Degree project

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Breathwork and Its Effect on Stress in Healthy Individuals: A Systematic Review

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Abstract

Stress is an ongoing and increasing problem at a societal and individual level. This systematic review aims to evaluate which effects breathwork has on stress in a healthy population. A literature search was done on Scopus, and Web of Science for peer-reviewed, published, and original research. In the search process, it was decided to include psychological and physiological assessments due to the narrow topic. Three studies met the inclusion criteria and were included in this review. The outcome measurements included were heart-rate variability (HRV) which measures the activity in the autonomic nervous system (ANS) and salivary cortisol to measure cortisol as a biomarker of psychological stress, as well as the questionnaires PSS, PANAS, and STAI. There are many different breathwork techniques, in this review the focus is on controlled, slow deep breathing techniques e.g. diaphragmatic breathing, resonance breathing, and cyclic sighing. All three studies showed significant improvement in stress in the breathing groups. These findings suggest that breathwork can be valuable in reducing stress in healthy individuals. Furthermore, more research is needed before conclusions on the general population can be drawn.

Keywords: Breathwork, controlled breathing, stress, heart-rate-variability, salivary cortisol

Breathwork and Its Effect on Stress in Healthy Individuals

Lifestyle changes, urbanization, and competitiveness have made stress, anxiety, depression, and psychosomatic illnesses an inevitable factor in human life (Chaitanya et al., 2022). Stress is a continuous major health problem in modern society worldwide. According to Gallup (2022), in 2021, 115 countries and areas participated in a survey that asked adults if they experienced specific negative experiences on the day before the survey (e.g. stress). A record-high 40% of adults experienced more stress than ever the in the past 16 years. According to Folkhälsomyndigheten (2023), in Sweden, 15% of the population between the ages of 16 and 84 stated that they felt stressed in 2021. Moreover, stress-related work absence has increased rapidly since 2010 in Sweden, according to Försäkringskassan (2023), further, the latest statistics show an increase of 13 percent between July 2019 and July 2022. To prevent chronic dysfunctional consequences, recovery from stress is important.

The human nervous system is complex and contains the central and peripheral nervous systems. The autonomic nervous system (ANS) is a part of the peripheral nervous system (PNS) which itself contains the sympathetic and parasympathetic nervous systems (Seo & Lee, 2010). The ANS performs and regulates automatic bodily functions associated with heart rate, breathing, digestion, and the hormonal system and initiates the stress and relaxation response (Seo & Lee, 2010). This system is sensitive to stress. Further, during stress, sympathetic dominance changes different bodily functions such as, increase blood flow to the muscles, decrease digestive functioning, and faster shallow breathing (Gazzaniga et al., 2013). Sympathetic dominance can according to van Dixhoord (1998), be altered and decreased with the use of conscious breathing and instead increased parasympathetic or vagal activity. Parasympathetic dominance control functions such as rest, digestion, reproduction, and repair (Gazzaniga et al., 2013).

Breathwork practices have emerged for centuries as a reported intervention with many health benefits which will be discussed later. Breathwork is today widely applied as a treatment and symptom alleviation of clinical populations with e.g. PTSD (de Witt & Cruz, 2021), phobias (Bonn et al., 1984), and other stress-related emotional disorders. Furthermore, longitudinal and cross-sectional studies have focused on how breathwork could benefit individuals with certain conditions, such as pregnancy (Aalami et al., 2016), and employees suffering from burnout (Salyers et al., 2011), while the general healthy population remains largely dismissed. The result of studies of breathing practice in mental and physical health are inconsistent because of methodological limitations in the experimental design, limited sample sizes, and a lack of measurable breathing feedback (Balban et al., 2023). According to Ma et al. (2017), most studies have investigated physiological effects, emotional benefits, and cognitive benefits separately, which inhibits an understanding of the possible benefits for both mental and physiological health in breathing mechanisms. The neurobiology of breath has been studied both in animals and humans (Lavretsky & Feldman, 2021), but little is known about the effect and amount of practice needed on different breathing techniques. One of the questions in this thesis is; how can an evidence-based intervention prevent stress from stagnating, in an effective, close-by, less expensive way?

The Stress Response

The body always aims for homeostasis (balance) between the sympathetic and parasympathetic nervous systems. It is a natural and involuntary process for optimal functioning in humans (Seo & Lee, 2010). Homeostasis is disrupted during a stress response by sympathetic dominance and eventually a balanced post-stressor. Further, when the stressful event has passed, the nervous system can revert and retain homeostasis. However, chronic stress can disturb this balance and cause health issues (Seo & Lee, 2010).

The stress response occurs when an individual perceives a demand where the response consists of physiological action and involves the release of hormones (e.g. norepinephrine and cortisol). These hormones have numerous biochemical and physiological effects, such as increased heart rate and blood pressure, sympathetic-over parasympathetic-nervous system dominance (the so-called fight or flight response) (Seo & Lee, 2010; Vanderhasselt & Ottaviani, 2022). The release of cortisol is controlled by the paraventricular nucleus of the hypothalamus and cortisol is the hormone that gets the most attention in research on stress (Seo & Lee, 2010). Cortisol is involved in processes that function primarily on immediate survival and homeostasis (McEwen & Gianaros, 2010). The stress response activates regulatory centers in the central nervous system (CNS), that stimulate both the ANS and hypothalamic-pituitary-adrenal (HPA) axis (Seo & Lee, 2010). The neuroendocrine system is the primary mediator of physiological changes during stress, in which the HPA axis regulates the secretion of cortisol and the sympathetic adrenomedullary system regulates catecholamine secretion from the adrenal glands (Dhama et al., 2019).

