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INFLUENCE OF ACADEMIC STRESS AND SEASON ON 24-HOUR MEAN CONCENTRATIONS OF ACTH, CORTISOL, AND β-ENDORPHIN

WILLIAM B. MALARKEY,[1-5] DENNIS K. PEARL,[5,6] LAURENCE M. DEMERS,[9] JANICE K. KIECOLT-GLASER,[4,7,8] and RONALD GLASER[1,3-5]

Departments of [1]Internal Medicine, [2]Medical Biochemistry, [3]Medical Microbiology and Immunology, [4]Behavioral Medicine Research Program, [5]Comprehensive Cancer Center, [6]Statistics, [7]Psychiatry, and [8]Psychology, The Ohio State University Medical Center, Columbus, Ohio 43210, USA and [9]Department of Pathology, Pennsylvania State University, M.S. Hershey Medical Center, Hershey, Pennsylvania 17033, USA

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SUMMARY

We investigated the influence of a common stressful event, i.e., academic examinations, on the 24-h mean concentration of adrenocorticotropic hormone (ACTH), cortisol, and/or β-endorphin. In addition, we evaluated the effect of season on the endocrine response to this stressor. We studied medical students ($n = 55$), screened for a variety of health and life style factors, from three consecutive medical school classes 1 month before, during, and 2 weeks following examinations. Hourly blood samples were obtained from an indwelling catheter and two serum pools were made (0800–2200h = day and 2300–0700h = night). Examinations produced a significant ($p < .001$) increase in perceived stress scores. In addition, we found a significant ($p < .001$) effect of examination stress on the increase in mean daytime but not nocturnal ACTH levels during autumn, but not during the spring. In contrast, the examination stress did not significantly effect day or night mean cortisol levels from baseline to examination week. We further divided the students by whether their perceived stress scores increased during examination week and fell during recovery (Group 1) or whether their perceived stress scores did not follow the expected pattern (Group 2). We found that in the Group 1 students who perceived the most stress, cortisol levels significantly increased ($p < .001$) from baseline to examination. Therefore, the nature of the stressor and the state of the responder were of equal importance in the observed cortisol response during examinations among these students. Further, academic stress had no significant effect on β-endorphin levels. Finally, we found that the mean day and night ACTH levels were higher ($p < .001$) in the spring than in the fall; a seasonal influence on cortisol and β-endorphin concentrations, however, was not observed. In summary, we have demonstrated that stress associated with the taking of examinations produces a dissociation among mean 24-h levels of ACTH, cortisol, and β-endorphin. In addition, daytime cortisol levels increased during examinations only in the group of students whose perceived stress scores increased. Further, a seasonal influence on ACTH secretion was suggested by these results with higher levels observed in the spring than in the fall.

Keywords—Academic stress; ACTH; Cortisol; β-Endorphin; Seasonal.

INTRODUCTION

STRESS ALTERS ENDOCRINE as well as immune function (Kiecolt–Glaser et al., 1992) and bidirectional feedback appears to be operative between the endocrine and immune system

Address correspondence and reprint requests to: Dr. William B. Malarkey, N-1106 Doan Hall, 410 West Tenth Avenue, Columbus, OH 43210, USA.

(Besedovsky et al., 1985; Blalock et al., 1985). Cortisol down regulates the immune system at many different loci. This includes inhibition by cortisol of interleukin-1 (IL-1) and interleukin-2 (IL-2) production as well as inhibition of the ability of monocytes to present antigen (Munck & Guyre, 1991). ACTH and β-endorphin have independent effects on the immune system. For example, ACTH binds to human lymphocytes (Smith et al., 1987) and can block interferon production by murine macrophages and splenocytes (Koff & Dunegan, 1985), whereas β-endorphin can either enhance or diminish cellular immune function (Shavit, 1991).

