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AIRWAY HYDROGEN PEROXIDE DURING A NATURALISTIC PERIOD OF SUSTAINED PSYCHOLOGICAL STRESS IN INDIVIDUALS WITH ASTHMA AND HEALTHY CONTROLS

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AIRWAY HYDROGEN PEROXIDE DURING A NATURALISTIC PERIOD OF SUSTAINED PSYCHOLOGICAL STRESS IN INDIVIDUALS WITH ASTHMA AND HEALTHY CONTROLS

A Dissertation Presented to the Faculty of the Dedman College Southern Methodist University In Partial Fulfillment of the Requirements For the Degree of Doctor of Philosophy In Clinical Psychology By Chelsey A. Werchan B.A., Psychology, Trinity University, San Antonio, TX M.A., Clinical Health Psychology, Texas State University, San Marcos, TX M.A., Clinical Psychology, Southern Methodist University, Dallas, TX
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Author has no conflicts of interest to disclose.

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ABSTRACT

Reactive oxygen species in the form of exhaled hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and nitric oxide (NO) are important biological markers of inflammation and immune response in the airways of both asthmatics and healthy individuals. Previous research has linked psychological distress to changes in these processes in the airways, which can impact overall disease management outcomes. The current study examined changes in H\textsubscript{2}O\textsubscript{2}, NO, mood, and other physiological measures during times of prolonged stress using a naturalistic paradigm. Both healthy and asthmatic participants were assessed at three separate time periods; once during a low stress period mid-semester, and twice during the week of final examinations. At each session, participants completed questionnaires regarding mood, social support, stress, cold symptoms, and physical activity. They also provided saliva samples, a blood pressure measurement, and several measures of lung function and airway physiology. Online surveys of stress and cold symptoms were completed one week after final examinations. Mixed models analyses were used to examine changes mood and physiological variables both between and within participants across time. Significant changes were found for stress, negative affect, H\textsubscript{2}O\textsubscript{2}, and NO. Implications for these results are discussed as well as directions for future research endeavors.
Oxygen molecules in the body readily bind with free electrons, resulting in either helpful enzyme reactions or the formation of harmful reactive oxygen species (ROS). Hydrogen peroxide is a type of ROS that has been suggested as a potential biomarker indicating oxidative stress (Teng et al., 2011). Oxidative stress is defined as an increase in oxidants in the body that outbalance antioxidant effects (Horvath, et al., 1998). Greater amounts of ROS are indicative of a potential imbalance with antioxidants in the body and therefore, oxidative stress. Thus, while oxidative stress cannot be directly measured, indirect measurement is often attempted through measurement of ROS.

Oxidative stress has been associated with inflammatory processes and damage to DNA, proteins, lipids, and other molecules and tissues in the body (Wolkowitz, Epel, Reus, & Mellon, 2010). It has also been linked to increased cellular aging and the pathophysiology of many diseases. Research suggests that a variety of internal and external factors may influence oxidative stress either through the development of free oxygen radicals or by affecting antioxidant status (Moller, Wallin, Knudsen, 1996).

Due to difficulty with directly assessing oxidative stress, researchers have utilized different measurements of oxidative reactions (Bettenridge, 2000). One such method involves measurement of hydrogen peroxide ($\text{H}_2\text{O}_2$) in exhaled breath condensate (Teng, et al., 2011). $\text{H}_2\text{O}_2$ in exhaled breath has been assessed in a variety of respiratory diseases as a non-invasive marker of inflammation (Schleiss et al., 2000). Elevated $\text{H}_2\text{O}_2$ has been linked to chronic respiratory diseases and respiratory infections such as the common cold (Jöbsis, Schellekens, Fakkel-Kroesbergen, Raatgeep, & de Jongste, 2001).

Past studies have examined the impact of psychological stress and mood on the development of ROS. In an investigation of workload and perceived stress in healthy workers,
Irie et al. (2001) found that perceived workload and stress levels in female employees were associated with increased levels of oxidative DNA damage. Interestingly, such oxidative damage was not associated with actual workload, suggesting a psychological component to this association. Psychological stress may be related to oxidative stress through a number of mechanisms and research has yet to fully explain such processes.

Additionally, there is research linking depression to oxidative stress (Moylan, et al., 2014). Depression pathogenesis is likely a complex interaction of feedback loops with oxidative processes, activation of immune-inflammatory processes, and decreased levels of antioxidants. Subsequently, depression has been linked to several long-term health consequences. Decreased oxidative stress markers in animals have been linked with administration of antidepressants (Maes, Galecki, Chang, & Berk, 2011). However, little is known about reactive oxygen species (ROS) production in the airways in response to psychological factors. It is possible that excessive production of free radicals in psychological stress and depression can pave the way for stress-induced disease exacerbations, such as in asthma. Therefore, the presence of depressed mood is an important consideration for research examining ROS processes in asthma as well as other chronic illnesses.