Dysfunction of the HPA axis is one important biological mechanism in stress-related illness, as the regulation of cortisol secretion elevates (Dhama et al., 2019). According to Brown et al. (2013), depression, anxiety, PTSD, and other psychiatric disorders are worsened by stress and are characterized by dominance in the sympathetic nervous system (SNS) and decreased (low) PNS. Also, associated with these disorders is an under activity of the inhibitory neurotransmitter and gamma amino-butyric acid. Related to the elevated SNS-related activity, is the increase of neural activity in the dorsal anterior cingulate cortex, the anterior insula, and the amygdala (Brown et al., 2013). According to Muscatell and Eisenberger (2012), this is likely due to these regions being involved in the system of threat processing. A review by Kim et al. (2015), discusses stress and its relationship with the hippocampus and argues that stress has been involved in the impairment of performance in memory tasks in the majority of studies. Further, the structural volume has also been reduced in the hippocampus following stress, which is argued to be related to memory performance (Kim et al., 2015; Piccolo & Noble, 2018).

Short-term exposure to a stressor can be beneficial as it might evoke extra focus and positive arousal. Further, one has to recover when such a stressor has disappeared. If not, stressors may trigger negative cognitions that are perseverative, and ultimately prolong the physiological stress response, which can cause chronic fatigue and other psychological and physiological diseases (Vanderhasselt & Ottaviani, 2022). Once chronic fatigue has developed it takes a longer time to recover, and people generally stay more sensitive to stress.

Measurements of Stress

Stress can be measured in different ways, along with the various benefits of each measurement tool. Physiological markers show the potential effects on the brain, and body which include e.g. HRV or salivary cortisol. Psychological measurements usually include self-report scales e.g. PSS, which assess the participants' subjective experience of stress.

Heart rate variability (HRV) is a well-established method to assess stress levels. In a meta-analysis on stress and HRV, Kim et al. (2018) explain how the beat-to-beat alterations in heart rate comprise sympathetic and parasympathetic nerve activities of the heart and are used to quantify cardiac autonomic regulation. HRV can measure time-domain and frequency-domain variables. The time- domain measures the time interval of heartbeats or variation over time, also explained as the interbeat interval (IBI), which is the period between heartbeats (Kim et al., 2018; Shaffer & Ginsberg, 2017). This domain involves parameters such as the mean normal-to-normal (NN) variance and the interval between that variance. The standard deviation of the NN interval (SDNN) is an index of physiological resilience against stress, whereby the SDNN value increases when HRV is large and irregular. The variables impacted by the PNS, as they reflect beat-to-beat changes, is the percentage of adjacent NN intervals that differ from each other by more than 50 ms (pNN50) (Kim et al., 2018; Shaffer & Ginsberg, 2017). The frequency domain provides information about how power is distributed in low-frequency (LF), and high-frequency (HF) bands, which allows an autonomic balance to be quantified at any given time (Kim et al., 2018; Shaffer & Ginsberg, 2017). The LF measures SNS activity and HF measures PNS, as it reflects the vagus nerve activity (Kim et al., 2018). According to Shaffer and Ginsberg (2017), the LF band (0.04–0.15 Hz) is comprised of rhythms with periods between 7 and 25 seconds and is influenced by breathing from 3 to 9 breaths per minute. Within a 5 min sample, there are between 12 to 45 complete periods of oscillation. The HF band (0.15–0.40 Hz) is affected by breathing from 9 to 24 breaths per minute. The ratio of LF to HF power may estimate the ratio between SNS and PNS activity under controlled conditions. The total power is the variance of all NN intervals (Kim et al., 2018). HRV is an important physiological biomarker of well-being, mood, and adaptation. Improved HRV indicates better health, mood, and adaptation to stress (Chaitanya et al., 2022).

Cortisol can be measured accurately in saliva and is a widely accepted biomarker of stress (Bigert et al., 2005). Salivary cortisol levels have been reported to range between 10.2–27.3 ng/mL in the morning and 2.2–4.1 ng/mL at night for healthy adults (Laudat et al., 1988). The HPA system is activated during a stress response that induces the secretion of cortisol, and in this regard, salivary cortisol can be a reliable estimate of stress-induced HPA activity (Dhama et al., 2019).

The Perceived Stress Scale (PSS), is the most used measurement tool to assess stress levels in situations over the last month (Cohen et al., 1983). The scale contains 10 items with four options ranging from 0= never and 4= very often. Examples of statements could be “In the last month, how have you felt that things were going your way?” or “In the last month, how often have you felt nervous or stressed?”.

Related to, but not synonymous with stress, is negative affect (NA), which is a component of our mood. In a study conducted by Mroczek and Almeida (2004), the results indicate that NA has been shown to correlate with stress. Therefore, is the Positive and Negative Affect Schedule (PANAS) included in this thesis. PANAS rates mood on 10 items, ranging from 1 = very slightly or not at all to 5 = extremely (Watson et al., 1988). PANAS is considered a valid and reliable measurement tool for the emotional aspect of well-being and measures one’s mood at the moment but can be used over a time period to administer any changes in well-being. The questionnaire asks about emotions experienced over the last week, such as “alert”, “irritated”, “scared” etc.

