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The Links of Respiration with Physical Fitness, Muscle Tension, and Executive Functioning

DOCTORAL DISSERTATION

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Biophysics (N 011)

VILNIUS 2023

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Kvėpavimo ryšys su fiziniu parengimu, raumenų įtempimu ir vykdymo funkcija

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LIST OF ABBREVIATIONS AND DEFINITIONS

AM – abdominal motion

AM/(AM+TM) – abdominal contribution to the sum of abdominal motion and thoracic motion

EtCO₂ – End-tidal carbon dioxide partial pressure

FAST – fast breathing

IE ratio – inhalation exhalation duration ratio

MINDFUL – mindful breathing

MUSIC – listening to music

RMS – root mean square

RR – respiration rate

sEMG – surface electromyography

SLOW – slow breathing

SpO₂ – oxygen saturation

Stroop color word test – Stroop test

SARs – Pulmonary stretch receptors

TM – thoracic motion

INTRODUCTION

Globally, the size of the aging population is increasing rapidly (Li et al. 2019). According to the 2022 UN World Population Prospects, it was anticipated that the percentage of the world's population aged ≥ 65 years will rise from 10% in 2022 to 16% by 2050 (United Nations 2022). To combat the challenges associated with aging and limited healthcare resources, medical professionals have begun advocating for the concept of “exercise is medicine” and promoting exercise as an essential component of healthcare (Sallis 2015).

Abdominal breathing, also called diaphragmatic breathing, is a popular exercise that is attracting more and more attention for health improvement (Anderson and Bliven 2017), and it has been found to be beneficial for both physical and mental health (Hamasaki 2020; Ma et al. 2017). The diaphragm muscle fibers interdigitate with transversus abdominis that plays a crucial role in trunk stability (Lynders 2019). It also link with the psoas and the quadratus lumborum muscles, which jointly create an anatomical structure that aids in supporting lumbopelvic stability (Rivera et al. 2010). Meanwhile, trunk/lumbopelvic stability are an important aspect of physical fitness (e.g., maintaining body balance) (Cha 2018; Haruyama, Kawakami, and Otsuka 2017; Yang et al. 2021). Therefore, people with more abdominal movement during spontaneous breathing are supposed to have a better physical health.

However, it remains unclear whether healthy individuals, especially those in the middle-aged population, should alter their breathing to a more abdominal-dominant pattern to prevent age-related health deterioration. Furthermore, it is not yet known whether increasing the proportion of abdominal movement adversely affects our health. These questions should be a cause for concern, as a large number of individuals practice abdominal breathing worldwide. It is certain that physical performance begins to decline at approximately 30 years of age (Milanović et al. 2013; Allen and Hopkins 2015). Understanding how respiratory movements change with age can provide greater confidence in answering the aforementioned question and allow for more targeted exercise recommendations.

Evidence exists about age-related alterations in respiratory movement. One study observed that the abdominal contribution to spontaneous breathing increased with aging, and the researchers suggested that the rise in abdominal contribution could compensate for the reduced thoracic activity in order to maintain the level of tidal volume (Mendes et al. 2020). Nevertheless, several previous studies reported no systematic age effects on respiratory movements during quiet breathing (Sharp et al. 1975; Verschakelen and Demedts 1995;

Ragnarsdóttir and Kristinsdóttir 2006; Kaneko and Horie 2012; Britto et al. 2009). Notably, the sample sizes were relatively small in all previous studies, and the biggest sample comprised 120 healthy subjects, combining two genders (Verschakelen and Demedts 1995). Sample size substantially affects statistical results (Faber and Fonseca 2014), and men and women are different physiologically. To confirm the findings and adhere to the principles of precision medicine, it is necessary to increase the sample size and analyze data for men and women separately.

Additionally, understanding the correlation between respiration and one's health can provide health professionals with additional information to guide exercise interventions. Respiratory patterns, such as respiration rate, the inhalation/exhalation ratio (I/E ratio), and respiratory movement, have an impact on our physical and mental condition. For example, RR serves as a crucial indicator of an individual's health status and is highly responsive to various stressors, including cognitive load, physical exertion, and exercise-induced fatigue (Nicolò et al. 2020a). A systematic review summarized that intentionally reducing the RR yielded positive effects such as enhanced ease, comfort, relaxation, and positive energy, while also reducing feelings of anxiety, dejection, anger, hostility, and confusion (Zaccaro et al. 2018a). Regarding I/E ratios, experiments have shown that deliberately altering the I/E ratio can modify psychological states, such as enhancing the cardiac vagal tone (Bae et al. 2021b). However, the relationship between spontaneous respiratory patterns and physical health remains unclear. Only one relevant study was found, which revealed a significant correlation between the motion of the diaphragm during spontaneous breathing and balance maintenance (Kocjan et al. 2018a). Uncovering the links between spontaneous respiratory patterns and physical fitness (e.g. muscular strength, muscular endurance, reaction time, flexibility, and cardiorespiratory endurance) can provide with additional information to guide exercise interventions.

While addressing issues related to aging and physical health, office workers, as a growing population, also encounter health-related concerns. Intensive and long-lasting office work is a common cause of muscular and mental disorders due to workplace stressors (Janwantanakul et al. 2008). Many workplace stressors, such as high work and memory demands, mental load, and time pressure are risk factors for shoulder and arm pain (Paulien M. Bongers, Kremer, and Laak 2002; Goh et al. 2015). These stressors increase sustained low-level muscle activity (P. M. Bongers et al. 2006; Roman-Liu et al. 2013), which chronically increases muscle tension, impairs body alignment, causes muscular pain, and even leads to headaches (Chowdhury 2012; Lundberg et al. 1999; Sambataro et al. 2019). One explanation of the

increased muscle tension under stress is that stress elevates sympathetic activity, initiates the release of catecholamines, and further facilitates muscle contraction (Melin and Lundberg 1997). Another group of scientists suggested that stress-induced hyperventilation leads to excessive exhalation of CO₂, which raises pH levels, and that elevated H_p levels facilitate muscle contraction (Schleifer, Ley, and Spalding 2002). Thus, reducing stress may be able to reduce muscle tension during intensive office work. In addition to increased muscle tension, chronic stress impairs cognitive function (Marin et al. 2011). Executive functions encompass cognitive processes that work for purposeful and goal-directed behavior, and perceived stress has a profound negative impact on executive functions (Kleen et al. 2006; Ohman et al. 2007).

It has been shown that microbreaks at work (i.e., short breaks of less than 10-15 min) can benefit physical and psychological well-being (Henning et al. 1997; Mclean et al. 2001). Workplace physical activity significantly reduced general musculoskeletal pain, including neck and shoulder pain (Moreira-Silva et al. 2016). Three months of workplace exercise had a moderate effect on executive function compared to the control group (da Silva et al. 2021). This points to the necessity of having work breaks for physical exercise.

Mindful breathing (MINDFUL, merely being aware of breath) and Slow breathing (SLOW, with a speed of 6 reps/min) are the two commonly used breathing exercises used in stress reduction. For instance, MINDFUL reduced emotional volatility in response to negative stimuli, reduced test anxiety (Cho et al. 2016), and even 5 min of breathing could reduce distress (Beng et al. 2016a). MINDFUL increased alpha power and enhanced error-related alpha suppression during the subsequent Stroop task, indicating enhanced error-monitoring (Bing-Canar et al., 2016). A study using intracranial electroencephalography demonstrated respiration-locked oscillations during attentive breathing (mindful breathing) with stronger power in the anterior cingulate cortex, premotor cortex, insula, and hippocampus (Herrero et al. 2018a), which are regions supposed to be involved in executive function (Carter et al., 1999; Rizzolatti et al., 2002; Eichenbaum, 2004). SLOW (device-guided, 5–6 breaths/min) is currently a Federal Drug Administration-approved treatment indicated by the American Heart Association for relaxation (Larson et al. 2020). Taking slow, deep breaths activates slowly adapting pulmonary stretch receptors (SARs), which play a key role in ending the inhalation phase and facilitating exhalation through the Breuer–Hering reflex (Kubin et al. 2006). These SARs are also involved in regulating the tone of airway smooth muscles, controlling systemic vascular tone and heart rate, and affecting the pathophysiology of restrictive lung diseases (Schelegle 2003). Therefore, intentional deep breathing could potentially influence the

autonomic nervous system through the activity of SARs in the lungs (Noble and Hochman 2019). Many studies have found that SLOW increased ease, comfort, relaxation, and positive energy and reduced anxiety, dejection, anger, hostility, and confusion (Zaccaro et al. 2018b). Besides, the executive function was improved after slow-paced breathing (4.5 sec inhalation and 5.5 sec exhalation) compared to after natural breathing, with higher scores observed for Stroop interference accuracy (Sylvain Laborde et al. 2021).

In addition to MINDFUL and SLOW, FAST can increase neuronal excitability that facilitates muscle contraction, and may be beneficial for cognitive functions. FAST increased ventilation and raised pH (Barrett et al. 2019). Higher pH increased Ca^{2+} and Na^{+} currents, lowered the threshold of the action potential, and shortened the refractory periods of action potentials, which facilitated muscle contraction (Tombaugh et al., 1996; Lu et al., 2012). Enhanced cognitive performance was associated with increased cortical excitability (Salehinejad et al. 2021a). One study compared the effect of 12 weeks of practice of slow and rapid types of pranayama breathing and found that both types of breathing were beneficial for cognitive functions, with fast pranayama having additional effects on executive function (Sharma, M, et al. 2014). Besides, listening to music (MUSIC) is also a common way to relax during work breaks. In a survey conducted by a large North American recruitment firm, 79% of the respondents felt that MUSIC improved their work satisfaction and productivity (Haake 2011).

To the best of our knowledge, the acute effects of different breathing techniques on muscle tension has not been investigated, and few studies have elucidated the efficacy of different breathing exercises and MUSIC on executive function (the detailed information can be seen in the Literature Review in Chapter 2). By the way, examining cardiorespiratory activity can help us gain a better understanding of the impact of these breathing exercises.

In summary, respiratory exercises have enormous therapeutic potential, and these exercises are significant in addressing the challenges posed by the accelerating aging population and the increasingly common issues of musculoskeletal and mental stress among office workers. Therefore, we conducted two experiments aimed to provide healthcare professionals with data for prescribing exercises more accurately and effectively. In the first experiment, we explored the association between spontaneous respiratory patterns and physical fitness at different ages. The second was aimed to examine the acute effects of breathing exercises on muscle tension, executive function, as well as cardiorespiratory activities.

General aim:

To gain a comprehensive understanding of the links between respiration and physical fitness, muscle tension, and executive functioning.

The practical importance of the research

1. Revealing the links of respiratory patterns with physical fitness not only enhances our understanding of breathing exercises but also helps us target specific breathing exercises for health improvement.

2. Through the examination of various respiration techniques, we can optimize our choices for more effective use of breathing exercises.

3. Our research findings in the field of respiration can provide a theoretical basis for incorporating breathing into movement interventions.

Scientific Novelty

1. This is the first study that exploited the links between respiratory patterns during spontaneous breathing and the most physical fitness components, including body composition, muscle power, muscle endurance, balance, flexibility, visuomotor reaction time, and cardiorespiratory endurance.

2. For the first time, study investigated and compared the acute impacts of MINDFUL, SLOW, FAST, and MUSIC on muscle tension and executive function. Simultaneously, we examined physiological responses, such as respiration rate (RR), oxygen saturation (SpO₂), and end-tidal carbon dioxide (EtCO₂), during these breathing exercises and under conditions of psychological stress (Stroop Test), which have not been compared previously.

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1. THE LINKS BETWEEN SPONTANEOUS RESPIRATORY PATTERNS AND PHYSICAL FITNESS AT DIFFERENT AGES

1.1. Literature Review

As the first part of this research, the primary aim is to explore the relationships between spontaneous respiration patterns and physical fitness, including visuomotor reaction time that related to executive function. We also investigated variations in respiratory patterns across different ages. To support this, the literature review will focus on topics such as spontaneous respiration, respiratory patterns, physical fitness, and executive functions. It will also provide an overview of existing research on the connection between respiratory patterns and physical fitness, as well as studies examining respiratory patterns among various age groups. Finally, we will outline the methods proposed to achieve the objectives.

1.1.1. Spontaneous respiration and respiratory patterns

The primary purpose of respiration is to facilitate the entry of air into the alveolar space, allowing the alveolar gases to equilibrate with those in the blood circulating through peri-alveolar capillaries (Mauri et al. 2017). Spontaneous breathing is the term used to describe the natural and unassisted process of respiration, and the pressure gradient is generated only by the work of respiratory muscles (Mauri et al. 2017).

1.1.1.1. Respiratory muscles

The diaphragm muscle is the main muscle of ventilation (Laurent and Shapiro 2006). A realistic two-dimensional model of the human trunk was used to quantitatively analyze the relative contribution to breathing mechanics of respiratory muscles, and the result clearly showed that the diaphragm muscle performed 60–80% of the inspiratory work during spontaneous breathing (Ratnovsky and Elad 2005).

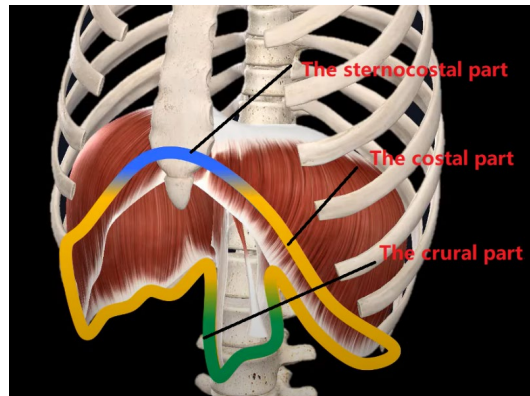


Figure 1. The human diaphragm. The figure adapted with permission from Taim Talks Med.

Anatomically, the diaphragm consists of three parts: the sternocostal, the costal, and crural (Figure 1) (Saladin 2014). The sternocostal part originates from the posterior of the xiphoid process, through the xiphisternal junction, and merges into the central tendon. The costal part emerges from the internal surfaces of the lower six costal cartilages and their adjacent ribs, interdigitating with the transversus abdominis muscle. Given that the transversus abdominis plays a crucial role in core stability (Lynders 2019), any dysfunction in the diaphragm can adversely affect lumbar instability. The crural part is rooted in two aponeurotic arches and the upper lumbar vertebrae (Koulouris and Dimitroulis 2001). The apex and crural regions contribute to the position of the diaphragm (Kolar et al. 2010). And the postural role of these regions of the diaphragm is evident from the fact that the fibers originate from the second and third lumbar vertebrae, descend to the medial arcuate ligaments (linked to the psoas) and the lateral arcuate ligaments (linked to the quadratus lumborum), creating an anatomical structure that aids in supporting the spine (Rivera et al. 2010). That can be a reason that the training of the diaphragm was able to improve lumbopelvic stability and reduce pain intensity in people with low back pain (Dülger et al. 2018; Vicente-Campos et al. 2021).

In addition to the diaphragm, external intercostal muscles are also a group of main inspiratory muscles (Xiao et al. 2012). The external intercostal muscles originate from the inferior surfaces of the proximal ribs and are inserted on the superior and distal parts of the next lower rib (Figure 2). When scalenes fix rib 1, external intercostals elevate and protract ribs 2–12, expanding the thoracic cavity and creating a partial vacuum causing an inflow of air (Saladin 2014).

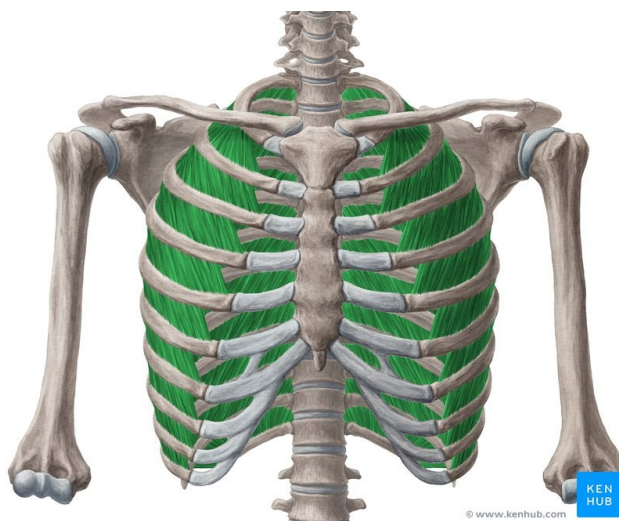


Figure 2. External intercostal muscles. The figure adapted with permission from www.kenhub.com.

In spontaneous breathing, the diaphragm and external intercostal muscles contract to enlarge the thoracic cavity. This decrease in intra-thoracic pressure enables air to enter the lungs. Meanwhile, the abdominal cavity also expands horizontally, as well as in anterior and posterior directions, due to increased intra-abdominal pressure caused by diaphragm contraction. Therefore, both thoracic and abdominal diameters increase in the anterior and posterior directions during inhalation and decrease during exhalation (Higashino, Miyata, and Kudo 2022). By the way, intra-abdominal pressure provides stability to the lumbar spine (Cholewicki, Juluru, and McGill 1999), illustrating another mechanism by which respiration influences physical health.

Except for the main inspiratory muscles, there are many accessory inspiratory muscles, such as scalenus, sternocleidomastoid, trapezius, and pectoralis, which work during forced breathing, or abnormal breathing patterns (Figure 3) (Saladin 2014). One type of irregular breathing is thoracic breathing, characterized by the predominant use of accessory respiratory muscles such as the sternocleidomastoids, upper trapezius, and scalene muscles. In this pattern, these muscles take over the function performed by main inspiratory muscles (Whittaker 2002). Overactivity of these accessory muscles has been linked to neck pain (Kapreli et al. 2009), scapular dyskinesis (CliftonSmith and Rowley 2011), and trigger point formation (Thompson 2001).

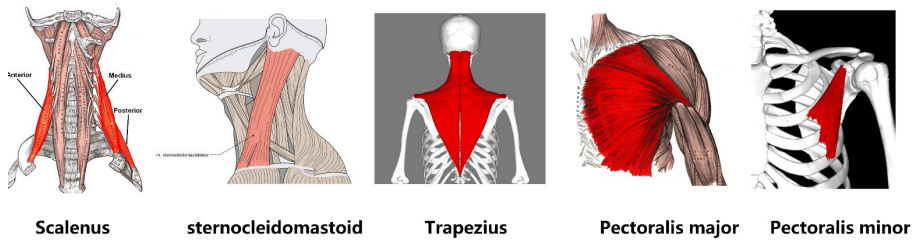


Figure 3. Accessory muscles for inhalation. The figures are in the public domain, downloaded from <https://commons.wikimedia.org>. Please see the Appendix for detailed information.

Normal expiration represents a passive process as it relies on the elastic recoil of the muscles and lungs (Bennett et al. 2020). During normal exhalation, both the external intercostals and the diaphragm relax. The forced exhalation requires accessory muscles for exhalation (Figure 4), such as internal intercostal muscles, abdominal muscles, serratus anterior and posterior inferior. The dysfunctional breathing patterns involve expiratory muscles. For example, fluent signing, which can result from a lack of oxygen, activates accessory respiratory muscles to expel more air, promoting deeper respiration (Vlemincx et al. 2009).

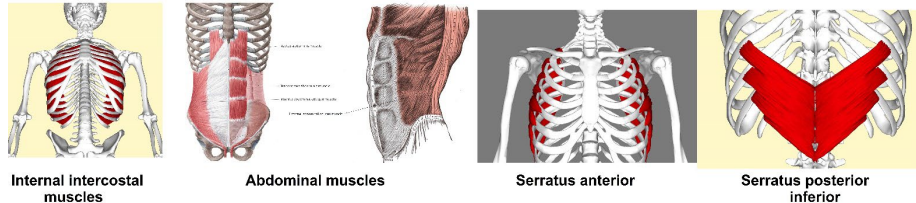


Figure 4. Accessory muscles for exhalation. The figures are in the public domain, downloaded from <https://commons.wikimedia.org>. Please see the Appendix No.1 for detailed information.

1.1.1.2. Initiation of spontaneous breathing

As mentioned above, spontaneous breath results from a phase of active contraction of inspiratory pump muscles (the diaphragm and external intercostals). Although the initiation is uncertain, more scientists support the hypothesis that rhythmic respiration is initiated by a small group of synaptically coupled pacemaker cells in the pre-Bötzinger complex (pre-BÖTC), that are located in the upper part of the ventral respiratory group, on either side of the medulla between the nucleus ambiguus and the lateral reticular nucleus (Barrett et al. 2019; Rybak et al. 2014) (Figure 5).

For smooth and regular respiration, pontine respiratory groups and medullary respiratory groups (dorsal respiratory group and ventral respiratory group) work on it. Pontine respiratory nuclei provide synaptic input to medullary arrhythmogenic circuits to shape and adapt the breathing pattern (Dutschmann and Dick 2012). The pontine respiratory groups also receive vagal afferents relating to lung volume and modulates respiratory frequency (Carlson, 2019). During inhalation, the stretching of the lungs initiates impulses in the afferent fibers of the pulmonary vagus nerve. These impulses inhibit inspiratory discharge. If the vagus nerve are cut, the depth of inspiration is increased after and apneusis develops (Barrett et al. 2019). Since the vagus nerve represents the main component of the parasympathetic nervous system, the stretching of the lungs might activate the parasympathetic nervous system.

Receiving sensory information from peripheral chemoreceptors and pulmonary mechanoreceptors, the dorsal respiratory group of the nucleus tractus solitarius controls inspiratory muscles through output via the phrenic nerves and external intercostal nerves (Guyenet 2014). Much like the dorsal respiratory group, the rostral portion of the ventral respiratory group provides output to the inspiratory muscles. Conversely, the caudal portion provides output to the expiratory muscles via the internal intercostal nerves (Alheid and McCrimmon 2008).

