

ORIGINAL RESEARCH

Stress-related biobehavioral profile of senior nursing students

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Received: February 26, 2015

Accepted: March 25, 2015

Online Published: April 27, 2015

DOI: 10.5430/jnep.v5n6p129

URL: <http://dx.doi.org/10.5430/jnep.v5n6p129>

ABSTRACT

Objective: The purpose of the study was to determine the levels of psychosocial (stress, moods, and loneliness) and salivary biomarker responses (cortisol, alpha-amylase, C-reactive protein, Interleukin-1 β , estradiol, and testosterone) and their associations in senior nursing students. Because of diversity in student characteristics, we also examined group differences by age, prior degree status, and curricular tracks.

Methods: In a cross-sectional study, 77 graduating baccalaureate nursing students completed questionnaires and provided a saliva sample via passive drool during fall semester, 2013. All data were collected between 8:00 am and noon. Biomarker levels were assessed with enzyme-linked immunoassays, and biological data were transformed prior to data analyses as needed.

Results: On average, psychosocial and biological responses seem to be within normal ranges. One third of students, however, showed moderately high or high levels of stress. Stress was significantly and inversely correlated with estradiol, $r = -.25$, $p < .04$, and alpha amylase, $r = -.31$, $p < .007$. Anger and confusion were significantly and positively correlated with testosterone, $r = .24$ to $.27$, $p < .05$. Despite the diversity, there were no significant psychosocial or biological differences between groups.

Conclusions: Although average psychosocial and biological responses seem unremarkable, a subset of students showed relatively high levels of stress. Several psychosocial factors were significantly correlated with biological responses, suggesting biobehavioral interactions to influence the health. Regular stress assessment and campus resources may facilitate early stress management to minimize potential long-term adverse health outcomes.

Key Words: Stress, Moods, Loneliness, Biomarker responses

1. INTRODUCTION

The rigor of nursing education continues to rise to meet the demand of increasingly more complex and challenging healthcare needs. Nursing students are under significant stress to achieve both academic and clinical competencies to be able to care for diverse groups of people under differing healthcare conditions and settings. Although academic and professional stress has been studied in student popula-

tions,^[1] few studies have included biological assessments. A biobehavioral approach with concurrent assessments of psychosocial and biological responses is likely to enhance the understanding of the overall well-being of students who are the next generation of workforce.

1.1 Nursing curriculum

In nursing education, the two fundamental components of curriculum include didactics and clinical practice that can be

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delivered in various formats. At this university, baccalaureate nursing curriculum is offered over two years of education in two different tracks, called traditional versus pacesetter. Both tracks cover the same materials over time but in a different sequence, and this approach was implemented to find a better way of providing nursing education more efficiently. Students are randomly assigned to one of the tracks at entry into the nursing program. The students in the traditional track take didactic courses and matching clinical practicum in the same semester, whereas the students in the pacesetter track take all didactic courses first, followed by a clinical practicum in their last semester.^[2] Clinical practicum for both tracks includes the completion of numerous care plans and written reports at each clinical rotation in different practice settings, and graduating students also are required to take national examinations (Health Education Systems Inc. [HESI]) prior to graduation, in addition to regular nursing curricular requirements. Upon successful graduation, students again need to pass another national licensing examination (NCLEX-RN) in order to work as a Registered Nurse. These demands place considerable stress on nursing students, particularly during their graduation semester.

1.2 Psychosocial concerns among students

Psychological stress, anxiety, depression, inadequate social support and loneliness are common concerns among students, and students frequently express feelings of fatigue. Stressors may be of physical, mental, or emotional in nature, and these stressors can affect psychosocial perception and ultimately physical and mental health of the person. Negative moods, such as anxiety and depression, are closely associated with stress and fatigue, and they together can contribute to poor performance, low well-being, and even thoughts of suicide.^[3,4] Academic stress-related depression and anxiety are common in college students as well as in students in professional degree programs. In veterinary schools, 49%-69% of the students reported significant levels of depressive symptoms at and above the clinical-cut off level. Academic stress negatively contributed to depression and anxiety symptoms, life satisfaction, academic performance, and general health of the students.^[5] In medical students, 14.3% reported moderate to severe depression, and depression was stigmatized.^[6] Loneliness is a newer concept of interest that has been explored little in college-age students. A person with loneliness experiences perceived lack of intimacy and companionship, which is related to social isolation, disconnection, and not fitting in. Loneliness in old age predicted mortality and increased the risk of heart conditions.^[7-9] Also, lonelier people express more depression and fatigue than those who are less lonely.^[10,11]