Anxiety is a more specific feeling that is considered relevant in the context of measuring stress. Anxiety and stress share similar symptoms during the activation of the sympathetic nervous system (Spielberger et al., 1983). Therefore, is the State-trait Anxiety Inventory (STAI), also included in this thesis. It is a commonly used assessment tool, that clearly differentiates between temporary feelings of anxiety and long-lasting ones. It contains 20 items each for state and trait anxiety, on a scale ranging from 1= almost never to 4= almost always. The STAI includes statements such as “I have disturbing thoughts”, “I feel like a failure”, “I am a steady person” etc.

The Breathing Process

Breathing is mostly an automatic unconscious bodily function that oxygenates and carbon dioxide off-gases the body (Balban et al., 2023). Further, breathing can easily be consciously altered for different purposes. According to Caldwell and Victoria (2011), it is understood that breathing in oxygen is a fuel source for the metabolism of the body and without it, death is inevitable within minutes.

Humans breathe about 18 times per minute, which is about 25800 times a day. According to Russo et al. (2017), the normal respiratory rate is within the range of 10–20 breaths per minute (0.16–0.33 Hz). Inhalation is the process that causes air to enter the lungs, and exhalation is the process that causes air to leave the lungs. Contraction and relaxation of the diaphragm and intercostal muscles (located between the ribs) cause most of the pressure changes that result in inhalation and exhalation (Downey, 2011). In general, two muscle groups are used during normal inhalation: the diaphragm and the external intercostal muscles (De Troyer & Boriek, 2011). Additional muscles can be used during deeper breaths. Inhalation is an active state, where the lungs expand to the contraction of the diaphragm. The contraction of the external intercostal muscles moves the ribs upward and outward, causing the rib cage to expand (Downey, 2011). Further, the expansion of the thoracic cavity forces the lungs to stretch and expand. Normal exhalation is generally passive, meaning that energy is not required to push air out of the lungs, as the diaphragm and intercostal muscles relax following inhalation (De Troyer & Boriek, 2011).

A respiratory cycle is one sequence of inhalation and exhalation. Normal respiration also called “tidal breathing” has a relatively constant rate and inhalation and exhalation volumes. It is known that the heart rate increases during inhalation and decreases during exhalation in a respiratory cycle (Billman, 2011). This phenomenon is called respiratory sinus arrhythmia (RSA), the result of multiple central and peripheral mechanisms (Olexova et al., 2020). RSA is considered a noninvasive marker of cognitive and emotional regulation. Different emotional and cognitive states alter the depth and frequency of breathing, which likewise impacts the emotional state, in part by the regulation of carbon dioxide levels (Olexova et al., 2020). The balance of the oxygen and carbon dioxide levels in the blood regulates critical bodily functions, such as blood pressure and the balancing of SNS and PNS. Porges (2007), links emotional and stress-related disorders to decreased vagal activity in the heart, which could be indexed by a reduced RSA. Further, it has been found that the vagus is inhibited during inhalation and uninhibited during exhalation (Zhang et al., 1997).

Disturbance in the breathing ratio, shallow breathing restricted to the upper chest, or hypo- or hyper-tonicity of the diaphragm are several definitions of dysfunctional breathing (Caldwell & Victoria, 2011; Courtney, 2009). Further, it can be caused by psychological, physical, and behavioral states and attitudes (Caldwell & Victoria, 2011; Courtney, 2009). Controlled breathing directly influences the respiratory rate, which can cause immediate physiological and psychological calming effects by increasing vagal tone during slow exhalation. This is a normal phenomenon related to the effects of breathing on intrathoracic pressure, diaphragmatic movement, heart volume/blood flow rates, and compensatory shifts in vagal activation (Balban et al., 2023; Latha & Lakshmi, 2022).

Breathwork

In Eastern cultures, the practice of controlling one's breath in order to enhance or restore one's health has been used for thousands of years. According to Russo et al. (2017), the popularity of breathwork in the West has risen since the mid-1900s, due to more interest in holistic approaches in healthcare.

According to Caldwell and Victoria (2011), conscious breathing or intentional breathing can be defined as the act of becoming aware of one's breath to alter it, which is an essential component of breathwork. It is well known, that conscious control of breathing affects heart rate variability and blood pressure, decreases stress, changes brain activity, and regulates cognitive and emotional processes where the respiratory rhythm has an important role (Heck et al., 2022; Van Diest et al., 2014). Slow breathing, is a collective term for techniques that are associated with up to 10 breaths per minute. EEG in slow breathing in healthy subjects has shown an increase in alpha power and a decrease in theta power related to emotional control and well-being (Zaccaro et al., 2018). Further, an fMRI study by Critchley et al. (2015), showed increased activity in cortical (e.g., prefrontal, motor, and parietal cortices) and subcortical (e.g., pons, thalamus, sub-parabrachial nucleus, periaqueductal grey, and hypothalamus) structures, a trend for increased alertness and relaxation, but also a decrease in anxiety and depression. According to the review by Zhang et al. (1997), it is suggested that prolonged practice of slow breathing is necessary to achieve long-term shifts in parasympathetic dominance. The following breathing techniques are examples of being consciously aware of the breath, to alter it.