Investigations that have examined the influence of various psychological stressors on endocrine secretion in human studies have produced conflicting results. Differences in sampling intervals which have not taken into account the pulsatile nature of hormonal release, diurnal variation, and the intensity of the stressor may account for some of the conflicting data reported. Another question raised by previous studies in humans is whether psychological stress significantly influences 24-h concentrations of various hormones. Our first attempt to clarify the impact of a common life event stressor on 24-h endocrine secretion was to evaluate growth hormone (GH) and prolactin (PRL) levels in medical students before, during and after examinations (Malarkey et al., 1991). Although GH and PRL secretion increase following a variety of stressful stimuli, we found that academic stress did not significantly alter the 24-h mean concentrations of these hormones. In addition we observed a seasonal influence on GH secretion, with consistently higher values in the fall than in the spring which could not be explained by the stress of examinations (Malarkey et al., 1991). This latter study has been extended and has provided an opportunity to examine the influence of academic stress and season on other peptides. ACTH, cortisol, and β-endorphin are released by a variety of stressful stimuli but in humans the type, magnitude, and duration of the stimulus required for their release has not been clarified. In this study we have evaluated 24-h levels of ACTH, cortisol, and β-endorphin in 55 male first-year medical students from three sequential classes. These studies were performed before, during and after major examinations in the fall and spring. We evaluated the influence of a commonplace stressor and season on the mean 24-h levels of ACTH, cortisol and β-endorphin.

MATERIAL AND METHODS

Subjects

The study sample consisted of 55 male medical students between the ages of 21 and 30 years (70% were 22 years old). Students were recruited via a letter sent 6 weeks before medical school began and/or during orientation week. Students who had a history of anxiety episodes or previously received counselling or medication for any psychological disorder were excluded from the study. Also, a history of any recent symptoms of illness or a history of a chronic disorder were excluded from the study. They were nonsmokers and consumed less than five alcoholic drinks/week. They were not well-conditioned athletes and did not use chronic medications or recreational drugs and were within ±20% of ideal body weight. In three consecutive freshman classes, we evaluated 13, 22, and 20 students. Examinations were given over 3 days for preclinical medical students.

Protocol

After obtaining informed consent, each subject was studied 3–4 weeks before a major academic examination series, within 24-h of the examination or on the day of the examination, and 2 weeks after the examination. Three meals which were similar for each subject were consumed daily by the students and all their activities were confined to the Clinical Research Center or the classroom. Hourly blood samples were obtained for ACTH, cortisol, and β-endorphin levels from an indwelling catheter with a heparin well and placed in tubes containing EDTA. The daytime specimens were collected hourly outside the student lecture hall while the specimens collected after 1700h were obtained in the Clinical Research Center. These specimens were allocated into two serum pools. The 0800–2200h specimens constitute the day pool and the 2300–0700h specimens constitute the night pool. This sampling sequence was followed both in the fall and the spring. Thus, a total of six 24-h evaluations were completed for each subject over the course of an academic year.

During each 24-h admission period subjects were asked to complete a 14-question Perceived Stress Scale (PSS). The Perceived Stress Scale (Cohen & Williamson, 1988) assesses global perceptions of stress and measures the degree to which individuals appraise situations in their daily lives as unpredictable, uncontrollable, and overloading. The PSS provided better predictions of psychological symptoms, physical symptom reports, and health service utilization than life event scales (Cohen & Williamson, 1988). While the PSS may have some item overlap with depression measures (e.g., "How often have you felt nervous or stressed?"), the PSS has been shown to be independent of depression.

Assays

ACTH levels were determined by an immunoradiometric assay using materials supplied by Nichols Institute (Capistrano, CA) and all samples were measured in duplicate. This assay has a sensitivity of .1 pmol/l, has <1% cross reactivity with potential interfering peptides and an intra- and interassay coefficient of variation of 4 and 8%, respectively. The plasma cortisol levels were assayed using the fluorescent polarization method on the Abbott TDX (Chicago, IL). The cortisol assay has a sensitivity of 25 nmol/l, has minimal or no cross reactivity with potential interfering peptides, and has intra- and interassay coefficient of variation of 5 and 9%, respectively. β-Endorphin was assayed with a direct IRMA method with reagents supplied by Nichols Institute (Capistrano, CA). This assay has a sensitivity of 5 pmol/l, has <1% cross reactivity with possibly interfering peptides, and has an intra- and interassay coefficient of variation of 6 and 9%, respectively. All of the pooled specimens from the fall were evaluated together and the spring specimens were handled in a similar fashion to decrease confounding by interassay variability. Similar internal control specimens were used in the fall and spring assays.

