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DETERMINING THE VIABILITY OF BREATHWORK AND MEDITATION AS INTERVENTION TECHNIQUES FOR STRESS IN THE FORENSIC SCIENCE COMMUNITY

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DETERMINING THE VIABILITY OF BREATHWORK AND MEDITATION AS INTERVENTION TECHNIQUES FOR STRESS IN THE FORENSIC SCIENCE COMMUNITY

A THESIS APPROVED FOR THE
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Abstract

The aim of this project was to determine the viability of breathwork and meditation as intervention techniques for stress in the forensic science community. Practitioners in the field and students studying forensic science to become future practitioners were included as participants. The General Well-Being Schedule (GWBS) and the Perceived Stress Scale (PSS) were used to assess the psychological changes in stress and stress responses. Salivary cortisol and cortisone measurements provided statistics to analyze hormonal changes in stress.

Participants of the study were requested to partake in practicing cyclic physiological sighing and mindfulness meditation for 30 days, depending on their random assignment into these intervention groups. Subjects in the breathwork group were required to practice the method for five minutes every day, and those in the meditation group had to meditate for 15 minutes every day during the study. They provided survey responses and saliva samples before and after the intervention period, and the hypothesis was that these measurements would reflect a reduction in stress levels. To account for external variables such as previous experience with these methods, exercise habits, religious/spiritual influences, frequency of practice, length of practice during each session, and time of day of practice, these were used to create participant subsets that were analyzed for statistically significant changes ($p < 0.05$).

The data showed that all participants who had previously practiced breathwork ($p = 0.012$) and students who had previously meditated ($p = 0.032$), regardless of their randomly assigned intervention in this study, had noticed statistically significant changes in their perceptions of stress. The mean difference of scores on the PSS for the two intervention techniques was calculated to be $- 2.4 \pm 5.0$ for breathwork and $- 2.5 \pm 5.0$ for meditation. The GWBS responses and the salivary measurements did not reflect any statistically significant positive changes in the direction of stress reduction. In most cases, there appeared to be an increase in scores and concentrations of the participants. The inference from the statistics analyzed in this study would suggest that practicing these interventions for 30 days only reduced one's perception and response to stress. The inner well-being and hormonal measurements either stayed the same or increased in some cases, which could be attributed to multiple factors specific to stressors and the nuances of the cortisol-cortisone dynamic in saliva. The data also suggests that breathwork is more efficient in improving perceptions of stress among this population. The positive changes in college students makes a case for integrating these methods as teachings for healthy coping mechanisms of stress in forensic science curricula.

Meditation and breathwork have a track record of improving stress levels in most cases. Therefore, future research efforts should look at implementing these interventions in this population for longer intervention periods to assess if their inner well-being and hormonal responses change significantly. Studies should also be performed to understand the enzymatic activity that converts cortisol to cortisone under the variables of stress, breathwork, and meditation.

Keywords: Stress, forensic science, meditation, breathwork.

Introduction

The world of forensic science is a marriage between the world of science and that of law enforcement. Over the last few decades, this field has grown in prominence, and dramatized television shows and media have supported this growth. These dramatized representations have led to many choosing this field as their career. Although forensic science constantly adapts to technological changes, there is room for improvement in many other aspects.

One such area is the well-being of the future and current workforce. Stress is associated with school, work, and many other facets of life. However, the stressors associated with a career in forensic science are far more unusual and traumatic than those of a traditional office job. Forensic scientists are exposed to the darkest aspects of humanity, be it in the form of incriminating digital evidence, violent acts of murder at a crime scene, or the psychopathy of a criminal. A multitude of such circumstances can take a toll on the human mind and body. Stress, vicarious trauma, post-traumatic stress disorder (PTSD), and burnout are a few conditions that impact the forensic science workforce.

A group of researchers in Mexico raised concerns regarding burnout among law enforcement from prolonged exposure to stressors and how that could lead to sleep, diet, and general health-related conditions (Torres-Vences et al., 2022). In another study, forensic physicians were surveyed for burnout levels, and results suggested significant increases in emotional exhaustion, depersonalization, and decreased perceptions of personal accomplishment. The root cause of burnout in this study was attributed to the stressful nature of the duties of the job (Sehsah et al., 2021). It is common knowledge that stress is pervasive and can impact daily activities and affect a person's decision-making abilities. Decisions made by a forensic practitioner subsequently can have enormous weight for a victim or suspect and have real implications for the criminal justice system. Hence, it is essential to also focus on improving the mental well-being of the forensic science community and reducing their stress levels. In this case, the forensic science community includes the active forensic science workforce and the students studying forensic science.

Breathwork and meditation are two techniques recognized for ages and that have been tested and researched over the past few decades. This research study will explore how breathwork and meditation can help to mitigate stress in the forensic science community. The topics that will be discussed in this document are the increasing stress levels of the workforce, the role breathwork and meditation play in stress relief, a method developed to test the viability of these exercises within the forensic science community, results obtained from those tests, and future steps that can be taken to ensure the development of a holistically healthy workforce.

Literature Review

Stress

The National Institute of Mental Health (NIMH), a subsection of the National Institutes of Health (NIH) in the United States, describes stress as a response to an external cause (I'm So Stressed Out! Fact Sheet, 2022). Mind, a mental health-based charitable group in London, describes stress as a human reaction to pressure or threats. These pressures or threats can arise from different parts of our lives and contribute to mental health problems if not assessed and relieved frequently (What Is Stress?, 2022). Stress can be seen as a net positive or negative depending on daily experiences. However, in these unprecedented times, mental health issues are rising daily. Stress has always been part of the human experience, and people mostly struggle to deal with it in their personal lives.

Stress can be a consequence of many things, such as relationship/domestic issues, work, school, financial troubles, or a combination of these. However, a person's individual experience and daily stressors can vary drastically. One such variation in this case is the exposure to the forensic science environment as a student or technician/agent. Some of the visuals and scenarios one encounters in a classroom, crime scene, or laboratory are not easily digested by the human mind.

Stress: Fighting or Flying

While there has been an increased focus on the human factors associated with the field of forensic science, it has been chiefly directed toward cognitive biases as opposed to workplace wellness and stress (Jeanguenat & Dror, 2018). Some workplace stressors include workload volume, technological distractions, the adversarial nature of the legal system, and disturbing and unnerving crime scenes and case details.

The "fight or flight" response associated with stress results from external stressors, causing the human brain to react and prepare for the upcoming challenge. This response engages the sympathetic nervous system, which, in turn, leads the body to undergo hormonal fluctuations, increased pulse rate, and suppressive actions to limit digestive and immune system activity. Some benefits arise from stress in small doses, referred to as eustress, a term coined by Hungarian endocrinologist Hans Selye (Tocino-Smith, 2019). It can help push someone to meet deadlines and complete their tasks. However, a person's performance can be negatively affected when stress is a common occurrence, and there is little respite from it due to its repetitive or constant nature. Chronic stress, as it is known, can cause a multitude of mental and physical health issues over time (Jeanguenat & Dror, 2018). Chronic stress within the field of forensic science can create an unhealthy work environment and affect the outcomes of the various tasks performed by practitioners.

Stress and Burnout

One consequence of prolonged stress is burnout, which has been studied in different groups of people within the law enforcement and forensic science community. Burnout comprises three components, namely, depersonalization (lack of empathy and sense of detachment from work), emotional exhaustion (mental or physical fatigue), and personal accomplishment/fulfillment (feelings of insecurity and inadequacy). These components are usually measured and analyzed to contextualize the burnout levels experienced by participants in studies (Torres-Vences et al., 2022).

A study performed in Egypt on forensic physicians over two months found that the participants suffered from various burnout subscales due to work stressors. The three subscales of burnout- depersonalization, emotional exhaustion, and personal accomplishments - were analyzed in this study, and 51.9%, 72.9%, and 75.9% of the participating physicians combated moderate to high levels of burnout in the respective scales. In addition, highly stressful job duties that occurred at least five times a month were categorized as an independent predictor of increased emotional exhaustion (Sehsah et al., 2021). Table 1 shows the stressors identified by the participants of this study. Some of the stressors include, but are not limited to, defending reports in court, examination of sexual assault victims, and exhumation and examination of corpses.

The percentages referenced in the paragraph above were gathered through a self-administered questionnaire, including the Maslach Burnout Inventory and the Brief COPE Inventory (Sehsah et al., 2021). These questions were structured as Likert scales wherein the physicians shared their responses on varying levels of burnout from personal experience. The researchers reported that higher levels of emotional exhaustion and depersonalization were experienced by male physicians, whereas a reduced sense of personal accomplishment was experienced by female participants (Sehsah et al., 2021)

Table 1: Stressful job duties reported in a study of forensic physicians (Sehsah et al., 2021).

Forensic Examiners (n = 87)	n (%) *
Defending reports in court	56 (64.4)
Attending legal execution procedures	49 (56.3)
Exhumation of corpses, examination, and autopsy	45 (51.7)
Examination of victims of sexual assault	39 (44.8)
Examination of children as victims of physical or sexual abuse	32 (36.8)
Deciding on medical malpractice cases	24 (27.6)
Performing autopsies	21 (24.1)
Examination of victims of domestic violence	13 (14.9)
Identification and determination of the cause of death in mass casualties	12 (13.8)
Deciding on the medical release of prisoners	11 (12.6)
Evaluation of the mental state of accused in criminal cases	9 (10.3)
Examination of injured persons to determine severity and disability	7 (8.0)
Examination of homicidal, work-related, and accidental injuries	7 (8.0)
Forensic Pathologists (n = 46)	n (%) *
Defending reports in court	37 (80.4)
Preparation and examination of corpses specimens	21 (45.7)
Examining crime scene evidence	6 (13.0)
Paternity cases	6 (13.0)
Laboratory examination of blood, urine, semen, and abortus	5 (10.9)

*Responses are not mutually exclusive

A study in Mexico assessed burnout and the related factors among police workforces. Some stressors experienced by police forces include the use of force, a risk to individual and collective safety, and decision-making in critical situations. In this study, Torres-Vences et al. (2022) found that 44.16% of the police force surveyed showed high levels of emotional exhaustion. Data collected from the study also indicated that 49.29% of the experimental group faced high levels of depersonalization, and 41.03% presented high levels of reduced personal accomplishment. The researchers referenced that chronic stress is connected to burnout and that the stressors experienced by participants in the study affected the data gathered in their study. The hypothalamic-pituitary-adrenal (HPA) axis, pivotal in the body's hormonal system, plays a significant role in responding to chronic stress. While factors external to the work environment can play a role in deteriorating health among police officers, it is interesting to note that health issues such as increased BMI, cardiovascular diseases, and gastrointestinal disorders are faced by those also dealing with burnout. These glands and their relation to stress will be discussed in a different section, but it was highlighted here to describe the consequences of improper functioning concerning a person's health (Torres-Vences et al., 2022).

The researchers of the study in Mexico suggested a correlation between the Body Mass Index (BMI) and all three burnout subscales, as they found that 8 out of 10 police officers were either overweight or obese (Torres-Vences et al., 2022). While there is insufficient evidence to make well-founded claims connecting burnout and deteriorating health, it is interesting to note how chronic stress can affect the functioning of the HPA axis. The glands release glucocorticoid hormones due to stress, which can increase a person's appetite. Subsequently, increased appetite and elevated food intake can easily cause one to gain weight and become overweight and obese over time. It is common knowledge that obesity leads to multiple health conditions, for example, cardiovascular and gastrointestinal disorders. The impact of stress on the holistic well-being of a human being, including a forensic technician or crime scene investigator, can be witnessed through the inferences of this study. Another consequence of the said web of stress was explored in a survey of forensic investigators in South Korea.

Stress and Post Traumatic Stress Disorder

Individuals with Post-Traumatic Stress Disorder (PTSD) are troubled by traumatic incidents they experience due to pain, terror, and helplessness. Stressors at crime scenes are often a part of the experience of forensic investigators, and these consistent exposures can result in PTSD in the workforce (Yoo et al., 2013). In a study published in 2013, Yoo et al. aimed to understand the factors influencing post-traumatic stress (PTS) among forensic science investigators in the Korean Republic. Data points were collected from 111 participants through a self-administered survey. The characteristics analyzed along with PTS included emotional intelligence, job-related factors such as rankings and duties, and death anxiety.

Based on the survey responses, Yoo et al. (2013) found the primary factors that influenced PTS were death anxiety (anxiousness associated with the inevitable nature of death), length of a career as a forensic investigator, homicide experience, and exposure, Type A personality, and fatigue. Those who had increased death anxiety, lower emotional intelligence, shorter careers as investigators, and had been exposed to homicides at least 3-4 times a week were found to be more vulnerable to PTS. The horrific nature of specific scenarios and the consistent experience of shock can drastically affect the mental health of an investigator and lead to them having short careers, as these experiences can impact their personal lives (Yoo et al., 2013). While these studies have provided evidence for how stress can affect the forensic science workforce, some studies have also analyzed the cascading effect of stress on decision-making and the possible errors that could arise from decisions made.

Stress: Decisions and Errors

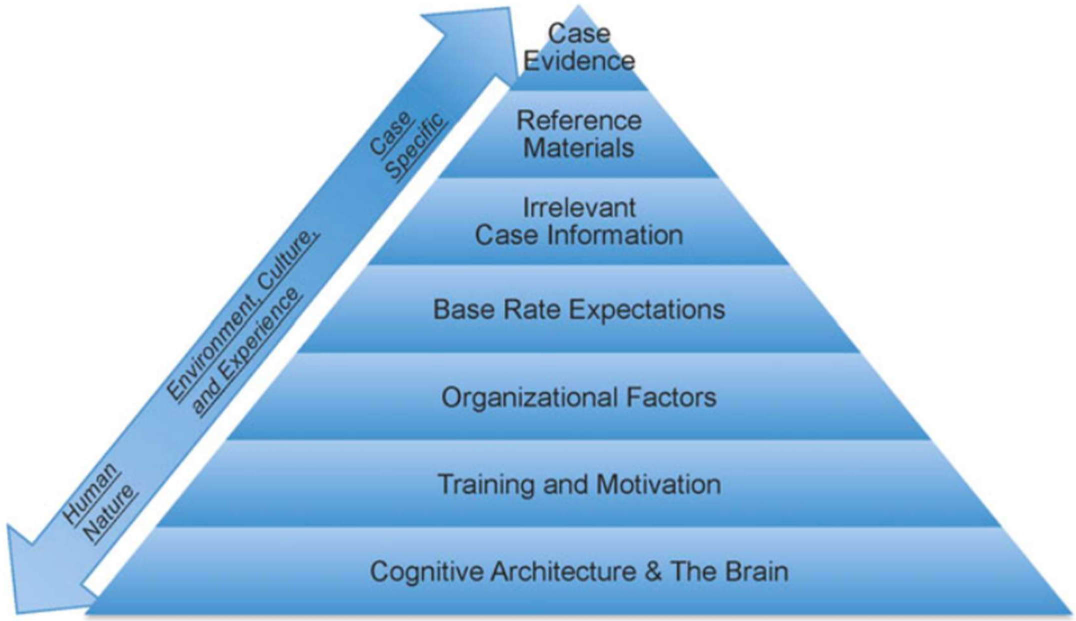
The impact of stress on the decision-making abilities of a forensic expert has not been extensively studied (Almazrouei et al., 2021). However, a few studies and reviews have been performed to connect the two and analyze the possible impact stress has on the errors and decisions made by forensic experts.

The analysis of evidence and the decisions made by a forensic scientist include some amount of human perception and subjectivity (Busey et al., 2022). According to Busey and his fellow researchers (2022), factors that guide the decision-making process in forensics for examiners are ill-defined in that there is minimal guidance from management or policymakers to assist with the decision-making process. Subsequently, the strength of the evidence must be clarified so that detectives and juries can draw fitting conclusions from the information presented to them. Concerning the decisions made, there is also an expectation of zero errors, which is improbable, if not impossible (Busey et al., 2022).

"Honest" mistakes made by forensic practitioners are often not tolerated because of the high standards set. The steps taken to resolve conflicts that arise from incorrect reporting can also seem hostile and combative as the blame is placed on a person, and there can be a lack of open and honest communication (Jeanguenat & Dror, 2018). The stress reaction often compromises one's decision-making abilities because of the fear that comes with deciding something important, and it also can make an individual defensive and disorganized (Jeanguenat & Dror, 2018).

Almazrouei and fellow researchers (2021) surveyed 41 forensic examiners from two different agencies about the sources of stress and their impact on decision-making processes. When responses to the questions about stress and decision-making were collated, the examiners were divided in their opinions. For example, 39% of the participants responded that stress would often affect their judgment, whereas 22% suggested that stress did not impact their ability to discern and decide on the job. The remainder of the participants were unsure if stress influenced their judgment and decision-making skills. Figure 1 presents different sources that may affect a forensic expert's decision-making skills. The image offers a frame of reference for the challenges faced by an expert when it comes to deciding the outcome of evidence and case material.

Figure 1: Taxonomy of different sources that affect decision-making (Jeanguenat & Dror, 2018).



While the data provided above is insufficient to draw firm conclusions, there is room for future research to explore the correlation between stress and decision-making within the scope of forensic science. That being noted, there is room for more research to be performed to examine mindfulness meditation and breathwork techniques and their role in reducing stress and improving the overall well-being of the forensic science community. Mindfulness meditation and breathwork can improve health factors, enhance focus and decision-making, and reduce stress (Jeanguenat & Dror, 2018). Therefore, the following section will explore breathwork, meditation, and mindfulness practices and their benefits in other fields to suggest research opportunities within the world of forensic science.

Stress, Breathwork, and Meditation

While meditation and breathwork can be confused as being the same, there are differences in how breathing plays a role in both exercises. Meditation largely involves being aware of the present moment to reach a settled and focused state of mind. There is little to no manipulation involved when one inhales and exhales during the practice of meditation. On the other hand, breathwork exercises involve the manipulation of the inhalation-exhalation cycle in different ways to modulate one's mood and state of mind (Breathwork Vs Meditation, 2021).

Breathwork has been practiced in different forms in various cultures and societies around the world. These techniques usually involve the modulation of inhalations and exhalations over a short time performed regularly to seek improved health and well-being. There are different patterns to breathwork exercises in terms of regulating the time of inhalation compared to the time of exhalation and the force of breathing in some exercises. Indo-Tibetan cultures have practiced breathwork in the form of pranayama (prana: the life force; yama: taking control) for centuries to improve wellness ("Pranayama Breathing Techniques," 2023).

Meditation is also an age-old practice that has been part of human society for centuries. Practicing it through mindfulness, visualization, and numerous other techniques, individuals have majorly benefited from meditation. The likes of Steve Jobs, the founder of Apple, has spoken about the practice and how it has helped him. Similarly, countless technology firms like Google and Facebook have integrated the practice into their routines (Sokoler, 2018). The Search Inside Yourself program at Google, led by Chade-Meng Tan, a software engineer by trade, found meditation to be a huge source of leadership growth. The exercise also helped staffers calm their minds, seek clarity, regulate their emotions, and boost creativity (*This Is How Google Creates Happier, More Productive Employees*, 2016).

Yet, in some of the most stressful fields of work, like forensic science and law enforcement, employers have been relatively slow to add breathwork and meditation into their routines to improve the well-being of their employees. There are studies within law enforcement that have been and are continually being performed to analyze the benefits. However, forensic science is still behind in studying how this practice could impact the workforce. This review will serve as an attempt to assess the possibilities of stress relief being provided through a breathwork pattern known as cyclic sighing and mindfulness meditation.

Breathwork and Sighing

Sometimes, breathwork and meditation are lumped together as the same practice. However, some differences separate the two practices from each other. According to MindOwl, a start-up offering mindfulness and meditation training based in London, breathwork exercises seek to impact the autonomic nervous system by modulating the levels of oxygen and carbon dioxide inhaled and exhaled, respectively. Breathwork can be used as a tool to alter physical and mental feelings (Breathwork Vs Meditation, 2021).

While breathwork exercises have not been extensively researched in many aspects, more studies have been performed since the turn of the millennium, which has helped contextualize ancient teachings such as pranayama in the world of modern science. As an intervention technique, breathwork is a non-pharmaceutical alternative that is safe to perform, requires minimal education to practice, and can be practiced anywhere (Shaw-Metz, 2023). These exercises reduce the negative effects that stem from stressful events by triggering the parasympathetic nervous response, which brings the practitioner of these exercises to a relaxed state and helps to avoid the negative consequences of the fight-or-flight response. Studies have shown that specifically engaging the diaphragm while breathing in moments of heightened stress can minimize the effects on the sympathetic nervous system and cause the parasympathetic nervous system to act to promote wellness and balance of physical and psychological states. The motion of the diaphragm in this context influences the autonomic nervous system through phrenic and vagus nerve innervation and alters metabolic and cardiovascular homeostasis (Shaw-Metz, 2023).

Research has found that instructed deep breathing has provided psychological relief, and spontaneous sighs and breathholds give practitioners physiological relief. Physiological relief is suggested to occur due to sighing resetting the system. Sighing contributes to a sense of temporary relief, which can subsequently enable one to cope with stressors and emotions. The immediate impact of sighing as a mechanism for relief may lead one to repeatedly perform breathwork involving sighing (Vlemincx et al., 2016). A recent study performed by Stanford researchers found cyclic sighing to be a greater improver of mood when performed for 5 minutes, which will be explored in the following subsection.

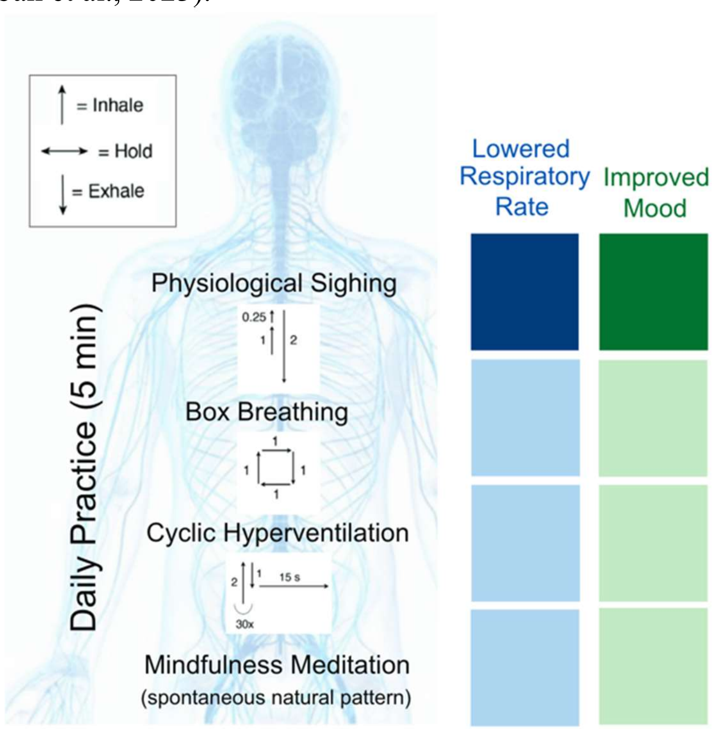
Cyclic Sighing and Stress Relief

Researchers at Stanford University compared three breathwork exercises and mindfulness meditation performed over a period of five minutes to analyze the effect they had on enhancing mood and reducing physiological arousal, i.e., the triggered response to external stressors. The study had participants performing these exercises daily for a period of one month. Cyclic sighing was found to be the most beneficial of the four intervention techniques discussed. The other two breathwork exercises included box breathing and cyclic hyperventilation The breathing patterns specific to these methods are described in Figure 2, wherein the upward arrows symbolize an inhalation and the downward arrows represent an exhalation (Balban et al., 2023).

One round of cyclic sighing is represented by two consecutive inhalations to fill the lungs, followed by one prolonged exhalation. The graphical representation of this cycle is represented in

Figure 2. The second inhalation is shorter than the first as there is little room to fill the lungs. For this study, the inhalations were performed nasally, whereas the exhalation was through the mouth. However, the researchers also provided participants with the option to exhale through the nose. The participants were required to complete the Positive and Negative Affect Schedule (PANAS) and the State-Trait Anxiety Inventory (STAI) and were provided with WHOOP straps, a health-tracking device to measure physiological measures such as respiratory rate and heart rate. Ninety percent of the participants reported that they had positive experiences while performing the exercises, whereas the remainder suggested that they had negative experiences. From the data obtained, they were able to determine that cyclic sighing was the most impactful intervention technique. Figure 2 illustrates the order of most to least impactful exercises tested in this study (Balban et al., 2023).

