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## RELIABILITY OF BREATH-HOLDING TESTS WITH POTENTIAL FOR USE IN SPORTS PRACTICE

Lörinczi František <sup>\*1 ABCDEFG</sup>, Lörincziová Drahomíra <sup>2 ABDEF</sup>,  
Vanderka Marián <sup>3 ABDEF</sup>

### Authors' contribution:

- A. Study design/planning
- B. Data collection/entry
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- D. Data interpretation
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<sup>1,3</sup> Comenius University Bratislava, Faculty of Physical Education and Sport, Slovakia  
<sup>2</sup> University of Economics in Bratislava, Physical Education and Sports Centre, Slovakia

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\*Author for Correspondence: frantisek.lorinczi@uniba.sk

### Abstract:

**Background:** Breathing is a crucial yet often overlooked phenomenon in sports practice. Simple, quick, cost-free, and equipment-free breath-holding tests allow for the assessment of chemosensitivity of athletes. The purpose of this study was to verify the reliability of two breath-holding tests: static breath-holding test (SBHT) and walking breath-holding test (WBHT).

**Materials and Methods:** The study assessed acute (intra-rater; ICC<sub>2,1</sub>) and inter-day (inter-rater; ICC<sub>2,k</sub>) test reliability. The research sample consisted of 207 individuals (74 adult males (31.12 ± 9.84 years), 41 adult females (32.41 ± 9.66 years), 67 adolescent males (13.73 ± 1.34 years), and 25 adolescent females (14.28 ± 1.81 years). While adolescents performed only the SBHT, the majority of adults completed both tests. An intraclass correlation coefficient (ICC) was employed to determine test-retest reliability. Pearson correlation coefficient (r) was used to detect correlations between SBHT and WBHT.

**Results:** In summary, the acute reliability of both SBHT and WBHT was significant (p < 0.01) and excellent (ICC<sub>2,1</sub> = 0.947 and 0.971, respectively). For SBHT, both acute and inter-day reliability were higher in adults compared to adolescents (ICC<sub>2,1</sub> = 0.947 vs. 0.892; ICC<sub>2,k</sub> = 0.908 vs. 0.852). The inter-day reliability of SBHT was significant (p < 0.01) and good (ICC<sub>2,k</sub> = 0.895) (excellent for adults; ICC<sub>2,k</sub> = 0.908). The inter-day reliability of WBHT was significant (p < 0.01) and excellent (ICC<sub>2,k</sub> = 0.953). A significant positive strong correlation was noted (r = 0.61; p < 0.01) between SBHT and WBHT.

**Conclusions:** Both SBHT and WBHT appear to be highly reliable breath-holding tests suitable for sports practice. Higher reliability was noted in groups of adults compared to adolescents. There is a significant correlation between SBHT and WBHT, which points to common determinants of the tests.

### Introduction

Comprehensive and targeted sports diagnostics contribute to optimizing training loads and preventing potential injuries or health issues [1]. Currently, a skewed trend is observed, with either basic diagnostics missing, or excessive testing being conducted. A holistic approach is popular even in diagnostics but despite the facts that around 90% of athletes display biomechanically dysfunctional breathing patterns [2,3] and incorrect breathing patterns may affect up to seventy percent of the population [4], breathing is often overlooked and underdiagnosed [5].

Dysfunctional breathing patterns may not be directly tied to an individual's fitness, but are associated with pain, motor limitations, dysfunctional movement patterns [6], sleep disorders [7], higher prevalence of ADHD and impairment of cognitive abilities [8], and severe pathophysiological changes in dental structures and oral health [9–12], and

can limit endurance performance [13] and lead to upper respiratory illnesses [14]. Upper respiratory illnesses are the most common medical conditions affecting elite athletes (14), potentially causing more acute disability among athletes than all other diseases combined [15]. The prevalence is higher in elite [14] and winter-sport athletes (with up to 79% among elite cross-country skiers) [16]. Also, the number of Olympic athletes with asthma and/or exercise-induced bronchoconstriction is increasing (reaching around 17.2% in 2008) [17]. Despite these implications, coaches and athletes rarely prioritize breathing.

