Examining the Mediating Role of CO2 in Symptom Change During Capnometry-Assisted Respiratory Training (CART) Using a Transdiagnostic Sample

Anni Hasratian
Southern Methodist University, ahasratian@smu.edu

Follow this and additional works at: https://scholar.smu.edu/hum_sci_psychology_etds

Part of the Clinical Psychology Commons

Recommended Citation
Hasratian, Anni, "Examining the Mediating Role of CO2 in Symptom Change During Capnometry-Assisted Respiratory Training (CART) Using a Transdiagnostic Sample" (2023). Psychology Theses and Dissertations. 44.
https://scholar.smu.edu/hum_sci_psychology_etds/44

This Dissertation is brought to you for free and open access by the Psychology at SMU Scholar. It has been accepted for inclusion in Psychology Theses and Dissertations by an authorized administrator of SMU Scholar. For more information, please visit http://digitalrepository.smu.edu
EXAMINING THE MEDIATING ROLE OF CO₂ IN SYMPTOM CHANGE DURING
CAPNOMETRY-ASSISTED RESPIRATORY TRAINING (CART)
USING A TRANSDIAGNOSTIC SAMPLE

Approved by:

Alicia Meuret, Ph.D.
Professor of Psychology

Sahib Khalsa, Ph.D.
Associate Professor of Psychology

Thomas Ritz, Ph.D.
Professor of Psychology

David Rosenfield, Ph.D.
Professor of Psychology
EXAMINING THE MEDIATING ROLE OF CO₂ IN SYMPTOM CHANGE DURING CAPNOMETRY-ASSISTED RESPIRATORY TRAINING (CART) USING A TRANSDIAGNOSTIC SAMPLE

A Dissertation Presented to the Graduate Faculty of the Deadman College Southern Methodist University in Partial Fulfillment of the Requirements for the degree of Doctor of Philosophy with a Major in Clinical Psychology by
Anni Hasratian, M.A.

B.A., Psychology, University of California, Irvine M.A., Psychology, Pepperdine University M.A., Psychology, Southern Methodist University

May 13, 2023
Much research supports the relation between respiration and mood, and more particularly, between hypocapnia and negative affect. A review of breathing trainings has demonstrated that traditional breathing trainings do not target CO$_2$ directly, which maintains or worsens chronic hypocapnia. Capnometry-Assisted Respiratory Training (CART) was developed to directly target increasing CO$_2$ with patients with panic disorder through the use of a biofeedback device. It is less known whether the positive effects of CART generalize to other negative mood states, such as non-panic anxiety, since hyperventilation is a well-known response to stress and anxiety. Further, the relation between CO$_2$ and depression has been less extensively examined in therapeutic contexts, which would be of great importance given the high comorbidity rate of anxiety and depression. The current study aimed to examine the mediating role of CO$_2$ in symptom change during CART using a transdiagnostic sample. Over the course of two study cycles (2015-2018; 2018-2021), participants ($N = 175$) were randomized to the Negative Affect Treatment (NAT; $n = 94$) and the Positive Affect Treatment ($n = 81$). As part of the intervention, participants in NAT completed one module of CART ($n = 82$). Results indicated that increases in baseline PCO$_2$ fully mediated changes in anxiety and depression at the next
session, but not in stress or negative affect. Our findings support previous research on the
efficacy of CART in increasing PCO$_2$, which demonstrates a mediating role in reducing anxiety
symptoms, extending generalizability of past findings to transdiagnostic populations. Further,
our study is the first to find a causal relation between CO$_2$ and symptoms of depression, in that
increases in baseline PCO$_2$ led to increases in symptoms of depression. Clinical implications and
future directions are discussed.
TABLE OF CONTENTS

LIST OF FIGURES .................................................................................................................. VIII

LIST OF TABLES .................................................................................................................. IX

LIST OF ABBREVIATIONS ...................................................................................................... X

ACKNOWLEDGMENTS ............................................................................................................ XI

CHAPTER 1: INTRODUCTION ........................................................................................................ 1

  1.1 CAPNOMETRY ASSISTED RESPIRATORY TRAINING.......................................................... 5
  1.2 CART STUDIES .................................................................................................................. 6
  1.3 CURRENT STUDY ............................................................................................................. 14

CHAPTER 2: METHOD ................................................................................................................. 15

  2.1 PARTICIPANTS .................................................................................................................. 15
  2.2 PROCEDURE ..................................................................................................................... 17
  2.3 INTERVENTIONS .............................................................................................................. 18
     a. Capnometry-Assisted Respiratory Training. ................................................................. 18
  2.4 MEASURES ....................................................................................................................... 19
     a. Depression, Anxiety, and Stress ................................................................................... 20
     b. Negative Affect ............................................................................................................. 20
     c. Demographic Information .......................................................................................... 21
  2.5 DISRUPTIONS DUE TO COVID-19 .................................................................................. 21
  2.6 STATISTICAL ANALYSES ............................................................................................... 22
LIST OF FIGURES

Figure 1 Flowchart of study procedures...............................................................38

Figure 2 Cross Lag Mediation Models with outcome variables of anxiety (a), depression (b), stress (c), and negative affect (d).................................................................39

Figure 3 Carbon dioxide (CO₂) (a) and Respiration Rate (b) outcome in Capnometry-Assisted Respiratory Training...........................................................41
LIST OF TABLES

Table 1 Participant demographics .................................................................42
Table 2 Participant pre-treatment (session 0) scores................................................43
Table 3 Participant DASS and PANAS Negative scores prior to module 3.....................43
LIST OF ABBREVIATIONS

AiA……………Apnea-induced Anxiety
BDI……………Beck Depression Inventory
Bpm……………Breaths per minute
CART…………Capnometry-Assisted Respiratory Training
CBT……………Cognitive Behavioral Therapy
CT……………Cognitive Therapy
CO₂…………Carbon Dioxide
COPD…………Chronic obstructive pulmonary disease
DASS-21……..Depression Anxiety Stress Scales-21
MCAR…………Missing Completely at Random
MDD………….Major Depressive Disorder
MLM…………Multilevel Modeling
mm Hg…………Millimeters of Mercury
NAT…………Negative Affect Treatment
PANAS………..Positive Affect Negative Affect Schedule
PAT……………Positive Affect Treatment
PDD…………..Persistent Depressive Disorder
PCO₂…………Partial Pressure of Carbon Dioxide
PTSD…………Posttraumatic Stress Disorder
RCT………….Randomized Controlled Trial
RDoC…………Research Domain Criteria
RR……………Respiration Rate
SPSS…………Statistical Package for the Social Sciences
SMU…………Southern Methodist University
TAD…………..Treatment for Affective Dimensions
TVP………….Time-Varying Predictor
UCLA…………University of California, Los Angeles
ACKNOWLEDGMENTS

First, I would like to thank my parents for all that they have done and continue to do. This accomplishment would not be possible if it were not for their endless love and understanding—I share this honor with them both. Thank you to my siblings, Menua and Arpi, for always being on my team. I would not be where I am today without their support and pep talks. I am thankful for my nephew Theodore, who brings so much happiness and light into my life. I also extend my deepest appreciation to all my loved ones and dear friends who have continued to support my goals. They all have helped me in more ways than I can say. Thank you to my advisors and “doctor parents” Drs. Alicia Meuret and Thomas Ritz, whose guidance throughout the years helped me become a better researcher and clinician. Thank you to my dissertation committee for their thoughtful feedback, guidance, and time. I appreciate them sharing their insight and knowledge with me. Lastly, I am grateful for my faithful companion and late-night study buddy, Roxi, who was always there for me and brought me much joy throughout my graduate studies.
CHAPTER 1:
Introduction

Respiration has long been linked to mood. This relation has been most notable regarding negative mood states such as fear, anxiety, and stress (see Boiten et al., 1994 for a review). More specifically, the connection between respiration and mood has been seen with regard to carbon dioxide (CO$_2$) and the fight-or-flight response (i.e., panic, fear). The fight-or-flight response is an automatic physiological reaction to an event that is perceived as harmful, threatening, stressful or frightening (Cannon, 1915). Consequently, when the fight-or-flight response is triggered, it is accompanied by faster and/or deeper breathes, which can lead to hyperventilation.

Hyperventilation typically occurs when one breathes more than metabolic demands causing hypocapnia, or a reduction in partial pressure CO$_2$ (PCO$_2$; Gardner, 1996). Hypocapnic hyperventilation is determined by a range of PCO$_2$ levels between 30-35 mmHg (Sharma & Hashmi, 2021), and severe hypocapnia is indicated when PCO$_2$ levels fall below 30 mmHg (Bass & Gardner, 1985). Hypocapnia can cause a host of problems that have been linked to physical (Curley et al., 2010) and mental (Sikter et al., 2009) illnesses. For example, hypocapnia can decrease perfusion to the heart, damaging the organ (Laffey & Kavanagh, 2002).

Symptoms of hypocapnia include lightheadedness, dizziness, faintness, paresthesias, labored breathing (dyspnea), and chest pain (Pal & Chen, 2014). Research on dyspnea more specifically has shown that high levels of negative affect significantly contribute to the subjective perception of dyspnea (Hayen et al., 2013). Studies have also shown that both
subjective reports of negative affective states (Wientjes et al., 1986) and more psychosomatic symptoms like shortness of breath (Bass and Gardner, 1985; Van den Hout et al., 1992) have been associated with lower PCO$_2$ levels, suggesting that the link between negative affect and psychosomatic complaints could be explained by hypocapnia. Further, neuroimaging studies have demonstrated that negative affectivity also impacts the neural processing of respiratory sensations (Chan et al., 2015; Chenivesse et al., 2014).

