Determining the Relationship of Acute Stress, Anxiety, and Salivary α-Amylase Level With Performance of Student Nurse Anesthetists During Human-Based Anesthesia Simulator Training

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Managing stress for student nurse anesthetists represents a multifaceted educational concern for anesthesia educators. Our purpose was to determine the relationship between physiologic measures of stress and performance of student nurse anesthetists during anesthesia simulator training.

Following institutional review board approval, 18 students were enrolled from a nurse anesthesia program. A prospective descriptive design was used to compare baseline, acute, and recovery measurements of stress with performance scores of students during an induction and intubation sequence in a patient simulator. Performance scores were stratified into low-, moderate-, and high-performing groups based on scores received from trained observers.

A statistically significant difference in physiologic measures of stress was detected between baseline and acute levels of salivary α-amylase (P = .017), heart rate (P = .003), and anxiety levels (P = .001). No significant differences were found when measures of stress were compared with performance of low, moderate, or high performers.

This investigation revealed remarkable findings regarding the relationship between stress and student performance. Analysis of the descriptive statistics and means of each group suggests that low performers have increased stress and perform poorly, whereas high performers have increased stress and perform superbly, and moderate performers have modest stress and perform moderately.

Keywords: Anesthesia simulator training, salivary α-amylase, State-Trait Anxiety Inventory, stress, student nurse anesthetist.

Many nurse anesthesia educational programs have incorporated the use of human-based anesthesia simulators to prepare their students for transition from the didactic to the clinical phase. Anesthesia simulators allow students to use higher order critical thinking and enhance behavioral skills. When anesthesia simulator training is initiated in the didactic phase of the graduate curriculum, students often report the benefits as they move into their clinical phase of training. The use of anesthesia simulators may also serve as a source of stress to student nurse anesthetists. Moderate levels of stress are essential for effective learning; however, excessive levels of stress and anxiety have been found to have a negative impact on a student's academic and psychomotor performance. To date, little information is available to determine the relationship between stress and performance in student nurse anesthetists.

Neurophysiologic Effects of Stress

Stress is a combination of behavioral and physiologic reactions. If analyzed on a continuum, stress at one extreme can produce a detrimental experience for one person, whereas at the opposite extreme, it may produce a favorable experience for another. Changes in homeostasis, defined as continued internal stability despite changes in the environment, incorporate an increase in the consumption of oxygen and glucose in response to released catecholamines due to activation of the sympathetic-adrenal-medullary system. If stress is prolonged, glucocorticoids are released from the adrenal cortex under activation by the more sluggish response of the hypothalamic-pituitary-adrenal axis. Educational literature has reported the experience of stress in students and provides evidence that stress impairs academic performance.

Previous investigations analyzing physiologic responses to stress and performance have examined changes in heart rate, blood pressure, the presence of per-
spirations, and plasma catecholamine levels.\textsuperscript{9,12,13} The measurement of salivary α-amylase, a valid and highly reliable method to measure the physiologic response to acute stress, has been incorporated into a multitude of biobehavioral research studies.\textsuperscript{8,9}

Salivary α-amylase is a major secretory protein found in saliva and aids in the initial digestion of starch.\textsuperscript{14,15} Release of salivary α-amylase is regulated by autonomic innervation.\textsuperscript{14,16} Recent investigations have demonstrated that salivary α-amylase levels significantly increase as a result of acute stress.\textsuperscript{7,8,13,17,18} Chatterton and colleagues\textsuperscript{19,20} linked levels of salivary α-amylase to sympathetic activation during physically and psychologically stressful conditions. The level of salivary α-amylase was observed to increase in an investigation that used written examinations as a psychological stressor. Increases in the level of salivary α-amylase from psychological and physical stressful conditions were found to be closely associated with changes in norepinephrine concentrations.\textsuperscript{20} Furthermore, Xiao and colleagues\textsuperscript{21} used salivary α-amylase to measure physiologic stress during human simulated trauma management. Of the 3 emergency cases simulated consecutively, 1 was a routine case with no acute events and 2 were target trauma cases with acute complications. The mean baseline salivary amylase level was 205 U/mL. The mean levels of salivary α-amylase for the 4 other sampling points were 307, 315, 207 mL, and 315 U/mL. All subjects in the investigation had significantly higher levels of salivary α-amylase ($P < .01$) compared with resting baseline control measurements.\textsuperscript{21}

Investigations analyzing the efficacy of salivary α-amylase levels for detecting a stress response determined that exposure to β-adrenergic agonists stimulated the release of salivary α-amylase without increasing salivary flow.\textsuperscript{22} Conversely, the administration of a β-adrenergic antagonist, such as propranolol, decreased the release of salivary α-amylase.\textsuperscript{23} This pattern of evidence supports the link between increases in catecholamine levels and the release of α-amylase from the salivary glands during sympathetic activation.\textsuperscript{24}

In this investigation, \textit{acute stress} was operationally defined as increases in the salivary α-amylase level and heart rate.

