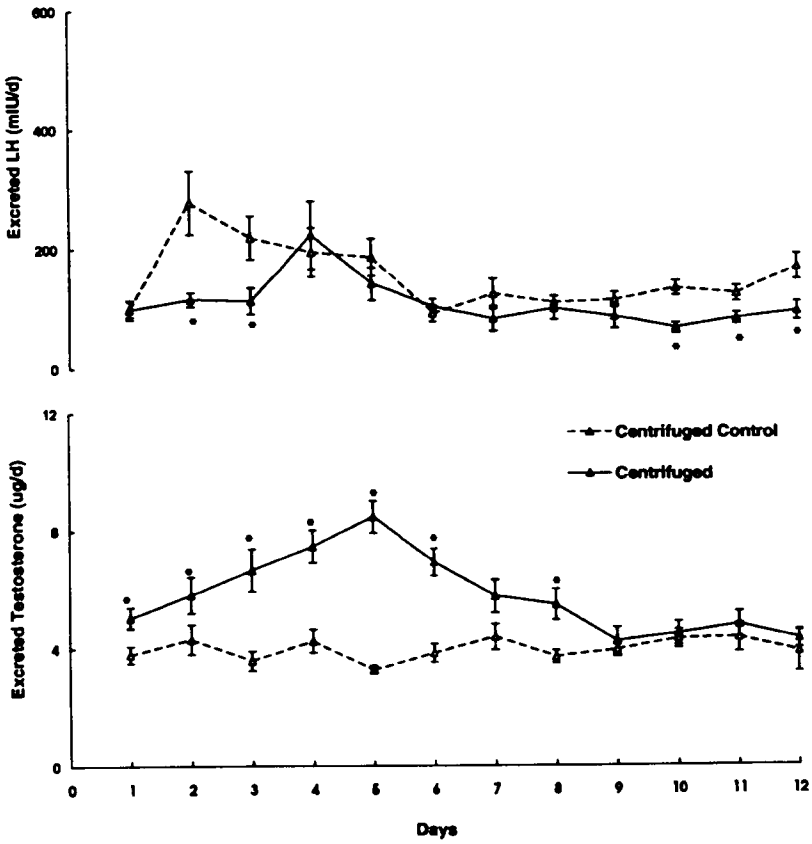

Figure 2. Effects of $+1\Delta g$ induced by centrifugation at $2g$ on excreted LH and T for centrifuged animals and their controls. Significant ($P < 0.05$) differences are designated by an asterisk (*). Means (± SE) are reported.

or (ii) the LH values measured not being representative of the "true" levels during centrifugation. The present study would support the second aforementioned contention that plasma LH values were not representative of the "true" levels during centrifugation. The advantage of excreted LH and T measured in the present study, as compared with previous studies that used plasma concentrations, is that it incorporated the integration of the stimulus over a 24-hr period as opposed to a single plasma sample of a hormone that is episodically released (7).

The pituitary-gonadal axis has previously been reported to be influenced by alterations in gravity (1–4). Veeramachaneni *et al.* (13) indicated that testicular function was not affected during increased gravity. The present study not only supports this contention, but further suggests that the pituitary-gonadal axis is potentiated in response to an increase in gravity. In response to $+1\Delta g$ induced by postspaceflight and by centrifugation, the increases in excreted T were proportional to increases in excreted LH as indicated by similar slopes of the correlations between excreted LH and T (Fig. 3). Although the pituitary-gonadal responses to increased gravity were similar, the differences in the *y*-intercepts between the two functions indicate that for a given concentration of LH, a higher level of T secretion exists during centrifugation compared with postspaceflight. This off-set in response to increased gravity suggests that an increase in pituitary-gonadal sensitivity exists due to centrifugation. The increase in excreted T over the first 3 days of centrifugation despite lack of an increase in excreted LH within the centrifuged animals is intriguing. This initial dissociation between excreted LH and T may suggest either an increased peripheral sensitivity to LH or the involvement of other mechanisms other than LH stimulation for T release during centrifugation.

