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Breath-holding test in evaluation of peripheral chemoreflex sensitivity in healthy subjects

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ABSTRACT

The aim of the study was to determine the feasibility of using a breath-holding test in assessing the sensitivity of the peripheral chemoreflex compared with the single-breath carbon dioxide test.

The study involved 48 healthy volunteers between the ages of 18–29 years. The breath-holding test was performed followed by the single-breath carbon dioxide test on the next day. A month after the first tests, these tests were repeated to evaluate their reproducibility

The coefficient of variability in the single-breath carbon dioxide test ranged from 0 to 32% with a mean of $10 \pm 7\%$. The mean coefficient of variability of the breath-holding test was $6 \pm 4\%$ (0–19%).

A significant inverse correlation between the results of the two tests was noted following analysis ($r = -0.82$, $p < 0.05$).

Conclusion: A breath-holding test after deep inspiration reflects the sensitivity of the peripheral chemoreflex as defined by the single-breath carbon dioxide test in healthy subjects.

1. Introduction

The role of the sensitivity of the peripheral chemoreflex in the pathogenesis of various conditions has garnered much attention in recent years. A significant increase in the sensitivity of the peripheral chemoreflex was noted in severe chronic heart failure, arterial hypertension, and sleep apnoea syndrome (Kara et al., 2003). Mechanisms for increasing the sensitivity of this chemoreflex in chronic heart failure were studied especially carefully in recent years due to the fact that the degree of increase is directly correlated with the severity of the cardiovascular disorder (Narkiewicz et al., 1999). The pathogenesis of hypersensitivity of the peripheral chemoreflex in severe forms of heart failure is complex and not yet fully understood. The main mechanisms are thought to be the elevation of angiotensin II, concomitant upregulation of the AT1 receptor in the carotid bodies (Li et al., 2006), and an impairment of nitric oxide production (Sun et al., 1999). Dysregulation of the effects of nitric oxide and angiotensin causes a change in the level of functioning potassium channels responsible for sensitivity to hypoxic stimulus in the cells of the carotid glomus (Buckler et al., 2000; Fuller et al., 2005). In patients with severe arterial hypertension the sensitivity of the peripheral chemoreflex is also increased (Kara et al., 2003), which is associated with morphological changes in the structures of the carotid glomus and decreased baroreflex sensitivity. In recent literature, there is evidence of increasing the sensitivity of peripheral chemoreceptors to hypoxic stimuli in patients with chronic obstructive pulmonary disease and patients with chronic kidney disease. However, the mechanism of this phenomenon has not been studied.

Regardless of the reasons for increasing the sensitivity of the peripheral chemoreceptors, increased afferent impulses from the carotid glomus lead to a number of changes in the status of the autonomic nervous system and regulation of the respiratory and cardiovascular systems. The result is an increase in the tone of the sympathetic nervous system and inhibition of the baroreflex regulation of the cardiovascular system, which is the result of integrated mechanisms of interaction between the baro- and chemoreflex. The sensitivity of the arterial baroreflex is inversely proportional to the sensitivity of the peripheral chemoreflex (Ponikowski et al., 1997). Thus, in the progression of chronic diseases associated with changes in the reflex regulation of the cardiorespiratory system, there is reduced tolerance to changes in blood pressure and increased risk of haemodynamic disturbances. Therefore, evaluating the sensitivity of the peripheral chemoreflex, we are able to predict the likelihood of developing respiratory and cardiovascular disorders during the treatment of these patients, disorders during surgery under general anaesthesia, and predict the outcome of the disease.

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The evaluation of the sensitivity of the peripheral chemoreflex is traditionally associated with a hypoxic test (Cormack et al., 1957; Edelman et al., 1973; Weil et al., 1970). However, persistent hypoxia occurs in these techniques, which can lead to respiratory depression due to central effects (Chua and Coats, 1995). Furthermore, there is a potential risk of adverse incidents related to hypoxia, especially in high-risk patients. The single-breath of carbon dioxide method designed by (McClean et al., 1988) is an alternative method of evaluating the sensitivity of the peripheral chemoreflex and is relatively safe when compared with hypoxic tests. However, this method can also be associated with gas exchange disorders and requires complex equipment, limiting its application in routine practice.

Breath-holding after inhalation is an alternative method that can potentially provide information on the status of reflex regulation of the cardiorespiratory system. The duration of the voluntary breath-holding depends on several factors, with the sensitivity of the peripheral chemoreflex as the main factor.

The aim of the study was to determine the feasibility of a breath-holding test in assessing the sensitivity of the peripheral chemoreflex compared with a single-breath carbon dioxide test.

2. Material and methods

The study involved 48 healthy volunteers between the ages of 18–29 years (27 men, 21 women). No subjects had a history of chronic respiratory or cardiovascular disease, alcohol abuse, or smoking. Before the study, all patients were weighed, the body mass index was calculated, and respiratory function was evaluated using spirometry (Table 1).