Individuals with dysfunctional breathing often struggle to hold their breath (an inability to hold breath for at least 20 seconds usually indicates the presence of a breathing disorder) [3]. Voluntary breath-hold time is considered an indirect indicator of sensitivity to CO<sub>2</sub> accumulation and can be deemed an objective measure of breathlessness [18,19]. Carbon dioxide accumulation is recognized as the primary stimulus for breathing [20,21]. Breath-holding causes a decline in O<sub>2</sub> and a rise of CO<sub>2</sub>, and passing the breakpoint initiates the onset of respiratory movements, including diaphragmatic, chest, and throat movements [22]. The duration of breath-holding is mainly influenced by chemoreceptors and mechanoreceptors [23–25]. While well-trained individuals experience discomfort related to hypercapnia, they can tolerate it [26].

SBHT finds application in various fields, particularly in medicine [25,27,28]. However, its application in sports is innovative. To date, only the reliability of SBHT has been examined on smaller samples (ICC<sub>2,1</sub> = 0.93 (25); ICC<sub>2,1</sub> = 0.943 [27]; one year test-retest ICC<sub>A,2</sub> = 0.798 (28); inter-tester reliability ICC<sub>3,2</sub> = 0.88 (3)), but WBHT is yet to be unexamined. The inter-rater reliability of WBHT and the inter-day reliability of both tests remain unverified.

Based on the aforementioned facts, there is an increasing demand in sports practice for valid and reliable tests to diagnose aspects of athletes' breathing. This demand can be fulfilled with easy-to-perform and free tests, suitable for almost everyone. This research is therefore highly actual and original.

The purpose of this study was to verify the reliability of two breath-holding tests: static breath-holding test (SBHT) and walking breath-holding test (WBHT).

## Material and Methods

### Research sample

207 individuals voluntarily participated in the study (see Table 1 for characteristics). Both genders were represented. The age range was wide (10 – 59 years). Non-probability convenience sampling was used to create a research sample. Most people from the sample came from Slovakia. Subjects had no heart or respiratory health issues.

**Table 1.** Characteristics of the research sample

age group	gender	n	age (years)		
			mean ± SD	max	min
adults	male	74	31.12 ± 9.84	59	19
	female	41	32.41 ± 9.66	58	18
adolescents	male	67	13.73 ± 1.34	17	10
	female	25	14.28 ± 1.81	17	11

### Description of the tests

Both tests are based on the Oxygen Advantage® methodology. Static breath-holding test (SBHT), involves an easy breath hold after exhalation. Instructions are as follows: “In the seated position, take a normal breath in through your nose and breathe out through your nose. Pinch your nose and start your timer (at the moment when breath holding starts). Time the number of seconds until you feel the first definite desire to breathe, or the first stresses of your body urging you to breathe (these sensations may include the need to swallow, contractions of the diaphragm, or a constriction of the airways). Release your nose, stop the timer, and continue breathing normally. Inhalation at the end of the breath hold should be calm [29]”.

Walking breath-holding test (WBHT) measures the uppermost limit of tolerance to breathlessness. Instructions are as follows: “Take a normal breath in through your nose and exhale normally through your nose. Hold your breath, pinch your nose, and start walking. Walk at a normal pace (2 – 3 steps per second), while holding your breath. Count the maximum number of paces that you can walk with your breath held [29]”.

These tests could be considered valid for the detection of chemosensitivity to CO<sub>2</sub> [19,29]. While SBHT measures chemosensitivity at rest, WBHT measures the level of tolerance to hypercapnia during simple movement [29].

### Familiarization

During the familiarization phase, the conditions of both tests were explained to the participants. Firstly, the participants observed an examiner, who was trained for this testing by an Oxygen Advantage certified instructor, perform the test. Subsequently, participants executed the actual test themselves under supervision of the instructor. Particularly, the SBHT test was practiced multiple times, as a high level of interoception is required to achieve an objective result.

