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Abstract

The Anxiety and Depression Association of America defines stress as the response to a threat in a situation, whereas anxiety is the reaction to the stress[1]. This means the acute "fight-or-flight" stress response ends once the situation is resolved, but anxiety is the resulting, long-term worry that may be

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manifested in such symptoms as headaches, high blood pressure, heart palpitations, and insomnia. Marshall et al. (2008) reported mental health-related... **Read More**

The Relationship Between Salivary Measures and Perceived Stress and Anxiety in First Semester Pharmacy Students

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INTRODUCTION

The Anxiety and Depression Association of America defines stress as the response to a threat in a situation, whereas anxiety is the reaction to the stress[1]. This means the acute "fight-or-flight" stress response ends once the situation is resolved, but anxiety is the resulting, long-term worry that may be manifested in such symptoms as headaches, high blood pressure, heart palpitations, and insomnia. Marshall et al. (2008) reported mental health–related quality of life scores for third-year student pharmacists were significantly below U.S. mean scores for individuals aged 20 to 34 years old, and, as stress increased, their scores decreased[2]. Votta and Benau (2013) found year in pharmacy program correlated negatively with stress levels, with first year student pharmacists being most stressed[3].
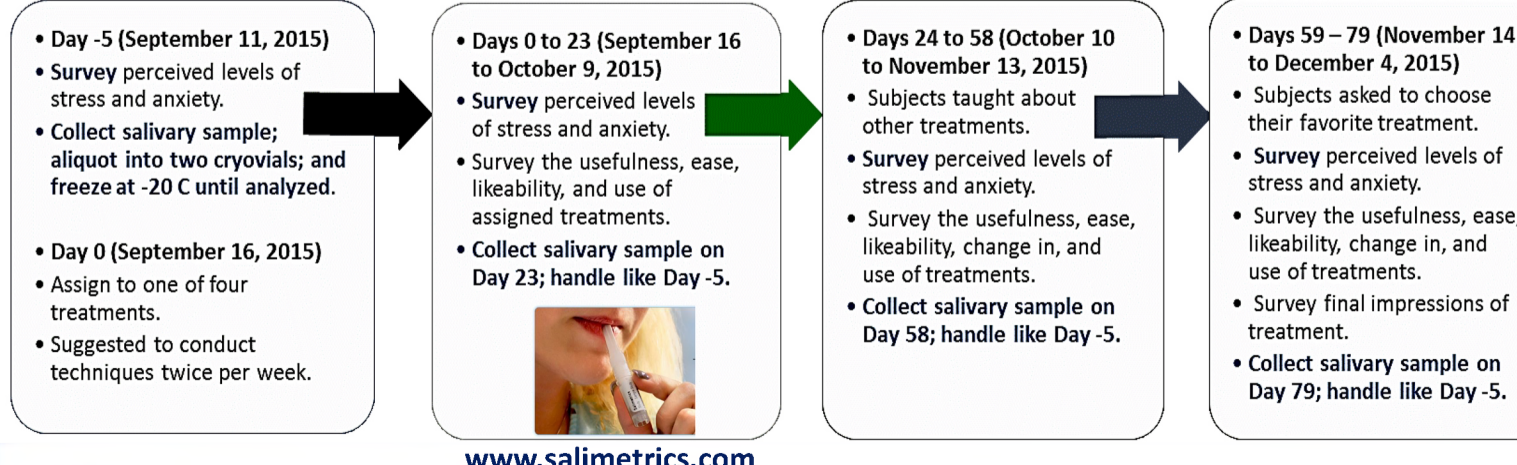
Preliminary evaluation of survey data from this study found first year, first semester student pharmacists perceived to have experienced significantly more stress and anxiety later in the semester as compared to the start[4]. Therefore, it is hypothesized that physiological measures of stress will also increase over the semester, and therefore be directly related to survey measures of stress and anxiety. Lajaunie et al. (2016) found no clear preference among the comparator and three relaxation techniques in this study, other than student pharmacists rated the comparator (Power Posing) as being easiest to conduct[4]. In contrast, it is hypothesized that treatment differences will be found when using changes in physiological measures to more objectively assess effects on student pharmacists. Ultimately, findings will be used to encourage student pharmacists to mitigate stress and anxiety levels.

OBJECTIVES

1. To determine cortisol and testosterone levels and alpha-amylase activity in salivary samples collected from first semester, student pharmacists over the semester.