Figure 2: Graphical representation of the breathing techniques and their impact on mood and respiratory rate (Balban et al., 2023).



While mindfulness meditation did not fare well in comparison to the breathwork exercises in this study, the time spent on meditation by participants was considerably lower than periods of 15-30 minutes, which is usually performed for increased benefits. Comparing five minutes spent on cyclic sighing to 15 minutes of mindfulness meditation for this project could help provide a different perspective.

Mindfulness Meditation

According to VeryWell Mind, an informational website about mental health, mindfulness meditation teaches one to slow down, notice pacing thoughts, and calm the mind. It requires one to be present in the moment to be aware of their thoughts and improve focus through attention, awareness, and deep breathing (What Is Mindfulness Meditation?, 2022). Treatments and therapies associated with mindfulness and meditation practices have entered the Western market over the past two decades, which has increased the interest in studying the benefits offered by said therapies (Abbott & Lack, 2020).

Mindfulness-Based Stress Reduction (MBSR) is a standardized meditation training program developed by Jon Kabat-Zinn, an American professor, in 2005. This program was rooted in practices of Theravada Buddhism and essentially cultivated moment-to-moment nonjudgmental awareness through meditation. The three modes of meditation ascribed to this protocol are body scanning, sitting meditation, and gentle hatha yoga (Allen et al., 2021). The process of body scanning focuses one's attention on the entire body by moving said attention from head to toe and noticing any sensations that may arise. Sitting meditation is a mode where an individual is prescribed to focus on their breath and observe the continuous flow of thoughts and distractions without judgment. Lastly, hatha yoga includes breathing exercises, stretches, and movements that benefit the musculoskeletal system (Allen et al., 2021).

While MBSR was initially developed for chronic pain, researchers found that the protocol reported positive health benefits among clinical and non-clinical populations (Khoury et al., 2015). A meta-analysis of research studies evaluating the effects of MBSR on figuratively healthy people was performed by Khoury et al. (2015), and they inferred that the program had a considerable impact on stress reduction in general. However, there are limiting factors associated with the conclusions made in this study due to the high variability between each of the 29 studies reviewed for the meta-analysis. On the other hand, more research has been conducted over the past decade to evaluate the role mindfulness-based meditation programs can play in a law enforcement setting. One such study was performed on officers of the Madison Police Department in the state of Wisconsin.

Mindfulness Meditation and Law Enforcement

As noted previously, forensic science and law enforcement professionals deal with many traumatic stressors. More effort is being directed toward helping these professionals deal with these issues through internal programs. One such program has been recently implemented as a study by the University of Wisconsin-Madison. The researchers aimed to analyze the acceptance and practicality of an 8-week mindfulness training program in the Madison Police Department and evaluate how it could affect the symptoms of PTSD experienced by the officers. Multiple surveys that assessed sleep patterns, stress, burnout, PTSD symptoms, and well-being were administered to the officers two weeks before the program began and three weeks following the final class (Grupe et al., 2021). The mindfulness program offered in this study reflected the MBSR designed by Kabat-Zinn and the Mindfulness-Based Resilience Training (MBRT) program that was tailor-made for law enforcement officers.

The study's data showed that the organized classes had roughly an 85% attendance rate. However, some officers found the process of having to frequently provide data tedious and discomforting. Nevertheless, there was a considerable reduction in perceived stress and PTSD symptoms following the program. Follow-up assessments administered five months after the program was completed indicated that the effects of mindfulness training persisted for an extended period. Hyperarousal was one of the significant symptoms of PTSD that had dropped considerably due to the program utilized in this study (Grupe et al., 2021). Therefore, the study indicated promising results for applications in other fields with high stressors.

Measuring Stress

Stress is often discussed as a part of everyday life. However, as mentioned previously, the stressors associated with forensic science are significantly different from those associated with other fields of employment due to the traumatic experiences that the workforce may face regularly. While the focus on the impact of stress has increased, the diagnosis of stress-related disorders is not a perfect procedure and is currently based on surveys, observations, and interviews.

Quantitative Psychological Surveys

Stress questionnaires and surveys are standardized tools often used to deduce one's perceived stress levels, identify potential stressors, and understand the effects of said stressors on aspects of the human experience (Neurolaunch Editorial Team, 2024). These questionnaires are built to accurately assess stress and enable people to take the necessary steps to combat stressors effectively. They traditionally use Likert scales, which are psychometric scales offering a range of responses, for example, from “strongly disagree” to “strongly agree,” to help express any subtleties associated with feelings of stress. These nuances expressed through the scale facilitate the quantitative analysis of results. Surveys tend to include open and close-ended questions. Open-ended questions provide survey takers the opportunity to provide more detailed and qualitative information about their experiences, whereas close-ended questions generate standardized responses that are easier to statistically analyze (Neurolaunch Editorial Team, 2024).

Surveys include paired questions that generally mean the same thing but phrase them positively and negatively. Researchers add these questions to ensure that respondents provide consistent responses. The opposing question is reverse-coded in this context, as it expresses the same variant in the opposite metric (Bobbitt, 2021). Stress questionnaires support individuals, healthcare professionals, organizations, and researchers alike as they detect stress, track stress changes over time, and guide stress management strategies (Neurolaunch Editorial Team, 2024). While psychological surveys provide context for stress assessment, hormonal responses offer crucial physiological data. Like other psychological/psychiatric conditions, stress triggers a hormonal response in our bodies that can affect our behavior and performance in everyday life.

Cortisol

Cortisol, a glucocorticoid, is one of the many compounds secreted in response to stress. It is the most commonly viewed human stress biomarker (Giacomello et al., 2020). When a person encounters mental or physical stressors, the adrenal cortex produces cortisol in high amounts. The metabolic process is activated due to cortisol secretion, which provides the body with additional energy by releasing glucose into the bloodstream. It also affects mental reactions by elevating levels of adrenaline and noradrenaline, also known as stress hormones. Due to these reactions in the body, the additional energy generated helps a person combat stressful situations (Chojnowska et al., 2021). The cortisol concentration in different matrices can depend on factors such as sex, age, population, and circadian rhythm. Stressors can also be categorized as acute or chronic, depending on their impact on a person's cortisol levels. A spike in cortisol levels reaches its peak

20-30 minutes after a person experiences an acute stressor. The HPA axis acts to signal the production of cortisol. This response is reduced when an individual is exposed to chronic stress, producing lower volumes of cortisol. These characteristics make cortisol an ideal assessment tool for stress levels over varying periods (Giacomello et al., 2020).

There are many examples of acute and chronic stress in the field of forensic science. However, stressors have not been researched thoroughly within this population. For the feasibility of this study, a biological fluid that can be collected through a non-invasive method is required to analyze the variations of cortisol in those who work and study in the field of forensic science. A matrix that has been proven to be an excellent medium to analyze multiple human biomarkers in the recent decade is saliva.

Saliva and Salivary Cortisol

Saliva has become the biological fluid of interest due to its advantages in clinical testing and diagnostics. The sample collection process is significantly noninvasive compared to collecting blood or urine. Additionally, it involves minimal risk of contracting infectious viruses such as HIV and Hepatitis B. Saliva as a matrix can reflect the well-being of a person through cortisol analysis, and the less intrusive nature of sample collection and preservation makes it an effective method for analyzing a wide range of characteristics (Punyadeera & Slowey, 2019).

Saliva is produced by three significant glands, paratoid, submandibular, and sublingual, and 400 minor glands in the oral cavity. In addition, it carries proteins that may either be produced within the cavity or are gathered from interactions with blood. According to Punyadeera & Slowey (2019), a healthy individual is estimated to generate between half a liter to one and a half liters of saliva daily. However, these volumes can be affected by physiological and pathological processes of the body both quantitatively and qualitatively.

As mentioned previously, cortisol is produced by the adrenal cortex, which is triggered by the signals of the HPA axis. A healthy individual usually has 30-160 ng/mL and 1.0-1.6 ng/mL of cortisol at night in blood and saliva, respectively (Steckl & Ray, 2018). According to Steckl & Ray (2018), in their study to detect stress biomarkers in biological samples, cortisol was the only biomarker with large concentrations relative to the bodily fluids assessed, namely, blood, urine, sweat, and saliva. Salivary cortisol is also used to diagnose Cushing syndrome, a metabolic disorder caused due to elevated production of corticosteroid hormones by the adrenal cortex (Raff & Phillips, 2019).

Cortisol is mainly produced late at night, which leads to serum levels being higher between 7 am and 8 am. As the day progresses into the evening, the cortisol concentration reduces drastically, and only 10% of the cortisol present in the morning remains in the body (Chojnowska et al., 2021). However, Raff & Phillips (2019) discuss the importance of another analyte when testing for cortisol levels in saliva.

Salivary Cortisol and Salivary Cortisone

Since saliva is the biological sample of interest for this project, the availability of cortisol in the matrix needs to be assessed in more detail. Preliminary reviews articulated the presence of another analyte of interest, cortisone. The biochemical activity associated with cortisol and cortisone is largely guided by the presence of the 11-β-hydroxysteroid-dehydrogenase (11β-HSD) enzyme. Due to this enzymatic activity, cortisone is present in higher concentrations in saliva than cortisol (Raff & Phillips, 2019).

To understand this relationship, it is important to investigate the activity of cortisol in serum to understand its presence in saliva. In the context of serum cortisol, Debono et al. (2016) state in their study that cortisol is present in various forms, namely cortisol-binding globulin (CBG), albumin-bound cortisol, and free cortisol. CBG accounts for 80% of all cortisol present in blood and is mainly a source of transportation of the hormone in the body. In contrast, free cortisol only accounts for 10% of total cortisol and is the variant that is biologically active. 11β-HSD type 2 (11β-HSD-2) is present in the salivary glands and swiftly deactivates cortisol by converting it to cortisone. This conversion is a protective measure to prevent cortisol from occupying receptors that are specific to other steroidal hormones in the kidneys, colon, placenta, and saliva (Kargl et al., 2017). If one had to compare the ratio of cortisol to cortisone in different matrices, the concentration of the latter is higher than the former by a factor of six in saliva, whereas the opposite is true for serum by a factor of four.

Debono et al. (2016) inferred from previous studies that salivary cortisone largely represented serum-free cortisol and explored to determine the relationship between serum cortisol, which includes all forms of cortisol described in this paragraph, and salivary cortisol and cortisone. The study analyzed samples collected from fourteen healthy males and concluded that there were stronger correlations between serum cortisol, and salivary cortisol and cortisone. This confirms the production of salivary cortisone from serum-free cortisol due to the oxidation of the latter by 11β-HSD-2. While there were no female samples to report from in this instance, the researchers opine that estrogen could increase the concentrations of the bound forms of cortisol in serum in women. While this doesn’t affect salivary cortisone production from serum-free cortisol, it does reduce the presence of the latter in serum and subsequently also leaves the correlation of serum cortisol to salivary cortisone in women slightly ambiguous.

However, a recent study conducted in the United Kingdom (El-Farhan et al., 2024) assessed the impact of adrenocorticotropic hormone (ACTH) stimulation on salivary cortisol measurements. ACTH is the adrenal signal referenced in previous sections, which triggers the release of cortisol by the adrenal glands. This hormone is released by the pituitary gland, which is part of the HPA axis that controls this entire mechanism. The concentrations of CBG can be affected by multiple factors in men and women, but this study suggested that there was no significant concentration difference in salivary cortisol between the two genders. Unfortunately, El-Farhan et al. (2024) did not test salivary cortisone in this study and referenced the previous study by Debono et al. (2016) to make a case for the dynamic of the compound with serum cortisol. Therefore, it is safe to assume that the relationship between salivary cortisone and serum cortisol is still slightly ambiguous when considering wider populations influenced by different external factors. But Debono et al. (2016) and Raff & Phillips (2019) imply that cortisone is present in higher concentrations than cortisol in saliva, and they strongly suggest that serum-free cortisol (active cortisol) triggers the production of the former. Hence, it is better to infer that cortisone solely reflects active cortisol and doesn't include protein-bound variants of the stress hormone until more in-depth research suggests otherwise. Regardless, the activity of the oxidative enzyme 11-β-HSD-2 can also be affected by many other variables such as sleep, diet, and exercise, which in turn can impact the presence of cortisone in saliva. The direct influence of meditation and breathwork on this enzyme is also largely unknown. Hence, the concentrations of cortisol and cortisone calculated from saliva samples would be relative to the upregulation or downregulation of this enzyme in every participant. On the other hand, the direct correlation between stress and cortisone has not been researched extensively at the time this project was conducted. Therefore, any inferences made may need to be researched further in the future for a better understanding of the hormonal dynamics of stress relative to saliva. In terms of testing these analytes in saliva, liquid chromatography-tandem mass spectrometry (LC/MS-MS) has appeared at the forefront of the methodology used by most research studies in the past decade.

Saliva and LC/MS-MS

As referenced previously, saliva has become a biological sample of interest, and recent studies have tested its use to measure health outcomes and diseases. However, saliva is a biological matrix with contaminants that could interfere with analyzing the analytes of interest. Several studies have been performed to formulate the ideal method to determine the most accurate concentrations of cortisol and cortisone. One such study used liquid chromatography-tandem mass spectrometry (LC/MS-MS) and solid-phase extraction (SPE) combined with a relatively easy saliva collection method.

Antonelli et al. (2015) developed a method using the Oasis HLB SPE cartridges manufactured by Waters Corporation and a collection device known as Salivette, manufactured by Sarstedt, to build a reliable method for testing cortisol and cortisone relative to Cushing Syndrome. SPE is a sample preparation technique that is used to isolate target analytes by passing the sample through a sorbent mass of specific polarity and washing away any interfering contaminants. The pre-treated sample volume in this study was indicated to be 300 µL of saliva. The calibrators and samples were combined with internal standards specific to the analytes. The SPE-treated sample was then processed through liquid chromatography-tandem mass spectrometry (LC/MS-MS) to analyze cortisol and cortisone simultaneously. In this study, the researchers used an Agilent HPLC series 1200 liquid chromatograph with the Agilent 6430, a triple quadrupole mass spectrometer equipped with an electrospray ionization source in positive ion mode. They used an analytical column combined with an on-line SPE (mechanism connected to the column) containing carbon-18 as the stationary phase (Antonelli et al., 2015).

In chromatography-tandem mass spectrometry, the stationary phase helps separate and retain the molecules of interest from a complex mixture. Carbon-18 (octadecyl carbon) chains are non-polar masses combined with silica that are widely used for their chemical properties and reliability for a wide range of molecules. Polar mobile phases that combine the use of water and methanol with formic acid carry the sample through the stationary phase and aid in separating specific chemical compounds. The gradient of each mobile phase passing through the column is adjusted to carry the sample, and the retained molecule in the stationary phase is picked up and transported to the mass spectrometer for ionization, fragmentation, and detection. The triple-quadrupole mass spectrometer functions on the principle of detecting molecular masses (mass-to-charge ratios) to distinguish between compounds. The detection process is preceded by the ionization of the parent molecule (quantifier) that is passed on from the chromatograph. With the parent molecule being ionized using electrospray ionization, the instrument filters out any molecules that do not possess the required mass-to-charge ratios before they are fragmented. The preset mass-to-charge ratios of the fragmented molecules (daughter molecule or qualifiers) are then detected at the end to identify specific analytes. These ionization mechanisms are guided by different types of voltages, which are often specific to the ion source, type, and manufacturer of the mass spectrometer.

Internal standards are deuterated versions of the analytes being tested and are often used in chromatography-tandem mass spectrometry methods. While analytes often contain protium, the most common isotope of hydrogen, internal standards (IS) are chemically synthesized with deuterium, a heavier isotope of hydrogen. They behave like the undeuterated analyte and primarily account for any issues that may be caused by matrix effects. In this context, the chemical properties of saliva obtained from different sources may vary and subsequently affect the expression of analytes when they separate from mixtures, which are classified as matrix effects. Internal standards monitor the times at which separation may occur (retention time) and balance for any intra-sample ion suppression or enhancement. Internal standards correct for any abnormalities caused by matrix effects and aid in accurately determining analyte concentrations.

While Antonelli et al. (2015) do not specify the mobile phases used or their flow rate, they do specify that the total run time for each sample was approximately seven minutes and that quantitative analysis was performed in multiple reaction monitoring (MRM) mode. From the development, validation, and statistical analysis of their method, the reference intervals for healthy adults were determined to be 3.0-21.0 nmol/L for morning salivary cortisol and 10.4-42.4 nmol/L for morning salivary cortisone. They opine that LC-MS/MS analysis methods avoid the drawbacks of traditional immunoassays, as the latter has lower specificity for potential cross-reactivity that interferences may cause. On the other hand, LC-MS/MS simplifies the process of measuring multiple analytes simultaneously.

This was confirmed in another study as Raff & Phillips (2019) measured salivary cortisol and cortisone concentrations relative to collection times for the diagnostic analysis of Cushing syndrome and used both liquid chromatography-tandem mass spectrometry (LC/MS-MS) and Enzyme Immunoassay (EIA) to determine if the former was reliable with small volumes of saliva. The study worked with sample volumes as low as 50 µL and determined that reliable results were generated with higher sensitivity for lower cortisol concentrations. The chromatography apparatus used in this study was the 1290 Infinity High-Performance Liquid Chromatograph and a triple quadrupole mass spectrometer, with the ion source being electrospray ionization produced by Agilent Technologies. The standards for cortisol and cortisone were prepared in a solution of 50:50 volumetric ratio of methanol to water. The column used for the resolution of analytes in this study was the Poroshell 120 EC-C18 produced by Agilent Technologies, at a temperature of 50°C with a 0.3 µm inline filter. The injection volume and mobile phase flow rate were set at 10 µL and 0.4

mL/minute. 5 mM ammonium formate solvent was used for gradient elution in both water and methanol. The mass-to-charge ratio (m/z) monitored by the mass spectrometer for cortisol was quantified and qualified at 363.2/121.1 m/z and 363.2/91.1 m/z, respectively. Similarly, for cortisone, the ratios for the quantifier and qualifier were 361.2/163.1 and 361.2/121.1. Cortisol-d4 and cortisone-d7 were the internal standards used in this methodology, and they were analyzed at ion transitions 367.2/121.1 and 369.2/169.1, respectively. The analytical range of cortisol and cortisone detection was set to 0.1-89.4 nmol/L for non-diluted samples and up to 27,600 nmol/L for diluted testing samples (Raff & Phillips, 2019).

As mentioned previously, cortisol is in smaller amounts in saliva than cortisone, which was corroborated by Debono et al. (2016) in their study. However, the authors do indicate that it can be challenging to detect significantly low cortisol concentrations, simultaneously making the case for the use of cortisone.

Objectives and Hypothesis

This study aims to determine the viability of breathwork and meditation as intervention techniques to help alleviate stress within the forensic science community. Those who work in the field and are students in college in forensic science programs are exposed to unique stressors that are seldom seen in other fields unrelated to law enforcement, first response teams, and clinicians. While each member of the forensic science community may experience the stressors of the field differently, their psychological and physiological well-being must be supported. In a field associated with fact-finding and assisting the court of law in seeking justice, ensuring that the workforce can make good decisions without being impacted by stress is imperative.

The impact of stress on law enforcement and forensic science has been explored in-depth in previous sections. The workforce and forensic science students in college are regularly exposed to unique stressors that can affect their physical and mental well-being in the long term. Breathwork and meditation have been found to positively impact one's ability to deal with stress in multiple studies. However, when this study was conceptualized, little to no studies had been performed to view the impact of these intervention techniques among forensic practitioners. The shorter practice times associated with these techniques are conducive to regular practice and irregular/busy work schedules. The stress levels of participants in this study were determined with salivary cortisol and cortisone measurements and quantitative psychological surveys. This approach to gathering measurements was taken to observe the possible differences between the participant's perception of stress in comparison to their body's response to the stressors experienced.

Implementing the intervention techniques is expected to reduce participant stress and improve their response to stressors. The data gathered through the surveys, and salivary measurements are anticipated to reflect the positive change created by breathwork and meditation. The data is also expected to provide some insight into the most effective and efficient exercise, the variables that impact said exercises, their real-time impact on the workforce, and the potential use of these interventions in curricula to arm students who aim to enter the forensic science workforce with healthy coping mechanisms.

Methods

Procedure

The project proposal was submitted to the university's Institutional Review Board (IRB) and was approved through an expedited review before recruitment. The intervention groups were separated into sets of cyclic physiological sighing and mindfulness meditation participants. Participants in the former group were required to practice the prescribed exercise for five minutes daily during the 30-day intervention period, whereas those in the meditation group had to practice the exercise for 15 minutes for the same duration of days. The stress measures were collected using quantitative psychological surveys administered through Qualtrics and salivary cortisol/cortisone measurements before and after the 30-day intervention period. The Perceived Stress Scale (PSS) and the General Well-Being Schedule (GWBS) were used for assessment purposes, and the saliva samples were collected using the Cortisol Salivette manufactured by Sarstedt. The salivary components were tested and calculated using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Each participant was required to submit a consent form before they were randomly assigned to either intervention group. These consent forms were administered digitally using Qualtrics (see Appendix A). They were subsequently asked to pick up a piece of paper from a bowl that listed a participant number, unique ID, and an intervention technique allotment. These unique IDs were structured in the following format: SST-BW-XYZ and SST-MT-XYZ, wherein SST stood for stress study, MT for meditation, BW for breathwork, and XYZ for an assortment of three-digit numbers starting from 100. The participant number was used to keep track of intake during the recruitment period, and the unique ID was used to connect survey responses and saliva samples to each participant while keeping their personal information anonymous. Information sheets specific to the designated method (attached in Appendices F and G) from the previous step were given to each participant. This included pointers for the participants to follow during the intervention period and a QR code/link to a Google Drive folder. The digital folder contained a video explaining the exercise guidelines and protocol to be followed, as well as guided soundtracks to assist with the practice of the intervention technique.

Upon receiving the information sheets, participants were asked to rinse their mouths for priming before saliva collection. For ideal sample collection, they were asked to provide their saliva at least 60 minutes after brushing their teeth, consuming a meal, and/or taking oral medication to minimize the presence of interfering substances. They were then requested to complete the pre-intervention survey. After completing the survey, the participants were asked to provide their saliva samples.

Once the first set of required surveys and samples were collected, participants were informed of the post-intervention sample collection date. Participants also received calendar invites to serve as reminders for meeting dates/times. Individuals were requested to meet and provide their second set of samples around the same time of day as their first sample collection to maintain consistency with the diurnal cortisol fluctuations throughout the day. In this instance, diurnal fluctuations refer to the increase of cortisol concentrations in waking hours and its subsequent decrease throughout the day. A buffer of seven days from the completion of the 30-day intervention period was set for the post-intervention collections to account for scheduling conflicts due to weekends or participant unavailability. During the intervention period, subjects received weekly emails as reminders to practice breathwork or meditation. During the scheduled meeting after the end of the 30-day intervention period, participants were required to use their unique IDs to complete their return survey and label their second saliva sample using said ID.

Participant Samples

Participant stress levels were analyzed with quantitative surveys and salivary cortisol and cortisone measurements. The Perceived Stress Scale (PSS) and the General Well-Being Schedule (GWBS) were combined with some demographic and contextual questions on Qualtrics surveys. Participants were asked to take these surveys and provide saliva samples before and after the completion of the 30-day intervention period.

Quantitative Psychological Surveys

The pre-intervention survey included demographic questions about gender, race, age, exercise habits, religious/spiritual inclinations, previous experiences with interventions (listed in Appendix B), and the GWBS and PSS questionnaires. The post-intervention survey contained the same questionnaires included in the pre-intervention survey and questions about the frequency of practice, use of guided soundtracks, time spent in each practice session, and future use of the assigned intervention exercise. These questions are listed under Appendix C.

The Perceived Stress Scale (PSS) was developed in 1983 by Cohen et al. and is still used to assess individual stress levels. These responses are believed to show the balance between a person's exposure to different life events and their ability to respond to them (Harris et al., 2023). Three versions of this scale were developed with 14, 10, and 4 questions. For this project, the 10-question survey was used to gather data about the participants' perceived stress levels. The questions are answered with the help of the five-point Likert scale that begins at "Never" (0) and goes to "Very Often" (4). Questions 4, 5, 7, and 8 are scored using a reversed grading, i.e., 0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0. The scores were then tabulated out of 40, and each participant was distributed into low-stress, moderate-stress, or high-stress subsets. People who scored 0-13 were labeled as low-stress, 14-26 as moderate-stress, and 27-40 as high-stress. This questionnaire is attached under Appendix E.