In the late 1930’s, the term hyperventilation syndrome was introduced to describe symptoms of hypocapnia (Kerr et al., 1938). Inspired by the hyperventilation syndrome literature, a small number of trials emerged as one of the first to attempt correction of hypocapnia through therapeutic approaches. The first study was conducted by Folgering and colleagues (1980) using a nonclinical sample ($N = 10$). In this single-group trial, participants breathed room air through a face mask. Participants sat in a chair facing an oscilloscope that displayed the PCO$_2$ values, serving as visual feedback. They also heard sounds when their measured PCO$_2$ was higher than preset values. Participants were instructed to pay attention to the oscilloscope and try to increase their PCO$_2$. Additional trials of audio and visual feedback, and audio feedback only, trials followed. Participants returned up to two weeks later and repeated the same procedure. Results indicated that all participants were able to increase PCO$_2$, making this the first study to demonstrate that visual and auditory feedback can increase PCO$_2$. In a randomized controlled trial with 20 participants with hyperventilation syndrome (PCO$_2 < 35$ mm Hg), van Doorn et al. (1982) examined the efficacy of respiratory training sessions in increasing PCO$_2$. Participants in the control group received respiratory training only, whereas those in the experiment group also received PCO$_2$ feedback during the trainings. Results demonstrated that the experimental group was successful in increasing PCO$_2$, although not all participants reached
PCO$_2$ levels in the normal range (> 37 mm Hg). Participants in the respiratory training + PCO$_2$ feedback group also had greater reductions in hyperventilation syndrome symptoms compared to the control group. The authors suggested that increases in PCO$_2$ may play a crucial role in symptom reduction. Finally, Fried et al. (1984) examined the effects of a diaphragmic respiration training with PCO$_2$ biofeedback in an uncontrolled trial. They used a sample of 18 individuals with moderate to severe chronic hyperventilation and idiopathic seizures refractory. During the training, PCO$_2$ was displayed on a video monitor and participants were asked to maintain a specific respiratory pattern (12-14 breaths per minute) with the goal to reach 5% PCO$_2$. Findings demonstrated that respiration rate and PCO$_2$ improved. The authors concluded that an essential element in the therapeutic correction of abnormal respiration involves CO$_2$ biofeedback and not just adjusting respiration rate. Taken together, these early studies highlighted key findings about the role of PCO$_2$ in correcting hypocapnia.

Hyperventilation has been commonly evaluated in the context of the fight-or-flight response, which has long been associated with panic disorder (see Meuret & Ritz, 2010 for a review). As such, much attention has been directed to hyperventilation in the context of panic disorder. Respiratory abnormalities, such as lower PCO$_2$, have been a strong contender of the development and maintenance of panic disorder (Nardi et al., 2009) and have been shown to be a distinct characteristic of panic compared to other anxiety disorders such as generalized anxiety and specific phobia (Grassi et al., 2014). Further, shortness of breath has been found to be one of the more highly endorsed symptoms in individuals with panic (Meuret et al., 2006).

Theories postulating the causal relation between respiratory symptoms and panic further illustrate this connection. Ley’s (1985) Hyperventilation Theory proposes that panic attacks are caused by acute hypocapnia, which are not limited to the attack itself, but may come before or
after. This in turn gives rise to chronic hypocapnia occurring outside of an individual’s awareness. According to this theory, one would presume that a reduction in hyperventilation would be an effective strategy in alleviating panic symptoms. Klein’s (1993) Suffocation False Alarm Theory, on the other hand, recognizes hypocapnia as an agent of panic but does not attribute it as the primary cause. Rather, hyperventilation happens as a response to the misfiring of the “suffocation alarm system.” Here, the system fires at a lower threshold when PCO$_2$ rises, leading to disproportional breathlessness. In Klein’s theory, chronic hyperventilation may be viewed as serving a functional role by keeping PCO$_2$ low to avoid triggering the suffocation alarm.

In addition, the neuroanatomical hypothesis of panic considered the role of brain stem mechanism in the fear network, expanding the perspective of panic disorder beyond respiratory systems (Gorman et al., 2000). Specifically, this model identifies three distinct components of panic which activate specific sites of the central nervous system, including the brainstem, limbic system, and prefrontal cortex. Here, the authors identify a “fear network” centered in the amygdala as mediating fear-related responses (Gorman et al., 2000). Recently, the Apnea-induced Anxiety (AiA) model was proposed as an extension of Klein’s false suffocation alarm theory to apply to a broader range of fear and anxiety states (Feinstein et al., 2022). The AiA model proposes that the amygdala inhibits the chemoreceptive system during apnea, causing individuals to not realize that they temporarily stopped breathing. This in turns triggers a real suffocation alarm that seemingly emerged out of the blue. However, the underlying neurobiological mechanisms associated with the experience of these affective states still remains largely unknown, with other systems, such as the hypothalamic orexin system, gaining attention in their role in respiratory regulation (e.g., Ritz, 2022).
1.1 Capnometry Assisted Respiratory Training

Despite evidence for the relation between CO₂ and panic, a review of the application of breathing trainings for panic disorder uncovered that traditional breathing trainings (e.g., abdominal breathing, paced breathing) have not targeted CO₂ regulation (Meuret et al., 2003). Although some studies have suggested that traditional breathing trainings can be beneficial (e.g., Bonn et al., 1984; Chen et al., 2017; Hagman et al., 2011), there is reason to believe that these traditional approaches may in fact perpetuate hyperventilation. This is because common recommendations during respiratory distress, such as taking slower and deeper breathes, results in expelling too much CO₂, which may exacerbate hypocapnia (Meuret & Ritz, 2021).

Recognizing that traditional breathing trainings have not targeted CO₂ regulation, Meuret and colleagues (2008) developed a novel intervention aimed to directly manipulate PCO₂ in individuals suffering from panic. This approach, termed Capnometry-Assisted Respiratory Training (CART, formerly BRT), was developed to target hypocapnia directly. CART is a five-session training aimed to increase PCO₂ to the normative range (37-43 mm Hg) with the use of a portable capnometer. This treatment uses immediate feedback to teach participants how to raise their PCO₂. CART is a manualized treatment that includes four key components: education about the role of breathing in symptom exacerbation; bringing awareness to problematic respiratory patterns (i.e., negative impact of sustained levels of low CO₂); teaching techniques to control respiration; and twice daily at-home breathing exercise trainings. CART exercises comprise of 17-minute recordings that include three parts: a two-minute baseline period, a 10-minute paced breathing period accompanied by pacing tones, and a 5-minute transition phase without the tones. During the baseline phase, individuals sit quietly with their eyes closed. The pacing phase involves monitoring of one’s PCO₂ and respiration rate, and the transition phase requires
individuals to try to maintain similar breathing patterns while receiving feedback of their PCO\textsubscript{2} and respiratory rate. Each week’s exercises correspond to descending respiratory rates, beginning with 13 breaths per minute in the first week, followed by 11, 9, and 6 breaths per minute in the subsequent weeks. The efficacy of CART has been tested in seven randomized controlled trials (RCT) to date.

1.2 CART Studies

In the CART pilot study, thirty-seven participants with panic disorder, with or without agoraphobia, were randomly assigned to CART or a delayed-treatment group (Meuret et al., 2008). At the time of this pilot study, only one uncontrolled trial had examined PCO\textsubscript{2} as an outcome measure (Salkovskis et al., 1986). All participants met diagnostic criteria for panic disorder, as determined by the Structured Clinical Interview for DSM-4 Disorders (First et al., 1994). Participants’ baseline PCO\textsubscript{2} values were in the hypocapnia range (PCO\textsubscript{2} < 35 mmHg).

Participants in the CART condition received four weeks of five-session CART protocol. Attrition was low, with all participants completing treatment, and approximately 12% dropping out by the 12-month follow-up. Participants also had high homework compliance, with 91.3% of the home exercises completed. Results indicated that those in the CART condition reported significant improvements in panic disorder severity, agoraphobic avoidance, anxiety sensitivity, and respiratory measures. Findings also indicated that the CART group reported significant reduction in depressive symptoms, as measured by the Beck Depression Inventory (BDI; Beck et al., 1961), compared to the waitlist control. Of note, baseline BDI scores for both groups were consistent with minimal depressive symptomology range ($M_{\text{CART}} = 11.15$, $SD = 8.41$; $M_{\text{Control}} = 13.47$, $SD = 7.51$). Overall, the CART pilot study demonstrated preliminary evidence that raising
end-tidal PCO$_2$ by means of a capnometry feedback was therapeutically beneficial for individuals with panic disorder.

Although these findings were promising, some criticisms about breathing trainings continued to exist in the field. These critiques included symptom misappraisal (Salkovski et al., 1986) and whether the mechanism at core was in fact perceived control (Garssen et al., 1992). To address previous methodological gaps, Meuret et al. (2010) employed a study examining the processes for CART vs. Cognitive Therapy (CT) for panic disorder. This study improved and extended previous research in several ways. First, the two interventions focused solely on the manipulation of the proposed mediator, that is PCO$_2$ for CART and symptom appraisal for CT. Second, the authors used psychological and biological markers to assess the intervention-specific mediators. This allowed for the exploration of whether results were consistent across multiple dimensions. Finally, perceived control, a mediator that was not specific to a particular intervention, was also examined.

In Meuret et al.’s (2010) randomized controlled trial, 41 participants with panic disorder and agoraphobia were randomly assigned to CART or CT. The CT intervention included four components of education about the relation between panic symptoms and catastrophic thoughts, identifying negative thoughts, forming alternative, more helpful thoughts, and between-session homework. The authors found that reductions in panic symptom severity, panic-related cognitions, and improvements in perceived control were significant in both CART and CT. However, CART led to corrections from baseline hypocapnia to normative levels compared to the cognitive training group. Further, PCO$_2$ unidirectionally mediated and preceded changes in symptom appraisal, and perceived control was unidirectionally associated with changes in panic symptom severity. On the other hand, for the cognitive training group, reductions in symptom
appraisal were bidirectionally associated with perceived control and panic symptom severity. Perceived control was also bidirectionally related to panic symptom severity in both treatment conditions. These results disproved the notion that therapeutic change in CART was due to corrections in misappraisal or perceived control.

Using the above-mentioned theories by Ley (1985) and Klein (1994), Kim and colleagues (2012) utilized CART to test the effectiveness of raising PCO₂ (hyperventilation theory) or lower PCO₂ (false-suffocation alarm theory). Participants with panic disorder (N = 74) were randomized to one of three groups: hypercapnia (raise-CO₂) therapy, hypocapnia (lower-CO₂) therapy, or waitlist. The two active therapies were identical apart from the instructed target PCO₂ levels, which was 40 mm Hg for the raise-CO₂ group and 30 mm Hg for the lower-CO₂ group. Results indicated that both active groups demonstrated significant decreases in panic severity compared to the waitlist group, and treatment effects were maintained at the 6-month follow up. At the 6-month follow up, the lower-CO₂ group showed PCO₂ significantly lowered to close to 30 mmHg, whereas no significant increases in PCO₂ was found in the raise-CO₂ group. This suggested that elements common for both therapies (e.g., education about how panic symptoms are part of a normal stress response), rather than their effect on CO₂ levels, were the driving factor in treatment successes.