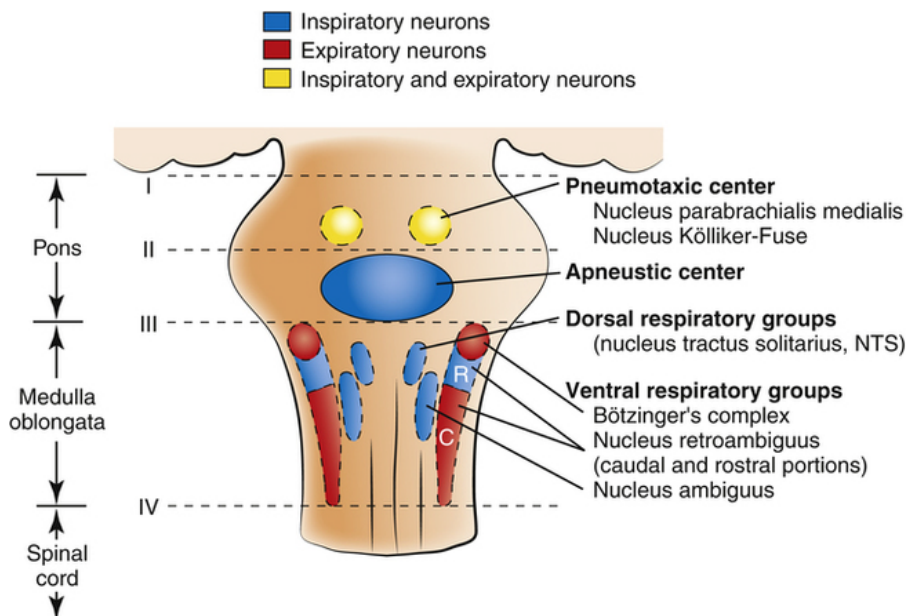


Figure 5. Respiratory Center (Dorsal view of the brainstem). The figure adapted with permission from www.clinicalgate.com/control-of-ventilation.

1.1.1.3. Regulation of respiration

Regulation of respiration refers to the mechanisms that the body uses to control the rate and depth of breathing, which involves complex interactions between the respiratory centers in the brain, chemoreceptors and mechanoreceptors in various parts of the body, as well as input from the peripheral nervous system (Brinkman, Toro, and Sharma 2023).

Chemoreceptors detect the chemical changes in the blood (PaCO_2 , PaO_2 , pH) and alter our breathing to restore homeostasis (Barrett et al. 2019). There are peripheral and central chemoreceptors. The central chemoreceptors are located on the ventral surface of the medulla. They are sensitive to H^+ , however, H^+ is difficult to pass through the blood-brain barrier, whereas it is easy for CO_2 . So, when PaCO_2 is higher than normal, CO_2 will diffuse from blood vessels to cerebrospinal fluid. Then it binds water to form H_2CO_3 and dissociates to H^+ and HCO_3^- . So that the H^+ can stimulate the receptors in the medulla (McConnell 2013). That is why respiration is sensitive to CO_2 . The peripheral chemoreceptors are located on the carotid and aortic bodies. The aortic bodies receive stimulation and transfer excitation through the vagus nerve, while the signal from the carotid body transfers through the glossopharyngeal nerve (Marshall 1994). A study found that rising 2 mmHg of PaCO_2 can activate central chemoreceptors while activation of peripheral chemoreceptors needs 10 mmHg (Guyenet, Stornetta, and Bayliss 2010). Differently, the central chemoreceptor is not sensitive to O_2 at all, but peripheral chemoreceptors work obviously when PaO_2 is lower than 80mmHg. In general, PaCO_2 affects ventilation more than pH and PO_2 (Khonsary 2017). Importantly, increasing alveolar ventilation to about twice normal raises the pH of the extracellular fluid by about 0.23 (Khonsary 2017), such that people are using respiration to alter physiological conditions via changing pH.

Mechanoreceptors are a type of somatosensory receptors that relay extracellular stimulus to intracellular signal transduction through mechanically gated ion channels. The external stimuli are usually in the form of touch, pressure, stretching, sound waves, and motion (Iheanacho and Vellipuram 2022). Pulmonary stretch receptors (SARs) are mechanoreceptors. Slow-deep inhalation activates slowly adapting SARs, which end inspiration and facilitate expiration as part of the Breuer–Hering reflex (Kubin et al. 2006). SARs have been implicated in the regulation of airway smooth muscle tone, the regulation of systemic vascular tone and heart rate, and the pathophysiology of restrictive lung disease (Schelegle 2003). The model for breathing-dependent relaxation assumes a physiological process of SAR

pathways through the nucleus tractus solitarius (NTS) with connectivity to central autonomic networks. Thus, voluntary deep breathing may modulate the autonomic nervous system via SAR activity in the lungs, resulting in eliciting resonant and coherent features in neuromechanical interactions that optimize physiological and brain states (Noble and Hochman 2019).

1.1.1.4. Respiratory patterns

Clinically, the doctors are suggested to observe the respiratory patterns on rate, depth, ratio of inspiration to expiration, sequence of chest wall movement during inspiration and expiration, comfort, presence of accessory muscle use, and symmetry (Jaime and Michele 2013). The normal breathing rate for adults ranges from 12 to 20 breaths per minute (Lim et al. 2023; Lovett et al. 2005). The normal respiratory time ratio varies from 1:1.5 to 1:2 during spontaneous breathing (Y.-P. Wang et al. 2013). No standard was found for the ratio of abdominal motion to thoracic motion, or the contribution of abdominal movement to the total respiratory movement. The pattern of respiration includes a range of parameters, including rate, inhalation and exhalation duration, tidal volume, and depth (Benchetrit 2000). In the present study, the respiration patterns we focus on are respiration rate (RR), inhalation exhalation duration ratio (I/E ratio), and abdominal contribution to total respiratory movements ($AM/(AM+TM)$, where "AM" is the abbreviation for abdominal motion and "TM" is the abbreviation for thoracic motion).

1.1.2. Physical fitness and executive function

Physical fitness encompasses both health-related and skill-related fitness. Health-related fitness includes muscular strength, endurance, flexibility, cardiorespiratory endurance, and body composition; skill-related fitness includes speed, power, agility, balance, coordination, and reaction time (Fahey, Insel, and Roth 2018). Thorough physical fitness tests provide a comprehensive assessment of one's overall physical well-being.

Executive function encompasses cognitive processes that work for purposeful and goal-directed behavior (Banich 2009). Inhibition, working memory, and cognitive flexibility serve as the three foundational executive functions. These fundamental skills pave the way for more complex executive functions like decision-making, problem-solving, and planning (Diamond 2014). In addition, a study found a negative correlation between reaction time and executive function, that both between-person ($b = -21.2, p < 0.001$) and within-person ($b = -13.2, p < 0.001$) sources of simple reaction time were

uniquely related to executive function performance (Willoughby et al. 2020). In the first part of our study, we conducted a test of visuomotor reaction time to reveal executive function. In the second part, executive function was assessed using the Stroop color word test.

1.1.3. The current state of research on the correlation between respiratory patterns during spontaneous breathing and physical fitness

There are plentiful researches on the effects of voluntarily controlled respiration patterns on physical fitness and executive function, and they found that breathing exercises with well-designed and altered respiratory patterns are beneficial for physical and mental health (Nicolò et al. 2020b; Zaccaro et al. 2018c; Luciano Bernardi et al. 1998; Ublosakka-Jones et al. 2018; Manandhar and Pramanik 2019; Sakamoto, Naito, and Chow 2014; Jacob et al. 2015; Bae et al. 2021a; Van Diest et al. 2014; Anderson and Bliven 2017; Yufan Lu et al. 2020; Barbosa et al. 2017; Martarelli et al. 2011; Nelson 2012), which will be presented and discussed in the second part.

However, despite employing various search strategies on Google Scholar, PubMed and Web of Science, we found only one study that demonstrated a positive correlation between diaphragm motion during spontaneous breathing and balance (only investigated on balance) (Kocjan et al. 2018a). The researching results show that scientific research on the links of respiratory patterns during spontaneous breathing with physical fitness is scarce.

1.1.4. The current state of research on respiratory patterns at different ages

We found six relevant pieces of research regarding spontaneous respiratory movements and age. One study was found that observed an increase in the abdominal contribution to spontaneous respiratory movement with aging, while the thoracic contribution decreased (Mendes et al. 2020). Whereas other five studies reported no systematic age effects on respiratory movements during quiet breathing (Sharp et al. 1975; Verschakelen and Demedts 1995; Ragnarsdóttir and Kristinsdóttir 2006; Kaneko and Horie 2012; Britto et al. 2009). However, the sample sizes were relatively small in all previous studies, and the biggest sample comprised 120 healthy subjects, combining two genders (Verschakelen and Demedts 1995). Sample size substantially affects statistical results (Faber and Fonseca 2014), and men and women are different physiologically. To confirm the findings and adhere to the principles of

precision medicine, it is necessary to increase the sample size and analyze data for men and women separately.

With respect to the I/E ratio during spontaneous breathing at different ages, no relevant study was found from Google Scholar, PubMed, and Web of Science.

Regarding RR, there wasn't a significant difference between the three age groups (15 ± 3 reps/min for 20-39 and 40-59 age groups, 16 ± 3 reps/min for 60-80 age group) (Verônica F. Parreira et al. 2010). Most reference values for respiratory rate in adults range from 10 to 20 breaths per minute (Chattopadhyay and Chowdhury 2016; Annesley 2020; McGrath, Pyke, and Taenzer 2017). This information shows that RR does not change significantly with aging for adults.

Therefore, regarding the change of respiratory patterns with aging, the main focus was on respiratory movements and the I/E ratio.

1.1.5. The current state of research on respiratory patterns at different genders

Here are some key findings from the comparison of men and women's respiratory patterns. A review of studies on respiratory function found that women having a higher respiratory rate and lower tidal volume than men (LoMauro and Aliverti 2018a). Some other Studies found women's abdominal movements during spontaneous breathing were less than men's, or the contribution of thoracic movements were larger in women than in men (Kaneko and Horie 2012; Mendes et al. 2020). With respect to the I/E ratio, it was 1:1.21 for men and 1:1.14 for women, and there is no significant difference in terms of statistics (Ragnarsdóttir and Kristinsdóttir 2006). The men's vital capacities were greater than women's (LoMauro and Aliverti 2018b).

In short, relative to men, women have higher respiration rates, lower tidal volume, larger thoracic movement, less vital capacity, and similar inhalation/exhalation ratios during spontaneous breathing.

The differences in respiration patterns might be due to anatomical structure. Women possess smaller airways and lungs compared to men (LoMauro and Aliverti 2018b). Women have a greater inclination of the ribs and lower radial dimension of the rib cage than men, which is considered an adaptation and evolution of the reproductive system to accommodate a growing fetus during pregnancy (Bellemare, Jeanneret, and Couture 2003). Meanwhile, women have smaller rib cages compared to men (Bellemare, Jeanneret, and Couture 2003).

1.1.6. Rationale

Breathing patterns have a profound impact not only on the effectiveness of ventilation but also on motor, cardiovascular, autonomic nervous activities, and executive function. Intentionally changing respiration patterns has been found to significantly impact physical well-being and executive function. However, the links of spontaneous respiration patterns with physical well-being unclear.

Objectives:

1. To investigate the links of temporal parameters of respiration with physical fitness. The primary outcomes are respiration rate (RR) and inhalation/exhalation ratio (IE ratio), with inhalation duration (ID) and exhalation duration (ED) as secondary outcomes.

2. To investigate the links of respiratory movements with physical fitness. The primary outcome is abdominal contribution to the total respiratory movement (AM/(AM+TM)), with abdominal motion (AM) and thoracic motion (TM) as secondary outcomes.

3. To examine the respiratory patterns at different ages. The primary outcomes are RR, IE ratio, AM/(AM+TM), with vital capacity as a secondary outcome.

1.2. Materials and Methods

In this part, the sampling method and sample size will be presented first. Then, we explain detailed information on how we collected and processed the data. In the end, we will show the statistical analysis.

1.2.1. Study design and participants

This is a cross-sectional correlational study. A total of 610 healthy adults (aged 20–59) were enrolled through convenience sampling from six communities in Haidian District in Beijing. The inclusion criteria for participants were as follows: 20–59 years old, capable of understanding and responding to the interview questions, having completed the Physical Activity Readiness Questionnaire and meeting all requirements, and providing written informed consent. Exclusion criteria: pregnancy or lactation; the presence of a mental illness; recent or ongoing acute diseases without physical recovery; consumption of coffee or tea within 2 hours prior to the tests; having a RR exceeding ± 2 times the standard deviations from the average in their age group (subjects excluded if $RR < \text{mean} - 2 \times SD$ or $RR > \text{mean} + 2 \times SD$);

did not perform ten consecutively stable respiratory cycles from a two-minute respiration test. The final study sample consisted of 564 participants, 163 men (age = 41 ± 11 , BMI = 25.8 ± 4.1) and 401 women (age: 42 ± 9 , BMI: 23.3 ± 3.5).

The study was approved by the research ethics committee of Beijing Sport University (Approval number: 2021079H), and all subjects were informed of the risks of the tests prior to signing the informed consent document.

1.2.2. Data collection and processing

We collected respiration data using two respiration belts, calculated the respiratory movements and respiratory duration, and tested physical fitness using an electronic physical fitness assessment system.

1.2.2.1. Respiratory Movements Testing and Data Processing

Respiration testing

Respiration was recorded by two respiration belts (Vernier, Beaverton, OR, USA). Each belt is a strap of fabric with a resistive stretch sensor embedded into it and provides ground truth respiration rate signals (Ross et al. 2021). Prior to the test, participants were instructed to remain seated quietly for 5 minutes. Then, the participants stood up, and the experimenter tied the belt at the level of the xiphoid process and navel of the participants until the light on the belt turned green according to the user's instructions (Figure 6). Throughout the two minutes test, the participants were asked to watch a neutral video featuring slow-swimming fishes in the sea. The video was displayed on a Xiaomi Pad (11 inches, Xiaomi, Beijing, China), positioned in front of the participant's face at a distance ranging from 50 to 80 cm.

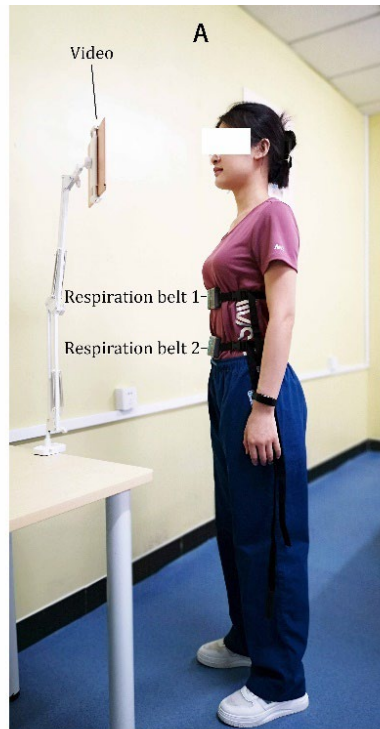


Figure 6. Respiration testing. Respiration belt 1 was fastened at the height of the xiphoid; respiration belt 2 was fastened at the height of the navel; a neutral video was playing. Photographer: Wenming Liang.

Determination of respiration period used for analysis

Different authors employed various approaches to determine the number of respiratory cycles for analysis, ranging from three satisfactory readings to six minutes of breathing cycles (Kocjan et al. 2018b; Szczygieł et al. 2018; Teixeira-Salmela et al. 2005). We observed that the respiration waves became more regular after 30 seconds from the beginning. Consequently, we chose ten consecutive respiration cycles that demonstrated consistent stability, minimal motion artifact, and baseline wander after the initial 30 seconds of the testing period.

Respiratory duration processing

A Matlab App Designer program (Matlab 2022a, Mathworks, Natick, MA, USA) was used to process the raw data and target the maximum peaks (indicating the end of inspiration) and minimum troughs (indicating the end of expiration). Safeguards were implemented to decrease the chance of flagging false minimum and maximum values in data with higher noises. As shown in Figure 7, the inhalation duration (ID) was calculated by subtracting

the time at the peak from the preceding trough and then averaging across all ten summed IDs. Exhalation duration (ED) was calculated by subtracting the time of trough from the peak time that preceded it and averaging across all ten summed EDs. The IE ratio was calculated by dividing the ID by the ED. The RR was determined as 60 seconds divided by the time used for one respiration cycle, which was calculated from the time of the 11th peak minus the time of the 1st peak, divided by 10 ($RR = 60 / (P_{11} - P_1) / 10$).

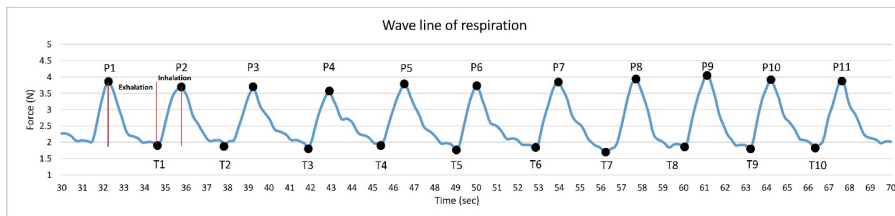


Figure 7. An example of recording of respiration from the belt at xiphoid and schematic representation of stage identification. P, peak; T, trough. Sec, second; N, Newton. The figure is produced by author.

Respiratory movements processing

Data were imported from the Vernier Graphical Analysis (Vernier, Beaverton, OR, USA) to OriginPro 9.0 (OriginLab, Northampton, MA, USA) for extracting peaks and troughs of breathing waves. Afterwards, the peaks and troughs were imported to Excel (Excel, Microsoft, Redmond, USA) for calculating abdominal motion (AM) and thoracic motion (TM). AM and TM were calculated separately, and the values of AM and TM were determined as the ten averaged peak (P) forces minus ten average trough (T) forces (motion = $(P_1 + P_2 \dots + P_{10}) / 10 - (T_1 + T_2 \dots + T_{10}) / 10$) (Figure 8). The signals were presented as force (unit = Newton) with a sampling frequency of 10 Hz.

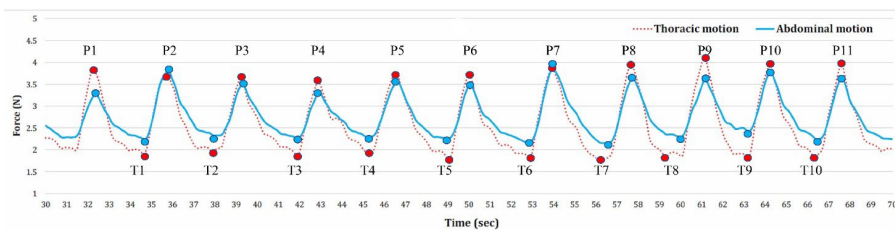


Figure 8. Two wave lines of respiration from the belts at the level of the xiphoid process and navel. The force was generated by the stretch of respiratory movement; solid line represents the abdominal motion; dotted line represents the thoracic motion. P = peak; T = trough. The figure is produced by author.

1.2.2.2. Physical Fitness Testing

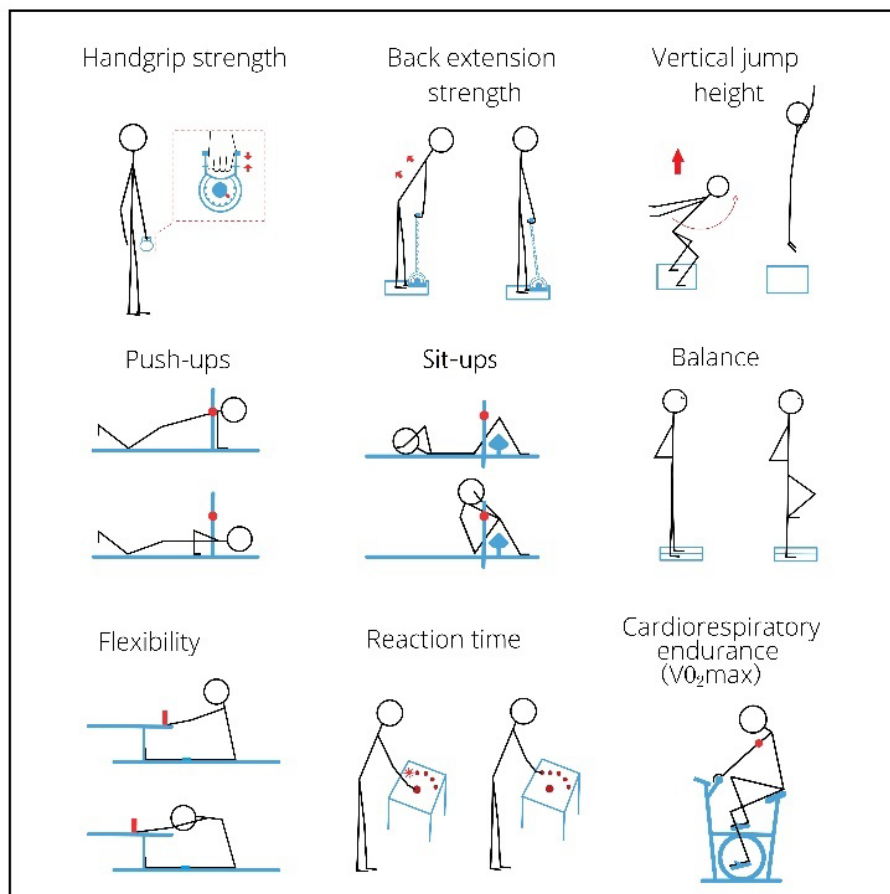


Figure 9. An illustration of the tests of physical fitness. The figure is used with permission from Dovilė Gelažiūtė.

Physical fitness tests (Figure 9) were conducted on an electronic physical fitness assessment system (Jianmin, Xindonghuateng Sports Equipment Co., Ltd., Beijing, China). Every participant had a card that each testing equipment could sense, and all testing results were stored directly in this system. The system was approved by the Sports Equipment Approval Committee at the General Administration of Sport, and the protocol of measurements was made and implemented according to the book *National Physical Fitness Testing and Evaluation* (Zhang, He, and Xu 2017).

Body height measurement: Participants had their body height measured with shoes off using an electronic body height measuring instrument (Jianmin GMCS-SGJ3, Xindonghuateng, Beijing, China). As most of the equipment for

the physical fitness tests were made by this company, we will state the brand “Jianmin” and the model, hereafter. Accuracy was set at 0.1 cm.

Body weight measurement: Participants had their body weight measured wearing light clothes with shoes off on an electronic weighing scale (Jianmin GMCS-RCS3). Accuracy was set at 0.1 kg.

Body fat test: The body fat test was performed on a body composition analyzer (Jian min GMCS-TZL3) with bare feet standing on two electrode plates and hands holding two electrode handles for one minute. Accuracy was set at 0.1 kg.

Waist and hip circumference measurement: Waist and hip circumference was measured by an electronic circumference measuring ruler (Jianmin GMCS-WD3) at the height of the navel and the widest part of the buttocks. The accuracy was set at 0.1 cm.

Muscular strength tests: Prior to the strength test, subjects were informed about all testing procedures and were recommended to engage in a 5 min warm-up. Then, participants stood and held a handgrip dynamometer (Jianmin GMCS-WCS3) in the dominant hand, 10–20 cm away from the thigh with the palm facing toward the thigh. Two minutes after the handgrip strength test, subjects participated in a back extension test with another dynamometer (Jianmin GMCS-BLJ3) that consisted of a plate for standing on, a bar for hand holding, and a chain connecting the plate and bar. The subject stood on the plate with hands dropped down and fingers straightened in front of two thighs. Then, the researcher adjusted the length of the chain and set the bar at the height of the tips of the middle fingers of subjects. Subjects flexed their hips and held the bar (keeping the arms, legs and trunk straight), and then slowly lifted up the bar with as much power as they could. The muscular strength tests were performed twice, and the best result was recorded with an accuracy of 0.1 kg.