The General Well-Being Schedule (GWBS) was developed in 1977 for the U.S. Health and Nutrition Examination Survey and contains 18 questions (General Well-Being (GWB) Schedule, n.d.). Fourteen of the 18 questions are set up to use six-point response scales, whereas the last four questions use 0-10 rating scales (Form: General Well-Being Schedule - 18 Item [NHANES], n.d.). This survey focuses on one's subjective feelings of distress and internal well-being and assesses how a participant may feel about their "inner personal state" (General Well-Being (GWB) Schedule, n.d.). The study is split into six sub-scores, which are categorized as follows: anxiety (questions 2, 5, 8, 16), depression (questions 4, 12, 18), positive well-being (1, 6, 11), self-control (questions 3, 7, 13), vitality (questions 9, 14, 17), and general health (questions 10 and 15). Questions 1, 3, 6, 7, 9, 11, 13, 15, and 16 are reverse scored, and 14 is subtracted from the final score to bring it within the range of 0-110. Depending on the range in which the scores of the individuals fell, they were allocated into severe distress, moderate distress, and positive well-being subcategories. Subjects who scored between 0-60 were labeled under severe distress, 61-72 under moderate distress, and 73-110 under positive well-being. This questionnaire is attached under Appendix D.

Saliva Collection

Saliva samples for this study were collected using the Sarstedt Cortisol Salivette. The salivette contains a transparent tube with a conical bottom, an insert, an absorbent cotton roll, and a blue cap. Individuals were asked to chew on the cotton roll for one minute to ensure the collection of optimal sample volume. Then, the salivette tubes were appropriately labeled and isolated in individually labeled biohazard bags to prevent any mixing, misidentification, and contamination.

After that, samples were transported in ice boxes with ice packs and stored in the lab freezer on campus at -20°C. The oldest sample tested was collected roughly five months prior to the date of testing due to discrepancies at the time that recruitment was started and method development for testing began. At the time the collection mechanism for this project was finalized, Sarstedt had only conducted one study that had stored these collection devices at freezing temperatures for 24 days (Dubberke et al., n.d.). However, Quest Diagnostics, a global medical research company, runs salivary cortisol tests using the same collection device, and their sample storage requirements suggest that cortisol remains stable when stored at freezing temperatures for six months (*Cortisol, LC/MS, Saliva | Test Detail | Quest Diagnostics*, n.d.).

Before testing, these tubes were thawed for 45-60 minutes at room temperature. Sarstedt's guidelines call for the salivette to be centrifuged at 1000 x g (g-force/gravitational force) for two minutes. For this study, the tubes were centrifuged at 1500 x g (g-force/gravitational force) for four minutes. The insert that carries the saturated cotton roll contains an opening on the bottom, which supports the collection of saliva at the bottom of the tube after centrifugation. The conical bottom helps separate any mucus and particles that may have been present in the saliva, with the clear specimen sitting on top of the conical portion. Following the separation of saliva from the cotton roll, the insert that aided in saliva separation and the roll itself were discarded, and the tubes were transported in ice boxes to the testing lab. The instructions for use document of the cortisol salivette has been attached to Appendix H for reference.

Saliva Testing

The cortisol and cortisone measurements in saliva were tested at In-House Labs LLC (testing lab) in Oklahoma City, Oklahoma. This facility is a clinical toxicology lab that tests oral fluid and urine biological matrices. Their liquid chromatograph tandem mass spectrometer was used for this study, and a method was developed and validated before participant samples were tested.

Instrumentation

The testing lab uses a Shimadzu Ultra-Fast Liquid Chromatograph (UFLC) combined with a Sciex 4500 Triple Quadrupole Mass Spectrometer, and the sample preparation and analysis protocol were built specifically for these instruments. Based on preliminary research conducted in the method development phase, recent studies used instruments that were more sensitive to hormonal assays like cortisol in saliva, as the concentrations can be comparatively low. Simultaneously, some articles referenced in the literature review of this project mention the difficulty in measuring salivary cortisol at significantly low concentrations. Adding cortisone to the testing protocol supported the gathering of more reliable data as it expresses the enzymatic activity occurring in salivary glands and is reflective of free cortisol levels observed in blood serum, as mentioned previously. The addition of cortisone to the testing parameters is also consistent with the research on using cortisol measurements in saliva for diagnostic purposes.

A Phenomenex Kinetex 2.6 µm C18 column (50 x 2.1 mm, 100 Å) was used to separate cortisol and cortisone in the saliva samples. Mobile Phase A (MPA) was prepared using 5.0 mM (0.315 g) ammonium formate (GFS chemicals) in HPLC-grade water (Sigma Aldrich), and Mobile Phase B (MPB) was made using the same mass of ammonium formate in HPLC-grade methanol (Sigma Aldrich). The injection volume was set at 50 µL, as preliminary testing with different injection volumes suggested that a higher injection volume was best for peak intensity. The flow rate was set at 0.25 mL/minute, and the gradient for the mobile phases at the start of the method was set to 50% for each, with MPB concentration increasing as the method progressed for a period of five minutes. The detailed adjustment of the mobile phase gradients is listed in Table 2 for reference. A needle rinse solution of HPLC water, acetonitrile, acetone, and isopropyl alcohol in the concentration ratio of 2:1:1:1 was used before and after the injection of every sample.

Table 2: Mobile Phases gradient parameters for the Shimadzu Ultra-Fast Liquid Chromatograph

Time (minutes)	Module	Event	Parameters
0.50	Pumps	Pump B concentration	50
3.00	Pumps	Pump B concentration	68
3.75	Pumps	Pump B concentration	95
4.75	Pumps	Pump B concentration	95
4.76	Pumps	Pump B concentration	50
5.26	Controller	Stop	N/A

The mass spectrometer used electrospray ionization as an ion source. It was set to detect two mass transitions (Q1 and Q3) with specific molecular masses. These masses and other parameters specific to the instrument are included in Table 3. After the precursor/parent molecule, Q1, is ionized and broken up into a daughter molecule, Q3, within the triple-quadrupole, they are detected relative to their retention times (RT) for cortisol and cortisone. Mass transition Q1 (interchangeable with T1 as transition 1) was used as the quantifier, and mass transition Q3 (interchangeable with T2 as transition 2) was used as the qualifier, wherein both needed to be detected for each sample to consider the analyte to be present.

Table 3: Mass Spectrometer Parameters for the Sciex 4500 Triple Quad

Analyte	Q1 (Daltons)	Q3 (Daltons)	RT (seconds)	DP (Volts)	EP (Volts)	CE (Volts)	CXP (Volts)
Cortisol-D4	367.1	121.1	1.00	141	10	36	20
Cortisone-D8	369.2	163.1	0.97	120	10	20	10
Cortisol 1	363.1	121.1	1.00	141	10	36	20
Cortisol 2	363.1	115.1	1.00	141	10	155	18
Cortisone 1	361.2	163.1	0.97	120	10	20	10
Cortisone 2	361.2	121.1	0.97	120	10	32	10

Legend: DP: Declustering Potential; EP: Entrance Potential; CE: Collision Energy; CXP: Collision Cell Exit Potential

Calibration, Standards, and Quality Controls

For this study, a 1.0 mg/mL cortisol stock solution in methanol was purchased from Cerilliant. At the time of method development, cortisone stock solutions were not readily available, and a powdered form of cortisone was purchased from MP Biomedicals. Methanolic stock solutions with a concentration of 100 µg/mL were prepared from these sources for cortisol and cortisone. These stocks were used to prepare two combined secondary stock solutions in methanol with a resulting concentration of 225 ng/mL cortisol and cortisone. One secondary stock solution was labeled and used as a standard mix, while the other was used as a quality control mix. The standard mix was used to make seven standard substock solutions of varying concentrations ranging from high to low using serial dilution in a 50:50 water-to-methanol solution. Similarly, the quality control (QC) mix was used to prepare three quality control substock solutions of varying concentrations to represent low, mid, and high points on the standard curve using serial dilution in a 50:50 water-to-methanol solution. The resultant standard and QC substocks of varying concentrations were diluted with a 50:50 water-methanol solution and aliquoted into vials as standard curve and QC stock solutions for use during sample preparation. The concentrations of the 7-point calibration curve and QC solutions are tabulated in Table 4. The 7-point curve serves the purpose of calibrating at wide-ranging concentrations of cortisol and cortisone, and the three QCs confirm the accuracy of the standard curve at low, mid, and high concentrations. The stock and substock preparation and dilution sheets made for this project have been attached to Appendix I.

Powdered forms of internal standards (IS) cortisol-d4 (labeled hydrocortisone-d4, an alternate name for cortisol) and cortisone-d8 were purchased from Cayman Chemical and Cambridge Isotope Laboratories, respectively. Based on the solubility of both substances, they were diluted to concentrations of 0.5 mg/mL and 1.0 mg/mL in methanol. A combined secondary stock solution of these internal standards was prepared using methanol. The resultant internal standard concentrations of cortisol and cortisone were 10.00 µg/mL and 75.00 µg/mL. The difference in concentrations can be attributed to the response of cortisol and cortisone that was detected in preliminary tests that were run, which was also suggested by the studies referenced in the review. The internal standard concentrations without these dilution factors would have been significantly high, which in turn would have made it challenging to calculate lower concentrations in saliva. The preparation volumes for the internal standard can be found attached with the standards and QCs sheets in Appendix I.

Table 4: Concentrations of combined standards and quality control solutions

Standards and QCs	Cortisol Concentration (ng/mL)	Cortisone Concentration (ng/mL)
Standard 1	0.75	0.75
Standard 2	1.50	1.50
Standard 3	4.50	4.50
Standard 4	7.50	7.50
Standard 5	15.00	15.00
Standard 6	37.50	37.50
Standard 7	75.00	75.00
Quality Control 1	1.05	1.05
Quality Control 2	15.00	15.00
Quality Control 3	60.00	60.00

Sample Preparation

Samples, standards, and QCs for this project were prepared with 1mL, 30 mg sorbent mass Strata-X Reversed Phase SPE cartridges manufactured by Phenomenex to ensure analyte isolation for testing purposes. This brand of cartridges is like the Oasis HLB cartridges referenced previously and uses a non-polar sorbent mass. A vacuum manifold was initially tested but provided inconsistent results for the sample volumes used in this project. Therefore, the samples were prepared manually by using a cartridge-compatible syringe and adapter, which was purchased through Phenomenex. The protocol for processing samples was developed using Phenomenex's Sample Preparation Catalog. The SPE cartridges were first conditioned with 1 mL of methanol with a flow rate of 4-5 mL/minute, which is the same as 4-5 drops/second. They were then equilibrated using 1 mL of water at the same flow rate. The cartridges were loaded with 250 µL of standard, QC, or saliva sample and 10 µL of IS mix. This combination was allowed to flow through the sorbent mass under the influence of gravity. This was done to avoid the rapid flow through the cartridge when inserting the adapter and syringe, as the pressure from insertion was sufficient to elute such small volumes. After the sample/standard/QC passed through the sorbent mass, the cartridge was washed with 1 mL of a 95:5 water-methanol wash solution. This step removed any contaminants that may have been present and isolated cortisol and cortisone in the silica mass. The wash step was performed at a flow rate of 1-2 mL/minute to ensure that the solution had enough time to pick up any unnecessary molecules. The cartridge was then eluted with a 0.1% formic acid solution in methanol. This solution was added in increments of 125 µL twice and allowed to elute under gravity to guarantee that all the cortisol and cortisone present in the cartridge were in the final solution. 200 µL of eluted methanol solution was transferred to an autosampler vial and loaded on the LC-MS/MS autosampler tray.

Method Validation

Once the sample preparation steps were developed, method validation for this study was performed. These validations were constructed based on the protocol of In-House Labs LLC, which is accredited by the Commission on Office Laboratory Accreditation (COLA). However, some validation studies were adapted to fit this project as this method differed from the instrumentation and methodologies used at the testing lab.

This method was tested for its accuracy, linearity, intra-assay results, inter-day results, LOD/LOQ, carryover, SPE cartridge recovery, matrix effects, and retention time stability. The accuracy of this method was validated by preparing and running three sets of each quality control (QC1, QC2, QC3) to generate at least 8 data points for each solution. The mean of the data for each QC was compared to its expected concentration listed in Table 4. Each quality control met the acceptance criterion of ± 20% accuracy from expected concentrations. The intra-assay tests were run by preparing a 30 ng/mL cortisol and cortisone concentration solution and injecting the same sample 25 times. The 25 data points gathered were required to have peak areas within ≤ 15% CV (coefficient of variation), and this criterion was satisfied. The inter-day data points were obtained from the accuracy study, and the concentrations were required to meet an acceptance criterion of ≤ 15% CV. The calculated concentrations of all three QCs passed the required metric. The linearity of this method was tested by preparing and running three sets of each standard. The mean of the data points for each standard had to meet the acceptance criteria of ± 20% expected concentration, and the individual points had to be within ≤ 15% CV, both of which were achieved.

The limit of detection (LOD) for this method was tested by setting the concentration of standard 1 (0.75 ng/mL) as the limit of quantitation (LOQ), and the lowest limit needed to be at least ≤ 25% of LOQ. To determine the LOD, two sets of eight samples of concentrations around the LOQ were prepared. Each set of samples was run three times for six data points per sample, and the most common concentration distinguishable from the baseline was deemed to be accepted as the LOD. From the data gathered, 0.1875 ng/mL was found to be the most frequent concentration, which was 25% of LOQ for both analytes.

To assess for potential carryover from high positives, five sets of standard 7 (concentration: 75 ng/mL) and the solvent blanks immediately following the standard were used from the linearity study. For carryover to be present, the solvent blank peak area had to be ≤ 5% of standard 7 peak area. No visibly distinguishable peaks were found, and the acceptance criterion for carryover was met.

To ensure sufficient recovery of cortisol and cortisone from the SPE cartridges, both saliva and 50:50 water-methanol were tested to account for the participant samples and the standards and QCs, respectively. Eight samples for each matrix were prepared, and four of them were combined with higher and lower cortisol and cortisone concentration solutions before passing them through the SPE cartridges. The other four samples were spiked with the same higher and lower concentration solutions after they were prepared using the cartridges. Each sample was run on the LCMS in triplicates to obtain 6 data points in total. The concentrations of the two types of samples for each matrix were compared using the percentage difference of the average concentrations and were required to meet ≥ 80% recovery. This was met for all tested samples in this validation study.

Since there was a difference in the matrices used for calibration curve solutions, which were run before participant samples, a matrix effects validation study was performed to ensure that there were no significant differences between the concentrations calculated in saliva samples that were tested against calibration curve solutions prepared using 50:50 water-methanol. Two sources of saliva samples collected using the Salivettes were split into five aliquots for a high-spike and low-spike set. Both sets of samples were spiked with known concentrations of cortisol and cortisone with an expected resultant concentration required to be met within ± 20% difference. This criterion was met for both samples within a concentration difference of ± 10% for both analytes.

The retention time stability of cortisol and cortisone was determined using a combination of samples from the other validation studies. Fifty data points were used for each analyte and required to have a standard deviation of less than 0.1. This acceptance criterion was satisfied for cortisol and cortisone with the same standard deviation across both assays. A validation study document that combines the results of all these tests has been attached to Appendix J.

While validation tests specific to the use and storage of the Cortisol Salivette were not performed, the evaluation report generated by the manufacturer and the storage requirements determined by Quest Diagnostics, as mentioned above, provided sufficient information for it to be acceptable for use. Tests run by Sarstedt determined that the average saliva recovery volume was 1.1 ± 0.3 mL in healthy adults. Other tests run to ensure cortisol recovery rates with low sample volumes were shown to have close to 100% recovery. While this collection device has not been tested for cortisone specifically, its reliable use in other studies for the analyte provides context for its use in this study. The evaluation report of the device has been appended to Appendix K.

Sample Testing

After the validation studies were completed and the required acceptance criteria were met, samples were prepared using the preparation steps described above. Analyst, a mass spectrometry software developed by AB Sciex, was used to build and set the parameters and to build batches for all the method validation and sample preparation runs. The data generated from running the samples were then seamlessly imported to MultiQuant, which is a quantitation software also made by AB Sciex used to build and assess calibration curves, analyze chromatograms generated, and gather the calculated concentrations of the samples tested.

The metrics of acceptance for calibration curves and quality controls run with each batch of participant samples were derived from the testing practices of In-House Labs LLC. The calibration curves for transition 1 (T1) of each analyte, when tested with each batch of participant samples, were required to meet $R^2 \leq 0.990$ under the Wagner regression model, which is tailored specifically for data generated from mass spectrometry. The calculated concentration of each standard and quality control had to be within ± 20% of the expected concentration. If any standard was being flagged outside of those requirements, the curve was adjusted accordingly by removing no more than two standards in one run. The rules regarding removing two standards were as follows: combinations of standard 1 and standard 7, standard 1 and standard 2, and standard 6 and standard 7 could not be removed to fix a curve. If any QC was outside of ± 40% of the expected concentration even after adjusting the curve by removing standards, the run would be marked as failed. While a standard or two may have been removed to adjust the curve in the different batches run, the possible issues of QC failure were not encountered while testing participant samples for this study. A reference calibration curve for cortisol and cortisone has been described in Figure 3.

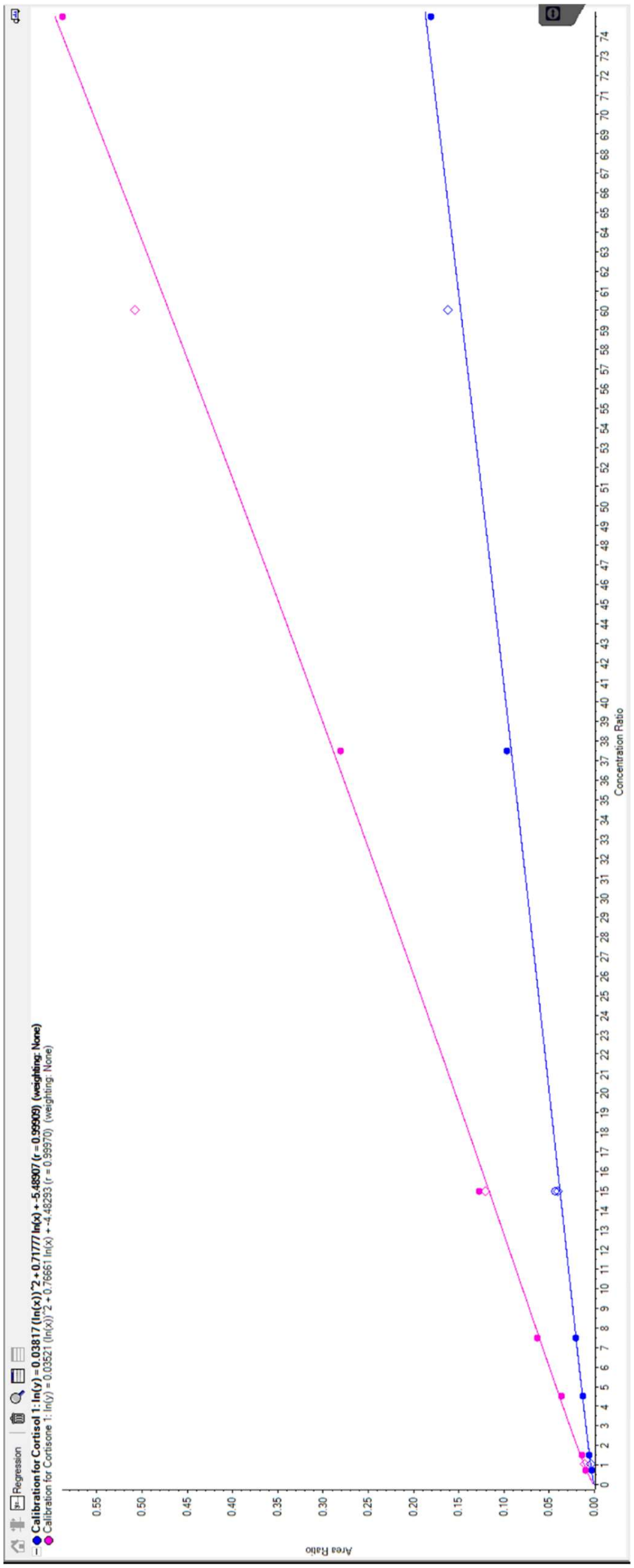


Figure 3: Calibration curve of cortisol and cortisone from a participant sample run.

The LC column, which was used to build the method, run the validation studies, and test the participant samples, stopped functioning optimally after 1000 injections, which were built up because of preliminary testing. A new column of the same variant as referenced above was purchased and replaced, and there was an expected change in retention times compared to the old column. Cortisol eluted at a retention time of 1.05 seconds, and cortisone eluted at 1.02 seconds. The same retention times applied to both transitions and the internal standards.

The testing lab uses concentrations generated from T1 as reportable values for their clinical testing, and the same principle was used to test the samples for this study. While both transitions needed to show peaks for the analyte to be present, the concentration values generated from T1 for each participant sample were used to analyze variations between pre-intervention and post-intervention samples.

Results

All the statistical analyses for this study were performed with the assistance of Project Scholar, a program that helps students at the University of Central Oklahoma with data analysis for research projects. For the purposes of testing the hypothesis of this study, the differences in stress measurements from pre-intervention and post-intervention samples were calculated by subtracting the former from the latter. The data points for the survey responses and the cortisol/cortisone concentrations were analyzed using paired t-tests (t-values in figures below) and ANOVA (F-values in figures below) where appropriate. A chi-square test was performed for one survey question regarding participant interest in continuing to practice these interventions after the study. The p-value for these measurements was set at 0.05 for the study, and test values equal to or less than that were considered statistically significant. Statistical analysis for this study was performed in R 4.4.1.

Participants

Based on the sample size of participants in the Stanford study of breathwork and meditation referenced previously, the goal was to recruit 120 participants. This goal also helped account for potential dropouts from the study. The pool of participants was sourced from the active forensic science workforce in the Oklahoma City metro area and Tulsa and students who declared their major to be forensic science at the University of Central Oklahoma at the time of recruitment. At the end of participant intake to start the intervention period, the data consisted of measurements from 126 subjects who were either students, professionals, or both. Table 5 describes the breakdown of students by their classification, and Table 6 the breakdown of professionals by their profession. The total number of participants in both tables adds up to 136 because it includes 10 participants who were both students and professionals at the time of participation.

Table 5: Number of student participants based on classification

Student Classification	Number of participants
Freshman	26
Sophomore	12
Junior	25
Senior	18
Graduate Student	21
Total	102

Table 6: Number of professional participants based on profession

Profession	Number of participants
Crime Scene Investigator	8
Chemist	3
Toxicologist	3
Digital Analyst	4
Latent Print Examiner	3
DNA Technician	1
Death Investigator	4
Forensic Pathologist	5
Communications Specialist	2
Criminal Intelligence Analyst	1
Total	34

Within the total population, 94 participants ($n_s = 94$) completed the pre-intervention and post-intervention survey, and 92 participants ($n_c = 92$) had salivary cortisol and cortisone measurements from before and after the intervention period. The difference in numbers can be attributed to the participants not returning to provide their second set of samples, incomplete survey responses, low saliva sample volumes, and scheduling conflicts that led to only survey responses being collected for a few participants.

