THE EFFECTS OF ACUTE YOGA ON ANXIETY SYMPTOMS IN RESPONSE TO A
CARBON DIOXIDE INHALATION TASK IN WOMEN

BY

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DISSERTATION

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Abstract

Background: Acute exercise is becoming an increasingly popular approach for treating anxiety symptoms in individuals with clinical and/or subclinical levels of anxiety. However, there has been limited empirical effort in studying such anxiolytic effects in individuals of this specific target population (i.e., those with elevated anxiety). This has consequently led to floor effects in the literature. There further has been inadequate focus on women, who are significantly more affected by these symptoms. Finally, yoga has not been adequately studied for such effects, although it has become a mainstream alternative health practice approach by many individuals.

Objectives: The purpose of this study was to examine the efficacy of a single bout of vinyasa-style yoga versus a stretching control condition for improving immediate and delayed cognitive and physical anxiety symptoms induced by a 5-minute, 7.5.% CO_2-inhalation protocol in women with self-reported high anxiety sensitivity.

Methods: In a within-subjects design, 18 women with elevated anxiety sensitivity completed 1 baseline session, and 2 experimental conditions in a randomized, counterbalanced order. Yoga and control conditions consisted of 40 minutes of guided vinyasa-style yoga and light stretching, respectively. Participants completed the 7.5% CO_2-inhalation task before, immediately after and 1 hour after the experimental conditions and filled out questionnaires on state anxiety, panic and anger before and after the inhalation. Respiratory measures (i.e., respiration rate, ventilation, tidal volume, CO_2 production) and self-reported overall anxiety data were collected via a metabolic cart attached to the mouthpiece during the inhalation task. Repeated measures ANOVAs were conducted for all outcome measures.

Results: Based on the results of the 3-way ANOVA, there was no evidence for a differential pattern of change in self-reported or respiratory outcomes in response to the inhalation task

between the 2 conditions ($p>.05$). There was a significant main effect of inhalation (i.e., from pre- to post-inhalation) on the self-reported panic and anxiety symptoms in both conditions ($p<.05$). Finally, collapsed over exposure and condition, there was a slight reduction in cognitive anxiety over time (i.e., from baseline to immediately post and 1-hour post-inhalation task).

Conclusion: A light-to-moderate intensity vinyasa-style yoga does not appear to be more efficacious than a light stretching session for improving symptoms of anxiety and panic in response to the anxiogenic 5-minute, 7.5% CO_2-air mixture inhalation task. However, there appears to be an overall effect of general physical activity for attenuating cognitions of anxiety, irrespective of the physiological responses. Furthermore, the inhalation task administered in the present study appears to be a reliable method for mimicking both acute panic and more generalized anxiety state symptoms under laboratory conditions.

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CHAPTER 1: INTRODUCTION

Anxiety disorders (ADs) are among the most prevalent forms of mental illness, affecting approximately 26% of the US population (Kessler et al., 2005) and costing approximately $43 annually (National Institute of Mental Health; NIMH, 2001).Women are twice as likely as men to be diagnosed with or suffer from clinical and subclinical levels of anxiety, and yet they are only half as likely to receive treatment for it. Furthermore, even though currently used behavioral and drug therapies can be effective (Hofmann & Smits, 2008; Mitte et al., 2005), only 15% of those diagnosed receive the even minimally adequate treatment they need (Mohr et al., 2010; Wang et al., 2002; 2005) due to a variety of reasons (e.g., high cost, lack of access, stigma). In fact, epidemiological studies indicate that ADs are often not recognized and that the doctors tend to overlook anxiety (Ormel et al., 1990). The lack of recognition was reported to be an even bigger problem for those with less severe symptoms related to their AD (Ormel et al., 1990). This is concerning, as ADs significantly increase the risk for other conditions such as depressive disorder, heart disease and diabetes (Shearer, 2007), and has been reported to be just as disabling as other disorders, such as major depressive disorder (Wittchen et al., 2000). These collectively underscore the necessity to find more accessible, alternative ways to effectively address symptoms of clinical and subclinical anxiety.

Exercise has been suggested as one such alternative method to help alleviate symptoms of anxiety. A recent meta-analysis (Ensari et al., 2015) of 36 randomized controlled trials (RCTs) on the anxiolytic effect of acute exercise concluded that there was a small but statistically significant (Hedge's g=0.16) beneficial effect of exercise. The moderator analyses indicated that exercise of high intensity and using the treadmill in particular, yielded larger effects ($p<0.05$). However, the authors concluded that the findings from this meta-analysis should be interpreted

with caution due to 2 major limitations of the literature; (1) Participants in these studies often did not have high baseline anxiety levels or a clinical anxiety disorder, resulting in floor effects and thus disabling researchers to determine the true effect of exercise on anxiety-reduction; and (2) the exercise stimulus (e.g., intensity, duration, type) was often not described in sufficient detail or lacked clear rationale. Accordingly, such acute exercise studies can help more accurately determine exactly what kind of exercise stimuli is best for reducing symptoms of anxiety in prone individuals. Once these effects are determined via acute bouts of exercise, such findings can then inform the design of chronic/longitudinal exercise interventions to compare their efficacy to other forms of therapy (i.e., behavioral and drug therapy). Accordingly, these limitations need to be addressed in well-designed studies, given the already existing evidence that exercise can be beneficial for anxiolysis.

Yoga as an exercise modality has been getting more common in the western parts of the world as an alternative treatment approach for a variety of negative health symptoms (e.g., pain, fatigue, mood) (National Center for Complementary and Alternative Medicine, NCCAM; 2007). Yoga practice involves physical postures that mimic stretching, balance and strength exercises. Depending on the style of the yoga, there can also be substantial focus on the breathing, deliberate attention on linking breath to movement and meditation within the practice. This is an important component of yoga as it pertains to anxiolysis. That is, some studies have reported that individuals suffering from anxiety disorders may misconstrue experiences that others may judge as normal response in such a way as to elicit intense feelings of panic and fear, i.e., increased anxiety sensitivity (AS) (Taylor, 1996; Taylor et al., 1991; Sandin et al., 2001). Accordingly, a regular practice of yoga involving greater focus on breathing and connecting it to movement may produce improvements in mood and anxiety symptoms by reducing the tendency to react to

negative mental and physical states with ruminative thought or maladaptive behavior)(i.e., desensitization). Furthermore, a possibly additive anxiolytic benefit of a faster-paced (e.g., moderate intensity) style of yoga (i.e., vinyasa) has not been investigated by previous research. Given the previous meta-analytic evidence on the efficacy of high intensity exercise for anxiolysis (Ensari et al., 2015), it is important to delineate such effects to determine if this style of yoga is a better method for alleviating anxiety symptoms in individuals who are prone to problematic anxiety levels.

One such approach to studying the anxiolytic effects of acute exercise is by using a well-established, validated paradigm of anxiety (and panic) induction, such as the CO_2-inhalation challenge (Bailey et al., 2007). The use of the CO_2-inhalation protocol allows countering the potential floor effects commonly observed in the previous studies (Ensari et al., 2015). This method has been validated to reliably induce panic and more general anxiety symptoms in both healthy and clinical populations (Bailey and Nutt, 2008). However, only 5 studies on exercise have been published to date that use the CO_2-inhalation paradigm as an anxiety induction method to study anxiolysis (i.e., Esquivel et al., 2002, 2008, 2012; Smits et al., 2009; Broman-Fulks et al., 2015). Furthermore, these studies have all utilized a 35% CO_2 single, vital-capacity inhalation procedure to induce/mimic symptoms of panic disorder in their participants. However, no studies thus far have used a longer (e.g., ~5 minutes) CO_2-inhalation protocol to mimic symptoms of Generalized Anxiety Disorder (GAD) in a similar setup. Accordingly, findings from these studies limit our ability to assess the true anxiolytic effects of exercise, as the anxiogenic effect of this stimulus might not be an accurate representation of most real life anxiety inducing situations (i.e., those that last longer than a few seconds) and other types of anxiety disorders (e.g., GAD). Indeed, it is possible that the longer duration is a better

representation of real life anxiety situations and therefore the response to an exercise stimulus might be more relevant. This needs to be addressed in subsequent investigations of the anxiolytic effect of acute exercise.

The proposed study further enrolled a sample of only women. Two reasons provided the basis for recruiting a sample consisting only of women: first, women are significantly more likely to suffer from a clinical GAD diagnosis and/or subclinical symptoms of anxiety and second, anxiogenic responses to the CO_2-inhalation protocol can vary between men and women, with women exhibiting greater anxiety symptoms. Some studies have indicated that this might be due to hormonal reasons (Ben-Zion et al., 1999; Perna et al., 2005). Controlling for this potential confounder can aid in the determination of a more isolated effect of exercise in response to the CO_2-inhalation challenge. Accordingly, the proposed study attempted to control for the phase of the menstrual cycle phase (i.e., luteal vs. non-luteal).

Aims and Hypotheses

The proposed study aimed to fill in the gaps in the current literature regarding acute exercise and anxiety symptoms by investigating the immediate and delayed anxiolytic effects of a single session of guided vinyasa-style yoga in response to 5 minutes of anxiogenic CO_2-inhalation protocol in a sample of women with high AS. A sample with a score of 25 or higher (i.e., 0.75 SD > published population norms) on the ASI were targeted based on the previous reports that this is a robust, consistent predictor of anxiogenic response to the CO_2-inhalation task (Smits et al., 2009).

Accordingly, the present study had several hypotheses:

Primary hypotheses:

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1) Yoga would significantly attenuate the anxiogenic responses to the CO_2-inhalation in comparison to the control condition. This hypothesis was partially based on the greater emphasis on the breathing meditation component inherent in yoga, which might make the participants more cognizant of their breathing and therefore respond differently to the CO_2-inhalation after this condition. In addition, it might be due to the greater cognitive engagement required while doing yoga than engaging in other exercise modalities (e.g., walking/running), activities and sitting quietly (e.g., during the control condition).

Secondary hypotheses:

1) Yoga condition would lead to slightly greater decreases in the post-CO_2 inhalation self-reported anger scores response in comparison to the control condition. This change effect would be greater in those with greater baseline depressive and anxiety symptom levels. This is based on the previous findings that anger has been consistently reported to co-occur in individuals with anxiety and depressive symptoms (Fava et al., 1998; Gould et al., 1996).

2) An acute improvement in the respiratory profile would accompany the self-reported improvements proposed in primary results. Specifically, respiratory rate (RR) and ventilation (VE) were expected to show an attenuation while tidal volume (Vt) was expected to increase, from before to immediately- and 1 hour-post-condition during the CO_2-inhalation tasks in the yoga condition. This was based on previously reported findings from individuals with sensitivity to this type of CO2-inhalation protocols (i.e., Schaeffer, 1958). These collectively could point to an acute, improved respiratory response profile (i.e., respiratory efficiency) as a result of the yoga intervention (vs the stretching control condition) in this sample of individuals with high AS (i.e., "high-responders").

This study can help determine the true effects of a bout of yoga, an exercise modality that is becoming exceedingly popular but has not been studied as extensively as others, under more ecologically valid conditions (i.e., biologically induced anxiety and in individuals with high AS). These results can subsequently inform the design of effective, targeted yoga and exercise interventions for individuals with clinical and subclinical symptoms of mood disturbance. Furthermore, the assessment of the physiological response can help determine whether behavioral and physiological responses to the inhalation task parallel each other or whether there are incongruences in individuals with AS. For example, no significant change in physiological response but significant improvement (i.e., reduction) in self-reported anxiety and panic symptoms might indicate cognitive adaptation to hyperventilation in this population. Accordingly, these results would collectively further underscore the feasibility of using yoga to target anxiety and panic problems. Ultimately, such findings can provide patients with anxiety disorders and health care professional working with such patients with guidelines for effectively using yoga as an exercise modality to effectively target their symptoms.

CHAPTER 2: LITERATURE REVIEW

The proposed randomized controlled study evaluated the efficacy of a single bout of guided vinyasa-style yoga for improving anxiety and related mood symptoms (e.g., anger) in a sample of women with self-reported AS. The subsequent sections review the following: prevalence of anxiety disorders (ADs) and generalized anxiety disorders (GADs) in the general population and comparisons between men and women, risk factors, antecedents and consequences associated with GADs, current treatment modalities and exercise as an alternative approach for treating anxiety symptoms, yoga as an exercise modality and alternative approach for treating anxiety symptoms, CO_2 inhalation protocol as a biological anxiety model, and the association between exercise-induced anxiolysis in response to CO_2-inhalation challenges. Finally, possible mechanisms through which CO_2 might be mimicking symptoms of generalized anxiety and how yoga might be countering this effect are also addressed.

Anxiety Disorders and Generalized Anxiety Disorder

Anxiety disorders (ADs) affect approximately ¼ of the US population (Kessler et al., 2005). Epidemiological studies have reported that they have the highest overall prevalence rate among psychiatric disorders, with a 12-month rate of 18.1% and a lifetime rate of 28.8% (Kessler et al., 2005; Kessler et al., 2005a; Ramos et al., 2003). GAD is the most frequently presented class of ADs in primary care (i.e., ~22% of patients with anxiety complaints). According to the Fifth Edition of the Diagnostic and Statistical Manual (DSM-V) (American Psychiatric Association, 2013) and the Tenth Edition of the International Classification of Diseases (ICD-10) (World Health Organization, 1992), GAD is characterized by excessive and inappropriate worrying that is persistent (lasting some months in ICD-10, six months or longer in DSM-V) and not restricted to particular circumstances. Patients with GAD have physical and

psychological symptoms (restlessness, fatigue, difficulty concentrating, irritability, muscle tension and disturbed sleep). GAD can be comorbid with major depression (but not arise solely in its context), panic disorder, phobic anxiety disorders and OCD in DSM-V, but must not meet full criteria for these in ICD-10.

GAD has an estimated prevalence of 8% in the general population and a remission rate of less than 20% within a 5-10 year period (Ballenger et al., 2001). Furthermore, the prevalence rates for GADs have remained consistent over time, in comparison to other psychiatric disorders, such as phobias and depressive disorders (Wittchen, 2002). Other studies have suggested that when compared with chronic medical disorders, there is evidence that GAD is just as equally disabling (Kessler, 2005a). Finally, given that the typical onset occurs in early adulthood based on a median diagnosis age of 26.6 (McLean et al, 2011), ADs clearly result in significant reduction in quality of life for all those diagnosed. Evidently, all of these aspects of the disease collectively affect various domains of functioning in individuals, including quality of life (Kroenke et al., 2007), cardiovascular health and social life (Wittchen et al., 2000). It further is a nation-wide problem, with significant job productivity/work impairment (Ludman et al., 2006; Sareen et al., 2005) and cost more than 43$ billion annually in the US alone (NIMH, 2001).

There is a large body of evidence that ADs, and especially GAD, pose a significantly larger problem for women than for men. For example, women are twice more likely than men to be diagnosed with an AD in adulthood (Pigott, 2003; Vesga-Lopez et al., 2008). Some authors have reported lifetime and 12-month prevalence rates of any AD as 1.7 and 1.79 times greater in women than in men (McLean et al., 2011). Furthermore, women with a lifetime AD diagnosis are more likely to also be diagnosed with another AD or depressive disorder (Saunders et al., 2012; McLean et al., 2011); and a greater illness burden is associated with AD in women than in

men, suggesting that ADs are more disabling in women than in men. These collectively underscore the importance of addressing this public health issue by targeting women for adequate and proper treatment of ADs.

Traditional treatment methods of pharmacotherapy (e.g., anxiolytics, antidepressants) and behavioral therapy (e.g., cognitive behavioral therapy (CBT)) have been indicated as moderately effective in treating ADs (Mitte, 2005; Mitte et al., 2005; Hofmann & Smits, 2008). Review of the literature suggests that these 2 modalities appear to be comparable in their efficacy for treating GAD, based on moderate effect sizes of d=0.60 and d=0.70 for pharmacotherapy and CBT, respectively (Gould et al., 1997). However, there are limitations to these approaches, in particular with regards to response, relapse and side effects. For example, 14–43% of the patients have been reported not to respond to treatment and 18–48% to relapse within 6 months (Barlow et al., 2000; Foa et al., 2005). In addition, these therapeutic approaches are associated with a range of unpleasant side-effects and limitations (e.g. high financial costs, inaccessibility, inconsistent response rates, and high relapse rates after discontinuation). For example, one study reported that scores on measures of quality of life (QOL) for these patients remained lower than those of the normal population even after successful treatment (e.g., Safren et al., 1997). This is a concern as low QOL may also represent a risk factor for relapse after successful treatment among anxiety disorder patients (Olatunji et al., 2007). A review published on the efficacy of pharmacotherapy concluded that there is still an important, unmet need for a fast-acting anxiolytic agent lacking unwanted side-effects of classical, full agonist, non-selective benzodiazepines (BZ) (Whiting, 2006). Furthermore, it has been reported that less than 15% of individuals with an AD diagnosis receive minimally adequate treatment (Mohr et al., 2010; Wang et al., 2005). Other studies have reported that not only men are more likely than women to

have access to and use medical services, women are significantly less likely to receive necessary medical services from health care professionals when compared to men (Wang et al., 2002; 2005). Collectively, these underscore the importance of identifying alternative symptom management methods to target symptoms of anxiety both in clinical and non-clinical populations, and in particular, for women.

Risk factors and antecedents of Generalized Anxiety Disorder

Several genetic/biological (e.g., impaired serotonergic neurotransmission, dysregulated corticotropin-releasing factor; CRF) and environmental (e.g., early life experiences, trauma) factors have been indicated in subsequent development of GAD in life. In general, it is thought that a genetic disposition coupled with early stress in critical phases of development may result in a phenotype that is neurobiologically vulnerable to stress and may lower an individual's threshold for developing clinical and subclinical levels of anxiety symptomology upon further stress exposure (Allgulander, 2010; Behar et al., 2009; Heim & Nemeroff, 1999). Therefore, the manifestation of clinical and subclinical anxiety symptomology can be thought of the confluence of dynamic, reciprocal interactions between genetic vulnerability and environmental stressors.

While the possibility of a genetic basis for GAD has been studied by several cohort studies and meta-analyses (e.g., Hettema et al., 2001; Torgersen, 1983), the results appear to be mixed. For example, one meta-analysis indicated 31.6% variance explained by familial aggregation (mostly due to genetic heredity) based on a best-fitting model to predict GAD (Hettema et al., 2001). The same model further predicted common familial environment only in women, and the rest of the variance due to non-shared environmental factors. Yet others have reported lower rates (i.e., 14.3% variance) when the GAD definition is more restrictive (Roy et al., 1995). Similarly, a large cohort twin study of monozygotic and dizygotic twins indicated that

the evidence for hereditary genetic factors in the development of GAD do not appear to be as strong as other risk factors (i.e., environmental and developmental) (Torgersen, 1983). Finally, studies using molecular genetic mapping methods to identify GAD-relevant loci have reported serotonin to be an important neurotransmitter. For example, one study reported that functional variations in the promoter of the serotonin transporter gene (the 5HTTLPR polymorphism) is associated with enhanced amygdala reactivity to the processing of emotional stimuli (Hariri et al., 2002), which is an important characteristic feature of GAD. Similarly, others reported that reduced expression of the 5HTTLPR was associated with increased limbic reactivity to emotional stimuli (Dannlowski et al., 2007). These findings collectively provide evidence for a genetic disposition to developing GAD, which might then exacerbate the impact of environmental risk factors.