Muscular power test: The countermovement jump with arm swing was adopted to test vertical jump height. Participants stood on a timing mat (Jianmin GMCS-ZTJ3) that contained an internal calculator for recording the individual’s time in the air and computing how high their jump was. Participants jumped two times, and the highest value was recorded with an accuracy of 0.1 cm.

Muscular endurance tests: Participants faced the floor with their weight distributed to the hands (straight) and knees (bent for 90°) on push-up counting equipment (Jianmin GMCS-FWC3), and one assistant adjusted two laser detectors to the height of participants’ shoulders. Then, participants performed the common push-up method for one minute. The system recorded when participants pushed their trunks up and their shoulders reached that height.

The number of sit-ups performed in one minute was recorded in a similar way with sit-ups counting equipment (Jianmin GMCS-YWQZ3).

Balance test: Participants stood with arms akimbo in two spots on balance testing equipment (Jianmin GMCS-DJZL3). Once they were ready, they closed their eyes and lifted one foot. The system started recording the time when the participant lifted their foot and stopped the recording when another foot moved away. The accuracy of standing time was set at 0.1 s.

Flexibility test: Participants took off their shoes and sat on the equipment (Jianmin GMCS-TQQ3) with legs stretched out against one box, and the assistant fastened their knees to the equipment to keep their knees straight. With their palms facing downwards, participants reached forward along the measuring bar as far as possible. The test was performed twice, and the longer distance was recorded with an accuracy of 0.1 cm.

Reaction time test: The panel for testing reaction time (Jianmin GMCS-FYS3) has one starting button and five signal buttons. Participants stood in front of the panel and kept their dominant hand on the starting button. One of the signal buttons lit up randomly during each trial, and participants pushed the lit-up signal button as fast as possible. Five trials were performed for each round, and the average time of the five trials was used as the result. Participants participated in two rounds and the shorter time was recorded with an accuracy of 1 millisecond (ms).

Cardiorespiratory endurance test: The maximal oxygen consumption ($VO_2\text{max}$) was estimated using a YMCA submaximal cycle ergometer (Beekley et al. 2004). After a five-minute rest, participants sat on the cycle ergometer (Jianmin GMCS-GLC3) with an optical heart rate sensor (Polar OH1, Polar Electro Oy, Kempele, Finland) worn around the upper arm. The sensor was connected to the physical fitness assessment system via Bluetooth, so the system could calculate the heart rate and add workloads. The test lasted seven minutes with 30 s for the baseline heart rate, three minutes for the first stage heart rate, another three minutes for the second stage heart rate, and the final 30 s for cooling down. A detailed calculation method can be found in the study conducted by Nuria Garatachea et al. (Garatachea et al. 2007).

Vital capacity test: Participants stood upright and held the handle of the spirometer (Jianmin GMCS-FHL). They took a full inspiration and then exhaled slowly and maximally. The test was performed twice, and the higher value was recorded with an accuracy of 1 mL.

1.2.3. Statistical analysis

Links between respiratory patterns and physical fitness

Body size is generally believed to be a confounding factor in the outcomes of physical performance tests (Bazett-Jones et al. 2011; Jaric 2003). Absolute values from physical tests might introduce bias to existing muscle strength for clinical reference. For instance, handgrip strength/body weight was a better clinical predictor of functional impairments than absolute value (Barbat-Artigas et al. 2013); muscle strength, normalized for body weight, height, and fat mass is superior to absolute muscle strength as a predictor of cardiometabolic risk (de Lima, Sui, and Silva 2021). Thus, normalizing physical performance for body size is necessary. The most often employed equation was:

$$P_n = P/M^b$$

where P is the physical performance; P_n is the normalized physical performance; M is the body mass; and b is the allometric value (Jaric 2002; Markovic and Jaric 2004). Markovic and Jaric proposed three allometric values for three types of physical performance: $b = 0.67$ for the tests of exertion of external force (e.g., hand grip strength), $b = 0$ for the tests of rapid movements (i.e., jump height), and $b = -0.33$ for the tests of supporting body weight (push-ups, pull-ups, maintaining strength demanding postures in gymnastics or yoga) (Markovic and Jaric 2004).

The present study normalized handgrip strength and back extensor strength using 0.67 as the allometric parameter. Vertical jump height was found to be a body size-independent index (Kons et al. 2018; Markovic and Jaric 2007), which was not likely to require normalization for body size (Jaric 2002). Therefore, the original jump height was used. Results for push-ups, sit-ups, and single-leg stance duration (balance) were negatively correlated with body weight, so the allometric parameter (-0.33) was employed in Equation (1). Flexibility and visual reaction time were not significantly correlated with body weight. These two physical outcomes could be regarded as body size-independent; thus, the original values were used. For VO_{2max} , the components for its calculation include age and body weight, which means it has already been normalized, and no further normalization is needed. Thus, the original VO_{2max} values were used.

Muscle strength is substantially affected by aging (Lexell 1995). In the present study, we divided participants into young and middle-aged groups: young group from 20 to 39 years old and middle-aged group from 40 to 59 years old). This division was for two purposes: to eliminate the effect of age

and to detect the specific age range in which respiratory movements might be associated with physical fitness. Nevertheless, age is still significantly correlated with some parameters (jump height, sit-ups, balance, and reaction time for middle-aged men; jump height, sit-ups, and VO₂max for young women; grip, jump, sit-ups, balance, and VO₂max for middle-aged women). To further eliminate the influence of age, we generated residuals that combined age with relevant parameters using linear regression. We used the generated residuals to conduct the correlational and comparative tests during statistical analysis. This method (using residuals) is exactly the same as the semipartial correlation processes that eliminate one confounding factor's effect (S. Kim et al. 2012).

Since the distributions of many parameters were skewed according to the Kolmogorov-Smirnov test, the Mann-Whitney U Test was conducted to compare two age groups, and the data were presented as the median and interquartile range (IQR).

The Spearman correlation test was employed for correlation calculation. The analysis was conducted in two gender groups and two age groups separately. The linear regression method was used to generate residuals between age and the physical fitness components, for which it had a significant correlation with age.

Furthermore, to discover the differences in physical fitness between higher and lower RR, IE ratio, and AM/(AM+TM), we split each of these three respiratory parameters into two parts based on the median values. Finally, we classified participants into two groups based on two parameters (inhalation and exhalation duration) using the K-means clustering method and compared the physical fitness of these two groups. This process was conducted solely to verify the results.

The correlation between respiratory patterns and age

Multiple regression analysis was used to test the links between the respiratory patterns and age, with adjustments for body size. The RR, IE ratio, AM/(AM + TM), ID, ED, AM, TM, and vital capacity were set as the dependent variables tested in separate analyses, while the age, height, weight, waist circumference, and hip circumference were the independent variables included in every test. Additionally, to prevent multicollinearity with height, weight, waist circumference, and hip circumference, we included the age, BMI, and waist-hip ratio as the independent variables and tested them with all the dependent variables. The final model was determined from the adjusted coefficient (R²) and the statistical significance. To determine the statistical quality of the model, the multicollinearity was verified by the variance

inflation factor, as well as the homogeneity and normal distribution of the residuals by graphic visual analysis.

Significance level and the strength of effect size

The significance level was set at $P < 0.05$. The strength of the effect size (Rho) from the Spearman correlation test was adopted as negligible ($Rho < 0.20$), weak ($0.21 < Rho < 0.40$), moderate ($0.41 < Rho < 0.60$), strong ($0.61 < Rho < 0.80$), and very strong ($0.81 < Rho < 1.00$) (Prion and Haerling 2014). The effect sizes (r) from the Mann-Whitney U Test were derived from the z -values divided by the square root of the sample size (Fritz, Morris, and Richler 2012), and it was referred to as small ($r < 0.2$), medium ($0.2 < r < 0.5$), and large ($r > 0.5$) according to other studies (J.-S. Park et al. 2019). All data were calculated and analyzed using Excel 365 (Excel, Microsoft, Redmond, WA) and SPSS 20.0 (IBM Corp., Armonk, NY, USA).

1.3. Results

To comprehensively demonstrate the relationship between respiratory patterns and physical fitness, we initially presented the data and compared two age groups. Subsequently, we showcased the associations between temporal parameters of respiration and physical fitness, respiratory movements and physical fitness, and the correlation between respiratory patterns and age.

1.3.1. Data description and comparison of all parameters between age groups

The comparison of all study parameters between age groups for men is presented in Table 1 and for women in Table 2. As shown in Table 1, men's RR, ID, ED, and IE ratio were not significantly different between the young and middle aged groups. However, body height, weight, hip circumference, AM, AM+TM, AM/(AM+TM), back extension strength, vertical jump height, number of sit-ups in one minute, and balance were higher in younger men, while reaction time was longer in older men.

Table 1. Data presentation and comparison between age groups in men

All parameters	Age1	n1	Median (IQR)1	Age2	n2	Median (IQR)2	z	P	r
Age (year)	20 - 39	70	32.0 (10.0)	40 - 59	93	49.0 (10.5)	10.9	0.000	0.86
Height(cm)		70	176 (8.45)		93	172 (8.55)	2.95	0.003	0.23
Weight (kg)		70	80.4 (18.2)		93	73.9 (14.4)	2.31	0.021	0.18
BMI (kg/m ²)		70	25.7 (5.29)		93	25.2 (4.47)	1.30	0.193	0.10
WC (cm)		67	89.8 (13.0)		90	92.0 (10.7)	1.36	0.173	0.11
HC (cm)		67	102 (9.80)		90	97.8 (9.38)	2.18	0.029	0.17
WH ratio		67	0.89 (0.09)		90	0.93 (0.07)	3.79	0.000	0.30
Body fat (%)		67	24.9 (7.32)		91	24.2 (6.90)	1.06	0.290	0.08
TM (N)		70	1.99 (1.86)		93	2.24 (1.84)	-1.00	0.318	0.08
AM (N)		70	2.29 (1.88)		93	3.42 (2.31)	-3.82	0.000	0.30
AM+TM (N)		70	4.36 (2.65)		93	5.88 (3.51)	-3.19	0.001	0.25
AM/(AM+TM)		70	0.29 (0.25)		93	0.40 (0.22)	-3.88	0.000	0.30
RR (rep/min)		70	15.2 (4.52)		93	17.1 (5.52)	1.22	0.224	0.10
ID (s)		70	1.53 (0.53)		93	1.41 (0.70)	1.27	0.205	0.10
ED (s)		70	2.19 (0.71)		93	2.03 (0.72)	1.05	0.292	0.08
IE ratio		70	0.70 (0.23)		93	0.73 (0.18)	0.14	0.891	0.01
Grip strength (kg)		69	43.5 (11.0)		93	41.5 (9.80)	1.65	0.099	0.13
Back strength (kg)		69	120 (35.2)		93	111 (41.0)	2.32	0.021	0.18
Jump height (cm)		70	37.0 (12.5)		93	27.5 (12.5)	5.57	0.000	0.44
Push-ups (rep/min)		65	20.0 (19.5)		79	18.0 (15.0)	1.38	0.168	0.11
Sit-ups (rep/min)		67	28.0 (11.0)		75	20.0 (11.0)	3.65	0.000	0.31
Balance (s)		70	14.4 (20.9)		90	11.3 (13.5)	2.04	0.042	0.16
Flexibility (cm)		69	2.90 (15.3)		89	3.30 (14.1)	0.59	0.556	0.05
Reaction time (s)		68	0.52 (0.09)		85	0.58 (0.12)	3.84	0.000	0.31
VO ₂ max (mL/(kg×min))		44	39.9 (10.8)		52	40.4 (12.6)	-0.31	0.760	0.03

Note: Age¹, n¹, and Median (IQR)¹ are for the 20-39 age group, while age², n², and Median (IQR)² are for the 40-59 age group. AM, abdominal motion; BMI, body mass index; cm, centimeter; ED, exhalation duration; HC, Hip circumference; ID, inhalation duration; IE ratio, inhalation exhalation ratio; kg, kilogram; ml, milliliter; N, Newton; TM, thoracic motion; WC, waist circumference; WH ratio, waist/hip circumference ratio; VO₂ max, the maximum rate of oxygen consumption.

As for female participants (Table 2), RR, ID, ED, and IE ratio were also not significantly different between the young and middle-aged groups, as well as respiratory movements. But, waist circumference, waist-hip circumference, body fat percentage, back extension strength, and reaction time were higher in older women. At the same time, vertical jump height, number of sit-ups, balance, and VO₂max were higher in younger women.

Table 2. Data presentation and comparison between age groups in women

All parameters	Age ¹	n ¹	Median (IQR) ¹	Age ²	n ²	Median (IQR) ²	z	p	r
Age (year)	20 - 39	154	34 (8)	40 - 59	247	48 (10)	16.9	0.000	0.84
Height (cm)		154	161 (7.12)		247	161 (6.70)	0.37	0.710	0.02
Weight (kg)		154	58.3 (13.4)		247	61.1 (11.8)	1.85	0.065	0.09
BMI (kg/m ²)		154	22.2 (5.15)		247	23.0 (4.11)	1.96	0.050	0.10
WC (cm)		151	73.3 (13.6)		245	77.5 (11.9)	3.15	0.002	0.16
HC (cm)		151	93.7 (9.50)		245	94.6 (9.05)	0.61	0.540	0.03
WH ratio		151	0.79 (0.08)		245	0.82 (0.08)	3.75	0.000	0.19
Body fat (%)		151	27.6 (9.07)		244	29.7 (6.79)	2.21	0.027	0.11
TM (N)		154	2.28 (0.92)		247	2.37 (1.58)	-0.85	0.394	0.04
AM (N)		154	0.90 (0.68)		247	0.93 (0.89)	-0.99	0.321	0.05
AM+TM (N)		154	3.15 (1.46)		247	3.35 (2.29)	-0.69	0.490	0.03
AM/(AM+TM)		154	0.26 (0.21)		247	0.30 (0.18)	-0.88	0.379	0.04
RR (rep/min)		154	17.2 (4.39)		247	17.1 (4.83)	0.68	0.494	0.03
ID (s)		152	1.40 (0.44)		247	1.38 (0.44)	0.30	0.760	0.02
ED (s)		152	2.08 (0.61)		247	2.15 (0.68)	1.32	0.188	0.07
IE ratio		152	0.69 (0.18)		247	0.66 (0.17)	1.65	0.099	0.08
Grip strength (kg)		150	24.6 (6.60)		244	25.8 (7.25)	1.65	0.100	0.08
Back strength (kg)		153	62.3 (23.7)		241	66.5 (22.9)	2.15	0.031	0.11
Jump height (cm)		153	22.6 (6.80)		243	19.6 (6.50)	5.63	0.000	0.28
Push-ups (rep/min)		141	17.0 (14.5)		229	15.0 (14.0)	1.32	0.186	0.07
Sit-ups (rep/min)	141	23.0 (13.0)	218	17.0 (12.3)	5.12	0.000	0.27		
Balance (s)	153	22.0 (25.5)	246	15.2 (21.9)	3.49	0.000	0.17		

All parameters	Age ¹	n ¹	Median (IQR) ¹	Age ²	n ²	Median (IQR) ²	z	p	r
Flexibility (cm)	20 - 39	150	8.65 (13.9)	40 - 59	245	10.9 (12.8)	1.88	0.061	0.09
Reaction time (s)		148	0.57 (0.08)		245	0.60 (0.11)	3.72	0.000	0.19
VO₂max (mL/(kg×min))		116	41.5 (11.2)		192	34.3 (8.03)	6.51	0.000	0.37

Note: Age¹, n¹, and Median (IQR)¹ are for the 20-39 age group, while age², n², and Median (IQR)² are for the 40-59 age group. AM, abdominal motion; BMI, body mass index; cm, centimeter; ED, exhalation duration; HC, Hip circumference; ID, inhalation duration; IE ratio, inhalation exhalation ratio; kg, kilogram; ml, milliliter; N, Newton; TM, thoracic motion; WC, waist circumference; WH ratio, waist/hip circumference ratio; VO₂ max, the maximum rate of oxygen consumption.

Overall, significant differences in respiratory movements and various components of physical fitness were observed between the two age groups. Additionally, it's well-established that there are physical differences between men and women. These two factors led us to analyze the data by stratifying it into separate groups based on both age and gender.

1.3.2. Links between temporal parameters of respiration and physical fitness

1.3.2.1. Simple correlation between temporal parameters of respiration and physical fitness

For young men, there was no significant correlation between age and physical fitness components. Whereas, for middle-aged men, age was significantly correlated with vertical jump height ($n = 93$, $Rho = -0.451$, $P < 0.001$), sit-ups ($n = 75$, $Rho = -0.272$, $P = 0.018$), balance ($n=90$, $Rho = -0.405$, $P < 0.001$), and reaction time ($n = 85$, $Rho = 0.242$, $P = 0.025$). Therefore, the generated residues of these four performances were used for the correlational test. All respiration parameters were not correlated with body size.

As shown in Table 3, men's RR had a weak and positive correlation with visuomotor reaction time from the two age groups, which indicated that male participants with lower RR might have a faster reaction time. Besides, the middle-aged men's RR had negative correlation with vertical jump height, which means middle-aged men with lower RR might have higher jump height. IE ratio had a weak and positive correlation with back extension strength and the number of sit-ups in middle-aged men, which indicated that middle-aged men with longer inhalation than exhalation might have better strength in the back, abdominal, and hip flexor muscles. For ID, it had a positive and weak correlation with the number of sit-ups in young men, and back extension strength & Reaction time in middle-aged men. ED had a negative and weak correlation with visual reaction time in young men, and had a positive correlation with vertical jump height in middle-aged men.

Table 3. Correlation between temporal parameters of respiration and physical fitness in men

Parameters (men)	n ¹	RR ¹ Rho	ID ¹ Rho	ED ¹ Rho	IE ratio ¹ Rho	n ²	RR ² Rho	ID ² Rho	ED ² Rho	IE ratio ² Rho
Grip (kg)	69	0.021	0.064	0.015	0.033	93	-0.169	0.149	0.142	0.106
Back (kg)	69	-0.151	0.171	0.203	-0.038	93	-0.192	.229*	0.112	.221*
Jump (cm)	70	-0.157	0.210	0.116	0.094	93	-.239*	.189	.227*	0.033
Push-ups (rep/min)	Age group ₁ 65	0.068	0.060	-0.090	0.132	Age group ₂ 79	-0.175	0.177	0.109	0.142
Sit-ups (rep/min)	67	-0.240	.242*	0.157	0.113	75	-0.120	0.200	0.030	.245*
Balance (s)	70	-0.123	0.147	0.071	0.003	90	-0.090	0.054	0.124	-0.082
Flexibility (cm)	69	-0.092	0.068	0.104	0.051	89	-0.128	0.175	0.028	0.194
Reaction time (s)	68	.278*	-0.189	-.291*	0.109	85	.246*	-.240*	-0.170	-0.170
VO ₂ max (mL/(kg×min))	44	0.025	0.039	0.021	0.072	55	-0.069	0.054	0.072	-0.063

Note: Age¹, n¹, and Median (IQR)¹ are for the 20-39 age group, while age², n², and Median (IQR)² are for the 40-59 age group. ED, exhalation duration; ID, inhalation duration; IE ratio, inhalation exhalation ratio; kg, kilogram; ml, milliliter; N, Newton; RR, respiration rate; VO₂ max, the maximum rate of oxygen consumption. * $P < 0.05$.

With respect to women, as shown in Table 4, there was no correlation between the respiration patterns and any of physical fitness components (only RR and sit-ups were significantly correlated, but the coefficient was negligible).

Table 4. Correlation between temporal parameters of respiration and physical fitness in women

Parameters (women)	n ¹	RR ¹ Rho	ID ¹ Rho	ED ¹ Rho	IE ratio ¹ Rho	n ²	RR ² Rho	ID ² Rho	ED ² Rho	IE ratio ² Rho		
Grip (kg)	148	-0.065	0.076	0.019	0.070	244	0.005	0.039	-0.065	0.109		
Back (kg)	151	-0.065	0.099	0.001	0.106	241	-0.017	0.069	-0.015	0.093		
Jump (cm)	151	0.025	0.084	-0.056	0.124	241	-0.109	0.120	0.081	0.078		
Push-ups (rep/min)	Age Group ¹	139	0.084	-0.102	-0.097	-0.062	Age Group ²	229	-0.063	0.031	0.044	-0.003
Sit-ups (rep/min)	139	0.102	-0.067	-0.115	0.004	218	-0.133*	0.119	0.122	0.030		
Balance (s)	151	-0.061	0.092	0.037	0.062	246	-0.082	0.117	0.041	0.099		
Flexibility (cm)	148	-0.016	-0.009	-0.012	0.006	245	-0.031	0.052	-0.023	0.114		
Reaction time (s)	146	0.102	-0.078	-0.064	-0.055	245	0.062	-0.063	-0.088	0.029		
VO ₂ max (mL/(kg×min))	114	-0.046	0.030	0.039	0.030	192	-0.112	0.103	0.088	0.022		

Note: Age¹, n¹, and Median (IQR)¹ are for the 20-39 age group, while age², n², and Median (IQR)² are for the 40-59 age group. ED, exhalation duration; ID, inhalation duration; IE ratio, inhalation exhalation ratio; kg, kilogram; ml, milliliter; N, Newton; RR, respiration rate; VO₂ max, the maximum rate of oxygen consumption. * $P < 0.05$.

1.3.2.2. The comparison of physical fitness components between groups with lower and higher respiratory parameters.

For further exploration of the link between temporal parameters of respiration and physical fitness, each parameter was divided into two groups using the median split method. This division was performed to compare physical fitness between the lower and higher respiratory parameter groups. The comparison results indicated that there were no significant differences between the two groups in terms of age, height, weight, BMI, waist circumference, hip circumference, and waist-hip circumference ratio. However, as shown in Table 5 (only significant results are presented), men with lower RR had a significantly faster reaction in younger and older age groups. Additionally, middle-aged men with lower RR jumped higher. There was no significant difference for men with higher or with lower IE ratio. Men with longer ED had shorter reaction time in the younger age group and jumped higher in the older age group. Men with longer ID jumped higher in both age groups.

Table 5. The comparison of physical fitness components between groups with lower and higher respiratory parameters in men.