Tolin et al. (2017) aimed to replicate and extend previous CART studies by examining the feasibility and effectiveness of CART in naturalistic clinical settings. Previous studies had been conducted under more rigorous circumstances, utilizing RCT designs in academic institutions. Sixty-nine participants with a primary panic disorder diagnosis, as determined by the Mini International Diagnostic Interview (Sheehan et al., 1998), were enrolled in the study. The CART sessions followed the same manualized protocol as previous studies. Results showed that
the average increase in baseline PCO$_2$ from pre- to post-treatment was significant, raising post-treatment PCO$_2$ to the normocapnic range. These findings supported replication of previous findings in a more diverse, outpatient clinical setting. Though the study did not utilize a control group, their findings demonstrated feasibility of implementing CART in a more naturalistic treatment setting.

Although CART was initially developed and tested with individuals with panic, preliminary findings indicated that CART could also be beneficial for patients with asthma (Meuret et al., 2007). In addition to connections to panic, hyperventilation has also been linked to asthma (see Meuret & Ritz, 2010 for a review). Hyperventilation has been associated with perception of a reduced health status above and beyond other asthma symptoms, such as congestion and fatigue, and low perceived control of asthma has been suggested to mediate this relation (Ritz et al., 2008). Further, laboratory studies using physiological measures reported lower PCO$_2$ levels in participants with asthma compared to healthy controls (Osborne et al., 2000). A review of the effectiveness of direct (e.g., respiratory resistance biofeedback) and indirect (e.g., heart rate variability) biofeedback techniques as an adjunctive treatment for asthma found that, although some of these techniques were beneficial for symptom reduction and improvements in quality of life, these techniques were not effective in improving hypocapnia (Ritz et al., 2004). This is in part because slow-breathing techniques did not examine changes in CO$_2$, thus basal PCO$_2$ remained in the hypocapnic range. In studies that did measure changes in PCO$_2$ levels, such as those that have used the “Buteyko method,” results on whether PCO$_2$ increased at the end of treatment were inconsistent (Bruton & Lewith, 2005). Overall, many breathing interventions for asthma have lacked direct manipulation of PCO$_2$, with only a handful assessing PCO$_2$ (see Ritz & Roth, 2003 for a review). To that extent, the use of CART with
patients with asthma was demonstrated to be successful in increasing PCO₂ (Meuret et al., 2007), with a subsequent study supporting those findings (Ritz et al., 2014). These findings suggest that CART shows promise as a useful adjunctive treatment to medication management of asthma (Jeter et al., 2012).

In addition to examining PCO₂ as an outcome, changes in PCO₂ have also been found to act as a mediator. Using the sample from their 2008 publication, Meuret et al. (2009) examined whether changes in PCO₂ mediate changes in fear of bodily sensation for individuals with panic disorder. The results indicated that PCO₂, not respiration rate, was a partial mediator of the changes in anxiety sensitivity. Increased PCO₂ levels reduced fear of bodily sensations in participants with panic disorder. These results not only emphasize the important role of PCO₂, as it also led to changes in respiration rate, but questioned the importance of respiration rate that is typically stressed in breathing trainings.

To date, only one study has tested the efficacy of CART outside of panic disorder and asthma. Jamison and colleagues (2019) conducted a RCT of CART in Veterans with Posttraumatic Stress Disorder (PTSD) hyperarousal symptoms. Based on research indicating that almost one-third of individuals with PTSD report panic attacks (Cougle et al., 2010), and that cues associated with trauma may become conditioned as triggers for panic symptoms and physiological activation (Nixon et al., 2004), Jamison et al. (2019) sought to examine whether participants with PTSD would have decreased hyperarousal after CART treatment compared to waitlist controls. Eighty Veterans with PTSD were randomized to CART or waitlist. The authors noted that attrition with this population was especially high compared to previous CART trials. Also, although participants in CART showed a reduction in respiratory rate, PCO₂ did not increase. Of note, baseline PCO₂ was within the normal range for most participants. Results also
indicated that CART was not successful in reducing symptoms of hyperarousal. The authors concluded that this might be because physiological arousal of PTSD may differ from arousal (e.g., hypocapnia) targeted by CART. This brings to question whether the positive effects of CART would generalize to other negative mood states such as non-panic anxiety since hyperventilation is a well-known response to stress and anxiety (Suess et al., 1980; see Grossman, 1983 for a review).

While evidence suggests that respiratory abnormalities are specific for panic disorder compared to other anxiety disorders (Coscì & Mansueto, 2020; Grassi et al., 2014), decreases in CO₂ have been observed in nonclinical samples in response to stress and anxiety. For example, under high-stress conditions (i.e., math performance and verbal memory recall), adolescents with high text anxiety displayed lower levels of PCO₂ and faster respiration rate than students with low test anxiety (Ley & Yelich, 1998). Similarly, music students with high music performance anxiety were found to have lower PCO₂ levels before private and public performances compared to musicians with low music performance anxiety (Studer et al., 2012). These patterns have been demonstrated with clinical samples as well. A study examining females with high- vs. low-trait anxiety found that high-trait anxiety was associated with stronger reductions in PCO₂ to images of suggested risk of suffocation (Van Diest et al., 2005). In a cross-sectional study using capnograph and spirometry to measure respiratory indices, Maleki et al. (2021) found that individuals diagnosed with generalized anxiety had significantly lower CO₂ and higher respiration rate compared to healthy controls. This may be in part related to the fact that generalized anxiety involves continued hypervigilance to internal and external cues that may signal future danger (Borkovec, 2002), and mental representations of threat have been shown to elicit fight-or-flight responses in anticipation of the stressor (Brosschot et al., 2010).
As demonstrated above, respiration, and hyperventilation more specifically, has been linked to many negative mood states typically associated with anxiety. However, the relation between CO$_2$ and depression has been less extensively examined, therefore much of our knowledge in the area is speculatory. For instance, hyperventilation has been most typically considered to be triggered by situations with high arousal and high negative valence. Yet, it has been suggested that arousal, more so than valence, plays a key role in hyperventilation. In an experimental study using script-driven imagery, Van Diest and colleagues (2001) examined the importance of arousal (high/low) and valence (negative/positive) as triggers for hyperventilation responses. Participants imagined themselves in eight different situations varying along the arousal and valence dimensions. Decreases in PCO$_2$ occurred in all scripts except the relaxation (positive valence, low arousal) and depressive (negative valence, low arousal) scripts. The authors concluded that arousal played a more crucial role in PCO$_2$ drops than valence as hyperventilation occurred during imagery of all high-arousal scripts regardless of their valence.

Additionally, CO$_2$ has been suggested to be more elevated during certain negative mood states, such as sadness. Kreibig et al. (2007) investigated responses to fear and sadness inducing films across a broad range of physiological measures, including respiration. Here, they found that physiological response patterns under fear and sadness were generated by different kinds of activation of the autonomic nervous system. Specifically, respiration rate decreased in the sadness condition and increased in fear, and PCO$_2$ decreased in fear and increased in sadness. This signified fast and shallow breathing (i.e., hyperventilation) during fear and slow and shallow breathing (i.e., hypoventilation) during sadness. This study was the first at the time to find increases in PCO$_2$ during sadness induction. Conversely, depression has been reported to be associated with increased respiration and decreased PCO$_2$ (Boiten et al., 1994). For example,
Damas-Mora et al. (1982) reported CO₂ within the hypocapnic range for individuals with depression who were admitted to a psychiatric hospital. Patients with depression took more breaths per minute compared to healthy controls and had lower resting CO₂ compared to the control group, who was within the normal range (37.8 mmHg). The authors noted that the low CO₂ observed among patients with depression draws attention to a possible alteration in central respiratory regulation. Further, depressive mood has been found to uniquely predict respiratory sensations of obstruction and arrest of breathing after controlling for anxious mood (Petersen & Ritz, 2009). Although evidence suggests a link between depression and respiration, the direction, or the impact, is yet to be determined. In a review of the role of the hypothalamus in modulating respiration, research mainly from animal studies have found that hypothalamus dysfunction causes abnormal breathing and hypoventilation (Fukushi et al., 2019). As the hypothalamus is part of the hypothalamic-pituitary-adrenal (HPA) axis, and abnormalities of the HPA axis has been linked to depression (Dean & Keshavan, 2017), perhaps there may be a relation between depression and respiratory dysregulation.

The current study adds to findings that link CO₂ regulation also to depressive symptoms. Gaining a better understanding of the relation between depressive symptoms and CO₂ would be helpful for tailoring transdiagnostic treatments across various clinical presentations. Although no study to date has directly examined the efficacy of CART in driving symptom changes in individuals with depression, post-treatment reductions in depression were reported in previous studies (Meuret et al., 2008; Tolin et al., 2017), with approximately 20% of participants with comorbid major depressive disorder diagnoses in one study (Tolin et al., 2017). An examination of whether these findings replicate would be of great importance given that anxiety and depression often co-occur (Brady & Kendall, 1992; Gorman, 1996).
1.3 Current Study

The overall objective of the current study was to examine the mediating role of CO$_2$ in symptom change during CART using a transdiagnostic sample from a larger RCT. The use of a transdiagnostic sample is a favorable approach as a large of body of research supports the dimensionality of anxiety and depression (e.g., Bjelland et al., 2009; Brown & Barlow, 2005) as well as shared components of both disorders (e.g., Brown & Barlow, 2009).

Participants enrolled in the study were randomized to one of two conditions: Negative Affect Treatment (NAT) or Positive Affect Treatment (PAT). Participants randomized to NAT completed 4-weeks of CART training as part of the intervention. The proposed study will be the first to evaluate the benefits of CART in individuals with varying levels of anxiety and depression. Using data from two waves, we investigated the following aims:

Aim 1: To examine the efficacy of CART in improving psychological well-being. We hypothesized that individuals in the NAT condition would demonstrate weekly improvements in the primary outcome (anxiety) and the secondary outcomes (depression, stress, and negative affect) during CART compared to participants in PAT (compassion module). We also explored whether the rate of improvement in psychological outcomes throughout the course of treatment differed during module three with the introduction of the respiratory training. We hypothesized that improvements in the primary outcome (anxiety) and secondary outcomes (depression, stress) would be greater during CART for the NAT condition compared to the previous modules.