Fraction of exhaled nitric oxide (FeNO) is also used as a marker of airway inflammation (Yates, 2001). The exact functions and mechanisms of FeNO are diverse and studies indicate it also serves an important role in immune functioning (Xu, Zheng, Dweik, & Erzurum, 2006). Research indicates that levels of NO are linked with psychological processes, stress, and mood (Ritz & Trueba, 2014). Previously, our laboratory has explored levels of exhaled NO, salivary cortisol, and lung function during times of acute stress. Results indicated increases in FeNO following an acute stress task and smaller changes were seen with larger increases in salivary
cortisol (Ritz, Ayala, Trueba, Vance, & Auchus, 2011). However, it is unclear to what degree such increases reflect an increase in airway inflammation or increases in immune function in response to potential threat (stressor).

Changes in FeNO may also depend on the type of stressor (acute vs. chronic). Some research suggests that chronic or sustained stressors is linked to decreases in FeNO, as well as decreases in lung function (Ritz, Trueba, Liu, Auchus, & Rosenfield, 2015). These contrasting findings would seem to suggest different types of stressors activate different biological pathways. Further research is needed to explore such pathways and how they might be influenced.

How NO and H$_2$O$_2$ are associated with each other remains to be thoroughly explored. Measurement of NO in FeNO provides only an indirect measure of levels of NO in the lungs. More specifically, FeNO provides a measure of NO production in the lungs minus the consumption of NO throughout the airways, particularly through oxidative processes (Nguyen et al., 2005). Research suggests that increases in oxidative processes would decrease measurable levels of exhaled NO as they would most likely result in greater consumption of NO in the airways. The consumptive nature of ROS could explain the paradoxical decreases of FeNO observed in prior studies. Given this reasoning, we would expect that increases in H$_2$O$_2$ in exhaled breath condensate would be associated with decreases in FeNO. The molecular processes described above would suggest that both levels of NO and H$_2$O$_2$ can be indicative of oxidative stress and may prove useful in monitoring airway inflammation, particularly in chronic airway diseases, such as asthma.

Asthma is a chronic respiratory disease characterized by airway hyperreactivity, bronchoconstriction, chronic inflammation, and airflow limitation (GINA, 2018). Symptoms
may include dyspnea, cough, wheezing, and chest tightness, as well as limitations in functioning due to such symptoms. Asthma is estimated to affect 300 million people worldwide and is increasing in prevalence (GINA appendix, 2018). The disease is associated with a high social and economic burden. Such costs vary depending on prevalence, disease control, ability to prevent exacerbations, and cost of treatment and medications. Poorer disease control is associated with greater number of exacerbations, higher productivity loss, and greater reductions in quality of life. Given the high prevalence and burden of this chronic disease, research often aims to improve maintenance of the disease and prevent exacerbations through a variety of interventions and monitoring techniques.

In asthma, the severity and frequency of airway inflammation influence overall disease course and outcomes. The ability to accurately assess inflammatory processes in the airways is therefore important from a disease management standpoint. Given the difficulty of directly assessing lung tissue for inflammation and other cellular processes, biological sciences have sought alternative methods for assessing these processes non-invasively.

Horvath and colleagues (1998) sought to examine airway inflammation in asthma using both NO and H₂O₂. In comparing healthy participants to steroid-naïve asthmatics, stable steroid-treated asthmatics, and unstable steroid-treated asthmatics, the study found that both NO and exhaled H₂O₂ were higher in steroid-naïve asthmatics but reduced in stable steroid-treated asthmatics. Additionally, patients who were steroid-treated but unstable demonstrated higher H₂O₂ levels but lower NO in comparison to healthy participants. The researchers concluded that both NO and H₂O₂ may serve complimentary roles in monitoring airway inflammation in patients with asthma. Research is needed to further explore how each of these measurements may be useful in monitoring asthmatic inflammation.
Airway compromise in asthma, including inflammation, may also occur as a result of psychological stress. Perceptions of increased stress are associated with greater asthma symptomatology and reduction in respiratory flow rates due to bronchoconstriction (Isenberg et al., 1992; Ritz, 2012; Wright, Rodriguez & Cohen, 1998). Beyond the short-term changes in lung function through the ANS and ventilation, slower developing and longer lasting effects on asthmatic airways pathology through inflammatory processes have also been shown to be susceptible to psychological processes. For example, chronic stress is associated with enhanced cytokine production that orchestrates allergic inflammation of the airway (e.g., Liu et al., 2002). However, paradoxically both baseline and changes in FeNO have been associated negatively with stress and depressed mood (Ritz et al., 2015). Individuals with higher baseline depressed mood show greater decreases in FeNO during prolonged stress and attenuated increases in FeNO during periods of acute stress.