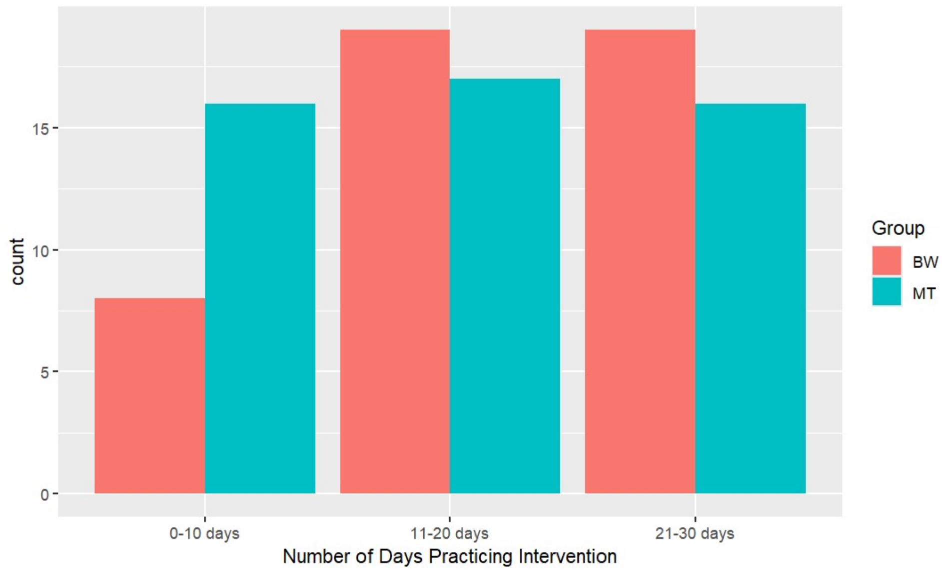
Survey Responses

Within the population of participants who completed the study, 45 (47.9%) participants were assigned to breathwork-cyclic sighing, and 49 (52.1%) participants were in the meditation group. Regarding occupation, 65 were students, and 29 were either professionals or both students and professionals. Twenty identified as male, 69 as female, and 5 people identified as other or preferred not to specify their gender identity. The age breakdown for the participants was as follows: 63.8% of them were between 18-24, 18.1% were between 25-34, and 18.1% were 35 or older.

The pre-intervention survey asked participants about their exercise habits; 32 did not exercise at all, 45 exercised 1-3 times a week, and 17 exercised at least 4 days a week. Regarding the subjects' religious/spiritual practices, 56.4% mentioned that they were religious and/or spiritual, 38.3% were not religious or spiritual, and 5.3% preferred not to say. When asked if they had practiced breathwork and meditation previously, 38 answered that they had done the former, and 39 responded that they had performed the latter exercise prior to participating in this project.

The post-intervention survey asked participants the number of days they practiced their allotted exercise; 24 people did it for 0-10 days, 35 for 11-20 days, and 35 for 21-30 days. The figure below graphically represents the breakdown of participants against the number of days the exercises were practiced. Seventy-five subjects meditated or performed cyclic sighing for the prescribed amount of time on each day that they practiced the intervention techniques, whereas 19 did it for varying time periods on either extreme of the prescribed time set for this study. At the end of the second survey, participants were asked if they would practice their assigned exercise after the study; 45.7% responded with maybe or no, and 54.3% said yes.

Figure 4: Breakdown of participants practicing their intervention by number of days



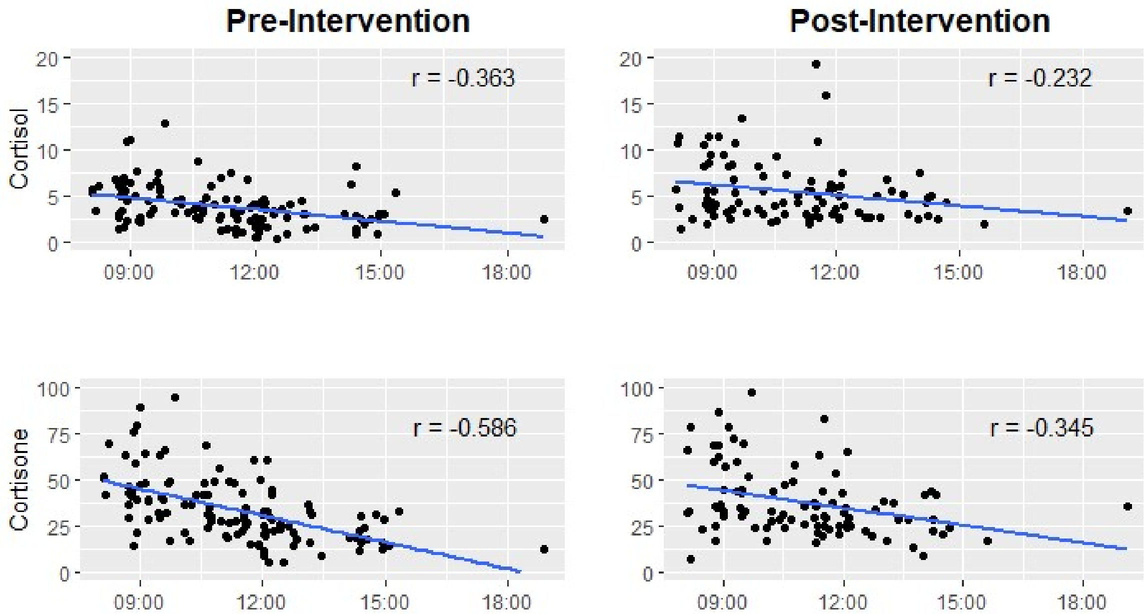
Salivary Cortisol/Cortisone Measurements

As mentioned above, 92 participants had saliva samples that provided data points to observe the changes in salivary cortisol and cortisone levels. The difference between the survey data and the salivary measurements of two of the participants was caused due to low sample volume and led to each intervention subset losing one participant. The two people were under the subset of professional/both student and professional, and one of them identified as male, and the other preferred not to specify their gender. This subtraction affected the demographic subsets in the following manner: 31 subjects did not exercise, 44 exercised 1-3 times a week, and 17 exercised at least 4 days a week. Regarding the subjects' religious/spiritual practices, 53 stated they were religious and/or spiritual, 34 were not religious or spiritual, and 5 preferred not to say. Regarding their previous experience with either intervention technique, 37 answered that they had done breathwork exercises previously, and 38 had meditated before.

In the post-intervention survey, the breakdown of days the subjects practiced their exercise was as follows: 22 people did it for 0-10 days, 35 for 11-20 days, and 35 for 21-30 days; 74 subjects meditated or performed cyclic sighing for the prescribed amount of time on each day that they practiced the intervention techniques, whereas 18 did it for varying time periods. The reduction in samples for salivary measurements subsequently subtracted two participants who stated that they would practice their intervention technique after the research study.

The correlation of cortisol and cortisone to the time of day that each of the samples was collected was plotted to check if the diurnal pattern of concentration changes was consistent with the suggestions of current literature. These concentrations were shown to follow the expected pattern for the pre-intervention and post-intervention sets of samples. The correlation values are negative on each graph, which suggests an inverse relationship between the time of day and the concentrations of these salivary assays. These plots include salivary concentrations of all participants who provided samples for this study, notwithstanding their completion of the prescribed intervention period. Due to the high variability in the concentrations of cortisol and cortisone for each participant, these r values are much closer to zero, which suggests a weak correlation between the two variables.

Figure 5: Correlation of salivary cortisol and cortisone concentrations relative to collection times



Summary Statistics

Among the 94 participants who provided both sets of samples, the survey scores for the General Well-Being Schedule (GWBS) before implementing the intervention showed that 3.2% were in the moderate distress category, whereas 96.8% were in the severe distress category. After the intervention, one participant scored in the moderate distress category, whereas 98.9% scored under the severe distress category. The mean of the pre-intervention scores for all participants was calculated to be 52.8 with a standard deviation of ± 5.1, and the values post-intervention were 53.3 with a standard deviation of ± 4.5. Within the intervention techniques, subjects in the breathwork group had average pre-intervention scores of 53.1 ± 4.5 and average post-intervention scores of 52.9 ± 5.0. Similarly, the meditation subset had pre-intervention scores of 52.5 ± 5.7 and post-intervention scores of 53.7 ± 4.1. There was limited variation in the scores depending on factors such as the number of days the interventions were practiced, the exercise habits of the participants, their religious/spiritual leanings, or their previous experiences with breathwork and meditation.

Figure 6 contains a detailed breakdown of the summary statistics generated from the General Well-Being Schedule.

The Perceived Stress Scale scores obtained prior to the intervention period placed 18 participants in the low-stress category, 70 in the moderate-stress category, and 6 in the high-stress category. After 30 days of practicing breathwork or meditation, 28 participants were categorized to be in the low-stress category, 63 in the moderate-stress group, and 3 in the high-stress group. The average participant score before the intervention began was 18.3 ± 5.4, and the same post-intervention was 15.9 ± 5.3. The mean of the pre-intervention participant scores for those who meditated was 17.8 ± 5.5. The post-intervention scores within the same subset averaged out to be 15.3 ± 5.5. Regarding the breathwork population, the averages before and after the intervention month were determined to be 18.9 ± 5.3 and 16.5 ± 5.1, respectively. While the drop in scores was not large, the mean difference between the pre-intervention and post-intervention scores reflected a reduction, and the same was reflected across different variables, some of which include age, gender, days practiced, exercise habits, religious/spiritual inclinations, and prior experience with breathwork and meditation, to list a few. The summary statistics obtained from the PSS scores are elaborated on in Figure 7.

Figure 6: Summary Statistics of General Well-Being Schedule

		Pre-Intervention		Post-Intervention		Difference	
Variable	***n***	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
All Subjects	94	52.8	5.1	53.3	4.5	0.5	5.2
Intervention Group							
Breathwork	45	53.1	4.5	52.9	5.0	-0.2	5.3
Meditation	49	52.5	5.7	53.7	4.1	1.2	5.1
Gender							
Male	20	53.1	4.4	53.5	4.0	0.4	4.9
Female	69	52.6	5.2	53.2	4.7	0.6	5.4
Other/Prefer to not say	5	53.5	7.4	53.5	3.6	0.0	4.4
Age Group							
18-24 years	60	52.2	5.5	52.8	4.7	0.7	5.6
25-34 years	17	53.1	4.5	54.0	4.7	1.0	4.8
35+ years	17	54.8	3.8	54.2	3.6	-0.5	4.0
Race/Ethnicity							
White	68	52.7	4.6	52.9	4.5	0.2	5.2
Black	4	49.8	9.2	53.4	1.6	3.6	8.1
Native American	5	55.0	7.4	54.5	5.9	-0.5	3.7
Asian	3	49.7	2.9	56.8	3.5	7.2	3.8
Hispanic	10	54.5	4.4	53.9	5.0	-0.6	4.6
Other/Prefer to not say	4	52.5	8.4	54.1	5.1	1.6	3.4
Professional/Student							
Professional/Both	29	53.9	4.0	54.2	3.7	0.3	4.6
Student	65	52.3	5.5	52.9	4.8	0.6	5.5
Frequency of Exercise							
0 times/week	32	51.9	5.4	53.4	4.6	1.6	4.8
1-3 times/week	45	53.0	5.0	52.9	4.7	-0.2	4.8
4+ times/week	17	53.9	5.0	54.2	4.0	0.4	6.7
Religous/Spiritual							
Yes	53	52.6	4.9	53.3	4.5	0.8	5.3
No	36	52.9	5.7	53.0	4.7	0.1	5.2
Prefer to not say	5	54.3	3.5	54.9	2.1	0.6	4.7
Previously Practiced Breathwork							
Yes	38	51.7	5.3	53.0	4.7	1.3	6.2
No	56	53.5	4.9	53.5	4.4	0.0	4.4
Previously Practiced Meditation							
Yes	39	51.4	5.5	52.9	4.2	1.5	6.0
No	55	53.8	4.7	53.6	4.8	-0.2	4.5
Number of Days Practicing Intervention							
0-10 days	24	54.4	5.2	54.6	3.1	0.2	4.3
11-20 days	35	52.0	5.6	53.9	4.6	1.9	5.6
21-30 days	35	52.5	4.5	51.8	4.9	-0.7	5.1
Length of Intervention							
Prescribed Time	75	53.0	4.6	52.9	4.4	-0.1	4.8
Other	19	51.9	6.9	54.7	4.8	2.9	6.2
Time of Day Intervention Practiced							
Daytime	12	52.8	3.7	54.8	2.5	2.0	4.1
Nighttime	45	52.7	4.9	52.9	4.5	0.3	5.0
Both	37	52.9	5.9	53.3	5.0	0.3	5.7
Used Guided Soundtracks							
Yes	21	53.8	5.5	53.6	4.4	-0.2	3.8
No	51	52.6	4.4	53.0	4.1	0.4	5.0
Other	22	52.2	6.3	53.7	5.5	1.5	6.7
Will Use Intervention Technique in the Future							
Maybe/No	43	53.2	4.5	54.3	4.3	1.1	5.2
Yes	51	52.4	5.6	52.4	4.5	0.0	5.2

Figure 7: Summary Statistics of Perceived Stress Scale

		Pre-Intervention		Post-Intervention		Difference	
Variable	*n*	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
All Subjects	94	18.3	5.4	15.9	5.3	-2.4	5.0
Intervention Group							
Breathwork	45	18.9	5.3	16.5	5.1	-2.4	5.0
Meditation	49	17.8	5.5	15.3	5.5	-2.5	5.0
Gender							
Male	20	14.3	3.7	11.4	3.9	-2.9	3.4
Female	69	19.3	5.4	17.0	5.1	-2.3	5.5
Other/Prefer to not say	5	20.6	5.1	17.6	4.5	-3.0	2.3
Age Group							
18-24 years	60	18.6	5.1	15.8	4.7	-2.7	4.8
25-34 years	17	19.2	5.5	16.6	7.4	-2.6	6.4
35+ years	17	16.6	6.4	15.2	5.2	-1.4	4.1
Race/Ethnicity							
White	68	18.5	5.7	15.9	5.4	-2.6	5.5
Black	4	17.2	3.1	13.2	4.6	-4.0	5.1
Native American	5	19.6	7.6	17.8	4.4	-1.8	4.1
Asian	3	19.0	6.6	17.3	8.7	-1.7	2.3
Hispanic	10	15.9	4.0	15.3	5.8	-0.6	3.0
Other/Prefer to not say	4	19.8	1.7	15.8	1.5	-4.0	1.4
Professional/Student							
Professional/Both	29	16.4	5.2	15.7	4.9	-0.8	4.0
Student	65	19.2	5.3	16.0	5.6	-3.2	5.2
Frequency of Exercise							
0 times/week	32	17.9	4.8	16.1	6.1	-1.8	5.4
1-3 times/week	45	19.2	5.5	16.7	4.3	-2.6	4.9
4+ times/week	17	16.6	6.0	13.3	5.7	-3.4	4.4
Religous/Spiritual							
Yes	53	17.8	4.8	15.7	5.3	-2.1	4.2
No	36	18.8	6.0	15.6	5.4	-3.2	6.1
Prefer to not say	5	20.4	7.4	19.2	4.4	-1.2	4.7
Previously Practiced Breathwork							
Yes	38	20.3	5.7	16.3	4.7	-3.9	4.1
No	56	17.0	4.9	15.6	5.8	-1.4	5.3
Previously Practiced Meditation							
Yes	39	19.6	5.7	16.1	4.8	-3.5	4.7
No	55	17.4	5.1	15.7	5.7	-1.7	5.1
Number of Days Practicing Intervention							
0-10 days	24	18.7	6.2	16.9	6.0	-1.8	5.1
11-20 days	35	17.7	5.0	15.1	4.7	-2.6	4.9
21-30 days	35	18.7	5.4	16.0	5.5	-2.7	5.1
Length of Intervention							
Prescribed Time	75	17.8	4.8	15.7	5.1	-2.1	4.5
Other	19	20.2	7.3	16.4	6.2	-3.8	6.7
Time of Day Intervention Practiced							
Daytime	12	17.4	6.6	15.0	5.3	-2.4	3.7
Nighttime	45	19.1	4.9	15.9	5.9	-3.2	5.1
Both	37	17.6	5.7	16.1	4.7	-1.5	5.2
Used Guided Soundtracks							
Yes	21	18.8	4.9	14.8	5.3	-4.0	5.0
No	51	17.4	5.2	15.9	5.6	-1.5	5.1
Other	22	20.0	6.2	16.8	4.6	-3.2	4.5
Will Use Intervention Technique in the Future							
Maybe/No	43	17.9	5.5	16.5	5.5	-1.4	4.5
Yes	51	18.7	5.4	15.3	5.2	-3.4	5.3

The mean cortisol concentration before the intervention period for 92 participants was 4.1 ± 2.3 ng/mL, and after the intervention period was 5.4 ± 3.3 ng/mL. For those who practiced breathwork, the averages were 3.8 ± 2.3 ng/mL and 5.6 ± 3.3 ng/mL before and after the intervention period. Likewise, the cortisol concentrations for participants who meditated averaged 4.3 ± 2.4 ng/mL and 5.2 ± 3.2 ng/mL before and after the intervention period. The pre-intervention mean cortisone concentration in all subjects was calculated to be 35.7 ± 18.3 ng/mL, and the post-intervention mean concentration was 37.8 ± 18.6 ng/mL. For the breathwork subgroup, the pre-intervention and post-intervention mean cortisone concentration was 32.6 ± 17.0 ng/mL and 37.6 ± 18.0 ng/mL, respectively. The mean cortisone concentrations for the meditation group barely changed between either end of the one-month window, as the mean difference was - 0.5 ng/mL with a standard deviation of ± 15.0. The breakdown of these concentrations across the variables is displayed in Figures 8 and 9 for cortisol and cortisone, respectively.

As mentioned in the literature review, multiple factors affect the expression of cortisone in relation to cortisol in human saliva. As such, summary statistics specific to the ratio of cortisol to cortisone were also generated for analysis. The mean of the ratio of cortisol to cortisone in saliva was calculated to be 0.124 ± 0.069 before the intervention period and found to be 0.149 ± 0.067 after the intervention period. For the breathwork population, the mean of the ratios was 0.128 ± 0.072 and 0.157 ± 0.070, and for the meditation population, the said values were 0.121 ± 0.067 and 0.141 ± 0.064 before and after the intervention period. There was no noticeable pattern of change in these concentrations with respect to variables such as exercise habits, previous experiences with these interventions, and religious/spiritual influences. These ratios are shown in Figure 10.

Figure 8: Summary Statistics of Cortisol Concentrations

Variable	*n*	Pre-Intervention mean	Pre-Intervention sd	Post-Intervention mean	Post-Intervention sd	Difference mean	Difference sd
All Subjects	92	4.1	2.3	5.4	3.3	1.3	2.9
Intervention Group							
Breathwork	44	3.8	2.3	5.6	3.3	1.8	2.9
Meditation	48	4.3	2.4	5.2	3.2	0.9	2.9
Gender							
Male	19	3.9	2.2	4.6	3.0	0.6	3.2
Female	69	4.0	2.4	5.6	3.3	1.5	2.9
Other/Prefer to not say	4	4.9	2.0	6.1	4.0	1.2	2.4
Age Group							
18-24 years	59	4.1	2.5	5.7	3.3	1.7	2.8
25-34 years	16	4.4	2.0	5.0	2.9	0.6	3.6
35+ years	17	3.8	2.3	4.7	3.5	0.9	2.7
Race/Ethnicity							
White	67	3.9	2.1	5.4	3.4	1.5	3.0
Black	4	4.3	4.6	4.1	1.4	-0.2	5.2
Native American	4	3.8	1.9	5.2	2.4	1.4	1.7
Asian	2	4.6	2.2	5.0	2.5	0.4	4.7
Hispanic	11	3.9	2.2	4.8	2.6	0.9	2.3
Other/Prefer to not say	4	7.4	3.9	8.7	4.6	1.3	2.5
Professional/Student							
Professional/Both	27	4.0	2.2	4.3	3.1	0.4	2.8
Student	65	4.1	2.4	5.8	3.3	1.7	2.9
Frequency of Exercise							
0 times/week	31	4.1	2.4	5.7	3.0	1.7	2.8
1-3 times/week	44	4.2	2.5	5.7	3.7	1.4	3.3
4+ times/week	17	3.6	1.8	4.1	2.1	0.5	1.9
Religous/Spiritual							
Yes	53	3.8	1.9	5.0	2.8	1.2	2.8
No	34	4.3	2.9	6.0	3.9	1.6	3.2
Prefer to not say	5	4.9	2.5	5.3	3.9	0.4	1.9
Previously Practiced Breathwork							
Yes	37	4.3	2.4	5.6	3.0	1.3	3.0
No	55	3.9	2.3	5.3	3.4	1.4	2.9
Previously Practiced Meditation							
Yes	38	4.4	2.9	5.2	3.0	0.8	2.6
No	54	3.8	1.8	5.5	3.5	1.7	3.1
Number of Days Practicing Intervention							
0-10 days	22	4.2	2.2	5.0	3.4	0.8	3.2
11-20 days	35	4.0	2.4	5.4	3.5	1.5	2.9
21-30 days	35	4.0	2.4	5.5	3.0	1.5	2.8
Length of Intervention							
Prescribed Time	74	3.8	2.2	5.2	3.1	1.4	2.8
Other	18	5.0	2.8	6.1	3.8	1.1	3.4
Time of Day Intervention Practiced							
Daytime	11	3.6	2.1	5.0	3.9	1.3	3.5
Nighttime	45	4.0	2.5	5.2	3.0	1.2	3.0
Both	36	4.3	2.2	5.8	3.5	1.5	2.8
Used Guided Soundtracks							
Yes	20	4.2	2.9	5.6	3.2	1.4	2.0
No	50	3.8	2.0	5.3	3.4	1.5	3.3
Other	22	4.5	2.5	5.4	3.2	1.0	2.9
Will Use Intervention Technique in the Future							
Maybe/No	43	3.8	2.1	5.3	3.1	1.5	2.6
Yes	49	4.3	2.5	5.4	3.4	1.1	3.2