1.3 Biobehavioral interactions

Psychosocial factors are known to alter biological responses, particularly of the neuroendocrine and immune systems.^[12-14] The activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system can be assessed from non-invasive samples of saliva. Salivary cortisol represents a free fraction of biologically active cortisol, and its levels and diurnal patterns have been assessed in association with acute and chronic stress.^[15] In university students, salivary cortisol was elevated before and after an oral presentation and on the day of the presentation. For written exams, salivary cortisol was elevated before the exam and was high on the day of the exam,^[16] indicating increased cortisol responses to anticipatory and actual stress in academic settings. Salivary α -amylase (sAA) has been regarded as a reliable surrogate marker of sympathetic nervous system activity in response to acute stress.^[17] For immune responses, stress and mood disturbance are known to elevate inflammatory mediators, such as interleukin (IL)-1^[14,18] and C-reactive protein (CRP).^[19,20] In teen age adolescents, daily interpersonal stress predicted an elevation of CRP levels months later,^[19] and chronic childhood stress was associated with elevated CRP levels in adulthood.^[21] Similarly, stress was positively associated with CRP in middle-aged and older adults as well.^[22]

Depression and anxiety disorders are more common in females than in males, and sex hormones are thought to play a major role.^[23] The hormone 17 beta-estradiol (E2) was found to be beneficial in decreasing anxiety and depression in young individuals and animals.^[24] Similarly, testosterone is thought to be protective against anxiety and depression.^[25,26] Females with a current depressive or anxiety disorder had lower salivary testosterone levels than controls in a community sample,^[27] and higher free testosterone levels were significantly associated with a decreased risk for depressive symptoms in men.^[28]

Loneliness has been less studied, but lonely people showed high inflammatory responses,^[29,30] and loneliness has been associated with elevated pro-inflammatory gene expression and increased risk for morbidity and mortality.^[31] Chronic inflammation, in turn, can increase risks for developing or progressing diseases, such as cardiovascular and metabolic diseases and even certain types of cancer over time.^[32,33] Similarly, loneliness was associated with an increased cortisol response,^[34] and daily solitude was associated with a significant increase in cortisol levels among female undergraduate students.^[35]

In summary, nursing is a rewarding profession, but nursing education and training can be considerably demanding and

stressful. During training, it is not uncommon for students to express high stress and emotional distress. Nursing students are a diverse group representing a wide range of age and prior academic preparation. Many students pursue nursing education as a second degree. In addition, the current curriculum at the study site includes two different curricular tracks with the students randomly assigned to either track. Given the known importance of bibehavioral interactions in a person's well-being, psychosocial and biological profiling seems to be important to better understand a potentially stressed population of senior nursing students. Furthermore, we sought to examine if diversity in students' background and curricular approach influence their bibehavioral responses.

1.4 Objectives

The major objectives of this study were as follows: (1) determine the levels of psychosocial variables (stress, moods, and loneliness) and salivary biomarker responses (cortisol, sAA, CRP, IL-1 β , estradiol, and testosterone) and their associations; and (2) compare the levels of psychosocial and biological responses between students in two different baccalaureate curricula tracks (pacesetter vs. traditional), two age groups (younger than 25 vs. equal to or older than 25), and two academic preparation levels (a prior professional or college degree vs. no prior degree) at entry to nursing education and in senior graduating nursing students at a baccalaureate degree nursing program.

2. METHODS

2.1 Design

A cross-sectional descriptive study was conducted during fall semester 2013.

2.2 Sample

Seventy seven undergraduate senior nursing students participated in the study. Power analysis indicated that the sample size of 82 would provide power of .80 with two-tailed test based on a small effect size of correlation coefficient of 0.3 and alpha level 0.05 using G*Power 3.17 program.

Inclusion criteria were as follows: (1) currently a college student enrolled in nursing program; (2) no known psychiatric or physical illness that required active treatment; (3) not taking corticosteroid, antibiotics, or anti-depressants; (4) no current infection; and (5) aged 18 - 45 years. Exclusion criteria were: (1) currently receiving structured psychotherapy; (2) inability to produce saliva; and (3) current or substance abuse within the past 3 months.