Environmental factors, and in particular those leading to early childhood stress/trauma, have been indicated to have a strong influence on the physiological and cognitive manifestations of GAD later in life (Klein, 1980). The cardiorespiratory fitness (CRF) dysregulation theory is one of the most commonly accepted mechanisms to explain the mediation of this biopsychological pathway. First, early life stress and/or adverse experiences can result in persistent central CRF, which is the central coordinator of the endocrinological, autonomic, immunological and behavioral stress response. CRF neurons originate from the amygdala and project to the locus coeruleus of the Pons, which is involved with physiological responses to stress and panic. Specifically, CRF produces many responses that mimic acute stress, including increase in HR, mean arterial pressure and catecholamines, disrupted sleep-wake cycles, and increased locomotor activity (Heim & Nemeroff, 1999). These are all symptoms often experiences by those with an AD. For example, according to a birth cohort study (Moffit et al.,

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2010), patients with a pure GAD diagnosis significantly differed from healthy controls on certain risk factors at baseline, including; maternal internalizing symptoms, low socioeconomic status, maltreatment, childhood internalizing symptoms, and conduct problems. These respondents also had higher scores on inhibited temperament and greater negative affect. Similarly, other studies have reported childhood trauma and insecure attachment as significant correlates of subsequent development GAD (Borkovec et al., 2004). The insecure attachment (Bowlby, 1982) theory of GAD suggests that these individuals might grow up to perceive the world as a dangerous place, and lack adequate resources to cope with uncertain events (Cassidy et al., 2009). These findings should be interpreted with caution; however, as they lack temporal sequence and therefore require further empirical evidence for making conclusive statements.

In conclusion, it is evident that these risk factors are in constant interaction with each other in the etiology of GAD. Anxiety behavior is an expression of adaptive brain systems that are related to survival, and therefore the genes influencing these behaviors most likely act by increasing the sensitivity of neural circuits of harm avoidance and threat detection (Smoller et al., 2008). It is possible that this starting point leads to the aforementioned changes in the CRF and/or neurotransmitter systems in the CNS. These persistent, dynamic changes possible then interact with the environmental factors to manifest a full clinical GAD. Collectively, these findings have important implications for use of exercise training for targeting these symptoms, based on the neurobiological and cognitive pathways through which exercise has been indicated to induce changes in the CNS (e.g., Petruzzello et al., 1991). That is, studies have been indicating for decades that both acute and chronic physical activity can influence the factors discussed above. This underscores the importance of carrying out studies that investigate the underlying mechanism of the anxiolytic effect of exercise in prone individuals.

Physical Activity and Anxiety Disorders

Strong associations between the physical activity habits and anxiety symptoms, as well as the suggestion of physical exercise as an alternative avenue for addressing these symptoms have been reported in the literature (DeBoer et al., 2012; Jayakody et al., 2014). Accordingly, investigating patterns of physical activity in individuals with clinical and subclinical levels of anxiety is necessary so as to determine how they can be helped to increase their physical activity (PA) levels. However, there has been limited research that focuses on the PA and sedentary behavior patterns in individuals with pure GADs or even ADs. Instead, most data come from heterogenous clinical populations (i.e., samples of individuals with various different psychiatric symptoms and diagnoses). From these available data, there is evidence based on both self-reported and objectively quantified data that individuals with clinical and subclinical levels of anxiety are more sedentary than their non-anxious counterparts (Chapman et al., 2015; de Wit et al., 2011; Helgadottir et al., 2015). For example, in one cross-sectional study, researchers studied the PA behavior of 142 participants who reported symptoms of anxiety and/or mental disorders (e.g., depression). All participants filled out PA questionnaires on time spent walking for transport, walking for recreation, and moderate-to-vigorous PA (MVPA), and sedentary behavior (e.g., TV watching, work, computer use). Participants also wore an accelerometer for 7 days. Estimates of time spent in sedentary behavior, light activity and MVPA were calculated in bout durations. Results indicated that approximately 70% of participants reported activity levels that met the PA guidelines; however, only a small proportion of activity was accumulated in bouts of 10 minutes or more (Chapman et al., 2015). Furthermore, the questionnaires indicated that the participants were highly sedentary. Specifically, participants spent on average, two-thirds of their waking time sedentary, and a third of that was accumulated in prolonged bouts. Finally, the

authors reported that self-reported PA poorly correlated with accelerometer-based PA data. In another similar study conducted with individuals with depressive and/or anxiety disorders, authors characterized PA and sedentary behavior in a sample of 165 adults who were in outpatient treatment (Helgadottir et al., 2015). All participants scored their mental health symptoms using the Montgomery-Åsberg Depression Rating Scale (MADRS) and were interviewed using the Mini International Neuropsychiatric Interview (MINI) to assess their anxiety and depressive symptoms. Participants also wore an accelerometer for 7 days for objective quantification of their habitual PA levels. Results indicated that; 1) only one-fifth of the sample followed public health guidelines for PA, 2) each point increase in the MADRS was associated with a 2.4 minute reduction in light PA, independent of MVPA and sedentary time, and finally 3) MADRS was positively associated with the number of sedentary bouts (Helgadottir et al., 2015). The authors noted that there weren't any significant differences in PA or SB patterns between depressed and anxious individuals; and these findings are in accordance which those of Chapman et al. (2015). Finally, the authors reported that these individuals spent considerably more time in SB compared to the general population. Lastly, another epidemiological study demonstrated that after controlling for PA level and sociodemographic factors, severity of symptom was positively associated with sedentary behavior involving computer and TV consumption (de Wit et al., 2011). Interestingly, the authors of this study reported that individuals with ADs spent more time watching TV, compared to those with depressive or co-morbid depressive/anxiety disorder. Taken together, these findings are highly relevant to public health and health care practitioners. First, there is a need for conducting further epidemiological research to objectively quantify these patterns of behavior in individuals with pure GADs and ADs in general. Second, they demonstrate that there is a large potential for

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treatment for individuals with ADs who present with a range of symptoms related to their disease by monitoring and manipulating their PA and SB levels.

Exercise and Anxiety Disorders

An increasing body of research indicates that chronic/regular leisure-time physical activity (i.e., exercise training) that is correctly targeted and prescribed may be an effective form of intervention for individuals with clinical and subclinical symptoms of anxiety (Asmudsen et al., 2013; Herring et al., 2007). For example, one meta-analysis reported an effect size of 0.94 for the anxiolytic effect of exercise training when compared to no-exercise control conditions, based on data collected from samples (N=11) with high trait anxiety (Stich, 1998). The same meta-analysis reported that this value increases to 0.99 when only those with a formal diagnosis AD (N=7) are included in the analysis (Stich, 1998). This magnitude of change should be interpreted with caution, however, due to the small number of studies included in the analysis. Based on the available data, certain characteristics of the exercise training and anxiety assessment have been suggested for maximal effects, including; exercise-training interventions of shorter than 12 weeks in duration, using training sessions of at least 30 minutes and using a time reference longer than the previous week to assess anxiety symptomology (Herring et al., 2007). These findings support those from a previous meta-analysis that focused on samples with high trait anxiety (Petruzzello et al., 1991). Collectively, these findings provide important information for guiding the design of future interventions targeted toward individuals with GAD and in particular, women with GAD.

There is some evidence suggesting that exercise can be as effective as other traditional forms of treatment, such as CBT and drug therapy (Broocks et al., 1998; Fremont and Craighead, 1987; McEntee & Halgin, 1999). However, studies comparing exercise training to

pharmacotherapy and psychotherapy for their anxiolytic effects are significantly limited in number in comparison to those that investigate their antidepressant effects for individuals with depression. Therefore, there is a critical need for such comparative assessment in samples with ADs (and subsequently GADs), given the aforementioned limitations of drug and behavioral therapy (i.e., accessibility, relapse likelihood, side-effects) and the benefits of exercise. That is, exercise is accessible to everyone, it does not require a medical professional (beyond getting a medical clearance for starting exercise for those with possible risks for physical activity (PA) participation), and it does not bear financial costs. Furthermore, exercise is associated with positive side effects (e.g., improvements in multiple components of fitness, immune and heart health) and these aspects collectively make exercise an even more exciting potential treatment approach for ADs and a generally desirable everyday tool for all individuals for managing anxiety symptoms. This underscores the importance of conducting well-designed, high-quality randomized controlled trials (RCTs) to examine such comparative effects and subsequently determine the true effect of exercise on clinical and subclinical anxiety symptoms to help inform exercise intervention designs and clinical practice.

Acute Exercise and Anxiety

To design high-quality longitudinal studies (i.e., RCTs) that use the correct exercise stimulus (i.e., exercise prescription) in their exercise training intervention, it is beneficial to first investigate the immediate effects of exercise on anxiety symptoms. To that end, meta-analytic evidence indicates an anxiolytic effect of just a single bout of (i.e., "acute") exercise for individuals with high anxiety levels based on effect sizes of 0.24 (Petruzzello et al., 1991) and 0.16 (Ensari et al., 2015). Specifically, studies of acute exercise interventions indicate that using a quiet rest control condition, high exercise intensity, the treadmill as a modality, the 10-item

STAI for state anxiety assessment; with female, sedentary and diseased samples yielded larger effects for acute exercise. These meta-analyses and systematic reviews collectively agree on the under-sampling of participants with high baseline anxiety symptoms to counter possible floor effects, improper prescription of exercise treatment and lack of appropriate no-exercise control condition. Furthermore, some authors (e.g., Ensari et al., 2015) concluded that these results need to be cross-validated in subsequent studies and that even though the results are insightful, they should be interpreted with caution considering the small sample of studies included within some of the categories in the analysis (e.g., too few exercise modalities beyond cycle ergometer and treadmill walking, too few samples with high baseline anxiety levels). These collectively underscore the need for further investigation of alternative exercise modalities that have not been as extensively investigated for samples with increased proneness to symptoms of anxiety (i.e., individuals with clinical or subclinical anxiety symptoms) to understand the true anxiolytic effect of acute exercise stimuli.

One aspect of exercise that needs to be determined for anxiolysis is the intensity of the exercise stimulus. Some meta-analytic findings indicate the moderate intensity might be most effective for anxiety relief (Petruzzello et al., 1991); however, more recent meta-analytic evidence indicates that higher intensity might be more effective (Ensari et al., 2015). Some studies suggest that both low and high intensity exercise can be beneficial for reducing AS; however, the specific domains of anxiety and its associated symptoms (e.g., physiological vs psychological vs cognitive symptoms) might respond differently to different intensities. For example, Broman-Fulks et al. (2004) investigated the anxiolytic effects of light and high intensity treadmill walking on AS in 54 participants with elevated AS. All participants completed six 20-min treadmill exercise sessions at either a high-intensity aerobic (N=29) or low-intensity

(N=25) level. Self-ratings on AS, fear of physiological sensations associated with anxiety, and general anxiety were obtained at pre-treatment, post-treatment, and one-week follow-up. Results indicated that both high- and low-intensity exercise reduced AS (Broman-Fulks et al., 2015). However, high-intensity exercise caused more rapid reductions in the overall measure of AS and produced more treatment responders than low-intensity exercise. Furthermore, only high-intensity exercise reduced fear of anxiety-related bodily sensations. These findings suggest that there is potential merit in the further investigation of higher exercise intensities for better determination of such immediate effects.

Another aspect that warrants further investigation is the determination of the modalities of exercise that are most efficacious for anxiolysis. Systematic and meta-analytic reviews agree that there is generally a disproportionate focus on cycle ergometer followed by treadmill walking as the modality of exercise for investigation of its anxiolytic effects (Ensari et al., 2015; Petruzzello et al., 1991). This consequently makes it difficult for researchers to make comparisons between studies based on unbiased effect sizes. In addition, some of these less studied modalities have been indicated to be more popular among responders (e.g., walking, running, yoga) or to have other important health benefits (e.g., increased strength). It is therefore important to further investigate these lesser studied alternative modalities as it would allow us to determine if some of these modalities are as effective as or more so than the already more thoroughly-investigated modalities (e.g., cycling) for anxiolysis. For example, one such modality worth exploring is yoga and accordingly, yoga-based exercises.

Yoga as an Exercise Modality

The most widely cited and accepted definition of yoga is as an ancient discipline designed to bring balance and health to the physical, mental, emotional and spiritual dimensions

of the individual (Iyengar, 1976). Yoga is often depicted as a tree and has 8 components: Universal ethics, individual ethics, physical postures (i.e., "asanas"), breath control, sense control, concentration and bliss. Despite this multi-faceted nature of yoga, most people usually think of the physical aspects, followed by the concentration (i.e., meditation) aspect (Ross and Thomas, 2010).

Yoga has long been a popular practice in its birth place of India; however, it has gained considerable popularity in the western world in the recent years for its wide range of self-reported benefits; such as fatigue, pain, sleep and mood in healthy and ill populations (Oken et al., 2004; Oken et al., 2006; Ross & Thomas, 2010; Yurtkuran et al., 2007). According to a survey conducted in 2007 by the National Center for Complementary and Alternative Medicine (NCCAM), yoga is the 6th most commonly used complementary health practice among adults in the US (NCCIH, 2015). Despite this self-reported acknowledgment of the wide range of benefits of yoga; there is currently a small body of research focusing on the possible anxiolytic effects of yoga, and in particular, vinyasa yoga.

For example, Coeytaux et al. (2014) acknowledged that there are considerably more studies investigating benefits of yoga for conditions such as depressive symptoms and chronic back pain, in comparison to its potential anxiolytic effects. The authors conducted a comprehensive evidence-mapping meta-review, and concluded that there is inconclusive evidence for the anxiolytic effects of yoga based on the lack of sufficient good-quality studies (Coeytaux et al., 2014). Specifically, the authors noted that RCTs that address yoga interventions for GAD or panic disorder (PD) were lacking in the published literature. The two systematic reviews the authors were able to identify (da Silva et al., 2009; Cabral, 2011) were rated poor quality based on methodology (e.g., lack of proper diagnostic criteria for diagnosis of GAD or

PD, control group, randomization). The authors concluded that the limited evidence provided from non-randomized, lower-quality studies indicates a possible anxiolytic effect for exercise and therefore they can serve as a foundation for higher-quality RCTs addressing this question. These collectively point to the need to conduct high-quality RCTs to delineate the true anxiolytic effect of yoga. Similarly, a qualitative review (Ross & Thomas, 2010) comparing the health benefits of yoga to other forms of exercise reported that overall, yoga was just as or more effective than exercise for improving a variety of symptoms including heart rate variability (HRV), cholesterol, fatigue, negative affect, stress and sleep disturbance. It should be noted that although this review does not include any data on anxiety symptoms specifically, it does provide some rationale for further investigation of yoga for anxiolysis for the purposes of the current study. This is based on the literature indicating strong associations between some of the reported symptoms (e.g., HRV, negative affect, stress, sleep disturbance) and anxiety symptomology (e.g., Carney et al., 2003; Hughes et al., 2010).

This qualitative review (i.e., Ross & Thomas, 2010) noted several important limitations with regards to the current literature on the study of the efficacy of yoga. Specifically, with the exception of one study (Oken et al. 2006), none of the studies included in the analysis used a randomized, controlled design to test their hypotheses (Ross & Thomas, 2010). Similarly, the authors noted that the studies did not specify which poses were included within their yoga protocol. This is an important limitation for two reasons. First, provision of a detailed yoga protocol can help future researchers replicate their findings to test if specific yoga poses are more beneficial than others and if so, for which symptoms they are more beneficial. Second, there are several different types of yoga (hatha, iyengar, ashtanga…etc.), which differ in their emphasis on the various components of yoga such as asana, breathing and meditation. Furthermore, there are

variations in the way certain poses are conducted (e.g., type, duration, speed) among these styles. The relative effects of these different aspects on the said health outcomes, and in particular anxiety symptoms for the purposes of the current study, have not been adequately answered thus far.

There are some practical reasons for advocating yoga as an alternative exercise modality as well. First, yoga does not require any special equipment, attire or accessories; and it can be practiced almost anywhere. Second, almost all yoga poses can be modified to meet the needs and restrictions of the practitioner. This means that almost all injuries and/or physical limitations can be accommodated in a yoga practice. These qualities make yoga a very practical health approach for managing a wide range of symptoms and for the purposes of the present study, particularly symptoms of anxiety. Therefore, determination of the specific characteristics (i.e., intensity, duration, style) of yoga that might render most beneficial for anxiolysis has significant potential benefits for this target population. Yet, despite these benefits, yoga as an exercise modality has not been extensively investigated in high-quality studies (i.e., randomized controlled designs conducted with individuals who experience anxiety symptoms at baseline). Furthermore, the published studies of this kind have not investigated vinyasa yoga which, given its higher intensity compared to other styles, is an ideal style to study for experimental models of anxiety. Finally, investigation of this relationship can initiate the attempts at trying to understand the mechanism(s) behind the anxiolytic effect of yoga. Ultimately, this is useful to understand why and how yoga (or other exercise modalities) induce its anxiolytic effects in prone individuals.

Key issues in developing yoga protocols for research

Several issues need to be addressed with regards to the use of yoga protocols in research. Unfortunately, despite its increasing popularity and potential advantages as an exercise modality,

previous literature provides limited guidance to assist researchers in developing research and treatment protocols on yoga. Especially given the complex, multi-faceted (i.e., 8 different components) nature of this discipline, it is important to consider some key domains when developing testable yoga protocols for research purposes. A qualitative review discussing guidelines for developing such interventions (Sherman, 2012) stated that we currently lack the necessary empirical data for establishing robust, reproducible protocols of yoga and described eight domains to be addressed for accomplishing this in studies of efficacy testing: 1) style, 2) dose and delivery, 3) components, 4) specific class sequence, 5) dealing with modifications, 6) selection of instructors, 7) facilitation of home practice, 8) measurement of intervention adherence over time. These aspects should be decided on *a priori* based on the condition and the goals of the study, and provision of this information in publication is necessary for proper replication of the protocol by other researchers. This can subsequently allow for development of reproducible, effective yoga protocols for therapeutic purposes. Finally, these recommendations need to be considered within the context of the proposed study; that is, establishing the parameters of these domains for the development of the optimal yoga sequence for acute anxiolysis.

Anxiety Sensitivity as a measure of symptom severity

It is important to delineate the differences between trait anxiety (TA) and anxiety sensitivity (AS) for the purposes of the proposed study, as they represent two different, but related constructs (Taylor, 1996; Taylor et al., 1991; Sandin et al., 2001). Theoretically, AS is linked to the underlying belief that anxiety symptoms have catastrophic consequences. Specifically, AS is the fear of anxiety and physical sensations related to anxiety, and the belief that the experiences of anxiety/fear and related physical sensations have harmful somatic,

psychological or social consequences (Reiss & McNally, 1985; Reiss, Peterson, Gursky & McNally, 1986). Therefore, when put in a stress-provoking situation, an individual with high AS is expected to experience greater levels of panic and overall anxiety symptoms in response to physical sensations triggered by the CO_2-inhalation protocol, which is then more likely to set off a cycle that further exacerbates these symptoms (Bouton et al. 2001; Clark, 1986).

High AS has also been reported in individuals with generalized anxiety disorder (GAD; e.g., Deacon & Abramowitz, 2006) and in non-clinical worriers (e.g., Viana & Rabian, 2008). It is possible that the fear of uncontrollable psychological symptoms (i.e., worry) is characterized in both individuals with high AS and those with GAD (Rector et al., 2007). Consistent with this hypothesis, some studies suggest that GAD might be characterized by high AS cognitive concerns (Rector et al., 2007). Accordingly, the rationale for screening for AS is three-fold. First, it prevents the occurrence of possible floor-effects that have been reported as a systematic limitation in the literature on the anxiolytic effect of acute exercise (Ensari et al., 2015). Second, AS has been indicated to be a risk factor for ADs and panic disorders (McNally, 1990, 1996; Reiss, 1991; Sandin, Chorot & McNally, 1996; Taylor, Koch & Crockett, 1991; Taylor, Koch & McNally, 1992); and therefore is a strong predictor of whether the individual will respond to a CO_2-inhalation challenge. Finally, previous studies have reported that self-reported AS score is a robust predictor of how likely an individual is to react to the presumably anxiogenic CO_2-inhalation task (e.g., Smits et al., 2009). Therefore, recruiting a sample with high self-reported AS levels can ensure that the participants are more likely show anxiety symptoms upon exposure to the 7.5% CO_2-air mixture. Accordingly, we targeted a sample of women who scored at least a 25 on the Anxiety Sensitivity Index (ASI), which is ~1standard deviation (SD) above the population norm (i.e., 19). This cut-off score has previously been suggested as an indication of

"problematic anxiety symptoms" (Peterson & Phlemn, 1999; Taylor et al., 2007) and has been used by previous studies investigating exercise effects on anxiety as a cut-off point as well (e.g., Broman-Fulks et al., 2004).