The duration the animals spent in their respective environment prior to exposure to increased gravity may have contributed to the observed difference in the time hormone concentrations returned to control levels between postspaceflight and centrifuged animals. The spaceflight rats were exposed to 14 days of microgravity prior to returning to Earth's $1g$ environment, whereas the centrifuged animals were continuously at $1g$ until the beginning of centrifugation. In postspaceflight animals, LH and T returned to control concentrations on Day 4, whereas T in centrifuged animals did not return to control levels until Day 9. The difference between the two parallel curves may also be an artifact of the gravitational environment experienced by each group prior to exposure to increased gravity. Therefore, the observed off-set between the two parallel curves may have resulted from the pituitary-gonadal axis in spaceflight rats adapting differentially to microgravity as previously suggested (1, 5). Other factors such as vendor source of the animals (14) or laboratory environment (15) could also contribute to the observed differences.

In conclusion, excreted LH and T are correlated during increases in gravity induced by postspaceflight and by centrifugation following an initial stabilization phase. This is in contrast to previous reports indicating a dissociation in LH

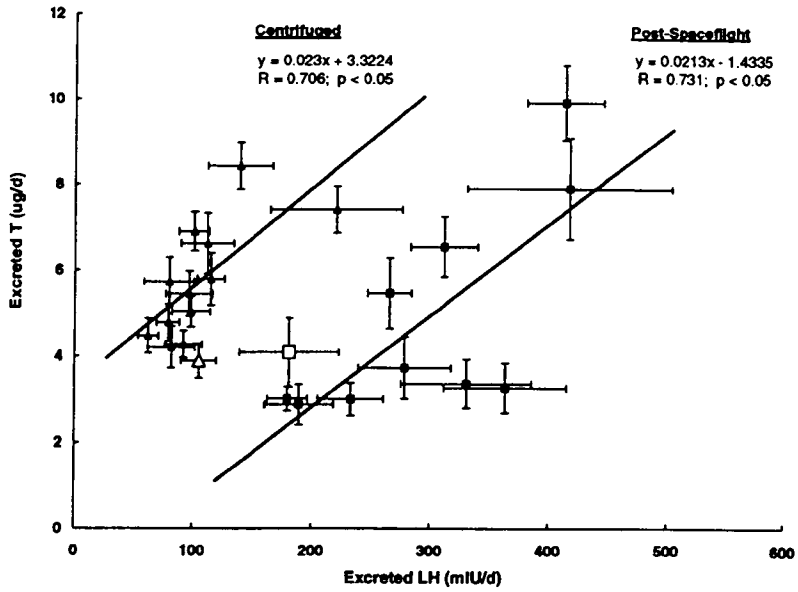

Figure 3. Positive correlations between excreted LH and T for both postspaceflight and centrifuged animals. Large open symbols are pooled means (± SE) for each treatment's control group determined on the first day of the study. Correlations were considered significant at $P < 0.05$.

and T during altered gravity (3, 4). The dissociation between excreted LH and T during the first 3 days of centrifugation is opposite to that observed in humans during spaceflight where urinary T was decreased despite increased circulating LH (4). The use of urine samples in the present study may provide a more comprehensive analysis of the function of the pituitary-gonadal axis on a daily basis in response to increased gravity than plasma concentrations. The sensitivity of the relationship between LH and T appears to be increased in the animals used in the centrifugation experiment. Adaptation of the pituitary-gonadal axis to the animal's respective environment prior to an increased gravitational load may have contributed to the observed differences. A better understanding of the pituitary-gonadal axis in response to altered gravity is an important step in evaluating the integration between this axis and other systems such as those influencing muscle and bone metabolism since these tissues are affected by gravity as well. The alterations in these systems point to an overlying influence of gravitational forces on the evolution, development, and function of organ systems, which may be mediated *via* the hypothalamic-pituitary-gonadal axis.

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