Resonance breathing (RB), is breathing at a slow rate (4.5 to 6.5 breaths/minute), to maximize their RSA (Steffen et al., 2017). The length of inhalation and exhalation are equal with only a breath hold between them (Brown et al., 2013). According to Steffen et al. (2017), self-training in resonance breathing lowers stress, and blood pressure and improves mood. Further, RB has been shown to reduce anxiety, and depression through the vagal pathway which in turn affects the activity in the locus coeruleus, orbitofrontal cortex, insula, amygdala, and hippocampus (Brown et al., 2013).

Diaphragmatic breathing (DB), is defined as an efficient integrative body–mind training for dealing with stress and psychosomatic conditions. Furthermore, a deeper inhale and exhale than normal involves an expansion of the belly and construction of the diaphragm which consequently decreases the respiration frequency (Caldwell & Victoria, 2011; Ma et al., 2017). Most research on DB has been investigated in association with other interventions, such as meditation (Paul et al., 2007), and yoga (Robinson et al., 2021). According to the review by Russo et al. (2017), studies in DB have reported increased efficiency of venous return, which refers to the flow of blood back to the heart, due to the anatomical fact that the diaphragm is connected to and supports the heart.

Cyclic sighing (CS), is a controlled breathing technique emphasizing the exhalation process. This technique is described as a slow inhale to expand the lungs, followed by a shorter inhale to fill the lungs, and then fully exhaling the breath slowly in a sigh (Balban et al., 2023).

These three breathwork techniques instruct the practitioner to breathe in through the nose. Studies have shown support for a different effect in the CNS in nasal breathing, compared to mouth breathing. Nasal breathing synchronizes activity in the olfactory cortex, the amygdala, and the hippocampus, unlike mouth breathing, which has implications for stress management and the treatment of anxiety (Zelano et al., 2016).

The Aim

This thesis will provide a systematic review to estimate the potential effect breathwork has on stress in healthy subjects and the potential way for one to alter stress responses in a positive way. The focus will be on studies including healthy participants, with or without stress-related symptoms. Studies investigating the effect breathwork has on stress and studies containing measurements on HRV or salivary cortisol will be included. Controlled slow breathing interventions (e.g., RB, DB, and CS), will be used. There will be a comparison of the potential effects of pre-and post-intervention in randomized control trials. Stress is a continuously growing problem, therefore, it is beneficial for both society and the individual in finding evidence-based, easy-to-apply, and accessible interventions to use. Contrary to e.g. therapy, breathing requires less effort, only requires oneself, costs less, and techniques can be easily accessed by e.g. the internet.

Methods

Search Strategy

To get an overview of the subject, we started off with different combinations of keywords (breathing, breathwork, connected breath and PTSD, trauma, stress and HRV, heart rate variability, NIRS, cortisol). After reading about measurements used in stress-related research combined with breathing interventions, the following search string was formulated: (breath* AND stress AND (HRV OR “heart rate variability” OR “salivary cortisol” OR cortisol)).

Quotation marks were changed to curly brackets in Scopus and the original search string was used in Web of Science. The final search was done on February 24th and gave 2107 hits on the two databases (Scopus n=1304 and Web of Science n=803). The records of the articles were exported to the software Rayyan (Ouzzani et al., 2016). First, 469 duplicates were removed. Second, 1638 articles were screened by title and abstract, whereas, 1617 articles were removed, due to not meeting the inclusion criteria. The remaining 21 articles were left for full-text evaluation. In the final screening, 18 articles were excluded, due to not fulfilling the inclusion criteria. Finally, three studies were included in this systematic review (see Figure 1).