Carbon Dioxide (CO$_2$) Inhalation Method as a Model of Anxiety

There are various experimental paradigms to study the human model of ADs, which attempt to induce acute anxiety symptoms that mimic real-life symptoms experienced by those with these conditions. Cognitive methods of anxiety induction include administration of stressful cognitive tasks, using anxiety-provoking vignettes and the Trier Social Stressor Test (TSST)). Biological methods of anxiety induction include administration of cholecystokinin tetra peptide (CCK$_4$), infusion of sodium lactate, caffeine ingestion and the breathing CO$_2$-enriched air mixture through a nasofacial mask or a mouthpiece. Of these methods, CO$_2$-inhalation challenge has received considerable attention (Gorman et al., 2001; Van den Bergh et al., 1993; Eifert et al., 2003; Olantunji et al., 2009; Bernstein et al., 2009; Lejuez et al., 1998).

CO$_2$-inhalation results in a range of acute physical (e.g. tachycardia, breathlessness, sweating, dizziness) and psychological (e.g. anxiety, nervousness, urge to escape, fear) symptoms. In general, concentrations of CO$_2$ used in research with human subjects typically range from 2.2% to 65% CO$_2$ mixtures, with higher concentrations of CO$_2$ producing greater anxiety and panic symptoms. Similarly, duration of administration also varies among studies; with single and double inhalations often used to mimic panic symptoms and longer inhalations of up to 20 minutes utilized to mimic more generalized states of anxiety (Bailey & Nutt, 2008). The main advantage of using CO$_2$-enriched air is that it is a safe, noninvasive, and reliable means of producing clinically-relevant sensations that remit within several minutes upon return to breathing normal room air. Furthermore, it produces symptoms that are similar to those observed

in individuals with anxiety disorders and that the administration can be safely and easily controlled. Consequently, it is frequently used as a realistic and valid model for studying anxiety response in humans without causing any permanent adverse effects.

There are 2 general clusters of conditions studied using the CO_2-inhalation protocol; panic symptoms (i.e., for experimental modelling of panic disorders) and general anxiety symptoms (i.e., for experimental modelling of other types of anxiety disorders). The CO_2-inhalation protocol has been more commonly used for its ability to reliably induce panic like symptoms that complies with the formal criteria of a clinical panic disorder with just a single or double inhalation of 35% CO_2-air mixture. On the other hand, fewer studies have investigated its ability to mimic more general anxiety symptoms to investigate the possible existence of similar effects on symptoms of GAD. To this effect, these studies have assessed the applicability of inhalation procedures that involve lower concentrations of CO_2-air mixtures (e.g. 5%, 7%, 7.5%) inhaled for longer durations (e.g., 10-20 minutes). These studies have collectively reported that both clinical and community samples can display signs of generalized anxiety (e.g., Poma et al., 2005; Bailey et al., 2005). Literature reviews on the CO_2 challenge protocol (e.g., Colasanti et al. 2012; Vickers et al., 2012) conclude that up to 20 minutes of 7.5% CO_2 and a few breaths of 35% CO_2 are safe and reliable procedures for inducing symptoms of panic, distress and anxiety that resemble real life symptoms; as well as increased blood pressure and heart rate (i.e., increased autonomic arousal). As such, this model of anxiety has been used in psychiatric research for two decades without any unexpected adverse event (Bailey et al., 2008; Papadopoulos et al., 2008).

Application of the CO_2 inhalation protocol provides further advantage with respect to our ability to assess the neurobiological basis of general anxiety symptoms. A variety of

neurotransmitter systems (e.g., gamma amino butyric acid; GABA, serotonin, epinephrine; NE, growth hormone; GH) have been investigated in an attempt to understand the central mechanisms that might be regulating CO_2-induced anxiogenesis, and the serotonergic and GABA-ergic neurotransmitter pathways have received most support thus far.

There is converging evidence from experimental studies that the midbrain serotonergic neurons acting as CO_2-chemoreceptors might be the underlying mechanism via which CO_2 inhalation might be inducing its effects (e.g., Bailey et al., 2007; Ben-Zion et al. 1999; Bocola et al., 1998; Corcoran et al., 2009). For example, one study (Bailey et al., 2007) reported that administration of paroxetine (a selective serotonin reuptake inhibitor; SSRI) inhibits responses to 7.5% CO_2; whereas other studies (Ben-Zion et al., 1999; Meiri et al., 2001) observed that serotonin antagonists effectively enhance the anxiety induced by CO_2 inhalation. Similarly, a central role of the GABA-A receptor has also been proposed to explain CO_2-induced anxiogenesis, based on findings from studies of benzodiazepines with clinical and volunteer participants. For example, in one proof-of-concept study comparing a full GABA-A receptor agonist (i.e., alprazolam), and a selective GABA-A receptor agonist (i.e., zolpidem) to a placebo, both drugs attenuated subjective feelings of anxiety and tension in response to the 20 minutes of 7.5% CO_2-inhalation (Bailey et al., 2008). These findings are in line with those of earlier studies that reported attenuated panicogenic effect of GABA-A receptor agonist (i.e., alprazolam) in response to 20 minutes of 7% CO_2-inhalation in both individuals with panic disorders and healthy volunteers (Woods et al., 1986, 1989). Of note, even though the panic disorder (PD) participants in Woods et al. (1986) reported significantly reduced ($p<0.05$) somatic symptom (i.e., choking sensation, dizziness, hot/cold flashes, faintness, weakness) severity from pre- to post-inhalation after being treated with the GABA-A receptor agonist, there were no significant

changes in the ventilatory response (i.e., $\Delta VE/\Delta pCO_2$) between treated and untreated

participants. Specifically, there were no correlations between somatic symptoms during

rebreathing and ventilatory response. This suggests that the locus of behavioral sensitivity to CO_2

in vulnerable individuals is likely some site other than the central chemoreceptors (Woods et al.,

1986). These findings are interesting because they are partially in contrast with those of another

study (Bocola et al., 1998) that reported that at baseline, individuals with PD differed

significantly in ventilatory response in comparison to healthy individuals, though the other

ventilatory parameters were comparable. Furthermore, the control participants did not experience

any significant panic symptoms in response to a 5-minute 7% CO_2-air mixture inhalation

(Bocola et al., 1998). A main limitation of these studies, for the purposes of the current study, is

that they were conducted with individuals with PD, not GAD. Accordingly, these findings should

be interpreted with caution and require further replication before reaching any conclusions.

In conclusion, application of a CO_2-inhalation protocol for modeling GAD in a variety of

experimental designs (e.g., exercise testing) has multiple advantages. First, it provides evidence

for its feasibility for modeling real-life, sustained, GAD-like symptoms in volunteer participants.

This can then allow researchers to use this protocol to assess the level of anxiety reduction of a

multitude of potential anxiolytic methods for individuals with AS. Next, this method presumably

affects the central nervous system directly, unlike other anxiogenic methods (e.g., lactate

infusion), and therefore it allows for testing of the central neural mechanisms through which

exercise could be inducing its anxiolytic effects. After it is validated in acute exercise studies,

this protocol can be used in chronic exercise training studies in a pre-/post-intervention design to

investigate if susceptibility to the anxiogenic effects of the CO_2-inhalation protocol is alleviated

after exercise training. Collectively, these provide further justification for undertaking the proposed study.

Acute exercise, CO$_2$ Inhalation and Anxiolysis

To date, there have been only 5 published studies that investigate the anxiolytic effect of acute exercise in response to a CO$_2$-inhalation challenge (Broman-Fulks et al., 2015; Esquivel et al., 2002, 2008, 2012; Smits et al., 2009). First, a pilot study (Esquivel et al., 2002) investigated the anti-panic effects of acute cycling at an intensity reaching >6m$_M$ of blood lactate elevation in healthy adults in response to a vital capacity single-inhalation of 35% CO$_2$-air mixture. In the control condition, participants cycled at a "minimal" intensity (i.e., no elevation in blood lactate levels). The results indicated that participants in the active exercise condition reported attenuated panic responses after the CO$_2$-inhalation challenge in comparison to those in the control condition. Interestingly, there were no correlations between blood lactate levels and the reduction in CO$_2$-induced symptomology (Esquivel et al., 2002). However, this study has several limitations. First, the exercise control condition involved cycling, even though it was of "minimal exertion", and so it is difficult to make an isolated attribution of the results to the cycling manipulation alone. Next, the exercise group had higher panic scores pre-CO$_2$ inhalation than did the controls. Therefore, this higher baseline may have influenced the difference in CO2-caused PSL increase.

The authors of this study undertook a follow-up study that investigated the anti-panic effects of acute cycling in response to a vital capacity single inhalation 35% CO$_2$ inhalation (Esquivel et al., 2008). Eighteen patients with PD performed either moderate-vigorous intensity cycling or "very light intensity" cycling (i.e., control condition) on an ergometer. The duration of the exercise was variable; with the goal of the exercise stimulus being reaching 80-90% of their

age-predicted maximal heart rate (HR_{max}). On average, women exercised at 100W and men exercised at 150W, at 70rpm. Participants either exercised for 15 mins, or until exhaustion. The Visual Analog Anxiety Scale (VAS-A) and the Panic Symptom List-IV (PSL-IV) were used to assess panic symptoms before and after the CO_2-inhalation challenge. A meaningful change in score was determined as a change of at least 4 points on the PSL-IV (Esquivel et al., 2008). The investigators also assessed blood lactate levels. The results indicated that the panicogenic response to CO_2-inhalation was significantly attenuated after the moderate/vigorous cycling, in comparison to the very light cycling condition. Specifically, 3 out of 10 and 8 out of 8 individuals in the exercise vs control conditions, respectively, had significant levels of increase on their PSL scores (Esquivel et al., 2008). Interestingly, the VAS-A scores were not significantly different between conditions ($p>0.05$). Finally, blood lactate levels were significantly increased after the moderate/vigorous intensity cycling ($p<0.05$), and this was comparable to the levels reached in previous studies in which participants received lactate infusions (Liebowitz et al., 1984). This is an important finding because there is some evidence that lactate can be anxiogenic (Cowley et al., 1988), and yet the self-reported measures on anxiety and panic symptoms were reduced in the moderate/vigorous cycling condition. This study has several limitations. In addition to the small sample size and the between subjects-design, the exercise manipulation was not clearly described or based on an exercise prescription or rationale. Essentially, participants exercised at different intensities (the intensity was based on age-predicted HR_{max}) and of different durations (15 minutes or until exhaustion). There were also no standardized warm-up or cool-down periods built into the exercise. However, this study provides important preliminary data on the potential beneficial effects of acute exercise for improving panic and anxiety symptoms in clinical populations.

The same authors conducted another follow-up study (Esquivel et al., 2012), investigating the antipanic effects of moderate/vigorous cycling (in comparison to a control condition of very light cycling) in response to a single or double inhalation of 35% CO2 air mixture. Thirty-one healthy participants completed 4 sessions in a randomized order: either moderate/vigorous or very light intensity cycling, and either 1 or 2 inhalation of 35%CO2 air mixture. All participants rated their anxiety and panic symptoms using the Visual Analog Discomfort Scale (for assessing discomfort), Visual Analog Fatigue Scale (for assessing fear) and the Panic Symptom List-IV (assessing acute panic symptoms). The results indicated that the symptom-reducing effect were largest in response to the 2 inhalation condition, based on the smaller increases in the fear and discomfort scores after the moderate/vigorous cycling condition in response to the double CO_2-inhalation ($p<0.05$) (Esquivel et al., 2012). On the other hand, there weren't significant differences in the post-exercise PSL scores between conditions ($p>0.05$); however, this might have been due to the baseline differences between groups. Finally, the results also indicated the when the effect of exercise alone was assessed, moderate/vigorous intensity cycling more effectively attenuated the panic and discomfort, but not fear (Esquivel et al., 2012). Similar to the findings from the previous 2 studies, this study also indicated that exercise alone increased the scores in the many items of the PSL related to physical symptoms that are in common with panic symptoms. Accordingly, this study suggests that the physical symptoms induced by exercise that mimic panic/anxiety are not necessarily accompanied by psychological symptoms of panic/anxiety. Collectively, these findings underscore the promise that exercise holds for effectively targeting clinical and subclinical symptoms of panic/anxiety without causing the individual any significantly perceived distress about their physical symptoms after exercise.

A similar study was conducted with 92 individuals without history of panic attacks (Smits et al., 2009). In a between-subjects design, participants completed a single vital capacity inhalation of 35% CO_2-air mixture before and after being randomized to either a 20-minute moderate-intensity (i.e., 70% of HR_{max}) treadmill walking condition or seated quiet rest. Gender and measures of negative affectivity (NA) and AS were included in the data analysis as control variables. The results indicated that exercise significantly reduced the anxiogenic response to the CO_2-inhalation challenge after the exercise ($p<0.05$). Furthermore, the effect size remained in the moderate-to-large range (i.e., $\eta_p^2>0.07$) even after controlling for gender, AS and NA. Specifically, the effects of exercise on response to challenge did not vary as a function of gender, but approached significance where women reported greater increase in symptom severity as a result of the challenge than men ($p=0.07$). Similarly, including cardiorespiratory fitness as a covariate did not change the observed results. Of note, the authors of this study have also demonstrated that AS appears to be a robust predictor of CO_2 reactivity; that is, higher the scores on ASI, greater the panicogenic and anxiogenic response to CO_2 inhalation challenge. This finding provides the rationale for consideration of ASI scores in the prospective sample for inclusion in the current study.

Finally, a recent study compared the relative effects of a single bout of aerobic vs resistance training on symptoms of panic and anxiety (Broman-Fulks et al., 2015) in healthy, sedentary individuals. Seventy-seven participants were randomized to complete 20 minutes of moderate-intensity (i.e., 70% of age-predicted HR_{max}) aerobic exercise (N=25), resistance training (N=26) or rest (N=26), followed by a single vital capacity inhalation of 35% CO_2 air mixture. All participants completed the STAI-S, ASI, API, Distress Tolerance Scale (DTS) and the Discomfort Intolerance Scale (DIS) at baseline, after exercise/rest and after the 35% CO_2-

inhalation challenge. The results indicated that ASI scores were significantly reduced after both aerobic resistance exercise, with more than 20% of the aerobic exercisers and more than 25% of the resistance exercisers exhibiting a clinically meaningful decrease in AS and also significantly less reactivity to the CO_2 inhalation (Broman-Fulks et al., 2015). The same pattern was observed on the API scores, with both exercise conditions resulting in comparable reductions in scores and reactivity ($p=0.009$ from baseline to post exercise, $p<0.001$ from post-exercise to post-CO_2-inhalation). There were no significant improvements on the other scales from pre- to post-exercise ($p>0.05$). On the other hand, rest condition yielded non-significant trends toward larger increases ($p=0.15$ from post-rest to post CO_2 inhalation) (Broman-Fulks et al., 2015). Interestingly, no significant interactions were observed on the scores of DIS, DTS and STAI-S ($p>0.10$ for all). This might suggest that the API and the ASI are more sensitive and applicable scales for measuring responses to panic/anxiety induced by inhaling CO_2-air mixtures. Alternatively, it is possible that the construct of "distress" is not relevant for this type of protocol. An interesting aspect of this study is that authors administer the ASI, which measures a trait construct, acutely. It appears that the participants' perceptions of their sensitivity to anxiety cues were slightly altered after the single exercise bout. This is unexpected, as by definition, trait measures are not expected to change in such a short amount of time or upon exposure to a single time-point event. Therefore, the results of this questionnaire should be interpreted with caution. Collectively, findings from this study provide further evidence acute exercise, regardless of modality (i.e., aerobic, anaerobic), can provide relief from symptoms of anxiety and panic in response to an acute stressor (i.e., CO_2-air mixture inhalation).

Accordingly, there are several differences between these studies and the present study, and therefore possess limitations in their ability to examine the interactions between acute

exercise and GAD symptoms. First, all of these studies used an air mixture with a CO_2

concentration of 35%, which is a model of PD, but not GAD. This limits our ability to make

inferences about the latter, as the literature agrees on the substantially different effects on the

body when exposed the 35% CO_2 concentration in comparison to lower (e.g., 7.5%)

concentrations. Second, none of these studies measured delayed responses to the CO_2 inhalation

but instead only assessed it immediately post-condition. Therefore, there are currently no

published data that describe exercise-induced delayed anxiolysis in response to a CO_2-inhalation

challenge. Third, cycle ergometer and treadmill walking are the primary exercise modalities

employed in their designs, with only 1 of the studies (Broman-Fulks et al., 2015) using resistance

training as one of the exercise conditions. Given the meta-analytic results that there is a

disproportionate sample of published studies that use these modalities, alternative exercise

modalities warrant further investigation for their potential anxiolytic effects. Another limitation

is that 3 of these studies (Esquivel et al., 2002, 2008, 2012) did not use a no-exercise control

condition. In these studies, the control condition consists of very light cycling at no resistance,

which by definition, still constitutes exercise behavior, therefore limiting its ability to provide as

a valid control condition for an exercise stimulus. Finally, it should be noted for these studies

that the higher intensity cycling was significantly more effective than the very light cycling (i.e.,

control condition) for reducing the panicogenic response to the CO_2-inhalation task, providing

further evidence that higher intensities are more efficacious for reducing symptoms of anxiety

and panic.

Yoga and CO_2 Inhalation and Anxiolysis

There are several mechanisms suggested in the literature to explain the anxiolytic effect

of exercise. These include physiological models (e.g., thermogenic effect, endorphin hypothesis,

afferent affect) and cognitive/psychological models (e.g., self-efficacy, physiological toughness, the opponent-process theory, time-out hypothesis). These theories have been supported by research encompassing the past several decades (Petruzzello et al., 1991). The studies investigating these mechanisms have mostly used walking and cycling as the exercise modality in their designs and it is timely, given the increased interest and the practical aspects that these mechanisms are also tested using a well-designed, reproducible yoga protocol. While a direct assessment of such a causal mechanism is beyond the scope of the present study, it is important to discuss some possibilities as much as the current evidence allows.

A preliminary study by Streeter et al. (2010) investigated the changes in levels of GABA in the CNS after 12 weeks of iyengar yoga in comparison to walking. Yoga-naïve participants underwent proton-Magnetic Resonance Spectroscopy (pMRS) before and 12 weeks after the intervention, as well as for a 3rd time after completing a single session of yoga or walking after their 2nd scan. The results indicated that yoga was significantly and positively correlated with improved self-reported anxiety levels and also GABA concentration as suggested by the scan analyses (Streeter et al., 2010). Specifically, there was a 13% increase in the GABA levels after a bout of yoga when the participants have been trained for 12 weeks. This suggests that the physiological effects of yoga might be occurring later than the psychological effects experienced by the individual. Overall, this was first study to demonstrate that 1) increased thalamic GABA levels are associated with improved mood and decreased anxiety, and 2) yoga postures are associated with a positive correlation between acute increases in thalamic GABA levels. These findings are in line with others that investigate this relationship using pharmacological agents (Bailey & Nutt, 2008). The ability of yoga to induce these changes might be due to improved (i.e., increased) activity in the parasympathetic nervous system (PNS), which is one of the

mechanisms previously proposed (Petruzzello et al., 1991). However, these interpretations should be made with caution as this study had some limitations. First, the acute bout of walking and yoga were done after 12 weeks of chronic exercise training, and therefore it is impossible to conclusive attribute these observed effects to the acute bout of yoga (or walking). Second, the intervention was semi-home based, such that participants were encouraged to practice on their own for the 2nd part of the intervention. During this time, participants averaged 1 session/week of yoga; therefore it is possible that with more frequent weekly sessions that there might have been larger effects observed in the 2nd time point assessment. Finally, in addition to the hypothalamus, there are other brain areas (e.g., raphe nuclei, midbrain periaqueductal grey (dPAG) have been implicated in anxiety and associated mood symptoms (e.g., depressive). Therefore, assessment of GABA in these areas might yield different patterns of change both over time and acutely.