2.3 Setting and recruitment

Participants were recruited from a baccalaureate degree nursing program in the Southern region of the United States by

posting flyers, classroom and Facebook announcements and by word of mouth. After a brief introduction of the purpose, if students were interested, more detailed information of the study was provided, and written informed consent was obtained prior to collecting data and saliva samples. No incentive was given.

2.4 Data collection

Data were collected between 8:00 and 12:00 am around class attendance hours. Participants rinsed their mouth with water per instruction, started to fill out the questionnaires, produced saliva samples, and completed the questionnaires. Restrooms were readily available for rinsing the mouth as needed, and personal space was provided in and around the classrooms. Saliva samples (approximately 1-2 ml) were collected via passive drool, marking the start and stop time recorded to adjust saliva flow rate for alpha amylase assessment. All saliva samples were placed in ice inside a cooler and transported to the bioscience laboratory in the same building within 30 min to an hour. Samples were stored at -80°C until batch-assayed with enzyme-linked immunoassay kits for specific biomarkers (Salimetrics, PA). The study protocol was approved by the Institutional Review Committee of the university.

2.5 Instruments

For stress, after considering several stress measures that may best fit college students, we chose the College Readjustment Rating Scale (CRRS) based on item relevance. The CRRS contains 30 life events relevant to college students. Participants were asked to mark the event if it occurred within the last 6 months, and the scale provided a weighted score for each event. The sum of the scores indicated stress level, with higher scores indicating greater stress.^[36]

Mood disturbance was measured by a short version of the Profile of Mood States (POMS). The short version of the 37-item POMS minimizes subject burden but has $\alpha > .95$ correlation coefficient with the original scale.^[37] Participants rated each item on a 5-point Likert scale that ranged from 0 = not at all to 5 = extremely. The six dimensions of the inventory are Tension-Anxiety, Depression-Dejection, Anger-Hostility, Fatigue-Inertia, Vigor-Activity, and Confusion-Bewilderment. Cronbach's α ranged from .76 to .90 for subdimensions and .93 for total score for this study.

Loneliness was measured with the Revised-University of California at Los Angeles (R-UCLA) Loneliness Scale to measure the feelings of social isolation and dissatisfaction with social interactions.^[38] The 20-item, Likert style questionnaire contains 10 positively worded items and 10 negatively worded items. After reversing the negatively worded items, all items were summed for a total score ranging 20 -

80. Higher scores indicate higher levels of loneliness. Cronbach's α was .87 for this study.

2.6 Biological assays

On the day of assay, saliva samples were thawed and centrifuged at 3000 rpm for 15 min. All assays were performed in duplicate using reliable commercially available immunoassay kits following the manufacturer's instructions (Salimetrics, LLC, State College, PA). Salivary alpha-amylase (sAA) is a good surrogate marker for sympathetic nervous system activity to indicate physical and psychological stress.^[39] Salivary assays of cortisol, sAA, IL-1 β , CRP, estradiol, and testosterone have shown high sensitivity and high precision (Salimetrics, LLC). The coefficients of variation for intra-assay and inter-assay precision were 1.9% – 4.4% for all biomarkers in this study.

Background Information was collected for descriptive purpose and included age, gender, race/ethnicity, religion, source of finance, marital status, living status, medications, and general health conditions.

2.7 Data analysis

Data were analyzed using IBM SPSS Statistical Package v. 20 (Research Triangle Park, NC). Biological data were log or square root transformed to generate normal distributions of the data prior to data analyses. Data were examined using descriptive statistics, and the associations between psychosocial factors and salivary biomarkers were calculated using Pearson correlation coefficients. Group comparisons were performed using student *t*-tests for independent samples or non-parametric tests when appropriate.

3. RESULTS

3.1 Characteristics of the participants

As shown in Table 1, the mean age of the participants was 26.6 ± 5.8 years with body mass indices within normal range (mean 23.1 ± 3.7). Although the sample consisted of mostly female students, 24% were male nursing students. The majority were single (71%), Caucasian (53%), living with the family (59%), and received tuition support from the family (41%). Nearly 40% of the students had a prior degree in various areas, and 61% were in the traditional curricular track. About 30% of the students reported having comorbidities, including asthma, migraine, and thyroid problems. Over 70% of the students expressed moderate to high satisfaction with the education program, with 18% missing data.