Inclusion and Exclusion Criteria

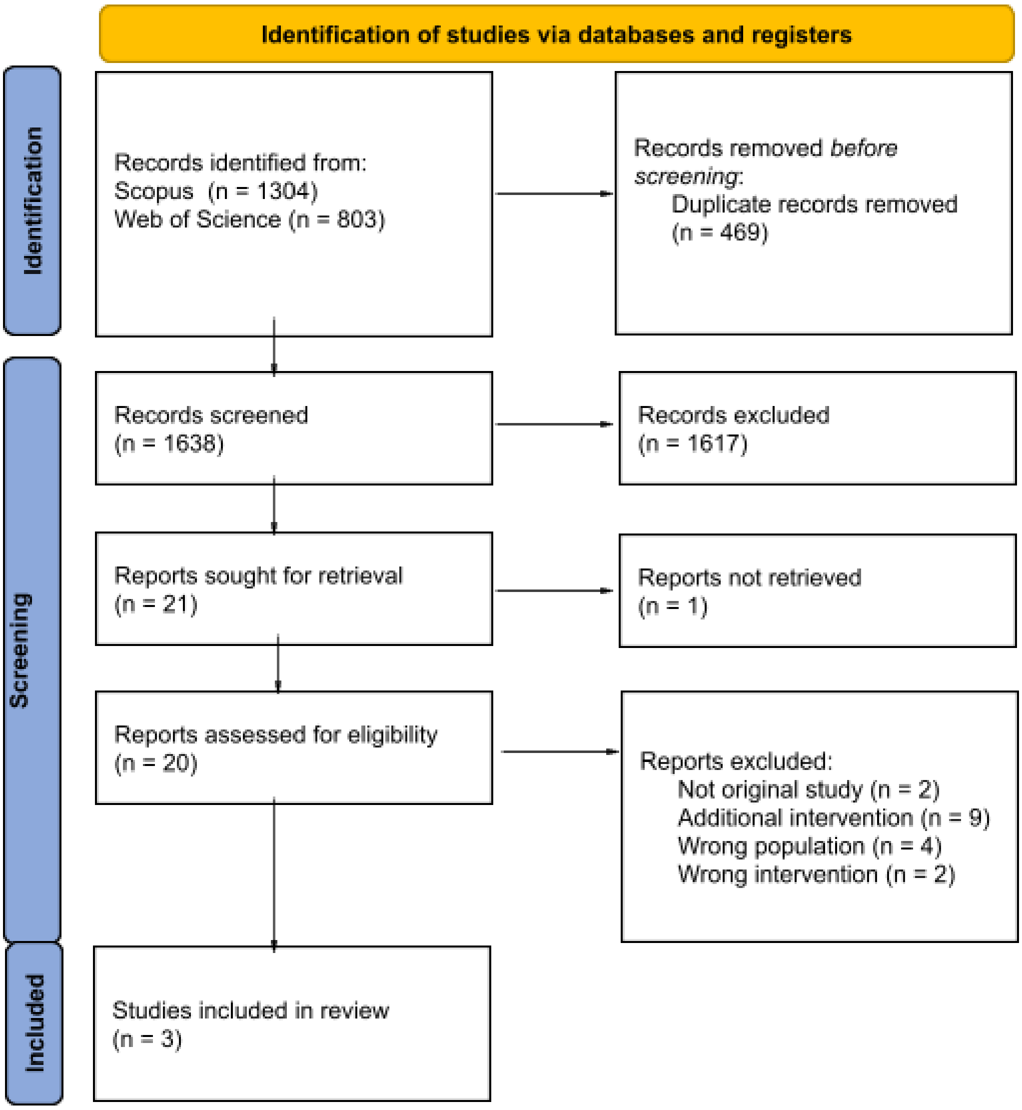
The inclusion criteria for the participants are, only healthy adults (18+) from all ethnicities and countries. Thereby, studies on children, animals, and clinical populations are excluded. Studies including athletes and experienced breathwork practitioners were excluded, as the aim of this thesis was to investigate the effect of breathing interventions in the general healthy population. Participants can be with or without stress-related issues. The inclusion criteria for the intervention are studies with a breathing-based intervention (e.g., resonance breathing, diaphragmatic breathing, and cyclic sighing), without any additional intervention. Interventions that affected breathing as a by-product (e.g., mindfulness, music, yoga, and exercise) were excluded, as well as, distractions of the main intervention. The primary goal is to find studies that measure the physiological outcome related to stress, with HRV or salivary cortisol. In the search process, we discovered that studies included PANAS or the PSS, or STAI in the context of stress. Given the reasons mentioned in the introduction, we decided to include them further in the process. Studies must be in English, peer-reviewed, published, and original research. Only studies available through the university database or open access are included.

Data Extraction

The data extracted from the included studies are the following seven categories; the first author with the date of publication, study design, sample characteristics, control group, intervention and duration of the intervention, outcome measurement, and the main findings (see Table 1 and 2).

Figure 1

PRISMA flow chart



Note: The literature search process is illustrated in a PRISMA 2020 flow chart (Page et al., 2021).

Result

The studies in this systematic review showed mixed results, where some results were significant while others were not. In addition to the significant result, there are indications that controlled slow breathing has beneficial effects on stress.

Study Characteristics

This review includes three studies. The sample size varied from 40 (Chaitanya et al., 2022; Ma et al., 2017) to 54 (Balban et al., 2023), with a total of 134 participants who finalized the studies and are included in the data analysis. The studies presented the age of participants, ranging from 18-81 years (see Table 1). The population of two of the studies included both men and women (Balban et al., 2023; Ma et al., 2017), one study only included men (Chaitanya et al., 2022), and all were healthy with no psychological or physical disorders. The studies were conducted in three different countries, the USA (Balban et al., 2023), India (Chaitanya et al., 2022), and China (Ma et al., 2017). All three studies used randomized control trials and comparisons between groups (Balban et al., 2023; Chaitanya et al., 2022; Ma et al., 2017) to investigate the effects of breathwork on stress-related characteristics. The studies used different breathwork interventions such as cyclic sighing (Balban et al., 2023), resonance breathing (Chaitanya et al., 2022), and diaphragmatic breathing (Ma et al., 2017). The amount of time breathing across all the studies varied from 5 to 20 minutes (see Table 1). Two studies collected data at baseline, pre- and post-breathing sessions, and post-study (Balban et al., 2023; Ma et al., 2017) while Chaitanya et al. (2022) collected data at baseline and post-study (see Table 2).

Balban et al. (2023) conducted a study comparing the possible effects of different breathwork techniques and a control group with mindfulness meditation. This review will account for one of the breathing techniques in the study, CS, where 30 subjects were randomized into. This technique begins with a slow inhalation to expand the lungs followed by a shorter inhale to fill the lungs and a slow complete exhalation. The control group consisted of 24 participants and they only did mindfulness meditation (breathe naturally through the nose and focus on the forehead). The resting heart rate and respiratory rate were measured daily with WHOOP (2023). A WHOOP strap uses a wrist-worn LED photoplethysmography to monitor HR and calculates resting heart rate and HRV during deep sleep through their proprietary algorithms.