The findings of Streeter et al. (2010) have further significance for the purposes of the current study. As mentioned earlier, one of the proposed mechanisms of the CO_2-induced anxiolysis is that of GABA-ergic neurotransmitter system. That is, there is some evidence that inhaling CO2-enhanced air mixtures increase anxiety symptoms in sensitive individuals significantly influences GABA concentrations (Johnson et al., 2005; Nutt & Bailey, 2008). However, the results are not necessarily in the expected direction; these studies report an increase in GABA levels after CO_2 exposure, which is in the opposite direction of previous studies that presumed that the anxiolytic effects were induced through an increase in GABA concentrations in the CNS. Collectively, these findings underscore the need for further research to delineate mechanisms through which acute yoga might be anxiety reducing and other mood-improving effects.

Desensitization to changes in respiration or an improved respiratory control induced by the acute yoga is another possible mechanistic explanation for why yoga might help attenuate the anxiogenic and panicogenic response to CO_2-enriched air inhalation. This suggestion is based on a limited number of previous studies in the literature investigating the respiratory responses to inhaling air mixtures with similar CO_2 concentrations (e.g., 7-7.5%). For example, one such study investigated the respiratory responses to inhaling 15 minutes of 7.5% CO_2-air mixture in a sample of 42 healthy adults and concluded that based on inter-individual differences, they could be separated into "high" vs "low" ventilation groups (Schaefer, 1958). That is, those classified as "low ventilators" had greater tidal volume (Vt) but lower respiratory rate (RR) than those classified as "high ventilators". The group differences were also apparent in the physical symptoms reported by the participants (e.g., dizziness, headaches, dyspnea) and as such, these symptoms were reported considerably more frequently and intensely by the participants categorized into the "high" ventilation group. Similarly, another study investigated the respiratory response to 7% CO_2-inhalation in patients with panic and anxiety disorders in comparison to healthy control participants (Papp et al., 1997). The authors reported that RR significantly increased in response to the inhalation task in the former group of individuals. These individuals were also reported to experience heightened respiratory sensitivity to the inhalation task (Papp et al., 1997). Collectively these studies indicate that individuals who possess cognitive worry (i.e., a primary feature of trait anxiety) may also be considerably more sensitive to changes in their breathing patterns (e.g., hyperventilation) and therefore respond with greater negative symptoms. Based on these prior reports, it would make sense to expect the sample in the current study to also exhibit similar symptoms. That is, that they would have relatively higher RR and lower Vt, which may potentially be amenable to acute change.

Accordingly, a breathing-focused physical exercise (i.e., vinyasa yoga), might be an ideal way to target these symptoms in this particular population.

The Present Study

The present study is novel in several ways. First, the CO_2-inhalation protocol as a biological human model of general anxiety and/or panic has never been investigated by inclusion of 2 time points after an exercise stimulus (i.e., immediately after and 1 hour after). Previous studies examining the anxiolytic effect of acute exercise have thus far only administered a single or double CO_2-inhalation challenge immediately after the exercise stimulus (Esquivel et al., 2002, 2008, 2012; Smits et al., 2009; Broman-Fulks et al., 2015). Investigation of whether the anxiolytic effects of a single bout of exercise sustains at later time points (i.e., 1 hour post-condition) can help determine the duration of such anxiolytic effects and subsequently help inform future exercise study designs. Second, this is the 1st study to combine a longer breathing period (i.e., 5 minutes) and 7.5% CO_2-air mixture to induce anxiety symptoms, with the exception of another, slightly modified study (i.e., Bocola et al., 1998) that used a 5 minute-inhalation of 7% CO_2-air mixture concentration. Previous studies have safely and successfully employed at least 1 of these components, but not both at the same time using a sample that has high baseline AS. The present study used a 7.5% CO_2-air mixture based on previous studies that have successfully administered various CO_2 concentrations below 20% as a proxy for anxiety symptoms (Van den Bergh et al., 1993; Eifert et al., 2003; Olantunji et al., 2009; Bernstein et al., 2009).

Next, this is the first study that we are aware of that used vinyasa-style yoga, which is selected for its higher intensity in comparison to more commonly studied styles of yoga in the literature thus far (e.g. hatha, restorative, kundalini), as well as it is focus on synchronizing each

movement with an inhale or a exhale. Vinyasa yoga, also known as flow-yoga, coordinates breath patterns with a series of asanas (i.e., "1 move/breath") and is therefore considered to be a more challenging form of yoga. During Vinyasa yoga, the practitioner moves from position to position while either inhaling or exhaling at a much faster pace than experienced in Hatha yoga (Cowen and Adams, 2007), or other styles (e.g., kundalini, iyengar). As an exercise modality, this faster pace makes vinyasa yoga a higher intensity form of exercise. Given the meta-analytic evidence that high intensity exercise is optimal for observing anxiolytic effects after an acute bout of exercise, it is evident that vinyasa yoga is an excellent candidate for efficacy testing. Furthermore, the present study took into account the published recommendations on how to design and implement yoga protocols for research purposes (Sherman et al., 2012). Based on these suggested guidelines, the following characteristics have been determined in the proposed yoga protocol: faster pace (i.e., in an attempt to achieve a moderate-to-higher intensity); a single, 40 minute bout (i.e., acute, ecologically valid manipulation); components including asana, breathing and meditation (with the primary focus on the asana).

Similarly, the current study compared vinyasa-style yoga in comparison to an active control condition (i.e., light stretching). Previously conducted similar studies have not made such comparisons. We are aware of 1 study (Broman-Fulks et al., 2015) that compared resistance training to treadmill walking; and 1 other study that compared treadmill walking to a no-exercise control condition (Smits et al., 2009). The other 3 studies investigating the anxiolytic effects of exercise on the CO_2-inhalation challenge have used a single cycle ergometer exercise stimulus (Esquivel et al., 2002, 2008, 2012). Furthermore, 3 of these studies (Esquivel et al., 2002, 2008, 2012) have not employed a true no-exercise control condition. Accordingly, the proposed study

aims to investigate the efficacy of such a yoga-exercise protocol in response to a biological anxiogenic (i.e., CO_2-inhalation) in a randomized, controlled study.

Finally, none of the previous 5 studies have used a women-only sample to test their anxiolysis hypothesis, which accordingly limits our ability make inferences regarding women based on their findings. This is an important gap in the literature to address for 2 reasons. First, given the significantly increased prevalence of ADs and anxiety symptoms in women compared to men, a greater emphasis on women samples is justified. Second, there is some evidence that menstrual cycle phase influences the response to the CO_2-inhalation challenge, but only among women with high AS. Specifically, it has been reported that women who score high on AS report increased cognitive panic symptoms in response to the challenge during the premenstrual phase compared to the follicular phase, and compared to women who score lower on AS assessed in either cycle phase (Nillni et al., 2012; Perna et al., 1995). Given the small body of currently-existing research on this topic, and the general under-representation of women in health research that is often attributed to difficulty of controlling for hormonal fluctuations, it is timely to include this variable in an experimental design that investigates a mood outcome measure (i.e., anxiety) that affects women twice as much as men.

CHAPTER 3: METHODS AND MATERIALS

Participants

Prospective participants were identified from the University of Illinois at Urbana-Champaign campus with the goal of recruiting 18 women participants in total. Sample size was selected based on previously conducted studies of CO_2-inhalation and anxiety (Bailey et al., 2007; Diaper et al., 2012). These studies indicated that for a within-subjects study of anxiolytic effects on 7.5% CO_2-inhalation, to detect a significant difference at alpha=0.05 level, and a sample size (N) of 13 would be required. To allow for variation in parameters (i.e., to control for potential baseline trait differences), increasing this number to 18 has been recommended (Diaper et al., 2012). Fliers advertising the study were distributed through classes not taught by any members of the experimental team. Fliers were also posted on bulletin boards in the common campus spaces, such as building hallways. Once a prospective participant expressed interest via email or phone, the study protocol was described to them via telephone. If the individual was interested, the screening for the following inclusion criteria were carried out: (a) women between 18 and 54 years of age; (b) willingness and ability to do yoga poses and complete multiple mood assessments; (c) a score of 25 or higher on the ASI (indicating clinically meaningful anxiety sensitivity); (d) no current use of drugs, cigarettes or psychotropic medication or adrenergic blockers; (e) no history or current diagnosis of asthma or other breathing-related disorders; (f) willing to avoid any caffeine, alcohol and moderate-to-vigorous exercise 12 hours prior to each testing session; (g) not pregnant, lactating or having plans of getting pregnant in the next 6 months; (h) no current or history of panic attacks, (i) no current or history of alcohol or drug abuse, (j) no current or history of medical conditions that might be aggravated by study procedures (e.g., yoga, CO_2-inhalation protocol) including cardiovascular disorders (e.g., cardiac

arrhythmia), renal disorders, respiratory disorders (e.g., asthma, lung fibrosis), high blood pressure, epilepsy, stroke or seizures; (k) not practicing yoga for more than 2 times a week for the past 6 months; (l) no current or past experience of panic attacks and (m) no abnormal resting or exercising electrocardiogram (ECG) diagnosed by a clinician, (n) low risk for contraindications of maximal exercise testing. The low risk for contraindications was based on no more than a single "yes" response on the Physical Activity Readiness Questionnaire (PAR-Q).

This study targeted women with self-reported AS (via the study advertisement distributed across the campus) to increase the probability of recruiting a sufficient sample of women who adequately respond to the CO_2-inhalation protocol (i.e., to avoid floor effects). This was based on the previous findings that the ASI score is a strong, reliable predictor of CO_2-reactivity (Broman-Fulks et al., 2015; Smits et al., 2009). This method for identification of the desired sample has been used by previous studies (Broman-Fulks et al., 2004) and has been suggested as indication of "possible problems" with respect to anxiety symptoms (Peterson & Plehn, 1998; Peterson and Plehn, 1999). Accordingly, only individuals whose self-reported ASI scores during the screening were 25 or higher were enrolled based on this inclusion criteria. A score of 25 or higher is ~0.75 SD above the population norms for adults (i.e., 19) and has been suggested as indicative of clinically meaningful AS (Taylor, 1982). Furthermore, to account for the possible effects of the hormones on the anxiogenic responses to the CO_2-air mixture, the phase of the menstrual cycle was accounted for (i.e., luteal vs non-luteal), which was based on the approach previously used by other researchers (i.e., Perna et al., 2005). Finally, participants were instructed to abstain from alcohol, caffeine, and any moderate-to-vigorous physical activity starting 12 hours before each session.

Baseline assessment measures.

1. Spielberger State-Trait Anxiety Inventory (STAI). The STAI (Spielberger et al., 1983) has 2 subscales (Trait and State Anxiety subscales) that measure overall (i.e., "Trait") and current (i.e., "State") anxiety levels in respondents. Both subscales have 20 items that are rated on a scale of 1 to 4 and the total scores range from 20 to 80, with higher scores indicating greater frequency of trait and state anxiety. It is one of the most commonly used anxiety assessment scales to date and it can be used in both clinical and community samples. It has demonstrated good internal consistency, test–retest reliability in the STAI-Trait subscale, sensitivity to detection of stress in the STAI-State subscale, and convergent and discriminant validity (Barnes et al., 2002; Hishinuma et al., 2000; Kabacoff, Segal, Hersen, & Van Hasselt, 1997; Spielberger, 1989; Vautier, 2004).

2. Hospital Anxiety Depression Scale (HADS). The HADS (Zigmond & Snaith, 1983) will be used to assess general symptoms of anxiety and depression. The HADS contains 14 items; 7 items measure anxiety and 7 items measure depression. Total scores range from 0 to 21 for each subscale and higher scores indicate greater frequency of anxiety and depression symptoms in the past 2 weeks. A cut-off score of 7 has been suggested to indicate clinically meaningful levels of anxiety and/or depressive symptoms.

3. Anxiety Sensitivity Index (ASI). The ASI is a 16-item self-report measure designed to assess fear of anxiety sensations. It includes items such as ''When I notice that my heart is beating rapidly", "I worry that I might have a heart attack,", ''When I am nervous, I worry that I might be mentally ill."). Items are rated on a 5-point Likert-type scale (0= very little to 4=very much). Initial evaluation of the ASI has indicated that it possesses sound psychometric properties, including good alpha scores (ranging from .78 to .91) and adequate convergent, discriminant,

and criterion-related validity (Peterson and Reiss, 1992; Reiss et al., 1986). This scale was used during screening to target and enroll individuals who score a 25 or higher on the scale (i.e., ~1 SD above the population norm and indicative of clinically meaningful anxiety sensitivity).

4. *Spielberger State-Trait Anger Expression Inventory (STAXI)*. The STAXI (Spielberger et al., 1988) includes five subscales: State Anger (15 items), Trait Anger (10 items), Anger-in, Anger-out, and Anger Control (8 items each). The Trait Anger subscale assesses the appraisal of one's typical mood (e.g., "I am quick tempered") with items evaluated in terms of their frequency (ranging from 0= Almost Never to 3= Almost Always) whereas the State Anger subscale (SAS) assesses current mood (e.g., "I am furious") with items evaluated with respect to their intensity (ranging from 0= "Not at all" to 3="Very much so"). The SAS possesses high internal consistency coefficients (ranging from .86 to .95) and good convergent and discriminant validity (Spielberger, Gorsuch, Lushene, & Jacobs, 1983). The other 3 subscales (i.e., Anger-in, Anger-out, and Anger Control) include 8 items each that rated on a scale of 0 ("Not at all") to 3 ("very much so") with higher overall scores indicating higher frequency of symptoms. All 3 subscales have been reported to be internally consistent and able to assess responses to hypothetical anger-provoking scenarios (Spielberger, 1988).

5. *Generalized Anxiety Disorder Scale (GAD-7)* The GAD-7 is a 7-item self-reported measure of frequency of GAD. Each item is scored on a scale of 0 ("not at all") to 3 ("Nearly every day") based on the presence of symptoms in the past 2 weeks. Higher scores indicate higher frequency of anxiety symptoms. The GAD-7 has been reported to have strong criterion validity for identifying probable cases of GAD and ability to assess symptom severity, as indicated by the strong positive correlations between the scale scores and functional impairment (Spitzer et al., 2006). Scores of 5, 10, and 15 have been suggested as reasonable cut points representing mild,

moderate, and severe levels of anxiety (Spitzer et al., 2006). Finally, the scale has good test-retest reliability, as well as criterion, construct, factorial, and procedural validity (Spitzer et al., 2006).

6. Trait Mindfulness And Attention Scale (MAAS-T). The MAAS-T is a 15-item self-reported measure of trait mindfulness, which is defined as "a receptive state of mind in which attention, informed by a sensitive awareness of what is occurring in the present, simply observes what is taking place" (Brown and Ryan, 2003). Each item is scored on a scale of 1 (almost always) to 6 (Almost never). Total score is calculated by averaging all the items and higher scores reflect higher levels of trait (i.e., dispositional) mindfulness. It has been validated for use in a variety of samples including college students, community adults (Brown & Ryan, 2003), and for individuals with cancer (Carlson & Brown, 2005). Internal consistency levels (Cronbach's alphas) generally range from .80 to .90. The MAAS has demonstrated high test-retest reliability, discriminant and convergent validity, known-groups validity, and criterion validity (Brown & Ryan, 2003; Carlson & Brown, 2005; Siegling & Petrides, 2013). The MAAS-T is one of the most commonly used scales to measure mindfulness and it has been used in samples with depressive and/or anxiety symptoms (e.g., Baer et al., 2006; Christopher & Gilbert, 2010). The scores on the MAAS-T were accounted for when assessing the responses to the CO_2-inhalation task. This allowed us to assess whether higher trait mindfulness is associated with more attenuated anxiety symptoms when exposed to this anxiogenic task.

7. *Anxiety Sensitivity Index-3 (ASI-3)*. The ASI-3 (Taylor et al., 2007) is an 18-item, revised version of the 16-item original ASI. There is some preliminary evidence that the revised scale might have better psychometric properties than the original, 16-item scale (Taylor et al., 2007). It measures 3 components of AS: cognitive, psychological and physical anxiety. Cut-off scores of

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23and 17 have been recommended by previous researchers for identification of high and moderate-to-high AS individuals, respectively (Allan et al., 2014). It has been validated for use in both healthy and clinical samples (Kemper et al., 2011; Olthius et al., 2002). Participants filled out this questionnaire during the baseline session (unlike the original ASI, which is used as a screening questionnaire). This served 2 purposes. First, it allowed for comparison of responses between the 2 scales among participants. Second, it allowed for determination of whether participants vary their responses when reporting verbally to somebody else (i.e., the experimenter/screener) versus in writing in person.

Primary outcome measures

1. Acute Panic Inventory (API) (Liebowitz et al., 1984) consists of 17 items that assess the presence of current panic-related symptomology using a 4-point Likert-type scale (0=not present to 3=severe). These are primarily somatic symptoms such as palpitations, rapid or difficulty breathing, or sweating. The API is one of the most commonly used questionnaires in studies that involve inducing panic symptoms and CO_2 challenges (e.g., Colasanti et al. 2012; Broman-Fulks et al 2015). Finally, it has sound psychometric properties (Liebowitz et al., 1984). The API has been indicated to be robust in differentiating panic attacks from other forms of stress and anxiety Dillon et al., 1986). This scale will be used in the current study to allow for making distinctions between panic-specific and more general anxiety symptoms experienced by the participants.

2. The 20-item Spielberger State Anxiety Inventory (SAI). The psychometric properties of the STAI have been described above (Spielberger, 1983). Each item is rated on a 4-point Likert-type scale (1=not at all to 4=very much so), with overall scores ranging from 10 to 40. Higher scores indicate higher levels of current/state anxiety.

3. The Spielberger State Anger Subscale (SAS). The SAS is described above.

4. Visual Analog Scale-Anxiety (VAS-A). The VAS-A is a 1-item scale that is rated on 100 mm line, anchored from 0 (''not at all'') to 100 (''the most ever''). These types of scales have been indicated to provide a good estimate of the rapid changes in aspects of mood states (Bond and Lader, 1974). The VAS-A has been validated to effectively capture acute changes in anxiety in clinical populations (Williams et al., 2010). In addition, it is a quick and easy way of assessing such changes while the participant is engaging in a potentially uncomfortable activity (i.e., breathing CO_2-enriched air mixture through a mouthpiece) that might make filling out questionnaire more difficult and distracting. An increase of at least 25% on the VAS-A has been reported to be indicative of significant clinical symptoms of panic and anxiety (Klaassen et al., 1998).

Other Outcome Measures

State Mindfulness And Attention Scale (state MAAS). The state MAAS is a 5-item scale designed to assess the short-term or current expression of a core characteristic of mindfulness (Brown & Ryan, 2003). The state MAAS includes items drawn from the MAAS-T (e.g., "I'm finding it difficult to stay focused on what's happening in the present"). As opposed to the MAAS-T, the state version addresses the questions based on a specific time point (i.e., "at the time of ____"), therefore allowing the experimenter to assess mindfulness in relation to a specific task (e.g., while doing yoga). Each item is scored on a scale of 0 (Not at all) to 5 (very much) and the overall state MAAS score is calculated by averaging the sum of the individual item scores. It has been reported to have adequate psychometric properties (e.g., Cronbach's alpha = .92) and has been validated for use with college student and community adults (Brown & Ryan, 2003). Trait MAAS scores have been reported to predict state MAAS scores, which in turn have been

associated with psychological wellbeing outcomes (Brown & Ryan, 2003). Accordingly, there is evidence for both the construct and incremental validity of the state measure. This scale was used to assess the level of mindfulness experienced by the participants during the yoga vs stretching sessions.