Analysis of Data

Inferential statements in this report were made using a repeated measures analysis of variance (ANOVA) style statistical model including within-subjects terms for season (fall or spring) and week (baseline, examination, or recovery) and between-subjects terms for cohort (years 1–3). For some analyses an additional between-subjects term was incorporated to compare the group of students who showed the expected pattern of increasing PSS scores at examination followed by a decrease at follow-up with those who did not follow this pattern. The data were analyzed on a logarithmic scale to make inferences regarding relative changes more relevant and to insure a more equal variance across the groups being compared. However, performing the analyses in the absolute scale does not change the fundamental results stated. In any case, an examination of

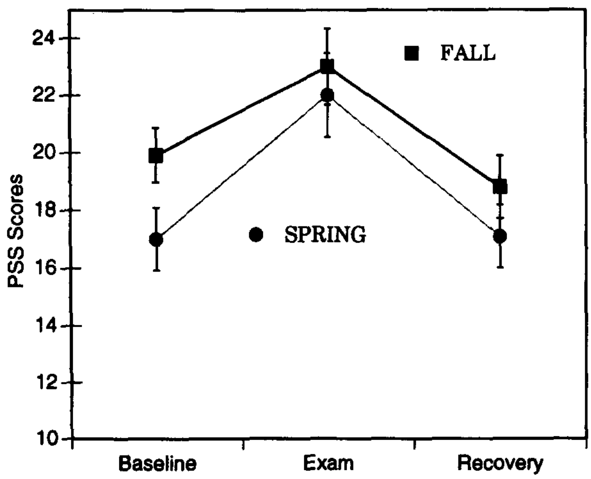

FIG. 1: Mean ± SEM perceived stress scale (PSS) scores on 55 male medical students. Note the significant ($p < .001$) influence of examinations on PSS scores in the fall and spring.

residual plots did not contradict the validity of the methods used in particular the normality of the transformed data. Finally, we required a conservative p-value of $< .01$ for significance tests to reduce the multiple comparisons problem of falsely declaring positive results.

RESULTS

Perceived Stress Scale (PSS)

The mean ± SE of PSS scores for the medical students in the fall were 19.3 ± 1.0, 23 ± 1.4, and 18.9 ± 1.1 at baseline, examination, and 2 weeks post-examination, respectively. The comparable PSS values in spring were 17.0 ± 1.1, 22 ± 1.4, and 17.1 ± 1.2. Both examination PSS values were significantly different ($p < .001$) from baseline (Fig. 1). In the fall, 36 of 54 individuals (67%) had an increase in their PSS values between the baseline and examination evaluation. Thirty of 54 (56%) had a typical pattern of stress rising from baseline to examination and then decreasing at the 2-week follow up evaluation. In the spring 69% of the students had an increase in PSS scores from baseline to examination. There was no significant correlation (r^2 values all < 0.07) between PSS scores and day or night ACTH, cortisol or β-endorphin levels.

24-h ACTH Concentrations

Academic stress significantly ($p < .001$) enhanced mean ± SE daytime ACTH levels but not nocturnal concentrations in the fall (Fig. 2). However, a significant increase in nocturnal ACTH levels from baseline to recovery was observed in the fall ($p < .05$). The fall ACTH changes occurred in each of the 3 years of study (Fig. 4). These changes were not observed in the spring (Fig. 2). In addition a significant ($p < .001$) seasonal effect on 24-h ACTH levels was also observed with higher values being observed in the spring (Fig. 2).