2. To evaluate correlations between salivary measures and self-reported levels of stress and anxiety.

3. To investigate differences in comparator and relaxation techniques using change in salivary components as the dependent variable.
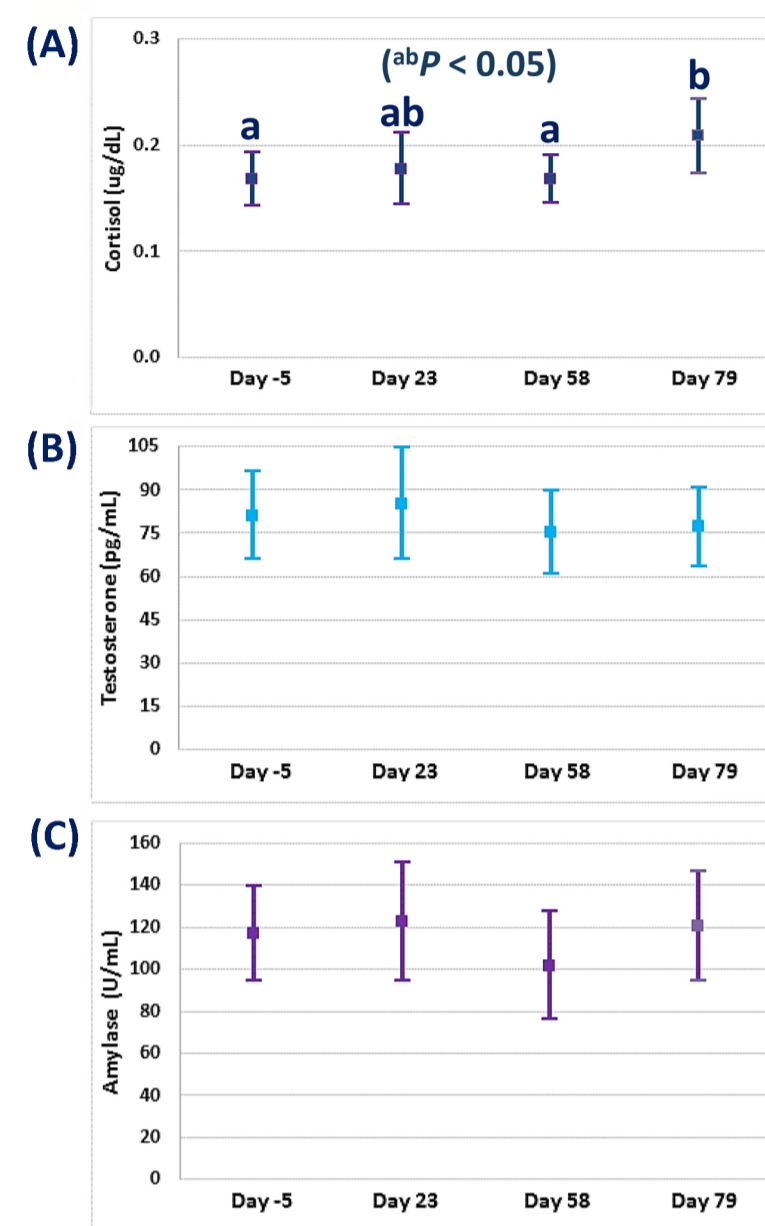
METHODS

- **Population**: 41 Student Pharmacists (22 females and 19 males) from the first year, first semester of pharmacy school in Fall 2015.
- **Treatments**:
  - **Comparator: Power Posing (initial n = 10)** – Holding an open pose[5].
  - **Interventions (relaxation techniques):**
    - **Body Scan Meditation(n = 10)** – Guided systematic muscle relaxation.
    - **Mindfulness Meditation (n = 11)** – Focusing on the sound of a bell.
    - **4 x 4 Meditation (n = 10)** – Breathing slowly in and out for four counts.
- **Protocol:**

- Day -5 (September 11, 2015)
- Survey perceived levels of stress and anxiety.
- Collect salivary sample; aliquot into two cryovials; and freeze at -20 C until analyzed.
- Day 0 (September 16, 2015)
- Assign to one of four treatments.
- Suggested to conduct techniques twice per week.

- Days 0 to 23 (September 16 to October 9, 2015)
- Survey perceived levels of stress and anxiety.
- Survey the usefulness, ease, likeability, and use of assigned treatments.
- Collect salivary sample on Day 23; handle like Day -5.

- Days 24 to 58 (October 10 to November 13, 2015)
- Subjects taught about other treatments.
- Survey perceived levels of stress and anxiety.
- Survey the usefulness, ease, likeability, change in, and use of treatments.
- Collect salivary sample on Day 58; handle like Day -5.

- Days 59 – 79 (November 14 to December 4, 2015)
- Subjects asked to choose their favorite treatment.
- Survey perceived levels of stress and anxiety.
- Survey the usefulness, ease, likeability, change in, and use of treatments.
- Survey final impressions of treatment.
- Collect salivary sample on Day 79; handle like Day -5.

www.salimetrics.com

RESULTS

❖ Objective 1:

Figure 1. The means and 95% confidence intervals of physiological measures in salivary samples as determined over time in first year, first semester student pharmacists for (A) Cortisol (Time effect, P = 0.088), (B) Testosterone (Time effect, P = 0.274), and (C) Amylase (Time effect, P = 0.121). Days relate to the date when treatments were assigned to student pharmacists, with baseline measures taken 5 days prior to then – see Protocol in Methods for specific dates.

(A) Cortisol (ug/dL) — (^{ab}P < 0.05); Day -5: a, Day 23: ab, Day 58: a, Day 79: b

(B) Testosterone (pg/mL) — Day -5, Day 23, Day 58, Day 79

(C) Amylase (U/mL) — Day -5, Day 23, Day 58, Day 79

❖ Objective 2:

Table 1. Spearman correlation coefficients for physiological measures in salivary samples as related to ratings for stress and anxiety since starting Pharmacy School in first year, first semester student pharmacists (Note: Higher ratings indicate higher self-reported stress or anxiety).