Respiratory outcomes. Expired air during the CO_2-inhalation procedure was collected via a computer-based ventilatory expired gas analysis system (TrueOne 2400, ParvoMedics, Sandy, UT). This allowed the experimenter to collect data on changes in respiratory response to the CO_2-inhalation task before, immediately and 1 hour after the intervention (i.e., yoga or stretching control). Specifically, tidal volume (Vt), respiratory rate (RR), ventilation (VE), and CO_2 production (VCO_2) were monitored. They were assessed both as peak values reached during the final minute of the inhalation task and for a more comprehensive response profile, as 30-second intervals of the entire inhalation period (i.e., 5 minutes).

Manipulation checks

Heart Rate (HR) and Ratings of Perceived exertion (RPE). HR and RPE were monitored during the yoga and stretching sessions to assess the intensity of the sessions. Accordingly, these 2 variables served as manipulation checks for the *a priori*-determined moderate intensity level for the yoga session and the very light intensity level of the stretching (i.e., active control) session.

Fraction of expired CO_2 ($FECO_2$). $FECO_2$ was monitored as a way to confirm the 7.5% CO_2 inhaled. That is, it served as a manipulation check for the enhanced CO_2 concentration that the participants were breathing an air mixture with ~7.5% CO_2. Therefore, it was expected to increase to at least 7.5% during these time points.

Procedures and Experimental Sessions

The procedures were approved by the University of Illinois Institutional Review Board and all participants provided written informed consent. The timeline of study events is provided in Figure 1. The study procedures consisted of 3 testing sessions conducted in our laboratory and each session was separated by one week. The first testing session was a baseline session, and the other sessions (i.e., sessions 2-3) consisted of the experimental conditions (i.e., yoga and stretching). Each session lasted approximately 2 hours. Participants received a remuneration of $10 for baseline and $20 for each experimental session for a total of $50. In the event that they do not complete a session, remuneration was prorated.

During the baseline session, participants first underwent the CO_2-inhalation task. The purpose of this 1st trial was to habituate the participants to the procedure and accordingly to avoid an anticipatory effects (i.e., a falsely exaggerated anxiety/discomfort response in anticipation of the task). Accordingly, they were asked to provide current mood symptoms pre- and post-inhalation using the 4 state mood questionnaires (SAS, SAI, VAS-A, API). Afterwards, they completed a battery of baseline questionnaires on mood (i.e., STAI, STAXI, HADS, GAD-7, VAS-A, API, ASI-3, MAAS-T), as well as information on demographics, and medication use. We also collected data regarding the menstrual phase of the participant, as this factor has been previously indicated to be influential on the anxiogenic response to the CO_2-inhalation protocol in high AS individuals (Nillni et al., 2012; Perna et al., 1995).

CO_2-inhalation protocol. After being seated in a comfortable chair, participants were given a standardized set of instructions regarding the CO_2-inhalation protocol: "*Next, we will do the CO_2-inhalation task. This task involves you wearing a mouthpiece and a nose clip similar to the one you wore during the IET. You will be breathing through this mouthpiece for the next ~3 minutes. It is important that you continue to take normal, full breaths during this period and not*

try to hold your breath. You will first be breathing ambient air to get familiarized with the task.

After 30 seconds, you will start receiving CO_2-enriched air that may produce physical and

mental sensations, such as racing heart, sweaty palms, dizziness and out of breath. These are

common symptoms during this task and we will be asking you to rate your overall anxiety level

on the visual scale after each minute of breathing the CO2-enriched air. As a reminder, you can

terminate this task by simply removing the mouthpiece if you feel unbearable discomfort. Since

you will have the mouthpiece in, you will not be able to talk. So please ask any questions you

might have before we begin". Afterwards, participants were fitted with a 3-way T-shaped

mouthpiece (Hans-Rudolph, Kansas) and a nose clip. The inhalation-end of the mouthpiece will

be attached to a breathing tube that is connected to a 100-Liter Douglas bag. The Douglas bag

was attached to a 3-way T-shape stopcock (Hans-Rudolph, Kansas) that is connected to a gas

canister filled with 7.5% CO_2/ 21% O_2/ 71.5% N mixture (Airgas Inc., IL) via a breathing tube.

The 3-way stopcock has a valve switch that allows the experimenter to control the air flow

between the gas canister, Douglas bag and the mouthpiece via breathing tubes. The exhalation-

end of the mouthpiece was attached to a pneumotachograph/integrator system (Parvo Medics

TrueOne 2400, Sandy, UT) to monitor breathing patterns and measure exhaled gases. The first

30 seconds of the inhalation procedure served as the habituation period during which participants

breathed ambient air. The purpose of this was to get the participants habituated to the breathing

procedure through the mouthpiece and ensure proper breathing without letting any air leak out of

the mouthpiece. Similar habituation methods have been used by previous studies (Pappens et al.

2012; Seddon et al., 2011) for this purpose. At the end of the habituation period, the

experimenter switched the stopcock valve so that the participant started breathing the

decompressed 7.5% CO_2-air mixture from the gas collection bag for the next 5 minutes. Previous

studies have used a wide range of durations for this protocol (i.e., 90 seconds to 20 minutes) and durations of around 5 minutes have previously been successfully and safely used (Pappens et al., 2012) without any adverse, sustaining side-effects for the participant. At the end of each testing session (i.e., after 3rd CO_2-inhalation task), participants were asked to stay seated until they felt well enough to leave the research laboratory. Previous studies have reported that participants return to baseline levels within several minutes upon termination of the CO_2-inhalation challenge regardless of the CO_2 concentration or the duration of the breathing (e.g., Kaye et al. et al., 2004; Pappens et al., 2012, Bullis et al., 2010, Dripps & Comroe, 1946, Ben-Zion et al., 1999). Combined with our experiences from the pilot-testing of our protocol, we expected this time period to be no longer than 5 minutes. The experimenter stayed with the participant during this period to ensure the safety and well-being of the participant and also checked their heart rate to make sure that the participant's heart rate has gone back to baseline. As expected, none of the participants required extra time to recuperate and were able to leave the research facilities soon after (i.e., within 5 minutes) the termination of the study.

Experimental conditions. The subsequent experimental conditions (i.e. sessions 2-3) occurred in a within-subjects design and the order of sessions were randomized and counterbalanced across participants. Participants initially completed the same state mood battery of questionnaires on anxiety, anger and panic symptoms (i.e., SAS, SAI, VAS-A, API) while seated in a comfortable chair before and after the 1st inhalation task. Participants then completed the assigned experimental condition (i.e., guided yoga, or light stretching) and complete the same battery of questionnaires again within 5-minutes after the condition (i.e., immediately post-condition), as well as the state MAAS (i.e., MAAS-S). At this point (i.e., immediately after the yoga/stretching protocol, before the 2nd CO_2-inhalation task), participants were also asked to

report their peak RPE (i.e., "the highest level of exertion they felt during the session"). After the 2nd inhalation task, participants completed the same battery of questionnaires. For the assessment of delayed response to CO_2-inhalation task, participants then went through a waiting period of ~40 minutes (i.e., 1 hour from the end of yoga/control condition), followed by a final CO_2-inhalation task and completion of the same battery of questionnaires pre- and post-inhalation procedure.

Yoga intervention. The yoga session began with 5 minutes of meditation/deep breathing, 20 minutes of Vinyasa yoga, and ended with a 5-minute period of "Savasana" or "Corpse Pose". The specific sequence was determined based on: 1) ecological validity, i.e., most commonly used poses in a community vinyasa yoga class, 2) the fundamentals of this type of yoga (e.g., sun salutations), 3) logical sequence that can be delivered with safety within 40 minutes (e.g., avoidance of any poses that might be too advances for a beginner and therefore become unsafe/hazardous). Finally, modifications were offered on a per need basis. That is, every pose in the sequence in the proposed study included a priori-determined alternative without compromising the main goal of the pose. Therefore, in the event that a participant was unable to carry out a pose in the way it is originally intended, they would still be able to participate and do it to the best of their ability. However, none of the participants enrolled in the study required any modifications, except for the use of a block to sit on during the meditation/deep breathing part at the beginning for some of the participants.

The Vinyasa yoga sequence started with cat and cow poses (i.e., spinal warm-up on hands and knees), followed by several repetitions of sun salutations A and B each. Following the sun salutations, sequences included the fundamental poses that form the majority of the sequence: Tadasana (Mountain Pose), Urdhva Hastasana (Upward Salute), Uttanasana (Standing

Forward Bend), Low Lunge (Anjaneyasana), Plank Pose, Chaturanga Dandasana (Four-Limbed Staff Pose), Bhujangasana (Cobra pose), Urdhva Mukha Svanasana (Upward-Facing Dog Pose), Adho Mukha Svanasana (Downward-Facing Dog Pose)); and ended with a series of other postures (i.e., child's pose, warrior-1, warrior-2, extended side-angle pose, forward bends, seated twists, balancing poses) that involve isometric muscle contraction and relaxation of different muscle groups in conjunction with regulated breathing. These poses are among the most commonly practiced poses by yoga practitioners and therefore were selected based on their ecologic validity with respect to the traditional vinyasa yoga classes offered at a regular yoga studio. Participants mirrored the yoga instructor (i.e., the experimenter), who performed the postures for the participants and guided them throughout the sequence, as well spotted them when necessary. Yoga blocks and bolsters were provided for the participants; they were further assisted by the experimenter if and when necessary. The intensity during each acute yoga session was monitored based on the assessment of HR using a Polar heart rate monitor (Polar Electro Oy, Finland)) and the RPE scale. HR was recorded every 10 minutes and RPE was recorded at the end of the session by asking the question "At the peak of the session, how hard were you working?" to the participant at the end of the session. The yoga protocol was developed based several factors. First, the sequence was created so that it closely mimics a traditional vinyasa yoga class that is offered at a community yoga class. Next, the included poses are considered fundamental in this style of yoga (e.g., doing a vinyasa between every pose, matching the pace of the breath to the pace of the pose). Similarly, attention was given to provision of a well-rounded protocol by including a variety of poses that are meant to target different components of yoga (e.g., balance, stretching, endurance) based on the teachings of commercially published books on yoga sequencing (e.g., Stephens, 2012). Finally, the protocol took into account previously used

yoga sequences for other research projects in our research laboratory (e.g., Sandroff, 2015), which had been developed in collaboration by the experimenters and a licensed yoga teacher who works as a yoga instructor at yoga studios across the United States. The session was delivered to the participants on a one-on-one basis by the experimenter (IE) who has 6 years of experience as a yoga practitioner and 3 years of experience as a meditation and stretching instructor assisting both healthy individuals and those with neurological diseases (i.e., multiple sclerosis).

Control condition. The control condition involved the participants completing a stretching protocol involving movement, attention and social contact for 40 minutes to match the active yoga condition. This is an active form of control condition and is considered to be ideal in mind-body and exercise studies as it more closely mimics the active condition (Kinser and Robbins, 2013). Therefore, this allowed for the attribution of any observed difference in the inhalation task responses to the active yoga component. Second, this has been suggested in the literature as a more ethical approach as it allows the participants to receive similar treatments and also similar amounts of attention and interaction from the experimenter under both conditions (i.e., experimental and control) (Kinser & Robbins, 2013).

During the 40-minute stretching protocol, participants did some light stretching either while sitting or lying on the floor (e.g., body twists, leg/hamstring stretches, arm and shoulder stretches), as well as some sitting and lying poses that resemble the breathing meditation component provided in the yoga condition. However, no instructions on any type of yogic or otherwise meditative breathing were given or mentioned to the participant. The stretching poses have been previously used in exercise training studies in our research laboratory with other populations (i.e., multiple sclerosis). This is an ecologically valid control condition for studies of

acute exercise and mood, as it accounts for the social aspect of the intervention, as well as the passage of time and its potential transient effect on mood.

   Post-condition CO_2-inhalation protocols and the resting period. The CO_2-inhalation protocol has been described above and the same steps were followed for the administration immediately post- and 1 hour-post condition. Participants were then given a seated resting period of approximately 40 minutes between the 2nd and 3rd inhalation tasks (i.e., 1 hour between the end of the intervention and the 3rd inhalation task). During this time, they were seated in a comfortable chair in the main research area and were allowed to read materials provided by the research team, use their cellphones for communication purposes only (i.e., using the internet or listening to music were not allowed) and use the restroom if needed. At the end of the resting period (i.e., 1 hour post-condition), participants went through the CO_2-inhalation protocol for a 2nd time, preceded and followed by the completion of the same battery of questionnaires. This marked the end of the study session. The experimenter followed the protocol described above regarding the release of the participant from the research facility.

Data Analysis

   All data analyses were conducted using SPSS v. 22.0. Descriptive statistics for demographic and clinical characteristics are provided as mean (SD) throughout text, table and figures. Separate 2(condition) × 3(assessment time point) × 2 (pre- and post-exposure) repeated-measures analyses of variance (ANOVAs) were conducted for identifying the interactions and main effects of assessment time point (pre-, immediately post-, 1-hour post-condition) and condition (yoga and light stretching/control) on self-report measures of anxiety, panic, and anger from pre- to post-exposure (i.e., CO_2-inhalation task). Respiratory data (i.e., Vt, RR, and VE)

were analyzed 2 separate ways: First, by taking the peak value from the last minute of the inhalation task (i.e., between minutes 4 and 5) and second, by taking the 30-second averages of the entire 5-minute exposure period to capture a more comprehensive profile of the fluctuations during this task. Accordingly, separate repeated measures ANOVAs were conducted for both sets of analyses and are reported in text (condition: yoga, control; × assessment time point: T1, T2, T3) and tables (condition: yoga, control; × assessment time point: T1, T2, T3; x ten 30-second intervals over entire task). Finally, effect sizes (ES) were computed as partial eta squared (η_p^2) for all scores reported in the ANOVAs and the suggested norms of 0.01 (small), 0.06 (medium), and .14 (large) for determination of the size of the ES (Field, 2013).

CHAPTER 4: RESULTS

Participant recruitment and enrollment

A total of 93 women contacted our research laboratory via email or phone expressing interest in potentially participating in the study. These individuals were provided with more details on the study, as well as the informed consent document. Of those, 46 individuals expressed further interest and were screened based on the previously described list of screening criteria. Twenty-four individuals were qualified. Two individuals decided not to continue after the screening due to the time commitment required and therefore 22 participants were scheduled for testing. Of these, 3 participants could not complete the full 5 minutes of breathing of the 7.5% CO_2-air mixture during the baseline session and were therefore disqualified. One participant dropped out after the 1st experimental session due to time-commitment issues. Accordingly, 18 participants completed the entire study and so the final sample size for the data analyses is 18 women with a score of 25 or higher on the ASI.

Participant characteristics

The sample had a mean age of 22 years (SD=5); 16 of the participants were undergraduate students on the university campus. Eleven participants (i.e., 61%) were using a hormone-based birth control method (e.g., pill or shot), and there was an even split in the menstrual cycle phase of the participants at the time of their testing (i.e., 9 on the luteal phase and 9 on the non-luteal phase). The mean score on the ASI was 32.2 (SD=6), which is approximately 2 SD above the normative value for the general adult population (i.e., 19). On the other hand, the mean score on the ASI-3 was 24.9 (SD=10.3). A score of 17 on the ASI-3 is considered having moderate-to-high AS and a score of 24 or higher is considered having high ASI. Accordingly, we have successfully managed to recruit a sample of women with clinically

meaningful levels of trait sensitivity for anxiety and panic symptoms. The mean score on the

GLTEQ was 40.9, which is considered highly physically active and meeting physical activity

guidelines (PAGs) according to published guidelines (USHHS, 2013). The mean score on the

MAAS-T was 3.8 (.9), which is very close to the normative mean score reported for college-aged

adult populations (i.e., M=3.8, SD=.7) (Brown and Ryan, 2003). The mean score on the TAI was

46.4 (SD=10.3), which is approximately 1.4 SD above the population normative score reported

for adult women (i.e., M=34.8, SD=9.2) (Spielberger et al., 1983). The mean HADS-D score was

5.2 (3.9) and the mean HADS-A score was 9.8 (4.7). The cut-off score on the HADS for

clinically meaningful anxiety or depressive symptomology is considered 7; accordingly, the

sample had clinically meaningful levels of anxiety symptoms, but not depressive symptoms. The

mean GAD-7 score was 8.4 (SD=4.8), which is slightly lower than the cut-off score (i.e., 10)

suggested for diagnosis of clinically meaningful generalized anxiety disorder symptoms. Finally,

mean trait anger score was 16.0 (4.4), which is considered to be on the low end of the trait anger

spectrum based on the normative mean scores for college-aged adult populations (Spielberger et

al., 1988). In summary, the sample in the study had sufficient levels of high trait anxiety, as well

as sensitivity to their anxiety symptoms, but without significant levels of any other negative

mood symptoms (i.e., anger or depressive symptoms).

Manipulation checks for exercise intensity and anxiety/panic induction

> *Heart Rate (HR) and Rating of Perceived Exertion (RPE) during yoga and stretching*

protocols. A 2×3 (i.e., condition: yoga, control; $\times$ assessment time point during session: 10th

min, 20th min, 30th min) ANOVA indicated no statistically significant interaction on HR,

indicating that HR did not change in a differential manner across the 3 time points per condition.

However, there was a significant main effect of condition on HR [F(1,16)=5.85, η_p^2=.27, $p<.05$].

The mean HR was 90 (SD=19.3) bpm during the yoga session and 78 (SD=14.9) bpm during the control session. Peak RPE values reported for yoga and control session were compared using paired samples t-tests. The results indicated a statistically significant difference between the 2 conditions [t(17)=5.18, $p<.001$]. Mean RPE was 12 (SD=1.8) for the yoga session and 9.8 (SD=2.6) for the control session. Collectively, these HR and RPE values suggest that participants were working at a light-to-moderate intensity during the yoga condition and at a very light intensity during the control condition. Accordingly, the yoga session selected for the study did not fully reach the initially targeted intensity level (i.e., moderate) for this intervention based on RPE and HR combined; however, it did approach moderate intensity based only on the RPE value.

FECO$_2$ during CO$_2$-inhalation task. The average values for FECO$_2$ ranged between 7.95% and 7.99% across all time points in both conditions. This was expected considering that the air mixture consisted of 7.5% CO_2. The 2 × 3 (i.e., condition: yoga, control; assessment time point: T1, T2, T3) ANOVA indicated no significant interaction or main effects on FECO$_2$ ($p>.05$ for all). Accordingly, there is evidence that all participants inhaled the air mixture with the increased CO_2 concentration without holding their breath, or air leaks from the mouthpiece or the gas collection bag. Furthermore, the lack of interaction or main effects indicates that this was achieved at all assessment time points and in both conditions.

Baseline session: Reliability of CO$_2$-inhalation task as anxiogenic/panicogenic

The mean scores (SD) on the self-reported measures from pre- to post-inhalation task during the two sessions at baseline are provided in Table 2. There was a significant interaction between assessment time point (1st vs 2nd inhalation task) and exposure (i.e., pre- to post-

inhalation task) on the API (F(1,17)=7.76, $\eta_p{}^2$=.31, p<.05) and the SAI (F(1,17)=7.94, $\eta_p{}^2$=.32 , p<.05). This indicates that the change (i.e., increase) in scores from pre- to post-exposure showed differential patterns at T1 vs T2 assessment points. Specifically, the increase in API scores from pre- to post-exposure was smaller for T2 as the pre-exposure was slightly higher (i.e., from 1.39 to 8.83 and 3.28 to 8.61 at T1 and T2, respectively). Similarly for the SAI, the increase from pre- to post-exposure was smaller at T2 (i.e., 33.78 to 46.61 and 39.78 to 43.44 at T1 and T2, respectively). . However, there were no such interactions on the VAS-A (F(1.17)=.44, $\eta_p{}^2$=.03, p>.05) or the SAS (F(1,17)=.026, $\eta_p{}^2$=.00, p>.05). This indicates that the change in scores from pre- to post-exposure did not vary across the levels of assessment time points

There was a significant main effect of exposure on the API (F(1,1)=52.86, $\eta_p{}^2$=.76, p<.001), SAI (F(1,1)=20.06, $\eta_p{}^2$=.54, p<.001), and the VAS-A (F(1,1)=13.37, $\eta_p{}^2$=.44, p<.05). These collectively indicate that participants reported significantly elevated physical and psychological symptoms of panic and anxiety from pre- to post-exposure independent of assessment time points. This further suggests a lack of habituation effect to the inhalation task over time. On the other hand, there was no significant main effect of assessment time point for any of the scales (p>.05), indicating a lack of significant difference in scores between the 2 time points. There further was no significant main effect of exposure for the SAS (p>.05), indicating that the inhalation task did not have any statistically significant effect on the anger scores independent of assessment time points. Specifically, the state anger scores were very close to 15 (i.e., lowest score possible on the SAS) at T1, and remained so all throughout the baseline session (i.e., pre- and post-exposure at both assessment time points).