The study conducted by Chaitanya et al. (2022) compared deep breathing at resonance frequencies with a control group with normal breathing. In the experiment group, each participant had to find their RB rate. The subjects were asked to breathe for two minutes at a given frequency from 4.5 to 7 breaths per minute with an increase of 0.5 breaths per minute using the Android application “paced breathing” to keep it as constant as possible. The RB rate was set when the breathing rate and HRV were at the subjects' total power. In the breathing group, the participants were given supervised training for breathing at their resonance frequency for eight sessions, two each week, and practiced at home in the morning. After four weeks of training, they practiced 20 minutes of RB for another four weeks. The control group breathed normally with no further instructions at the same time interval. During every session, the subject was asked to lie comfortably on a couch and relax for 10 minutes. An electrocardiogram (ECG) recording was done for 10 minutes for short-term HRV analysis by following the recommended standard procedure by the task force on HRV (Malik et al., 1996). The ECG was acquired using a computer-based digital data acquisition system using Power

Lab® electrocardiographs (AD Instruments, Sydney, Australia, cited in Chaitanya et al., 2022). HRV was assessed using time-domain and frequency-domain analysis using a standard three-lead ECG (Malik et al., 1996) placed on their torso.

In the study by Ma et al. (2017), they compared a DB group with a control group with no intervention. The intervention group learned essential skills on how to breathe as deeply as possible and exhale slowly, til almost empty, in a self-controlled manner, under the guidance of a breathing coach. While breathing this way the subjects were asked to focus on their breathing and sensations in the body. All participants were seated in a comfortable chair and kept their eyes closed during the process and breathed through their noses. An assistant supervised their recordings and obtained the help of the coach if the recording vibrated or if the breathing frequency became higher than 6 breaths per minute, 2 minutes after the intervention began. The breathing intervention involved 15 min resting breathing (normal breathing with closed eyes) and 15 min DB in each session (with verbal guidance from the breathing coach). All interventions and tests were performed in a sunny, soundproof, open-air conference room at the IT company the participants were from, not in the laboratory.

Table 1

Characteristics of Included Studies

First Author & publication date	Study design	Sample characteristics	Control group	Intervention and Duration of Intervention	Outcome measurements
Balban et al. (2023)	Randomized control study	(n=30) intervention group (n=24) control group Age, M + SD (range) 27.97 +- 13.46 (18-81)	Yes	CS 5 min intervention x 28 days	PANAS STAI HRV
Chaitanya et al. (2022)	Randomized control study	Men (n = 40), 18-30 years.	Yes	RB Supervised training of 20 min, two each week, plus self-practice each morning. After four weeks of training, they practiced for 20 min for another four weeks.	HRV: SDNN (ms) pNN50 (ms) LF LF/HF Total power PSSl
Ma et al. (2017)	Randomized control study	N=40 Intervention group: men (n = 10) women (n= 10) Age 30.16 ± 5.11 control group: men (n= 10) women (n= 10) Age 28.25 ± 3.91	Yes	DB 15-minute resting breathing and 15-min diaphragmatic breathing (each session) 20 sessions over 8 weeks.	PANAS Cortisol test

Note: M= Mean age; SD= Standard Deviation. CS= Cyclic sighing; RB= Resonance breathing; DB= Diaphragmatic breathing. HRV= Heart Rate Variability; SDNN= The standard deviation of the normal-to-normal interval; PNN50= The proportion derived by dividing NN50 by the total number of NN intervals; LF= Low Frequency; HF= High Frequency. PANAS= Positive and Negative Affect Schedule (Watson et al., 1988); STAI= State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983); PSS= Perceived Stress Scale (Cohen et al., 1983).

Stress Measurements

This review identified two different outcome measurements of physiological stress: activity in the ANS measured with HRV (Kim et al., 2018) and stress-induced HPA activity measured by salivary cortisol (Dhama et al., 2019). Further, three different questionnaires PSS, PANAS, and STAI measured subjective stress-related factors.

HRV and Salivary Cortisol

HRV were measured for their total power, LF component, HF component, LF/HF ratio, SDNN, and pNN50.

In the study conducted by Chaitanya et al. (2022), a significant improvement in the HRV parameters SDNN, pNN50, LF, LF/HF, and total power was found in the intervention group after practicing RB for four weeks. The control group showed no difference (see Table 2). The result indicates an increased parasympathetic and decreased sympathetic activity after practicing resonance breathing every day for four weeks. In the study conducted by Balban et al. (2023), the HRV showed no significant results (no data was reported).

In the study by Ma et al. (2017), salivary cortisol was collected between 11:00 and 12:00 to control for the variation in cortisol levels over the circadian rhythm, before and after every DB intervention. The result showed a significant decrease in salivary cortisol after the DB session compared to the control group. The concentration result showed a significant interaction between time and group (see Table 2).

PSS, PANAS, and STAI

The three questionnaires were used between the three studies to measure stress-related factors in pre-and post-intervention periods as well as in between intervention sessions.

PSS was given at the baseline and after eight weeks in the intervention group and the control group (Chaitanya et al., 2022). The perceived stress score decreased significantly after eight weeks of RB compared to baseline scores as well as the control group (see Table 2).

PANAS was conducted pre-and post-study and after the daily intervention. Their result showed a significant increase in PA, further, a significant decrease in NA, in both the CS group and the mindfulness meditation group (Balban et al., 2023). For the within-participant variance in the daily mood changes, there were no differences between the two groups in NA changes. The CS group also had a significant interaction with the days on protocol, the daily PA increase was more significant the more days subjects had been on the protocol (Balban et al., 2023). Furthermore, DB demonstrated a significant reduction in NA score after the intervention compared with the control group (Ma et al., 2017). There was no detected significance in PA in either group (see Table 2).