Figure 9: Summary Statistics of Cortisone Concentrations

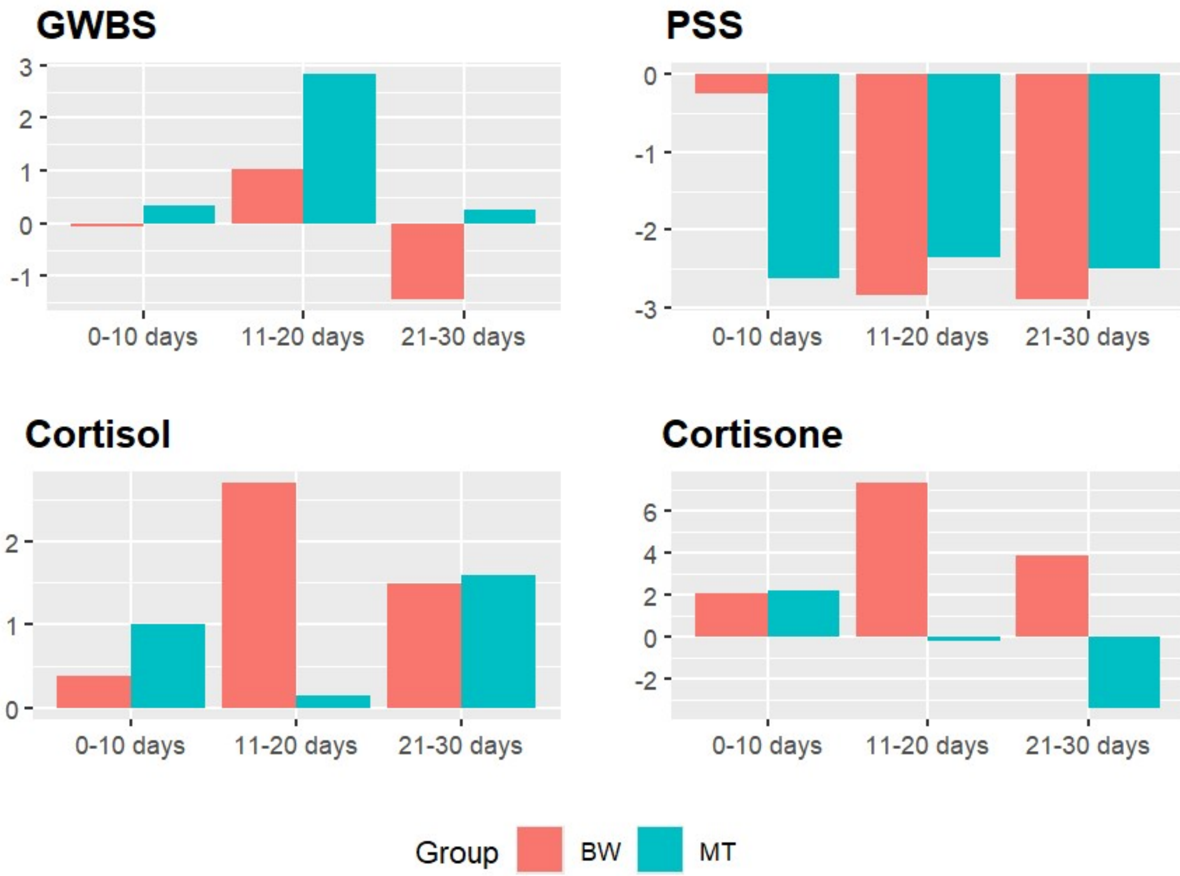
		Pre-Intervention		Post-Intervention		Difference	
Variable	*n*	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
All Subjects	92	35.7	18.3	37.8	18.6	2.1	17.5
Intervention Group							
Breathwork	44	32.6	17.0	37.6	18.0	5.0	19.6
Meditation	48	38.5	19.1	38.0	19.2	-0.5	15.0
Gender							
Male	19	35.7	16.7	32.9	10.4	-2.8	14.1
Female	69	36.0	19.2	39.3	20.0	3.3	18.0
Other/Prefer to not say	4	30.5	9.6	35.8	23.0	5.3	22.7
Age Group							
18-24 years	59	37.3	18.9	39.8	18.8	2.5	15.2
25-34 years	16	31.3	17.0	36.6	22.3	5.3	24.7
35+ years	17	34.2	17.6	32.0	12.4	-2.2	17.3
Race/Ethnicity							
White	67	34.4	17.0	38.1	18.0	3.8	17.4
Black	4	41.4	35.8	36.1	18.4	-5.2	19.5
Native American	4	31.6	12.8	32.1	12.5	0.5	19.0
Asian	2	48.5	21.6	40.6	4.1	-7.9	17.5
Hispanic	11	35.8	15.4	31.2	17.1	-4.6	16.3
Other/Prefer to not say	4	49.1	30.6	56.7	33.6	7.6	21.3
Professional/Student							
Professional/Both	27	33.8	17.5	31.3	13.8	-2.5	15.2
Student	65	36.4	18.7	40.5	19.7	4.1	18.1
Frequency of Exercise							
0 times/week	31	38.2	19.0	40.1	21.0	1.9	16.0
1-3 times/week	44	35.2	18.9	39.0	18.7	3.8	19.7
4+ times/week	17	32.3	15.5	30.7	11.4	-1.6	13.9
Religous/Spiritual							
Yes	53	35.9	17.1	36.4	16.6	0.4	15.9
No	34	34.8	20.4	39.9	20.6	5.1	17.8
Prefer to not say	5	38.9	18.7	38.6	26.5	-0.2	30.5
Previously Practiced Breathwork							
Yes	37	35.8	17.6	40.3	19.7	4.5	20.4
No	55	35.6	18.9	36.1	17.7	0.6	15.2
Previously Practiced Meditation							
Yes	38	38.2	21.8	39.7	20.6	1.5	18.4
No	54	33.9	15.3	36.5	17.0	2.6	17.0
Number of Days Practicing Intervention							
0-10 days	22	34.8	21.2	37.0	17.9	2.2	19.0
11-20 days	35	33.9	15.1	37.6	17.1	3.7	12.3
21-30 days	35	38.0	19.5	38.6	20.7	0.6	20.9
Length of Intervention							
Prescribed Time	74	35.0	16.9	37.0	18.3	2.0	18.2
Other	18	38.5	23.5	41.0	20.0	2.5	14.6
Time of Day Intervention Practiced							
Daytime	11	31.2	19.1	34.8	15.3	3.6	20.9
Nighttime	45	36.2	18.4	39.2	20.3	3.0	18.7
Both	36	36.4	18.2	37.0	17.4	0.6	15.1
Used Guided Soundtracks							
Yes	20	37.5	21.3	42.3	20.4	4.8	18.8
No	50	32.9	16.4	35.3	16.6	2.4	18.3
Other	22	40.3	19.1	39.5	20.7	-0.9	14.3
Will Use Intervention Technique in the Future							
Maybe/No	43	33.4	17.3	37.8	18.5	4.4	14.5
Yes	49	37.6	19.1	37.8	18.8	0.1	19.7

Figure 10: Summary Statistics of Concentration Ratio of Cortisol to Cortisone

		Pre-Intervention		Post-Intervention		Difference	
Variable	*n*	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
All Subjects	92	0.124	0.069	0.149	0.067	0.025	0.084
Intervention Group							
Breathwork	44	0.128	0.072	0.157	0.070	0.030	0.069
Meditation	48	0.121	0.067	0.141	0.064	0.020	0.095
Gender							
Male	19	0.120	0.076	0.142	0.079	0.022	0.102
Female	69	0.123	0.069	0.149	0.062	0.026	0.076
Other/Prefer to not say	4	0.164	0.052	0.178	0.105	0.014	0.130
Age Group							
18-24 years	59	0.113	0.054	0.150	0.068	0.037	0.064
25-34 years	16	0.162	0.095	0.149	0.049	-0.014	0.104
35+ years	17	0.127	0.080	0.144	0.081	0.018	0.113
Race/Ethnicity							
White	67	0.125	0.072	0.147	0.070	0.021	0.083
Black	4	0.105	0.053	0.129	0.054	0.024	0.097
Native American	4	0.145	0.131	0.157	0.016	0.012	0.118
Asian	2	0.094	0.003	0.127	0.075	0.033	0.078
Hispanic	11	0.110	0.039	0.162	0.051	0.052	0.069
Other/Prefer to not say	4	0.159	0.051	0.173	0.112	0.015	0.132
Professional/Student							
Professional/Both	27	0.135	0.089	0.141	0.071	0.006	0.111
Student	65	0.120	0.060	0.152	0.066	0.032	0.069
Frequency of Exercise							
0 times/week	31	0.119	0.072	0.151	0.057	0.032	0.093
1-3 times/week	44	0.129	0.065	0.152	0.079	0.024	0.077
4+ times/week	17	0.123	0.078	0.137	0.051	0.013	0.085
Religous/Spiritual							
Yes	53	0.112	0.052	0.147	0.068	0.035	0.083
No	34	0.141	0.089	0.152	0.071	0.011	0.083
Prefer to not say	5	0.141	0.068	0.145	0.037	0.003	0.091
Previously Practiced Breathwork							
Yes	37	0.129	0.067	0.143	0.048	0.014	0.085
No	55	0.121	0.071	0.153	0.078	0.032	0.083
Previously Practiced Meditation							
Yes	38	0.120	0.057	0.142	0.060	0.022	0.083
No	54	0.127	0.077	0.154	0.072	0.027	0.084
Number of Days Practicing Intervention							
0-10 days	22	0.145	0.074	0.140	0.073	-0.004	0.113
11-20 days	35	0.124	0.082	0.150	0.077	0.026	0.083
21-30 days	35	0.111	0.049	0.153	0.054	0.042	0.054
Length of Intervention							
Prescribed Time	74	0.118	0.068	0.147	0.064	0.029	0.074
Other	18	0.150	0.072	0.155	0.082	0.005	0.117
Time of Day Intervention Practiced							
Daytime	11	0.131	0.078	0.148	0.097	0.016	0.134
Nighttime	45	0.116	0.063	0.137	0.046	0.021	0.070
Both	36	0.132	0.075	0.164	0.078	0.032	0.083
Used Guided Soundtracks							
Yes	20	0.118	0.072	0.137	0.062	0.019	0.087
No	50	0.131	0.072	0.158	0.077	0.027	0.087
Other	22	0.115	0.063	0.139	0.042	0.024	0.074
Will Use Intervention Technique in the Future							
Maybe/No	43	0.126	0.070	0.145	0.062	0.019	0.090
Yes	49	0.122	0.070	0.152	0.072	0.030	0.078

The mean change in each measure except cortisol-to-cortisone ratios was graphed against the number of days the participants practiced their randomly assigned interventions, as expressed in Figure 11. While the cortisol concentrations and the GWBS scores reflect some inconsistencies, the PSS scores and the cortisone concentrations show that the differences increase as the number of days each intervention was practiced increases. For the PSS scores, there is minimal change for those who practiced breathwork for 0-10 days. However, the mean decrease in scores is close to three points for those who practiced cyclic sighing for 11-20 days, and it stays the same for those who practiced for 21-30 days. For meditation, there is a positive change (mean decrease) of roughly 2.5 points among the subjects who meditated for 0-10 days, but the change seems to reduce to under 2.5 points for those who meditated for 11-20 days. The mean decrease is exactly 2.5 for those who meditated for 21-30 days. Regarding cortisone, as the number of days spent meditating increases, the positive change in the mean difference of the pre-intervention and post-intervention concentrations also increases. On the other hand, breathwork seems more inconsistent as the cortisone levels seem to negatively change (mean increase) between the populations that perform cyclic sighing for 0-10 days and 11-20 days. The mean concentrations seem to drop between those who have done their assigned breathwork exercise for 11-20 days and those who have done it for 21-30 days. The combination of these data points was not tested for statistical significance, but the emerging patterns are worth noting for future analysis.

Figure 11: Mean Changes in Measures by Number of Days Practicing the Intervention



Hypothesis Tests

With there being multiple variables that can impact the influence of breathwork and meditation on those who practice it, hypothesis tests were run on both students and professional participants by considering their exercise habits, religious/spiritual inclinations, number of days within the intervention period the assigned method was practiced, the time spent during each practice of the intervention method, and their previous experience of practicing either technique. Only two of these tests were statistically significant at the 0.05 level for the survey scores. Regarding the scores from the Perceived Stress Scale (PSS), students and professionals from both intervention groups who had previously practiced breathwork saw a mean decrease of 3.9 points compared to a mean decrease of 1.4 points among those who had not practiced breathwork before participating in this project ($p = 0.012$). Similarly, the mean decrease in PSS scores for students and professionals who had previously practiced meditation in comparison to those who had not meditated before this study derived a *p*-value of 0.072 ($t = -1.82$). The mean decrease in perceived stress for students within these subsets was significant, with a *p*-value of 0.032 ($t = -2.2$). The paired t-test of scores from the General Well-Being Schedule (GWBS) for the same subset of students showed a mean increase with $t = 1.9$ and $p = 0.066$. While not statistically significant, the data shows a decrease in GWBS scores for those who practiced the exercises for the prescribed amount of time. For all subjects of the study, the *p*-value was 0.065 ($t = -1.95$), and within students the *p*-value was 0.052 ($t = -2.09$). Figure 12 includes all the hypothesis tests performed using both survey questionnaires.

Figure 12: Hypothesis Tests for the Mean Difference in GWBS and PSS scores

	GWBS Scores		PSS Scores	
	test statistic	p-value	test statistic	p-value
Intervention Group				
All Subjects	$t = -1.3$	0.198	$t = 0.09$	0.931
Students	$t = -0.82$	0.413	$t = 0.3$	0.765
Professionals/Both	$t = -1.18$	0.248	$t = -0.19$	0.847
Gender				
All Subjects	$t = -0.17$	0.868	$t = -0.56$	0.58
Students	$t = -0.44$	0.67	$t = -0.47$	0.648
Professionals/Both	$t = 0.36$	0.721	$t = -1.72$	0.1
Age Group				
All Subjects	$F = 0.43$	0.649	$F = 0.49$	0.613
Students	$F = 0.03$	0.971	$F = 0.77$	0.468
Professionals/Both	$F = 0.58$	0.568	$F = 0.04$	0.961
Frequency of Exercise				
All Subjects	$F = 1.48$	0.215	$F = 0.48$	0.751
Students	$F = 1.37$	0.253	$F = 0.4$	0.808
Professionals/Both	$F = 0.43$	0.787	$F = 1.5$	0.234
Religious/Spiritual				
All Subjects	$t = 0.54$	0.588	$t = 0.98$	0.333
Students	$t = 0.27$	0.786	$t = 1.28$	0.207
Professionals/Both	$t = 0.63$	0.544	$t = -0.86$	0.402
Previously Practiced Breathwork				
All Subjects	$t = 1.13$	0.263	$t = -2.58$	0.012
Students	$t = 0.99$	0.325	$t = -1.75$	0.086
Professionals/Both	$t = 0.49$	0.631	$t = -1.82$	0.086
Previously Practiced Meditation				
All Subjects	$t = 1.46$	0.149	$t = -1.82$	0.072
Students	$t = 1.9$	0.066	$t = -2.2$	0.032
Professionals/Both	$t = -0.43$	0.67	$t = -0.49$	0.628
Number of Days Practicing Intervention				
All Subjects	$F = 2.27$	0.109	$F = 0.24$	0.785
Students	$F = 1.48$	0.235	$F = 0.24$	0.789
Professionals/Both	$F = 1.09$	0.351	$F = 0.74$	0.488
Length of Intervention				
All Subjects	$t = -1.95$	0.064	$t = 1.08$	0.291
Students	$t = -2.09$	0.052	$t = 1.02$	0.322
Professionals/Both	$t = 0.01$	0.994	$t = 0.17$	0.872
Time of Day Intervention Practiced				
All Subjects	$F = 0.6$	0.553	$F = 1.19$	0.309
Students	$F = 0.02$	0.984	$F = 0.91$	0.409
Professionals/Both	$F = 0.55$	0.584	$F = 0.38$	0.687

Note:
p-values less than 0.05 are highlighted in dark gray
p-values between 0.05 and 0.10 are highlighted in light gray

The mean differences in cortisol, cortisone, and the ratio of cortisol to cortisone were analyzed with the hypothesis tests, and all the data points that were statistically significant at the 0.05 level indicated an increase in concentrations. Variations in concentrations calculated among students of different age groups contributed to statistically significant data points. Cortisone concentrations of the saliva samples obtained from students saw a mean increase of 1.94 ng/mL among 18-24 year olds, 12.56 ng/mL among 25-34 year olds, and 28.92 ng/mL among those who were 35 years or older ($F = 3.39$, p-value = 0.040). The cortisol-to-cortisone ratio saw a significant change, with a mean increase of 0.04 among students aged 18-24 years, 0.03 for those aged 25-34 years, and a mean decrease of 0.13 among students 35+ years old ($F = 7.09$, p-value = 0.002). However, the mean values may not be accurate within this subset as these changes are affected by low sample size in the older age categories. There were 8 students between the ages of 25-34 and only 2 students who were 35 or older. One other score ranging between p-values of 0.05 and 0.10 was also within the age group variable, but accounts for all participants ($F = 2.46$, $p = 0.091$) has been highlighted in light gray in Figure 13, which includes the hypothesis tests performed using all three variations of salivary stress measurements.

Figure 13: Hypothesis Tests for the Mean Difference in Cortisol, Cortisone, and the Ratio of Cortisol to Cortisone

	Cortisol		Cortisone		Ratio	
	test statistic	*p*-value	test statistic	*p*-value	test statistic	*p*-value
Intervention Group						
All Subjects	$t = 1.5$	0.137	$t = 1.51$	0.135	$t = 0.53$	0.595
Students	$t = 1.66$	0.102	$t = 1.29$	0.201	$t = 0.67$	0.508
Professionals/Both	$t = 0.08$	0.939	$t = 0.63$	0.535	$t = 0.01$	0.991
Gender						
All Subjects	$t = -1.13$	0.267	$t = -1.57$	0.125	$t = -0.16$	0.876
Students	$t = -0.86$	0.408	$t = -0.33$	0.746	$t = -0.38$	0.715
Professionals/Both	$t = -0.06$	0.956	$t = -1.12$	0.274	$t = 0.42$	0.681
Age Group						
All Subjects	$F = 1.05$	0.353	$F = 0.79$	0.457	$F = 2.46$	0.091
Students	$F = 0.15$	0.863	$F = 3.39$	0.04	$F = 7.09$	0.002
Professionals/Both	$F = 1.23$	0.311	$F = 2.11$	0.143	$F = 2.02$	0.155
Frequency of Exercise						
All Subjects	$F = 0.77$	0.55	$F = 0.65$	0.627	$F = 0.75$	0.563
Students	$F = 0.51$	0.725	$F = 1.07$	0.377	$F = 1.11$	0.361
Professionals/Both	$F = 0.4$	0.806	$F = 0.43$	0.786	$F = 1.18$	0.349
Religious/Spiritual						
All Subjects	$t = -0.61$	0.542	$t = -1.25$	0.214	$t = 1.3$	0.198
Students	$t = -0.9$	0.375	$t = -0.6$	0.553	$t = 0.54$	0.592
Professionals/Both	$t = 0.72$	0.479	$t = -1.65$	0.113	$t = 1.56$	0.142
Previously Practiced Breathwork						
All Subjects	$t = -0.13$	0.897	$t = 1$	0.32	$t = -0.96$	0.342
Students	$t = 0.36$	0.719	$t = 1.24$	0.22	$t = -1.34$	0.186
Professionals/Both	$t = -1.04$	0.311	$t = -0.35$	0.732	$t = -0.13$	0.901
Previously Practiced Meditation						
All Subjects	$t = -1.38$	0.171	$t = -0.29$	0.776	$t = -0.29$	0.771
Students	$t = -1.13$	0.266	$t = 0.06$	0.956	$t = -0.86$	0.396
Professionals/Both	$t = -0.26$	0.801	$t = -0.23$	0.819	$t = 0.72$	0.481
Number of Days Practicing Intervention						
All Subjects	$F = 0.45$	0.637	$F = 0.27$	0.762	$F = 2.11$	0.127
Students	$F = 1.11$	0.336	$F = 0.21$	0.812	$F = 1.54$	0.222
Professionals/Both	$F = 0.09$	0.914	$F = 1.96$	0.162	$F = 0.96$	0.398
Length of Intervention						
All Subjects	$t = 0.29$	0.776	$t = -0.12$	0.909	$t = 0.83$	0.414
Students	$t = 1.1$	0.283	$t = 0.17$	0.866	$t = 1.51$	0.147
Professionals/Both	$t = -0.57$	0.607	$t = -0.25$	0.817	$t = -0.01$	0.992
Time of Day Intervention Practiced						
All Subjects	$F = 0.08$	0.927	$F = 0.22$	0.801	$F = 0.22$	0.802
Students	$F = 0.37$	0.69	$F = 0.3$	0.745	$F = 0.95$	0.395
Professionals/Both	$F = 0.42$	0.658	$F = 0.3$	0.739	$F = 1.27$	0.29

Note:
p-values less than 0.05 are highlighted in dark gray
p-values between 0.05 and 0.10 are highlighted in light gray

Discussion

This study sought to determine the viability of breathwork and meditation as intervention techniques for stress in the forensic science community. The measurements used to calculate changes in stress were psychological surveys and salivary cortisol and cortisone measurements. The General Well-Being Schedule (GWBS) and the Perceived Stress Scale (PSS) were combined on Qualtrics surveys with supporting demographic questions and administered to participants before the intervention and after thirty days of implementing the intervention. Saliva samples before and after the intervention period were collected from participants around the same time that both surveys were submitted. The scoring metrics of each questionnaire were used to categorize the changes in perceived stress and inner well-being, whereas measurements of salivary cortisol and cortisone gathered from testing samples using liquid chromatography-tandem mass spectrometry provided a hormonal perspective on the variation in stress levels between the two ends of the intervention period. Out of the participants who completed the study by providing both sets of samples, 47.8% were randomly assigned to the breathwork-cyclic sighing group, and 52.2% were assigned to the meditation group.

Statistical Analysis

As referenced in the results section above, the hypothesis tests for data obtained from this participant pool were performed using paired t-tests and ANOVA tests where necessary. These analysis methods were able to describe statistically significant changes among the subjects with the applied external variables. However, with the repeated measures in this study that were tested against multiple groups, it was initially thought that a multiple regression or a mixed-effects model would have been an effective statistical test. These methods would have accounted for various groups simultaneously compared to the tabulations used. The use of the current approach came down to two factors. The first factor is the number of research questions posed relative to the hypothesis of this study, and the second factor is the study's sample size in relation to the research questions posed at the time of statistical analysis.

In the first case, the aim was to assess the change in stress levels after breathwork and meditation were implemented in the forensic science community. However, to understand the influence of external variables such as exercise habits, religious/spiritual influences, previous experience practicing meditation/breathwork, the number of days spent practicing the intervention, the time spent during each intervention practice session, and the time of day that the intervention was practiced, the changes in stress levels of the participants that fell into each of these groups needed to be tested. This connects to the second factor of the sample size relative to these research questions. The variables of each group caused the sample size to be broken up into smaller groups, which was not conducive for use in the other models. The smaller sample sizes due to multiple variable groups being inserted into the analysis make it harder to analyze these data points for statistical significance with multiple regression/mixed-effect models when compared to their use with the statistical tests applied for this study. Therefore, the tests used were suitable for the data gathered for this study.

The external variable groups, such as exercise, religious/spiritual leanings, and previous experience with interventions, when statistically tested relative to the influence of breathwork and meditation in this study, can profoundly impact how one may experience stress. The influence of physical exercise on stress and mental health has been researched extensively and shown to have positive benefits. In the context of religion/spiritual practices, these are a source of grounding, faith, and acceptance of daily outcomes for many people in the world, which in turn can considerably affect their outlook toward stress. Analyzing the frequency of practice during the intervention period, the minutes spent practicing the methods during each session, and the time of day that they were practicing the methods play a role in the reduction of stress as well. The assumption prior to testing was that the higher the frequency of practice, the better the impact of breathwork/meditation. Similarly, the minutes spent practicing during each session can affect the data obtained from everyone. In terms of the time of day that a participant practiced these interventions, meditation practices can often be affected by fatigue, and practicing at nighttime or before bedtime can lead to someone falling asleep instead of meditating, which is counter-productive to experiencing the act of meditation. The latter variable and its subsequent impact are based on anecdotal evidence and may not have much bearing in a scientific argument unless tested. Lastly, previous experiences with practicing either intervention can also significantly impact one's relation to stress, and it was deemed necessary to consider that for statistical analysis. Assessing and combining these variables using paired t-tests and ANOVA matrices within this population was conducive to identifying important variables affecting stress responses.

Survey Responses

The scores tabulated from the Well-Being Schedule did not make for a favorable reading when compared to the Perceived Stress Scale. That could be attributed to two errors in importing the questions to the Qualtrics survey, which affected scoring for the specific subsets of questions. The first missing item was question 11, and to statistically normalize the error, responses to this question were imputed by averaging the other items (questions #1 and #6) that belonged to the same positive well-being subset. The other error arose in building the Likert scale for questions #15-18, as the source survey is scored on a 0-10 scale, and these questions were built on a 1-10 scale on the Qualtrics survey. The results gathered from the GWBS appeared to show increased distress scores among participants, as the mean difference between the pre-intervention and post-intervention scores increased. However, the change in the number of participants in the moderate distress and severe distress categories was considerably minute within the sample size.

The errors in transferring the survey may have affected the validity of the scores by a small margin. However, the corrections should normalize the scores tabulated from the responses submitted by the participants. The data from the GWBS could be an indicator of the general distress that subjects experienced during the period that they participated in this study. The lack of statistically significant change in any variable groups hints at their state not changing drastically for better or slightly deteriorating in some cases over the period of a month that they participated in the study. The scores are a determinant of one's subjective state of personal well-being, and the influence of external stressors that may have been experienced by participants during the study could be a contributing factor as well. The latter inference is made from conversations with participants during the second sample collection meetings, wherein they would mention certain things that may have affected their stress levels and their concern for how their numbers may look in this study. However, a marked decrease in the perceived stress of participants was observed from the data gathered.

This study used the 10-question variant of the Perceived Stress Scale, which showed decreased mean differences between the pre-intervention and post-intervention scores. It could be inferred that the perception of stress for all subsets and tested variables noticed a drop. However, only two subsets showed statistically significant changes with p-values < 0.05. Students and forensic science professionals who had practiced breathwork before participating in this study were shown to have a substantial reduction in scores. Students who had previously meditated before participating in this study also had a significant reduction in survey scores. There was a decrease in perceived stress levels among professionals who had meditated prior to taking part in this study, but the p-value was 0.072. When the mean change in PSS scores was compared to the number of days the intervention was practiced, the scores of those who practiced breathwork were shown to continuously reduce as the number of days spent performing cyclic sighing increased. Regarding meditation on the same plot, those who practiced the intervention for 0-10 of the thirty days had a higher decrease in their mean scores. The mean decrease between subjects who meditated for 11-20 days, and 21-30 days remained the same. The reduction in stress perception ascertains that both intervention techniques positively impacted the populations tested. The summary statistics implied that both meditation and cyclic sighing had about the same decrease in mean scores, with them being -2.5 ± 5.0 and -2.4 ± 5.0, respectively.