In all participants, the breath-holding test was performed in the morning before breakfast. The single-breath carbon dioxide test was performed the next day. A month after the first tests, these tests were repeated to evaluate their reproducibility. The study was approved by the local ethics committee. All subjects provided signed informed consent to both tests.

The single-breath carbon dioxide test was performed as follows. The participant's nose was clamped using a soft grip. Breathing through the mouth was monitored using a mouthpiece connected to a pneumatic respiratory valve separating the inhaled gas mixture from exhaled. The inspiratory port was connected to a T-shaped valve in such a way that ventilation is carried out either from a rubber bag or a 2 L tank, which was filled after each inhalation of the gas mixture containing 13% CO_2 or atmospheric air. After a brief period of quiet air breathing (approximately 5 min), in the expiratory phase the T-shaped valve was switched to breathing a mixture with high CO_2 content so that the next breath was taken using this mixture. The valve was then switched to atmospheric air. On average, 10 breaths of the hypercapnic mixture were taken with intervals of 2 min. Minute ventilation was determined from inspiration to inspiration (Volumeter Blease, United Kingdom). The CO_2 fraction in the exhaled mixture was measured using a sidestream gas analyser (Nihon Kohden, Japan). The average minute ventilation, calculated from the data of the last five breaths before breathing the hypercapnic mixture as the control (MVc). Likewise, the average $FetCO_2$ was determined during these breaths and used as the control $FetCO_2$ (c). The ventilation response to a hypercapnic stimulus was determined as the average of the two highest rates of MV (s) (the MV during the first 20 s after the stimulus were excluded to minimize the contribution of central chemoreception). Post-stimulus $FetCO_2$ (s) was also assessed during these cycles. The ventilation response to breathing a hypercapnic mixture was calculated by the formula: (MV (s) – MV (c))/(($FetCO_2$ (s) – $FetCO_2$ (c)) × (P_{atm} – 47)), where P_{atm} represents the atmospheric pressure in mmHg, and 47 is the saturated water vapour pressure in mmHg.

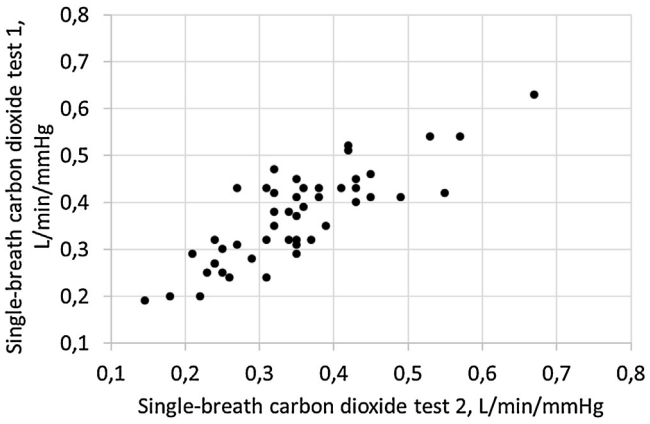

Fig. 1. The reproducibility of the single-breath carbon dioxide test.

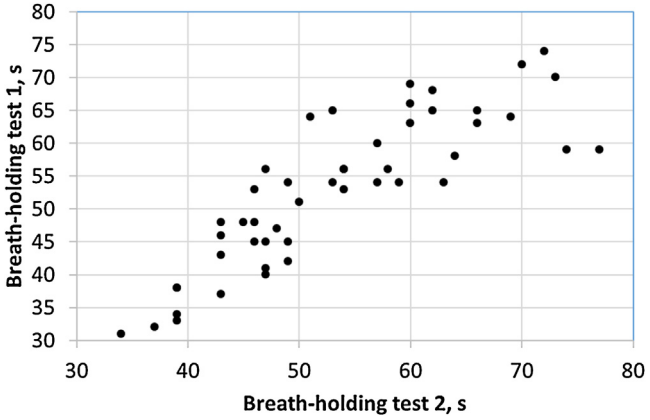

Fig. 2. The reproducibility of the breath-holding test.

The median of all 10 episodes was taken as the sensitivity of the peripheral chemoreflex, expressed in L/min/mmHg.

The breath-holding test was performed as follows: voluntary breath-holding duration was assessed three times, with 10 min intervals. After inspiration of a volume equal to 2/3 of the vital lung capacity, the participant was asked to hold their breath and the duration of voluntary apnoea was measured from the beginning of the voluntary inspiration until reflex contractions of the diaphragm were noted by palpation. A mean value of the duration of the three samples was calculated.

Normally distributed data are presented as mean ± standard deviation and non-normally distributed data as median (25th–75th percentile). To assess the relationship between the two methods, correlational analysis was performed. The coefficient of variation was also calculated to assess the reproducibility of the tests. The independent *t*-test was used to compare coefficients of variation.

3. Results

The average sensitivity of the peripheral chemoreflex measured with the single-breath carbon dioxide test was 0.35 ± 0.10 L/min/mmHg. The average breath-holding duration was 53 ± 10 s.