3.2 Psychosocial and biological profiles

The levels of psychosocial and biological responses are summarized in Table 2. Stress levels measured with CRRS were relatively low given a mean of 123.6, although the actual

scores ranged up to 581. The mood disturbance total score was not particularly high, with a mean of 34.7. However, three individual dimensions of the POMS, lack of vitality, anxiety, and fatigue, showed a mean level higher than 5.0. The loneliness score was moderate with a mean of 34.3.

The levels of biological responses indicated that the range of the values for each biomarker was wide, suggesting substantial inter-individual variability in biological responses. The mean values, however, seem to be within the expected range for those markers for which reference values are available from the manufacturer of biological assays (cortisol, estradiol, and testosterone).

3.3 Correlations between psychosocial variables and biomarkers

For psychosocial variables (see Table 3), the CRRS stress score was significantly and positively correlated with fatigue and confusion dimensions of the POMS, $r = .32, p = .01$ and $r = .27, p = .05$, but not with anxiety, depression, or anger. As expected, the total mood disturbance score was significantly and positively correlated with each subdimension of the POMS, $r = .74$ to $.83, p < .01$, except for correlation with the lack of vigor dimension, which showed a significant but was the lowest correlation, $r = .35, p < .01$. Loneliness was significantly and positively correlated with depression, anger, and confusion, $r = .28$ to $.39, p = .05$ to $.01$.

For correlations between psychosocial responses and biological responses (see Table 4), significant correlations were noted in a few pairs. The stress (CRRS) score showed significant inverse relationships with estradiol, $r = -.25, p < .04$, and alpha amylase levels, $r = -.31, p < .007$. Anger and confusion scores from the POMS were significantly and positively correlated with testosterone levels, $r = .24$ to $.27, p < .05$. The fatigue score from the POMS was significantly and negatively correlated with alpha amylase, $r = -.25, p < .03$.

3.4 Comparisons between subgroups

Overall, there were minimal differences between the two groups of different curricula tracks, age, and prior degree status in both psychosocial and biological responses (see Table 5).

The main difference was noted in the total CRRS stress score in that students with a prior degree showed a significantly higher level of stress score than students without a prior degree, $p = .039$. The levels of CRP and estradiol showed a tendency for the younger group to have higher CRP and lower estradiol levels, $p = .07$, but neither reached statistical significance.

Table 1. Characteristics of the participants (N = 76)

| Variable | Category | N (SD or %) |
|--|---|--|
| Gender | Male | 18 (23.7%) |
| | Female | 58 (76.3%) |
| Age (years) | Total | 26.6 (5.8) |
| | Male | 28.5 (5.3) |
| | Female | 26.5 (5.5) |
| BMI | Total | 23.1 (3.7) |
| | Males | 25.1 (4.0) |
| Finance (Who pays for your tuition and living cost?) | Females | 22.5 (3.5) |
| | Family (parents, grandparents, etc.) | 31 (40.8%) |
| | Myself | 17 (22.4%) |
| | Full-time work | 1 (1.3%) |
| | Part-time work | 5 (6.6%) |
| Marital Status | Loan | 14 (18.4%) |
| | Missing data | 8 (10.5%) |
| | Single | 54 (71.1%) |
| Ethnicity | Married | 19 (25.0%) |
| | Divorced | 3 (3.9%) |
| | Hispanic | 14 (18.5%) |
| Living Arrangement | Asian | 17 (22.4%) |
| | Caucasian | 40 (52.6%) |
| | African American | 1 (1.3%) |
| | Others | 4 (5.2%) |
| Physical Activity | Alone | 14 (18.4%) |
| | With Family | 45 (59.2%) |
| | With Friends | 5 (6.6%) |
| | Other | 1 (1.3%) |
| | Missing Data | 11 (14.5%) |
| Other Degrees | Rarely or never | 9 (11.8%) |
| | Moderate activities < 30 min/d or 5x/wk | 39 (51.3%) |
| | Moderate activities > 30 min/d and ≥ 5/wk | 10 (13.2%) |
| | Vigorous activities > 30 min/d or ≥ 3/wk | 17 (22.4%) |
| | Missing Data | 1 (1.3%) |
| Educational Track | Yes | 30 (39.5%) |
| | No | 44 (57.9%) |
| | Missing | 2 (2.6%) |
| | Types | Psychology=6 Biology=8 Health-related=4 Arts and Science=8 Finance-related=3 |
| Satisfaction with Nursing Program-total (1-10 highest) | Traditional | 46 (60.5%) |
| | Pacesetter | 29 (38.2%) |
| | Missing Data | 1 (1.3%) |
| Traditional (n=46) | 1-4 | 8 (10.5%) |
| | 5-7 | 29 (38.2%) |
| | 8-10 | 25 (32.9%) |
| | Missing Data | 14 (18.4%) |
| Pacesetter (n=29) | 1-4 | 6 (13.0%) |
| | 5-7 | 21 (45.6%) |
| | 8-10 | 13 (28.3%) |
| | Missing Data | 6 (13.0%) |
| Birth Control Pills (females) | 1-4 | 2 (6.9%) |
| | 5-7 | 8 (27.6%) |
| | 8-10 | 12 (41.4%) |
| | Missing Data | 7 (24.1%) |
| Comorbidity | Yes | 19 (32.8%) |
| | No | 39 (67.2%) |
| | None | 29 (38.2%) |
| | Asthma | 7 (9.2%) |
| | Thyroid | 6 (7.9%) |
| | Migraine | 2 (2.6%) |
| | Gum Disease | 3 (3.9%) |
| Others (ADHD, GERD, Depression) | 5 (6.6%) | |
| | Missing Data | 24 (31.6%) |