Analyzing the data from both questionnaires against each other makes for interesting reading as they suggest an improvement in the participants' response to stressors, but their inner personal state did not change in the same period. Distress in this context was on the higher end for participants on either side of the intervention period. This could suggest that the stressors in their lives have not changed or that they may have experienced new stressors, but their reaction to those stressors has changed by practicing these intervention techniques. This was anecdotally confirmed by a few participants who shared their experiences of practicing these exercises and their own opinions of their reactions to certain stressors that occurred during the intervention period. While the anecdotes of the participants alone do not prove the hypothesis to be true, it does show some signs that the exercises have positively affected some subjects over a period of 30 days. The data does show that students who practiced the exercise assigned to them (either breathwork or meditation) for the prescribed time showed a reduction in their GWBS, which was not statistically significant, but the p-value was 0.052. However, when compared to other hypotheses tests run, this may be lower in consideration as the sample size variations may have generated means that were not accurate.

Another contributor to consider in these survey responses is the influence of memory errors and cognitive and response biases. Literature shows that self-report measures in psychological surveys are dependent on the participant's willingness to be honest and their insight on the questions they are being asked when completing a survey. It is possible that a participant may have lied in some of their responses to minimize any negative feelings (Abbott & Lack, 2020). However, with the surveys being anonymous and not directly tied to their identity, the likelihood of participants lying may be low as there is minimal exposure to any negative judgments that may arise in a social construct.

Salivary Cortisol/Cortisone Measurements

The concentrations of cortisol and cortisone obtained from the saliva samples tested did not really show any statistically significant trends that were parallel with the hypothesis of this study. The data points that did show statistical significance were reflective of concentration increases in cortisone. Not much can be read into that significance as the mean may not be accurate due to the lower sample size within the age group subset of students. The measurements gathered were quite irregular and elevated in most cases compared to concentrations in samples provided at the start of the intervention period. When this project was conceptualized, the pre-intervention sample was set as the baseline for each participant, and the post-intervention sample was expected to drop in cortisol concentration. However, as the method development literature reviews were conducted, it was understood that cortisone would be in higher volumes in saliva compared to cortisol. It was also understood that the enzymatic activity in saliva would affect the amount of cortisone present relative to cortisol. As referenced in the review sections specific to these assays, endocrinologists have studied cortisone in saliva enough to know that it is reflective of cortisol in human blood serum.

Cortisone was added to the testing protocol after understanding its importance in saliva, and the method was developed to determine these concentrations together. However, the data obtained, and the current literature indicate a lack of studies that assess the relationship between salivary cortisone, enzyme 11β-HSD-2, and the stress response at large. It is known that an external stressor triggers bodily reactions in the nervous system to release cortisol into the bloodstream. With the expectation of lesser cortisol being secreted into the bloodstream because of meditation and breathwork, one could assume that the amount of salivary cortisone in that instance would also be reduced. In this case, with the numbers increasing in most cases, it is safe to assume that enzymatic activity is partial towards generating more of the inactive cortisone from the excess of cortisol formed. However, the difference between acute stress, chronic stress, and its expression on 11β-HSD-2 varies. A study performed on pregnant rats suggested that 11β-HSD-2 in placental tissues produces more cortisone when exposed to acute stressors, but the same enzyme drastically loses its ability to convert cortisol when under chronic stress. The damage caused by chronic stress on the enzyme reduces its ability to create cortisone in situations of acute stress (Welberg et al., 2005). The conversion in this study and in the human body protects tissues from being damaged by increased exposure to cortisol. While not much stock can be placed on comparing pregnant rats to forensic science professionals and students, understanding the behavior of the enzyme to some extent helps contextualize the relationship between the two analytes and the results obtained from testing participant samples.

Another consideration to be made with the numbers calculated for cortisol is that when the concentrations got lower than 1-2 ng/mL, there was considerably low intensity for both transitions of the analyte on the chromatograms. When concentrations dropped to 0.5 ng/mL, finding distinguishable peaks to calculate concentrations accurately was challenging. Low intensity, matrix effect, and instrument sensitivity in salivary matrices are major contributors to this issue. However, not too many samples were in that range, so only a few samples may have been affected. However, the cortisone numbers generally helped account for that, as there were no intensity issues in observing peaks and calculating concentrations for the cortisone analyte.

The trend in cortisone concentrations dropping over time for those who practiced meditation in Figure 13 should certainly be investigated in future studies, specifically regarding analyzing the direct effects of stress on cortisone before and after interventions such as breathwork and meditation. With many variables affecting the oxidative enzyme in each participant, it is hard to establish any cause and effect with this specific trend. A better case can be made for this argument once the literature and evidence become extensive. This is specific to testing methods that may use saliva for testing, as cortisone is readily available compared to cortisol in this matrix.

The stability of samples and the collection times relative to the study are other important factors to break down, as this could have been one of the many contributing factors to the irregularities noticed in concentration. As mentioned under the methods section, a buffer of seven days was placed from the time that participants had to meet the researcher to provide their second set of samples. This was mainly done to eliminate scheduling conflicts that arose due to the collection days falling on weekends or during days when participants would not be available to meet. Additionally, a timeframe of one hour was set in terms of collection times matching or being close to the hour of the time that the first sample was provided. Only two participants provided saliva samples outside of the one-hour buffer by a difference of another hour; these samples were included at the time of testing as the numbers were expected to drop due to the diurnal pattern of cortisol concentration changing in the body. Those two numbers within a population size of 90+ participants could not have drastically changed the mean concentrations calculated. In regards to sample and assay stability, cortisol collected using salivettes was found to be stable for six months when frozen and 21 days when refrigerated (*Cortisol, LC/MS, Saliva | Test Detail | Quest Diagnostics*, n.d.). The samples were split into three batches for testing, and each batch was thawed, centrifuged, refrigerated, and tested within 24-48 hours of being pulled out of the freezer. Hence, the samples could not have degraded dramatically to affect the concentrations calculated.

The ratio of cortisol to cortisone was used as a measure of change to assess and run hypothesis tests because of the ratio being individual to each participant. The studies referenced in the literature review suggested that cortisone was higher in concentration than cortisol by a factor of six in saliva samples. However, that ratio was not the same in this study, as the average of the ratios suggests that the factor is closer to eight in pre-intervention samples and about seven in post-intervention samples. The likely cause for these ratios not adhering to the suggestions of the literature is the considerably larger sample sizes, as the articles that mention this ratio have tested 50 or fewer samples when compared to the 92 of this study. None of the data obtained from the ratios was statistically significant, so any erroneous data points generated from ratio inconsistencies were not influential on the conclusions made about the outcome of the interventions in this study.

Part of the bodily response and secretion of cortisol is associated with situational stressors. One influential factor considered to be a contributor was the situational stressors of exams for students and casework for professionals, which may have affected participant cortisol/cortisone data in this study. There were instances during the study when meeting dates for students, and professionals were around times that they had exams or were actively working on a case when they stepped aside to provide a sample. One suggestion was that those situations may have caused an increase in post-intervention concentrations. However, in this study we cannot assess the impact of these external factors because the cortisol and cortisone concentrations were not collected and measured daily and analyzed for any possible trends. It may be imperative that future research takes the influence of these stressors into account when analyzing the impact of these interventions on specific populations.

Another argument could be made that the data obtained from the subjects may compute more with the General Well-Being Schedule in comparison to the Perceived Stress Scale. In the context of understanding hormonal responses versus psychological responses to the implementation of these intervention techniques in the 30-day period, it could be argued that the interventions were only successful in changing the perceptions of stress among participants. Whereas their internal state, both hormonally and psychologically, did not change relative to the intervention period.

Intervention Techniques

Balban et al. (2023) compared meditation, cyclic sighing, and two other breathwork exercises to study changes in mood, anxiety, and physiological arousal when practiced for five minutes every day for a month. In that study, cyclic sighing had the most positive changes in metrics, and meditation had the least. The tweak of comparing fifteen minutes of meditation practice against five minutes of cyclic sighing over thirty days in this study appears to make the changes in the mean difference of PSS scores similar. Within the populations that had statistically significant changes, the participant pool of students and professionals who had previously practiced breathwork included subjects who were in both intervention groups in this study. Similarly, the pool of students who had previously practiced meditation and had statistically significant changes in their perceived stress levels also included students who were in both intervention groups in this study. By accounting for previous experience with these stress mitigation methods, it can be inferred that having previous experience with these exercises helped those participants notice considerable changes in their perception of stress. Despite that, their GWBS scores did not have much positive change, and their previous experience with either intervention technique was not helpful in reducing distress during their participation in this research study. This adds credence to the fact that both breathwork and meditation have a learning curve associated with them. If a comparison to learning algebra were to be made in this instance, someone who has practiced algebra for thirty days would be less proficient than someone who has had much more experience with answering questions using the quadratic equation. Similarly, an individual who has more experience with cyclic sighing or meditation in their everyday life may benefit from greater stress reduction.

One other comparative factor that was tested by tweaking the practice times of the interventions was the efficiency of each method relative to their benefits. Breathwork and meditation are two different exercises with the benefits they offer, apart from stress management, being very different. As mentioned in the review, the former is a method of breath manipulation, whereas the latter is a focus and awareness-driven exercise. Since the time spent on practicing cyclic sighing was less than that spent meditating, it could be inferred that the former is more efficient in improving reactions to stressors than the latter, as the changes were about the same within this population.

Data obtained from the survey responses also provides some insight into implementing these exercises into forensic science curricula to arm students with healthier coping mechanisms to combat workplace stressors before they join the workforce. With students benefiting from practicing both exercises for prolonged periods of time, it may be helpful to make them part of academic work by introducing these as avenues for improving feelings of stress. The data generated from this study in the context of students is more specific to stressors from being a college student and is a far cry from what the professionals experience in the field. However, forensic science courses have the tendency to be graphic compared to most other coursework on a college campus. As these courses build the foundations of this discipline in students, said foundations could also be informed with teachings pertaining to well-being.

Conclusions

This research study aimed to analyze the impact of breathwork and meditation on the stress levels of the forensic science community by measuring stress using psychological and hormonal metrics. The implementation of the Perceived Stress Scale and the General Well-Being Schedule suggests that participants underwent a change in their response to stressors, but their subjective state of well-being and distress did not change much, and it even increased in some cases. The cortisol and cortisone concentrations analyzed for this study did not show positive change.

The only positive statistically significant ($p < 0.05$) data points inferred that those who had practiced breathwork and meditation previously had noticed changes in their perceptions of stress because of the responses provided to the Perceived Stress Scale questionnaire. From the mean changes in stress perception, it was deduced that both intervention techniques performed similarly in having a positive impact. This evidence can help the forensic science workforce move towards using these healthy coping mechanisms. A breathwork exercise that could help a crime scene investigator manage their stress within five minutes is quite advantageous in scenarios when they may need it the most. The same would apply to every discipline and position within the forensic science workforce as a means of equipping practitioners with coping mechanisms and subsequently helping them to make decisions that are not influenced by stress. In saying that, the response-specific improvements directly caused by breathwork or meditation within the population of forensic science practitioners may need to be tested by future studies to obtain greater certainty on the influence of these exercises in the decision-making process within this population.

In the context of helping students build healthy coping mechanisms before they get into the field, the data shows both interventions to be helpful in reducing perceptions of stress. While students are affected by college-based stressors, forensic science courses have the tendency to be graphic compared to most other coursework on a college campus. As these courses build the foundations of this discipline in students, said foundations could also be informed with well-being teachings, especially in the day and age when people are generally struggling with mental health issues.

The data from this study is an indicator that more studies need to be performed to determine the use of salivary cortisone to measure stress in human beings. This would need to be combined with developing a larger understanding of how stressors affect enzymatic activity in the matrix. Once there is considerable validity to using cortisone for stress, assessing it against meditation and breathwork to understand its impact on the body could also be tested by researchers. In the context of the forensic workforce, a study that assesses the impact of these interventions for more than 30 days would also be pivotal to noticing a significant improvement in cortisol and cortisone levels and the sense of inner well-being. While there are studies out there that have already proven these intervention methods to positively impact these measurements, forensic practitioners may need to be assessed for longer periods. This could provide more insight into how these interventions impact stress and could benefit the forensic science community. Given the prevalence of mental health issues and the additional stressors inherent in forensic work, it is imperative to identify and implement strategies to alleviate these challenges.

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Appendices

Appendix A: Participant Informed Consent Form

UNIVERSITY OF CENTRAL OKLAHOMA
INFORMED CONSENT FORM

Research Project Title: Determining the Viability of Breathwork and Meditation as Intervention Techniques for Stress in the Forensic Science Community

Researcher: Prateek Shetty, Forensic Science MS Student

You are being invited to participate in a graduate student's research project at the Forensic Science Institute of the University of Central Oklahoma. Participation is voluntary and not required.

A. Purpose of this research: Stressors in the field of forensic science are unique and seldom experienced in everyday life. Workplace stress in this field has recently been explored through research to suggest that there are a lot of underlying stressors affecting the workforce. However, not much research has been directed at finding intervention techniques to alleviate workplace stress within the forensic science community. Hence, the purpose of this study is to determine and compare the impact of breathwork (cyclic sighing) and meditation on the stress of those in the forensic science community. Analyzing the effects of these exercises on the current workforce will provide real-time information about their impact whereas assessing students studying forensic science could potentially provide a framework for implementing these practices as part of curricula to support the future workforce.

B. Procedures/treatments involved: The participants will be randomly assigned into two groups, breathwork-cyclic sighing, and meditation. Those in the former group will practice the exercise for five minutes every day during the 30-day intervention period whereas those in the latter group will meditate for fifteen minutes every day during the same intervention period. The stress levels of the participants before and after the implementation of the interventions will be tested using psychological surveys and salivary cortisol measurements. The survey responses and demographic information will be gathered through a compiled Qualtrics questionnaire. The psychological surveys to be administered are the Perceived Stress Scale (PSS) and the General Well-Being Schedule (GWBS). Salivary cortisol will be analyzed using the protocol dictated by Raff and Phillips (2019) and samples will be collected using the Sarstedt Cortisol Salivette. The salivette contains an absorbent roll that must be placed in the mouth for approximately two to three minutes. The participants will be directed to place the sponge back into the tube after the required time has elapsed. The researcher will then collect the tubes and centrifuge them to isolate the saliva for testing. The separated saliva will then be extracted from the tube to assess cortisol levels. Interested candidates will have to schedule one meeting with the researcher before and after the intervention period. The participants will be requested by the researcher to complete the survey and provide a saliva sample during said scheduled meeting times. Meetings between the researcher and the students and professionals to gather samples will be held in the Forensic Science Institute and external office spaces respectively. The Qualtrics forms will only be accessible to the researcher and the primary advisor. The saliva samples will be frozen and stored in the STEM building laboratory and tested in In-House Labs LLC, an external laboratory in the Oklahoma City area. The researcher will transport the samples in appropriate storage conditions between storage and testing locations. The variations in cortisol will be tested using a Liquid Chromatograph Tandem Mass Spectrometer (LC-MS/MS) and compared with survey responses to investigate and collate perceived stress levels and bodily reactions to these intervention methods.

C. Expected length of participation: 32 days (30-day intervention period + 1-day pre-intervention data collection + 1-day post-intervention data collection)

D. Potential benefits: Developing frameworks of stress intervention techniques for the present and future forensic science workforce.

E. Potential risks or discomforts: Participants will be asked to disclose their feelings about their overall health through some questions of the General Well-Being Schedule Questionnaire. They will also be asked to provide basic information about themselves and their previous history of practicing breathwork and/or meditation if any. This information is required to quantify any potential variables that may influence the stress levels of an individual.

F. Medical/mental health contact information: None

G. Contact information for researchers:
Researcher: Prateek Shetty
Email: pshetty@uco.edu
Phone: 405-413-9646

Advising Professor: Ms. Keisha Jones
Email: kjones78@uco.edu

H. Contact information for UCO IRB:
UCO Office of Research Integrity and Compliance, NUC 341
ORIC manager: 4059745479
Email: irb@uco.edu

I. Explanation of confidentiality and privacy: Participants will be requested to provide information about their age, ethnicity, exercise habits, and health. At the end of the first survey, their response sheets and saliva samples will be tagged with a unique ID which will only be known to the researcher and primary advisor. This ID will be used to identify participants' samples and data obtained from the testing performed. No names, email addresses, or any other personal information will be used to properly identify surveys and samples. Names and email addresses of participants will be collected separately to contact them to gather surveys and samples after the completion of the intervention period, but these will not be tied to their survey/sample submissions. The data obtained from surveys submitted will be stored on the laptop of the researcher through the Qualtrics database and the saliva samples collected will be stored in the STEM building laboratory. The data from the chemical analysis of saliva will be transferred from the external lab's system to the researcher's laptop. After the completion of this study, all the data and samples will be safely destroyed.

J. Assurance of voluntary participation: Participation in this research is voluntary. No participant will be forced against their will to take part in this study. The primary advisor of the project is a faculty member/instructor at the University of Central Oklahoma's Forensic Science Institute but will not be involved in recruiting students for this project. The instructor will not have access to any identifiable information about the student participants until after the class has ended. Choosing to participate or abstain from participating in this project will not affect a student's class grade. Employers of the professionals participating in this study will also not have access to the data gathered from the latter. Choosing to participate or abstain from participating in this project will not affect the employment of a professional.

AFFIRMATION BY RESEARCH SUBJECT

I hereby voluntarily agree to participate in the research project described above and further understand the explanations and descriptions of the research project. I also understand that there is no penalty for refusal to participate and that I am free to withdraw my consent and participation in this project at any time without being penalized. I acknowledge that I am at least 18 years old and that I have read and fully understood this Informed Consent Form. I sign it freely and voluntarily. If you would like a copy of this consent form, please select the "Print" command and save this page as a PDF file before proceeding.

- ○ I consent to participate in this study.
- ○ I don't consent to participate in this study.

Display This Question:

If UNIVERSITY OF CENTRAL OKLAHOMA INFORMED CONSENT FORM Research Project Title: Determining the... = I consent to participate in this study.

Name

____

Display This Question:

If UNIVERSITY OF CENTRAL OKLAHOMA INFORMED CONSENT FORM Research Project Title: Determining the... = I consent to participate in this study.

Signature

Display This Question:

If UNIVERSITY OF CENTRAL OKLAHOMA INFORMED CONSENT FORM Research Project Title: Determining the... = I consent to participate in this study.

*

Email Address

____

Display This Question:

If UNIVERSITY OF CENTRAL OKLAHOMA INFORMED CONSENT FORM Research Project Title: Determining the... = I consent to participate in this study.

*

Date

____

Display This Question:

If UNIVERSITY OF CENTRAL OKLAHOMA INFORMED CONSENT FORM Research Project Title: Determining the... = I don't consent to participate in this study.

Since you do not consent to take part in this study, please close this survey page. Thank you for your time!

Skip To: End of Survey If Since you do not consent to take part in this study, please close this survey page. Thank you for... Displayed

Display This Question:

If UNIVERSITY OF CENTRAL OKLAHOMA INFORMED CONSENT FORM Research Project Title: Determining the... = I consent to participate in this study.

Please meet the researcher to follow the next steps to participate in this study!

Appendix B: Pre-Intervention Survey Questions (Psychological Surveys excluded)

1. Please enter your unique ID

____

2. How old are you?

- ◯ 18-24 Years
- ◯ 25-34 Years
- ◯ 35-44 Years
- ◯ 45-54 Years
- ◯ 55+ Years

3. How do you describe yourself?

- ◯ Male
- ◯ Female
- ◯ Non-binary / third gender
- ◯ Prefer to self-describe ____
- ◯ Prefer not to say

4. What is your ethnicity?

- ◯ White/European/Caucasian
- ◯ Black/African American/African/Carribean
- ◯ Hispanic/Latino American
- ◯ American Indian/Native American or Alaska Native
- ◯ Asian/Asian American
- ◯ Native Hawaiian or Other Pacific Islander
- ◯ Other
- ◯ Prefer not to say

5. Are you a student or professional in the forensic science field?

- ◯ Student
- ◯ Professional
- ◯ Both

Display This Question:

If Are you a student or professional in the forensic science field? = Student

Or Are you a student or professional in the forensic science field? = Both

6. What is your classification?

- Undergraduate: Freshman
- Undergraduate: Sophomore
- Undergraduate: Junior
- Undergraduate: Senior
- Graduate: First Year
- Graduate: Second Year
- Graduate: Second Year +

Display This Question:

If Are you a student or professional in the forensic science field? = Professional

Or Are you a student or professional in the forensic science field? = Both

7. What is your job title?

- Crime Scene Investigator
- DNA Analyst
- Chemist
- Toxicologist
- Digital Analyst
- Other

Display This Question:

If What is your job title? = Other

8. Please specify your job title:

---

9. How often do you exercise in a week?

- ◯ Little to no exercise
- ◯ Exercise 1-3 times/week
- ◯ Exercise 4-5 times/week
- ◯ Daily exercise or intense exercise 3-4 times/week
- ◯ Intense exercise 6-7 times/week
- ◯ Very intense exercise daily

10. Are you a religious/spiritual person?

- ◯ Yes
- ◯ No
- ◯ Prefer not to say

11. Have you previously practiced any types of breathwork exercise?

- ◯ Yes
- ◯ No

12. Have you previously practiced any types of meditation?

○ Yes

○ No

Appendix C: Post-Intervention Survey Questions (Psychological Surveys excluded)

1. Please enter your unique ID

____

2. How often did you practice the intervention technique?

- ○ 0-5 days
- ○ 6-10 days
- ○ 11-15 days
- ○ 16-20 days
- ○ 21-25 days
- ○ 26-30 days

3. On average, how long did you practice the intervention technique in each sitting?

- ○ Prescribed time
- ○ Other ______________________________________

4. When did you practice the intervention technique?

- ○ Daytime
- ○ Nighttime
- ○ Both

5. Did you use the guided soundtracks?

- Yes
- No
- Other ____

Display This Question:

If Did you use the guided soundtracks? = Yes

6. Did you use the guided soundtracks with background music?

- Yes
- No
- Other ____

7. Will you practice the intervention technique outside of this study?

- Yes
- Maybe
- No

Appendix D: General Well-Being Schedule (Sourced from National Institutes of Health CDE Depository)

1. How have you been feeling in general? (DURING THE PAST MONTH)
- In excellent spirits
- In very good spirits
- In good spirits mostly
- I've been up and down in spirits a lot
- In low spirits mostly
- In very low spirits

2. Have you been bothered by nervousness or your nerves? (DURING THE PAST MONTH)
- Extremely so -- to the point where I could not work or take care of things
- Very much so
- Quite a bit
- Some -- enough to bother me
- A little
- Not at all

3. Have you been in firm control of your behavior, thoughts, emotions, or feelings? (DURING THE PAST MONTH)
- Yes, definitely so
- Yes, for the most part
- Generally so
- Not too well
- No, and I am somewhat disturbed
- No, and I am very disturbed

4. Have you felt so sad, discourages, hopeless, or had so many problems that you wondered if anything was worthwhile? (DURING THE PAST MONTH)
- Extremely so -- to the point that I have just about given up
- Very much so
- Quite a bit
- Some -- enough to bother me
- A little bit
- Not at all

5. Have you been under or felt you were under any strain, stress, or pressure? (DURING THE PAST MONTH)
- Yes -- almost more than I could bear or stand
- Yes -- quite a bit of pressure
- Yes -- some - more than usual
- Yes -- some - but about usual
- Yes - a little

- Not at all

6. How happy, satisfied, or pleased have you been with your personal life? (DURING THE PAST MONTH)

- Extremely happy -- could not have been more satisfied or pleased
- Very happy
- Fairly happy
- Satisfied -- pleased
- Somewhat dissatisfied
- Very dissatisfied

7. Have you had any reason to wonder if you were losing your mind, or losing control over the way you act, talk, think, feel, or of your memory? (DURING THE PAST MONTH)

- Not at all
- Only a little
- Some -- but not enough to be concerned or worried about
- Some and I have been a little concerned
- Some and I am quite concerned
- Yes, very much so and I am very concerned

8. Have you been anxious, worried, or upset? (DURING THE PAST MONTH)

- Extremely so -- to the point of being sick, or almost sick
- Very much so
- Quite a bit
- Some -- enough to bother me
- A little bit
- Not at all

9. Have you been waking up fresh and rested? (DURING THE PAST MONTH)

- Every day
- Most every day
- Fairly often
- Less than half the time
- Rarely
- None of the time

10. Have you been bothered by any illness, bodily disorder, pains, or fears about your health? (DURING THE PAST MONTH)

- All of the time
- Most of the time
- A good bit of the time
- Some of the time
- A little of the time
- None of the time

11. Have you felt down hearted and blue? (DURING THE PAST MONTH)
- All of the time
- Most of the time
- A good bit of the time
- Some of the time
- A little of the time
- None of the time

12. Have you been feeling emotionally stable and sure of yourself? (DURING THE PAST MONTH)
- All of the time
- Most of the time
- A good bit of the time
- Some of the time
- A little of the time
- None of the time

13. Have you felt tired, worn out, used-up, or exhausted? (DURING THE PAST MONTH)
- All of the time
- Most of the time
- A good bit of the time
- Some of the time
- A little of the time
- None of the time

14. How concerned or worried about your HEALTH have you been? (DURING THE PAST MONTH)
- 1 - Not concerned at all
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10 - Very concerned

15. How RELAXED or TENSE have you been? (DURING THE PAST MONTH)
- 1 - Very relaxed
- 2
- 3
- 4
- 5

- 6
- 7
- 8
- 9
- 10 - Very tense

16. How much ENERGY, PEP, and VITALITY have you felt? (DURING THE PAST MONTH)

- 1 - No energy at all, listless
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10 - Very energetic, dynamic

17. How DEPRESSED or CHEERFUL have you been? (DURING THE PAST MONTH)

- 1 - Very depressed
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10 - Very cheerful

Appendix E: Perceived Stress Scale (Sourced from the State of New Hampshire Employee Assistance Program Page)

The five-point Likert scale responses include:

0: never 1: almost never 2: sometimes 3: fairly often 4: very often

The 10 questions on the survey are as follows:

1. In the last month, how often have you been upset because of something that happened unexpectedly?
2. In the last month, how often have you felt that you were unable to control the important things in your life?
3. In the last month, how often have you felt nervous and stressed?
4. In the last month, how often have you felt confident about your ability to handle your personal problems?
5. In the last month, how often have you felt that things were going your way?
6. In the last month, how often have you found that you could not cope with all the things that you had to do?
7. In the last month, how often have you been able to control irritations in your life?
8. In the last month, how often have you felt that you were on top of things?
9. In the last month, how often have you been angered because of things that happened that were outside of your control?
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

Appendix F: Breathwork Information Sheet

University of Central Oklahoma
Forensic Science Institute

Research Project: Determining the Viability of Breathwork and Meditation as Intervention Techniques for Stress in the Forensic Science Community

Breathwork: Information Sheet

1. Please enter the Unique ID (SST-BW-XYZ) assigned to you when requested on the survey(s).
2. Please make sure to take a picture of your Unique ID and keep track of it until you have completed your participation in this research project.
3. You will receive an email from the researcher 20 days after you have submitted samples for this study (i.e., 20 days after today). This email will be about scheduling a time for you to return to provide samples for testing after the 30-day intervention period.
4. Please follow the QR code (or link) below to a Google Drive folder. This folder contains reference files to your exercise during the 30-day intervention period.
    a. The video labeled "Breathwork Guidelines" has instructions to be followed to practice this exercise. Please ensure to watch this video before you start performing the exercise the first time.
    b. The two audio tracks are guided soundtracks that can be used to assist your practice but are not required to be used.
        i. Guided Breathwork-With Soundtrack
        ii. Guided Breathwork-Without Soundtrack
5. For easier access to the drive folder every day, it is recommended that you bookmark the link. If needed, you may also download the guided soundtracks.
6. Any questions during the intervention period can be directed to Prateek Shetty (pshetty@uco.edu) or Keisha Jones (kjones78@uco.edu).