**Acute Stress and Anxiety Impacts Performance**

The behavioral response to stress manifests as anxiety. Anxiety and stress are 2 concepts that are often used in the same context. For the purpose of this investigation, \textit{anxiety} is defined as an unpleasant inner emotional state characterized by feelings of fear, apprehension, and uncertainty from anticipation of a threatening event or situation.\textsuperscript{25} Anxiety can be further differentiated into state anxiety and trait anxiety. \textit{State anxiety} is a transient state of anxiety that refers to how a person feels “right now” or at this moment.” This type of anxiety can be compared with the acute physiologic stress response involving a temporary release of catecholamines.\textsuperscript{26} \textit{Trait anxiety} is defined as the existence of stable individual differences in the tendency to respond with state anxiety in the anticipation of a threatening situation. Trait anxiety refers to the general differences in personality between people and describes how a person generally feels on a regular basis. In this investigation, state and trait anxiety were operationally defined by a person’s score on the State-Trait Anxiety Inventory (STAI; state anxiety form Y-1 and trait anxiety form Y-2).

Previous investigations analyzing the effect of anxiety on performance have used a variety of mechanisms to induce this response. Stroop word video tests,\textsuperscript{27,28} electrical shock,\textsuperscript{29} trauma simulation,\textsuperscript{21} and human-based anesthesia simulators\textsuperscript{30} are mechanisms to provoke a state of anxiety and a stress response mediating changes in physiology. To evaluate performance, visual and auditory response time has been quantified.\textsuperscript{27} Subjects in other anxiety and performance investigations have been asked to perform simple drug calculations\textsuperscript{31} or a motor pinch task.\textsuperscript{29} More involved anxiety and performance investigations involved an evaluation of a subject’s proficiency in a series of clinical tasks.\textsuperscript{7,8,12}

Chronic anxiety has been implicated in affecting the formation of new memory in humans and animals.\textsuperscript{4} In addition, the behavioral effect of anxiety can impede motor performance. A 2001 investigation suggested that a person’s motor performance is influenced by the level of anxiety and the intensity of an imposed stressor\textsuperscript{29}, thus, a person’s state anxiety impacts performance.\textsuperscript{27,29}

In 2006, researchers in Metz-Borny, France, investigated the effects of moderate state anxiety levels and adverse mood states on visual and auditory response time in subjects with different levels of trait anxiety.\textsuperscript{27} Their results showed that moderate levels of state anxiety improved performance in auditory response time in subjects with very low trait anxiety and in visual response time in subjects with normal trait anxiety. This improved performance was attributed to a subject’s greater arousal and attention from visual stimuli.

A similar investigation suggested that cognitive and physiologic arousal increased with stressor intensity.\textsuperscript{28} However, a decrease in motor response steadiness in subjects with moderate levels of trait anxiety was observed. The acute stress generated from an electric shock stimulus evoked significant change in cognitive and physiologic arousal compared with baseline and control subjects. This observation was even more notable in subjects with moderate levels of trait anxiety. As cognitive and physiologic arousal increased with stressor intensity, performance and steadiness of the pinch grip were observed to decrease. Steadiness was markedly reduced with the highest stress intensity.
The present study is supported by several classic studies. In 1969, Fredericks and Mundy investigated the effect of perceived stress of dental students to academic performance and suggested a significant but weak negative correlation between anxiety and academic performance. Cecchini and Friedman reported a modest inverse relationship between state anxiety and grade point average. Westerman et al reported a weak inverse relationship between trait anxiety and student grades. In summary, these studies support an inverse relationship between academic performance and state and trait anxiety.

State anxiety, central fatigue, or confusion can produce a slowing of information-processing and motor strategies. Thus, moderate amounts of anxiety provide a constant catalyst that aids a learner's attention and ability to retain what has been taught. High trait anxiety and anxiety disorders have been associated with an exaggerated response to stressful stimuli and worse mental performance. To date, there are no investigations that incorporated the use of salivary α-amylase levels and the STAI to determine the relationship among acute stress, anxiety, and clinical performance. Previous investigations have used blood pressure, heart rate, perspiration, and cortisol levels; however, these measures of physiologic stress are highly variable among people and are inconsistent. Therefore, the purpose of this investigation was to determine the relationship between a physiologic measure of stress, the salivary α-amylase level, and performance of first-year nurse anesthesia students during anesthesia simulator training.