Note. ADHD = Attention deficit hyperactivity disorder; GERD = Gastroesophageal reflux disease.

Table 2. Mean levels of psychosocial and salivary biomarker responses

| Concept/Biomarker | Instrument | Score (possible) | Mean (SD) | Score range (actual) |
|----------------------|---------------|------------------|-----------------|----------------------|
| Stress | CRRS | 0 – 1440 | 123.63 (108.56) | 0 – 581 |
| | POMS | 0 – 148 | 34.72 (17.80) | 0 – 102 |
| Moods | Anxiety | 0 – 24 | 6.22 (4.94) | 0 – 23 |
| | Depression | 0 – 32 | 2.67 (3.95) | 0 – 21 |
| | Anger | 0 – 28 | 2.99 (4.05) | 0 – 22 |
| | Fatigue | 0 – 20 | 5.96 (3.85) | 0 – 16 |
| | Confusion | 0 – 20 | 4.12 (3.28) | 0 – 12 |
| | Lack of Vigor | 0 – 24 | 12.76 (4.47) | 0 – 24 |
| Loneliness | R-UCLA scale | 20 – 80 | 32.25 (8.28) | 21 – 56 |
| CRP (pg/ml) | | | 5603.7 (9825.6) | 968 – 70,641 |
| IL-1 beta (pg/ml) | | | 179.9 (183.2) | 1 – 886.5 |
| Cortisol (µg/dl) | | | .24 (.15) | .04 – .79 |
| AA (U/min) | | | 30.9 (31.1) | 2.1 – 195.6 |
| Estradiol (pg/ml) | | | 2.4 (1.8) | .01 – 11.3 |
| Testosterone (pg/ml) | | | 60.0 (45.4) | 13.9 – 246.6 |

Note. CRRS = College Readjustment Rating Scale; POMS = Profile of Mood States; R-UCLA = Revised-University of California at Los Angeles Loneliness Scale; CRP = C-reactive protein; IL = interleukin; AA = Alpha amylase.

Table 3. Pearson's correlations between psychological variables

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------------|---|------|--------|--------|--------|--------|--------|--------|--------|
| 1. Stress (CRRS) | 1 | .224 | .225 | .035 | .054 | .318** | .266* | .093 | .050 |
| 2. Moods-total | | 1 | .821** | .811** | .743** | .833** | .831** | .354** | .335** |
| 3. Anxiety | | | 1 | .596** | .537** | .650** | .763** | .030 | .170 |
| 4. Depression | | | | 1 | .665** | .637** | .635** | .067 | .306** |
| 5. Anger | | | | | 1 | .530** | .503** | .044 | .393** |
| 6. Fatigue | | | | | | 1 | .665** | .203 | .197 |
| 7. Confusion | | | | | | | 1 | .140 | .281* |
| 8. Lack of Vigor | | | | | | | | 1 | .141 |
| 9. Loneliness | | | | | | | | | 1 |