**Aims and Hypotheses**

The current study sought to expand on previous research on respiratory inflammation in asthma during times of stress. Research has yet to examine whether changes in reactive oxygen species production can be observed in the airways in response to psychological stressors and how such changes may differ for individuals with asthma. Such changes and their relation with other biological markers of inflammation (i.e. FeNO) may have important implications for disease management for asthma during times of stress. Specifically, we examined the temporal dynamics of, and the interrelationships between, reactive oxygen species in the form of H₂O₂ and mood, perceived stress, and FeNO using a naturalistic stress-induction paradigm of undergraduate final exams. Our first aim was to examine how H₂O₂ changes over time during stress and whether such changes are different between healthy and asthmatic participants. Based
on prior research findings, we expected significant increases in H$_2$O$_2$ during times of stress and for such changes to be greater in participants with asthma. The second aim was to examine whether changes in FeNO and H$_2$O$_2$ are related. Our expectation was that these two biomarkers would demonstrate a negative association within individuals. Specifically, we expected that decreases in FeNO during times of stress, as has been previously demonstrated (Höglund et al., 2006; Ritz et al., 2015; Trueba et al., 2013), would be negatively correlated with increases in H$_2$O$_2$ under the assumption that ROs would convert NO and thus lead to reduced FeNO (Nguyen et al., 2005). Finally, we explored depression as a potential moderator of changes in FeNO and H$_2$O$_2$, with the expectation that higher depressed mood will be related to a stronger increase in H$_2$O$_2$ and stronger decrease of FeNO.

**Methods**

**Study Design**

Participants were scheduled for 3 in-person assessments. The initial assessment session took place during the academic semester as a “low stress” period. The other two assessments took place during the week of final exams, with the early final exam assessment occurring sometime after the student’s first of at least three exams and the late final exam assessment occurring before their last final exam. Participants were also sent a follow-up survey via email one week after completion of final examinations.

**Participants**

Participants were undergraduate students at a university in the southwestern USA. Students participated either for research credits in the Psychology Subject Pool or for $35 compensation.
Prior to being scheduled for their initial lab session, all participants were screened via phone to ensure they met eligibility criteria. Participants met inclusion criteria if they were over the age of 18, able to speak and read English, had a physician’s diagnosis of asthma (or if not, were otherwise healthy), and had at least three final exams at the end of the academic semester in which they were participating. Exclusion criteria included current active smoking status, having a significant health condition other than asthma (i.e. seizures, stroke, heart attack, heart disease, thyroid problems, head injury or neurological disorder, diabetes, any other kind of respiratory disease), and use of antibiotics or corticosteroids within the past two months. Permission for this study was obtained from the Institutional Review Board (2015-109-RITT) and participants provided written informed consent.

Measures

Single item stress rating. Participants were asked to indicate on a 10-point scale how stressed they are at the present moment, from 1 = not at all to 10 = extremely.

General information and health questionnaire (GI-HQ). Participants provided information regarding health behaviors and medical history (i.e. smoking history, medical diagnoses, and medications).

Positive affect negative affect scale (PANAS). The PANAS is a psychometrically valid and reliable measure ($\alpha = .84$ to $.90$) which assesses a variety of positive and negative mood states and symptoms (Watson, Clark, & Tellegen, 1988). The measure consists of 20 mood state items rated on a 5-point scale from 1 = very slightly to 5 = extremely, as well as an additional 9 physical symptom items rated on a scale from 1 = not at all to 10 = extremely.

Hospital anxiety and depression scale (HADS). The HADS is a 14-item measure that assesses depressed and anxious mood (Zigmond & Snaith, 1983). A more recent literature review by
Bjelland et al. (2002) suggests that Cronbach’s alpha for the anxiety subscale ranges from .68 to .93 across different studies and from .67 to .90 for the depression subscale.

**Asthma-Relevant Questionnaires**

The following questionnaires were completed only by students with asthma:

**Asthma-related information questionnaire (AIQ).** This questionnaire consists of 24 items compiled ad-hoc to assess participants’ asthma history, treatment, and manifestation.