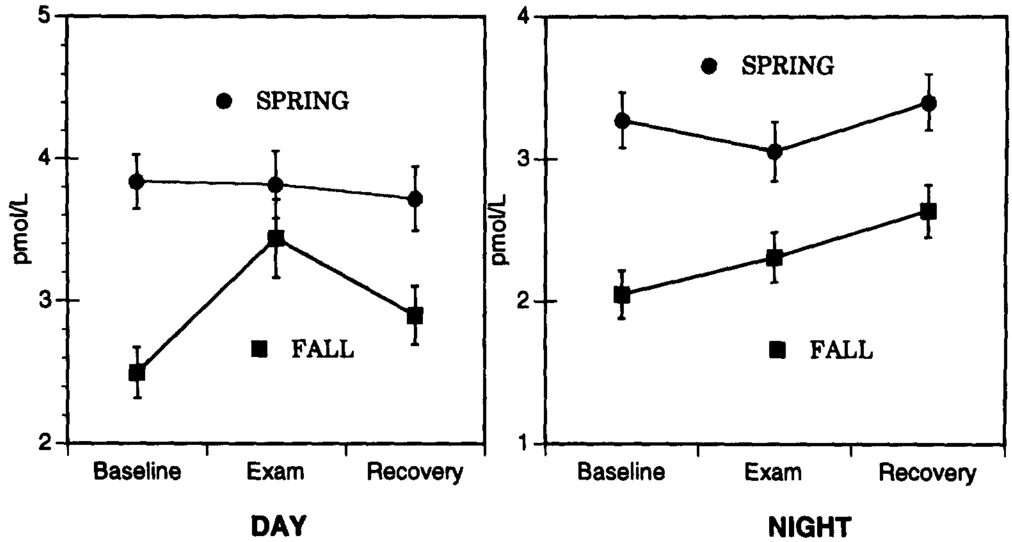

FIG. 2: Examination stress significantly ($p < .001$) increased mean ± SEM daytime but not nocturnal ACTH levels in 55 male medical students. Also, mean ACTH concentrations were significantly ($p < .001$) higher in the spring than in the fall. Each data point here and in Fig. 3 represents the mean ± SEM of 15 hourly samples obtained from 0800 to 2200h (day) and 9 hourly samples acquired from 2300 to 0700h (night).

24-h Cortisol and β-Endorphin Levels

Academic stress had no significant effect on mean ± SE daytime or nocturnal cortisol levels from baseline to examination week but significant ($p < .001$) decreases were observed from examination week to the recovery period in both the fall ($p < .001$) and

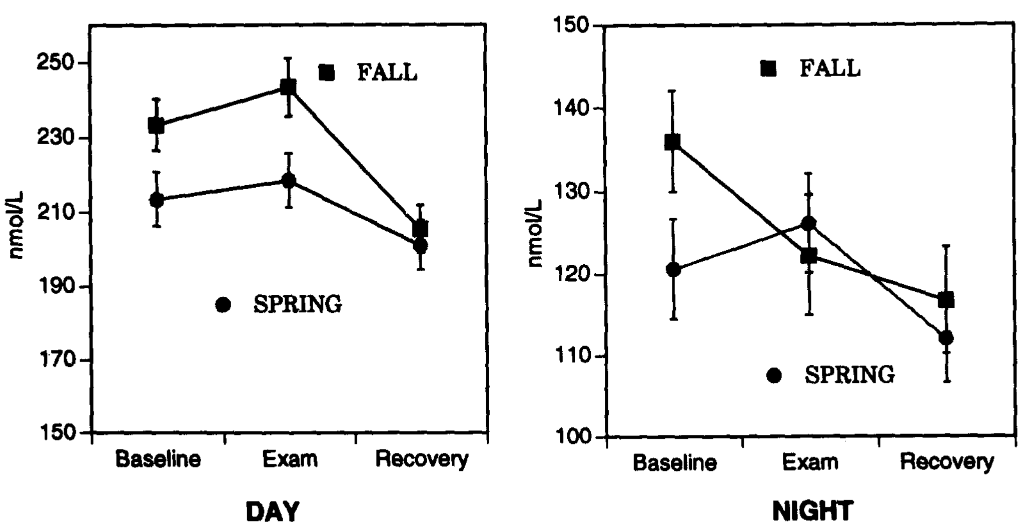

FIG. 3: Examination stress failed to significantly increase serum cortisol levels in 55 male medical students and a significant seasonal effect was not found.