Note. ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Table 4. Correlations between psychological and biological variables

| Psychological variable | Biological variable | R | p |
|------------------------|---------------------|------|--------|
| CRRS total score | Estradiol | -.25 | .039 |
| | AA | -.31 | < .007 |
| Anger | Testosterone | .27 | .02 |
| Fatigue (POMS) | AA | -.25 | .03 |
| Confusion | Testosterone | .24 | < .05 |

Note. AA = Alpha amylase; POMS = Profile of Mood States.

4. DISCUSSION

The primary purposes of this study were to describe the levels of biobehavioral responses and their associations and to

compare potential group differences by curricular track, age, and a prior degree on biobehavioral responses in graduating senior nursing students.

4.1 Psychosocial profile

Overall, the average levels of stress, mood disturbance, and loneliness were not particularly high. When stress was assessed in relation to the occurrence of stressful events specific to college students, the mean score of this study reflected a relatively low level of life stress with a low risk of having a serious health change.^[36] In comparison, a score between 150 and 299 is thought to indicate a 50-50 chance of a serious health change within the next two years, and an adoption of new coping strategies to deal with the stressful demands is recommended. However, a score of 300 and higher is

thought to indicate a high health risk, and a recommendation is made to seek a professional consultation for managing stress. In our sample, despite the relatively low mean score, we found that 26% of the students reported a score between 150 and 299, with 5% of the students reporting a score higher than 300. This means over 30% of students, in fact, reported high or moderately high levels of stress with potential risk of compromising health in the future. In previous studies

with college students, general stress level was reported to be normal^[40] or high,^[41] but because of differences in instrumentation, direct comparison is not appropriate. However, the fact that over 30% of the students reported a moderately high or very high stress scores indicates a need for considering a stress management program and for testing its potential efficacy in future studies.

Table 5. Group differences by prior degree, types of curriculum, and age groups (N = 76)

| Variable | Types of curriculum (traditional or pacesetter) | | Age (<25 or ≥25) | | Prior degree (yes or no) | |
|----------------------|---|----------|------------------|----------|--------------------------|----------|
| | <i>t</i> | <i>p</i> | <i>t</i> | <i>p</i> | <i>t</i> | <i>p</i> |
| Stress | | .46 | | .16 | | .039** |
| Moods (total) | -.08 | .93 | .79 | .43 | .33 | .74 |
| Anxiety | .34 | .74 | .98 | .33 | -.03 | .98 |
| Depression | | .40 | | .15 | | .94 |
| Anger | | .28 | | .93 | | .35 |
| Fatigue | .61 | .54 | .51 | .61 | .11 | .92 |
| Confusion | .81 | .42 | .30 | .77 | -.45 | .65 |
| Lack of vigor | -1.36 | .18 | -.06 | .95 | 1.27 | .21 |
| Loneliness | -.86 | .39 | .70 | .49 | 1.15 | .25 |
| CRP | .16 | .87 | 1.86 | .07 | 1.66 | .10 |
| IL-1 beta | -.79 | .43 | 1.65 | .10 | .62 | .53 |
| Cortisol | -.18 | .85 | .18 | .86 | .16 | .88 |
| Alpha Amylase | -.29 | .77 | -.92 | .36 | -1.02 | .31 |
| Estradiol | .18 | .86 | -1.84 | .07 | -1.10 | .28 |
| Testosterone | .70 | .48 | .92 | .36 | 1.39 | .17 |

Note. CRP = C-reactive protein; IL-1 = interleukin-1.

The CRRS stress score was not significantly related to self-reported anxiety, depression, anger, or total mood disturbance scores but was significantly and positively related to fatigue and confusion. These findings suggest that stress measured by the quantification of stressful events may not be equivalent to the measure of a person's perceived level of stress. Both dimensions of stress would have unique contributions to understanding human stress overall. In contrast to our findings, college-related activities as well as general life experiences were significant predictors for depression in college undergraduate students.^[42] In a large sample of undergraduate and graduate students (N = 870), life stressors significantly predicted depressive symptoms, which, in turn, predicted suicidality.^[43] Even at the professional degree levels, up to 69% of students reported significant levels of depressive symptoms, and academic stress was attributed to depression and anxiety symptoms, life satisfaction, academic performance, and general health.^[5] Furthermore, a recent systematic review indicated high prevalence of anxiety,

depression, and psychological distress in medical students, ranging from 6% to 97%, but little has been studied about the causes or consequences.^[44] Although depression and anxiety levels seem to be relatively low in our study, given potential serious consequences (*e.g.*, suicide)^[4] even with mild and moderate levels of depressive symptoms,^[3] regular monitoring and availability of counseling resources may be beneficial.