Aim 2: To examine to what extent changes in CO$_2$ mediated changes in psychological well-being for the NAT condition. We hypothesized that improvements in the primary (anxiety) and secondary (depression, stress, and negative affect) outcomes would be mediated by changes in weekly baseline PCO$_2$ levels over time.
CHAPTER 2:
Method

2.1 Participants

The current project was part of a multi-site RCT study, Treatment of Affective Disorders (TAD), conducted in two waves at the University of California, Los Angeles (UCLA) and Southern Methodist University (SMU). The first wave (henceforth referred to as TAD 1), was conducted between 2015 and 2018, and the paper discussing the main results from TAD 1 can be referenced for details including main study objectives, participant demographic characteristics, description of interventions, and detailed procedures (Craske, Meuret et al., 2019). The second wave (TAD 2) began in 2018 and ended in 2021 (Craske, Meuret et al., 2023). Procedures between the two waves were identical with two distinct changes in TAD 2, which will be discussed in greater detail below: there were slight adjustments to the eligibility criteria and there were additional laboratory assessments throughout treatment.

Participants for TAD 1 and 2 were recruited from the Greater Los Angeles and Dallas Metropolitan areas through community postings. Participants were invited to participate in a psychological treatment to reduce symptoms of depression, anxiety, or stress. A total of 96 participants were recruited for TAD 1 (UCLA: n = 42, 43.8%; SMU: n = 54, 56.2%) and 85 participants for TAD 2 (UCLA: n = 44, 51.8%; SMU: n = 41, 48.2%). A subset of the samples completed a module on CART as part of the NAT intervention (see Interventions subsection). In
TAD 1, 53 participants completed the CART sessions, and 29 participants completed the CART sessions in TAD 2 (Total N = 82). A power analysis conducted on RMASS2 (Hedeker et al., 1999) using available data (n = 67) (i.e., not number of participants who completed CART) indicated that the sample size was large enough to detect power based on a desired power of .80, alpha error rate of .05, and moderate to large effect sizes.

Individuals were eligible to participate in TAD if they were between the ages of 18 to 65 and English-speaking. Participants had to be seeking treatment for emotional distress as well as meet pre-determined cut-off scores on at least one scale of the Depression Anxiety Stress Scale-21 (DASS-21; Lovibond & Lovibond, 1995): severe DASS Depression (score ≥ 11), moderate DASS Anxiety (score ≥ 6), or moderate DASS Stress (score ≥ 10). Additionally, as a measure of clinical impairment, participants had to have a score of 5 or greater on at least one of the subscales of the Sheehan Disability Scale (Sheehan et al., 1996). Further inclusion criteria included either being stabilized on psychotropic medications\(^1\) or be medication-free, and a willingness to refrain from another psychosocial or pharmacological treatment until study completion. The two TAD waves differed on eligibility criteria in two areas. First, participants in TAD 2 had to also meet a certain cut-off score on the positive affect scale of the Positive and Negative Affect Schedule (PANAS-P; Watson et al., 1988). Specifically, a PANAS-P score of 24 or less, which places individuals in the 18\(^{th}\) percentile range or lower for the positive affect scale, was determined as the PANAS-P entry criteria for TAD 2. Although no explicit positive affect cut-off scores were established as an entry criterion for TAD 1, participants in this wave also reported low pre-treatment PANAS-P mean scores (between 10-13\(^{th}\) percentile range) (Craske, Meuret, et al., 2019). Second, TAD 1 required a BDI score less than 48, and a score less

---

\(^{1}\) 1 month for benzodiazepines and beta blockers, 3 months for heterocyclics and SSRIs
than or equal to 1 on the suicidal ideation item (BDI item #9). For TAD 2, no BDI specifications were selected as an inclusion criterion.

The exclusion criteria for both study waves were largely similar (e.g., history of serious, uncontrolled medical illness or instability, pregnancy, lifetime history of bipolar disorder, psychosis, mental retardation, or organic brain damage). The Structured Clinical Interview for DSM-5 (First et al., 2015) was used to determine the diagnostic exclusion criteria. The two waves differed on specificity for substance use and suicidal ideation. Specifically, TAD 1 excluded substance abuse and dependence within the last 6 months whereas TAD 2 excluded abuse within the past 6 months and dependence in past 12 months. Also, for TAD 2, only current active suicidal ideation was exclusionary rather than a history of suicide and active suicidal ideation and/or self-harm in the past year, as was the case with TAD 1. Further, TAD 2 included the use of 11 or more cigarettes per week as an exclusion. Although smoking status/frequency was assessed in TAD 1, it was not listed as an exclusion.

TAD 1 and 2 were approved by the UCLA and SMU ethics committees, and informed consent was obtained from all participants. Participants were compensated for each completed laboratory assessments, and the weekly therapy sessions were free of charge.

2.2 Procedure

In both TAD waves, participants underwent a screening process, diagnostic interview, randomization process, lab assessments before and after treatment, and 15 weekly therapy sessions. Further, participants in TAD 2 also completed two additional laboratory assessments at week 5 and week 10. The reason for this was due to the study aims, which evolved since the first wave. Specifically, the researchers wanted to have greater emphasis on potential biological and physiological markers of the negative and positive valence systems.
The initial screener was a telephone-based screener to assess study criteria. Participants who met initial study eligibility were invited to complete a clinical interview conducted by a trained study coordinator. The Structured Clinical Interview for DSM-5 (First et al., 2015) was used to determine the diagnostic exclusion criteria. Participants who met study criteria after this point were randomly assigned one of two study conditions, described below. Participants came in for pre-treatment physiological laboratory assessments, 15 weekly one-hour individual therapy sessions, and a post-treatment assessment. Participants in TAD 2 completed two additional laboratory assessments mid-treatment: week 5 and week 10 (after completion of second module). See Figure 1 for a flowchart of the TAD study procedures.

2.3 Interventions

Participants were randomized to one of two study conditions: NAT or PAT. Briefly, NAT targeted decreasing negative affect and PAT targeted increasing positive affect. Both treatments were 15 weeks long and composed of three modules that target behavior (module 1, sessions 1-7), cognitions (module 2, sessions 8-10), and arousal (NAT) or compassion (PAT) (module 3, sessions 11-14). The final session (session 15) addressed relapse prevention. Daily between-session exercises were assigned each week. Participants recorded their responses in workbooks provided by the study. For more details regarding the PAT and NAT conditions, see Craske, Meuret, et al. (2019).

a. Capnometry-Assisted Respiratory Training. Participants in NAT completed CART training during the third module of the intervention (week 11). Participants were lent a handheld capnometer for the duration of the four weeks. During CART session 1, study clinicians provided education about the fight-or-flight response, and the symptoms associated with it, as an adaptive system to deal with threat or danger. Clinicians explained how hyperventilation can
aggravate physical sensations even when individuals are not acutely stressed or anxious, and they discussed the connection of over-breathing and mood. Next, participants were introduced to CART, and the study clinicians explained the between-session exercises and the three parts to each exercise (baseline stage, pacing stage, and transition stage). Participants were instructed to complete mood ratings in their client workbooks before and after each exercise. Finally, participants practiced how to operate the capnometer and completed a shortened practice exercise in session (via an audio recording) with clinicians providing feedback during each stage. As part of their between-session practice, participants completed two exercises per day with paced tones set to 13 breaths per minute (14 total exercises).

CART sessions 2-4 consisted of the following format. Participants brought their capnometers with them to session, and the study clinicians extracted the data and printed graphs of each exercise using a software compatible with the capnometry devices. Together, clinicians and participants reviewed the between-session exercises and troubleshooting noticeable trends, such as low PCO$_2$ and high respiration rate, or drops in PCO$_2$ while respiration rate was on target. Lastly, participants completed an in-session practice with an audio recording corresponding to the new respiration rate for that week (11, 9, 6, respectively). Participants continued to practice the new set of tones twice daily as part of their between-session practices.

### 2.4 Measures

Throughout the course of the study, participants completed self-report measures at baseline (week 0), weekly throughout treatment (weeks 1-15), and post assessment (week 16). See Appendix A for the self-report measures included in the current study.

CO$_2$. End-tidal PCO$_2$ was assessed with portable capnometry devices (Tidal Wave Sp, Respironics for TAD 1; PC-900B Handheld Capnograph and Oximeter, CMI Health for TAD 2).
These devices sample exhaled gas through a nasal cannula and provide displays of PCO₂, respiration rate, oxygen, and pulse. PCO₂, respiration rate, oxygen, and pulse were recorded in 4-second intervals, and data from each exercise was extracted through a software compatible with the capnometry devices. The data was exported in an excel format, and formulas were applied to separate the files into the three exercise phases (i.e., baseline, pacing, transition), and average values were calculated.

**a. Depression, Anxiety, and Stress.** DASS-21 is a self-report measure of depression (e.g., hopelessness, dysphoria, anhedonia), anxiety (e.g., autonomic arousal/panic, situational anxiety/fear), and stress (e.g., nervous arousal, difficulty relaxing) over the past week. Participants rated 21 items on a 4-point scale (0 = did not apply to me at all to 3 = applied to me very much, or most of the time). The DASS subscales have demonstrated good internal consistency (α = .94 for Depression; α = .87 for Anxiety; α = .91 for Stress) and validity (Antony, Bieling, Cox, Enns, & Swinson, 1998). The DASS subscales have established cut-off scores indicating clinical severity levels ranging from minimal to extremely severe.

**b. Negative Affect.** The Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) is a 20-item questionnaire measuring positive and negative affect over the past week. Items were rated using a 5-point scale (1 = very slightly or not at all to 5 = extremely). Ten items correspond to the Positive Affect scale (PANAS-P) (α = .86-.90) and 10 items correspond to the Negative Affect scale (PANAS-N) (α = .84-.87) (Watson et al., 1988). PANAS has demonstrated good test-retest reliability over an 8-week time interval (r = .88 for PANAS-P; r = .85 for PANAS-N), and the correlation between PANAS-P and PANAS-N has been low, ranging from -.12 to -.23 (Watson et al., 1988). The PANAS includes clinical cut-off scores in the form of percentiles based on raw scores.
c. **Demographic Information.** At the baseline assessment (week 0), participants completed a demographics form which collected information about participants’ age, gender, race and ethnicity, socioeconomic status, etc. See Appendix A for the demographics form in its entirety. *CART Diaries.* As part of their daily practices, participants completed mood ratings for each CART exercise. Before each exercise, participants rated the severity of symptoms (e.g., anxious, depressed) on a 0 (none) to 10 (extreme) scale. They also noted their current CO₂ level, respiration rate, pulse, and oxygen prior to beginning the exercise. After completing the CART exercise, participants rated the severity of their symptoms on the same 0-10 scale. They indicated the highest and lowest CO₂ during the training as well as their respiration rate and pulse at the end of the training. See Appendix B for a sample CART diary workbook page.