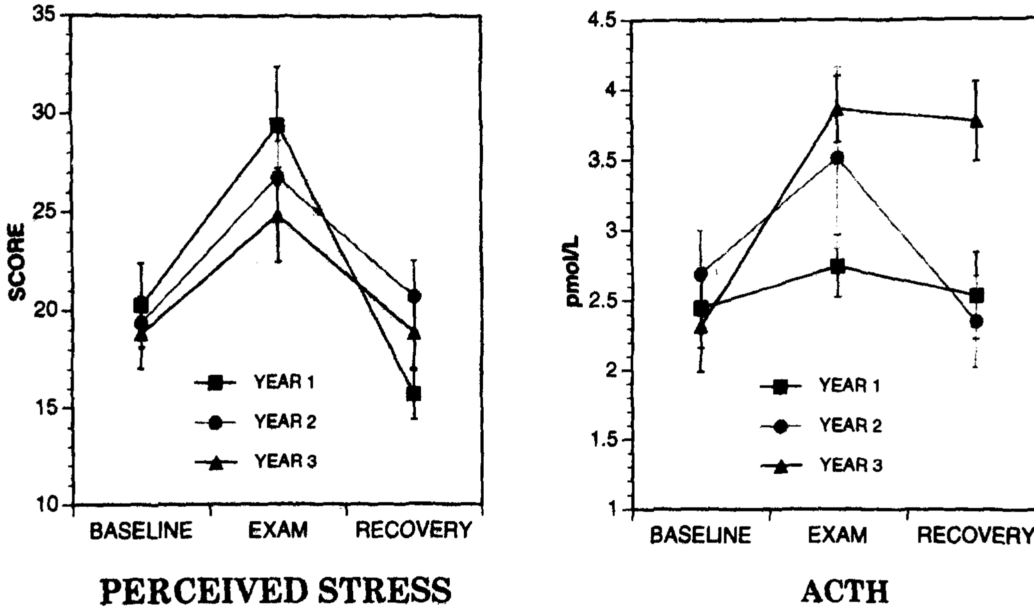

FIG. 4: Examination stress increased perceived stress scores and ACTH levels in 13, 22, and 20 students in years 1, 2, and 3, respectively.

spring ($p < .01$-daytime levels) (Fig. 3). The fall baseline cortisol level was elevated and significantly decreased by examination week ($p < .05$) (Fig. 3). This finding, however, was inconsistent and was produced by the year 2 class. When we divided the subjects evaluated in the fall by whether their PSS scores increased during examination week and fell during recovery week (Group 1) or whether their PSS scores did not follow this expected pattern (Group 2), differences in daytime but not nocturnal cortisol levels were seen (Fig. 5). In Group 1 ($n = 30$) fall daytime cortisol levels increased by 13.2 ± 5.1%