### Protocol

The testing protocol was conducted under the supervision of an examiner or another knowledgeable person. Both tests were administered on the same day, with the SBHT being conducted first. The participant was instructed to sit still for a minimum of 10 minutes to achieve a state of calmness. They were then asked to focus their attention on their breath to increase interoceptive awareness [30]. After a 10-minute sitting period, the examiner started a stopwatch upon the signal from the participant (initiating a breath hold and pinching the nose). The participants commenced the test when they were ready. The examiner ceased timing when the tested subject released his or her nose and took a breath. After completion of the first SBHT, the participant was given a 2-minute interval to regain composure before a repeated test. After the second repetition of SBHT, the participant was given another 2 minutes to rest before performing the initial WBHT. A second iteration of the WBHT was carried out after a 5 to 10-minute break. During these breaks, participants were required to remain calm and still. Participants were allowed to discontinue the test at any point if they experienced discomfort or refuse to participate in any of the tests.

### Statistics

The normality of data distribution was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The intraclass correlation coefficient (ICC) was employed to evaluate test-retest reliability. Both intra-rater reliability (test-retest on the same day) and inter-day reliability (test-retest on different days) were evaluated. Reliability was examined both separately for adolescents and adults and collectively. Similarly, the reliability for both genders was analyzed. Intra-rater reliability was computed based on results obtained within the same day. Inter-day reliability was calculated from the average results across separate days, with an interval of 2 to 7 days. ICC was calculated with 95% confidence intervals. ICC was computed using the following parameters: two-way random effects, absolute agreement, and a single rater for intra-rater reliability (ICC<sub>2,1</sub>), and multiple raters for inter-day reliability (ICC<sub>2,k</sub>). For results, average measures were employed. The standard error of the mean (SEM) was calculated based on the formula  $SEM = SD \times \sqrt{1 - ICC}$ , with SD representing the standard deviation of the measure [31]. Pearson correlation coefficient (r) was used to detect a correlation between SBHT and WBHT [32].

### Results

SBHT testing revealed the average result for the adult category  $20.21 \pm 6.24$  s, with the highest value being 39 s and the lowest value being 6 s. Furthermore, adolescents generally achieved lower values, with an average of  $17.58 \pm 5.33$  s (max 33; min 7). Regarding the WBHT results, relatively higher statistical deviations were observed. The average result for the adult category was  $45.26 \pm 18.53$  steps (max 116; min 14) (see Table 2).

The intra-rater (acute) reliability of SBHT was assessed using ICC<sub>2,1</sub> and overall was found to be significant ( $p < 0.01$ ) and excellent (ICC<sub>2,1</sub> = 0.931). This excellent reliability was also observed within the groups of adults (ICC<sub>2,1</sub> = 0.933 – 0.958). In adolescents, the ICC<sub>2,1</sub> indicated significant ( $p < 0.01$ ) and good reliability (ICC<sub>2,1</sub> = 0.860 – 0.897) (see Table 3).

For SBHT, inter-day reliability was deemed significant ( $p < 0.01$ ) and good (ICC<sub>2,k</sub> = 0.895). Similar reliability interpretations apply to the groups of adolescents (ICC<sub>2,k</sub> = 0.830 – 0.857). Among adults, ICC<sub>2,k</sub> showed significant and excellent reliability (ICC<sub>2,k</sub> = 0.904 – 0.913) (see Table 4).

**Table 2.** SBHT and WBHT results per category

Group	SBHT (s)			WBHT (steps)		
	(mean $\pm$ SD)	max	min	(mean $\pm$ SD)	max	min
Male adults	20.34 $\pm$ 5.25	36	8	46.61 $\pm$ 19.25	116	14
Female adults	19.96 $\pm$ 7.73	39	6	42.30 $\pm$ 16.56	93	16
Total adults	20.21 $\pm$ 6.24	39	6	45.26 $\pm$ 18.53	116	14
Male adolescents	17.69 $\pm$ 5.82	33	7			
Female adolescents	17.31 $\pm$ 3.75	27	8			
Total adolescents	17.58 $\pm$ 5.33	33	7			
Total (all participants)	19.04 $\pm$ 6.00	39	6			

**Table 3.** SBHT intra-rater (acute) reliability

Group	Number of pairs correlated	ICC <sub>2,1</sub>	Lower bound	Upper bound	sig.	SEM
Male adults	148	0.933	0.908	0.956	**	1.36
Female adults	82	0.958	0.936	0.973	**	1.58
Total adults	230	0.947	0.931	0.959	**	1.44
Male adolescents	134	0.897	0.855	0.927	**	1.87
Female adolescents	50	0.860	0.753	0.920	**	1.40
Total adolescents	184	0.892	0.856	0.919	**	1.75
Total (all participants)	414	0.931	0.916	0.943	**	1.58

sig. – significance; \*\* - ( $p < 0.01$ )