Link: https://tinyurl.com/FSISSTBW

Appendix G: Meditation Information Sheet

University of Central Oklahoma
Forensic Science Institute

Research Project: Determining the Viability of Breathwork and Meditation as Intervention Techniques for Stress in the Forensic Science Community

Meditation: Information Sheet

1. Please enter the Unique ID (SST-MT-XYZ) assigned to you when requested on the survey(s).
2. Please make sure to take a picture of your Unique ID and keep track of it until you have completed your participation in this research project.
3. You will receive an email from the researcher 20 days after you have submitted samples for this study (i.e., 20 days after today). This email will be about scheduling a time for you to return to provide samples for testing after the 30-day intervention period.
4. Please follow the QR code (or link) below to a Google Drive folder. This folder contains reference files to your exercise during the 30-day intervention period.
    a. The video labeled "Meditation Guidelines" has instructions to be followed to practice this exercise. Please ensure to watch this video before you start performing the exercise the first time.
    b. The two audio tracks are guided soundtracks that can be used to assist your practice but are not required to be used.
        i. Guided Meditation-With Soundtrack
        ii. Guided Meditation-Without Soundtrack
5. For easier access to the drive folder every day, it is recommended that you bookmark the link. If needed, you may also download the guided soundtracks.
6. Any questions during the intervention period can be directed to Prateek Shetty (pshetty@uco.edu) or Keisha Jones (kjones78@uco.edu).

Link: https://tinyurl.com/FSISSTMT

Appendix H: Cortisol Salivette Instructions for Use (Sarstedt)

Instructions for use

Salivette® Cortisol

Its crucial clinical significance makes cortisol one of the most important analytes that can be determined from saliva.
Saliva is optimally recovered using the **Salivette® Cortisol** (Art. No. 51.1534.500) specially designed for cortisol determination from saliva.

The **blue screw cap** visibly distinguishes the Salivette® Cortisol from the other swab versions.
The label provides sufficient writing space for important patient information and the collection time.

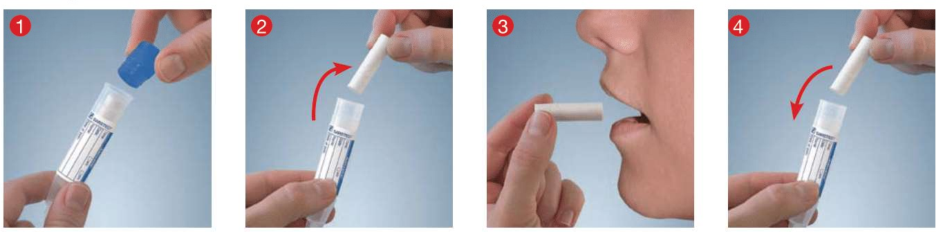
The cortisol recovery rate in the Salivette® Cortisol is proven to be almost 100 %, regardless of the cortisol concentration, the saliva volume or the measuring method applied.

Moreover, the dimensionally stable and **biocompatible synthetic swab** stands out for its superior absorption quality and virtually complete saliva recovery under the recommended centrifugation conditions.

A high saliva recovery rate after centrifugation is an important precondition for reliable analysis of even the smallest saliva volumes. Generally, a sample volume of just 50 µl or less is sufficient for cortisol determination. The average saliva volume usually recovered from adults is 1.1 ± 0.3 ml.

Salivette® – Instructions for use

The Salivette® Cortisol is designed to achieve precise analytical values from **small volumes** and/or samples with **very low cortisol levels.**



The patient removes the swab from the Salivette® (see Figs. 1 and 2) and places the swab in the mouth and chews it for about 60 seconds to stimulate salivation (see Fig. 3). Now the swab with the absorbed saliva is returned to the Salivette® (see Fig. 4)...

... and the stopper is replaced immediately (see Fig. 5). Centrifugation for 2 minutes at 1,000 x g yields a clear saliva sample in the conical tube (see Fig. 6). Particles and mucus strands are collected in the specially designed extended tip of the Salivette® tube (see Fig. 7). The closed insert containing the swab is then hygienically disposed. The saliva recovered can now be used for analysis (see Fig. 8).

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SARSTEDT

Appendix I: LCMS/MS Stock Preparation Sheets

Cortisol/Cortisone Method
Stock Preparation Sheets

1. Methanolic Stock Solutions

#	Standard	Stock Concentration (ug/mL)	Substock concentration (ug/mL)	Spike amount (uL)	Dilution amount (uL)	Final volume (uL)
1	Cortisol	1000	100	20	180	200
2	Cortisone	1000	100	20	180	200

2. Standard and Quality Control Secondary Stock Solutions

#	Standard	Stock Concentration (ug/mL)	Spike Volume (uL)	Resulting Concentration (ng/mL)	LOQ (ng/mL)
1	Cortisol	100	18	225	0.75
2	Cortisone	100	18	225	0.75
	Total spike volume (µL)		36		
	Methanol dilution volume (µL)		7964		
	Final volume (µL)		8000		

3. Standard Curve Substock Solutions

Standard Substock	Solution Used	Spike Volume (uL)	1:1 H20:MeOH Volume (uL)	Final Volume (uL)
Standard 7 Substock	Standard Mix	4158	0	4158
Standard 6 Substock	Standard 7 Substock	1958	1958	3916
Standard 5 Substock	Standard 6 Substock	1716	2574	4290
Standard 4 Substock	Standard 5 Substock	2090	2090	4180
Standard 3 Substock	Standard 4 Substock	1980	1320	3300
Standard 2 Substock	Standard 3 Substock	1100	2200	3300
Standard 1 Substock	Standard 2 Substock	1100	1100	2200
	1:1 H20:MeOH Volume (uL)		11242	

4. Standard Curve Stock Solutions

Standard	Solution Used	Spike Volume (uL)	1:1 H20:MeOH Volume (uL)	Final Volume (uL)
Standard 1	Standard 1 Substock	900	1800	2700
Standard 2	Standard 2 Substock	900	1800	2700
Standard 3	Standard 3 Substock	900	1800	2700
Standard 4	Standard 4 Substock	900	1800	2700
Standard 5	Standard 5 Substock	900	1800	2700
Standard 6	Standard 6 Substock	900	1800	2700
Standard 7	Standard 7 Substock	900	1800	2700
	1:1 H20:MeOH Volume (uL)		12600	

5. Quality Control Substock Solutions

QC Substock	Solution Used	Spike Volume (uL)	1:1 H20:MeOH Volume (uL)	Final Volume (uL)
QC 3 Substock	QC Mix	2232	558	2790
QC 2 Substock	QC 3 Substock	589	1767	2356
QC 1 Substock	QC 2 Substock	154	2046	2200
		1:1 H20:MeOH Volume (uL)	4371	

6. Quality Control Stock Solutions

QC	Solution Used	Spike Volume (uL)	1:1 H20:MeOH Volume (uL)	Final Volume (uL)
QC 1	QC 1 Substock	900	1800	2700
QC 2	QC 2 Substock	900	1800	2700
QC 3	QC 3 Substock	900	1800	2700
		1:1 H20:MeOH Volume (uL)	5400	

7. Internal Standard Solution

#	Internal Standard	Stock Concentration (µg/mL)	Spike Volume (µL)	Resulting Concentration (ug/mL)
1	Cortisol-D4	500	40	10
2	Cortisone-D8	1000	150	75
		Total spike volume (µL)	190	
		Methanol dilution volume (µL)	1810	
		Final volume (µL)	**2000**	

Appendix J: Cortisol/Cortisone Testing Method Validation

Cortisol/Cortisone Method
Method Validation Studies

- 1. Accuracy:
  - Prepared 3 sets of QC 1, QC 2, and QC3.
  - Ran each set of QC's 4 times each for at least 8 data points per QC.
  - The mean of the data for each QC was compared to the expected concentration.
  - *Acceptance criteria - ± 20% of expected concentration was met.*

QC 1:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	Accuracy
1	Cortisol	0.96	1.13	1.07	0.93	0.92	1.07	0.86	0.99	0.99	1.05	94%
2	Cortisone	1.13	1.07	1.06	0.98	0.96	0.99	0.97	0.96	1.01	1.05	97%

QC 2:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	Accuracy
1	Cortisol	15.51	15.87	14.82	14.76	15.26	14.68	16.27	16.07	15.41	15	103%
2	Cortisone	15.50	16.59	16.89	14.25	15.32	15.50	15.77	15.96	15.72	15	105%

QC 3:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	Accuracy
1	Cortisol	65.06	64.92	63.13	69.49	70.14	69.57	65.37	66.00	66.71	60	111%
2	Cortisone	66.37	65.76	68.33	70.07	68.60	69.76	68.21	68.95	68.26	60	114%

- 2. Intra-Assay:
  - Prepared 5 intra-assay samples and combined them in the same autosampler vial.
  - Ran the combined intra-assay sample for a total of 25 data points.
  - *Acceptance criteria - ≤ 15% CV of peak area was met*

Analyte	Cortisol	Cortisone
1	110538.1	80055.23
2	104181.2	72470.25
3	100339.7	71812.68
4	94770.57	69221.61
5	99563.72	68912.22
6	95112.49	68147.55
7	95066.34	68152.92
8	94640.31	66842.73
9	94935.11	66083.53
10	92477.07	68226.43
11	92741.54	68928.31
12	89525.37	67959.95
13	93532.79	67255.62
14	88156.77	68531.44
15	91380.63	65601.17
16	88600.56	67938.96
17	90013.63	69194.22
18	87562.13	67506.78
19	87888.65	64651.28
20	88950.89	67206.62
21	84934.11	64327.15
22	87238.41	65690
23	85052.08	61880.07
24	86612.83	66741.69
25	87127.58	63975.3
Mean	92437.7032	67892.5484
SD	6177.252023	3427.975931
CV%	7%	5%

- 3. Inter day:
  - Data from the "1. Accuracy" study was used as QCs were run on separate days.
  - *Acceptance criteria - ≤ 15% CV was met.*

QC 1:

#	Analyte	1	2	3	4	5	6	7	8	Mean	SD	CV%
1	Cortisol	0.96	1.13	1.07	0.93	0.92	1.07	0.86	0.99	0.99	0.09	9%
2	Cortisone	1.13	1.07	1.06	0.98	0.96	0.99	0.97	0.96	1.01	0.06	6%

QC 2:

#	Analyte	1	2	3	4	5	6	7	8	Mean	SD	CV%
1	Cortisol	15.51	15.87	14.82	14.76	15.26	14.68	16.27	16.07	15.41	0.62	4%
2	Cortisone	15.50	16.59	16.89	14.25	15.32	15.50	15.77	15.96	15.72	0.81	5%

QC 3:

#	Analyte	1	2	3	4	5	6	7	8	Mean	SD	CV%
1	Cortisol	65.06	64.92	63.13	69.49	70.14	69.57	65.37	66.00	66.71	2.64	4%
2	Cortisone	66.37	65.76	68.33	70.07	68.60	69.76	68.21	68.95	68.26	1.51	2%

- 4. Linearity:
  - Prepared 3 sets of standards 1-7.
  - Ran each set 3 times each for a total of 9 data points per standard.
  - The mean of the data for each standard was compared to the expected concentration.
  - *Acceptance criteria - ± 20% of expected concentration and ≤ 15% CV was met.*

Standard 1:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	SD	CV%	Accuracy
1	Cortisol	0.87	0.76	0.78	0.78	0.69	0.73	0.80	0.84	0.78	0.75	0.06	0.07	104%
2	Cortisone	0.77	0.83	0.85	0.70	0.81	0.80	0.72	0.73	0.78	0.75	0.06	0.07	104%

Standard 2:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	SD	CV%	Accuracy
1	Cortisol	1.27	1.58	1.47	1.48	1.76	1.58	1.44	1.33	1.49	1.5	0.15	0.10	99%
2	Cortisone	1.49	1.34	1.27	1.72	1.35	1.32	1.63	1.64	1.47	1.5	0.17	0.12	98%

Standard 3:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	SD	CV%	Accuracy
1	Cortisol	3.87	3.90	4.23	4.20	3.99	4.66	4.26	4.17	4.16	4.5	0.25	0.06	92%
2	Cortisone	4.00	3.87	4.16	4.15	4.34	4.97	4.44	4.03	4.25	4.5	0.34	0.08	94%

Standard 4:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	SD	CV%	Accuracy
1	Cortisol	7.95	7.43	7.50	7.14	7.58	7.01	6.92	7.49	7.38	7.5	0.34	0.05	98%
2	Cortisone	7.64	8.45	7.88	7.48	7.63	7.52	6.96	7.77	7.67	7.5	0.42	0.05	102%

Standard 5:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	SD	CV%	Accuracy
1	Cortisol	17.62	16.85	16.15	16.37	14.77	14.57	16.53	16.30	16.14	15	1.02	0.06	108%
2	Cortisone	16.99	16.55	16.99	14.77	15.57	14.17	15.30	15.08	15.68	15	1.06	0.07	105%

Standard 6:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	SD	CV%	Accuracy
1	Cortisol	36.20	37.30	37.09	39.02	40.33	40.27	41.11	40.04	38.92	37.5	1.82	0.05	104%
2	Cortisone	35.73	35.81	36.45	39.51	39.47	38.84	39.55	38.99	38.04	37.5	1.73	0.05	101%

Standard 7:

#	Analyte	1	2	3	4	5	6	7	8	Mean	Acutal	SD	CV%	Accuracy
1	Cortisol	73.00	73.38	74.08	71.99	72.42	72.85	69.50	70.30	72.19	75	1.56	0.02	96%
2	Cortisone	74.70	74.23	73.01	73.40	71.52	73.99	72.94	73.17	73.37	75	0.97	0.01	98%

- 5. LOD:
    - Prepared 2 sets of samples LOD 0.5%-100%.
    - Ran each set 3 times each for a total of 6 data points per LOD sample.
    - The most common concentration distinguishable from the baseline was accepted as the LOD.
    - *Acceptance criteria – ≤ 25% of LOQ was met.*

#	Analyte	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	% of LOQ	LOD (ng/mL)	LOQ (ng/mL)
1	Cortisol	25%	10%	50%	25%	25%	25%	25%	0.1875	0.75
2	Cortisone	50%	50%	25%	25%	25%	25%	25%	0.1875	0.75

- 6. Carryover:
    - 5 sets of standard 7 and the following solvent blanks ran in the "4. Linearity" study were used to determine if any carryover was present in solvent blanks.
    - *Acceptance criteria – solvent blank peak area ≤ 5% of standard 7 (S7) average peak area was met.*

#	Analyte	S7	Solvent	Carryover
1	Cortisol	283456.21	0	0%
2	Cortisone	227451.00	0	0%

- 7. Recovery of SPE cartridge:
  - Prepared the following:
    - 2 saliva samples spiked with a high-concentration solution before and after SPE elution.
    - 2 saliva samples spiked with a low-concentration solution before and after SPE elution.
    - 2 water/methanol samples spiked with a high-concentration solution before and after SPE elution.
    - 2 water/methanol samples spiked with a low-concentration solution before and after SPE elution.
  - Ran each set 3 times each for a total of 6 data points per set.
  - Samples spiked before SPE elution were compared to samples spiked after SPE elution at each concentration.
  - *Acceptance criteria - ≥ 80% recovery was met.*

Saliva samples (marked as oral fluid-OF):

High Spike OF samples														
#	Analyte	Spiked before SPE						Spiked after SPE						Recovery
		1	2	3	4	5	6	1	2	3	4	5	6	
1	Cortisol	30.55	32.36	30.05	30.22	27.98	26.00	31.47	34.89	32.94	31.14	33.86	33.76	89%
2	Cortisone	57.87	57.74	58.15	48.69	49.04	50.63	67.22	60.26	65.70	56.40	57.15	59.72	88%

Low Spike OF samples														
#	Analyte	Spiked before SPE						Spiked after SPE						Recovery
		1	2	3	4	5	6	1	2	3	4	5	6	
1	Cortisol	9.22	11.52	10.75	8.24	8.65	10.61	12.30	13.56	11.89	11.18	11.50	11.63	82%
2	Cortisone	38.62	38.00	36.78	29.18	27.05	27.36	44.35	45.62	44.18	40.68	35.44	35.16	80%

Average OF recovery		
#	Analyte	Average Recovery
1	Cortisol	86%
2	Cortisone	84%

Water/Methanol samples:

High Spike W/M samples														
#	Analyte	Spiked before SPE						Spiked after SPE						Recovery
		1	2	3	4	5	6	1	2	3	4	5	6	
1	Cortisol	24.74	25.00	25.30	25.77	25.96	24.09	28.19	27.31	26.62	27.23	26.53	26.20	93%
2	Cortisone	25.14	24.67	25.06	22.98	24.79	22.66	28.94	28.07	26.30	29.60	28.97	29.01	85%

Low Spike W/M samples														
#	Analyte	Spiked before SPE						Spiked after SPE						Recovery
		1	2	3	4	5	6	1	2	3	4	5	6	
1	Cortisol	4.96	4.67	4.67	4.49	4.45	4.85	5.56	5.75	5.09	5.50	5.16	5.20	87%
2	Cortisone	4.86	5.08	4.93	4.99	4.95	4.85	6.21	5.27	5.58	5.95	5.40	5.70	87%

Average W/M recovery		
#	Analyte	Average Recovery
1	Cortisol	90%
2	Cortisone	86%

- 8. Matrix effects:
  - Prepared the following:
    - 5 source saliva samples.
    - 5 high-concentration spiked saliva samples.
    - 5 low-concentration spiked saliva samples.
  - Each sample ran one time.
  - The percent difference between the mean sample concentrations and the expected concentration was calculated.
  - *Acceptance criteria - ± 20% percent difference from the expected concentration was met.*

Known concentrations of saliva samples (marked as oral fluid-OF):

OF samples						
Analyte	1	2	3	4	5	Mean
Cortisol	1.73	1.91	2.55	5.52	2.28	2.80
Cortisone	7.58	10.03	34.66	36.53	28.38	23.43

Concentration of samples after spiked with high-concentration solution:

High Spike OF samples						
Analyte	1	2	3	4	5	Mean
Cortisol	38.26	48.36	42.11	47.67	43.57	43.99
Cortisone	42.78	53.57	66.30	70.57	52.45	57.13

High-concentration spiked saliva samples with percent difference:

High Spike OF adjusted conc.								
Analyte	1	2	3	4	5	Mean	Expected	Difference
Cortisol	37.10	47.09	40.41	43.99	42.05	42.13	40.00	5%
Cortisone	37.72	46.88	43.18	46.21	33.52	41.50	40.00	4%

Concentration of samples after spiked with low-concentration solution:

Low Spike OF samples						
Analyte	1	2	3	4	5	Mean
Cortisol	7.50	7.59	9.29	9.16	8.39	8.39
Cortisone	12.24	13.46	27.58	34.74	25.69	22.74

Low-concentration spiked saliva samples with percent difference:

Low Spike OF adjusted conc.								
Analyte	1	2	3	4	5	Mean	Expected	Difference
Cortisol	6.34	6.32	7.59	5.47	6.87	6.52	7	-7%
Cortisone	7.19	6.77	4.46	10.37	6.76	7.11	7	2%

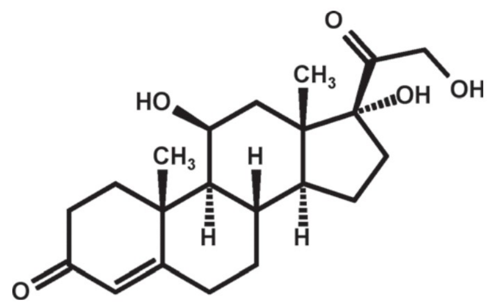
- 9. Retention time stability
  - 50 data points from other validation samples were used.
  - Calculated the standard deviations of the retention time for each analyte.
  - *Acceptance criteria - ≥ 0.1 standard deviation was met.*

#	Analyte	Sample #	1	2	3	4	5	6	7	8	9	10	Mean	SD
1	Cortisol	1-10	1.01	1.01	1.00	0.99	1.01	1.01	0.99	1.00	1.01	1.00	1.00	0.01
		11-20	1.00	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.01	0.99		
		21-30	1.00	1.00	1.00	1.00	0.99	1.01	1.00	1.00	1.01	1.00		
		31-40	1.01	0.99	0.98	1.00	1.00	1.01	0.99	1.00	1.00	1.00		
		41-50	0.99	0.98	0.99	1.00	1.00	0.98	0.98	0.97	0.97	0.98		
2	Cortisone	1-10	0.98	0.98	0.98	0.97	0.98	0.98	0.96	0.97	0.98	0.97	0.97	0.01
		11-20	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.98	0.96		
		21-30	0.97	0.98	0.97	0.97	0.97	0.99	0.98	0.97	0.98	0.97		
		31-40	0.98	0.97	0.96	0.98	0.98	0.98	0.97	0.97	0.97	0.97		
		41-50	0.96	0.96	0.98	0.97	0.97	0.95	0.95	0.94	0.94	0.95		

Appendix K: Cortisol Salivette Evaluation Report (Sarstedt)

Cortisol-Salivette®

Saliva Collection Device



Evaluation Report

by:

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With the rising diagnostic importance of analytes with rhythmically changing values (e.g. hormones or drugs) it is necessary to easily obtain high quality diagnostic samples. Many studies verify the suitability of ***saliva*** as sample material[1].