**Instruments**

- **Salivary α-Amylase.** Salivary α-amylase is an enzyme that is produced by the acinar cells of the salivary gland. Sympathetic stimulation causes high salivary α-amylase release from the parotid and submandibular acinar cells, whereas parasympathetic stimulation causes low salivary α-amylase release from the sublingual acinar cells. Nater and colleagues explored correlates of the diurnal pattern of salivary α-amylase activity. Saliva samples were collected directly after waking up, 30 and 60 minutes later, and each full hour until 9:00 AM by the subjects themselves. Results indicated that salivary α-amylase levels follow a diurnal pattern, with low levels in the morning and a steady increase in levels throughout the day. Levels of salivary α-amylase also rise in response to stressful conditions, including exercise, temperature extremes, and academic examinations. Past attempts were made to access catecholamines in the saliva, but it takes approximately 1 hour for norepinephrine to transfer from blood to saliva, which is too long to gain an accurate assessment of stress-induced changes.

Salivary α-amylase measurement presents a noninvasive and a highly reliable indicator of sympathetic-adrenomedullary activity and was used in this investigation as a physiologic indicator of the acute stress response.

- **State-Trait Anxiety Inventory.** To effectively measure anxiety, many investigations have used the STAI questionnaire. The STAI, developed by Charles D. Spielberger in 1983, measures state anxiety and trait anxiety. This tool exists in 2 forms (the Y-1 and the Y-2). State anxiety is measured with form Y1 and trait anxiety with form Y2. Each form has 20 questions. The minimum and maximum scores for each form are 20 and 80. A low score reflects low state or trait anxiety, and a high score reflects high state or trait anxiety. Investigations by Sanders and Lushington and Sandres incorporated the STAI to suggest that trait anxiety affects performance negatively; students who reported higher levels of anxiety were found by faculty and administration to have lower grades for clinical competency and contextual understanding. Similarly, an investigation by Dyers suggested that state anxiety affects physiology because the heart rate and blood pressure were observed to increase in investigation subjects. Furthermore, Noto and colleagues used salivary α-amylase levels and the STAI to demonstrate the effect of a mental arithmetic test on state anxiety levels. Results showed an increase in the state portion of the STAI score, indicating that the mental arithmetic test increased the subjects’ perceptions of anxiety. Similarly, Takai et al reported a significant correlation between the salivary α-amylase level and the score on the trait portion of the STAI with stressful video viewing.

- **Human Anesthesia Simulator.** Human anesthesia simulation has been used in several investigations to evoke a stress response. Anxiety and performance investigations have successfully used an anesthesia simulator to elicit changes in the salivary α-amylase level, heart rate, and blood pressure level and to evoke perspiration. Shimoda and Ikuta determined that forehead sweating developed in 78% of medical students when they attempted tracheal intubation on a simulator. Forehead sweating increased during subsequent attempts if the first attempt was not successful.

The present study was conducted at the Uniformed Services Simulation Center. The Uniformed Services University, Bethesda, Maryland, has a simulated operating room environment and uses a high-fidelity, life-sized, human patient computerized manikin manufactured by Medical Education Technologies, Inc. (Sarasota, Florida) with HPS 6.1 software. (HP Software, Palo Alto, California). The simulator software includes a program that mimics the physiology for normal induction. A simulation expert, who is seated behind 1-way glass, inputs commands and manages the mechanical interfaces. The mannequin’s voice is a speaker located inside the mannequin, with a microphone in the computer technician’s control room behind the 1-way glass. There is capability...
for communication between the simulated operating room and the control room via speakers. Three video cameras in the simulated operating room are controlled by the computer technician. The training session consists of preoxygenation, anesthesia induction, and tracheal intubation with laryngoscopy and initial maintenance of the anesthesia.

• **Assessment of Subjects’ Performance.** Successful intubation of a patient relies on established guidelines to promote safety and continuity of care. Although there is no one accepted standard performance tool to evaluate the induction and intubation of a patient, there are tasks common to all anesthesia inductions.