**2.5 Disruptions due to COVID-19**

From March 2020 to September 2020, TAD 2 experienced unexpected protocol changes due to the COVID-19 pandemic. Specifically, in person sessions were suspended due to state-wide stay-at-home and lockdown measures in California and Texas. For this reason, certain procedures were either altered or discontinued to comply with COVID-19 research compliance guidelines. First, participants did not come into the laboratory for the pre-, mid-, or post-treatment assessments. Consequently, physiological measures, such as CO₂ and respiration rate, were not collected. Instead, participants completed the self-report measure portion of the laboratory assessments via a RedCap link that was sent to their email. Second, weekly therapy sessions were transitioned from in-person to teletherapy sessions conducted through Zoom using HIPAA-compliant user accounts. Finally, participants in the NAT condition completed sessions 11-14 without feedback from the capnometer as they were unable to come into the laboratory to
pick up the devices \((n = 4)\). Thus, CO\(_2\) values were not collected during the CART exercises during this time.

2.6 Statistical Analyses

All analyses were conducted using SPSS Version 25. Data was screened for outliers prior to data analysis. Before conducting the main analyses, participant baseline characteristics were computed through frequencies and means of descriptive statistics (e.g., average age of participants, gender, race, ethnicity, and diagnoses). Next, we evaluated whether participants with missing data differed from those with complete data on any baseline level of the study variables. Then, pattern mixture modeling was performed to inspect whether growth curve parameters differed for those with missing data compared with those with complete data. Next, evidence of manipulation success was conducted using multilevel modeling (MLM) with time (weeks) as the level one predictor and end-tidal PCO\(_2\) and respiration rate as the outcome variables. Pre-treatment CO\(_2\) levels were determined by the first baseline of the first home exercises during week 1. Baseline levels of the primary and secondary outcomes were determined by the DASS Depression, Anxiety, and Stress subscale scores and PANAS-N scores prior to beginning CART.

_Hypothesis 1a:_ Individuals randomized to NAT will demonstrate weekly improvements in the primary outcome (anxiety) and the secondary outcomes (depression, stress, negative affect) during the CART module compared to the compassion module in the PAT condition.

To address the first aim, MLM was used. This method allowed all participants to be included in the analysis, regardless of missing data, increasing the ability to detect effects (Hollis & Campbell, 1999). Four analyses were conducted with time (weeks) as the level 1 predictor, and treatment condition (NAT, PAT) and baseline levels of anxiety, stress, depression, and
negative affect, respectively, serving as the level 2 predictors. The two TAD waves (TAD 1, TAD 2) were included as moderators. The DASS subscales and PANAS-N were the four dependent variables. Multiple covariance matrices were tested to determine the best-fitting growth curve models. As multiple tests were performed, data was corrected to control for false discovery rate using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995).

**Hypothesis 1b**: Rates of improvement in anxiety, depression, and stress throughout treatment will be greater during module 3 for the NAT condition compared to the previous modules.

To test this exploratory hypothesis, we utilized a two-phase piecewise growth curve model for the entire treatment (16 sessions) with discontinuity after session 11. Separate MLM analyses for each outcome measure (DASS Depression, Anxiety, Stress) were performed with time (phase 1 (time centered at 11), and phase 2) as the predictors and condition (NAT, PAT) as moderators.

**Hypothesis 2**: Changes in mean weekly baseline CO$_2$ levels will mediate the improvements in the primary outcome (anxiety) and the secondary outcomes (stress, depression, negative affect) over time.

To address Aim 2, a within-subject cross-lag mediation model was conducted using MLM in order to test for time precedence of PCO$_2$ on symptoms (see Figure 2). After determining the best-fitting growth curve models, separate analyses were performed with PCO$_2$ at time “t” predicting the primary outcome (DASS A) and secondary outcomes (DASS D, DASS S, and PANAS-N) at the next session (“t+1”), controlling for the respective outcome at time “t”. The original time-varying predictor (TVP) variables for PCO$_2$ and DASS subscales and PANAS-N were disaggregated into a mean and a deviation variable and included as separate variables for
each in predicting outcome (Hedeker & Gibbons, 2006; Hoffman, 2015; Wang & Maxwell, 2015). Differences in site was controlled for by including Site (coded 0 = SMU, 1 = UCLA) as a covariate across all analyses. An estimate of the indirect effect of time (a*b) as well as its significance was obtained using R*Mediation (Tofighi & MacKinnon, 2011). The mediated pathway was determined significant if the confidence interval did not include 0. To measure the effect size for the mediation, we tested what proportion of time’s effect could be accounted for by the effect of PCO₂ (P_M = a*b/c). Additionally, reverse mediation analyses were conducted where PCO₂ and the outcome of interest were reversed in order to test whether the reverse relation was responsible for the obtained mediation results (Moscovitch et al., 2005). Multiple tests were corrected using the Benjamini-Hochberg procedure to control for false discovery rate.
CHAPTER 3:

Results

3.1 Participant Characteristics

As shown in Table 1, participants identified as predominately White (53.1% in NAT; 62.7% in PAT), female (67.3% in NAT; 75.9% in PAT), and majority met criteria for at least one DSM-5 diagnosis (94.7% in NAT; 91.4% in PAT). Participants’ ages ranged from 18 to 64 years (NAT $M_{age} = 33.59$, $SD = 12.5$; PAT $M_{age} = 33.00$, $SD = 12.1$). Pre-treatment (i.e., Session 0) DASS subscales and PANAS-N levels did not differ between conditions (all $p$’s $>.05$, see Table 2). Prior to module 3 (i.e., Session 10), baseline PANAS-N, DASS D, and DASS A scores did not differ between conditions (all $p$’s $>.05$); although, participants in NAT had higher DASS S scores compared to PAT ($p = .029$) (Table 3). Of note, mean DASS S scores for both conditions were considered within normal range ($M_{NAT} = 6.82$; $M_{PAT} = 5.70$).

3.2 Missing Data

Missing data analyses utilizing Little’s MCAR test showed that the main study variables were missing completely at random ($\chi^2(13) = 12.14$, $p = .516$). Pattern mixture modeling was used to assess whether the growth curve was significantly different between those with missing data and those without missing data. None of the growth curve parameters were different for those with, versus those without, missing data ($p$’s $>.05$), which is consistent with data missing at random, suggesting that the MLM estimates are unbiased estimates of the true parameters.
3.3 CART Adherence

Participants were assigned a total of 52 CART home exercises, of which an average of 17.69 (SD = 14.2) exercises were completed, resulting in 34% adherence. Participants completed more exercises at SMU (M = 24.16, SD = 16.5) compared to UCLA (M = 12.76, SD = 8.54), resulting in 46.5% adherence at SMU and 24.5% adherence at UCLA (t(60) = 3.17, p = .002). No differences were found in number of total exercises completed in TAD 1 (M = 17.65, SD = 15.59) and TAD 2 (M = 22.40, SD = 13.59) (t(60) = -1.24, p = .220).

On average, participants followed the paced tones most closely during Week 1, which were set to 13 breaths per minute (bpm) (M = 13.8, SD = 1.58). Results indicated less adherence to the paced tones in the subsequent weeks that were set to 11, 9, and 6 bpm, respectively (MWek2 = 12.20, SDWek2 = 1.81; MWek3 = 11.06, SDWek3 = 1.96; MWek4 = 9.08, SDWek4 = 2.36).

3.4 CART Outcome

During each CART training phase, participants demonstrated increases in PCO₂ levels (PCO₂baseline t(62) = 3.22, p = .002; PCO₂tones t(61) = 60.61, p < .001; PCO₂transition t(62) = 4.65, p < .001) and decreases in respiration rate over time (RRbaseline t(62) = - 4.13, p < .001; RRtones t(55) = - 14.65, p < .001; RRtransition t(61) = - 7.82, p < .001) (see Figure 3). Baseline PCO₂ levels were below the normal range at the first CART exercise (M = 34.4, SD = 5.01) and increased at the last CART exercise (M = 36.6, SD = 5.72), demonstrating a significant increase in PCO₂ between participants’ first and last exercises (t(114) = -2.28, p = .025). Baseline respiration rate at the first CART exercise was approximately 16 bpm (M = 15.8, SD = 3.67) and 13 bpm at the last CART exercise (M = 13.5, SD = 4.06), which was a significant decrease in baseline respiration rate from the first to final CART exercise (t(116) = 3.31, p = .001).
3.5 Aim 1 Analyses

*Primary outcome:* Rates of improvement in DASS Anxiety did not significantly differ between conditions during CART ($b = -.02, t(117) = -.18, p = .858$). Higher baseline DASS Anxiety was related to higher anxiety through the module ($b = 2.38, t(116) = 13.95, p < .001$).

*Secondary outcomes:* During CART, DASS Depression ($b = -21.57, t(168) = -1.19, p = .237$), DASS Stress ($b = -.001, t(125) = .01, p = .994$), and PANAS-N ($b = 9.75, t(120) = .266, p = .791$) scores did not significantly improve between NAT and PAT. For all measures, higher baseline scores were related to higher depression ($b = 3.43, t(114) = 16.39, p < .001$), stress ($b = 3.21, t(121) = 15.96, p < .001$), and negative affect ($b = 4.58, t(130) = 14.15, p < .001$), respectively, throughout the final module.

3.6 Aim 1 Exploratory Analyses

*Primary outcome:* Rates of improvement in DASS Anxiety did not change significantly for the NAT condition during CART ($b = -.05, t(1896) = -.52, p = .605$). Anxiety symptoms significantly decreased during the behavioral and cognitive modules ($b = -.21, t(198) = -4.94, p < .001$) but not during CART ($b = -.003, t(1873) = -.07, p = .945$).

*Secondary outcomes:* Results demonstrated no significant changes in rates of improvement in DASS Depression or DASS Stress for the NAT condition during CART (DASS Depression: $b = .04, t(1899) = .27, p = .786$; DASS Stress: $b = .02, t(1912) = .16, p = .870$). DASS Depression significantly decreased during the behavioral and cognitive modules ($b = -.30, t(164) = -5.94, p < .001$) and did not significantly decrease during CART ($b = .05, t(1871) = .69, p = .491$). Similarly, DASS Stress significantly decreased during the behavioral and cognitive modules ($b = -.29, t(177) = -6.53, p < .001$) but not during the final module of CART ($b = .08, t(1883) = 1.21, p = .227$).
3.7 Aim 2 Analyses

*Primary outcome:* As shown in Figure 2a, increases in deviations in baseline PCO\(_2\) at the current session (time “t”) significantly predicted decreases in DASS Anxiety levels at the next session (time “t+1”) \((b = -.118, t(160) = -2.55, p = .024)\). The indirect effect of time on DASS Anxiety mediated by baseline PCO\(_2\) changes was significant \((ab = -.054; 95\% \text{ CI} [-0.114, -0.01], P_M = .46)\). Reverse mediation was not significant \((ab = -.005; 95\% \text{ CI} [-0.054, 0.039], P_M = .02)\), indicating a unidirectional relation.