There was a positive correlation between participant height and the single-breath carbon dioxide test result ($r = 0.47$, $p < 0.05$). No correlation was found between this test and other characteristics. We also found a positive correlation between the breath-holding duration and vital lung capacity ($r = 0.64$, $p < 0.05$).

The reproducibility of the test is shown in Figs. 1 and 2. The average sensitivity of the peripheral chemoreflex mea-

Table 1
Characteristics of the test subjects.

Number of subjects	Age, years	Weight, kg	Height, cm	FEV_1 (% predicted)	VLC (% predicted)
48 (27 men)	27 ± 5	72 ± 4	169 ± 7	98 ± 4	102 ± 3

FEV_1 – forced expiratory volume, VLC – vital lung capacity. Data are presented as mean ± standard deviation.

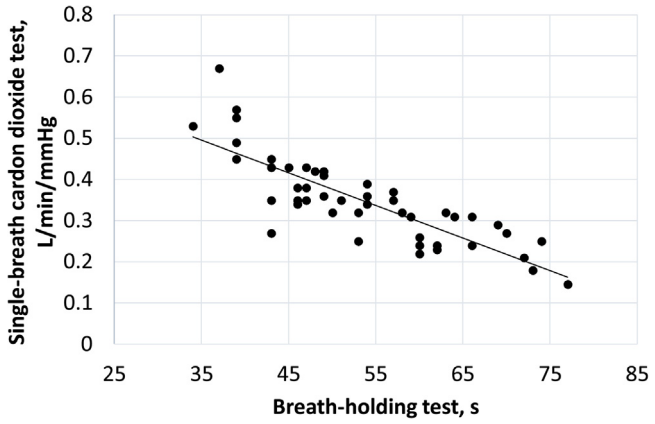

Fig. 3. Correlation between two tests.

sured with the second single-breath carbon dioxide test was 0.37 ± 0.10 L/min/mmHg. The average breath-holding duration was 52 ± 11 s The coefficient of variability in the single-breath carbon dioxide test ranged from 0 to 32% with a mean of 10 ± 7%. The coefficient of variability of the breath-holding test ranged from 0 to 19% with a mean of 6 ± 4% ($p < 0.05$).

During the correlation analysis a strong inverse correlation between the results of the two test paradigms was noted (-0.82, $R^2 = 0{,}68$, $p < 0.05$) (see Fig. 3). Linear correlation equation is $y = -0.00792\,x + 0.7726$.

4. Discussion

The results of repeated measurements indicate that both tests are reproducible, but that the results of the breath-holding test were significantly more reproducible. The single-breath carbon dioxide test was less reliable based on this parameter, but was also reproducible with a coefficient of variation of 10 ± 7%. Chua and Coats (1995) reported a 17.7% variability in this test's results (Chua and Coats, 1995), while McClean et al. (1988) recorded a 25% coefficient of variation in their work. However, it should be noted that these tests were performed in subjects of different ages (under 73 years), with the highest variability observed in older patients (McClean et al., 1988). Martinez (2008) studied the single-breath carbon dioxide test in older people and found a coefficient of variation of 36%. A study in patients with chronic heart failure (Maestri et al., 2013) identified the variability of this test as 20%. In general, the reproducibility of the single-breath carbon dioxide test is lower than the breath-holding test, which may be due to its greater technical complexity.

The correlation between the single-breath carbon dioxide test result and participant height observed in our study was consistent with the results obtained by Chua and Coats (1995), who also found a similar, albeit non-significant, relationship. This difference may be due to the fact that fewer subjects were involved in the previous study, potentially affecting the result. The results of our study demonstrated a positive correlation between the duration of breath-holding and vital lung capacity, which correlates with previously obtained data regarding the influence of lung volume on the duration of breath-holding (Whitelaw et al., 1987).

Our results show a strong inverse correlation between the results of two tests. The duration of breath-holding after deep inspiration is a function of several factors (Skow et al., 2015): chemoreception, mechanoreception (light stretching receptors), the impact of descending cortical respiratory drive, and a cognitive component. Of these, the first two are involuntary but the most important (Ilyukhina and Zabolotskikh, 2000; Parkes, 2006) The duration of breath-holding doubled during the breathing of the hyperoxic mixture or pre-hyperventilation (Klocke and Rahn, 1959). On the other hand, the breath-holding duration was reduced under hypoxemic and hypercapnic conditions (Godfrey and Campbell, 1969; Kelman and Wann, 1971). Given the above, the duration of breath-holding depends not only on the sensitivity of the peripheral chemoreflex, but also on baseline blood gas levels and metabolic rate, i.e. the rate of the gas exchange. Thus, the correlation found in healthy people may be somewhat different in situations that affect these factors (e.g. obesity, lung disease, hypothyroidism, hyperthyroidism, etc.). These situations and the relationship between the two tests in such conditions require further research.

5. Conclusion

A breath-holding test performed after a deep inspiration reflects the sensitivity of the peripheral chemoreflex as defined by the single-breath carbon dioxide test in healthy subjects. Due to its simplicity, reproducibility, and safety, it can be used routinely. Further studies are needed to determine the ability of this test in pathological conditions.

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