	Cortisol	Testosterone	Amylase
Stress since starting Pharmacy School (Day -5)	- 0.297	- 0.370*	- 0.197
Anxiety since starting Pharmacy School (Day -5)	- 0.229	- 0.100	- 0.263
Stress since starting Pharmacy School (Day 79)	- 0.020	- 0.116	- 0.021
Anxiety since starting Pharmacy School (Day 79)	+ 0.001	- 0.441**	+ 0.073

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

- **Lab Analysis**: Salivary samples were analyzed using commercial kits (Salimetrics, LLC) for Cortisol (an increase suggests an endocrine stress response[5]), Testosterone (an increase suggests increased feelings of power[5]), and alpha-Amylase (surrogate measure for Sympathetic Nervous System[6]) via a Synergy H1 Hybrid microplate reader (BioTek Instruments, Inc.). Intra-assay and inter-assay coefficients of variation were maintained below 10 and 15%, respectively, as recommended by Salimetrics (LLC).

- **Data Analysis:** All data were analyzed using IBM SPSS Advanced Statistics Software, version 23.
  - **Objective 1**: GLM Repeated Measures conducted, with Day of saliva collection used as within subjects variables to investigate changes in physiological measures over time.
  - **Objective 2**: Bivariate Spearman Correlations conducted on Day -5 and Day 79 between cortisol, testosterone, and alpha-amylase levels with self-reported stress and anxiety levels experienced since the start of school (where 1 = no stress or anxiety and 5 = severe stress or anxiety).
  - **Objective 3**: One-way ANOVA conducted, with percentage change in cortisol, testosterone, and alpha-amylase between each Day and Overall (Day -5 to 79) tested as dependent variables, and Treatments as independent variables.

❖ Objective 3:

Figure 2. The means and SE of overall percentage change in the physiological measure of salivary testosterone calculated from Day -5 to 79 in first year, first semester student pharmacists (Treatment effect, P = 0.078).

Percent Change in Testosterone — (^{abc}P < 0.10); Power Pose: a, Body Scan: bc, Mindfulness: b, 4 x 4: ac
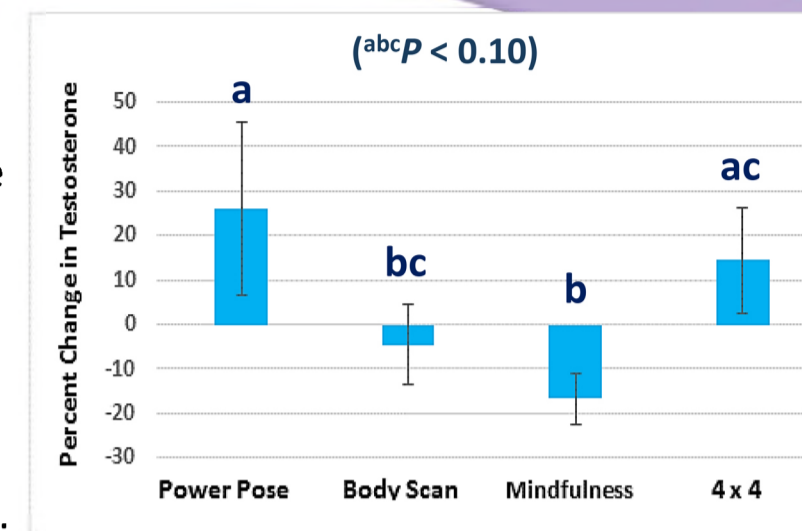
CONCLUSIONS

- Results suggests student pharmacists had physiologically (cortisol) and self-reported higher stress levels by the end of their first semester.
- The negative relationship between testosterone levels with stress and anxiety ratings may have resulted from first semester, first year student pharmacists experiencing decreased feelings of power[5,7].
- A percentage increase in testosterone over the course of the semester suggests positive impacts of Power Posing and 4 x 4 meditation on feelings of power[5,7]. However, student pharmacists rated 4 x 4 meditation to be the least useful, less relaxing, hardest to conduct, and least liked treatment in a related acute study[9].
- Overall, this study educated first year, first semester student pharmacists about techniques to lower stress and anxiety levels.

LIMITATIONS & FUTURE DIRECTIONS

- A high variation was observed in salivary measures. This also has been observed in other studies we have conducted[7,8].
- There was a lack of control over how often and when student pharmacists conducted treatments or other relaxation techniques.
- Future analysis will include:
  - Identifying outlier data points and other factors which may have significantly impacted variation in salivary measures.
  - Conducting statistical analysis using scores obtained from more objective survey instruments, which included the Kentucky Inventory of Mindfulness Skills scale, the Four Scale Anxiety Questionnaire, and the Perceived Stress Scale.
  - Conducting follow-up questionnaires to determine long-term application and usefulness of these or other relaxation techniques.

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