*Secondary outcomes:* Increases in deviations in baseline PCO\(_2\) at the current session significantly predicted increases in DASS Depression levels at the next session \((b = .183, t(160) = 2.65, p = .024)\) (Figure 2b). The indirect of time on DASS Depression mediated by baseline PCO\(_2\) was significant \((ab = .084; 95\% \text{ CI} [0.017, 0.175], P_M = .54)\) (Figure 2b). The reverse mediation was not significant \((ab = .005; 95\% \text{ CI} [-0.027, 0.044], P_M = .02)\), demonstrating a unidirectional relation. Regarding stress, changes in deviations in baseline PCO\(_2\) did not significantly predict changes in DASS Stress at the next session \((b = -.033, t(160) = -.54, p = .63)\) (Figure 2c). The indirect effect of time mediated through PCO\(_2\) was also not significant \((ab = -.015; 95\% \text{ CI} [-0.077, 0.041], P_M = .21)\). Finally, changes in deviations in baseline PCO\(_2\) did not significantly predict changes in PANAS-N levels \((b = -.064, t(160) = -.48, p = .63)\) at the next session (Figure 2d). Similarly, the indirect effect of time mediated through PCO\(_2\) was also not significant \((ab = -.029; 95\% \text{ CI} [-0.164, 0.094], P_M = .12)\).
CHAPTER 4:
Discussion

The present study sought to examine the mediating effects of CO$_2$ on a range of negative affect symptoms (e.g., anxiety, depression) during CART using a transdiagnostic sample. Moreover, this study was the first to examine the effectiveness of CART delivered within a larger intervention examining mechanisms of change across domains in the National Institute of Mental Health Research Domain Criteria (RDoC) Negative and Positive Valence Systems (Cuthbert & Insel, 2013). First, findings did not support the hypotheses formulated under Study Aim 1. Specifically, during module 3 when CART was administered, symptoms of anxiety, depression, stress, and negative affect did not improve more for NAT compared to PAT. In fact, symptoms were within the normal range at the start of module 3 and remained in the normal range through the end of treatment for both groups. Second, we did not find support for our exploratory analyses, which showed that the rates of improvement in depression, anxiety, and stress throughout treatment did not change during CART compared to the previous behavioral and cognitive modules. Rather, our results indicated that symptoms significantly decreased during the first two modules but not during the final module of CART. Finally, the findings provided partial support for the second study aim. In support of our hypothesis, and aligned with findings in the literature, changes in mean weekly baseline PCO$_2$ levels mediated changes in anxiety. In other words, increases in baseline PCO$_2$ predicted lower levels of anxiety at the next session, controlling for current anxiety levels. CO$_2$ was also found to have a mediating effect on
depression, although not in the predicted direction. Specifically, results indicated that increases in baseline PCO$_2$ predicted higher levels of depression at the next session, controlling for current depressive levels. Moreover, the causal relation between PCO$_2$ and anxiety, and PCO$_2$ and depression, were shown to be unidirectional. Counter to our hypotheses, deviations in PCO$_2$ did not predict changes in stress and negative affect at next session. These results may perhaps point to a specific distinction between CO$_2$ regulation and anxiety and depression, rather than negative affective states more broadly.

Regarding the first study aim, results did not confirm our hypotheses on the efficacy of CART in improving psychological well-being for NAT compared to PAT. However, these findings did not surprise us given the nature of the current study and the inevitable limitations. For example, because the current study was imbedded within a larger RCT, CART was administered at the final module. As a result, participants completed 10 weeks of cognitive and behavioral interventions before beginning the third module (CART/compassion). The literature reports less therapeutic change at the end of therapy compared to the start of therapy (Robinson et al., 2020), which may speak to less symptom reduction during module 3. Additionally, levels of anxiety, depression, and stress were within the normal range prior to the start of CART (Table 3) and remained within the normal range through the end of the treatment. Given these limitations, with regard to our first aim, we were unable to examine the effectiveness of CART as a stand-alone protocol or randomize the timing of the CART intervention in relation to the other modules. By doing so, we would have been able to assess whether the timing of the CART intervention impacted the degree of symptom reduction. Understanding the impact of CO$_2$ regulation on symptom reduction would help shed light on tailoring treatment planning based on symptom severity, baseline PCO$_2$ range, and individual treatment goals.
Further, the current study found partial support for Aim 2, in that PCO\textsubscript{2} fully mediated changes in anxiety symptoms. This not only replicates but extends previous CART findings in the literature. For example, PCO\textsubscript{2} has been found to be a partial mediator of change in anxiety sensitivity (Meuret et al., 2009) and a mediator of change in panic symptom severity (Meuret et al., 2010). While Meuret and colleagues’ findings were in the context of panic disorder, some evidence points to similarities in the symptom changes captured in the current study and those reported in the literature. Specifically, we found that changes in PCO\textsubscript{2} predicted changes in anxiety symptoms, as measured by DASS Anxiety, which has recently been identified as a potential Self-Report marker of the RDoC Negative Valence System domain construct of response to acute threat (i.e., panic, fear) (Hasratian et al., 2022). This would suggest that the items on DASS Anxiety also tap into panic-related arousal as endorsed in panic disorder, extending methodological generalizability through the use of an alternative, yet similar, symptom measure. Further, the effect sizes of mediation reported in our study are comparable to previous studies (Meuret et al., 2009; Meuret et al., 2010). Given that only 20% of the current study’s sample met diagnostic criteria for panic disorder, as compared to 100% reported in prior CART studies (e.g., Meuret et al., 2009; Meuret et al., 2010), our findings increase our confidence in the transdiagnostic effectiveness of the intervention in decreasing varying levels of panic-related anxiety and arousal symptoms as opposed to serving as a disorder-specific intervention.

Curious whether our results could provide further support of the unique role of PCO\textsubscript{2} in mediating anxiety symptoms, a post hoc exploratory cross-lag mediation analysis was conducted to examine the role of changes in respiration rate on symptom change. Findings from this analysis replicated those found by Meuret et al. (2009), in that changes in respiration rate did not
significantly predict later changes in anxiety \((ab = .001, 95\% \text{ CI } [-.021, .026])\), providing further support for the unique role of CO\(_2\) in driving change. This finding also emphasizes the questions raised in the literature about the relevance of targeting respiration rate as demonstrated in typical breathing trainings. More importantly, findings from our study highlights the critical need to target CO\(_2\) when implementing breathing trainings, a method seldom used in practice (Meuret et al., 2003). Raising awareness about the impact of hypocapnia and disseminating successful treatments that target CO\(_2\) regulation, such as CART, would benefit many patients. For instance, including CO\(_2\) readings as part of routine medical check-ups, could alert providers to patients with chronic hypocapnia who may benefit from breathing retraining. In clinical settings, portable capnometers can be used in adjunct when treating individuals with anxiety disorders. Taken together, our findings regarding the mediating role of CO\(_2\) in anxiety reduction not only replicates previous CART mediation study findings, but also demonstrates greater generalizability with regard to sample characteristics and clinical presentations.

Next, the current study contributes to the literature by finding support for the causal relation between changes in baseline PCO\(_2\) and report of depressive symptoms at the next session. Based on findings from previous studies, which reported significant reductions in symptoms of depression following CART (e.g., Meuret et al., 2008), we believed that CART would play a mediating role in depressive symptom reduction. Contrary to our hypothesis, changes in baseline PCO\(_2\) were related to increases in reported depressive symptomology at the next session. Given the high comorbidity between anxiety and depression, one may wonder whether changes in anxiety impacted the association between PCO\(_2\) and depression. Thus, an exploratory post hoc cross-lag analysis, controlling for anxiety changes within-individuals, was performed. Results indicated that even when controlling for anxiety symptoms, changes in PCO\(_2\)
significantly mediated changes in depression scores at the next session \((ab = .089; 95\% \text{ CI} [.022, 0.181])\), pointing to a distinct relation between CO\(_2\) and depression.

Furthermore, we recognize that previous CART studies reported depressive symptoms in the normal range at the start of the study (Meuret et al., 2008), whereas our sample represented varying levels of depression, with about half of the sample meeting diagnostic criteria for a current mood disorder at the start of the study (i.e., major depressive disorder (MDD), persistent depressive disorder (PDD)) (Table 2). Clinically significant levels of depression were part of the exclusion criteria in a past CART study with patients with asthma (Meuret et al., 2007). Thus, we examined whether the association of increases in PCO\(_2\) with subsequent worsening of depressive symptomology was limited to patients of our study who presented with a mood disorder diagnosis. We conducted a post hoc exploratory cross-lag mediation analysis separately for those with and without current mood disorder (i.e., MDD, PDD, or both). As predicted, for those who met diagnostic criteria for MDD, PDD, or both \((n = 30)\) at the start of therapy, we found that increases in PCO\(_2\) predicted higher levels of depressive symptoms at the next session \((ab = .127, 95\% \text{ CI} [.003, .295])\). On the other hand, in those without a diagnosed mood disorder \((n = 21)\), changes in PCO\(_2\) did not significantly predict changes in depressive symptoms at the next session \((ab = -.02, 95\% \text{ CI} [-.0147, .097])\). These findings indicate that there may be a unique relation between CO\(_2\) dysregulation and depression.