Age group	Respiratory Parameter	Physical fitness	Lower	n ¹	Higher	n ²	z	p	r
20 – 39	RR	Reaction time	0.50 (0.07)	36	0.55 (0.10)	32	3.23	0.001	0.39
	ID	Vertical Jump	32.8 (13.2)	35	38.9 (14.3)	35	1.63	0.102	0.20
	ED	Reaction time	0.54 (0.09)	34	0.50 (0.07)	34	2.69	0.007	0.33
40 – 59	RR	Reaction time	0.56 (0.12)	43	0.61 (0.11)	42	2.52	0.012	0.27
		Vertical Jump	30.1 (12.7)	47	24.6 (10.1)	46	3.10	0.002	0.32
	ID	Vertical Jump	25.2 (11.8)	47	29.0 (13.5)	46	2.19	0.028	0.23
		Flexibility	1.95 (12.9)	44	6.80 (17.0)	45	2.00	0.045	0.21
	ED	Vertical Jump	25.3 (11.5)	46	29.1 (12.6)	47	2.38	0.017	0.25

Note: n¹ represents the number of participants with lower values of the respiratory parameters, while n² for the higher value of the respiratory parameters. ED, exhalation duration; ID, inhalation duration; RR, respiration rate; r, effect size.

With respect to women’s respiratory parameters, we used the same way to split the RR, ID, ED, and IE ratio. Different from men's results, none of physical fitness components were significantly different between the lower and higher sets ($p > 0.05$). Thus, we did not present these results.

1.3.2.3 The comparison of physical fitness components in groups based on inhalation and exhalation duration

As we found significant correlation between RR and physical fitness, to further verify the results, we used K-means clustering to identify two groups based on ID and ED. The first cluster had relatively longer ID and ED, while the second had shorter ID and ED.

Age and body size were not significantly different between the two clusters. Table 6 shows the comparison results between these two clusters. The RR was significantly lower for both young and middle-aged men in the longer ID and ED cluster. Additionally, in the longer ID and ED cluster, the reaction time was significantly shorter in both age groups, and the vertical jump was significantly higher only in the middle aged aged group.

Table 6. The comparison of the parameters in groups based on inhalation and exhalation duration within two age groups of men

Age group	ALL parameters (men)	Longer ID and ED		Shorter ID and ED		z	p	r
		n	Median (IQR)	n	Median (IQR)			
20 – 39 men	RR	36	13.8 (2.75)	34	18.3 (2.27)	-6.99	0.000	0.84
	ID	36	1.81 (0.61)	34	1.29 (0.37)	-6.13	0.000	0.73
	ED	36	2.56 (0.57)	34	1.86 (0.34)	-6.85	0.000	0.82
	Grip (kg)	36	43.9 (10.8)	33	43.5 (13.1)	-1.17	0.244	0.14
	Back (kg)	36	127 (34.8)	33	115 (42.5)	-1.93	0.053	0.23
	Jump (cm)	36	39.1 (16.6)	34	34.4 (10.3)	-1.96	0.050	0.23
	Push-ups (rep/min)	36	20.0 (19.5)	29	20.0 (20.0)	-0.42	0.677	0.05
	Sit-ups (rep/min)	36	27.5 (11.5)	31	28.0 (14.0)	-1.14	0.254	0.14
	Balance (s)	36	17.8 (24.4)	34	13.6 (17.2)	-0.56	0.573	0.07
	Flexibility (cm)	36	4.90 (16.0)	33	0.50 (12.7)	-1.44	0.151	0.17
	Reaction time (s)	36	0.50 (0.07)	32	0.55 (0.08)	-2.76	0.006	0.33
VO ₂ max (mL/(kg×min))	26	38.6 (12.0)	18	40.9 (9.48)	-0.47	0.642	0.07	
40 – 59 men	RR	34	12.6 (3.19)	59	18.5 (3.29)	-7.94	0.000	0.82
	ID	34	2.03 (0.59)	59	1.27 (0.25)	-7.13	0.000	0.74
	ED	34	2.71 (0.58)	59	1.89 (0.32)	-7.45	0.000	0.77
	Grip (kg)	34	43.1 (8.78)	59	40.5 (9.30)	-1.43	0.152	0.15
	Back (kg)	34	118 (36.9)	59	104 (42.5)	-1.44	0.151	0.15
	Jump (cm)	34	30.7 (11.0)	59	25.2 (11.3)	-3.20	0.001	0.33
	Push-ups (rep/min)	33	20.0 (16.0)	46	16.5 (16.0)	-0.57	0.571	0.06
	Sit-ups (rep/min)	31	21.0 (18.0)	44	20.0 (11.8)	-1.34	0.180	0.15

Age group	ALL parameters (men)	Longer ID and ED		Shorter ID and ED		z	p	r
		n	Median (IQR)	n	Median (IQR)			
40 – 59 men	Balance (s)	33	12.7 (14.1)	57	8.50 (11.7)	-1.03	0.305	0.11
	Flexibility (cm)	34	6.10 (17.4)	55	2.70 (14.5)	-1.16	0.247	0.12
	Reaction time (s)	31	0.55 (0.12)	54	0.60 (0.13)	-2.52	0.012	0.27
	VO ₂ max (mL/(kg×min))	21	39.7 (11.7)	31	40.6 (13.8)	-0.45	0.654	0.06

Note: cm, centimeter; ED, exhalation duration; ID, inhalation duration; IE ratio, inhalation exhalation ratio; kg, kilogram; N, Newton; ml, milliliter; RR, respiration rate; s, second; VO₂ max, the maximum rate of oxygen consumption.

The cluster analysis also identified two sub-groups in female participants based on ID and ED. Age and body size were not significantly different between the two clusters. The first cluster had relatively longer ID and ED, and the second had shorter ID and ED. As shown in Table 7, women of both age groups had significantly lower RR in the longer ID and ED cluster. Only in middle-aged group, women with the longer ID and ED clusters had a higher number of sit-ups in one minute and better cardiopulmonary endurance (VO₂max), and the effect sizes were small ($r < 0.2$).

Table 7. The comparison of the parameters in groups based on inhalation and exhalation duration within two age groups of women

Age group	Parameters (women)	Longer ID and ED		Shorter ID and ED		Z	P	r
		n1	Median (IQR)	n2	Median (IQR)			
20–39 Women	RR	52	14.2 (3.05)	100	18.5 (2.93)	-9.91	0.000	0.80
	ID	52	1.72 (0.33)	100	1.23 (0.29)	-9.28	0.000	0.75
	ED	52	2.51 (0.59)	100	1.91 (0.35)	-8.88	0.000	0.72
	Grip (kg)	51	25.0 (6.30)	97	24.5 (6.75)	-0.50	0.620	0.04
	Back (kg)	51	61.5 (20.8)	100	63.2 (23.6)	-0.64	0.524	0.05
	Jump (cm)	52	22.9 (3.65)	99	22.0 (8.00)	-0.21	0.837	0.02
	Push-ups (rep/min)	52	15.5 (15.0)	87	17.0 (13.0)	-0.44	0.657	0.04
	Sit-ups (rep/min)	51	22.0 (12.0)	88	23.5 (14.8)	-0.66	0.509	0.06
	Balance (s)	52	27.5 (29.8)	99	20.5 (26.4)	-0.91	0.360	0.07
	Flexibility (cm)	52	8.45 (8.48)	96	9.05 (17.2)	-0.16	0.869	0.01
	Reaction time (s)	51	0.56 (0.08)	95	0.57 (0.10)	-1.11	0.269	0.09
VO ₂ max (mL/(kg×min))	38	43.5 (16.1)	76	40.0 (10.6)	-1.16	0.245	0.11	
40–59 women	RR	93	13.5 (3.11)	154	18.8 (3.13)	-12.94	0.000	0.82
	ID	93	1.71 (0.51)	154	1.23 (0.22)	-12.28	0.000	0.78

Age group	Parameters (women)	Longer ID and ED		Shorter ID and ED		Z	P	r
		n1	Median (IQR)	n2	Median (IQR)			
40–59 women	ED	93	2.68 (0.66)	154	1.91 (0.43)	-11.53	0.000	0.73
	Grip (kg)	92	25.5 (7.20)	152	25.8 (7.63)	-0.27	0.785	0.02
	Back (kg)	91	66.6 (25.0)	150	66.4 (22.0)	-0.14	0.889	0.01
	Jump (cm)	92	20.1 (6.15)	151	18.6 (7.00)	-1.84	0.065	0.12
	Push-ups (rep/min)	90	15.0 (16.0)	139	15.0 (13.0)	-0.81	0.417	0.05
	Sit-ups (rep/min)	81	20.0 (15.5)	137	16.0 (10.5)	-2.19	0.028	0.15
	Balance (s)	93	18.9 (26.5)	153	14.5 (16.2)	-1.90	0.057	0.12
	Flexibility (cm)	92	11.4 (13.4)	153	10.1 (12.6)	-1.22	0.221	0.08
	Reaction time (s)	92	0.59 (0.10)	153	0.60 (0.11)	-0.91	0.365	0.06
	VO₂ max (mL/(kg×min))	68	36.2 (7.35)	124	33.8 (7.35)	-2.21	0.027	0.16

Note: cm, centimeter; ED, exhalation duration; ID, inhalation duration; IE ratio, inhalation exhalation ratio; kg, kilogram; N, Newton; ml, milliliter; RR, respiration rate; s, second; VO₂ max, the maximum rate of oxygen consumption.

1.3.3. Links between respiratory movements and physical fitness

1.3.3.1. Correlation between respiratory movements and physical fitness in men

As shown in Table 8, for young men, AM/(AM+TM) was not significantly correlated with any components of physical fitness. For middle-aged men, AM/(AM+TM) was significantly correlated with VO₂ max, with weak correlation. Grip strength was significantly and positively correlated with TM and AM. Flexibility was negatively correlated with TM, and VO₂max was positively correlated with AM. Age and body size were not significantly correlated with AM/(AM+TM), except the correlation between height and AM/(AM+TM) (Rho = -0.243, P = 0.019).

Table 8. Correlation between respiration movement and physical fitness in men

Parameters (men)	n ¹	TM ¹ Rho	AM ¹ Rho	AM/(AM+TM) ¹ Rho	n ²	TM ² Rho	AM ² Rho	AM/(AM+TM) ² Rho
Grip (kg)	69	0.113	0.069	0.030	93	.250*	.227*	0.047
Back (kg)	69	-0.097	-0.213	-0.124	93	0.173	0.121	-0.021
Jump (cm)	70	0.034	0.000	-0.027	93	-0.042	-0.016	-0.016
Push-ups (rep/min)	Age ¹ 65	0.152	0.017	-0.096	Age ² 79	0.085	-0.041	-0.095
Sit-ups (rep/min)	67	0.053	-0.008	-0.084	75	0.024	-0.128	-0.134
Balance (s)	70	0.149	0.016	-0.053	90	0.043	-0.015	-0.003
Flexibility (cm)	69	0.093	0.041	-0.029	89	-.289**	-0.009	0.164
Reaction time (s)	68	-0.044	0.228	0.231	85	-0.001	0.028	0.064
VO ₂ max (mL/(kg×min))	44	0.125	.299*	0.200	52	-0.033	.310*	.279*

Note: Age¹, n¹, TM¹, AM¹, and AM/(AM+TM)¹ are for the 20-39 age group, while age², n², TM², AM², and AM/(AM+TM)² are for the 40-59 age group. AM, abdominal motion; kg, kilogram; ml, milliliter; N, Newton; TM, thoracic motion; VO₂ max, the maximum rate of oxygen consumption. * $P < 0.05$, ** $P < 0.01$.

As for young women, AM was significantly correlated with jump height with weak correlation. Except this, the remaining bivariate correlations were negligible ($Rho < 0.2$), as shown in Table 9. Age and body size were not significantly correlated with respiratory movement ($Rho < 0.2$).

Table 9. Correlation between respiratory movements and physical fitness in women

Parameters (women)	n ¹	TM ² Rho	AM ² Rho	AM/(AM+TM) ² Rho		n ²	TM ² Rho	AM ² Rho	AM/(AM+TM) ² Rho
Grip (kg)	148	0.078	0.093	0.041		244	0.101	.157*	0.115
Back (kg)	151	0.094	0.140	0.082		241	0.050	0.009	-0.012
Jump (cm)	151	0.111	.238**	0.141		241	0.090	.160*	.153*
Push-ups (rep/min)	Age¹ 139	0.021	0.063	0.026	Age²	229	0.104	.133*	0.098
Sit-ups (rep/min)	139	.185*	0.121	-0.031		218	0.010	0.094	0.109
Balance (s)	151	0.012	.178*	0.151		246	0.040	-0.037	-0.026
Flexibility (cm)	148	-0.084	0.120	0.107		245	0.009	0.039	0.027
Reaction time (s)	146	-0.072	-0.058	-0.009		245	-0.007	0.048	0.027
VO ₂ max (mL/(kg×min))	114	0.116	.186*	0.111		192	-0.072	-0.031	0.057

Note: Age¹, n¹, TM¹, AM¹, and AM/(AM+TM)¹ are for the 20-39 age group, while age², n², TM², AM², and AM/(AM+TM)² are for the 40-59 age group. AM, abdominal motion; BMI, body mass index; cm, centimeter; HC, Hip circumference; kg, kilogram; ml, milliliter; N, Newton; TM, thoracic motion; VO₂ max, the maximum rate of oxygen consumption; WC, waist circumference; WH ratio, waist/hip circumference ratio; * $P < 0.05$, ** $P < 0.01$.

1.3.3.2. The comparison of physical fitness components between groups with lower and higher respiratory movement parameters

For further exploration of the link between respiration movements and physical fitness, each parameter (AM, TM, and AM/(AM+TM)) was divided into two groups using the median split method. This division was performed to compare physical fitness between the groups with larger and smaller respiratory parameter values.

As shown in Table 10, for young men, according to AM/(AM+TM), only body height differed significantly between the two groups ($p = 0.021$, $r = 0.28$). According to AM, only relative $VO_2\max$ showed a significant difference between these two groups. According to TM, none of the physical fitness parameters differed significantly between the two groups. For middle-aged men, according to AM/(AM+TM), none of the physical fitness parameters showed significant differences between the two groups. In addition, participants with larger AM had better $VO_2\max$, while participants with larger TM had higher grip strength and better flexibility.

These results indicate that the AM/(AM+TM) were not linked with men's physical fitness. As the secondary outcomes, AM and TM are influenced by body size, here we only focus on AM/(AM+TM).

Table 10. The comparison of physical fitness components between groups with lower and higher respiratory movement parameters in men.

Age group	Respiratory Parameter (RP)	Physical fitness	Lower	n ¹	Higher	n ²	z	p	r
20–39	AM	VO ² max	37.4 (9.85)	18	41.5 (11.4)	26	-2.11	0.035	0.32
40–59	AM	Sit-ups	22.0 (12.5)	37	19.0 (11.3)	38	-2.00	0.045	0.23
		VO ² max	38.3 (10.8)	32	45.0 (19.7)	20	-2.37	0.018	0.33
	TM	Grip	40.5 (8.6)	47	43.2 (2.90)	46	-2.33	0.020	0.24
		Flexibility	6.65 (13.0)	44	1.50 (14.4)	45	-2.07	0.038	0.22

Note: AM represents abdominal motion, TM represents thoracic motion, and 'r' represents effect size. 'Lower' and 'n¹' refer to participants with lower values of respiratory parameters and the number of participants in this category, while 'higher' and 'n²' pertain to participants with higher values of respiratory parameters and their corresponding participant count. r, effect size.

As shown in Table 11, for young women, according to AM/(AM+TM), no significantly difference was found. Participants with more AM jumped higher and had better $VO_2\max$. According to TM, all physical fitness

parameters from the two groups were not significantly different. For middle-aged women, according to AM/(AM+TM), age was significantly different ($z = -2.14$, $p = 0.032$). We compared the residues and found participants with larger AM/(AM+TM) jumped higher. According to AM, there was no significant difference. Participants with larger TM had larger grip strength, higher jumps, and more push-ups. These results indicate that middle-aged women with larger AM/(AM+TM) might have better vertical jump ability.

Table 11. The comparison of physical fitness components between groups with lower and higher respiratory movement parameters in women.

Age group	Respiratory Parameter (RP)	Physical fitness	Lower	n ¹	Higher	n ²	z	p	r
20 – 39	AM	Jump	-1.43 (6.81)	77	0.83 (5.61)	76	-2.39	0.017	0.19
		VO ₂ max	-1.58 (10.4)	53	2.06 (10.5)	63	-2.44	0.015	0.23
40 – 59	TM	Grip	1.61 (1.42)	120	1.71 (1.50)	124	-2.11	0.035	0.13
		Jump	18.6 (15.7)	118	20.1 (17.4)	125	-2.47	0.014	0.16
		Push-ups	54.4 (27.0)	114	60.6 (32.4)	115	-2.03	0.043	0.13
	AM/(AM+TM)	Jump	-1.69 (-4.35)	122	0.49 (-2.42)	121	-3.21	0.001	0.21

Note: AM represents abdominal motion, TM represents thoracic motion, and 'r' represents effect size. 'Lower' and 'n¹' refer to participants with lower values of respiratory parameters and the number of participants in this category, while 'higher' and 'n²' pertain to participants with higher values of respiratory parameters and their corresponding participant count. r, effect size. The age variable had a significant impact on physical fitness, and the results showed a significant difference between calculations using the original values and those using the normalized values. Therefore, the table above presents the normalized values.

1.3.4. Correlation between respiratory patterns and age

In addition to studying respiratory patterns during spontaneous breathing, we also tested participants' vital capacity because it can represent their ability to maximally expand the thoracic cavity. By doing so, we aim to gain more knowledge about changes in respiratory movements.

1.3.4.1. Correlation between respiratory patterns and age in men

The RR, IE ratio, AM/(AM + TM), ID, ED, AM, TM, and vital capacity were set as the dependent variables tested in separate analyses, while the age, height, weight, waist circumference, and hip circumference were the independent variables included in every test. Additionally, to prevent

multicollinearity with height, weight, waist circumference, and hip circumference, we included the age, BMI, and waist–hip ratio as the independent variables and tested them with all the dependent variables.

All the respiratory parameters were not significantly linked with body weight, BMI, waist circumference, hip circumference, and waist–hip ratio ($p < 0.05$), which were not presented.

Men's RR, IE ratio, ID, and ED was not significantly correlated with age ($p < 0.05$), which are not presented in table.

Table 12 shows the results that were consistent with those from group comparison tests in Table 1, where the men's AM/(AM + TM) values increased with age. Further, both age and height were significantly associated with AM/(AM + TM), and the model explained 17.1% of the variance. In addition, AM/(AM + TM) was predicted by the regression model to increase by 0.4% each year. Age was significantly associated with AM, and the model explained 13.2% of the variance. AM was predicted to increase for 0.043 Newton each year. In contrast, the correlation of age with TM was not significant, which was also consistent with the results from the comparison tests. Regarding the vital capacity, as shown in Table 12, age, height, and waist circumference were significantly linked with vital capacity, and the model explained 33.3% of the variance. In addition, vital capacity was predicted to decrease by 36.9 mL each year.

Table 12. Regression analysis for the prediction of respiratory patterns in men.

Dependent Variables	Independent Variables	B	95% CI	β	p^β	R ²	F	p^F
AM	Age	0.043	0.026, 0.061	0.363	<0.001	0.132	24.345	<0.001
AM/(AM + TM)	Age	0.004	0.002, 0.006	0.265	<0.001	0.171	16.355	<0.001
	Height	-0.007	-0.010, -0.003	-0.264	<0.001			
Vital capacity	Age	-36.941	-47.067, -26.815	-0.479	<0.001	0.333	27.816	<0.001
	Height	29.111	11.411, 46.820	0.216	<0.001			
	Waist circumference	-10.456	-20.429, -0.482	-0.135	0.040			

Note: as the stepwise regression analysis was performed, the non-significant predictors were removed automatically. AM, abdominal motion; AM/(AM + TM), abdominal motion divided by the sum of the abdominal and thoracic motions. p^β is the p value of β , p^F is the p value from F.

1.3.4.2. Correlation between respiratory patterns and age in women

When testing the relationship between the women's respiration movements and their ages, the results showed a quadratic U-shaped model, and the trough was located at the age of 40. Therefore, we split the age into 2 parts—from 20 to 39 years old and from 40 to 59 years old—for linear regression analysis. As

shown in Table 13, although the AM/(AM + TM) was significantly associated with age, the model only explained 2.5% of the variance. The AM and TM values were not presented, as they were not significantly predicted by age and body size. These findings indicated that women’s spontaneous respiratory movements were not substantially associated with age. In contrast, their vital capacities decreased significantly with age. Age and height were linked with the women’s vital capacities, and the model explained 17.4% of the variance. In addition, the vital capacity was predicted to decrease by 21.8 mL each year. Similar to men's results, women's RR and IE ratio was also not significantly correlated with age ($p < 0.05$).

Table 13. Regression analysis for the prediction of the respiratory movements in women.

	Dependent Variables	Independent Variables	B	95% CI	β	p^β	R ²	F	p^F
20–39 years old	AM/(AM + TM)	Age	-0.005	-0.009, 0.000	-0.167	0.037	0.046	4.664	0.011
		Height	-0.005	-0.008, 0.000	-0.191	0.018			
40–59 years old	AM/(AM + TM)	Age	0.003	0.001, 0.006	0.163	0.011	0.022	6.585	0.011
20–59 years old	Vital capacity	Age	-21.08	-26.907, -15.250	-0.329	<0.001	0.174	41.65	<0.001
		Height	27.09	17.88, 37.93	0.254	<0.001			

Note: as the stepwise regression analysis was performed, the non-significant predictors were removed automatically. AM, abdominal motion; AM/(AM + TM), abdominal motion divided by the sum of the abdominal and thoracic motions. p^β is the p value of β , p^F is the p value from F.

1.4. Discussion

We will sequentially discuss the links between temporal parameters of respiration and physical fitness, the associations between respiratory movement and physical fitness, and the correlation between respiratory patterns and age.

1.4.1. Links between temporal parameters of respiration and physical fitness

Among young men (aged 20–39), those with a lower RR exhibited faster reaction speeds than those with a higher RR. Middle-aged men (aged 40–59) with a lower RR demonstrated both faster reaction speeds and stronger lower limb strength. Notably, young men with longer ED had shorter reaction times compared to those with shorter ED, while ID was not significantly linked with reaction time, indicating that exhalation contributed more than inhalation to the correlation between RR and visuomotor reaction time. In the case of women, our study revealed unsubstantial correlations between respiratory patterns and components of physical fitness.