**Asthma control questionnaire (ACQ).** The ACQ is a 7-item self-report questionnaire that assesses participants’ control over their asthma symptoms within the past week (Juniper, O’Byrne, Guyatt, Ferrie, & King, 1999). Asthma patients rate their symptoms on 6 items using a 7-point scale from 0 = never/no symptoms to 6= all the time/ severe symptoms, with higher overall scores indicating poorer asthma control. The final item is the participant’s FEV1% converted to a rating scale.

**Physiological measures.** Physiological measurements included fraction of exhaled nitric oxide (FeNO), exhaled breath condensate (EBC), peak expiratory flow (PEF), forced expiratory volume in one second (FEV1), and three saliva samples. Two saliva measurements were collected using cotton swabs (Sarstedt salivettes, Sarstedt, Germany) which participants held in their mouth for two minutes each to collect saliva. FeNO was measured by exhaling for 10 seconds at a steady rate into a hand-held monitor (NIOX mino, Aerocrine, Solna, Sweden). EBC was collected by having participants breathe normally through a tube that is encapsulated in a frozen metal cylinder that condenses the water vapor and collects the breath condensate (Rtube device, Respiratory Research Inc, Austin, Texas). Lastly, PEF and FEV1 values were obtained using a hand-held spirometer (AM2, Jaeger/Toennies) into which participants forcefully exhaled
from maximum inspiration. The best FEV1 value of three attempts and its corresponding PEF value were used for data analysis.

**Procedure**

At the initial low-stress assessment, all participants completed written informed consent. Any questions they had were addressed and they were given a copy of the consent form for their own records. Participants were asked to refrain from eating, drinking anything except water, and strenuous exercise for two hours prior to the assessment. Asthmatic participants were asked to refrain from bronchodilator use at least 6 hours prior to the session. Participants then completed questionnaires including background and demographic information, as well as psychological and health-related measures. All participants then completed the physiological measurements.

The other two assessment periods took place during participants’ week of final exams. Both assessment periods included the same protocol and physiological measurements as the non-stress period. The questionnaires that participants completed in the early exam period were the PANAS, and stress item. During the late exam period, which occurred sometime before each participant’s last exam, questionnaires were the PANAS, HADS, Stress item and the ACQ for asthma participants. One week after exams were completed, participants were emailed a link to a final set of questionnaires, which are not a subject of this report.

**Measurement of H$_2$O$_2$ in exhaled breath condensate and salivary cortisol.** Exhaled breath condensate samples were assessed using the Amplex Red assay (Life Technologies, Carlsbad, CA) following the manufacturer’s instructions. Calibration was performed and stock H$_2$O$_2$ solutions were measured in triplicate by monitoring peak fluorescence at 582 nm using an F-7000 Spectrophotometer (Hitachi, Tokyo, Japan). Calibrations were performed on the same day as exhaled breath condensate measurement (Quimbar, Krenek, & Lippert, 2016).
Cortisol saliva samples were frozen and salivettes were subsequently spun for 3 minutes to obtain saliva. Standards and quality controls (QCs) were spiked with phosphate-buffered saline. Samples, standards and QCs were precipitated with crash containing: methanol, formic acid (0.15%, final 0.1%) and D4 Cortisol (Sigma-Aldrich, final 10ng). Tubes were incubated at room temperature for 10 minutes and centrifuged. Aliquots of supernatant were analyzed by liquid chromatography-mass spectrometry. Values from three analytical runs were averaged. (Kroll, Brown, & Ritz, 2019).

**Data Analysis**

Initial data cleaning involved adding a water reference value of 200 to H$_2$O$_2$ values, and removing H$_2$O$_2$ values that were outliers (greater than 3 S.D. from mean) or had a value less than or equal to zero. This resulted in the exclusion of 5 total data points, 2 of which were negative values and 3 were positive outliers. Data distributions for all dependent variables were examined and those with skewness greater than 1.0 were transformed. Variables that required transformation were FeNO, cortisol, and HADS depression score at baseline. FeNO was log transformed and cortisol was log transformed with a constant of 10 added to eliminate occurrence of negative values. Depression scores on the HADS at baseline were square root transformed. Characteristics of healthy and asthmatic groups were compared using chi-square and t-tests. A power analysis using RMASS-2 (Hedeker, Gibbons & Waternaux, 1999) indicated power greater than 0.85 to detect a medium effect size (d= 0.5) with the current sample of 86 subjects and assuming a dropout rate of 12%.

Mixed model analyses were used to examine the effect of time and group (asthma vs. healthy) on H$_2$O$_2$, FeNO, salivary cortisol, lung function measurements (FEV$_1$% and PEF), negative affect, and momentary stress rating. For the H$_2$O$_2$ models, time was coded as
assessment number (1, 2, 3) whereas for the FeNO models, time was coded as assessment day from 0: baseline and then 1-7 for exam assessment day. Differences between condition (asthma vs. healthy) were examined as well as changes within individuals across time, controlling for demographic information (age, sex, race, BMI) and inhaled corticosteroid use (0: no inhaled corticosteroid use, 1: inhaled corticosteroid use)). For each model, an unstructured covariance matrix was used and non-significant control variables were dropped from the final models.