Prior findings associating CO\(_2\) with depression are inconsistent. For instance, some evidence points to respiratory abnormalities in depression, such as higher variability of the respiratory pattern (Zamoscik et al., 2018) and elevated resting CO\(_2\) levels (Mora et al., 1976). On the other hand, resting PCO\(_2\) levels were significantly lower in patients with a primary diagnosis of depression admitted to a psychiatric hospital compared to healthy controls (Damas-
Mora et al., 1982). Whereas correcting hypocapnia may lead to symptom reduction in these cases, our findings and those of others (e.g., Sikter et al., 2009) speak against the usefulness of PCO$_2$ biofeedback in depression. For example, research on obstructive sleep apnea (OSA), shows that PCO$_2$ is usually elevated during sleep (Ayappa et al., 2002). The literature points to up to 50-65% prevalence rate of depression reported in patients with OSA (Saunamaki & Jehkonen, 2007; Sikter et al., 2009), with signs of reduced symptoms of depression with routine CPAP use (Schwartz & Karatinos, 2007). It is possible that the reductions in basal CO$_2$, through treatment contributed to the improvements in depressive symptoms in these patients. While hypoxia, rather than hypercapnia, could have played a role in depression, one evidence in favor of the latter comes from research on patients with chronic obstructive pulmonary disease (COPD) who underwent oxygen therapy (Maurer et al., 2008, as cited in Sikter et al., 2009). Here, the researchers found that symptoms of depression increased significantly. Given the knowledge that oxygen therapy elevates CO$_2$ levels (e.g., Moloney et al., 2001; Pilcher et al., 2013), Sikter and colleagues (2009) concluded that elevations in CO$_2$ levels, and not hypoxia, were what drove an increase in depressive symptoms. Our findings of increases in baseline CO$_2$ predicting later increases in depressive symptoms, and that this was limited to patients with a depressive disorder diagnosis, are consistent with that.

4.1 Limitations of the Current Study

The current study presents limitations that are important to consider. First, similar to other CART studies, the present study was conducted in a controlled environment (i.e., RCT), which may affect generalizability of the intervention’s success. However, evidence of CART’s success in a non-controlled environment, as presented by Tolin et al. 2017, shows promise of its efficacy in clinical settings outside of academic and research institutions. Additionally, the
current study had to adapt to state-wide COVID-19 related lock-down and stay-at-home orders, prompting a sudden shift from standard protocol. Regardless of this interruption, results showed CART to be effective in increasing CO$_2$. As such, we are able to provide preliminary evidence for CART’s success in a telehealth, or at least a hybrid, format.

A second limitation is that treatment adherence for our sample was low compared to what has been reported in the literature. For example, the pilot CART study reported that 91.3% of the home exercises were completed (Meuret et al., 2008). In our study, participants completed an average of 34% of the home exercises. Further, results from the manipulation check indicated that participants did not follow the paced tones closely, demonstrating less adherence compared to prior CART studies. Although improvements in treatment adherence could likely contribute to greater positive impacts of CART, this does also highlight a potential strength. Specifically, our findings suggest that CART can be beneficial even if completing an average of one-third the prescribed exercises. Further investigation into this would allow for greater generalizability to patients in outpatient settings, who may be more likely to report lower homework compliance compared to previous clinical trials. Additionally, future studies could provide more refined recommendations regarding number of exercises found to initiate change.

4.2 Strengths and Future Direction

The current study joins the preceding aforementioned CART trials in demonstrating increases in baseline CO$_2$ from hypocapnic to the normative range. This is especially encouraging given the continued experience of hypocapnia reported across various clinical presentations (e.g., Clague et al., 2000). Further, our study is the first to provide support for CO$_2$ driving reductions in anxious arousal symptoms using a transdiagnostic sample, extending
generalizability to more diverse clinical presentations than previously reported (i.e., patients with panic disorder).

Given the continued strong evidence demonstrating that CO₂ drives reductions in anxiety, future studies would benefit from examining potential overlapping benefits of CART in different populations. For example, CART has been found to be beneficial with COPD (Norweg et al., 2021). As there has been a strong link shown between patients with COPD and heightened anxiety (e.g., Pelgrim et al., 2021), increasing basal CO₂ into the normative range by way of an intervention such as a CART may prove to be helpful in improving anxiety symptoms in addition to the documented COPD improvements.

Additionally, the successful nature of CO₂ driving reductions in anxiety prompts us to turn to examining the effectiveness of current treatments for anxiety disorders. Cognitive Behavioral Therapy (CBT) is among one of the most commonly used interventions when it comes to targeting anxiety (Curtiss et al., 2021). However, CBT for anxiety has been found to have a 50% effective response rate (Loerinc et al., 2015), indicating a need to incorporate additional strategies that are known to improve symptoms of anxiety. As further supported by our findings, CO₂ regulation is one such strategy that may positively impact symptom reduction. In a recent systematic review examining the efficacy of breathing training interventions with anxiety disorders, Banushi and colleagues (2023) reported that despite the strong relation between breathing practices and anxiety symptom improvements, breathing is still not targeted in gold standard treatments. Our findings highlight the importance of CO₂ regulation in the treatment of mental health disorders, in particular anxiety disorders. Not only do our findings support those in the literature regarding the positive impact of CART in increasing CO₂ into the normative range, but we also extend previous findings by reporting on the causal role of CO₂ in
reductions in anxiety symptoms in a transdiagnostic sample. This suggests that an important consideration would be to target CO$_2$ regulation as an essential component in the treatment of anxiety.

By growing greater awareness about the impact of hypocapnia on maintaining negative mood states, like anxiety, we allow for understanding about why interventions like CART could be helpful in correcting hypocapnia and improving symptoms. Implications of this would be wide as it would provide strong evidence for the benefits of using biofeedback devices (i.e., capnometer) to improve symptom functioning. This could, in turn, support future advocacy for policy changes in areas such as medical insurance coverage for biofeedback devices that are used as part of treatment.

Finally, our findings point to a relation between changes in baseline PCO$_2$ and depressive symptoms that require further attention. For participants who met diagnostic criteria for a depressive disorder, depressive symptoms worsened when baseline CO$_2$ increased, whereas the results were nonsignificant when filtering out current mood diagnoses, driving speculations about the unique relation between CO$_2$, respiration, hypocapnia, and depressive symptoms. Future studies may consider more in-depth measurement of respiratory function, including gas exchange, as part of depression interventions, to begin examining how respiration is linked to symptom change during the course of therapy.
APPENDIX

*Figure 1*. Flowchart of study procedures
Figure 2. Cross Lag Mediation Models with outcome variables of anxiety (a), depression (b), stress (c), and negative affect (d).
Note: PCO₂ = partial pressure of carbon dioxide; DASS = Depression Anxiety Stress Scale-21; PANAS = Positive and Negative Affect Schedule; N = Negative Affect Scale. *p < .05. **p < .01. ***p ≤ .001.
Figure 3. Carbon dioxide (CO₂) (a) and Respiration Rate (b) outcome in Capnometry-Assisted Respiratory Training

(a)

(b)
<table>
<thead>
<tr>
<th>Characteristics n (%)</th>
<th>Total (N=94)</th>
<th>TAD 1 (N=81)</th>
<th>TAD 2</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, M (SD), years</td>
<td>33.59 (12.5)</td>
<td>33.00 (12.1)</td>
<td>35.41 (11.7)</td>
<td>34.10 (12.8)</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>66 (67.3)</td>
<td>63 (75.9)</td>
<td>31 (56.4)</td>
<td>32 (78.0)</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>52 (53.1)</td>
<td>52 (62.7)</td>
<td>27 (49.1)</td>
<td>24 (58.5)</td>
</tr>
<tr>
<td>Black</td>
<td>7 (7.1)</td>
<td>6 (7.2)</td>
<td>4 (7.3)</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td>Asian</td>
<td>12 (12.2)</td>
<td>10 (12.0)</td>
<td>3 (5.5)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Alaska Native/</td>
<td>0 (0.0)</td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Native American/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indigenous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacific Islander/</td>
<td>1 (1.0)</td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Native Hawaiian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic (White)</td>
<td>15 (15.3)</td>
<td>9 (10.8)</td>
<td>6 (10.9)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Hispanic (Non-White)</td>
<td>9 (9.2)</td>
<td>11 (13.3)</td>
<td>5 (9.1)</td>
<td>7 (17.1)</td>
</tr>
<tr>
<td>Multiracial</td>
<td>5 (5.1)</td>
<td>3 (3.6)</td>
<td>4 (7.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (8.2)</td>
<td>1 (1.2)</td>
<td>1 (1.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Has a diagnosis</td>
<td>89 (94.7)</td>
<td>74 (91.4)</td>
<td>50 (94.3)</td>
<td>37 (92.5)</td>
</tr>
<tr>
<td>Major depressive</td>
<td>52 (53.1)</td>
<td>37 (44.6)</td>
<td>25 (45.5)</td>
<td>18 (43.9)</td>
</tr>
<tr>
<td>Persistent depressive</td>
<td>28 (28.6)</td>
<td>21 (25.3)</td>
<td>12 (21.8)</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td>Panic disorder</td>
<td>20 (20.4)</td>
<td>15 (18.1)</td>
<td>15 (27.3)</td>
<td>10 (24.4)</td>
</tr>
<tr>
<td>Agoraphobia</td>
<td>9 (9.2)</td>
<td>6 (7.2)</td>
<td>7 (12.7)</td>
<td>5 (12.2)</td>
</tr>
<tr>
<td>Social anxiety disorder</td>
<td>43 (43.9)</td>
<td>36 (43.4)</td>
<td>26 (47.3)</td>
<td>19 (46.3)</td>
</tr>
<tr>
<td>Specific phobia</td>
<td>11 (11.2)</td>
<td>10 (12.0)</td>
<td>7 (12.7)</td>
<td>7 (17.1)</td>
</tr>
<tr>
<td>Generalized anxiety disorder</td>
<td>52 (53.1)</td>
<td>33 (39.8)</td>
<td>36 (65.5)</td>
<td>18 (43.9)</td>
</tr>
<tr>
<td>Obsessive compulsive disorder</td>
<td>9 (9.2)</td>
<td>3 (3.6)</td>
<td>6 (10.9)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Attention deficit</td>
<td>7 (7.1)</td>
<td>11 (13.3)</td>
<td>2 (3.6)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Hyperactivity disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute Stress Disorder</td>
<td>1 (1.0)</td>
<td>0 (0.0)</td>
<td>1 (1.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Posttraumatic stress disorder</td>
<td>7 (7.1)</td>
<td>3 (3.6)</td>
<td>4 (7.3)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Adjustment disorder</td>
<td>1 (1.0)</td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

Note. Abbreviations: %, Percent; TAD, Treatment for Affective Dimensions; NAT, Negative Affect Treatment; PAT, Positive Affect Treatment. M, Mean, SD, Standard Deviation. \(^1\)Values represent n (%), unless otherwise indicated.
### Table 2. Participant pre-treatment (session 0) scores

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Total</th>
<th>TAD 1</th>
<th>TAD 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=94)</td>
<td>(N=81)</td>
<td>(N=81)</td>
</tr>
<tr>
<td></td>
<td>(N=51)</td>
<td>(N=39)</td>
<td>(N=43)</td>
</tr>
<tr>
<td></td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>range</td>
<td>range</td>
</tr>
<tr>
<td>DASS Depression</td>
<td>10.20</td>
<td>11.43</td>
<td>.178</td>
</tr>
<tr>
<td></td>
<td>(6.29)</td>
<td>(5.66)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>DASS Anxiety</td>
<td>6.94</td>
<td>8.14</td>
<td>.133</td>
</tr>
<tr>
<td></td>
<td>(5.29)</td>
<td>(5.20)</td>
<td>(0.20)</td>
</tr>
<tr>
<td>DASS Stress</td>
<td>11.00</td>
<td>11.81</td>
<td>.267</td>
</tr>
<tr>
<td></td>
<td>(4.91)</td>
<td>(4.74)</td>
<td>(0.20)</td>
</tr>
<tr>
<td>PANAS Negative</td>
<td>30.01</td>
<td>30.54</td>
<td>.643</td>
</tr>
<tr>
<td></td>
<td>(7.24)</td>
<td>(7.74)</td>
<td>(0.20)</td>
</tr>
</tbody>
</table>

Note. DASS, Depression Anxiety Stress Scale-21; PANAS, Positive and Negative Affect Schedule; NAT, Negative Affect Treatment; PAT, Positive Affect Treatment. M, Mean, SD, Standard Deviation.