The collection of saliva by spitting or drooling is not aesthetically pleasing. To stimulate the saliva flow, recommendations to chew on wax pellets or parafilm® can be found, although it is clear that some analytes can be lost through adsorption in this process[2].

The ***Salivette®*** saliva collection device provides a more hygienic alternative: The patient places the swab in the mouth where it easily absorbs saliva. Jaw movement- will stimulate additional saliva flow. The saturated swab is placed back into the Salivette®, capped and sent to the laboratory. Centrifugation for 2 minutes at 1000 x g delivers clear saliva. Any mucous material or solid substances (food debris) are collected in the specially designed conical tube base.
The simple design of the Salivette®, makes it easy for patients to collect their own samples throughout the day, which may be necessary for certain analytes.
The Salivette® is available with three different swab types: plain cotton, cotton treated with citric-acid to stimulate the saliva flow, and a new material specially developed for Cortisol determinations.

Due to its high clinical relevance, ***Cortisol*** is one of the most important analytes that can be determined from saliva[3]. The Salivette® with plain cotton swab is suitable[4] if the saliva volume reaches at least 1 ml. Also, when the cortisol level is in the trace range, values that are too low cannot be totally excluded.

The new Cortisol Salivette is designed to achieve precise analytical values from small volumes and samples with very low cortisol levels. The effectiveness of the ***Cortisol Salivette®*** is demonstrated below:

A high saliva recovery rate from the swab is an important requirement for reliable analysis of *small volume samples*, e.g. from children.

As shown in Fig. 1, the Cortisol Salivette® yields a high sample recovery rate upon centrifugation. For example, an 80% recovery rate is achieved for even a tiny 200 µl sample. This is more than sufficient, as cortisol can be determined from samples as small as 50 µl. Tests with adult volunteers revealed an average saliva recovery volume of 1.1 +/- 0.3 ml.

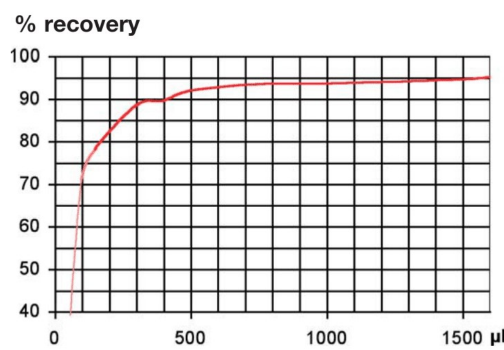


Fig. 1: Recovery rate of the added water volume in Cortisol Salivettes after centrifugation (2 min, 1,000 x g)

The efficacy of the Cortisol Salivette® is established not only in the recovered sample volume, but more importantly in the *volume of cortisol recovered*. There is always a risk of substance loss due to surface absorption with any absorbent material, which carries particular weight in the case of small volumes and low concentrations.

To have enough homogeneous sample material, various artificial saliva solutions (according to DIN 53160-1) were spiked with cortisol to defined concentrations. These artificial saliva solutions were pipetted onto the swabs in different volumes. The Cortisol concentrations in the centrifugates were determined by means of two commercially available kit systems: an established ELISA kit from Salimetrics, USA and a new LIA kit from IBL Hamburg[5].

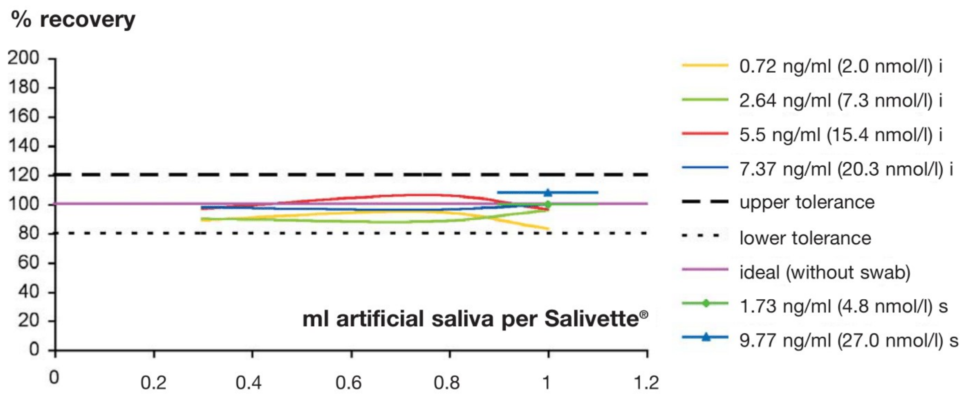
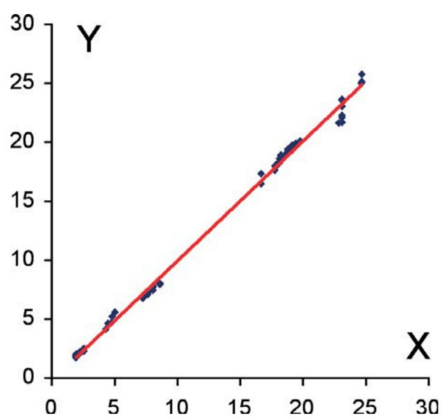


Fig. 2: Cortisol recovery rate at different artificial saliva volumes and cortisol concentrations
i: IBL LIA, s: Salimetrics EIA

As shown in Fig. 2, the cortisol recovery rate is always close to 100 %, regardless of the saliva volume, cortisol concentration or test procedure.

The general suitability of the Cortisol Salivette® is further demonstrated by the following correlation graph:



Y = 1.017·X - 0.3657
r = 0.9984
n = 70 samples

Fig. 3: Correlation graph for Cortisol from Cortisol Salivettes (y axis) and Cortisol from Salivettes without swab (x axis), in nmol/l

Conclusion:

The Cortisol Salivette® is an effective saliva collection device for diagnostic tests even from low volumes samples and/or samples with low Cortisol concentrations.

The Cortisol Salivette® was especially developed for the determination of salivary Cortisol. Its suitability for other salivary analytes is possible though cannot be assured. Users should perform suitability tests of their own.

References:

[1] Selected Reviews (see references there, too):
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Hodinka RL, Nagashunmugam T, Malamud D: Detection of Human Immunodeficiency Virus Antibodies in Oral Fluids, *Clin Diagn Lab Immun* **1998**, 5, 419-426.

[2] Danhof M, Breimer DD: Therapeutic Drug Monitoring in Saliva, *Clin Pharmacokinetics* **1978**, 3, 29-57.

[3] Kirschbaum C, Hellhammer DH: Salivary Cortisol in Psychoneuroendocrine Research: Recent Developments and Applications, *Psychoneuroendocinology* **1994**, 19, 313-333.
Hellhammer J, Fries E, Buss C, Engert V, Tuch A, Rutenberg D, Hellhammer D: Effects of Soy Lecithin Phosphatidic Acid and Phosphatidylserine Complex (PAS) on the Endocrine and Psychological Responses to Mental Stress, *Stress* **2004**, 7, 119-126.
Hellhammer J, Hellhammer D: Ein neuer Weg in der Stressdiagnostik: Neuropattern, in: Stubbe H, Follmann W (Hrsg.): Interventionen in der Angewandten Psychologie, Shaker Verlag Aachen, **2004**, S. 19-26.

[4] Roche Diagnostics: Product Information Summary: Elecsys® Cortisol in Saliva, Nr. 11875116122 (Modular Analytics E170), S. 5.

[5] Westermann J, Demir A, Herbst V: Determination of Cortisol in Saliva and Serum by a Luminescence-Enhanced Enzyme Immunoassay, *Clin Lab* **2004**, 50, 11-24.

Ordering information

Order No.	Length / Ø (mm)	Description	Packaging
51.1534	97 / 16,8	Cotton swab without preparation	500/case 100/bag
51.1534.001	97 / 16,8	Cotton swab with citric acid	500/case 100/bag
51.1534.500	97 / 16,8	Cortisol-Salivette®	500/case 100/inner box

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SARSTEDT

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Appendix L: Cortisol/Cortisone Collection Data

Unique ID	Date (1)	Time (1)	Cortisol (1)	Cortisone (1)	Date (2)	Time (2)	Cortisol (2)	Cortisone (2)
SST-BW-480	5/23/2024	10:14:00 AM	4.03	17.17	6/24/2024	9:01:00 AM	2.88	30.8
SST-BW-885	5/23/2024	10:45:00 AM	3.62	30.46	6/24/2024	9:33:00 AM	5.37	32.77
SST-BW-108	5/23/2024	11:32:00 AM	0.85	30.92	6/24/2024	10:32:00 AM	2.4	23.66
SST-MT-565	5/23/2024	11:38:00 AM	3.4	27.05	N/A	N/A	N/A	N/A
SST-MT-879	5/23/2024	11:32:00 AM	1.49	28.87	6/26/2024	8:30:00 AM	2.54	23.62
SST-BW-165	5/23/2024	2:56:00 PM	2.96	12.31	6/24/2024	1:45:00 PM	2.99	13.01
SST-BW-987	5/23/2024	2:55:00 PM	0.86	16.48	6/24/2024	10:31:00 AM	3.99	31.4
SST-MT-748	5/24/2024	9:44:00 AM	4.89	17.21	7/1/2024	3:36:00 PM	1.87	17.36
SST-MT-158	5/24/2024	11:10:00 AM	2.81	19.07	6/26/2024	9:00:00 AM	5.59	32.67
SST-BW-222	5/24/2024	11:42:00 AM	0.99	15.15	N/A	N/A	N/A	N/A
SST-MT-147	6/12/2024	11:54:00 AM	3.7	14.47	7/15/2024	12:06:00 PM	6.01	65.4
SST-BW-690	6/24/2024	8:55:00 AM	10.83	79.9	7/26/2024	8:54:00 AM	11.49	86.77
SST-MT-321	6/24/2024	9:00:00 AM	11.07	89.31	7/31/2024	8:54:00 AM	3.55	62.65
SST-MT-555	6/26/2024	8:45:00 AM	2.6	36.6	8/2/2024	9:03:00 AM	4.16	56.85
SST-MT-222	7/8/2024	8:39:00 AM	6.76	63.17	8/7/2024	8:46:00 AM	8.17	60.2
SST-BW-612	7/8/2024	8:49:00 AM	6.24	42.34	N/A	N/A	N/A	N/A
SST-BW-113	7/8/2024	8:52:00 AM	1.65	14.32	N/A	N/A	N/A	N/A
SST-MT-963	7/8/2024	9:00:00 AM	6.43	47.22	N/A	N/A	N/A	N/A
SST-MT-777	7/8/2024	9:38:00 AM	6.64	66.26	8/15/2024	8:08:00 AM	10.79	66.16
SST-MT-456	8/20/2024	8:16:00 AM	6.04	69.61	9/24/2024	8:11:00 AM	11.53	78.9
SST-MT-518	8/26/2024	9:51:00 AM	12.84	94.87	9/25/2024	9:43:00 AM	13.34	97.66
SST-BW-555	8/28/2024	10:05:00 AM	3.12	21.09	N/A	N/A	N/A	N/A
SST-MT-205	8/28/2024	10:06:00 AM	4.48	36.44	9/30/2024	10:12:00 AM	3.04	28.12
SST-MT-920	8/28/2024	10:15:00 AM	4.7	36.89	9/30/2024	10:05:00 AM	8.17	24.55
SST-MT-471	8/28/2024	10:34:00 AM	2.68	48.04	9/30/2024	10:13:00 AM	5.62	32.65
SST-BW-308	8/28/2024	10:32:00 AM	3.59	42.36	9/30/2024	10:14:00 AM	7.14	43.6
SST-BW-782	8/28/2024	10:37:00 AM	8.85	69.28	9/30/2024	10:33:00 AM	9.3	47.3
SST-BW-369	8/28/2024	10:41:00 AM	3.57	24.41	9/30/2024	10:42:00 AM	4.2	28.4
SST-BW-420	8/28/2024	10:41:00 AM	3.89	41.86	9/30/2024	10:45:00 AM	3.08	49.17
SST-BW-111	8/28/2024	10:56:00 AM	6.12	55.92	N/A	N/A	N/A	N/A
SST-MT-987	8/28/2024	10:50:00 AM	4.9	49.03	9/30/2024	10:48:00 AM	7.39	57.71
SST-MT-201	8/28/2024	11:00:00 AM	4.06	28.03	N/A	N/A	N/A	N/A
SST-BW-200	8/28/2024	11:11:00 AM	1.29	26.91	N/A	N/A	N/A	N/A
SST-BW-468	8/28/2024	11:24:00 AM	7.43	20.35	10/2/2024	11:30:00 AM	19.29	43.45
SST-MT-778	8/28/2024	11:30:00 AM	3.1	22.17	10/2/2024	11:40:00 AM	4.29	23.57
SST-MT-452	8/28/2024	11:30:00 AM	3.53	42.74	10/2/2024	11:30:00 AM	2.93	19.97
SST-BW-371	8/28/2024	11:35:00 AM	4.64	34.58	10/2/2024	11:32:00 AM	5.03	30.03
SST-BW-314	8/28/2024	11:35:00 AM	2.55	24.91	9/30/2024	11:50:00 AM	5.5	53.23
SST-BW-983	8/28/2024	11:54:00 AM	2.38	14.74	9/30/2024	11:22:00 AM	5.45	25.13
SST-MT-822	8/28/2024	11:59:00 AM	2.59	31.3	9/30/2024	11:17:00 AM	3.06	37.49
SST-BW-290	8/28/2024	11:58:00 AM	4.1	50.23	N/A	N/A	N/A	N/A
SST-BW-813	8/28/2024	12:03:00 PM	0.48	9.24	N/A	N/A	N/A	N/A
SST-BW-751	8/28/2024	12:01:00 PM	2.1	20.84	10/2/2024	11:54:00 AM	2.96	25.33
SST-MT-598	8/28/2024	12:01:00 PM	1.55	24.86	N/A	N/A	N/A	N/A
SST-MT-386	8/28/2024	12:05:00 PM	0.99	20.06	9/30/2024	11:20:00 AM	2.29	16.39

Unique ID	Date (1)	Time (1)	Cortisol (1)	Cortisone (1)	Date (2)	Time (2)	Cortisol (2)	Cortisone (2)
SST-BW-525	8/28/2024	12:04:00 PM	1.28	22.57	9/27/2024	12:49:00 PM	2.62	34.16
SST-BW-844	8/28/2024	12:06:00 PM	1.94	33.35	9/30/2024	11:21:00 AM	1.98	21.06
SST-MT-210	8/28/2024	12:03:00 PM	2.52	32.85	9/30/2024	11:20:00 AM	2.66	35.6
SST-MT-800	8/28/2024	12:08:00 PM	3.92	31.85	N/A	N/A	N/A	N/A
SST-MT-143	8/28/2024	12:10:00 PM	2.47	29.97	N/A	N/A	N/A	N/A
SST-MT-943	8/28/2024	12:08:00 PM	4.29	60.9	N/A	N/A	N/A	N/A
SST-MT-418	8/28/2024	12:25:00 PM	3.37	23.15	9/30/2024	12:05:00 PM	2.58	26.51
SST-BW-367	8/29/2024	6:52:00 PM	2.43	12.85	9/30/2024	7:05:00 PM	3.33	36.11
SST-BW-786	8/30/2024	8:10:00 AM	3.44	41.82	9/30/2024	8:12:00 AM	1.35	6.9
SST-BW-650	9/4/2024	8:45:00 AM	1.35	29.72	10/7/2024	10:06:00 AM	3.53	16.69
SST-MT-766	9/4/2024	8:47:00 AM	4.98	42.13	10/7/2024	8:58:00 AM	4.31	34.15
SST-BW-533	9/4/2024	8:51:00 AM	6.95	75.84	10/7/2024	9:00:00 AM	3.75	44.51
SST-MT-548	9/4/2024	9:14:00 AM	2.21	28.44	10/7/2024	10:26:00 AM	2.08	28.73
SST-MT-100	9/4/2024	9:43:00 AM	5.52	47.91	10/7/2024	9:38:00 AM	4.37	51.44
SST-MT-789	9/4/2024	11:07:00 AM	2.48	19.93	10/7/2024	11:03:00 AM	4.23	26.16
SST-MT-944	9/4/2024	11:11:00 AM	6.97	49.57	10/7/2024	11:03:00 AM	5.07	35.51
SST-MT-801	9/4/2024	11:48:00 AM	6.84	60.49	10/7/2024	11:44:00 AM	15.89	37.57
SST-BW-770	9/5/2024	8:45:00 AM	6.08	46.31	10/8/2024	8:50:00 AM	5.53	36.1
SST-MT-873	9/5/2024	8:49:00 AM	3.34	44.41	10/8/2024	8:51:00 AM	4.59	35.21
SST-BW-725	9/5/2024	8:56:00 AM	4.46	39.04	10/10/2024	8:56:00 AM	9.49	37.06
SST-MT-320	9/5/2024	9:27:00 AM	4.46	32.46	10/8/2024	9:25:00 AM	2.46	34.57
SST-MT-465	9/5/2024	9:30:00 AM	3.01	29.38	N/A	N/A	N/A	N/A
SST-BW-645	9/5/2024	9:33:00 AM	3.59	40.28	10/8/2024	9:15:00 AM	9.56	72.73
SST-MT-716	9/6/2024	10:38:00 AM	3.96	41.64	10/11/2024	11:31:00 AM	3.59	25.25
SST-BW-207	9/6/2024	10:40:00 AM	2.48	31.27	10/11/2024	12:05:00 PM	2.74	24.22
SST-BW-879	9/6/2024	10:42:00 AM	4.19	34.47	10/11/2024	12:04:00 PM	4.95	34.64
SST-BW-187	9/6/2024	10:45:00 AM	3.17	27.84	10/11/2024	12:08:00 PM	7.42	29.14
SST-BW-765	9/9/2024	8:07:00 AM	5.71	50.88	10/9/2024	8:10:00 AM	3.77	33.2
SST-MT-970	9/9/2024	8:07:00 AM	5.31	51.66	10/9/2024	8:06:00 AM	5.74	32.11
SST-MT-376	9/9/2024	9:08:00 AM	4.95	48.11	10/15/2024	9:08:00 AM	11.42	78.46
SST-MT-774	9/9/2024	9:14:00 AM	2.74	38.33	10/14/2024	9:28:00 AM	3.58	29.96
SST-MT-486	9/9/2024	9:09:00 AM	7.65	64.57	10/14/2024	9:22:00 AM	8.21	60.1
SST-BW-810	9/9/2024	9:13:00 AM	2.28	39.77	10/14/2024	9:29:00 AM	6.79	44.69
SST-BW-292	N/A	N/A	N/A	N/A	10/14/2024	9:28:00 AM	8.33	42.79
SST-MT-281	9/9/2024	9:30:00 AM	6.13	63.78	10/14/2024	9:21:00 AM	3.23	43.51
SST-MT-704	9/9/2024	1:07:00 PM	4.48	36.46	10/14/2024	1:38:00 PM	5.24	28.77
SST-BW-413	9/9/2024	2:58:00 PM	2.93	28.44	N/A	N/A	N/A	N/A
SST-MT-500	9/10/2024	1:10:00 PM	3.03	16.47	10/15/2024	1:05:00 PM	2.63	16.61
SST-BW-740	9/10/2024	12:38:00 PM	3.5	25.33	10/15/2024	12:42:00 PM	2.72	19.63
SST-MT-935	9/11/2024	8:43:00 AM	2.98	42.48	10/16/2024	8:47:00 AM	10.47	69.05
SST-BW-972	9/11/2024	8:48:00 AM	5.42	41.32	10/16/2024	8:50:00 AM	2.03	24.73
SST-BW-747	9/11/2024	8:53:00 AM	5.62	59	10/16/2024	8:53:00 AM	8.58	69.04
SST-BW-570	9/11/2024	8:57:00 AM	2.24	21.54	10/16/2024	8:48:00 AM	4.17	16.86
SST-MT-888	9/11/2024	9:34:00 AM	3.54	38.89	10/14/2024	9:48:00 AM	3.12	24.54

Unique ID	Date (1)	Time (1)	Cortisol (1)	Cortisone (1)	Date (2)	Time (2)	Cortisol (2)	Cortisone (2)
SST-BW-171	9/11/2024	9:40:00 AM	7.59	32.36	10/14/2024	9:31:00 AM	10.66	70.05
SST-BW-552	9/11/2024	9:44:00 AM	5.93	48.77	10/16/2024	11:18:00 AM	5.59	29.85
SST-BW-546	9/11/2024	10:23:00 AM	3.23	42.4	N/A	N/A	N/A	N/A
SST-MT-327	9/11/2024	11:20:00 AM	1.45	27.74	N/A	N/A	N/A	N/A
SST-BW-433	9/11/2024	12:30:00 PM	0.42	5.34	10/14/2024	11:32:00 AM	10.89	83.57
SST-BW-370	9/11/2024	12:27:00 PM	2.97	25.96	N/A	N/A	N/A	N/A
SST-MT-463	9/11/2024	12:01:00 PM	0.57	11.49	10/14/2024	11:34:00 AM	2.71	29.86
SST-BW-641	9/11/2024	12:13:00 PM	4.68	41.6	10/16/2024	11:58:00 AM	5.88	42.67
SST-BW-307	9/11/2024	12:15:00 PM	4.25	44.66	N/A	N/A	N/A	N/A
SST-MT-823	9/11/2024	12:17:00 PM	1.63	22.71	10/14/2024	12:34:00 PM	3.21	22.7
SST-BW-409	9/11/2024	1:26:00 PM	1.63	8.96	10/14/2024	1:22:00 PM	5.54	28.53
SST-MT-710	9/11/2024	1:13:00 PM	1.5	31.19	10/14/2024	2:00:00 PM	2.47	9.16
SST-MT-757	9/11/2024	12:45:00 PM	0.91	14.38	N/A	N/A	N/A	N/A
SST-MT-861	9/11/2024	12:45:00 PM	2.73	21.75	N/A	N/A	N/A	N/A
SST-MT-329	9/11/2024	12:15:00 PM	3.59	28.93	10/16/2024	12:00:00 PM	3.6	31.85
SST-BW-829	9/11/2024	2:23:00 PM	0.81	11.69	10/16/2024	2:15:00 PM	4.81	28.54
SST-MT-645	9/11/2024	12:09:00 PM	1.74	5.2	10/16/2024	12:12:00 PM	3.88	25.36
SST-MT-101	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SST-BW-128	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SST-BW-573	9/16/2024	11:18:00 AM	4.59	48.23	10/16/2024	11:23:00 AM	6.65	63.59
SST-MT-998	9/17/2024	1:09:00 PM	3.06	33.25	10/22/2024	1:14:00 PM	6.81	37.74
SST-BW-138	9/17/2024	2:08:00 PM	3.11	18.31	10/22/2024	1:00:00 PM	5.07	38.15
SST-MT-532	9/17/2024	2:35:00 PM	1.9	17.75	N/A	N/A	N/A	N/A
SST-MT-584	9/17/2024	2:45:00 PM	2.56	31.31	10/22/2024	2:40:00 PM	4.32	24.01
SST-MT-643	9/18/2024	2:23:00 PM	8.31	22.43	10/23/2024	2:15:00 PM	2.89	22.29
SST-BW-315	9/18/2024	2:25:00 PM	1.46	30.4	10/23/2024	2:12:00 PM	4.32	43.71
SST-BW-665	9/18/2024	2:18:00 PM	6.29	18.46	10/23/2024	2:02:00 PM	7.43	42.11
SST-MT-791	9/18/2024	2:25:00 PM	2.8	16.38	10/23/2024	2:29:00 PM	2.48	20.6
SST-MT-857	9/18/2024	2:30:00 PM	2.58	24.4	N/A	N/A	N/A	N/A
SST-BW-527	9/18/2024	3:05:00 PM	3.04	14.19	N/A	N/A	N/A	N/A
SST-MT-864	9/18/2024	3:20:00 PM	5.29	33.31	10/23/2024	2:19:00 PM	5.06	42.45
SST-BW-356	9/23/2024	12:02:00 PM	1.72	20.68	N/A	N/A	N/A	N/A
SST-BW-323	9/23/2024	12:50:00 PM	4.11	17.45	10/23/2024	11:53:00 AM	6.2	20.16