• **Objective Checklist.** Checklists are commonly used to objectively score performance during anesthesia simulator training.40-43 The present study used an objective checklist (Table) that is based on the assessment tool currently used by the nurse anesthesia faculty at the Uniformed Services University. It is a composite of standard induction and intubation criteria depicted in widely accepted textbooks of anesthesia. Currently, there is a lack of instruments with validity and reliability testing available to evaluate student performance of induction and intubation in an anesthesia simulator training environment. To date, there has been no validity and reliability testing for the objective checklist.

**Methods**

Following approval by the institutional review board, this prospective, descriptive, comparative investigation was performed on student nurse anesthetists while they were conducting a standard induction and intubation sequence in a high-fidelity patient simulator at the Uniformed Services University. Only military and federal health students enrolled in the nurse anesthesia program at the Uniformed Services University, class of 2009, were considered for inclusion. Students with any tendency for gingival bleeding or who were taking medication or over-the-counter supplements with adrenergic agonist or antagonist properties were asked not to volunteer because these medications could cause an elevation in salivary α-amylase activity. The convenience sample included 18 men and women. Based on Salimetrics (State College, Pennsylvania) recommendations for conditions for collection of salivary α-amylase samples, subjects were asked not to consume a high carbohydrate meal, caffeinated beverages or foods, or nicotine-containing products on the morning of the experiment. Studies show that the highly acidic aldehydes in tobacco smoke inactivate the α-amylase enzyme. Thus, contrary to the activation effects of nicotine on the sympathetic nervous system, exposure to tobacco products is associated with lower salivary α-amylase activity.16

The performance scores on the objective checklist for this investigation were not part of any grade that a student received. Following informed consent, routine demographic data and baseline control data were collected. Due to the diurnal pattern of salivary α-amylase levels, data collection was conducted between the hours of 5:00 and 11:00 AM. Subjects filled out the STAI (forms Y-1 and Y-2). This was the only time subjects completed form Y-2 to score trait anxiety. Heart rate and blood pressure were measured using a Dinamap PRO 1000 monitor (GE Healthcare, Wauwatosa, Wisconsin). A pulse oximeter was placed on the left index finger for 1 minute, and a blood pressure cuff was placed on the right upper arm. The presence or absence of sweat was recorded by direct observation of the subject’s forehead. Saliva sample 1 was collected by having subjects place a Salimetrics Oral Swab into their mouths between the upper cheek and gum for 1 minute. The swab with the whole saliva sample was placed into a plastic microtiter well that was frozen at −20°C until assay.

Each subject was videotaped while performing a standard induction sequence (approximately 15 minutes) in the high-fidelity patient simulator with a member of the anesthesia faculty in the laboratory to proctor the student and provide assistance when necessary. An anesthesia faculty member served as the operating room nurse to assist the subject with the intubation procedure as would normally occur during this process. On completion, the subject was escorted to a room located across from the simulator laboratory where he or she was seated in a comfortable chair. The acute measurements of heart rate, blood pressure, and presence or absence of sweat were recorded, and saliva sample 2 was collected for assay of the salivary α-amylase level. The subject then completed STAI form Y-1 only. This was the second measurement of the state anxiety level. The subject then relaxed in a chair for 20 minutes. Following the rest, recovery heart rate, blood pressure, and presence of absence of sweat were recorded, and saliva sample 3 was collected for assay of the salivary α-amylase level. The subject then completed the STAI form Y-1. This was the third measurement of the state anxiety level. Assessment of performance was by direct observation of the recorded induction and intubation sequence. Three experienced (> 7 years of practice) Certified Registered Nurse Anesthetists independently rated each induction video using the objective checklist. The 18 subjects were stratified into 3 groups based on their scores on the objective checklist. The top 6 scores made up the high performer group, the middle 6 made up the moderate performer group, and the lowest 6 made up the low performer group.

**Results**

Study subjects were 15 men and 3 women. Demographic data are given in the Table. The determination of effect size was based on work by Noto et al9 using a large effect size based on the minimum mean difference of 10.4, a
Statistically significant differences in physiologic measures of stress and anxiety were detected between baseline and acute levels of salivary α-amylase \( (P = .017) \).