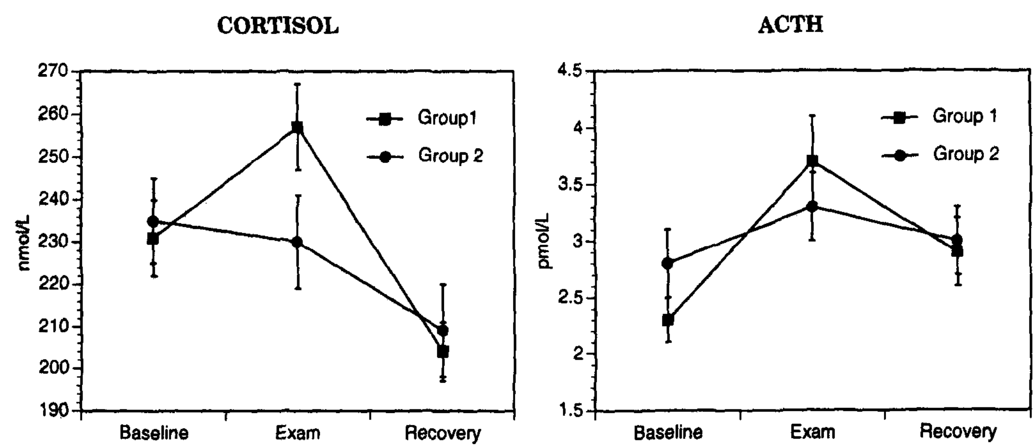

FIG. 5: In Group 1 (their PSS scores increased between baseline and examination weeks) the daytime cortisol levels significantly ($p < .01$) increased from baseline to examination and then significantly decreased ($p < .001$) from examination to recovery. No change was noted in Group 2 subjects (no significant change in PSS scores from baseline to examination). The ACTH increase in the Group 1 students was not significant.

TABLE I. TWENTY-FOUR HOUR β-ENDORPHIN LEVELS

Season	Day	Night
Fall		
Baseline	10.4 ± .6	10.1 ± .9
Exam	11.0 ± .9	9.8 ± .6
Recovery	11.8 ± .9	11.0 ± .9
Spring		
Baseline	9.8 ± 9	10.1 ± 1.2
Exam	12.6 ± 1.5	12.1 ± 1.4
Recovery	9.8 ± .9	10.4 ± 1.4

($p < .01$) from baseline to examination and fell 16.3 ± 4% ($p < .001$) from examination to recovery whereas in Group 2 ($n = 25$) baseline to examination cortisols did not increase, 1.2 ± 5%, and fell by 8.0 ± 4.7% ($p < .1$) from examination to recovery. Nocturnal cortisol levels were similar between groups; baseline 137 ± 9 and 135 ± 8, examination 121 ± 9 and 125 ± 12, recovery 117 ± 9 and 117 ± 10 in Groups 1 and 2, respectively. When ACTH levels were examined in Group 1 and 2 a nonsignificant increase in the level of changes reported above was found in Group 1 subjects (Fig. 5). In addition academic stress had no significant seasonal influence on cortisol levels (Fig. 3) and daytime or nocturnal β-endorphin levels were not significantly influenced by academic stress or season (Table I).

DISCUSSION

Examination stress produced a significant increase in medical student's perceived stress scores which was associated with an increase in ACTH levels during the day in the fall but not in the spring. This was a consistent finding noted in each of the three classes we evaluated. A change in mean ACTH levels of 38% (312–430 nmol/l) was observed from baseline to examination but was not of sufficient magnitude to alter the mean daytime cortisol level. In another human stress study which we have recently completed, we also observed significant increases in ACTH levels without a coincident increase in cortisol levels (Malarkey et al., 1994).

Dallman's laboratory has examined the issue of adrenal sensitivity to a controlled infusion of ACTH. They found that a 2 pmol/l change in ACTH levels produced a 55 nmol/l change in plasma corticosteroids in conscious dogs (Wood et al., 1982). If human adrenals have a similar sensitivity to ACTH, the peak ACTH increase in medical students in the fall of approximately 1 pmol/l would predict a 27 nmol/l increase in cortisol levels in the students. However, we observed a nonsignificant 10 nmol/l increase in fall cortisol levels. Our data would suggest that the ACTH levels we noted in the fall were below the threshold required for a significant increase in cortisol secretion, but studies similar to the animal research would have to be performed in humans before this conclusion could be validated.