Loneliness has been studied mostly in older adults with negative health consequences^[7-9] but sparsely in younger populations. In 384 Turkish college students, the mean level and range of loneliness from the same UCLA Loneliness Scale was nearly identical with our finding. In that study, loneliness was associated with internet addiction, and together with low self-esteem, loneliness was a source of time-management and health problems.^[45] In older adults, lonelier people were more depressed and fatigued than others,^[10,11] and anxiety was thought to mediate the effect of loneliness on depressive mood and poor sleep quality in college students.^[46]

Considering close associations between psychosocial variables and various potential adverse outcomes from increased psychosocial distress, regular assessment of psychosocial distress and counseling resources may improve the outcomes. Stress reduction therapies, in general, are effective in reducing depression, anxiety, and stress in students.^[47] Furthermore, web-based and computer-delivered interventions are found to be effective in reducing depression, anxiety, and stress in college students.^[48–50] Thus, technology-based availability of screening and management programs can be useful for selective students who experience high levels of stress and other emotional distress.

4.2 Biological profile

The overall profile of biomarkers was unremarkable. The average level of salivary cortisol in our sample was within the expected morning cortisol range for adults aged 21–30 years, based on the manufacturer's reference level (Salimetrics, LLC., State College, PA). The average flow adjusted sAA level seems to be lower than the level reported in a previous study, but our sample represented a much younger group (26.6 vs. 43.1 years) than the other study sample.^[51] Age related changes in sAA are not clear. For CRP, the mean level was similar to the level of healthy young college students with a mean age of 21.7 years.^[52] In another study with 18–22 years old college students, the baseline salivary CRP level seemed far lower than ours, but the saliva collection method differed (salivette use vs. passive drool in our study), which could have affected the measurement. It is known that different saliva collection methods may produce different results.^[53] When salivary CRP levels were compared with the serum levels, the two levels were significantly correlated ($r = .42$), with the correlation being stronger at higher levels of serum CRP ($r = .60$). Salivary Estradiol levels are expected to vary by the phase of menopausal cycle in women, whereas salivary testosterone levels, not surprisingly, are reported to be significantly higher in males than females. The majority in our sample were females in premenopausal state, and our sample means of these hormones appear to be within the expected ranges listed by the manufacturer of bioassay kits (Salimetrics, LLC.).

4.3 Association between psychosocial responses and hormones

Psychological stress is known to activate the HPA axis, raising cortisol production from the adrenal cortex, a small fraction of which can be detected in saliva.^[54] In university students, salivary cortisol was elevated before and after an oral presentation and on the day of the presentation ($p < .001$) as well as before a written exam ($p < .022$) and on the day of the exam ($p < .05$),^[16] indicating increased cortisol responses

to anticipatory and actual stress in academic settings. Even in very young children under 5 years old, early exposure to psychosocial adversity leads to increased cortisol reactivity.^[55] Our findings did not show a significant association between stress and salivary cortisol levels, possibly because of the way stress was assessed. The cumulative occurrence of stressful events per se may not sensitively reflect individual biological responses, because the stress weight assigned per event is not based on individual stress appraisal. Alternatively, it is possible that cortisol levels were influenced by other confounding factors, such as the different magnitude of HPA responsiveness, gene-environment interactions, and the levels of cortisol binding globulins,^[54] which were not assessed in this study. Therefore, the findings should be interpreted with caution.

Salivary alpha-amylase is a surrogate marker for sympathetic activity.^[17] Because stress typically activates sympathetic responses, stress and sAA are positively associated. Contrary to expectation, we found that the CRRS stress score was significantly but negatively associated with sAA. Others found an acute stress challenge induced a significant increase in sAA in healthy young adults,^[56] and changes in sAA reflected adrenergic dysregulation in patients with psychopathologies, particularly with anxiety-related conditions.^[57] However, not all findings in these associations have been unequivocal. Dental anxiety levels had no significant correlations with sAA or cortisol.^[51] The precise reason for negative correlation in our study is not clear and requires further investigation.