**Table 4.** SBHT inter-day reliability

Group	Number of pairs correlated	ICC <sub>2,k</sub>	Lower bound	Upper bound	sig.	SEM
Male adults	74	0.904	0.847	0.939	**	1.58
Female adults	41	0.913	0.836	0.954	**	2.24
Total adults	115	0.908	0.867	0.936	**	1.85
Male adolescents	67	0.857	0.766	0.912	**	2.10
Female adolescents	25	0.830	0.615	0.925	**	1.46
Total adolescents	92	0.852	0.777	0.902	**	1.95
Total (all participants)	207	0.895	0.861	0.920	**	1.88

For the WBHT, which was conducted only in adults, intra-rater reliability was determined to be significant ( $p < 0.01$ ) and excellent (ICC<sub>2,1</sub> = 0.970 – 0.971) (see Table 5).

**Table 5.** WBHT intra-rater (acute) reliability

Group	Number of pairs correlated	ICC <sub>2,1</sub>	Lower bound	Upper bound	sig.	SEM
Male adults	144	0.971	0.960	0.979	**	3.28
Female adults	66	0.970	0.951	0.982	**	2.87
Total adults	210	0.971	0.962	0.978	**	3.16

Inter-day reliability of the WBHT was also assessed as significant ( $p < 0.01$ ) and excellent ( $ICC_{2,k} = 0.952 - 0.956$ ) (see Table 6).

Table 6. WBHT inter-day reliability

Group	Number of pairs correlated	ICC <sub>2,k</sub>	Lower bound	Upper bound	sig.	SEM
Male adults	72	0.952	0.923	0.970	**	4.16
Female adults	33	0.956	0.910	0.978	**	3.46
Total adults	105	0.953	0.931	0.968	**	3.97

Using the Pearson coefficient of correlation, the correlations between SBHT and WBHT were evaluated as significant, positive, and strong ( $r = 0.61$ ;  $p < 0.01$ ).

Discussion

The research was conducted on a heterogeneous sample, encompassing both genders and a wide age range (10 – 59 years), which enhances the applicability of the results to a broader population.

The total intra-rater reliability of SBHT ( $ICC_{2,1} = 0.931$ ) was evaluated as significant and excellent, which is consistent with findings from previous studies [25,27]. The total inter-day reliability of SBHT was also significant and good (nearly excellent;  $ICC_{2,k} = 0.895$ ). While inter-day reliability of SBHT was excellent in adults, it was good in adolescents. The higher ICC values in adults are likely attributable to their potential higher level of interoception compared to adolescents [33]. Despite sex differences in interoceptive accuracy [34], no significant differences in ICC were found between genders.

Regarding MBT, which has yet to undergo verification, it has demonstrated significant and excellent reliability in both acute and inter-day measurements. Both SBHT and WBHT appear to be highly reliable tests, with acute test-retest reliability consistently higher than inter-day reliability, suggesting slight fluctuations over days. Fluctuation in the range of 1 – 10% of breath hold over a period of days seems to be logical as many factors affect chemosensitivity and ability to hold the breath (circadian rhythm, quality of sleep, level of stress, metabolism, etc. [35,36]). Furthermore, a significant positive correlation between SBHT and WBHT was observed ( $r = 0.61$ ), indicative of a strong correlation and shared determinants between the tests.