Primary Outcomes: Hypothesis 1 (Anxiety and panic symptoms)

When the phase of the menstrual cycle (i.e., luteal vs. non-luteal phase) was entered as a covariate factor into all the analyses, the results for the final ANCOVA models did not significantly differ from those of the ANOVA models. Accordingly, this variable was not included in the final models and only the results for the ANOVAs are reported in the subsequent sections.

The mean scores (SD) for the API, SAI, VAS-A and the SAS before and after CO_2 inhalation at each time point for both experimental conditions (i.e., yoga and control) are presented in Table 3. The results of the $2 \times 3 \times 2$ (condition × assessment time point × exposure) ANOVA models are presented in Table 5.

Acute Panic Inventory (API). There was no significant 3-way interaction between condition (i.e., yoga, control), assessment time point (i.e., T1, T2, T3) and exposure (i.e., pre- vs post- inhalation task) on API scores (p>.05). This lack of interaction indicates that participants' responses to the inhalation task did not differ across assessment time points for the 2 conditions. Similarly, none of the 2-way interactions or main effects of condition or time point was statistically significant (p>.05), indicating that the exposure effect was independent of condition and assessment time point. There was a statistically significant main effect of exposure (p<.001), indicating that the CO_2-inhalation task reliably induced symptoms of panic independent of condition and assessment time point (see Table 3).

State Anxiety Inventory (SAI). Based on the repeated measures ANOVA, there was no significant 3-way interaction between condition (i.e., yoga, control), assessment time point (i.e., T1, T2, T3) and exposure (i.e., pre- vs post- inhalation task) on SAI scores (p>.05). This again indicates that the anxiogenic response to the CO_2-inhalation task did not differ across assessment time points

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between the conditions. Specifically, the mean SAI scores increased from pre- to post-exposure at all assessment time points in both conditions. Similarly, there were no statistically significant 2-way interactions (see Table 3). Finally, the main effect of condition was not statistically significant ($p>.05$), and this indicates that the condition did not have an effect on the change in scores across time points. However, there was a significant main effect of both assessment time point and exposure on the SAI scores ($p<.001$).This indicates that the scores increased from pre- to post-exposure independent of condition or assessment time points. Similarly, a significant main effect of time point indicates that the scores, collapsed over exposure and condition, were significantly different at each assessment point (i.e., 32.25, 27.03 and 30.50 for T1, T2, and T3, respectively). .

Visual Analog Scale-Anxiety (VAS-A). Results of the repeated measures ANOVA indicated no statistically significant 3-way interaction between condition (i.e., yoga, control), assessment time point (i.e., T1, T2, T3) and exposure (i.e., pre- and post-inhalation task) ($p>.05$). This again indicates that the anxiogenic response (i.e., pre- to post-exposure) to the CO_2-inhalation task did not differ across assessment time points between the conditions. Specifically, the mean VAS-A scores increased from pre- to post-exposure at all assessment time points in both conditions. However, the 2-way interaction between assessment time point and exposure was significant ($p<.05$), indicating that the exposure effect varied across assessment time points. Specifically, the change from pre- to post-exposure slightly increased from T1 to T3 (i.e., Δ values of ~15, ~17 and ~21 for T1, T2 and T3, respectively). Furthermore, the main effects of assessment time point and exposure were both statistically significant at $p<.001$ (See Table 5). Collectively, these indicate that the inhalation task reliably induced symptoms of generalized anxiety from pre- to post-exposure based on the VAS-A, independent of condition (i.e., yoga or control). The lack of

main effect of condition (i.e., $p>.05$) indicates that this effect was independent of condition such that, in both conditions the pre-exposure scores at T2 and T3 were significantly lower than that of T1 (see Table 3).

Secondary Outcomes: Hypothesis 2 (anger)

State Anger Scale (SAS). Results of the repeated measures ANOVA indicated no significant interaction between condition (i.e., yoga, control), assessment time point (i.e., T1, T2, T3) and exposure ($p>.05$). This indicates that the change in SAS scores did not differ significantly across assessment time points or conditions. Similarly, none of the 2-way interactions or main effects were statistically significant ($p>.05$). Main effect of assessment time point approached statistical significance ($p=.06$) (See Table 5), indicating that there was a trend toward a differential pattern of change in anger scores in response to the exposure independent of condition and exposure (i.e., pre- and post-exposure scores) (See Table 2). Specifically, when collapsed over exposure and across conditions, there was a slight decrease from T1 to T3 in the mean scores (i.e., 15.49, 15.18, 15.17 for T1, T2, and T3, respectively). However, there was a large floor effect (i.e. mean (SD) scores of 15.22 (0.56) and 15.33 (0.46) for yoga and control conditions, respectively). In combination with the consistent changes in anxiety and panic symptom scores from pre- to post-inhalation tasks, these results collectively provide evidence for the construct validity for our anxiety induction model (i.e., 5 minutes of 7.5% CO_2-inhalation) in this study.

Secondary Outcomes: Hypothesis 3 (Respiratory measures)

The mean (SD) peak values for RR, Vt and VE at each assessment time point during the final (i.e., 5th) minute of exposure for both experimental conditions (i.e., yoga and control) are presented in Table 4. The results of the ANOVA did not differ significantly based on method of

data analysis (i.e., peak values during minute 5 of inhalation period vs. 30-second averages over the entire inhalation period), and both versions of the data analysis is presented in text and Tables 6 and 7. This lack of difference suggests that there were no differential pattern of responses to the CO_2-inhalation task based on the method of data analysis. Overall, RR and VE significantly increased over the course of the 5 minutes of exposure (based on the results of the 2 x 3 x 10 ANOVA: see Table 6); however, this did not differ significantly across the 3 assessment time points (i.e., T1, T2, T3) or the 2 conditions (i.e., yoga or control). Finally, there was a significant main effect of exposure (i.e., 10 30-second intervals) on all 3 outcomes based on the 2 x 3 10 ANOVA; but not based on the 2 x 3 ANOVA. This would make sense given the former analysis includes this as an additional factor and thus provides a more comprehensive profile for the respiratory response to the inhalation task. Accordingly, this method of analysis was the focus in the following sections on the description and discussion of these results.

Peak respiratory rate (RR). Results of the repeated measures ANOVA indicated no significant interaction between condition (i.e., yoga, control) and assessment time point (i.e., T1, T2, T3) [$F(2,32)=1.42$, $\eta_p^2=.08$, $p>.05$]. This suggests that RR at each time point did not significantly differ across conditions. There was a significant main effect of assessment time point [$F(2,32)=3.40$, $\eta_p^2=0.68$, $p<.05$], but not a significant main effect of condition ($p>.05$). This indicates that the RR differed across the 3 time points (i.e., T1, T2, T3), independent of the condition (See Table 4). Specifically, when collapsed over conditions, there was a very slight decrease in RR over time. However, this was no longer significant when all 5 minutes of exposure were included in the repeated measures ANOVA (See Table 6).

Peak tidal Volume (Vt). Results of the repeated measures ANOVA indicated no significant interaction between condition (i.e., yoga, control) and assessment time point (i.e., T1, T2, T3) [$F(2,342)=.99$, $\eta_p^2=.06$, $p>.05$]. This suggests that Vt at each time point did not differ significantly across conditions. Specifically, the values ranged from 1.74 to 1.77 in the yoga condition and from 1.72 to 1.76 in the control condition. There further were no significant main effects of condition or assessment time point ($p.>05$ for both). Specifically, the mean values collapsed over time were 1.76 lt and 1.74 lt for yoga and control conditions, respectively.

Peak ventilation (VE). Results of the repeated measures ANOVA indicated no significant interaction between condition (i.e., yoga, control) and assessment time point (i.e., T1, T2, T3) [$F(2,32)=.11$, $\eta_p^2=.01$, $p>.05$]. This indicates that peak ventilation did not differ across the 2 conditions or the 3 assessment time points. However, the mean values collapsed across the 3 assessment time points were not significantly different between the 2 conditions (i.e., ~47lt/min), as indicated by the statistically non-significant main effect of condition ($p>.05$). On the other hand, main effect of assessment time point approached significance [$F(2,32)=3.12$, $\eta_p^2=6.23$, $p=.06$]. This suggests that there was a change (i.e., slight increase) in VE at each assessment time point, which was independent of condition. However, this was no longer significant when all 5 minutes of exposure were included in the repeated measures ANOVA (See Table 6).

Other outcome measures

Minute-by-minute VAS-A. VAS-A assessments taken at the end of each minute of the CO_2 inhalation were analyzed in a 2 × 3 × 5 (i.e., condition; yoga, control; × assessment time point: T1, T2, T3; × minute of exposure: 1st through 5th) ANOVA. The results of the ANOVA indicated no significant 3-way or 2-way interactions ($p>.05$). This suggests that the change in

VAS-A over the course of exposure did not differ across the assessment points or the 2 conditions. However, there was a significant main effect of minute of exposure, such that the scores significantly increased over the course of the 5 minutes, and this was independent of condition or assessment time points. This change in scores over the course of the 5 minutes of exposure is presented in Figure 2.

Carbon dioxide production (VCO2). The 2 × 3 (i.e., condition × assessment time point) ANOVA results indicated that there were no statistically significant interactions or main effects of condition or assessment time point ($p > .05$) on the CO_2 production. This indicates that the CO_2 production was consistent across conditions and assessment time points. The mean VCO_2 values (SD) at T1, T2 and T3 were 3.02 (.80), 2.58 (1.81) and 3.04 (.87) for the yoga condition and 2.93 (.75), 2.92 (.78) and 2.99 (.78) for the control condition.

Mindfulness. Mean scores on the state mindfulness scale (i.e., MAAS-S) immediately after yoga (M=1.07(SD=1.04)) and control (M=1.17 (SD=.80)) did not significantly differ between conditions based on the results of the paired samples *t*-test ($T(17)=-.50, p > .05$). These scores suggest that the participants did not feel any more mindful after the yoga session when compared to after the stretching control condition. The mean score on the trait mindfulness (i.e., MAAS-T) was 3.78 (.9), which is consistent with what has been reported in this population (i.e., M= 3.83 (.7) (Brown and Ryan, 2003). In addition, when the mean MAAS-T scores were entered into the model as a covariate, it did not have a statistically significant effect on the results of the final ANOVA models for the self-reported outcome measures (i.e., API, SAI, SAS and VAS-A). This indicates that the response to the inhalation task was consistent across all time points and both conditions independent of the participants' self-reported trait mindfulness scores. However,

it is possible that the small variance in the scores might have influenced this lack of statistical significance.

In summary, there are 3 important main outcomes based on the results of this study. First, there was no differential effect of condition on the anxiolytic response. Specifically, the yoga intervention did not induce a larger attenuation in the anxiogenic response when compared to the control condition. This finding does not provide support for the 1st hypothesis and is further not in accordance with previously published similar studies. Second, there was an attenuation in the cognitive symptoms of anxiety, based on the change in the SAI and VAS-A scores, across the assessment time points when collapsed over condition and exposure. On the other hand, the respiratory outcomes did not parallel the self-reported outcomes, and this refutes the secondary hypothesis regarding the physiological/respiratory outcomes. That only the cognitions of general anxiety, but not panic or physiology of anxiety, attenuate over time indicates an overall effect of general physical activity and this is in line with previous studies. Finally, the inhalation task used in the present study was anxiety/panic-specific, based on the consistent, reliable change in both the self-reported and respiratory measures of anxiety and panic, but not anger, from pre- to post-exposure that was independent of condition and assessment time point. While this refutes the secondary hypothesis regarding acute anger, it does provide preliminary evidence for the construct validity of the model.

CHAPTER 5: DISCUSSION

Overview of the results

The purpose of this study was to investigate the acute anxiolytic effects of a vinyasa-style yoga session in response to an anxiogenic, 5-minute 7.5% CO_2-air mixture exposure in women with self-reported trait anxiety sensitivity (i.e., a score of ≥25 on the ASI). Overall, our results from the 3-way interactions provide no evidence for a differential change in anxiety or panic response between acute, light-to-moderate intensity yoga and an active control session involving light stretching for attenuating the response to a biological anxiogenic stimulus (i.e., CO_2-inhalation task) ($p>.05$). Similarly, respiratory response to the inhalation task did not significantly differ across any of the assessment time points between the 2 conditions. However, when collapsed over exposure and condition, there was a reduction in SAI and the VAS-A scores that sustained over time (i.e., a main effect of assessment time point). There were no such effects for the API scores (i.e., physical panic symptoms), however. Collectively, these results point to a reduction in cognitive aspects of anxiety irrespective of the physiological aspects of anxiety or panic in our sample in response to the CO_2-inhalation task after both conditions. This potentially indicates an effect of general physical activity, passage of time or time-out effect. However, given the lack of changes in panic and respiratory outcomes, it is unlikely to be a passage of time or time-out effect. That is, we would have expected to observe the same pattern of reduction across all outcome measures for a passage-of-time effect. Therefore, the specificity of the cognitive anxiety changes points to a more anxiety-specific effect for both yoga and stretching. Finally, there was a consistent, reliable increase in both physical panic and anxiety symptoms from pre- to post-exposure (i.e., exposure main effect), but not anger, in response to the anxiogenic stimulus at all assessment time points ($p<.05$), providing evidence to the construct

validity of the protocol. There were no significant changes in results for any of data analyses after controlling for phase of menstrual cycle (i.e., luteal vs non-luteal), and trait mindfulness scores.

Primary hypothesis

Our primary hypothesis that yoga would attenuate the self-reported anxiety response more so than the control condition was not supported, based on the lack of differences in self-reported outcome measures between the 2 conditions ($p > .05$). Our results suggest that in comparison to the control condition, participants did not experience any statistically significant attenuation in self-reported panic or anxiety after the yoga intervention in response to the CO_2-inhalation ($p > .05$). In addition, the increases in anxiety symptoms in response to the inhalation task were statistically significant at all time points, regardless of the condition ($p < .05$). Accordingly, the proposed hypotheses were only partially supported in that, participants experienced significant increases in physical panic and generalized anxiety symptoms in response to the inhalation task at all time points based on the scores on the API, SAI and VAS-A from pre- to post-exposure, as well as based on the minute-by-minute VAS-A assessments during the exposure, but this was not influenced by yoga. Findings on the VAS-A indicate an overall effect of physical activity on the cognitions of generalized anxiety. When collapsed over exposure and condition, the mean scores for each assessment time point were 23.32 (SD=14.91), 15.82 (SD=12.22) and 17.69 (SD=13.66), respectively. These translate to ESs (i.e., Cohen's *d*) of 0.55 and 0.39 from T1 to T2 and T1 to T3, respectively. These ESs would be considered of medium in magnitude are larger than what has been reported by previous meta-analyses (e.g., Ensari et al., 2015; Petruzzello et al., 1991) on the efficacy of acute exercise for reducing anxiety symptoms (e.g., Cohen's *d*=.24 and Hedge's *g*=.16, respectively). When collapsed over

condition and exposure, there was attenuation in the increase on SAI scores and this is discordant with those of others. For example, in the study by Broman-Fulks et al., (2015), SAI scores were the only scores (among other anxiety measures such as the ASI) that did not change after the resistance training session. The authors suggested that this might perhaps be due to a delayed anxiolytic response, which they could not capture (as they did not include a delayed response assessment). However, we addressed this in our study by assessing response at 1 hour post-intervention and provide preliminary evidence for an attenuation effect that is independent of condition and also irrespective of physical and physiological symptoms of anxiety. Given the lack of differential pattern of change in scores between conditions, it might be possible to suggest that the slightly anxiogenic effect of yoga might pertain to the stretching and/or meditation components, as those were also included in the control protocol. Accordingly, these findings confirm and provide an update on the previously reported tension-relieving effects of muscle stretching relaxation techniques in moderately anxious samples (e.g., Carlson et al., 1990; Bernstein & Borkovec, 1973).

Secondary hypothesis

Our secondary hypothesis regarding the anger response was not supported. There was no evidence for a differential pattern of change in scores from pre- to post-exposure at any assessment time points between the conditions (i.e., non-significant 3-way or 2-way interactions). Furthermore, there was a large floor effect (i.e., a score of ~15 at all points) in the anger response where, the scores remained low from pre- to post-exposure and throughout the assessment time points in both sessions. One rationale for including an anger measure in this study was to account for the influence of potential differences in anger levels between participants on the primary outcome measures. Previous researchers have reported significant

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associations between symptoms of anger and symptoms of anxiety and panic (e.g., Moscovitch et al., 2007). To our knowledge, this is the first study to account for this potential confounding influence and we report that anger does not appear to be prevalent in individuals with heightened AS or influence the anxiogenic or panicogenic response to the CO_2-inhalation task. It might be possible that this comorbidity previously reported by others is more likely in those with a clinically diagnosed condition (e.g., PD, OCD). Anger was not affected by the CO_2-exposure in our sample; and this makes the model more anxiety-specific in interpretation. Collectively, they provide construct validity of the CO_2-inhalation protocol as a method for inducing physical panic and cognitive symptoms of generalized anxiety.

There was partial evidence for the secondary hypothesis regarding the respiratory outcomes. Specifically, there were no 3-way interactions, indicating a lack of differential effect between yoga and stretching conditions for attenuation of respiratory rate or ventilation or increasing tidal volume. However, there was a significant main effect of exposure where the rate of respiration and ventilation increased over the course of the inhalation task ($p<.05$). These effects were consistent across time points and conditions, as indicated by the lack of 2-way interaction or main effects of condition or assessment time point ($p>.05$). This pattern of increase indicates that physiological changes do indeed parallel the self-reported panic symptoms in response to this experimental anxiogenic stimulus. However, they do not parallel the self-reported cognitive symptoms of anxiety. Collectively, these findings suggest that the acute cognitive improvements that are seen after a bout of yoga or stretching activities might be independent of the possibly inherent and centrally-mediated physiological responses to the CO_2-inhalation task. Findings of others who have investigated the respiratory response to 7.5% or other similar concentrations of CO_2-inhalation tasks (e.g., Shafer, 1958; Zandbergen et al., 1991)

and our results somewhat agree. For example, one such study divided a group of individuals into "high" vs "low" ventilators to the 7.5% CO_2-inhalation task based on the severity of their panic symptomology (Shafer, 1958). The results indicated that within the first 5 minutes of the inhalation task, high ventilators exhibited much greater RR but lower Vt when compared to low ventilators. The range of RR in the high ventilators in this study was 20-30 (means not reported), whereas the mean RR in our study ranged between 28 and 29. Another similar study reported a mean RR of 23 and Vt of ~1.6lt for "responders" (i.e., those who respond to the CO_2-inhalation with significantly increased ventilatory response) during the first 5 minutes of a 10-minute inhalation of 7% CO_2-inhalation (Papp et al., 1997). This reported Vt value is closer what is reported in the current study (i.e., 1.6-1.7lt), and accordingly provides further confirmatory evidence for the respiratory response that is typically observed in sensitive individuals (i.e., "responders" or "high ventilators). Another study compared ventilatory response to the Reading rebreathing method (a similar type of CO_2-inhalation protocol involving the participant breathing the same gas mixture from a gas collection bag) in healthy control participants vs patients with PD (Zandbergen et al., 1991). Their results indicated that the group with PD responded with greater VE (Range: 20-40 lt/min; means not reported) in comparison to the healthy control group (Range: 10-20lt/min, means not reported). The mean VE values we observed in our study had a range of 45.76-47.76 lt/min. The observed differences might have been due to factors such as sample characteristics, and the differences in the breathing protocols used. Overall, our findings build upon these by providing a comprehensive profile of both cognitions and physiology of anxiety in response to the CO_2-inhalation task before and after a bout of physical activity. Accordingly, it appears that the cognitive versus physiological responses to the CO_2-inhalation protocol might be independent of each other in individuals with heightened anxiety sensitivity.