Visuomotor reaction time relies on the intact functioning of sensory systems, cognitive processing, and sensorimotor coordination (Balakrishnan et al. 2014) while also being linked to factors such as arousal and attention. Kovacs et al. conducted a study revealing that increased arousal, induced by mental stress, significantly increased the reaction time (Kovacs and Bories 2010). Furthermore, attention was found to be closely linked to reaction time, with higher levels of attention resulting in shorter reaction times (Golmohammadi et al. 2021; Karwowski 2006). Our findings revealed that men with lower respiration rates exhibited faster visuomotor reaction speeds, potentially suggesting that men with lower RR (13.8 (2.75) for young men and 12.6 (3.19) for old men) have better visual motor coordination, higher attention levels, and/or experience lower mental stress than men with higher RR (18.3 (2.27) for young men and 18.5 (3.29) for old men). We have not found any other studies to verify our findings.

In addition to the results of reaction time, men with lower RR had significantly higher jump height but not on other muscular performance. The countermovement jump was tested in the present study, which requires a lower limb explosive power (Markovic et al. 2004; Martinez et al. 2016), as well as refined muscular coordination as it requires the activation of stretch reflex (or myotatic reflex, muscle stretch-shortening cycle) on the legs and arms (Król and Mynarski 2012; Petrigna et al. 2019). RR and tidal volume are negatively correlated (Wu et al. 2015), and the diaphragm muscle takes 60-80% work for tidal breathing (Ratnovsky and Elad 2005), which means a healthy person with lower RR should have greater tidal volume/activation of the diaphragm muscle than those with higher RR. The stability of the trunk is the basis of all functional movements (Cha 2018). The diaphragm muscle is one of the main core muscles for trunk stability (Huxel Bliven and Anderson 2013), as it works to control intra-abdominal pressure and reduces stress on the spine through cooperation with the abdominal and pelvic floor muscles (E. Kim and Lee 2013). Therefore, we suggest that people with lower RR have a better function of the diaphragm that optimizes core stability, facilitates body coordination, and results in a better countermovement jump.

One finding that has puzzled us is the unsubstantial difference in reaction time between women with slower breathing rates and those with faster breathing rates. So far, we do not have an explanation for this finding, and this requires further investigation.

Another perplexing finding is that individuals with different IE ratios did not exhibit substantial differences in physical fitness. Inhalation is primarily influenced by sympathetic activity, while exhalation is predominantly associated with parasympathetic activity. Lower IE ratios are often indicative

of a higher level of relaxation. However, despite these associations, differing IE ratios did not yield substantial effects on physical fitness. This observation raises the possibility that IE ratios may not hold much influence over physical fitness outcomes.

1.4.2. Links between respiratory movement and physical fitness

With respect to respiratory movement, we focused on the abdominal contribution to respiratory movements (AM/(AM+TM)). Regarding the link between men's AM/(AM+TM) and $VO_2\text{max}$, we cannot make a firm conclusion due to (1) two analysis methods yield different results and (2) the effect sizes and the sample size are both relatively small. Therefore, we suggest conducting further research to verify our findings in men. With respect to middle-aged women's abdominal contribution to respiratory movements and countermovement jump height, the correlation was significant, and the difference between higher value of abdominal contribution and lower value had significantly different jump height. Therefore, the results indicate older women (40-59) with more abdominal contribution to respiratory movements jumped higher.

A possible explanation for why older women with greater abdominal involvement jump higher could be related to the explosive power of the lower limbs, diaphragm strength, and core stability. As we previously discussed regarding the link between RR and vertical jump height, a countermovement jump requires lower limb explosive power and muscular coordination. The diaphragm serves as a major core muscle, contributing to trunk stability, which in turn enhances core stability, facilitates body coordination, and ultimately improves countermovement jump performance. However, this phenomenon was not observed in younger women. Our results show that AM/(AM+TM) were not significantly different between younger and older women. As of now, we don't have a clear explanation for this observation.

1.4.3. Correlation between respiratory patterns and age

Overall, we found that older men's AM/(AM+TM) were significantly larger than those in the younger men, whereas the changes in the women's respiratory movements were unsubstantial. In addition, the vital capacities/the ability to maximally expand the rib cage decreased with age for both men and women. We confirmed that the abdominal contribution was positively correlated with age for the male participants aged 20 to 59 years old. This finding was consistent with one past study (Mendes et al. 2020), but it was

inconsistent with other studies (Britto et al. 2009; Kaneko and Horie 2012; Ragnarsdóttir and Kristinsdóttir 2006; Sharp et al. 1975; Verschakelen and Demedts 1995). Due to the larger sample size of the present study, our results strengthen the existing findings. Regarding vital capacity, it decreased gradually and significantly with age, which was in line with the results of other studies (Britto et al. 2009; Kaneko and Horie 2012).

The chest wall compliance reduces in the process of aging (Lalley 2013), which can be caused by increased rigidity of the ribs (Mittman et al. 1965), thoracic kyphosis (Enright et al. 1994), and reductions in respiratory muscle strength (V. F. Parreira et al. 2005). Furthermore, the rib end-to-end separations and rib aspect ratios are seen to increase with age, producing elongated and flatter overall rib shapes in elderly populations (Holcombe, Wang, and Grotberg 2017). The barrel-shaped thoracic walls found in older populations reduces the mobility around the involved joints and constrains the activity of the ribs (Joshua, Shetty, and Pare 2014). The changes in muscle units could also be an influential factor. The muscle units are classified into slow-twitch (type S) and fast-twitch (type F) motor units, and type F motor units are further sub-classified into fast-twitch fatigue-resistant (type FR), fast-twitch fatigue-intermediate (type FInt), and fast-twitch fatigable (type FF) motor units (Elliott et al. 2016). During eupneic ventilation (spontaneous breathing), only type S and type FR motor units in the diaphragm are recruited, whereas forced ventilation requires additional recruitment of type FInt and type FF motor units (Gransee, Mantilla, and Sieck 2012; Greising et al. 2012). Sarcopenia is a common phenomenon in aging populations (Ligibel, Schmitz, and Berger 2020). Type S motor units are the ones most saved by sarcopenic processes, while type F are the ones most negatively affected (Elliott et al. 2016). Therefore, these changes in the chest wall compliance and muscle units deteriorate vital capacity/maximal chest expansion ability, whereas the thoracic motion during spontaneous breathing is not significantly affected.

Concerning the increase in abdominal motion during spontaneous breathing in advancing ages, Mendes et al. (2020) proposed that the change in abdominal motion was a compensatory mechanism triggered by decreasing thoracic movements (Mendes et al. 2020). The thickness of the diaphragm and its contractility are minimally affected by age, as diaphragm thickness in the zone of apposition remains stable throughout a wide age range (20–83), with a mean thickness of 3.3 mm, and diaphragm contractility also does not change significantly with age (Boon et al. 2013). Moreover, Özden et al. (2019) found that the diaphragm was significantly thicker in the older group (age: 71.3 ± 5.2 years; thickness: 2.3 ± 0.6 mm) than in younger adults (age: 26.9 ± 5.1 years; thickness: 2.0 ± 0.5 mm), and they suggested that the thickening of the

diaphragm could be attributed to substantial atrophy in the other core muscles in the older groups to preserve balance and posture (Özkal et al. 2019). Because the diaphragm is less influenced or even becomes thicker with age, its dome-like shape presses down on the abdominal cavity during inspiration, resulting in increased abdominal movement. In addition, Mendes et al. found for each year of increase in age (for mixed men and women between 21 and 85 years of age), the abdomen (navel level) percentage contribution was increased by 0.29%, the pulmonary rib cage (axilla level) percentage contribution was reduced by 0.20%, and the abdominal rib cage (xiphoid process level) percentage contribution reduced by 0.08% (Mendes et al. 2020). Our findings were consistent with the study by Mendes et al., and we agreed with them that the increased proportion of abdominal movement compensates for the limited movement of the chest. Differently, the present study stratified men and women and found that women's respiratory movements did not change substantially with age.

The changes in women's respiration movement were not substantial, although we observed a U-shaped change from 20 years old to 59 years old. Except for the finding from the study conducted by Mendes et al., wherein the abdominal contribution increased with age (for mixed men and women) (Mendes et al. 2020), other studies have consistently observed nonsignificant ($p > 0.05$) changes in women's abdominal contributions (Britto et al. 2009; Ragnarsdóttir and Kristinsdóttir 2006; Sharp et al. 1975; Verschakelen and Demedts 1995). Generally, we confirmed the results from most of the previous studies.

Women have a greater inclination of the ribs and lower radial dimension of the rib cage than men, which is considered an adaptation and evolution of the reproductive system to accommodate a growing fetus during pregnancy (Bellemare, Jeanneret, and Couture 2003). During pregnancy, the augmented tidal volume necessary to meet reproductive needs is mainly attained through an enhanced displacement of the ribcage without any consistent changes being detected in the abdominal contribution, as measured by magnetometers (LoMauro and Aliverti 2015). Therefore, the women exhibited prominent thoracic movements during quiet breathing. Due to the anatomical characteristics of women, they are more likely to use chest breathing. The degree of restriction of the thorax during the aging process for women should be lighter than that for men. As a result, the compensatory increase in diaphragmatic movement is less, and there is not much change in abdominal motion.

Women's respiratory movements with decreased abdominal contribution from 20 years of age to 39 years of age, and increased from 40 years of age to

59 years of age. Data variations can cause these results, but this phenomenon might also be influenced by sociopsychological factors, such as stress. A past survey (where the participants came from the region where the present study was conducted) revealed that younger people (20–39 years of age) have significantly higher occupational stress than older people (40–59 years of age), and women have significantly higher levels of occupational stress than men (Shen, Jiang, and Na 2018). When experiencing occupational stress, people may hyperventilate, causing biomechanical stress in their neck and shoulder region due to the activation of the sternocleidomastoid, scalene, and trapezius muscles, which tend to increase thoracic motion (Schleifer, Ley, and Spalding 2002). We assumed that when stress is reduced after 40 years of age, the activation of supplementary respiratory muscles (e.g., the sternocleidomastoid, scalene, and trapezius muscles) decreases, and the contribution of abdominal motion increases.

Regarding the changes in the women's vital capacities, the results were comparable to those of the men, showing decreases with age. The anatomical and physiological changes responsible for these decreases are likely the same as those discussed previously for men.

1.4.4. Limitations

The present study had at least three limitations. At first, the sample size was unbalanced between ages and genders. The female participants outnumbered the males and a larger proportion of elderly participants compared to young participants. Secondly, some male participants were not willing to have their push-ups and cardiopulmonary endurance tested. Therefore, the sample size of young men did not meet the estimated number for correlation test. Thirdly, information regarding the participants' smoking histories was not collected, which could have been a factor influencing their respiratory patterns.

1.5. Conclusions

1. Men with lower respiration rates had shorter reaction times. Middle-aged men with lower respiration rates had higher vertical jump heights.
2. Women's respiration rates and inhalation/exhalation ratios were not substantially interlinked with physical fitness.
3. The relationship between men's abdominal contribution and VO_2 max need further research to verify.

4. For middle-aged women, it appears that those with a greater abdominal contribution to respiratory movements tend to achieve higher countermovement jump heights.
5. Respiration rate and inhalation/exhalation ratio did not show a significant correlation with age. In contrast, men's abdominal contributions to total respiratory movements exhibited a significant correlation with age. For women, respiratory movements remained consistent across various age groups.
6. Vital capacities decreased with age for both men and women.

2. ACUTE EFFECT OF BREATHING EXERCISES ON MUSCLE TENSION AND EXECUTIVE FUNCTION

2.1. Literature Review

In the second part, the literature review will start with voluntary breathing, muscle tension, and executive function (the Strop Test). Then, the current state of research on the effect of acute breathing methods on muscle tension and executive function will be presented. Last but not least, the acute effect of breathing techniques on cardiorespiratory activities will also be presented as we planned to search for the physiological response to explain the results regarding muscle tension and executive function. Finally, we will outline the methods designed to achieve the objectives of this study.

2.1.1. Voluntary breathing and breathing exercises

The initiation of voluntary breathing and breathing exercises and their mechanisms are reviewed, focusing on mindful, slow, and fast breathings.

2.1.1.1. Initiation of voluntary breathing

The conscious control of breathing originates in the brain's cortex. When electrical stimulation was applied to the brain's cortical surface, it triggered a quick contraction of the diaphragm (Peckham and Knutson 2005). This showed that the primary motor cortex contains a representation of our respiratory muscles. Later research involving both percutaneous electrical stimulation and transcranial magnetic brain stimulation has revealed a high-speed connection from the motor cortex to the diaphragm (McKay et al. 2003). Each hemisphere of the diaphragm is represented primarily in the contralateral motor cortex (Maskill et al. 1991). Research employing positron emission tomography scans has illuminated specific regions in the motor cortex, thalamus, and cerebellum that are linked to conscious control of breathing (Colebatch et al. 1991; Fink et al. 1996; Ramsay et al. 1993), and these regions could also play a role in managing other types of purposeful breathing activities, such as respiration during speaking and physical exercise (McKay et al. 2003).

2.1.1.2. Breathing exercises and their mechanisms

There are many breathing techniques. Mindful breathing and slow breathing are popular two for relaxation. Additionally, fast breathing has a different effect that is worth comparing.

Mindful breathing

As a simple mind-body exercise, mindful breathing serves as a basis for more advanced mindfulness practice. So, before we talk about mindful breathing, it is better to have a clear understanding of mindfulness. The goal of mindfulness practicing is to center our attention on our breath, allowing us to feel its impact on our physical and mental condition (Lei Chui et al. 2021). A frequently referred definition of mindfulness is "paying attention in a particular way: on purpose, in the present moment, and non-judgmentally." (Kabat-Zinn 2005). Through consciously (intention) directing our focus (intention) and acceptance (attitude) toward our current experiences, we can more effectively tap into a broader and more adaptable set of coping strategies (Shapiro et al. 2006). Thus, mindfulness was summarized into three aspects: intention, attention, and attitude (Shapiro et al. 2006). Intentions direct where you focus, aiming to foster awareness (Batchelor 2011). Concerning attention, it entails closely watching one's ongoing internal and external experiences as they unfold moment by moment, including acting with awareness (Shapiro et al. 2006). Research found that "acting with awareness" (being conscious of one's actions) was significantly linked to positive improvements in all assessed outcomes for psychotic patients who took part in the mindfulness-based psychoeducation group (Chien et al. 2020). Cultivating awareness via mindfulness practice should be psychologically beneficial (Enkema et al. 2020). The fundamental attitude of mindfulness practice is non-judgment. Being non-judgmental means that you don't see something as 'good' or 'bad', 'right' or 'wrong'. Instead, you just observe it or experience it (Hall-remm 2007). Other key attitudes include patience, a beginner's mindset, trust, non-striving, acceptance, and the ability to let go (Kabat-Zinn 2005). Obviously, these attitudes are positive. Brown and Ryan showed that individuals with higher level of mindfulness reported significantly better behavior and self-regulation emotion (Brown and Ryan 2003).

Then, let's shed light on mindful breathing. There are various methods to practice mindfulness, with mindful breathing being a popular one. Mindful breathing requires you to be aware of the breath, without controlling the speed and depth, and without judgment of the quality (Burg and Michalak 2011a). Research has examined the immediate effects of mindful breathing, that five-

minute mindfulness breathing exercise led to immediate enhancements in both emotional well-being and physiological metrics among palliative care patients (Ng et al. 2016). Studies using intracranial electroencephalography (iEEG) and fMRI indicated that when healthy volunteers paid attention to their breathing, respiration–brain signal coupling (i.e., respiration-iEEG coherence and respiration fMRI signal synchronization) was increased in the insula, ACC, premotor cortex, and hippocampus (Herrero et al. 2018b; X. Wang et al. 2019). Mindful breathing increased alpha power (Bing-Canar, Pizzuto, and Compton 2016b), and it increased left-frontal activity related to positive feelings and approach motivation (Barnhofer et al. 2010). Thus, mindful breathing should be effective in reducing stress. The following paragraph discusses slow breathing, which has also been proven to be effective in decreasing stress.

Slow breathing

In medical terms, bradypnea is defined as a breathing rate of fewer than 10 breaths per minute in adults (Clini et al. 2018). In a review study, Marc A. Russo et al. defined slow breathing as a rate ranging between 4 to 10 breaths per minute (0.07–0.16 Hz) (Russo, Santarelli, and O’Rourke 2017). There are studies that found slow respiration at 6 breaths per minute in healthy humans to be the optimal rate for improving health. Controlled slow respiration at 6 breaths per min in healthy humans reduces the chemoreflex response to hypercapnia and hypoxia, compared with spontaneous respiration or controlled respiration at 15 breaths per min (L. Bernardi, Gabutti, et al. 2001). Slow breathing towards a rate of 6 breaths per min has been said to result in increased venous return (Dick et al. 2014). A recent study has also found that coupling of respiration and vasomotion (oscillations in vascular tone (i.e., arteriole diameter), which causes oscillations in capillary blood flow) becomes apparent when respiration is slowed, and at around 6 breaths per min, significantly greater coupling occurred in subjects with low initial blood oxygenation (Ovadia-Blechman et al. 2017). Another study of the effect of breathing rate on oxygen saturation and exercise performance has confirmed this by measuring arterial oxygen saturation during spontaneous respiration and respiration at 15, 6 and 3 breaths per min, during rest and during exercise, in healthy subjects and in chronic heart failure patients (Luciano Bernardi et al. 1998). Slow respiration at 6 breaths per min was found to be optimal for improving alveolar ventilation and reducing dead space in both groups in terms of increased arterial oxygen saturation and ease and sustainability in terms of respiratory effort.

SLOW (device-guided, 5–6 breaths/min) is currently a Federal Drug Administration-approved treatment indicated by the American Heart Association for relaxation (Larson et al. 2020). Taking slow, deep breaths activates slowly adapting pulmonary stretch receptors (SARs), which play a key role in ending the inhalation phase and facilitating exhalation through the Breuer–Hering reflex (Kubin et al. 2006). These SARs are also involved in regulating the tone of airway smooth muscles, controlling systemic vascular tone and heart rate, and affecting the pathophysiology of restrictive lung diseases (Schelegle 2003). One model for breathing-dependent relaxation suggests a physiological process of SARs pathways through the nucleus tractus solitarius (NTS) with connectivity to central autonomic networks (Birn, Murphy, and Bandettini 2008). Therefore, intentional deep breathing could potentially influence the autonomic nervous system through the activity of SARs in the lungs (Noble and Hochman 2019). Therefore, the present study chose the rate 6 reps/min for slow breathing.

Generally, for a healthy person, increased heart rate variability (HRV) indicates a relaxed state. HRV was found at the highest level when the breathing rate was 6 reps/min compared to that with spontaneous breathing and other breathing rates (L. Bernardi, Porta, et al. 2001; Radaelli et al. 2004). Although high frequency power in HRV (reflect vagal tone) is influenced by respiration rate (Sylvain Laborde, Mosley, and Thayer 2017), many studies have found that SLOW increased ease, comfort, relaxation, and positive energy and reduced anxiety, dejection, anger, hostility, and confusion (Zaccaro et al. 2018c). The executive function was improved after slow-paced breathing (4.5 sec inhalation and 5.5 sec exhalation) compared to after natural breathing, with higher scores observed for Stroop interference accuracy (S. Laborde et al. 2022). Meanwhile, the authors found that the improved executive function after slow breathing, was not mediated by RMSSD in HRV using bootstrapped mediation analyses (S. Laborde et al. 2022). Thus, other mediative factors, such as oxygenation and ventilation volume, should be tested.

Fast breathing

The normal breathing rate for an average adult is 12 to 20 breaths per minute. Tachypnea in adults is breathing more than 20 breaths per minute (S. B. Park and Khattar 2023).

Intentionally increasing the respiration rate has been a method to alter physical conditions. The Wim Hof breathing method is one that combines periods of hyperventilation followed by voluntary breath-holds at low lung volume (Citherlet et al. 2021). FAST can increase neuronal excitability that

facilitates muscle contraction, and may be beneficial for cognitive functions. FAST increased ventilation and raised pH (Barrett et al. 2019). Higher pH increased Ca^{2+} and Na^{+} currents, lowered the threshold of the action potential, and shortened the refractory periods of action potentials, which facilitated muscle contraction (Yunting Lu et al. 2012b; Tombaugh and Somjen 1996b).

Enhanced cognitive performance was associated with increased cortical excitability (Salehinejad et al. 2021b). One study compared the effect of 12 weeks of practice of slow and rapid types of pranayama breathing and found that both types of breathing were beneficial for cognitive functions, with fast pranayama having additional effects on executive function (Sharma, M., et al. 2014).

2.1.2. Muscle tension under psychological stress

Two terms, muscle tone and muscle tension, have been used interchangeably in scientific papers. Muscle tone is defined as "a state of a relaxed muscle under spontaneous excitation from the central nervous system." (Latash and Zatsiorsky 2016), and/or "the constant muscular activity that is necessary as a background to actual movement in order to maintain the basic attitude of the body, particularly against the force of gravity" (Carpenter 1996). Bernstein postulated that muscle tone serves as an adaptive feature of the neuromotor system, finely calibrating the responsiveness of sensory and motor cells to execute commands from higher-level movement coordinators that allows for effective control in active posture and movement tasks (Profeta and Turvey 2018). Meanwhile, muscle tension is defined as "the number of cross-bridges formed between actin and myosin determines the amount of tension that a muscle fiber can produce." (Biga et al. 2019). With the nuance of concept, muscle tone is produced automatically, while muscle tension can be produced automatically or voluntarily. We assume muscle tone is more accurate to explain the increased muscle stiffness under stressful conditions. But in neurology, muscle tone, as the resistance to passive stretch, is physiologically a complex interlaced network encompassing neural circuits in the brain, spinal cord, and muscle spindle, and disorders of muscle tone can arise from dysfunction in these pathways, manifesting as hypertonia or hypotonia (Ganguly et al. 2021). This adds to the connotation of "muscle tone", and using the term "muscle tone" can lead to ambiguity. Since many studies have used the term 'muscle tension' to describe the increase in tone caused by psychological stress (Chowdhury 2012; Lundberg et al. 1999; 1999; Roman-

Liu et al. 2013), eventually, we decided to use the term “muscle tension” in the present study.