power of .80 and an \( \alpha = .05 \). Sample Power 2.0 (SPSS Inc, Chicago, Illinois) used to establish that a total sample of 18 students was needed.
heart rate \((P = .003)\), and anxiety levels \((P = .001)\). Figure 1 graphically represents a comparison of salivary \(\alpha\)-amylase from baseline to acute measurements. The graph indicates a 68.8% increase in salivary \(\alpha\)-amylase levels compared with presimulation values. Figure 2 graphically represents a comparison of heart rate from baseline to acute measurements. The graph indicates a 14.0% increase in heart rate compared with presimulation values. Figure 3 represents the data as a comparison of anxiety level from baseline to acute measurements. The graph indicates a 28.0% increase in anxiety level compared with presimulation values. Analysis of variance was used to compare performance scores with salivary \(\alpha\)-amylase level, heart rate, and anxiety level among 3 groups: low, moderate, and high performers. The least significant difference procedure, a conservative post hoc test, was used to make comparisons between groups. Analysis revealed no significant difference when measures of stress were compared with performance of low, moderate, or high performers. Figure 4 illustrates an increase in salivary \(\alpha\)-amylase activity in the groups of low, moderate, and high performers. In the low-performance group, there was a 119% increase in salivary \(\alpha\)-amylase activity; in moderate performers, there was a 0.6% increase, and in high performers, there was a 114% increase.

**Discussion**

Our results show that human-based anesthesia simulators provoke an acute stress and anxiety response from student nurse anesthetists. Our findings that salivary \(\alpha\)-amylase levels, heart rate, and anxiety levels increased from baseline levels in response to stress are consistent with the findings of other investigations using similar methods.\(^7,^9,^{12,13,17,18}\) Our findings are also consistent with other works that investigated stress, anxiety, and performance in student populations.\(^2,^3\) Furthermore, our results imply that this stress response is variable among performance groups.

The relationship between stress and anxiety and performance exists on a continuum with several possibilities at the extremes. An inverse relationship that indicates increased performance in the light of decreased stress and anxiety and the opposite, decreased performance with increased stress and anxiety are possibilities. There may also be increased performance with increased stress and anxiety or decreased performance with decreased stress...
and anxiety. For the purposes of this study, performance was stratified into 3 basic groups: low, moderate, and high. This would include people who operate optimally during high states of stress and people who have low performance despite low levels of stress and anxiety. Our findings did not reflect a significant difference in stress and anxiety between performance groups and, thus, are inconsistent with research showing that stress impairs academic performance. This finding could be attributed to the study being powered to detect a change in the salivary α-amylase level rather than to 3 distinct performance groups. Analysis of the descriptive statistics and means of each group revealed remarkable findings regarding the relationship between stress and performance of students. There was a large increase from baseline to acute amylase scores in the low- and high-performing groups but only a small increase in the moderate-performing group. Statistical significance was not achieved within the strong-performing group, likely due to high between-subject variability, but it would be worth repeating the study with a larger sample to see if a significant difference is found. Implications for further research could include analysis into the difference between the low- and high-performing groups to distinguish trends and profiles necessary to develop strategies to assist students with this potentially career-ending phenomenon.

Limitations to the study included a small sample for comparing low, moderate, and high performers. In addition, performance was measured by using an objective checklist that requires further investigation regarding validity and reliability. The video recording of each student’s simulator experience was limited to 2 views on 1 screen for practicality purposes and may have affected the rater’s ability to adequately assess performance based on the detailed criteria in the objective checklist. Much care was taken in the timing of data collection to minimize confounding stress and anxiety for the students; however, outside life stressors (eg, family issues, road traffic, and finances) experienced by each student could not be controlled in this environment. An additional contributor to exogenous stress may have been the presence of anesthesia faculty members throughout the simulation. Some students might feel more stress than others with faculty members present during the simulation.

Furthermore, perceptions and physiologic responses to the experiences of simulator training are individually variable. Some students may not perceive the experience as stressful because they do not see simulator training as reflective of a real-life experience and do not take the training seriously. Future research warrants a recalculation of power to determine sample size necessary to detect the possible differences in low, moderate, and high performers. In addition, research measuring performance during scenarios that include unanticipated events requiring students to recall more difficult anesthesia concepts to intervene appropriately may cause more anxiety and result in different findings.
To our knowledge, this study is the first to incorporate the salivary α-amylase level as a measurement of physiologic stress with anesthesia simulation and assessment of student performance. Anesthesia educators have recognized the impact of excessive stress and anxiety on clinical performance for many years but have lacked the ability to identify at-risk students before the beginning of clinical training. Attrition of students who have recently graduated is often related to their inability to perform or recall information because of high levels of stress. Comments are reported such as, “I knew the information, but I was so stressed out I couldn’t remember it.” These results could be useful to educators seeking to identify students who may not perform well during the clinical phase of anesthesia education primarily due to the high levels of anxiety that the environment can provoke. Obtaining more knowledge related to the impact of acute stress on performance can only benefit and improve educational programs and lead to the development of strategies to provide interventions early in the education process to help students succeed.

REFERENCES


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