STAI scores were made by each participant before and after each breathwork protocol daily in the study conducted by Balban et al. (2023). Both the CS group and the mindfulness meditation group showed significant reductions in state anxiety. There were no significant changes in trait anxiety in any group, nor were there differences in trait anxiety change between the groups. For the within-participant variance in the daily changes in anxiety over time, there were no differences between the two groups in state anxiety (see Table 2).

Table 2

Significant Results of Included Studies

First Author & publication date	Main findings of the experimental group	Main findings of the control group
	Mean (SD)	Mean (SD)
Balban et al. (2023)	PA The average daily change per person was 1.89 ± 3.76 for CS, p=.025 Days protocol: 0.034 (0.05, 0.062) Type_BW: 1.893 (0.090, 3.700) Days on protocol*Type_BW: 0.056 (0.000, 0.113) NA The average daily change per person was 1.48 ± 1.69 for CS. State anxiety The average daily change per person was 3.85 ± 4.88 for CS.	PA The average daily change per person was 1.22 ± 2.34 for mindfulness meditation p=.06 NA The average daily change per person was 1.62 ± 1.91 for mindfulness meditation State anxiety The average daily change per person was 3.95 ± 4.16 for mindfulness meditation
Chaitanya et al. (2022)	SDNN (ms) Pre-intervention 66.69±33.03 Post-intervention 78.76±24.15 (p=.001) pNN50 (ms) Pre-intervention 30.39±30.39 Post-intervention 54.82±14.49 (p=.02) LF Pre-intervention 1587.6±2308 Post 2546.16±2119 (p=.04) LF/HF Pre-intervention 2.05±1.889 Post-intervention 0.25±0.1323 (p=.02) Total power Pre-intervention 3570.23±2829 Post-intervention 5543.38±2838 (p=.002) PSS Pre-intervention 27.65± Post-intervention 21±20.09 (p=.01)	
Ma et al. (2017)	NA score Pre-intervention 22 Post-intervention 19 *MD* = 2.55, *p* = 0.02	

Salivary cortisol concentration
First session:
1, Pre intervention: 6,25 nmol/L
2, Post intervention: 6,50 nmol/L
Last session:
3, Pre intervention: 5 nmol/L
4, Post intervention: ~ 4,90 nmol/L

Note: SD= Standard Deviation. PA= Positive affect. CS= Cyclic sighing. NA= Negative affect. SDNN= The standard deviation of the normal-to-normal interval; PNN50= The proportion derived by dividing NN50 by the total number of NN intervals; LF= Low-Frequency power (ms2); HF= High-Frequency power (ms2). PSS= Perceived Stress Scale (Cohen et al., 1983). MD= Mean ± Standard deviation.

Discussion

The aim of this systematic review was to investigate the potential effects breathwork may have on stress. Studies included both the physiological markers of stress such as HRV and salivary cortisol and subjective experience, assessed using self-report questionnaires. The three studies all demonstrated significant results relevant to our aim.

The results of Chaitanya et al. (2022) showed improved HRV in several parameters. After the four weeks of RB, the participants showed increased SDNN and pNN50, which is a sign of improved parasympathetic activity, indicating reduced stress levels. The authors argue that a specific sample size of young men was used, as HRV might differ between genders, due to different parasympathetic activities. The outcome of a larger and more diverse sample size in genders and ages is unknown. The results are however in line with a meta-analysis by Goessl et al. (2017), in which HRV biofeedback training shows an association with reduced stress and anxiety. In Balban et al. (2023), no significant changes in HRV were shown, however, there was a significant reduction in the respiratory rate in the CS group compared to the mindfulness meditation group. This indicates increased parasympathetic activity, but no data was reported. In the study by Ma et al. (2017), the level of cortisol significantly decreased after practicing DB, which indicates a reduced stress level. Further, the decrease in NA also indicates a successful reduction of stress. These results are in line with Tsiouli et al. (2014), in which cortisol levels decreased in parents of diabetic children, after 20 sessions of DB. Although, the study also included muscle relaxation, thus not providing a correlation with breathwork only.

The PSS score showed a significant reduction of stress, after four weeks, in both the RB group and the control group (Chaitanya et al., 2022). Due to significant PSS scores in both groups, the results could be put into question, as to what caused the changes. Since the PSS is a self-report measurement, it accounts for one’s subjective experience of stress and can not be considered an objective assessment. This is worth taking into consideration regarding all studies included. Regarding PANAS, a common effect between studies was a decrease in NA (Balban et al., 2023; Ma et al., 2017), which shows how breathwork might be useful in improving one's mood. Considering its usefulness in studies on stress, such as Mroczek and Almeida (2004), the results strengthen the argument, that the intervention was successful. PA was also shown to increase following CS, along with a decrease in state anxiety (Balban et al., 2023). Arguably, the intervention seems to have improved the well-being of the participants. The mindfulness meditation group also showed significant changes in the same reports, although, not as much in increasing PA, suggesting breathwork to be more effective. Thus as mentioned before, the breathing intervention is not the main intervention but could be a preparation or a latent component for the core intervention. Therefore, Ma et al. (2017) conducted this study to monitor breathing, as an independent mind–body intervention, to discuss health promotion after slowing down respiration. Moreover, this was achieved by their controlled breathing method, which combined a monitoring device and a breath coach supervision. According to Ma et al. (2017), DB is demonstrated as an effective relaxation technique with beneficial effects on both mental and physical health. It has since been reported, that athletes experienced feeling more relaxed following an intervention of DB, further suggesting that the intervention might have positive effects on stress (Hunt et al., 2018).