This study has several novel features that address the limitations in the existing literature on experimental models of anxiety using the CO_2-inhalation paradigm and acute exercise. These differences can be categorized as those relative to the inhalation task, physical activity intervention and sample. First, the previous 5 studies published on this topic have used a single or double inhalation of 35% CO2-air mixture, which is a panic induction protocol, not an anxiety-specific protocol. On the contrary, our goal was to induce symptoms of generalized anxiety symptoms and therefore we used a 7.5% CO_2 concentration and exposed our participants for 5 minutes. Based on the consistent increase in symptoms of anxiety (in addition to panic), but not anger, we provide evidence that this is a feasible model to study states of generalized anxiety and its related cognitions and physiology under experimental conditions. We further provide construct validity for this novel protocol given the lack of any significant changes in anger scores from pre- to post-exposure. To our knowledge, this is the first study to administer this specific protocol in an attempt to induce a more generalized anxiety state in participants and assess this state by administering both anxiety and panic measures, as well as anger measure, to discern the these different constructs that are often reported to overlap.

Similarly, we assessed change in response over time by administering the inhalation task both before and after the intervention and furthermore, by administering the mood questionnaires before, during and after each inhalation. On the contrary, previous studies have administered the CO_2-inhalation challenge only after the exercise intervention, which is a limitation of the previous studies as this prevents them from being able to compare the post-exercise response to a reference point (i.e., the T1 assessment) before exposure to any aspects of the study (e.g., intervention protocol, inhalation protocol). This is important to consider, especially given that the sessions were scheduled on different days, as there might be differences in physiological or

psychological measures between conditions at baseline, due to reasons unrelated to the experimental and study design (e.g., personal issues, hormonal fluctuations, sickness, outside stressors). Next, we assessed both immediate and delayed (i.e., 1-hour post-intervention) response, in an attempt to investigate the sustainability of the response over time. This makes the current study design stronger and allows for assessment of effect longevity. Similarly, we assessed self-reported anxiety response throughout the inhalation task, which allowed for comparisons between self-reported and physiological changes (e.g., ventilation, respiratory frequency) over the course of the inhalation. Assessment of symptoms during inhalation has been recommended by previous researchers (e.g., Bailey et al., 2005) for capturing a more comprehensive profile of the continuous fluctuations in the anxiogenic response. Given the lack of condition main effects in our study, however, it is difficult to infer whether the respiratory response profile might have followed a differential pattern with a higher-intensity physical activity intervention. For example, maybe an aerobic-type physical activity protocol that significantly increases heart rate might have resulted in more substantial changes in respiratory rate, ventilation and tidal volume afterwards. Since there were no statistically significant 3-way interactions; however, we are unable to comment on whether a session of yoga can help attenuate the anxiety response more effectively than a light stretching protocol over time.

Next, there were some important differences in the physical activity paradigm between this and previous studies. That is, our physical activity intervention was a light-to-moderate intensity yoga condition, whereas previous studies have used walking, cycling and resistance training as modality, and higher intensities than ours, in their designs. Indeed, previous studies using aerobic-type interventions such as cycling and walking in their designs (e.g., Broman-Fulks et al., 2015; Esquivel et al., 2008, 2012; Smits et al., 2009) have reported larger attenuation

in response to the CO_2-inhalation challenge after the exercise bout in comparison to the control condition. Consequently, this might have contributed to the smaller ESs we observed in this study, in comparison to some of the ESs reported in previous studies (e.g., $\eta_p^2=.52$ for AS and $\eta_p^2=.17$ for API, reported by Broman-Fulks et al., 2015). We further did not have a true, no-exercise control condition but instead, a light stretching control condition. In this regard, our design is similar to those of Esquivel et al. (2002, 2008, 2012). For example, one of those studies recruited a sample with a clinical diagnosis of panic disorder and reported significantly attenuated response to the CO_2-inhalation challenge after moderate-to-hard intensity cycling, but not after very light-intensity cycling (e.g., Esquivel et al., 2008). Accordingly, it is possible that the yoga protocol in the current study might have failed to induce greater benefits than the control stretching activity due to its lower intensity. Both meta-analytic (e.g., Ensari et al., 2015) and experimental studies of similar type (e.g., Broman-Fulks et al., 2015; Esquivel et al., 2008) point to the greater effects observed after higher-intensity exercise. A replication of our study using a higher intensity yoga protocol as the physical activity intervention to see if this can improve the attenuation of the anxiogenic response would be beneficial and subsequently allow for generation of practical recommendations for the target population (e.g., those with clinically meaningful levels of anxiety and panic symptoms and sensitivity).

Finally, our sample was a relatively unique one that consisted of otherwise healthy women with high sensitivity toward their physical anxiety and panic symptoms. Specifically, we recruited a sample with a mean ASI score of 32.2 (SD=6), which is approximately 1 SD above the threshold score of 25 that is indicated to suggest clinically meaningful AS (Taylor et al., 2007). Previous studies of acute exercise and CO_2-inhalation have included samples with lower mean ASI scores (e.g., Broman-Fulks et al., 2015; Smits et al., 2009). On the other hand, we

excluded those with current or past diagnosis of anxiety or panic disorder, and those who were using any psychotropic medication at the time of the study. Accordingly, this might help partially explain the discrepancy between our results and theirs. That is, it is possible that the anxiogenic response to the CO_2-inhalation task is not attenuated significantly in individuals with clinically meaningful levels of trait AS. This argument finds further support from previous literature suggesting that the panicogenic response to the CO_2-inhalation task is centrally-mediated, implicating the amygdala (Argyropoulos et al., 2002; Gorman et al., 2001), and that the physiological change (i.e., increased ventilation) is genetically determined (Kawakami et al., 1982) and involves the chemoreceptors (e.g., Zandbergen et al., 1991). If this is indeed true, then such an acute intervention like ours cannot realistically be expected to attenuate this response and would require the assessment of a longer intervention (e.g., 12 weeks). Based on the present findings, this theory of a centrally-mediated, genetically determined pattern might also be true for generalized anxiety. Accordingly, it might be possible to suggest that individuals with heightened sensitivity have a lower threshold for stimuli such as air mixtures with increased CO_2 concentration and based on the previously theories (Zandbergen et al., 1991), that this might under the influence of the chemoreceptors (i.e., "impaired chemoreceptor sensitivity"). Combined with the results of the self-reported data, it might be possible to suggest that the acute attenuation in anxiogenesis is independent of the physiological symptoms of anxiety. Obviously, longitudinal studies are required to clarify whether physiological symptoms would follow cognitive symptoms.

The inclusion of only women in the present sample is an important aspect of the study for 2 main reasons that deserve further elaboration. First, anxiety disorders are significantly more prevalent (i.e., ~2:1 prevalence) and debilitating (i.e., less likely to receive treatment and

associated with more comorbidities) in women than in men. Therefore, we acknowledge the importance of focusing research efforts to possibly find alternative approaches for targeting these negative symptoms, which may or may not be unique to this demographic, by designing a study that specifically targets women. Second, women are reportedly underrepresented in biomedical research and only recently have there been more deliberate efforts to include women participants in studies (Rieker & Bird, 2005). Frequently, this exclusion has been justified by the goal to provide a homogenous sample (i.e., therefore fewer potential between-subjects differences at baseline) and also the difficulties of adjusting for the hormonal and other related physiological fluctuations (e.g., those that occur as a part of the menstrual cycle) (e.g., Killien et al., 2004; Kim et al., 2000). This gap in the literature is particularly essential to consider for the purposes of this study. There is preliminary evidence that use of hormone-based contraception can alter the estrogen-progesterone fluctuations and consequently, can disable the anxiety-buffering effect of estrogen during the non-luteal phase. For example, women low on estrogen levels have been reported to have an impaired ability to attenuate their startle response when faced with a panicogenic (e.g., Glover et al., 2015). When faced with the CO_2-inhalation task, then, these women would be expected to show greater sensitivity than those who are not. Indeed, this luteal drop in estrogen levels has been reported by others to influence the panicogenic and/or anxiogenic response to the CO_2-inhalation task (e.g., Niilni et al., 1999; Perna et al., 1995). Interestingly, our results indicated no such effect, as the results for the final ANOVA model were not statistically different than the results of the ANCOVA with this variable included as a covariate factor. This discrepancy between our results and others might have been due to the differences in the sample characteristics. For example, another similar study compared the anxiogenic response to 5% and 7% CO_2-inhalation challenge in separate groups of women with

major depressive disorder (MDD), pre-menstrual dysphoric disorder (PMDD), panic disorder (PD) or those without (i.e., healthy control) (Gorman et al., 2001). The results of this study indicated that the elevation in anxiogenesis was comparable between those with PD and those with PMDD and furthermore, that these 2 groups responded with much greater anxiety than the other 2 groups (i.e., healthy control and those with MDD). However, the physiological response (i.e., change in respiratory rate and ventilation) was comparable across all groups. The authors concluded that these findings collectively point toward the existence of a central fear mechanism that involves an inherent conditioning to anxiogenic stimuli in some individuals. Our findings would seem to be in accordance with those of Gorman et al., (2001) and therefore their proposed mechanism could also apply to our sample. That is, it is possible that we have selected such a group of individuals (i.e., those with significant levels of AS) and therefore this hypothesized inherent, central fear mechanism overrides any possible anxiety-attenuating effect of the acute exercise bout in our participants. Taking into account our findings, as well as those aforementioned, it is clear that further research with a focus on samples consisting of women are needed to clarify these points.

Limitations and future directions

This study is not without limitations. First, the sample size was relatively small. Subsequently, the effect sizes (i.e., η_p^2) for interaction and main effects were in the small-to-moderate range in our study, whereas others before us have reported larger ESs (e.g., $\eta_p^2 = .17$ for a 3 X 3 ANOVA of API scores in the study by Broman-Fulks et al. (2015). Therefore, it is possible that statistically significant results might be observed with a larger sample size. Next, this study did not have a true, no-exercise control condition and therefore it is not possible to

conclude whether acute yoga is more efficacious for anxiety attenuation than seated quiet rest or an alternate no-exercise activity (e.g., reading). It is possible that the stretching protocol in the present study mimicked the yoga protocol too closely. In other words, the control condition was might have been overbuilt and therefore was too "active". To circumnavigate this problem in the future, having a meditation session instead might be another alternative as a control condition. This would then prevent the problem of a potentially "overbuilt" control condition that is too similar to the active condition and furthermore, control for the effect of the meditation component that is already inherent in the yoga condition. Similarly, both the yoga and stretching were conducted on a 1-on-1 basis; and it is possible that this made some of the participants feel self-conscious while doing the poses. Subsequently, the yoga session might not have been as anxiolytic for such participants as intended. Similarly, we recruited participants who were "yoga-naïve". Our selection criterion was doing yoga <2/week and most participants in our sample reported not doing any yoga at all in past 6 months at least. Accordingly, this lack of familiarity with the poses might then have led to a state of cognitive activation where, the participants were paying so much attention to trying to execute the poses that any possible anxiolytic effects were greatly reduced. There is some preliminary, indirect indication of this based on the relatively low MAAS-S scores reported after both conditions. It is possible that the mood-improving benefits of yoga start to occur after some familiarization with the practice. In this regard, conducting longitudinal studies to assess such potential benefits might help clarify this point. Alternatively, comparing these effects in those who are regular yoga-practitioners versus those who are not (i.e., "Yoga-naïve") would help elucidate familiarity is indeed an influential factor. Such familiarization would allow the individuals to flow through the poses at a faster pace and potentially achieve a higher heart rate. This would further help address whether it is the intensity

of the yoga that drives the anxiolytic effect. Furthermore, we did not control for PA levels at baseline and our sample consisted of physically active individuals who met the PA Guidelines (USDHHS, 2008). It is possible that replication of the current design with a sample of individuals who are sedentary might yield a different set of results and accordingly, comparison of those who are physically active versus those who are not would help clarify this question. Finally, there was a mix of participants in our sample who were using hormone-based contraceptive methods and those who were not (i.e., 11:7). As discussed above, this might have altered the natural ability of the body to regulate the possible anxiolytic effect of the yoga intervention in response to the CO_2-inhalation task in some of the participants due to their relatively lower estrogen levels. In this regard, recruiting a sample of women who are not using a hormone-based birth control method might better clarify whether the pattern of the anxiogenic response differs as a covariate of the menstrual cycle phase (e.g., an expected direction of greater attenuation in response during non-luteal phase). Similarly, it should also be noted that the determination of the luteal vs non-luteal phase at the time of testing was approximate and was based on the self-reported 1st day of menstrual period. Luteal phase can last anywhere between 6 and 14 days and accordingly; the categorization might have been slightly erroneous for some participants. Therefore, the results of the ANCOVA should be interpreted with caution.

It appears that both vinyasa-style yoga and light stretching-type activity might induce effects of general physical activity for attenuating anxiogenesis and might be feasible approaches to counter the effects of a future anxiogenic or panicogenic stressor for an individual (e.g., an upcoming interview, getting on a flight). This practical suggestion is based on the condition-independent attenuation in the generalized anxiety response over the course of the assessment time point (i.e., T1 through T3). These findings provide an update on and expand upon

previously reported beneficial effects of muscle relaxation techniques among individuals with self-reported anxiety (e.g., Carlson et al., 1990; Bernstein & Borkovec, 1973). The current results are further in line with previous research on the anxiolytic effects of physical activity (e.g., Ensari et al. 2015; Esquivel et al., 2012). Given the increasing popularity of yoga as an alternative treatment approach for a variety of health conditions and symptoms and as well the wide range of yoga styles commercially available, it is timely to investigate yoga as a physical activity modality in well-designed, rigorous experimental studies so as to delineate its true mood-improving effects.

CHAPTER 6: CONCLUSIONS

In conclusion, there was no evidence for a differential pattern of change between an acute bout of vinyasa style yoga and a stretching control condition for attenuating the anxiety and panic symptoms in response to the 5-minutes of 7.5% CO_2-inhalation task in women with high AS. However, there was an overall reduction in cognitive anxiety symptoms after both conditions, suggesting an overall effect of general physical activity for reducing cognitions of anxiety. Our results are partially in accordance with previous research in this area (e.g., Broman-Fulks et al., 2015; Smits et al., 2009), and this might be due to the methodological differences between studies. First, all previous studies investigated symptoms of panic, not anxiety, and accordingly have used a single or double-inhalation of 35% CO_2-air mixture (Broman-Fulks et al., 2015; Esquivel et al., 2002; 2009; 2012; Smits et al. 2009). Next, those studies did not assess change in scores (i.e., pre- to post-exposure). This is a more robust method for assessing change in response to an acute exercise stimulus, as it takes into account any potential fluctuations in pre-exposure scores across participants. Possible influence of the phase of menstrual cycle was also accounted for by including it as a covariate factor in the data analyses, and the results did not significantly change. Future studies should recruit larger samples and include a true, no-exercise control condition to account for the passage of time. Investigation of higher-intensity yoga protocols for would also elucidate whether this would improve the attenuation response. This study provides the first evidence that 5 minutes of 7.5% CO2-inhalation task is a reliable method to induce symptoms of both panic and more generalized anxiety in sensitive individuals and that there does not seem to be a habituation response to these effects either acutely or over several (i.e., 3-4) weeks. Accordingly, this method can be used for studying models of anxiety

and panic under laboratory conditions as a valid method for experimental induction of such symptoms.

CHAPTER 7: TABLES AND FIGURES

Table 1. Descriptive characteristics for the study sample (N=18). SD=Standard deviation.

Variable	Mean (SD)
Age (years)	22.1 (5.0)
Height (cm)	166.2 (4.9)
Weight (kg)	64.9 (11.4)
HADS-Anxiety	9.8 (4.7)
HADS-Depression	5.2 (3.9)
GADS-7	8.4 (4.8)
Trait Anxiety	46.4 (10.3)
Trait Anger	16.0 (4.4)
ASI-3 score	24.9 (10.3)
Trait Mindfulness	3.8 (.9)
GLTEQ score	40.9 (21.2)
Hormone-based contraceptive use (%)	11 (61%)

Table 2. Mean scores (SD) on the self-report measures to the 1st and 2nd inhalation tasks during the baseline session. API=Acute Panic Inventory, SAI=State Anxiety Inventory, SAS=State Anger Scale, VAS-A=Visual Analog Anxiety Scale.

Time point	API		SAI		SAS		VAS-A	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1st inhalation task	1.39 (1.50)	8.83 (4.63)	33.78 (8.97)	46.61 (10.49)	15.50 (0.86)	15.50 (91.04)	26.39 (18.29)	40.89 (21.33)
2nd inhalation task	3.28 (2.49)	8.61 (3.74)	39.78 (9.00)	43.44 (11.45)	15.33 (0.77)	15.39 (0.70)	24.83 (17.01)	36.83 (24.05)

Table 3. Mean scores (SD) on the self-report measures to the 1st, 2nd and 3rd inhalation tasks during the experimental (i.e., yoga) and control (i.e., stretching) sessions. API=Acute Panic Inventory, SAI=State Anxiety Inventory, SAS=State Anger Scale, VAS-A=Visual Analog Anxiety Scale.

Time point	API				SAI			
	Yoga		Control		Yoga		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1st inhalation task	1.00 (1.33)	8.39 (4.67)	1.06 (1.55)	8.89 (5.17)	31.55 (8.58)	43.06 (12.11)	32.94 (10.31)	43.17 (10.72)
2nd inhalation task	1.17 (2.20)	8.50 (5.87)	.72 (1.41)	6.89 (2.87)	26.83 (6.04)	39.67 (12.00)	27.22 (8.82)	40.06 (10.94)
3rd inhalation task	.83 (1.29)	8.67 (6.13)	.67 (.97)	7.61 (5.98)	29.17 (9.88)	42.17 (12.55)	31.83 (9.16)	41.11 (13.48)

(Table 3 cont.)

Time point	VAS-A				SAS			
	Yoga		Control		Yoga		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1st inhalation task	13.67 (12.43)	28.83 (21.60)	17.83 (13.76)	32.94 (22.10)	15.17 (.71)	15.39 (.14)	15.61 (.45)	15.78 (.32)
2nd inhalation task	6.72 (7.27)	24.00 (21.65)	8.17 (10.11)	24.39 (19.34)	15.06 (.06)	15.11 (.08)	15.33 (.33)	15.22 (.13)
3rd inhalation task	7.67 (9.25)	27.22 (22.17)	6.78 (7.10)	29.11 (24.85)	15.17 (.17)	15.06 (.06)	15.00 (.00)	15.44 (.29)

Table 4. ANOVA results for the Acute Panic Inventory (API), State Anxiety Inventory (SAI), Visual Analog Anxiety Scale (VAS-A), and the State Anger Scale (SAS) scores. Condition (yoga, control), Time point (Baseline, immediately post yoga/control, 1 hour post yoga/control), exposure (pre- and post-inhalation task).