The current study paid attention to muscle tension that is influenced by psychological stress. One explanation of the increased muscle tension under stress is that stress elevates sympathetic activity, initiates the release of catecholamines, and further facilitates muscle contraction (Melin and Lundberg 1997). Another group of scientists suggested that stress-induced hyperventilation leads to excessive exhalation of CO₂, which raises pH levels, and that elevated H_p levels facilitate muscle contraction (Schleifer, Ley, and Spalding 2002). Meanwhile, we propose another hypothesis (both 'muscle tension' and 'muscle tone' will be used to deduce our hypothesis). Muscle tone (the minimum involuntary muscle contraction for maintaining posture) is generated by muscle spindles by acting through the stretch reflex (Mukherjee and Chakravarty 2010). When muscles are stretched, the intrafusal muscles are also stretched, and the signal through Ia sensory fiber to the posterior horn activates alpha (α) motor neurons to contract the muscle to prevent damage. The sensitivity of the stretch reflex is modulated by gamma motor neurons, which adjust the tension level in the intrafusal muscle fibers of the muscle spindle (Purves 2004). Meanwhile, the central nervous system can directly regulate the sensitivity of muscle spindles via gamma (γ) motor neurons (Klein 2020). Heightened muscle tone has been linked to various central mechanisms, including neural pathways originating from the cerebellum, basal ganglia, and thalamus (Burke 1983; Kandel et al. 2012; Khonsary 2017). These structures are capable of altering the sensitivity of muscle spindles by regulating the inhibitory signals in the fusimotor loop, also known as the gamma motor loop (Eccles and Lundberg 1957). Electrically stimulating the orbital surface of the frontal lobe can trigger states of behavioral inhibition (Brutkowski 1965), while diffuse and nonreciprocal inhibition of muscle tone was achieved through electrical stimulation of the cortical inhibitory area in the cat (Sauerland et al. 1967). In humans, the right inferior PFC (rIPFC) seems to be specialized for inhibiting inappropriate motor responses (Aron, Robbins, and Poldrack 2004). During psychological stress, the amygdala triggers stress pathways in the hypothalamus and brainstem, leading to elevated levels of noradrenaline (NA) and dopamine (DA). This hampers the regulatory function of the prefrontal cortex while amplifying amygdala activity, creating a 'vicious cycle.' (Arnsten 2009). Thus, the impaired PFC might lose the ability to inhibit muscle tone/tension, resulting in an increase in muscle tone/tension.

2.1.3. Executive function and Stroop color-word test.

Executive functions encompass cognitive processes that work for purposeful and goal-directed behavior (Banich 2009). Inhibition, working memory, and cognitive flexibility serve as the three foundational executive functions. These fundamental skills pave the way for more complex executive functions like decision-making, problem-solving, and planning (Diamond 2014). Obviously, Executive function is critical for high work efficiency (Balconi, Angioletti, and Crivelli 2020).

The Stroop test, commonly employed among neuropsychological tools for assessing executive functions, measures the ability to inhibit cognitive interference by creating a stimulus incongruity effect (Braga et al. 2022; Scarpina and Tagini 2017). Inhibition involves controlling one's attention, behavior, thoughts, and emotions to resist a dominant impulse and instead act in a manner that's contextually appropriate (Diamond 2013). In general, the test helps researchers and clinicians to understand the complex interplay of cognitive processes involved in executive function, attention, and inhibitory control. The Stroop test procedure will be described in the MATERIALS AND METHODS section.

2.1.4. Current state of research on the effect of breathing exercises on muscle tension

There are many methods to reduce stress-induced high muscle tension, and breathing might be an effective one because mindful breathing and slow breathing have been proven to be able to reduce stress. However, studies regarding the effects of breathing on decreasing muscle tension are scarce.

Muscle tension dysphonia is a prevalent functional voice disorder that involves excessive muscle engagement while generating sound (Van Houtte, Van Lierde, and Claeys 2011). A study revealed that traditional singers with muscle tension dysphonia experienced significant improvements in laryngeal function, breathing performance, and vocal handicap when treated with a combination of abdominal breathing exercises and manual therapy (Ahmadi et al. 2022). However, the study did not test muscle tension. In addition, one study found a slight but significant decline in self-reported relief and physiological tension (used sEMG and electrodes were placed on the medial frontal forehead) after high anxiety-sensitive individuals took an instructed deep breath; however, there was no noticeable change in muscle tension following an instructed breath hold (Vlemincx, Van Diest, and Van den Bergh 2016). Because the activity of the upper trapezius muscle is more evident than

that of the most other body sites during mental tasks (Schleifer, Ley, and Spalding 2002), and given the prevalence of shoulder and neck pain among office workers, it's important to assess the muscle tension in the upper trapezius. Another research tested the effect of eight weeks respiratory relaxation exercise on muscle relaxation by self-evaluation scale and EMG: breathing relaxation exercise could relax muscles subjectively and reduce the level of myoelectricity (Mingjing 2018). Since this study spanned an 8-week intervention, there could be potential confounding factors; shorter, acute studies may minimize such interference.

It can be seen that there is a lack of research on using breathing exercises to reduce muscle tone. Moreover, the first article lacked objective indicators, such as electromyography, muscle tension tester, etc.; the second article conducted an 8-week intervention, and the interfering factors were uncontrolled. Acute studies can reduce interfering factors, but we have not found any related articles. To the best of our knowledge, the acute effects of different breathing techniques on muscle tension have not been investigated.

2.1.5. Current state of research on the effect of breathing exercises on executive function

Regarding mindful breathing, a study using intracranial electroencephalography demonstrated respiration-locked oscillations during attentive breathing (mindful breathing) with stronger power in the anterior cingulate cortex, premotor cortex, insula, and hippocampus (Herrero et al. 2018b), which are regions supposed to be involved in executive function (Carter, Botvinick, and Cohen 1999b; Rizzolatti, Fogassi, and Gallese 2002a; Eichenbaum 2004b). One study found mindful breathing increased alpha power and enhanced error-related alpha suppression during the subsequent Stroop task, indicating enhanced error-monitoring (Bing-Canar, Pizzuto, and Compton 2016b).

As mentioned in an article written by Laborde et al, the influence of slow-paced breathing on executive functioning has received little attention to date (S. Laborde et al. 2022). Then Laborde et al tested slow breathing (inhalation 4.5 s and Exhalation 5.5 s) for 5 min with 3 sets, and 1 min break at each set, compared to watching a neutral TV program. Results showed that performance on executive function tasks was better after slow-paced breathing compared to control, with higher scores observed for Stroop interference accuracy, but not Stroop interference reaction times. Meanwhile, the authors found that the improved executive function after slow breathing, was not mediated by RMSSD in HRV using bootstrapped mediation analysis (S.

Laborde et al. 2022). Thus, other mediative factors, such as oxygenation and ventilation volume, should be tested. Laborde et al. conducted another study and found that slow-paced breathing was able to improve inhibition after physical exertion via improving Stroop interference accuracy from color word test. Again, slow-paced breathing was not found to impact Stroop interference reaction time (Sylvain Laborde et al. 2019).

Although slow breathing and mindful breathing are two popular breathing methods for reducing stress and improving executive function, the effectiveness of these two breathing methods is unknown.

2.1.6. Rationale

Mindful and slow breathing exercises decrease psychological stress and improve mental health, whereas fast breathing increases neuronal excitability. This study aimed to explore the influence of 5 min of mindful breathing (MINDFUL), slow breathing (SLOW), fast breathing (FAST), and listening to music (MUSIC) on muscle tension and executive function during an intensive psychological task.

Main aim:

To explore the acute effect of breathing exercises on muscle tension and executive function.

Main objectives.

(1) To explore the acute effect of mindful breathing, slow breathing, fast breathing, and music listening on muscle tension and executive function.

(2) To examine the cardiorespiratory activities during different breathing exercises and the activities under psychological stress immediately after breathing exercises.

2.2. Materials and Methods

2.2.1. Participants

Forty-eight participants (men: $n = 24$, age = 30 ± 6 , BMI = 23.2 ± 1.7 ; women: $n = 24$, age = 29 ± 6 , BMI = 21 ± 1.3) were enrolled according to the estimations of sample size using G Power 3.1 (Heinrich Heine University Düsseldorf, Düsseldorf, Germany). Referring to prior studies (Springer et al. 2018; Howard et al. 2020; Redlich Bossy et al. 2020) and assuming an effect size of 0.25, alpha of 0.05, power of 0.8, and correlation coefficient of 0.6, 20

participants were required for each sex. Allowing for a 20% dropout rate, a total of 48 participants were required.

Inclusion criteria were as follows: (1) age 20–39 years; (2) BMI 18.9–24.9; (3) students or office workers; (4) ability to perform MINDFUL and SLOW (with obvious abdominal movement); (5) willingness to test at 3–10 days after the end of menstruation for women; (6) Perceived Stress Scale \leq

42 (Cohen, et al., 1983); and (7) written informed consent. Exclusion criteria were as follows: (1) mental or cardiorespiratory diseases; (2) skeletal muscle disorders in the lower back, shoulder, or arm with a score of pain (Visual Analogue Scale) higher than 3; (3) color vision disorder; and (4) unwillingness to complete the experiment. In addition, participants were asked not to consume caffeinated drinks for 2 h before the experiment.

The study was approved by the Medical Ethics Committee of Xiyuan Hospital, China Academy of Chinese Medical Sciences (Approval number: 2022XLA013-2).

2.2.2. Interventions

MINDFUL, SLOW, FAST, and MUSIC were the four interventions. For MINDFUL, participants were required to be aware of the breath, without controlling the speed and depth, and without judgment of the quality (Burg et al., 2011). The breath counting method was allowed for participants who felt it challenging to focus on their breath. SLOW was set at a speed of six breaths per minute, and obvious abdominal movement was required. An audio metronome was designed with a rising tone for inhalation and a falling tone for exhalation, setting the rate at 4 s for inhalation and 6 s for exhalation. Participants were instructed to gently expand their abdomen in addition to the natural expansion of the ribcage during inhaling, and relax their abdomen with attention on the area around the navel during exhaling. They were allowed to hold their breath naturally after exhalation to mimic natural breathing. The assessment of SLOW with obvious abdominal movement was conducted using two respiration belts. To determine the FAST rate, a respiration belt was used to measure RR during spontaneous breathing. FAST was then set at a speed that was 30% higher than an individual's regular breathing rate. During FAST, participants were also guided by an audio metronome. Regarding MUSIC, participants chose the relaxing music they preferred. Classical music (piano, Kiss the Rain; guzheng, Yun Shui Chan Xin) was provided, although participants could also listen to their own relaxing music.

2.2.3. Experimental Protocol

The same participants were tested in all conditions to limit inter-individual differences and minimize random noise (Sylvain Laborde, Mosley, and Thayer 2017). The durations of the interventions, the Stroop Test, and the rest between interventions were set at 5 min according to previous studies (Endo et al. 2013; Beng et al. 2016b; Kluger and Gross 2020). To ensure that the participants could perform breathing exercises properly, they received one-time online training one month before the testing day and were requested to practice for one month. All participants were invited to a social platform group where they could ask questions and exchange training videos, and instructors checked and guided participants' movement of SLOW during the one-month training period through video recordings and/or online meetings. Prior to the experiment, participants underwent two training sessions of the Stroop Test to reduce the learning effect. Then, participants sat on an adjustable chair in a standardized posture, with approximately 90 degrees flexion of the knees and hips, and a straight upper body (Figure 10); the computer screen was set at 50–80 cm in front of the face. The angle between the arm and the trunk was about 20 degrees, and the angle between the forearm and the arm was about 110 degrees, the junction of the front 1/3 of the forearm and the back 2/3 (tester made marks on participants' arms) was above the edge of the table. Two markers were put on the table beside the participants' forearms to eliminate the movement. During the experiment, participants performed a one-time baseline test (watching a neutral video for 5 min) and then completed 5 min of MUSIC, MINDFUL, SLOW, and FAST in a random sequence. The Stroop Test was performed after each intervention, including the baseline test, and was followed by a 5 min rest before performing the next intervention. While watching the video, listening to music, and performing breathing methods, participants overlapped their hands and rested the ulnar side on the table with palms toward the body and eyes closed. Before the Stroop Test, participants opened their eyes and waited for 10 s to “wake up.” At the resting time, participants were allowed to stand up, walk, and drink water. Sequence randomization used Latin Squares Design-William Design, as shown in Figure 11.

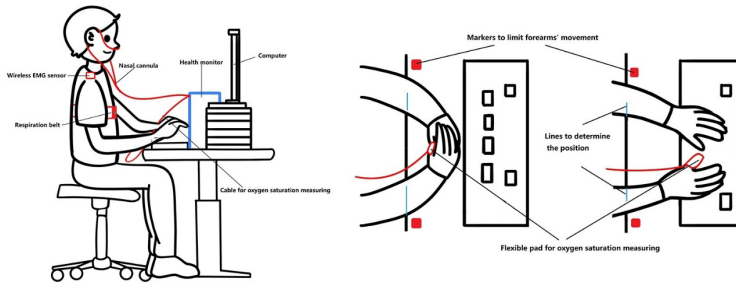


Figure 10. Postures during the experiment. The figure is used with permission from painter Ming Li.

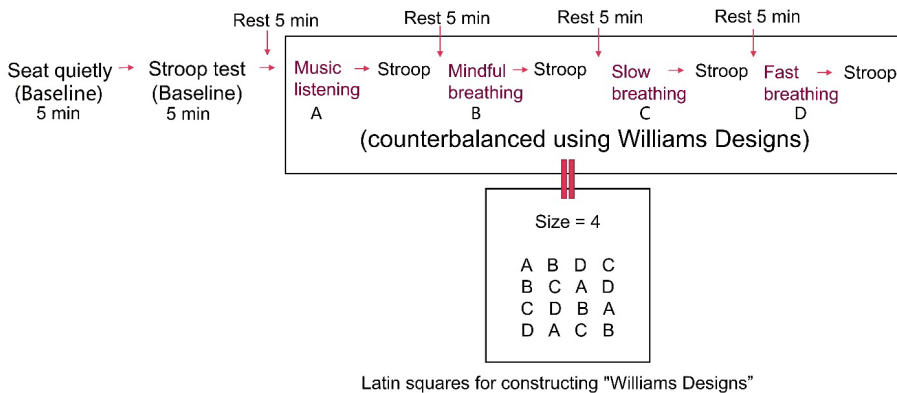


Figure 11. Experimental procedure.

2.2.4. Data collection

2.2.4.1. Muscle tension

Muscle tension/activity was measured using sEMG (Delsys Trigno wireless system, Natick, MA, USA). The skin was gently abraded and cleaned with an alcohol wipe, and hair was removed when necessary. Two rectangular Delsys Trigno EMG sensors (27 mm × 37 mm × 15 mm) with 99% silver electrode contacts were adhered to the upper trapezius (halfway along the line from the acromion to the spine on vertebra C7 on both left and right sides). We chose the upper trapezius muscle because its activity is more evident than that of most other body sites during mental tasks (Schleifer, Ley, and Spalding 2002). The wireless electrodes were suitable for our experiments since participants reported that the electrodes did not cause distraction. The sampling frequency was 2148 Hz, with an amplification gain of 1000. Raw sEMG data were band-pass filtered between 20 and 450 Hz using a fourth-order Butterworth filter,

and band-stop filtered at 50 Hz with aliases with sidebands of 0.25 Hz. The RMS was used to represent muscle tension.

To verify and consolidate the results, we used two methods to normalize the RMS values. The first used the mean value (average of four interventions for each participant) as a reference. Halaki et al., (2012) summarized that using the mean activation level of EMG could decrease the variability between individuals (Halaki et al. 2012). The second used the baseline as the reference. Roman-Liu et al., (2013b) conducted a study that tested muscle tension (using sEMG) under mental load with repeated measures, and claimed that taking the baseline as the reference value was the most appropriate reference (Roman-Liu et al. 2013). Normalization was performed by dividing muscle tension during the baseline condition by muscle tension during different breathing methods or Stroop Test and multiplied by 100.

2.2.4.2. Executive function

Executive function was determined from the accuracy and reaction time of the Stroop Test, tested on a computer using E-prime 3.0 (Psychology Software Tools, Pittsburgh, PA, USA). The test consisted of four words ('red,' 'blue,' 'green,' and 'yellow'), which were randomly displayed with a color that was the same as or different from the word's meaning. A series of color words were presented consecutively during the Stroop test. The participants were instructed to name the color of the displayed word as accurately and quickly as possible. A new color word was shown for 21 ms after one response or for 2000 ms without a response (no response within 2000 ms was recorded as an incorrect answer). There were 24 samples in each cycle, and the system randomly generated the sequence for every cycle. The accuracy was calculated by dividing the number of correct answers by the total number of color words, and then multiplying the result by 100.

2.2.4.3. Respiration rate

A respiration belt (Vernier, Beaverton, OR, USA) was secured around the chest, level with the xiphoid, to record respiration rate. This belt has been used in previous studies to provide ground truth data (Jakkaew and Onoye 2020). The signals were presented as a force with a sampling frequency of 10 Hz. There was a channel detecting inhalations and calculating the number of breaths per minute. The sample window for the calculation was 30 seconds, the advance interval was 10 seconds, and the value were updated every 10 seconds.

2.2.4.4. Oxygen saturation and end-tidal carbon dioxide partial pressure

A health monitor (Contec-CMS8000, Qinhuangdao, China) was used to record SpO₂ and EtCO₂. The SpO₂ measuring range was 0–100%, resolution 1%, and actualization interval 1 s. The flexible pad was worn on the right thumb since the participants needed the index and middle fingers to press the keys on the keyboard. The expired air was continuously collected using a disposable nasal cannula for measuring EtCO₂. The sampling gas flow rate was 50 ml/min±10 ml/min, resolution 0.1 mmHg (0–69 mmHg), actualization interval 1 s and the delay time 2–3 s. Atmospheric pressure was set at a default value of 760 mmHg. Routine calibration was not required, but 1–2 min of warm-up was implemented prior to each participant. Participants could opt out of the EtCO₂ test if the cannula was uncomfortable.

2.2.4.5. Preferred intervention

At the end of the test, participants selected their preferred intervention according to their general subjective feelings of relaxation and working efficiency.

2.2.5. Outcomes

The primary outcomes were as follows: muscle tension, represented by root mean square (RMS) values from surface electromyography (sEMG) recording; and executive function, represented by accuracy and reaction time from the Stroop Test. The secondary outcomes were RR from respiration belt, SpO₂ and EtCO₂ from a health monitor, and participants' preferred method.

2.2.6. Statistical Analysis

The Shapiro–Wilk test was performed to assess data distribution. The percentage of missing data was calculated and Little's MCAR Test was used to determine the missingness model. The effect of interventions from the average 5 min values and every minute's values were tested using Generalized Estimating Equations (GEE) on SPSS (IBM Corp., Armonk, NY, USA) since normal and skewed distributions were mixed throughout the data, and some could not be converted to normal distribution using log transform. The exchangeable correlation structure was chosen as the present study had a balanced design, and the value of goodness-of-fit was the highest. Significance was set at an alpha level of $P < 0.05$ and a highly significant level of $P < 0.01$, with Holm correction applied for post-hoc test. The Wilcoxon paired test was

used to evaluate the difference between the parameters when performing interventions and those while taking the Stroop Test, and the significance was set at $P < 0.05$. Effect sizes were calculated using Hedges' g (g) method. Participants' preference (percentage of favorable intervention) was analyzed using the chi-squared test on MedCalc (MedCalc Inc., Mariakerke, Belgium), and significance was set at $P < 0.05$, and $P < 0.01$ with Holm correction.

2.3. Results

According to the inclusion criteria, 48 participants were enrolled in this study. Three male subjects were excluded: one fell asleep during SLOW and MINDFUL, one was in bad condition (the error rate was over 40% in the Stroop Test), and one did not perform MINDFUL and SLOW as required. Two women were excluded: one reported a bad condition during the testing and another did not perform MINDFUL and SLOW as required. Therefore, 21 men and 22 women were included in the analysis. All missing data were missed completely at random (Little's MCAR test, $P > 0.05$).

According to the Latin square Williams Design, the participant performed each method according to the horizontal sequence as the first participant performed ABCD, the second performed BCAD, the third performed CDBA, the fourth DACB, and the fifth restarted from ABCD. In order to test for the effect of fatigue and/or learning, we compared each vertical sequence (ABCD, BCDA, DABC, CDAB) using the averaged 5 min values (Did not get it). Every post-hoc comparison's result was not significant as the lowest p -value was 0.127 from men's data and 0.074 from women's data. Therefore, we concluded that fatigue and/or learning effects were nonsignificant.

2.3.1. Muscle tension

Among the included participants, the sEMG signals of one man and two women were not recorded successfully. Thus, 20 men and 20 women were included in the analysis. Men's data was skewed after SLOW and FAST, while women's were normally distributed. Men had no missing data from the right upper trapezius and 2.5% from the left side; women had 2.5% missing data from the right side and no missing data from the left side. Outliers were defined as outside of median $- (3 \times \text{median absolute deviation})$ and median $+ (3 \times \text{median absolute deviation})$ (Trevino et al. 2015). Outliers were not removed because they were genuine observations. To verify the results, we analyzed the data after removing the outliers and found no significant differences from the results that retained outliers.

As depicted in Table 14, for both men and women, different interventions did not significantly affect muscle tension during the interventions and during the Stroop Test. However, there was a clear trend for men to have the lowest muscle tension during MINDFUL, and the tension decreased minute-by-minute. The muscle tension was relatively high after SLOW. The muscle tension in women was lower after MUSIC and FAST than that after MINDFUL and SLOW, and it was the lowest after FAST. In addition, the results were inconsistent between the left and right sides in both men and women.

The two normalization methods yielded similar results, as both had nonsignificant p-values and similar trends.

Table 14. Results of muscle tension.

Interventions	1 st min	2 nd min	3 rd min	4 th min	5 th min		Averaged 5min	Wald χ^2	<i>P</i>
Men's muscle activity of the right upper trapezius during the Stroop Test (normalized RMS, %)									
MUSIC	92±25	89±27	95±27	97±28	94±29		93±27	3.469	0.3248
MINDFUL	98±35	95±36	89±33	88±34	86±30		91±33		
SLOW	95±25	102±29	109±33	104±33	104±36		103±31		
FAST	116±58	111±47	110±40	110±49	116±56		113±49		
Men's muscle activity of the left upper trapezius during the Stroop Test (normalized RMS, %)									
MUSIC	92±34	91±29	103±34	107±38	109±38		101±35	1.630	0.5627
MINDFUL	93±29	92±36	96±43	93±42	88±31		92±36		
SLOW	105±28	109±39	109±36	107±36	106±33		107±34		
FAST	110±51	101±37	97±36	95±40	94±39		99±41		
Women's muscle activity of the right upper trapezius during the Stroop Test (normalized RMS, %)									
MUSIC	98±26	99±26	99±26	99±27	99±29		99±26	4.249	0.4883
MINDFUL	98±39	106±34	112±40	116±38	109±35		108±37		
SLOW	102±36	101±37	104±39	104±37	103±40		103±37		
FAST	93±38	87±36	90±37	92±37	90±37		90±36		
Women's muscle activity of the left upper trapezius during the Stroop Test (normalized RMS, %)									
MUSIC	95±28	95±29	94±28	97±27	97±26		96±27	1.661	0.6457
MINDFUL	101±39	103±37	105±34	105±33	102±31		103±34		
SLOW	113±39	104±36	104±35	105±35	106±35		106±35		
FAST	98±34	93±30	92±28	95±28	96±30		95±29		

Note: MUSIC: listening to music; MINDFUL: mindful breathing; SLOW: slow breathing; FAST: fast breathing.