It is becoming increasingly clear that the endocrine profile of stress hormones observed following a stressful stimulus depends on the nature and duration of the stimulus. For example, we and others have shown that short- or long-term physical stressors produce marked changes in a number of peptide and steroid hormones (Hagberg et al., 1988; Malarkey et al., 1993). In contrast, psychological stressors often produce more limited endocrine changes within normal physiological ranges such as the ACTH changes seen in this study (Kiecolt–Glaser et al., 1992). Certain "stress" hormones may not be influenced by stressful events such as examinations as we have demonstrated in this study for cortisol between baseline and examination and in a previous study for GH and PRL (Malarkey et al., 1991). One could argue that the cortisol response to examination stress may have been very brief and therefore our hourly sampling protocol may have missed the subtle changes or that only measuring pooled daytime and nocturnal samples may have diluted out the examination effect. The ACTH changes, however, were observed with this same protocol. In addition, in another study evaluating the influence of marital conflict stress on pituitary and adrenal hormones we have observed significant changes in ACTH, prolactin, and catecholamines but not with the cortisol levels (Malarkey et al., 1994). Hence our studies and those of other investigators suggest that at least in humans, there is a hierarchy of endocrine responses to stress with a stronger stimulus or one of longer duration being required for cortisol release then for promoting catecholamine and ACTH secretion.

A complicating factor in the interpretation of the cortisol data is the state of the individual who is being challenged by the stressful event. When we divided the students into two groups based on their perceived stress scores and evaluated cortisol levels in the most reactive students (PSS scores which increased from baseline to examination and fell from examination to recovery) we found the more reactive students had a significant increase in cortisol levels from baseline to examination. Recently we have examined cardiovascular reactivity following brief psychological stressors and have noted that high and low cardiovascular reactive subjects have differing endocrine and immune responses including cortisol reactivity (Sgoutas–Emch et al., 1994). When high and low cardiovascular reactivity was not utilized as dependent variables, no differences in cortisol levels to stress were noted. Therefore not only the nature of the stressor but also the state of the responder may be of critical importance in determining the endocrine profile to stress.

In this study, academic stress did not significantly alter β-endorphin levels. Although not well studied in psychological stress, β-endorphin secretion may be dissociated from the ACTH secretory response. For example, in an ultraendurance event we noted that several pituitary, thyroid, and adrenal hormones were significantly changed whereas β-endorphin levels were not significantly altered (Malarkey et al., 1993). This lack of response of β-endorphin to ultra-endurance stress has also been observed by others (Pestell et al., 1989). ACTH and β-endorphin are produced from the same precursor molecule, and the reason for this dissociation between the magnitude of the ACTH and β-endorphin elevations is not clear. Possible explanations include differential processing in the pituitary and/or metabolism of the two peptides.

We observed a seasonal influence on ACTH concentrations but not cortisol levels. Both daytime and nocturnal baseline ACTH levels were approximately 50% higher in the spring when compared with the fall. The PSS scores of the subjects, however, were similar in the fall and spring and therefore examination stress was not responsible for the higher spring ACTH levels. It is unclear if this seasonal effect was involved in reducing the examination influence on the spring ACTH levels.

A previous report with six male subjects found that 24-h ACTH levels were higher in the winter (December) than during the summer (July) (Walter–Van Cauter et al., 1981). The mean ACTH levels in that study were almost identical to our ACTH levels. They also found seasonal differences in cortisol levels which we did not observe in any of the 3 years that we performed the study. The reason for this difference is not clear, however, their study was performed in winter and summer whereas our data were collected in the fall and spring.

Our findings have implications for studies that evaluate the role of stress on the immune system in humans. An ACTH receptor is found on human mononuclear cells (Smith et al., 1987) and ACTH can exert immunological effects independent of its effect on cortisol secretion. For example, ACTH can block interferon production by murine macrophages and splenocytes (Koff & Dunegan, 1985). This raises the suggestion that pituitary hormones may directly influence immune competent cells, thereby linking the immune system to pituitary hormonal events.

In summary, we have demonstrated that examination stress produces a dissociation among mean 24-h levels of ACTH, cortisol, and β-endorphin. In addition daytime cortisol levels increased during examinations only in the group of students whose perceived stress scores increased. Further, a seasonal influence on ACTH secretion is suggested by these data with higher levels being observed in the spring than in the fall.

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