In terms of study quality, after studying the topic, it would seem that practice might be an important factor, considering that the techniques initially might be done incorrectly. Sufficient learning and habitual practicing seem to be important factors in Chaitanya et al. (2022) and Ma et al. (2017) given the intensity of the practice. Balban et al. (2023) fail to demonstrate any significant HRV results, not to mention, not reporting the numbers. Also to mention, the studies use different self-report scales. Chaitanya et al. (2022) use the PSS, which is the only one among the studies to directly measure stress. Although, the others can arguably be used for that purpose.

The combined results from the included studies indicate that breathwork might be helpful in stress reduction in healthy individuals. This is in line with the review by Brown et al. (2013), who argued that breathwork aside from alleviating symptoms of PTSD or panic disorders, has been shown to significantly improve HRV and state anxiety levels. Although the studies had also been investigating mindfulness, the variables showed correlations with breathing practice (Sherlin et al., 2009; Sherlin et al., 2010).

Ethical and Societal Aspects

One conflict of interest was reported by Balban et al. (2023), in which one of the author's researchers became an advisor to WHOOP, in 2022. WHOOP contributed wearable devices monitoring physiological data, which was a restriction due to COVID and reportedly, did not affect the study. The participants were all healthy, and none had any psychiatric or physiological disorders.

Stress is a continuous problem in today's society, which is shown in the growing number of sick leave caused by stress-related illnesses. If breathwork was more established as a coping strategy, it might on a larger scale reduce stress and diminish stress-related illnesses. Therefore, this line of research is valuable to both society and the individual. Breathwork is easily accessible anywhere and can be an inexpensive tool anyone around the world can use oneself when mastering the techniques. With these promising results, breathwork should be much more researched and be considered in the future to become a general aid for stress management, to prevent chronic stress and/or other pathology.

Limitations

A limitation of Balban et al. (2023) was that they did not present the gender distribution between participants in the different interventions, which makes conclusions on who was affected difficult. Another issue was the lack of monitoring of the participants' breathwork practice. This causes uncertainty about their individual performance level, which also creates issues when generalizing. The authors of the study discuss the issue and examples of how monitoring can be enforced in future studies. The data on HRV was neither available causing difficulties in discussing the results since it is unknown whether the HRV results were close to being significant or not. Another limitation is the use of self-report scales. These scales account for one's subjective experience of stress and can not be considered an objective assessment. This is worth taking into consideration regarding all studies included.

This thesis has several limitations. The most obvious is the small number of studies included which is a result of shortage in qualitative studies on the topic which cannot be answered to here. Not to mention, our narrow inclusion criteria, such as healthy populations and only peer-reviewed English articles. Another limitation is the limited access to full texts, as the university does not provide access to every published study. Not a major issue since only one study was inaccessible. The studies showed significant results, but since different measurements were used it was difficult to draw a conclusion with such limited comparisons and the amount of information. Only one study used cortisol which makes comparisons impossible. Although one could counter that it might indicate something if different measurements (e.g. salivary cortisol, HRV, and PSS) indicate reduced stress levels. Not to forget, these are only a combination of different variables indicating reduced stress.

Another limitation is the different definitions of breathwork and different terms, which causes difficulties in studying the subject as different researchers have their own definitions. In general, there is limited research on breathwork in the non-clinical population and the studies usually include other interventions as well.

Future Research

Future research should measure stress levels using broader measurements as stress varies between individuals. More studies should include salivary cortisol and HRV as we only found three studies with our inclusion criteria and since it would provide more objective assessments contrary to self-assessments, which are prone to individual biases.

Having Balban et al. (2022) in mind, the original study was intended as an exploratory study in preparation for a larger clinical trial. In addition, the authors are now planning a larger confirmatory trial that will be pre-registered. Following up on the intervention would also provide insight into any possible lasting effects.

Another suggestion is a larger sample size of participants. Our studies included limited numbers and one study only used men. Along with larger and diverse samples broader markers could complete the physiological differences between genders. Although, this would require two simultaneous analyses. Since most studies focus on the clinical population, more studies should investigate non-clinical or sub-clinical populations for preventative reasons. Including both men and women, it would show whether the interventions have the same possible effects on both genders.

In the future, the definitions of breathwork should be more established. The terms and techniques vary and cause confusion when studying the topic. Also, separating breathwork from mindfulness would be preferable since it causes difficulties in drawing conclusions from such studies. Many of the excluded studies in this search process used e.g. mindful breathing and it is uncertain if their possible outcomes were generated by the breathing aspect or simply being mindful.

Conclusion

This systematic review shows that breathwork exercises might be associated with reduced stress in healthy individuals. The changing HRV and cortisol levels along with the self-reports indicate that the interventions might have beneficial effects in reducing stress, as well as improving mood. Stress is a universal phenomenon and can easily evolve into clinical or chronic stress if not regulated or remains at a healthy level. More research is needed in order to provide the general population with evidence-based easily accessible breathing techniques for coping strategies for stress.

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