In order to assess whether changes in FeNO and H$_2$O$_2$ were related, H$_2$O$_2$ was included in the mixed model analysis as a potential time-varying predictor of changes in FeNO, focusing selectively on the within-subject portion of their covariance (association of FeNO changes over time with H$_2$O$_2$ changes). Other time-varying predictors that were examined included momentary stress, negative affect, cortisol, and positive affect.

To examine whether depression moderated changes in H$_2$O$_2$ and FeNO, the HADS depression score at baseline was included in the growth curve models as interactions with all growth curve parameters. Non-significant interactions were systematically removed to develop the finalized model. Season in which samples were collected was also examined as a potential moderator of changes over time. Additionally, for the sample of individuals with asthma, asthma severity, asthma control and medication category (0: no asthma medication, 1: rescue inhaler only, 2: inhaled corticosteroids) were examined as potential moderators of FeNO and H$_2$O$_2$ changes over time.

**Results**

**Sample characteristics**

The total sample consisted of 86 undergraduate students, of which 39 (45.3%) students reported a diagnosis of asthma. Baseline characteristics of healthy and asthmatic participants are presented in Table 1. The asthmatic and healthy groups did not significantly differ at baseline on
variables of interest, with the exception of higher FeNO levels in the asthma group, \( t(77)= 2.07, p = 0.042 \).

**Stress Experience and Negative Affect**

There was a significant effect of time on stress and negative affect, \( F(2, 77) = 15.36, p < .001 \); \( F(2, 77) = 22.00, p < .001 \) (Fig. 1 & 2). Stress experience demonstrated a significant increase from baseline to early exam session, \( b = -1.20, t(77) = -5.18, p < .001 \), followed by a significant decrease from early to late exam sessions, as participants were near the end of their final examinations, \( b = -.76, t(75) = -3.27, p = .002 \). There was no significant difference between baseline and late exam sessions \( (p = .136) \), indicating that students’ momentary stress had decreased to near baseline levels by their final session of the study. While similar in pattern to momentary stress, negative affect appeared to have a more enduring effect during final exams. Negative affect significantly decreased from early to late exam session, \( b = -3.23, t(74) = -5.54, p < .001 \); but was significantly higher in both early and late exam sessions compared to baseline, \( b = 4.75, t(79) = 6.23, p < .001 \); \( b = 1.52, t(78) = 2.19, p = .031 \).

**Effects of Exam Stress on \( \text{H}_2\text{O}_2 \)**

Mixed models analysis found no significant effect of time, group, or time by group interaction on exhaled hydrogen peroxide, \( p = .239 - .309 \) (Fig. 3).

**Effects of Exam Stress on FeNO and its Relation to \( \text{H}_2\text{O}_2 \)**

In examining the effect of exam stress on FeNO, significant main effects were found for group and time. At baseline, participants with asthma had significantly higher FeNO values compared to healthy participants, \( b = -3.9, t(85) = -3.11, p = .003 \) (Fig. 4). FeNO significantly increased across time for both groups, \( b = .0005, t(69) = 2.83, p = .006 \). There were also significant main effects for sex \( F(1, 83) = 4.60, p = .035 \), ethnicity \( F(1, 84) = 7.87, p = .006 \), and
inhaled corticosteroid use $F(1, 81) = 4.06, p = .047$. Non-white individuals, women, and those not taking inhaled corticosteroids had higher FeNO values. FeNO was not a significant time-varying covariate of H$_2$O$_2$, suggesting that H$_2$O$_2$ was not associated with FeNO over time, $p = 0.516$.

**Effects of Exam Stress on Cortisol and Lung Function**

There were no significant effects of time or condition on cortisol or FEV$_1$%. The model for prediction of cortisol included saliva collection time in order to control for diurnal changes in cortisol levels. There was a significant effect of time on PEF, $F(2, 82) = 3.41, p = .038$ (Fig. 5). PEF values significantly increased from baseline to the late exam session for the sample as a whole, $b = 24.16, t(81) = 2.68, p = .009$.