API	df	F	η_p^2	p
Condition	1	1.49	.08	
Time point	2	1.31	.07	
Exposure	1	55.53	.77	<.001
Condition*time point	2	1.27	.07	
Condition* Exposure	1	.88	.05	
Time point* Exposure	2	.64	.04	
Condition*time point* Exposure	2	1.25	.07	
SAI	df	F	η_p^2	p
Condition	1	.32	.02	
Time point	2	9.63	.36	<.001
Exposure	1	40.08	.70	<.001
Condition*time point	2	.52	.00	
Condition* Exposure	1	1.09	.06	
Time point*Exposure	2	.88	.05	
Condition*time point* Exposure	2	1.06	.06	
VAS-A	df	F	η_p^2	p
Condition	1	.94	.05	
Time point	2	14.31	.46	<.001
Exposure	1	21.52	.56	<.001
Condition*time point	2	1.84	.10	
Condition* Exposure	1	.06	.00	
Time point* Exposure	2	3.58	.17	<.05
Condition*time point* Exposure	2	.35	.02	

(Table 4 cont.)

SAS	df	F	η_p^2	p
Condition	1	1.85	.10	
Time point	2	3.07	.15	.06
Exposure	1	.73	.04	
Condition*time point	2	.97	.05	
Condition* Exposure	1	.16	.01	
Time point* Exposure	2	.50	.03	
Condition*time point* Exposure	2	.86	.05	

Table 5. Mean peak values (SD) during the 5th minute of inhalation for respiratory measures in response to the 1st, 2nd and 3rd inhalation tasks during the experimental (i.e., yoga) and control (i.e., stretching) sessions. RR=Respiratory Rate, VE=Ventilation, Vt=Tidal volume. All values are the peak values reached during the 5th minute of the CO_2-inhalation task.

Time point	RR		Vt (liters)		VE (liters/min)	
	Yoga	Control	Yoga	Control	Yoga	Control
1st inhalation task	29.11 (6.46)	28.89 (4.79)	1.74 (.35)	1.72 (.33)	47.26 (12.81)	46.21 (12.12)
2nd inhalation task	28.61 (7.73)	27.50 (4.74)	1.76 (.33)	1.76 (.32)	47.08 (13.55)	45.76 (12.49)
3rd inhalation task	28.72 (7.66)	28.28 (5.80)	1.77 (.32)	1.74 (.30)	47.74 (14.07)	47.15 (12.54)

Table 6. ANOVA results for respiratory rate (RR), tidal volume (Vt), ventilation (VE), and carbon dioxide production (VCO$_2$). Condition (yoga, control), assessment time point (Baseline, immediately post-condition, 1 hour post-condition), minute of exposure (30-second intervals averaged over the course of the entire 5-minute inhalation task).

RR	df	F	η_p^2	p
Condition	1	.46	.03	
Time point	2	2.56	.15	
Exposure	9	59.67	.80	<.001
Condition*time point	2	.50	.03	
Condition* Exposure	9	.88	.05	
Time point* Exposure	18	1.12	.07	
Condition*time point* Exposure	18	.60	.04	

Vt	df	F	η_p^2	p
Condition	1	1.11	.07	
Time point	2	1.57	.10	
Exposure	9	164.2	.92	<.001
Condition*time point	2	.03	.00	
Condition* Exposure	9	.94	.06	
Time point*Exposure	18	1.00	.06	
Condition*time point* Exposure	18	1.36	.08	

VE	df	F	η_p^2	p
Condition	1	1.38	.08	
Time point	2	1.13	.07	
Exposure	9	123.2	.89	<.001
Condition*time point	2	1.34	.08	
Condition* Exposure	9	.51	.03	
Time point* Exposure	18	1.49	.09	
Condition*time point*Exposure	18	1.44	.09	

(Table 6 cont.)

VCO$_2$	df	F	η_p^2	p
Condition	1	1.71	.10	
Time point	2	.43	.03	
Exposure	9	142.7	.91	<.001
Condition*time point	2	1.19	.07	
Condition* Exposure	9	.69	.04	
Time point* Exposure	18	1.15	.07	
Condition*time point* Exposure	18	1.32	.08	

Table 7. ANOVA results for peak respiratory rate (RR), tidal volume (Vt), and ventilation (VE) during the last minute of the inhalation task. Condition (yoga, control), Time point (Baseline, immediately post yoga/control, 1 hour post yoga/control). None of the F values were statistically significant (i.e., *p*>.05 for all values).

RR	df	F	η_p^2
Condition	1	.56	.03
Time point	2	1.24	.07
Condition*time point	2	.56	.03
Vt	**df**	**F**	η_p^2
Condition	1	.20	.01
Time point	2	1.61	.09
Condition*time point	2	.19	.01
VE	**df**	**F**	η_p^2
Condition	1	.61	.04
Time point	2	.70	.04
Condition*time point	2	.15	.10

Table 8. ANOVA results for minute-by-minute VAS-A rating during the 5-minute inhalation task. Condition (yoga, control), Time point (Baseline, immediately post yoga/control, 1 hour post yoga/control), Minute of exposure (minutes 1 through 5).

| During inhalation VAS-A	df	F	η_p^2	p
Condition	1	.36	.02	
Time point	2	10.31	.41	<.001
Minute of exposure	4	30.15	.67	<.001
Condition*time point	2	4.06	.21	<.05
Condition* Exposure	4	.87	.06	
Time point* Exposure	8	.30	.02	
Condition* Time point* Exposure	8	.65	.04	
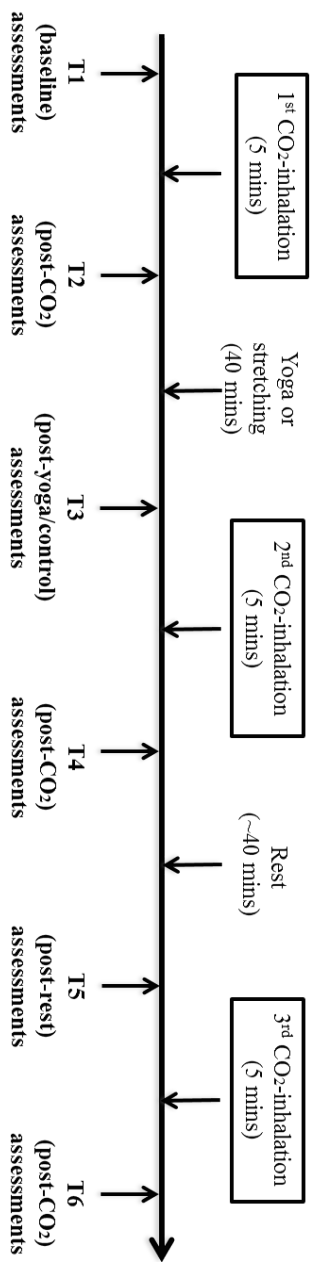
Figure 1. Timeline of study events.
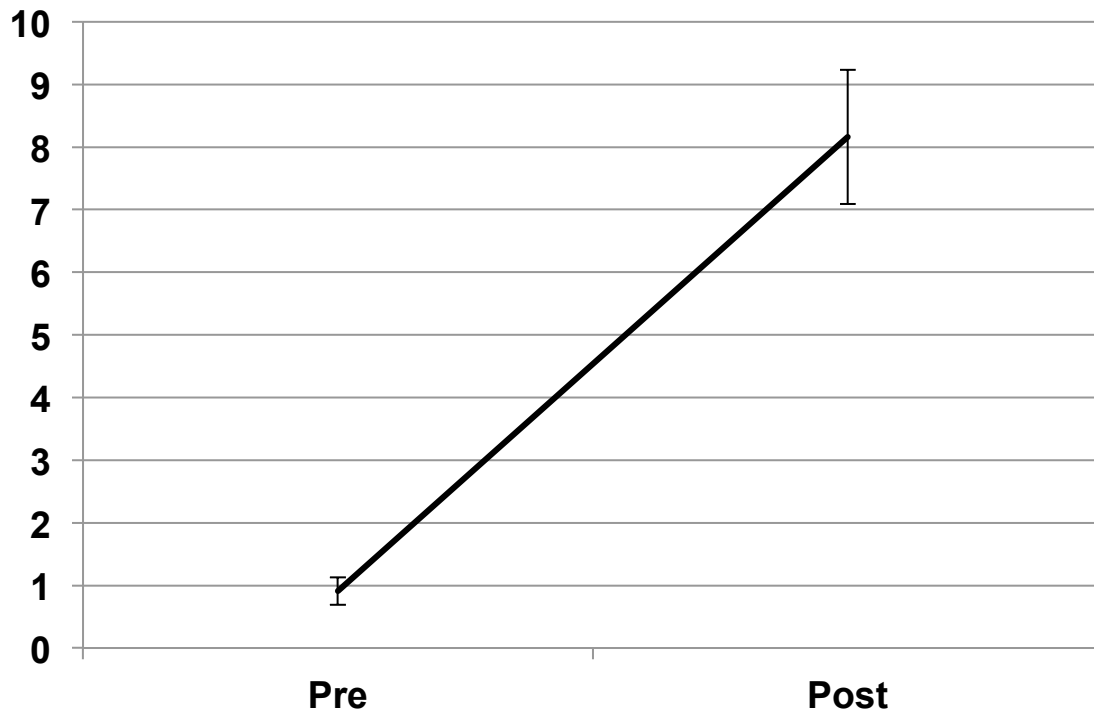
Figure 2. Mean API scores (represented on the y-axis) from pre- to post-exposure collapsed over condition and assessment time point. N=18. Error bars represent standard error (SE).
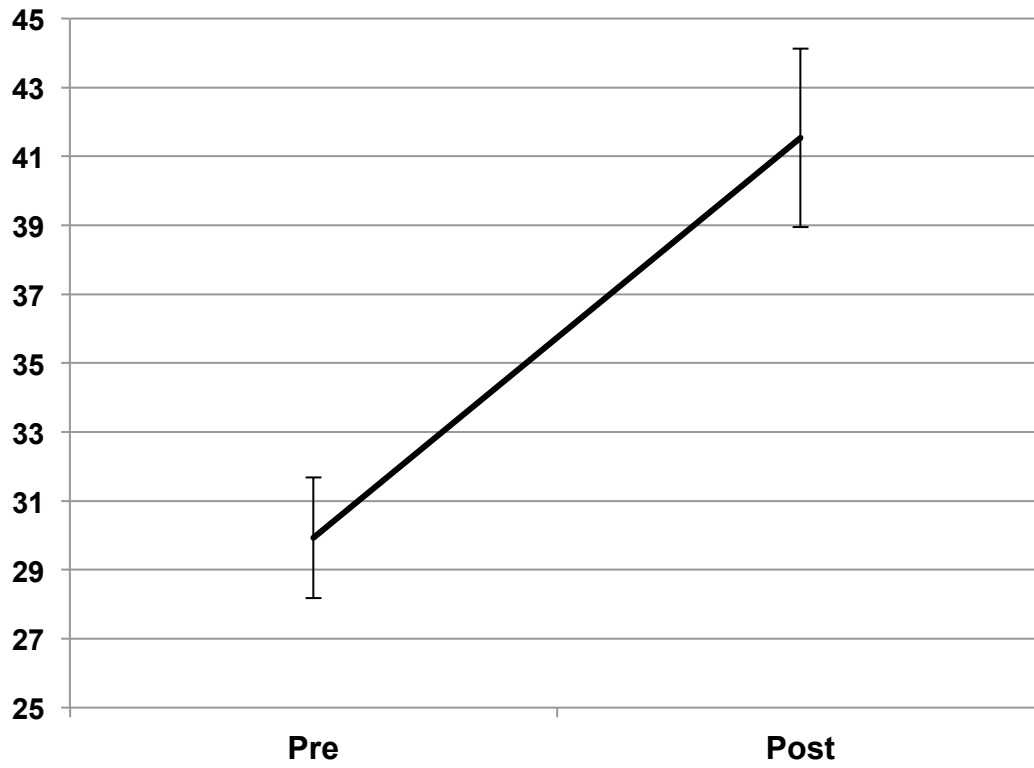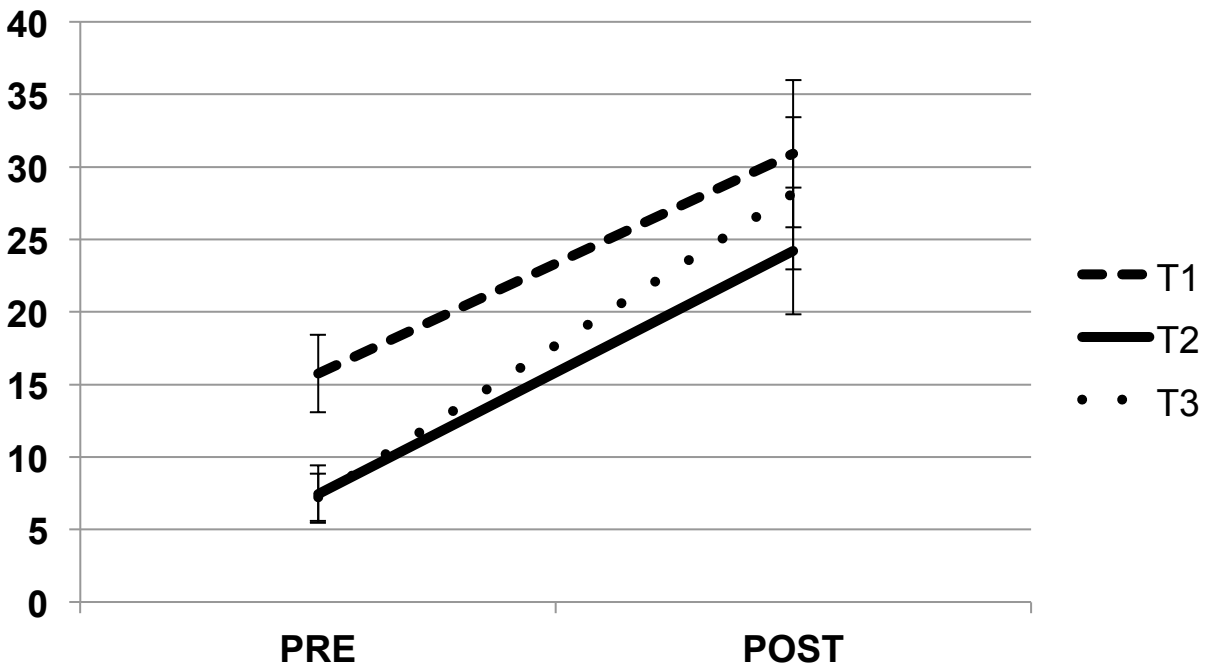
Figure 3. Mean SAI scores collapsed over condition and time point from pre- to post-exposure. N=18. Error bars represent standard error (SE).
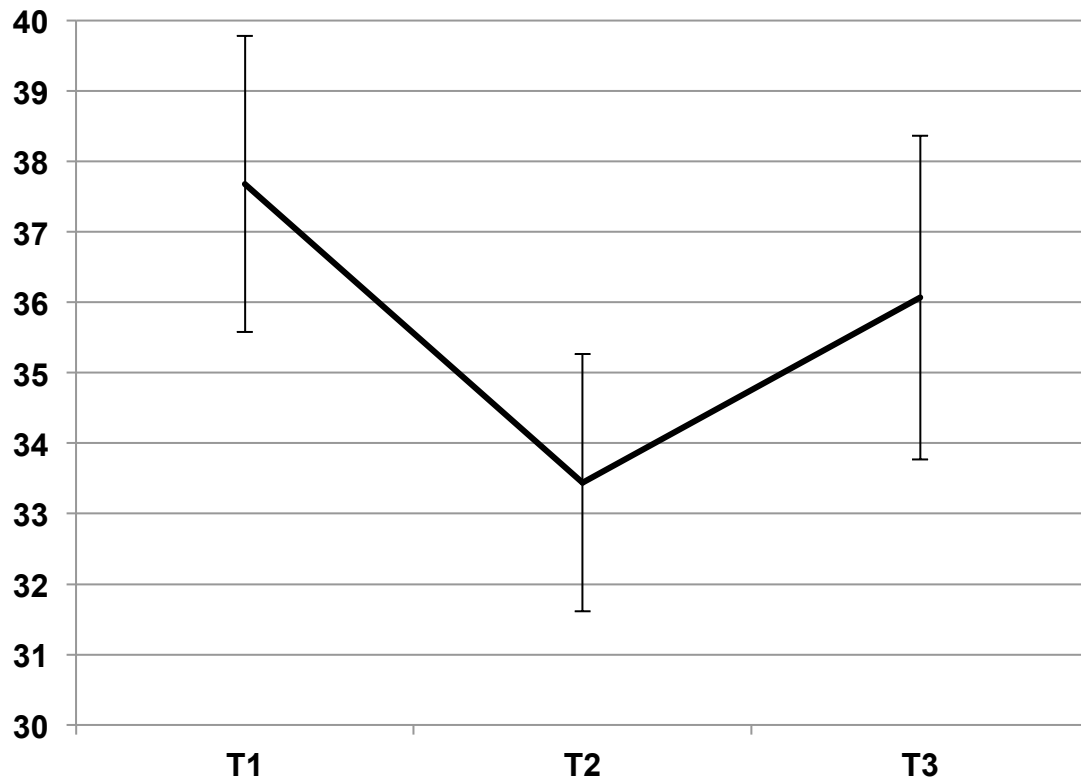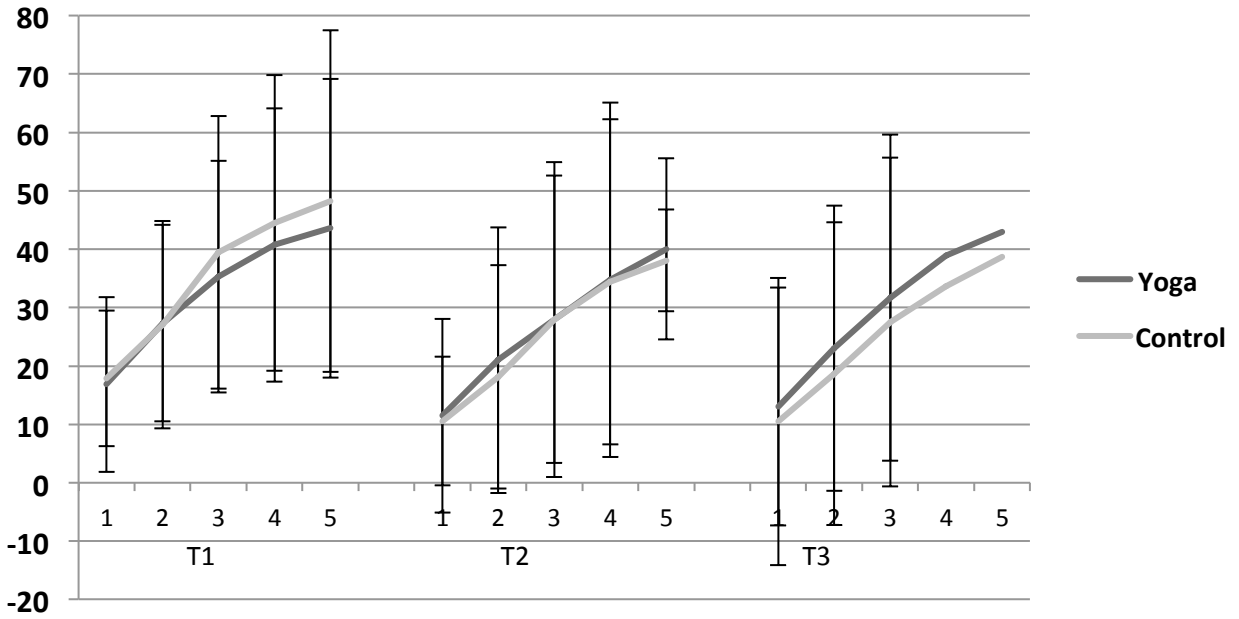
Figure 4. Mean SAI scores across the 3 assessment time points (i.e., T1, T2, T3) collapsed over condition and exposure. N=18. Error bars represent standard error (SE).

Figure 5. Mean VAS-A scores from pre- to post-exposure across the 3 assessment time points collapsed over condition. N=18. Error bars represent standard error (SE).

Figure 6. Mean minute-by-minute VAS-A ratings during each of the 5-minute CO_2-inhalation protocols. T1= pre-condition, T2=immediately post-condition, T3=1 hour post-condition. Error bars represent standard deviations.

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