The safety of these tests warrants further exploration. SBHT seems to be safe and suitable for adolescents, as the breath-holding duration does not approach maximum levels (no adverse physiological reactions were recorded during testing). The physiological urge to inhale emerges well before oxygen and CO<sub>2</sub> levels reach health-threatening values [26]. Ordinary individuals may lose consciousness if pO<sub>2</sub> drops below 27 mmHg and/or pCO<sub>2</sub> is within the range of 90-120 mmHg [37]. The brain is affected when SpO<sub>2</sub> falls below 80-85%, and cyanosis sets in below 67% [38]. After 20 seconds of static breath-holding, SpO<sub>2</sub> can significantly decrease to  $92.4 \pm 4.9$  [33], but the likelihood of approaching dangerous hypoxia levels during SBHT is minimal [3]. Under natural conditions, voluntarily holding the breath long enough to endanger health or lose consciousness is practically impossible [22]. The risks associated with breath-holding mainly pertain to hyperventilation before the hold itself [39,40] or breath-holding underwater under high pressure [41]. Both of these conditions are absent in SBHT and WBHT. However, during WBHT, when individuals hold their breath to the maximum, potential risks may arise, necessitating further investigation. Although no health-threatening situations arose during the study, WBHT should be employed judiciously by individuals free from heart or respiratory problems.

Evidence suggests that athletes and coaches should take into account the importance of breathing [42]. While carbon dioxide is a by-product of cellular respiration and the human body is sensitive to its increased levels [21], building resistance to hypercapnia is crucial in practice, especially for athletes, as CO<sub>2</sub> facilitates oxygen release from hemoglobin (Bohr effect) and enhances blood circulation [43]. Athletes with lower CO<sub>2</sub> tolerance switch sooner to overbreathing and mouth breathing while increasing intensity [44,45], which has further negative physiological consequences [44,46]. Hyperventilation and mouth breathing are associated with faster muscle fatigue [46,47], worse tissue oxygenation [43], and faster water loss [48], potentially leading to dehydration and cognitive and mental impairment [49]. In extreme cases, overbreathing and mouth breathing can lead to exercise-induced bronchoconstriction and a higher risk of asthma [50].

While SBHT is predominantly employed in the medical domain [25,27,28], both tests hold potential for use in sports practice. SBHT and WBHT can help coaches identify individuals with potential respiratory issues, given that an inability to hold breath for at least 20 seconds often indicates a breathing disorder [3]. Regular application of these breath-holding tests can assist coaches in monitoring changes in athletes' chemosensitivity. These tests are simple, quick, cost-free, and require no specialized equipment. Although high reliability is one of their advantages, the need for a certain level of interoception in SBHT and potential unknown risks in WBHT are noteworthy disadvantages.

The study limitations include testing resting values at different times of the day. For enhanced accuracy, it would be optimal to conduct testing in the morning after waking up, especially for SBHT. Additionally, monitoring the onset and rate of hypoxia and hypercapnia achieved during the tests, along with the resulting time and number of steps, would be valuable. Limitations of SBHT also include measurement errors, which could arise due to various factors (limited interoception, inadequate assessment of the signal to inhale, insufficient exhalation prior to breath-holding, etc.). Similarly, WBHT has its limitations, with the speed and step length being most common; participants may struggle to maintain these consistently throughout the measurements. Breathing is a finely tuned process, and breath-hold tolerance can be influenced by numerous confounding variables. Nevertheless, future endeavours should focus on crafting the most reliable and freely accessible tests for diagnosing parameters of the respiratory system.

## Conclusions

Both SBHT and WBHT are highly reliable breath-holding tests, as confirmed across both genders and over a wide age range of participants. Acute reliability consistently appears higher than inter-day reliability in all instances. For adults in general, significant and excellent reliability was established, while for adolescents, reliability was significant and good. Furthermore, a significantly strong correlation between SBHT and WBHT indicates shared determinants between these tests. Both SBHT and WBHT appear to be safe and suitable for not only medical applications but also sports practice; however, further examination is necessary to verify this suggestion.

## Abbreviations

SBHT – static breath-holding test

WBHT – walking breath-holding test

ICC – intraclass correlation coefficient

## Conflict of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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**Ethical approval and consent to participate:** The present study was approved by the ethics commission of the Faculty of Physical Education and Sport, Comenius University in Bratislava (under the number 6/2022), and conforms to the ethical guidelines of the Declaration of Helsinki 2000. All participants (legal representatives in the case of adolescents) provided witnessed written/oral informed consent to all authors prior to the study. The other research participants were witnesses.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study

**Data availability statement:** The data presented in this study are available on request from the corresponding author.

**Author Contributions:** All authors listed (FL, DL and MK) have made a direct and intellectual contribution to the work and consented with its publication. All authors have approved the submitted version and agree to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work.



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