**Associations of Stress, Negative Affect, and Cortisol with H$_2$O$_2$ and FeNO Over Time**

Other potential time-varying predictors of H$_2$O$_2$ and FeNO that were examined include momentary stress, negative affect, cortisol, positive affect and time of session. Momentary stress deviation score was a significant time-varying predictor of H$_2$O$_2$, $b = 13.37, t(109) = 2.13, p = .035$. Thus, while average stress levels did not significantly predict H$_2$O$_2$, individuals with higher stress levels had significantly higher values of H$_2$O$_2$. Momentary stress was not a significant predictor of FeNO, $p = .747$.

Negative affect deviation score approached significance as a time-varying predictor of H$_2$O$_2$, $p = .077$. There was a trend for those individuals with higher levels of negative affect to have higher H$_2$O$_2$ values. Negative affect was not a significant time-varying predictor of FeNO, $p = .984$. Additionally, positive affect scores were not a significant time-varying predictor for either H$_2$O$_2$ or FeNO, $p = .257$ & .096.

Cortisol was also not found to be a significant time-varying predictor for either H$_2$O$_2$ or FeNO, $p = .088$ & .774. However, time of saliva collection was included in these models as a
potential control variable for diurnal changes in cortisol and found to be a significant predictor of overall levels of H₂O₂, \( F(1, 92) = 8.01, p = .006 \). Saliva samples collected later in the day, and therefore also EBC collected later in the day, demonstrated higher H₂O₂ values, \( b = 0.14, t(92) = 2.83, p = .006 \).

**Effects of Depression on Hydrogen Peroxide and Nitric Oxide**

In examining baseline depression as a potential moderator of changes in H₂O₂ and FeNO, the finalized models indicated that depression was not a significant moderator of changes in either biomarker (\( p = .647 \) and .657, respectively).

**Effects of Season on Hydrogen Peroxide and Nitric Oxide**

Season in which data were collected was examined as a potential moderator of changes in H₂O₂ and FeNO (Fig. 6 and 7). Finalized models indicated that participants in Fall semesters had lower FeNO levels compared to those in the Spring semesters, \( b = -.24, t(88) = -2.07, p = .042 \). Additionally, participants in the Fall semesters had higher H₂O₂ values compared to those in the Spring semesters, \( b = 71.31, t(68) = 3.28, p = .002 \). The interaction between season and time for both models was not significant, indicating that changes in either biomarker over time did not differ depending on the season.

**Asthma-Specific Effects**

Asthma severity, asthma control, and type of asthma medication were assessed as potential moderators of H₂O₂ and FeNO for participants with asthma. Both severity and control were not significant moderators of changes in either biomarker over time (\( p = .512 - .839 \)).

Type of asthma medication was a significant moderator of H₂O₂. \( F(1, 30) = 9.80, p = .004 \), but not for FeNO, \( p = .175 \). Participants not prescribed any asthma-specific medication at
baseline had higher H$_2$O$_2$ levels than those prescribed any asthma medication, $b = -89.62$, $t(30) = -3.13$, $p = .004$ (Fig. 8).

**Discussion**

In the present study we sought to examine the relation of H$_2$O$_2$ to changes in psychological stress and airway inflammation, as well as its relation to another biomarker of inflammation, nitric oxide. We also examined how such biomarkers change in response to a naturalistic period of sustained stress and changes in mood, as well as how they may differ between individuals with and without asthma.

Participants in this study demonstrated a significant increase in stress rating from a baseline, low-stress timepoint to the early exam timepoint, followed by a significant decrease in stress rating from the early exam timepoint to the late exam timepoint. In addition, negative affect significantly changed over time, with a significant decrease from early to late exam period and both early and late exam period negative affect significantly higher than at baseline. These findings suggest that the naturalistic paradigm of undergraduate final examinations was effective at inducing stress and negative affect in the targeted population.

Based on previous research, we expected significant increases in H$_2$O$_2$ during final examination stress in undergraduate students and for such changes to be more pronounced in participants with asthma. Data findings indicated a non-significant increase in H$_2$O$_2$ from baseline to the first stress assessment, followed by a non-significant decrease from this first stress assessment to the second stress assessment. We did not find any significant difference for those with asthma compared to those without. While not significant, it is interesting to note that there was a mean level increase in H$_2$O$_2$ from baseline to the early stress period. The decrease in H$_2$O$_2$ from early to late exam stress periods aligns with the significant decrease in stress rating...
and negative affect. Interestingly, momentary stress rating was found to be a significant time-varying predictor of $H_2O_2$ in that individuals with higher than average stress ratings tended to have higher $H_2O_2$ measurements. Additionally, while cortisol levels were not found to be a time-varying predictor, inclusion of cortisol levels and time of saliva collection demonstrated a significant effect of sample collection time on $H_2O_2$ values. Samples collected later in the day tended to have higher $H_2O_2$ values overall.