### Table 3. Participant DASS and PANAS Negative scores prior to module 3

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Total</th>
<th>TAD 1</th>
<th>TAD 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=94)</td>
<td>(N=81)</td>
<td>(N=81)</td>
</tr>
<tr>
<td></td>
<td>(N=51)</td>
<td>(N=39)</td>
<td>(N=43)</td>
</tr>
<tr>
<td></td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>range</td>
<td>range</td>
</tr>
<tr>
<td>DASS Depression</td>
<td>4.97</td>
<td>4.67</td>
<td>.129</td>
</tr>
<tr>
<td></td>
<td>(5.1)</td>
<td>(4.2)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>DASS Anxiety</td>
<td>3.74</td>
<td>3.15</td>
<td>.644</td>
</tr>
<tr>
<td></td>
<td>(3.7)</td>
<td>(3.6)</td>
<td>(3.6)</td>
</tr>
<tr>
<td>DASS Stress</td>
<td>6.82</td>
<td>5.70</td>
<td>.029</td>
</tr>
<tr>
<td></td>
<td>(4.7)</td>
<td>(3.6)</td>
<td>(4.7)</td>
</tr>
<tr>
<td>PANAS Negative</td>
<td>20.52</td>
<td>18.84</td>
<td>.114</td>
</tr>
<tr>
<td></td>
<td>(7.4)</td>
<td>(6.3)</td>
<td>(7.4)</td>
</tr>
</tbody>
</table>

Note. DASS, Depression Anxiety Stress Scale-21; PANAS, Positive and Negative Affect Schedule; NAT, Negative Affect Treatment; PAT, Positive Affect Treatment. M, Mean, SD, Standard Deviation.
## Depression Anxiety Stress Scale-21 (DASS-21)

Please read each statement and circle a number 0, 1, 2 or 3 that indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

<table>
<thead>
<tr>
<th></th>
<th>Did not apply to me at all</th>
<th>Applied to me to some degree, or some of the time</th>
<th>Applied to me to a considerable degree, or a good part of the time</th>
<th>Applied to me very much, or most of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I found it hard to wind down</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>I was aware of dryness of my mouth</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>I couldn't seem to experience any positive feeling at all</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4.</td>
<td>I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>I found it difficult to work up the initiative to do things</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>I tended to over-react to situations</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7.</td>
<td>I experienced trembling (e.g., in the hands)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8.</td>
<td>I felt that I was using a lot of nervous energy</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9.</td>
<td>I was worried about situations in which I might panic and make a fool of myself</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10.</td>
<td>I felt that I had nothing to look forward to</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11.</td>
<td>I found myself getting agitated</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>12.</td>
<td>I found it difficult to relax</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>13.</td>
<td>I felt down-hearted and blue</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>14.</td>
<td>I was intolerant of anything that kept me from getting on with what I was doing</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15.</td>
<td>I felt I was close to panic</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>16.</td>
<td>I was unable to become enthusiastic about anything</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>17.</td>
<td>I felt I wasn't worth much as a person</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>I felt that I was rather touchy</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>18</td>
<td>I was aware of the action of my heart in the absence of physical exertion (e.g., sense of heart rate increase, heart missing a beat)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>I felt scared without any good reason</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>I felt that life was meaningless</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Positive and Negative Affect Schedule (PANAS)

This scale consists of a number of words that describe different feelings and emotions. Read each item and then circle the appropriate answer next to that word. Indicate to what extent you have felt this way during the past week.

<table>
<thead>
<tr>
<th></th>
<th>Very slightly or not at all</th>
<th>A little</th>
<th>Moderately</th>
<th>Quite a bit</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Interested</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Distressed</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Excited</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4.</td>
<td>Upset</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Strong</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6.</td>
<td>Guilty</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7.</td>
<td>Scared</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8.</td>
<td>Hostile</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9.</td>
<td>Enthusiastic</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10.</td>
<td>Proud</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11.</td>
<td>Irritable</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>12.</td>
<td>Alert</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>13.</td>
<td>Ashamed</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>14.</td>
<td>Inspired</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15.</td>
<td>Nervous</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>16.</td>
<td>Determined</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>17.</td>
<td>Attentive</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>18.</td>
<td>Jittery</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>19.</td>
<td>Active</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>20.</td>
<td>Afraid</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Patient Demographics

Age: ______ Date of Birth: ________ Height: ________ Weight: ________

Which group below most accurately describes your racial background? (check all that apply)
- □ Alaskan Native/Native American/Indigenous (please specify tribal affiliation if applicable) __________
- □ Asian
- □ Black
- □ Latino(a)/Hispanic (Non-White)
- □ Latino(a)/Hispanic (White)
- □ Pacific Islander/Native Hawaiian
- □ White
- □ Multiracial (please specify): __________
- □ Other (please specify): __________

Ethnicity typically emphasizes the common history, nationality, geographic distribution, language, cuisine or dress of groups of people rather than their racial background (such as Cuban, Haitian, Cambodian, African-American, Ukrainian, etc.). In your own words, with which ethnic group or groups do you identify? ___________________

1. What is your gender identity?
   - □ Male
   - □ Female
   - □ Transgendered
   - □ Other (please specify): ______

2. What is your sexual orientation?
   - □ Bisexual
   - □ Gay/Lesbian
   - □ Heterosexual
   - □ Other (please specify): ______

3. What is the highest grade you completed?
   - □ Less than high school
   - □ High school diploma or GED
   - □ Some college or a 20-year degree
   - □ 4-year college degree
   - □ Post-graduate work or degree

4. If you did NOT receive a high school diploma, what was the last grade you completed?

5. What is your current employment status?
   - □ Unemployed, not looking
   - □ Unemployed, looking
   - □ Employed part-time
   - □ Employed full-time
   - □ Retired
   - □ Homemaker

6. If employed, what do you do for a living? ______________

7. If retired or laid off, what did you do for a living? ______________

8. Are you currently a full-time student? □ Yes □ No

9. Which of the following best describes your yearly household income (including all persons who contribute)? (If retired, what was your monthly income?)
10. How many people are dependent on your yearly household income, including yourself? _____

11. Do you currently have health care insurance, including government-sponsored insurance such as Medicaid or MediCal? ☐Yes ☐No

12. Where you born in the United States? ☐Yes ☐No

13. If NO, how many years have you lived in the United States? _______

14. What language do you usually speak at home?
   ☐ English
   ☐ Spanish
   ☐ English and Spanish
   ☐ Other language(s): Please specify _______________

15. What is your current marital status?
   ☐ Married
   ☐ Single, but in a committed relationship
   ☐ Single, not in a current relationship
   ☐ Separated
   ☐ Divorced
   ☐ Widowed
   ☐

16. Are you currently taking medication? ☐Yes ☐No

Please list all medications you are taking regularly (including today) (prescribed or over the counter, for example: tranquilizers, sleeping pills, antidepressants, hormones, allergy medicine, aspirin, blood pressure medicine, St. John’s Wort, Melatonin, diet pills).

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose</th>
<th>Taken since/comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Date: __/__/__  

1. Breathing Exercise  

Time of first trial: __________

Instructions: Please rate the maximum severity of the symptoms you experience before and after you did your breathing exercises for 15 minutes.

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Strong</td>
<td>Extreme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Before you start with the breathing exercise:

| Relaxed | Shortness of breath | Racing/pounding heart |
| Worried | Trembling/shaking | Nausea/ upset stomach |
| Anxious | Choking sensations | Chest pain/or pressure |
| Happy | Fear of dying | Feeling of unreality/detachment |
| Excited/involved (pos.) | Sweating | Numbness/tingling |
| Sad/depressed | Hot flashes or chills | Unsteadiness/dizziness/faintness |
| Sighing | Awareness of breathing | Fear of losing control/going crazy |

My current CO₂ level is: ___ mmHg; respiration rate is: ___RR, pulse is: ___bpm; O₂ is: ___%  

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Strong</td>
<td>Extreme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After your breathing exercise:

| Relaxed | Shortness of breath | Racing/pounding heart |
| Worried | Trembling/shaking | Nausea/ upset stomach |
| Anxious | Choking sensations | Chest pain/or pressure |
| Happy | Fear of dying | Feeling of unreality/detachment |
| Excited/involved (pos.) | Sweating | Numbness/tingling |
| Sad/depressed | Hot flashes or chills | Unsteadiness/dizziness/faintness |
| Sighing | Awareness of breathing | Fear of losing control/going crazy |

What was the highest/lowest CO₂ level during the training:  

What was your respiration rate at the end of the training:  
What was your pulse at the end of the training:

highest CO₂ level: ____ mmHg  
lowest CO₂ level: ____ mmHg  
RR  
BPM
REFERENCES


https://doi.org/10.1016/j.resp.2018.07.003


https://doi.org/10.1378/chest.14-0665

https://doi.org/10.1080/10503307.2019.1566676

https://doi.org/10.1192/bjp.148.5.526

doi:10.1111/j.1600-0404.2007.00901.x

Schwartz, D. J., & Karatinos, G. (2007). For individuals with obstructive sleep apnea, institution of CPAP therapy is associated with an amelioration of symptoms of depression which is sustained long term. *Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine, 3*(6), 631–635.


https://doi.org/10.1097/00004850-199606003-00015