4.4 Association between psychosocial responses and inflammatory markers and sex steroids

Stress and mood disturbance can increase inflammatory responses, such as IL-1^[14,18] and CRP.^[19,20] Daily interpersonal stress predicted CRP elevation months later,^[19] and chronic childhood stress was associated with elevated CRP levels in adulthood.^[21] However, we did not find any significant association between stress and inflammatory markers. Similarly, CRP levels did not differ between depressed and healthy adolescents.^[58]

Instead, stress was significantly and negatively correlated with estradiol, whereas anger and confusion were significantly and positively correlated with testosterone. Estrogen is thought to be beneficial in decreasing anxiety and administration of estradiol reduced anxiety and depression-like behaviors.^[24] In an animal study, estrogen was found to protect against negative effects from repeated stress on certain cognitions.^[29] Our finding is consistent with the notion that estrogen has beneficial effect on psychosocial distress. Similarly, our finding on significant positive correlation be-

tween anger and testosterone is consistent with previous findings. Exposure to angry facial expressions induced a greater increase in salivary testosterone than exposure to happy expressions.^[60] Increased anger induction was associated with increased testosterone levels.^[61] In addition, testosterone is thought to be protective against anxiety and depression,^[25,28] and females with current depressive or anxiety disorders showed lower salivary testosterone levels than did controls.^[27] A meta-analysis indicated that testosterone therapy has a significant positive effect on depressed patients and the elderly,^[26] but we did not see any significant association between testosterone and anxiety or depression. It is possible that beneficial effects of testosterone may emerge only in high risk populations.

The salivary testosterone level showed a significant positive association with confusion in this study. Testosterone has not been directly assessed for its association with confusion, but a low testosterone level has been associated with low cognitive function, although the findings are not unequivocal.^[62,63] Testosterone supplements for six months failed to show beneficial effects on cognitive function.^[64] Most studies on testosterone have been conducted with older adults at high risk for declining cognitive function. Little is known about the effects or association of testosterone on potential cognitive impairment in young adults.

4.5 Group differences

Despite the diversity in student characteristics, we found very little differences between the groups of different curricular tracks, age, and prior degree status. Both psychosocial and biological responses were similar across all comparison groups. For age comparison, we stayed with an a priori criterion of age 25, a slightly different cut point from the actual mean age of 26.6, but the groups were nearly equally distributed (53% to 47%) and did not differ.

4.6 Limitations

Limitations of the study relate to a relatively small sample size and use of cross-sectional data collection. From this approach, interpretation of the findings is limited to the correlations, but not to causality. One time measurement may not

adequately represent persistent responses, both psychosocial and biological. The sample was limited to a group of senior graduating nursing students recruited from one university setting, which constrains the generalizability of the findings. Finally, the sensitivity of instruments may also be limited in reflecting variable biological responses. Despite the limitations, a biobehavioral research provides important insight to nursing students' psychosocial and biological profiles.

5. CONCLUSION

Nursing students are under intensive training for didactic and clinical practice to meet increasingly complex health care demands on graduation. Students frequently express feelings of stress and fatigue. The average levels of stress, moods, loneliness, and hormonal and inflammatory responses seem to be equivalent to the levels reported in other student populations. This may reflect that nursing students have learned to cope with intense demands over semesters. However, over 30% of the students reported relatively high levels of stress with potentially increased risk for compromising their health status. Given the known adverse consequences of chronic stress, it would be important for the university to regularly assess psychosocial and behavioral status and develop an action plan for students presenting high levels of stress. The plan may include a referral to campus counseling resources, encouragement to participation in a stress management program, providing a peer support system, and keeping on-going dialogues between faculty and students to find better ways to improving learning environment. Future research should include the evaluation of the efficacy of any program listed above as well as longitudinal follow-ups on students' biobehavioral profiles throughout nursing education. A long-term follow-up will reveal changes over time and may suggest an optimal time of intervention.

ACKNOWLEDGEMENTS

This study was supported by the Lee and Joseph D. Jamail Endowment to DK.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest statement.

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