Previous studies have found a significant decrease in FeNO during final examinations, which were more pronounced for those with asthma and depressive mood (Ritz, et al., 2015). Contrary to previous findings, the current study found that although FeNO was significantly higher in asthma participants at baseline, FeNO significantly increased in both groups across time and was not found to be related to baseline depressed mood. In comparing to previous studies in this area, it should be noted that the current asthmatic sample had greater disease severity (12.8% intermittent severity) compared to previous samples (48.6% intermittent, Ritz et al., 2015). Also, in contrast to prior findings, there were no significant changes in cortisol or FEV$_1$% over time. Again, these differences in findings may be a result of differing disease severity samples.

Additionally, although we attempted to counterbalance the sample across semesters, the final sample consisted of more participants from the Spring semester compared to the Fall semester. Given that respiratory viruses tend to be more prolific in the Fall and Winter seasons and environmental allergens more prolific in the Spring, this may have impacted the degree of inflammation in the overall sample, thus impacting the measurements of FeNO and possibly $H_2O_2$. In examining season as a potential moderator, it was found that participants in Fall semesters had lower FeNO levels and higher $H_2O_2$ values compared to those in the Spring.
semesters. Significantly higher FeNO values in the Spring may be the result of increased environmental allergens, such as pollen.

We did find a significant increase in PEF for the entire sample from baseline to the late stress assessment. This finding would likely support a sympathetic arousal in response to the stress of final examinations, as activation of the sympathetic nervous system results in airway dilation, which could lead to increased PEF values. Alternatively, the overall increase in PEF values may be a result of greater practice in healthy subjects, who are not typically trained in the maneuver of spirometry, given that PEF is more dependent on effort than FEV$_1$ (Ritz et al., 2002). In fact, examination of the data (Fig. 5) suggests that a majority of increase in PEF values for this sample are attributable to healthy individuals.

Contrary to our hypothesis, changes in FeNO were not related to changes in H$_2$O$_2$. This finding suggests that while FeNO and H$_2$O$_2$ may be linked at a molecular level, this relationship is likely influenced by a number of other factors. Both appeared to demonstrate increases during times of stress, but changes occurred at different times and to differing degrees. Thus, both appear to be impacted by the experience of sustained psychological stress but possibly through different physiological pathways. Further research would be necessary to elucidate factors associated with each individual biomarker.

Depression did not emerge as a significant moderator of changes in FeNO or H$_2$O$_2$, suggesting that baseline depressive symptoms do not influence how these two biomarkers change over time in response to sustained stress. The average baseline depression scores in the current sample were far below the cutoff scores for even mild depression. It is likely that this lack of variance in depressive scores resulted in a lack of power to detect any significant relations.
Importantly, medication use was a significant predictor of FeNO and H$_2$O$_2$. In alignment with previous studies, use of inhaled corticosteroids was related to lower levels of FeNO (Jones et al., 2002). Participants not prescribed any asthma-specific medication had higher H$_2$O$_2$ than those prescribed any asthma medications. Given this relation, it is important to further explore how different medications impact each of these biomarkers.

The present study has several strengths. To our knowledge, this is one of the first studies to examine changes in hydrogen peroxide in response to stress in a naturalistic setting. Previous studies that have examined hydrogen peroxide in exhaled breath have been cross-sectional in nature rather than longitudinal as in the current study. Additionally, this study attempted to examine potential psychosocial factors that impact the development of ROS, such as psychological stress, negative affect, and depressed mood. This is also one of the few studies to explore the relation between FeNO and H$_2$O$_2$, which deserves further exploration in future research, given that these biomarkers are indicated in the development of inflammation and oxidative stress.

The current study also has a number of potential limitations. There are likely numerous other factors that impact FeNO and H$_2$O$_2$ that we were not able to examine in the present study. For instance, research suggests that various environmental exposures and lifestyle factors, such as diet and exercise, can impact the development of ROS (Moller et al., 1996). Future research may focus on examining such factors and how they relate to FeNO and H$_2$O$_2$.