2.3.2. Executive function

Data from 21 men and 22 women were analyzed for executive function, with no missing data. The accuracy data for men and women were skewed, while reaction times were normally distributed.

Based on the average 5 min values, different interventions did not significantly affect mental activity in terms of accuracy and reaction time in both men and women, as shown in Figure 12. However, men's accuracy rate in the Stroop Test was highest after SLOW among all methods, and it was significantly higher after SLOW than after MUSIC and FAST at the fifth minute (SLOW vs MUSIC, Wald $\chi^2 = 9.44$, $P = 0.011$, $g = 0.622$; SLOW vs FAST, Wald $\chi^2 = 16.9$, $P < 0.001$, $g = 0.863$). This indicates that the SLOW intervention has a better potential to sustain the ability of inhibition control in men. Additionally, men's reaction time after the SLOW was stable with a slight decrease through 5 min task, and became the shortest at the fifth minute, which indicates participants' working speed after SLOW was sustained well relative to after other three methods.

MINDFUL and MUSIC showed comparable results in executive function in both women and men. Regarding FAST, there were clear trends showing a gradual decrease in accuracy rate and a gradual increase in reaction time in both women and men, but these changes were minimal, which suggests that FAST did not boost executive function but had an adverse impact on it.

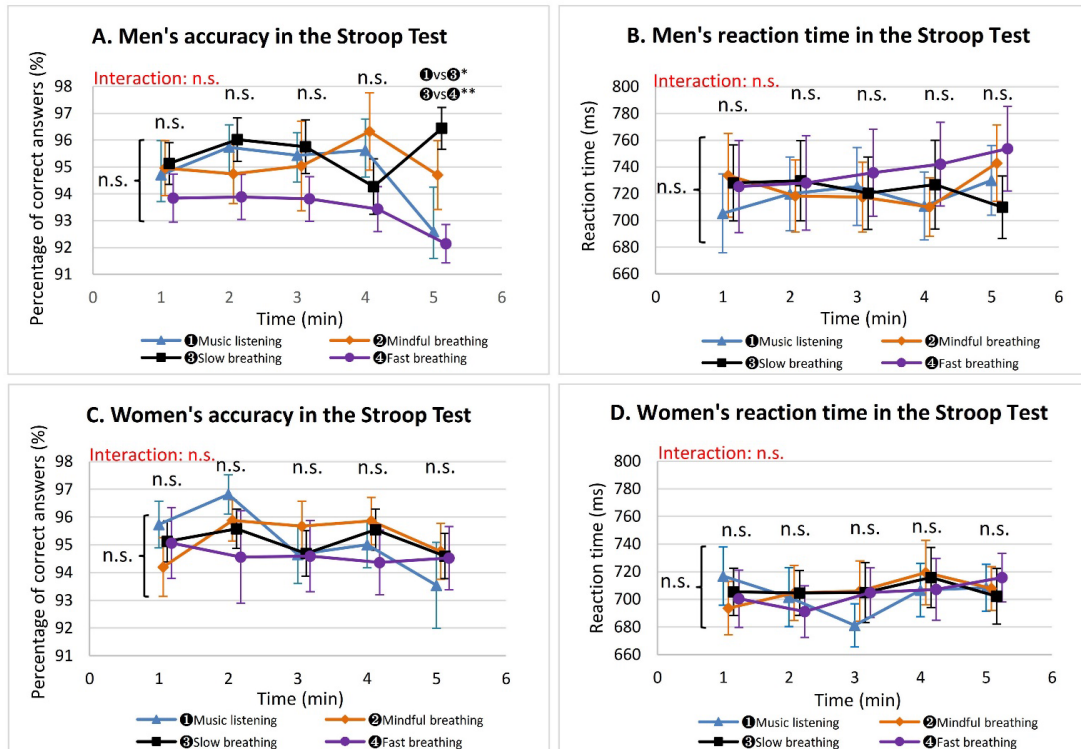


Figure 12. The results of executive function. “n. s.” beside the curly brackets denotes that no significant difference was found between each intervention based on the average 5 min values. “n. s.” or “yes vs yes” above each minute presents the results of the comparisons based on the values in that minute. The results of interactive effects were colored in red. * $P < 0.05$, ** $P < 0.01$. (A) Men's accuracy in the Stroop test. (B) Men's reaction time in the Stroop test. (C) Women's accuracy in the Stroop test. (D) Women's reaction time in the Stroop test. The figure is produced by author.

2.3.3. Respiration rate

One woman's respiration rate was not recorded successfully; therefore, data from 21 men and 21 women were analyzed. All data were normally distributed. Men did not have missing data; women had 1.1% of missing data.

As depicted in Figure 13A, men's RR was significantly different during different interventions (Wald $\chi^2 = 559.3$, $P < 0.001$). It was not surprising that the RR of FAST was faster than that of the other methods and SLOW was the slowest. Interestingly, the RR during MINDFUL was significantly lower than that during MUSIC (Wald $\chi^2 = 7.168$, $P = 0.007$, $g = 0.499$). During the Stroop Test, based on the average 5 min values, RR after SLOW was significantly lower than after MINDFUL (Wald $\chi^2 = 6.982$, $P = 0.049$, $g = 0.382$). There was a significant interaction effect on RR during the Stroop test after SLOW and after MUSIC (Wald $\chi^2 = 15.9$, $P = 0.019$). RR at the first minute after SLOW was significantly lower than after MUSIC ($P = 0.010$, $g = 0.544$) and MINDFUL ($P = 0.001$, $g = -0.609$), and the second minute after SLOW was also lower than MINDFUL at the second minute ($P = 0.043$, $g = 0.542$). There were no significant differences at and after the third minute. According to Figure 13A and the results obtained, it is evident that 5 minutes of SLOW reduced RR during stressful conditions for a longer duration than MUSIC and MINDFUL, although not beyond 5 minutes. From performing intervention to taking the Stroop Test, the average 5 min values for RR increased significantly in MUSIC (13.5 ± 3.62 vs 18.8 ± 3.03 , $t = -6.68$, $P < 0.001$, $g = 0.542$), in MINDFUL (11.6 ± 3.98 vs 18.7 ± 2.60 , $t = -6.80$, $P < 0.001$, $g = 2.105$), and in SLOW (6.02 ± 0.09 vs 17.5 ± 3.57 , $t = -14.7$, $P < 0.001$, $g = 4.516$). However, RR after FAST did not change significantly (17.5 ± 4.00 vs 18.2 ± 2.96 , $t = -0.70$, $P = 0.490$, $g = 0.198$).

The changes in women's RR are shown in Figure 13B. During the intervention periods, the RR was significantly different between interventions using the average 5 min values (Wald $\chi^2 = 841.6$, $P < 0.001$). Similar with men's results, RR during MINDFUL was also significantly lower than during MUSIC (Wald $\chi^2 = 6.509$, $P = 0.011$) in women. Furthermore, there are significant interactions between MINDFUL and SLOW (Wald $\chi^2 = 16.8$, $P = 0.013$) due to the increasing of RR during MINDFUL. During the Stroop Test, the results were different from those of men, as RR after various interventions were not different in average 5 min values, nor at each minute, except for the interactive effect after MUSIC and after FAST (Wald $\chi^2 = 16.8$, $P = 0.020$). From performing intervention to taking the Stroop Test, RR increased significantly in MUSIC (14.9 ± 3.8 vs 19.0 ± 3.9 , $z = -3.32$, $P < 0.001$,

g = 1.065), in MINDFUL (13.6±4.1 vs 18.1±3.8, z = -3.56, P = 0.001, g = 1.138), and in SLOW (6.00±0.1 vs 18.4±3.5, z = -4.02, P = 0.001, g = 5.008). Similar to men, RR during FAST did not change significantly (19.6±3.9 vs 18.3±3.5, z = -1.91, P = 0.0559, g = 0.351).

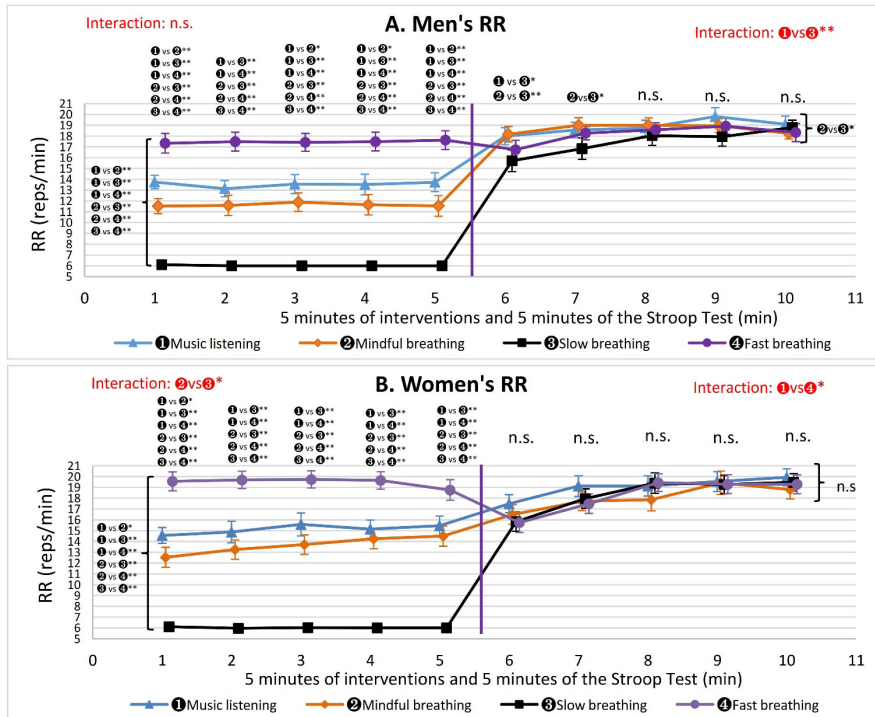


Figure 13. The results of respiration rate (RR). The markers beside the curly brackets, such as "n. s." or "1 vs 2*" etc., denote the results of comparison based on the average 5 min values. The markers above each minute present the results of the comparisons based on the values in that minute. The results of interactive effects were colored in red. * P < 0.05, ** P < 0.01. The figure is produced by author.

2.3.4. Oxygen saturation

SpO₂ was not recorded successfully for one man and one woman. Therefore, 20 men and 21 women were included in this analysis. The data from both men and women were normally distributed. Men had 5.8% missing values, whereas women had 3.7%.

For men, during the interventions, the SpO₂ was significantly different between MUSIC and SLOW (96.6±0.9 vs 97.4±1.3, Wald $\chi^2 = 9.53$, P = 0.012, g = 0.716), MUSIC and FAST (96.6±0.9 vs 97.4±1.0, Wald $\chi^2 = 8.79$, P = 0.015, g = 0.841), and MINDFUL and FAST (97.0±1.1 vs 97.4±1.0, Wald $\chi^2 = 7.64$, P = 0.023, g = 0.381) (Figure 14A). Besides, there was an interactive

effect between MUSIC and SLOW (Wald $\chi^2 = 21.2$, $P = 0.002$). In contrast, there was no significant difference between MUSIC and MINDFUL. (96.6 ± 0.9 vs 97.0 ± 1.1 , Wald $\chi^2 = 0.31$, $P = 0.502$, $g = 0.398$). During the Stroop Test, different interventions did not influence SpO₂ significantly for the average 5 min values and at each minute. SpO₂ decreased significantly from performing FAST to taking the Stroop Test (97.4 ± 1.03 vs 96.7 ± 0.99 , $t = 2.72$, $P = 0.014$, $g = 0.693$). The effects of the interventions lasted for one minute during the Stroop Test. SpO₂ dropped significantly from the second minute (compared to the first minute) after MUSIC ($P < 0.001$), MINDFUL ($P < 0.001$), SLOW ($P = 0.016$), and FAST ($P < 0.001$).

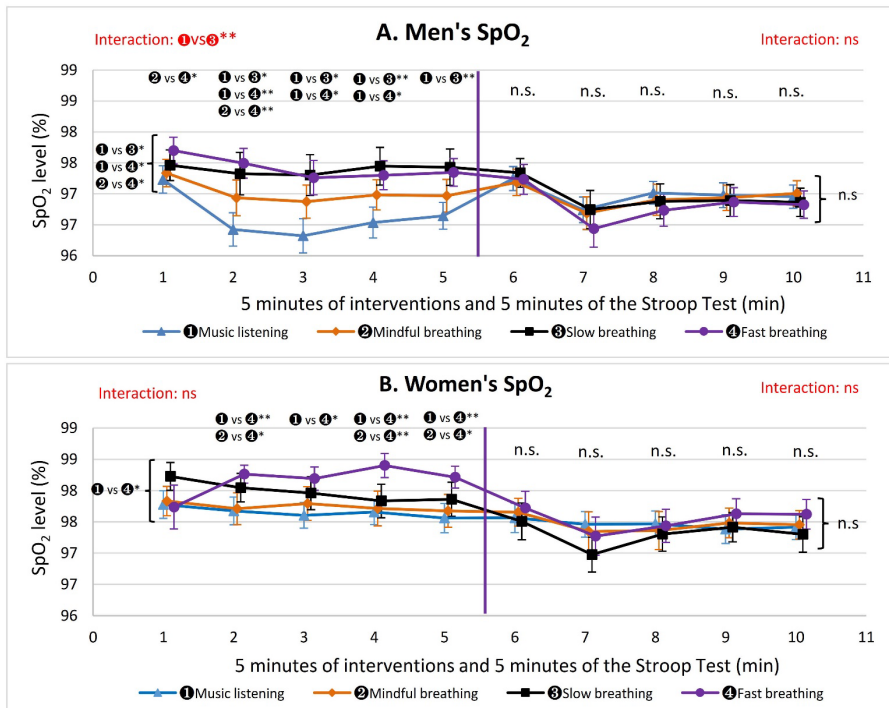


Figure 14. The results of oxygen saturation (SpO₂). The markers beside the curly brackets, such as "n. s." or "① vs ②*" etc., denote the results of comparison based on the average 5 min values. The markers above each minute present the results of the comparisons based on the values in that minute. The results of interactive effects were colored in red. * $P < 0.05$, ** $P < 0.01$. The figure is produced by author.

For women, during the different interventions, only MUSIC and FAST influenced SpO₂ differently (97.7 ± 0.95 vs 98.2 ± 0.73 , Wald $\chi^2 = 9.46$, $P = 0.012$, $g = 0.590$) (Figure 14B). During the Stroop Test, different interventions did not influence SpO₂ significantly. From performing intervention to taking the Stroop Test, SpO₂ decreased significantly after SLOW (97.9 ± 1.07 vs

97.3±1.17, $t = 3.17$, $P = 0.005$, $g = 0.535$) and FAST (98.2±0.73 vs 97.5±1.15, $t = 3.00$, $P = 0.007$, $g = 0.727$). During the Stroop Test, SpO₂ decreased significantly from the first minute to the second minute only after SLOW ($P = 0.008$).

2.3.5. End-tidal carbon dioxide partial pressure

Data were not recorded successfully for one man and two women. Therefore, data from 20 men and 20 women were analyzed. All data had a normal distribution except during the Stroop Test after FAST in women. There were 6% missing data from men and 2.5% from women.

The results for men are shown in Figure 15A. During the different interventions, the EtCO₂ were significantly different, using the average 5 min values, between FAST and MUSIC (35.3±5.29 vs 39.9±4.82, Wald $\chi^2 = 18.19$, $P < 0.001$, $g = 0.929$), FAST and MINDFUL (35.3±5.29 vs 40.0±5.80, Wald $\chi^2 = 19.22$, $P < 0.001$, $g = 0.865$), and FAST and SLOW (35.3±5.29 vs 38.5±6.36, Wald $\chi^2 = 7.02$, $P = 0.032$, $g = 0.547$). In addition, there were significant interactive effects between SLOW and MUSIC (Wald $\chi^2 = 48.8$, $P < 0.001$), between SLOW and MINDFUL (Wald $\chi^2 = 26.4$, $P < 0.001$), and between SLOW and FAST (Wald $\chi^2 = 26.2$, $P < 0.001$). Notably, the EtCO₂ during SLOW started to reduce from the second minute; it was significantly lower than that of MUSIC and MINDFUL at the fourth minute (37.0±6.70 vs 40.6±5.12, $P = 0.004$, $g = 0.547$; 37.0±6.70 vs 39.8±7.01, $P = 0.036$, $g = 0.408$), and at the fifth minute (37.0±6.70 vs 40.2±6.07, $P = 0.005$, $g = 0.472$; 37.0±6.70 vs 40.2±6.88, $P = 0.003$, $g = 0.447$). During the Stroop Test, different interventions did not influence EtCO₂ significantly based on the average 5 min values. Only at the first minute, EtCO₂ after FAST was significantly lower than that after MUSIC (39.0±3.76 vs 36.2±4.88, $P = 0.046$, $g = 0.642$). From performing intervention to taking the Stroop Test, EtCO₂ increased significantly only in FAST (35.3±5.29 vs 37.9±4.68, $t = -3.30$, $P = 0.023$, $g = 0.521$).

The results for women are shown in Figure 15B. The EtCO₂ during FAST (33.2±4.18) was significantly lower than that during the other three interventions (MUSIC: 37.1±2.87, Wald $\chi^2 = 18.66$, $P < 0.001$, $g = 1.088$; MINDFUL: 36.7±4.43, Wald $\chi^2 = 10.74$, $P = 0.004$, $g = 0.813$; SLOW: 35.9±5.70, Wald $\chi^2 = 12.32$, $P = 0.002$, $g = 0.540$), and there were no significant differences between MUSIC, MINDFUL and SLOW. Also, there were significant interactive effects between FAST and MUSIC (Wald $\chi^2 = 17.0$, $P = 0.011$), between FAST and MINDFUL (Wald $\chi^2 = 25.2$, $P < 0.001$), and between FAST and SLOW (Wald $\chi^2 = 16.4$, $P = 0.013$). During the Stroop

Test, different interventions did not influence EtCO₂ significantly for the average 5 min values. However, there was an interactive effect after MUSIC and after FAST (Wald $\chi^2 = 30.0$, $P < 0.001$), and EtCO₂ after FAST was significantly lower than that after MUSIC (33.8 ± 5.25 vs 36.3 ± 2.95 , $P = 0.002$, $g = 0.587$) only in the first minute. From performing the intervention to the Stroop Test, comparing the average 5 min values, EtCO₂ increased significantly only for FAST (33.2 ± 4.18 vs 35.8 ± 5.00 , $t = -3.49$, $P = 0.003$, $g = 0.564$).

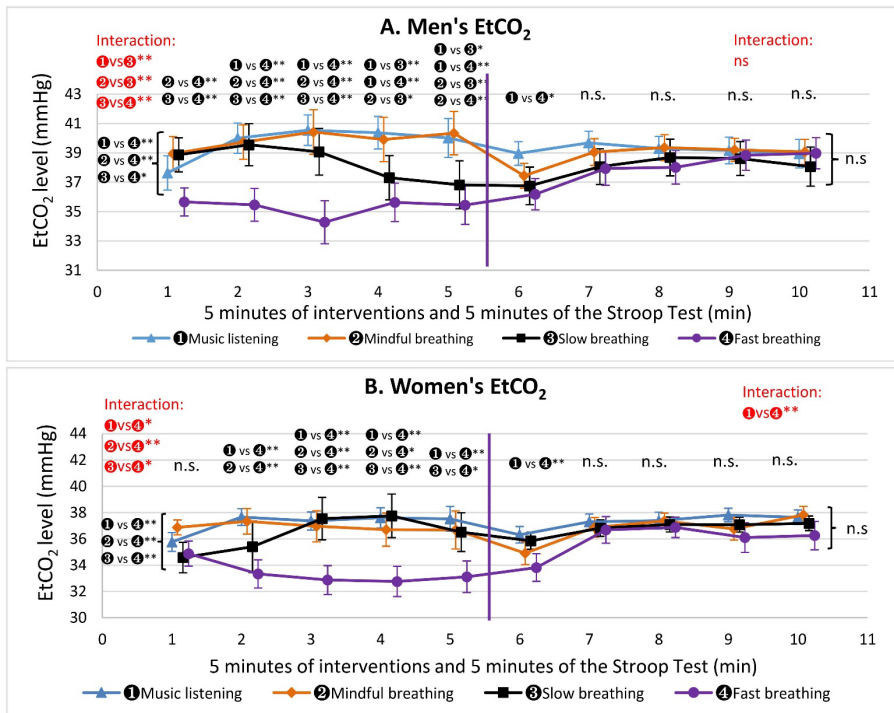


Figure 15. The results of end-tidal carbon dioxide partial pressure (EtCO₂). The markers beside the curly brackets, such as "n. s." or "1 vs 2**" etc., denote the results of comparison based on the average 5 min values. The markers above each minute present the results of the comparisons based on the values in that minute. The results of interactive effects were colored in red. * $P < 0.05$, ** $P < 0.01$. The figure is produced by author.

2.3.6. Preferred intervention

As shown in Figure 16A, 52% ($n = 11$) of the male participants preferred SLOW, 24% ($n = 5$) MUSIC, 19% ($n = 4$) MINDFUL, and 5% ($n = 1$) FAST. Statistically, the percentage of participants who preferred SLOW was significantly higher than that of participants who preferred FAST ($\chi^2 = 11.37$, $P = 0.004$). For the female participants, 48% ($n = 10$) preferred MUSIC, 24% ($n = 5$) MINDFUL, 14% ($n = 3$) SLOW, and 10% ($n = 2$) FAST (Figure 16B).

One woman could not firmly choose a favorite, so she did not respond. The percentage of female participants who preferred MUSIC was significantly higher than those who preferred FAST ($\chi^2 = 7.19, P = 0.0044$).

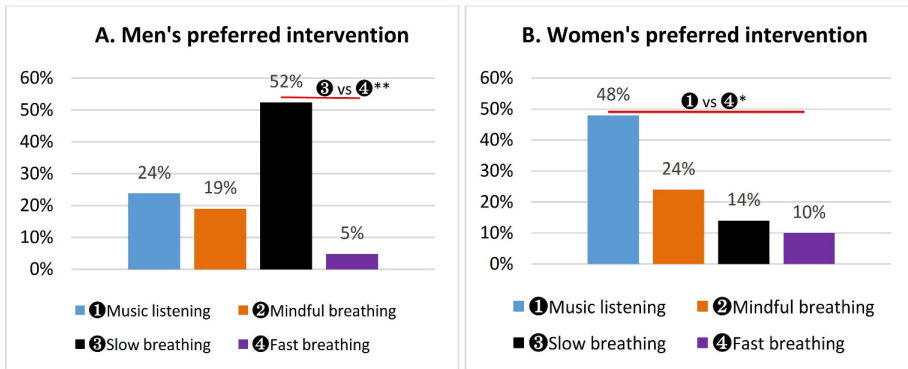


Figure 16. Participants' preferred intervention. * $P < 0.05$, ** $P < 0.01$. The figure is produced by author.

2.4. Discussion

The purpose of the current study was to test which method (between breathing techniques and music listening) is better for body relaxation and work efficacy such that it could be used alone or in combination with physical activity during work breaks. To the best of our knowledge, this is unprecedented research to investigate the effect of breathing techniques on muscle tension, even though we found different breathing methods did not influence muscle tension significantly. However, MINDFUL had the tendency to be better on muscle tension reduction under stressful condition for men, and SLOW showed superior potential over other methods on executive function. In addition, the results of RR, SpO₂, and EtCO₂ provided many interesting and meaningful physiological findings.

2.4.1. Muscle tension

One explanation for increased muscle tension under stress is that the elevated sympathetic activity initiates release of catecholamines, which further increases muscle tension (Melin and Lundberg 1997). Another study suggested that stress-induced hyperventilation causes excessive exhalation of CO₂, which increases pH and facilitates muscle contraction (Schleifer, Ley, and Spalding 2002). Mental stress in computer work increased muscle tension and brief MINDFUL or SLOW was able to reduce stress (Lundberg et al.

1999; Karthikeyan, Murugappan, and Yaacob 2012; Beng et al. 2016b). Therefore, we investigated the influence of different breathing methods and MUSIC on muscle tension.

Unexpectedly, but meaningfully, we found that 5 min of MINDFUL, SLOW, FAST, and MUSIC did not influence upper trapezius activity when taking the stressful psychological task, the Stroop Test. To verify the results, successful stress induction was confirmed, as RR, a stress indicator, increased when performing the Stroop Test. Second, two sEMG normalization methods (normalized from the mean and baseline) were performed, and there were no significant differences between each intervention.

According to the mean values, men's muscle tension was the lowest after MINDFUL from both the right and left sides of the upper trapezius, and the muscle tension was higher after SLOW than after MINDFUL and MUSIC. This implies that it might be difficult to relax physically while being energized mentally. The muscle tension in women was lower after MUSIC and FAST than that after SLOW and MINDFUL, while the lowest was after FAST. Oddly, FAST was the least favorable method. In addition, the muscle tension was inconsistent between the left and right sides. For example, men's muscle tension was the highest after FAST from the right side and the highest after SLOW from the left side. These odd and inconsistent results might be due to the influence of posture. We noticed that some participants' muscle tension changed substantially when their posture was altered. We strictly specified where the participants' arms were to rest. However, there were movements of the upper body during the Stroop Test. Wijsman et al., (2013) also stressed "EMG is influenced by many other factors, apart from stress. Static factors like body morphology and dynamic factors like posture influence the recorded EMG signals possibly even more than stress." (Wijsman et al. 2013)

2.4.2. Executive function

SLOW has been shown to cause a better accuracy, but not reaction time, on the Stroop Test compared to that of watching a neutral TV program (S. Laborde et al. 2022). In the present study, SLOW shown greater potential for sustaining executive function in men, as the accuracy rate during the average 5 min values was highest after slow breathing, with the accuracy rate in the fifth minute being significantly higher after slow breathing compared to music listening and fast breathing, and the reaction time being shortest after slow breathing. A resonance model proposed 6 reps/min for SLOW as being most beneficial. In this model, SLOW was related to heart rate, blood pressure, baroreflex modulation, and resonance characteristics of the cardiovascular

system (Sylvain Laborde et al. 2019; Lehrer and Gevirtz 2014). These processes should be able to strengthen homeostasis, improve gas exchange, and increase vagal afferents. Our findings support this model, as SpO₂ during SLOW was higher than during MUSIC.

Respiration interacts with the autonomic nervous system, as inhalation is associated with sympathetic activity and exhalation with parasympathetic activity (Jerath et al. 2006). SLOW increases parasympathetic outflow (Kromenacker et al. 2018), while decreased sympathetic activity and increased parasympathetic activity is likely associated with better cognitive performance (Forte, Favieri, and Casagrande 2019). In the present study, SLOW was set as 4 sec of inhalation and 6 sec exhalations, which was meant to increase parasympathetic activity, and the improved cognitive function might be caused by the activation of the parasympathetic outflow. But, as mentioned in the introduction, Laborde et al., 2021 observed an increased RMSSD in HRV (represents cardiac vagal activity/parasympathetic activity) during slow breathing (4.5 sec inhalation and 5.5 sec exhalation) and improved executive function after slow breathing. However, they found the improved executive function (compared to watching TV) was not mediated by RMSSD using bootstrapped mediation analyses (S. Laborde et al. 2022). Nevertheless, while slow breathing reduces stress (Zaccaro et al. 2018c), the Stroop test was stressful, and even mild acute uncontrollable stress can cause a rapid and dramatic loss of prefrontal cognitive abilities (Arnsten 2009). It is possible that SLOW improves executive function by alleviating the effects of stress and maintaining prefrontal neuronal function during stressful psychological tasks. The human body is a complex system. Oxygenation and respiration rate might also be the medicative factors, which will be discussed in the next part, “cardiorespiratory activity.”

Regarding the results in women, the measured interventions did not significantly affect their executive function. Women have shorter diaphragms, higher position of the sternum, greater inclination of the ribs and more chest movement than men during regular breathing (García-Martínez et al. 2016; LoMauro and Aliverti 2018a). Additionally, cultural influences may lead women to contract their abdominal muscles in order to appear slenderer. Therefore, we suggest that women might need longer time than men to practice SLOW (with obvious abdominal movement) to gain the same effect as men.

The effect of 5 min of MINDFUL on the performance of the executive test was not significantly different from MUSIC, even though the RR during MINDFUL was significantly lower than during MUSIC, and the SpO₂ was relatively higher than that of MUSIC.

FAST was the least effective intervention according to the Stroop Test results. We did not find a boost in mental ability, as we hypothesized, due to increased pH and neuronal excitability. The acute effects we found were not consistent with chronic effect, as a previous study shown that 12 weeks of fast yoga breathing significantly improved healthy young adults' executive function (Sharma, M., et al. 2014). Fast breathing often accompanies high-anxiety states, and it is possible that voluntarily increasing the breathing rate can trigger mechanism similar to those triggered by anxiety (Homma and Masaoka 2008; Masaoka and Homma 1997). In our study, some men felt nervous after FAST, which might due to the mild hyperventilation and that decreased performance in the Stroop Test.

Additionally, as inhalation and exhalation trigger different autonomic nervous activity, we suggest to conduct a study to compare the different effects of inhalation/exhalation ratios on executive function.

2.4.3. Cardiorespiratory activity

RR, SpO₂, and EtCO₂ were evaluated to monitor physiological responses and explain the results. Although not the primary focus, there were new findings, and some could meaningfully strengthen the results of previous studies.

Both men and women's RR during MINDFUL was lower than during MUSIC, which is in line with the finding that MINDFUL tended to result in a lower rate of respiration (Hunt et al. 2021). Paying attention to breathing strengthens breathing activity, even though we do not intend to influence it. In the Stroop Test, men's RR after SLOW was the lowest when compared to that of the other interventions. We propose two physiological mechanisms to explain these results. First, SLOW increased ventilation volume, which activated the regulation center in the medulla oblongata and inhibited the respiration rate (Kandel et al. 2012). Second, SLOW activated the parasympathetic nervous system (increased vagal tone) (Radaelli et al. 2004). The carry-over effect after SLOW decreased stress during the Stroop Test, so the respiration rate did not increase as much as it did after MINDFUL and MUSIC. The second proposed mechanism was strengthened by the results of FAST. FAST increased ventilation more than SLOW, and theoretically, it should inhibit RR more than SLOW during the Stroop Test, but it did not. Therefore, the reduction in RR after SLOW during the Stroop test was more likely due to the increased parasympathetic activity. Additionally, since RR is an indicator of stress, our findings are consistent with a previous report (Hunt et al. 2021) where SLOW was more effective than MINDFUL in stress reduction.

SpO₂ was higher during SLOW than during MUSIC and higher during FAST than during MUSIC and MINDFUL. There were slight differences in the mean and standard deviation between the SLOW and FAST groups. However, the EtCO₂, directly influenced by ventilation volume, was significantly different between the SLOW and FAST groups. These results confirmed SLOW's superior respiration efficiency compared to FAST, as hemoglobin oxygenation during SLOW can reach levels as high as those during FAST, but with less ventilation.

Consistently, the SpO₂ alterations by different interventions did not last longer than one minute during the Stroop Test. A similar result was also found in EtCO₂, where only FAST maintained EtCO₂ higher than MUSIC in the first minute. These two findings indicate that the carryover effect of breathing on SpO₂ and EtCO₂ was short.

2.4.4. Breathing method preference

Subjective preference should be a serious consideration when choosing a breathing technique because it directly reflects participants' physiological state. In this study, neither men nor women liked FAST, most men liked SLOW, and most women liked MUSIC. Participants chose according to their general subjective feelings of relaxation and working efficiency. The results mean women felt better after MUSIC, whereas men felt better after SLOW. Although 52% of men preferred SLOW, there were still 24% who preferred MINDFUL. Similarly, although 48% of women preferred MUSIC, there were still 24% who preferred MINDFUL. These findings demonstrate the diversity of participants' physiological responses after using different methods. Our findings can be used as a reference, but people should choose a breathing method (or music) according to their mental and physical states.

2.4.5. Limitations

This study has at least four limitations. First, we noticed that some participants' muscle tension changed substantially when their posture was altered. This phenomenon was difficult to control because some participants unconsciously adjusted their posture during the Stroop Test. Second, 5 min of MINDFUL might be too short to generate enough of an effect to influence cognitive function and muscle tension, although a previous study found that it significantly reduced distress scores. Thirdly, we did not ask participants the specific reasons for their preference ratings. Fourthly, although we used Latin square William's design to balance the influence of carryover effect, there

should be still carryover effect between each intervention. Future studies might consider testing interventions in different days.

2.5. Conclusions

1. Brief breathing exercises did not substantially affect muscle tension under psychological stress.
2. Slow breathing demonstrated greater potential for sustaining executive function in men, possibly via its superior respiration efficiency on SpO₂ and inhibition of RR.

3. GENERAL DISCUSSION

Respiration, a fundamental process for our life, can be initiated spontaneously or controlled voluntarily. To comprehensively understand the connections between respiration and general physical health (such as muscular performance and muscle tension) as well as mental health (executive function), we conducted two experiments. The first one explored the relationship between spontaneous respiratory patterns and physical fitness at different ages, while the second one examined the effects of voluntarily controlled breathing exercises on muscle tension, executive function, and cardiorespiratory activities. These two experiments provided valuable information for understanding respiration and its multiple and divergent functions. Also, the results of the two experiments can serve as mutual references to each other.

From the first experiment, we observed that older men exhibited larger abdominal contributions to respiratory movement. This increased abdominal movement appears to be a compensatory strategy in response to limited thoracic movement as individuals age. Additionally, we found that men's abdominal movement was not significantly correlated with physical fitness parameters, such as muscle power, muscle endurance, flexibility, balance, and cardiorespiratory endurance, nor with executive function (visual reaction time). Therefore, it is important to maintain normal thoracic expansion during abdominal breathing, as long-term abdominal breathing (expanding only the abdominal wall) can lead to a deterioration in the ability to expand the chest wall. Consequently, in the second experiment, we instructed participants to expand both the thoracic wall and abdomen during slow breathing exercises.

From the second experiment, slow breathing demonstrated greater potential for sustaining executive function in men. In the first experiment, we observed that male participants with lower respiration rates exhibited faster visual reactions, indicating that men with lower respiration rates possess better attention sustainability and visual-motor processing speed than those with higher respiration rates. These two experiments collectively lead to the conclusion: whether intentionally lowered or controlled spontaneously, a slower respiration rate is preferable for executive function compared to a faster rate for healthy individuals.

Interestingly, different breathing exercises did not significantly affect women's executive function in the second experiment, and women's spontaneous respiratory patterns were not correlated with age, executive function, and physical fitness (except the correlation between abdominal movement and jump height) in the first experiment. These results were

inconsistent with men's, which might be because women's anatomical structure for respiration and respiratory characteristics (more thoracic movement) are different from men's. Further research should consider these differences.

Solely observing the results of the first experiment, middle-aged men with lower respiration rates exhibited significantly better lower limb explosive power/motor coordination and faster reaction speed than those with higher respiration rates. When we carefully compare the results for men in the table, we can observe that middle-aged men with lower respiration rates performed better in most of the physical fitness components, including hand grip strength, back extension strength, number of push-ups and sit-ups in one minute, balance, and flexibility, even though the differences were not statistically significant. This leads to one conclusion: within the normal respiratory rate range (10-20 reps/min), middle-aged men with a relatively lower frequency (13.8 ± 2.75 reps/min) tend to have superior physical fitness compared to those with a higher frequency (18.3 ± 2.27 reps/min), which deserves attention. Healthcare professionals may consider various approaches, such as physical training or psychological treatments, to help reduce men's respiration rates for the sake of health preservation.

In addition, we found women's respiration rate and inhalation/exhalation ratio during spontaneous breathing were not substantially interlinked with physical fitness. For middle-aged women, it appears that those with a greater abdominal contribution to respiratory movements tend to achieve higher countermovement jump heights. As for men, their abdominal contribution to total respiratory movements during spontaneous breathing was not substantially linked with physical fitness.

Solely observing the results of the second experiment, it was found that brief breathing exercises did not significantly influence muscular activity (upper trapezius) during stressful work. One potential factor contributing to this result could be the influence of shoulder movement during the Stroop test. Another reason could be the limited duration of the breathing exercises, which may have played a role. Further research should consider refining the study's design. In addition, future studies should be cautious that slow breathing (6 reps/min) significantly decreases EtCO_2 , indicating a substantial increase in ventilation. From another perspective, slow breathing combined with movements might be ideal because the additional muscle activity produces more CO_2 , which can help mediate the decreased EtCO_2 .

Overall, our research has provided meaningful and practical findings for health preservation, and these findings hold significant reference value for future studies.

4. GENERAL CONCLUSIONS

1. Whether intentionally lowered or breathed spontaneously, a slower respiration rate is preferable for executive function compared to a faster rate for healthy individuals.

2. Middle-aged men (aged 40–59) with a relatively lower frequency (13.8 ± 2.75 reps/min) tend to have superior physical fitness compared to those with a higher frequency (18.3 ± 2.27 reps/min).

3. Men's abdominal contribution to total respiratory movements during spontaneous breathing was not substantially linked with physical fitness.

4. Women's respiration rate and inhalation/exhalation ratio during spontaneous breathing were not substantially interlinked with physical fitness.

5. Middle-aged women with larger abdominal contributions during spontaneous breathing might have better vertical jump ability.

6. Abdominal contributions to respiratory movement during spontaneous breathing were larger in older men than in younger men. Women's respiratory movements were not significantly different at the various ages.

7. Maximal chest expansion ability (vital capacity) decreased with age for both men and women.

8. Performing breathing exercises or listening to music before engaging in stressful work did not significantly impact muscle tension during the stressful work.

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LIST OF PUBLICATIONS

Publications in Journals with impact factor included in Clarative Analytics Web of Science database:

- Liang, W. M., Bai, Z. M., Aihemaiti, M., Yuan, L., Hong, Z. M., Xiao, J., Ren, F. F., & Rukšėnas, O. (2022). Women's Respiratory Movements during Spontaneous Breathing and Physical Fitness: A Cross-Sectional, Correlational Study. *International journal of environmental research and public health*, 19(19), 12007. <https://doi.org/10.3390/ijerph191912007>
- Liang, W. M., Xiao, J., Ren, F. F., Chen, Z. S., Li, C. R., Bai, Z. M., & Rukšėnas, O. (2023). Acute effect of breathing exercises on muscle tension and executive function under psychological stress. *Frontiers in psychology*, 14, 1155134. <https://doi.org/10.3389/fpsyg.2023.1155134>
- Bai, Z. M., Sun, Y. T., Liang, W. M., Truskauskaitė, I., Yan, M. E., Li, C. R., Xiao, J., Aihemaiti, M., Yuan, L., & Rukšėnas, O. (2023). Respiratory Movements at Different Ages. *Medicina (Kaunas, Lithuania)*, 59(6), 1024. <https://doi.org/10.3390/medicina59061024>

International conferences attended:

Oral presentation:

Wenming Liang, Osvaldas Rukšėnas, Zhenmin Bai, Maiwulamu Aihemaiti, Lei Yuan, Jing Xiao, Feifei Ren, Yang Zhu, Zishuai Chen. How Aging Affects Respiratory Movement. Arqus European University Alliance Research Focus Forum “Healthy Aging from a Multidisciplinary Perspective”, 27th-29th July, 2022, Vilnius University, Lithuania

Poster presentation:

- WM Liang, V. Mikuličiūtė, CR Li. Is Taichi / Qigong Exercise Better than other Exercises on Body Awareness and Psychological Well-Being: A Comparison Study. The 12th Conference of the Lithuanian Neuroscience Association, Vilnius, Lithuania, 6th of November, 2020.
- Skiauterytė L, Muntianaitė I, Liang WM, Rukšėnas O. Comparison Study of Taichi and Therapeutic Training Program on Cognitive Function and Life Satisfaction in Elderly Women, Virtual FENS Regional Meeting 2021, Krakow, Poland, 25-27 August 2021.
- WM. Liang, ZM. Bai, M. Aihemaiti, Lei Yuan, ZM. Hong, Jing Xiao, FF Ren, Yang Zhu, JQ. Qiao, ZS. Chen, Osvaldas Rukšėnas. Correlation Between Respiratory Motion During Spontaneous Breathing and Visuomotor

Reaction Time in Women FENS Forum 2022. 9-13 July 2022. Paris, France.

WM. Liang, ZM. Bai, M. Aihemaiti, L. Yuan, ZM. Hong, J. Xiao, O. Rukšėnas. Do People With More Abdominal Motion During Spontaneous Breathing Have Higher Maximal Oxygen Consumption? Participant of the VII International Scientific Conference "Actual Issues in the Development of Biology and Ecology", November 16 - 17, 2022, Vinnitsia, Ukraine.

WM. Liang, Jing Xiao, Feifei Ren, Zishuai Chen, Zhenmin Bai, O. Rukšėnas. Acute Effect of Breathing Exercises on Muscle Tension and Mental Activity Under Psychological Stress. the 14th International Conference of the Lithuanian Neuroscience Association, 25 November 2022, Vilnius, Lithuania.

CURRICULUM VITAE

Education Background

- 2019-2023, PhD in Biophysics, Vilnius University
- 2011-2015, MSc in Physical Education, Beijing Sport University
- 2004-2008, BSc in Physical Education, Beijing Sport University

Work experience:

- 2023 till now, junior research fellow at the Institute of Biosciences, Life Sciences Center, Vilnius University.
- 2016-2019, traditional Chinese exercise lecturer, Vilnius University.
- 2015, traditional Chinese exercises instructor, Xiyuan Hospital of China Academy of Chinese Medical Science.
- 2013-2014, traditional Chinese exercises instructor, Bergen University.
- 2008-2011, physical education teacher, Beijing Zhaitang Primary School.

Internship:

- 2021.07-12, experiment with colleagues at the School of Sports Medicine and Rehabilitation, Beijing Sport University.
- 2021.09-2022.06, internship and experiment at Xiyuan Hospital of China Academy of Chinese Medical Science.

Seminar delivered:

- Presented a seminar Respiration and Health during the event "National Health Day: Body Mind and Environment Purity" on 2021 April 7th organized by Vilnius University Health and Sports Center.
- Conducted an open lecture on the Basics of Taichi in the Sokio Zingsniu per Azija held on the 30th of May, 2021.

Additional Courses Completed:

- Completed the course Grant Writing, 26th of November, 2020.
- Completed the course Responsible Research through Supervision, Mentoring, and Working Together, from the 11th of January to the 15th of February, 2011.
- Completed the course Integrity H2020 European Student Convention 2021, 8th-9th September 2021,
- Completed the course Academic English Before Christmas, 14th-21th of December 2022.

Award:

- Third place at the Three Minute Thesis (3MT®) research communication competition. 24th of March, 2023, Vilnius University, Vilnius, Lithuania.

APPENDIX No.1

Copywrite regarding the figures

Figure 1. The human diaphragm, from Taim Talks Med with permission.

Figure 2. External intercostal muscles, from Kenhub with permission.

Figure 3. Accessory muscles for inhalation

- Scalenus. This image is in the public domain, downloaded from <https://commons.wikimedia.org/wiki/File:Scalenus.png>.
- Sternocleidomastoid. From Gray's Anatomy, written by Henry Gray, illustrated by Henry Vandyke Carter. downloaded from https://commons.wikimedia.org/wiki/File:Musculi_coli_sternocleidomastoideus.svg
- Trapezius. From BodyParts3D/Anatomography, downloaded from https://commons.wikimedia.org/wiki/File:Trapezius_back2.png.
- Pectoralis major. This image is in the public domain, downloaded from https://commons.wikimedia.org/wiki/File:Gray410_pectoralis_major.png
- Pectoralis major. From BodyParts3D/Anatomography, downloaded from https://commons.wikimedia.org/wiki/File:Pectoralis_minor_muscle_frontal.png
- **Figure 4.** Accessory muscles for exhalation
- Internal intercostal muscles. From BodyParts3D/Anatomography, downloaded from https://commons.wikimedia.org/wiki/File:Internal_intercostal_muscles_below.png
- Abdominal muscles. From Henry Gray (1918) Anatomy of the Human Body, downloaded from <https://commons.wikimedia.org/wiki/File:Gray392.png>
- Serratus Anterior. From BodyParts3D/Anatomography, downloaded from https://commons.wikimedia.org/wiki/File:Serratus_anterior_muscles_frontal.png
- Serratus posterior inferior. From BodyParts3D/Anatomography, downloaded from https://commons.wikimedia.org/wiki/File:Serratus_posterior_inferior_muscle_back3.png.

Figure 5. Respiratory Center. The figure is used with permission, <https://clinicalgate.com/control-of-ventilation/>

Figure 6. Taken by Wenming Liang, permitted by the Zeng Xie.

Figure 7. Wenming Liang own the copywrite.

Figure 8. Made by Wenming Liang.

Figure 9. An illustration of the tests of physical fitness. Drew by Dovilė Gelažiūtė, who agreed to the use of her drawing.

Figure 10-18. Wenming Liang own the copywrite.

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