Research continues to explore the impact of ROS on the development of inflammation and oxidative stress, with the overarching goal to better understand, prevent, and treat a variety of chronic diseases such as asthma. It is important that future research continue to explore potential psychosocial mechanisms behind the development of ROS as a means of developing
preventative and treatment strategies for chronic diseases. Stress management strategies may prove an impactful intervention for the development of ROS but additional research is needed to explore this possibility.
References


Table 1. Participant Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Asthma (N = 39)</th>
<th>Healthy (N = 47)</th>
<th>P-value for sample differences *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>19.7 (1.4)</td>
<td>20.3 (2.0)</td>
<td>0.117</td>
</tr>
<tr>
<td>Sex, female, n (%)</td>
<td>28 (71.8)</td>
<td>34 (72.3)</td>
<td>0.572</td>
</tr>
<tr>
<td>BMI</td>
<td>22.3 (3.0)</td>
<td>23.4 (4.7)</td>
<td>0.291</td>
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<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>White/Caucasian</td>
<td>26 (66.7)</td>
<td>29 (61.7)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>6 (15.4)</td>
<td>5 (10.6)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>4 (10.3)</td>
<td>7 (14.9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (7.7)</td>
<td>6 (12.8)</td>
<td></td>
</tr>
<tr>
<td>H₂O₂, nM</td>
<td>323.4 (110.2)</td>
<td>339.6 (126.0)</td>
<td>0.605</td>
</tr>
<tr>
<td>FeNO, ppb</td>
<td>30.2 (29.5)</td>
<td>20.1 (13.2)</td>
<td>0.042</td>
</tr>
<tr>
<td>FEV₁ % predicted</td>
<td>99.3 (20.5)</td>
<td>97.3 (16.2)</td>
<td>0.692</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>2.5 (2.4)</td>
<td>2.3 (2.0)</td>
<td>0.931</td>
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<td>PEF, L/min</td>
<td>431.9 (117.9)</td>
<td>413.2 (126.4)</td>
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<td>HADS_D (0 - 15)</td>
<td>4.0 (2.8)</td>
<td>3.7 (3.6)</td>
<td>0.675</td>
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<tr>
<td>PANAS_NA (10 - 43)</td>
<td>14.6 (6.1)</td>
<td>15.0 (6.5)</td>
<td>0.743</td>
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<td>PANAS_PA (10 – 43)</td>
<td>26.5 (6.6)</td>
<td>26.9 (6.9)</td>
<td>0.762</td>
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<tr>
<td>Momentary stress (1-10)</td>
<td>4.99 (2.29)</td>
<td>4.65 (2.25)</td>
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<tr>
<td>ACQ (0 – 2.83)</td>
<td>1.0 (0.7)</td>
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<td>Asthma Severity b, n (%)</td>
<td></td>
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<tr>
<td>Intermittent</td>
<td>5 (12.8)</td>
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</tr>
<tr>
<td>Mild persistent</td>
<td>11 (28.2)</td>
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<tr>
<td>Moderate persistent</td>
<td>4 (10.3)</td>
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<td>Medication, n (%)</td>
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<tr>
<td>SABA</td>
<td>19 (16.2)</td>
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</tr>
<tr>
<td>ICS</td>
<td>6 (15.4)</td>
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<td></td>
</tr>
<tr>
<td>Combination LABA + ICS</td>
<td>3 (2.6)</td>
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<tr>
<td>Leukotriene Modifiers</td>
<td>2 (1.7)</td>
<td>NA</td>
<td></td>
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</tbody>
</table>

Data presented as mean (SD) unless otherwise indicated

*Definition of abbreviations: BMI = body mass index; H₂O₂ = hydrogen peroxide; FeNO = fractional exhaled nitric oxide; FEV₁ =forced expiratory volume in 1 second; PEF = peak expiratory flow; HADS_D = Hospital Anxiety and Depression Scale Depression; PANAS_NA = Positive Affect Negative Affect Scale, negative subscale; ACQ = Asthma Control Questionnaire; SABA = short-acting β-agonist bronchodilators; LABA = long acting β-agonist bronchodilators; ICS = inhaled corticosteroids; NA = not applicable, not assessed in the control group

*P-values for sample differences from t-test or χ²-test

b Severity ratings based on NHLBI Guidelines and determined for those not on maintenance medication or well-controlled on maintenance medication

*p < 0.05
Fig. 1 Changes in Momentary Stress Ratings
Fig. 2 Changes in Negative Affect

![Graph showing changes in negative affect over time]

- Y-axis: PANAS Negative Affect (10 - 50)
- X-axis: Non-Stress, Early Exams, Late Exams
Fig. 3 Changes in H$_2$O$_2$ concentrations in exhaled breath
Fig. 4 Changes in FeNO values
Fig. 5 Changes in PEF values
Fig. 6 Changes in FeNO Between Seasons and Group

Changes in FeNOlog Between Season for Asthma Group

Changes in FeNO Between Season for Healthy Group
Fig. 7 Changes in $\text{H}_2\text{O}_2$ Between Seasons and Group

Changes in $\text{H}_2\text{O}_2$ Between Seasons for Asthma Group

Changes in $\text{H}_2\text{O}_2$ Between Seasons for Healthy Group
Fig. 8 Changes in H$_2$O$_2$ by Asthma Medication