Original Article

Validation of the 4-Second Exercise Test in the Orthostatic Position

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Objective

To test the operational viability of and validate the 4-second exercise test (4sET) protocol in the orthostatic position (ORTHO).

Methods

The ORTHO protocol, an alternative to the conventional protocol (CYCLO), was used. The ORTHO protocol consists of performing sudden exercise in the orthostatic position – accelerated stationary walking (alternate upward flexion of the thighs) – from the fourth to the eighth second of a 12-second maximum inspiratory apnea, instead of rapid cycling without load. The adimensional cardiac vagal index (CVI) was calculated using the ratio between the longest RR interval (RRB) – the one immediately before, or the first during exercise – and the shortest RR interval during exercise – usually the last (RRC) – measured on electrocardiographic tracings at a 10-ms resolution. Forty-seven individuals (40±17 years, 169±9 cm, 72±14 kg) of both sexes, healthy or unhealthy, randomly underwent 3 consecutive repetitions of the 2 protocols, the first being performed only to acquaint patients with the procedure.

Results

Although differences in the CVI were found in both protocols (1.48±0.04 vs 1.42±0.04; P<0.001), no physiological relevance was observed. In 5 (11%) cases, a clinically significant difference between the ORTHO and CYCLO protocols was observed for CVI. The results of RRB, RRC, and CVI in the 2 protocols were strongly correlated, being 0.84, 0.85, and 0.93, respectively (P<0.001).

Conclusion

The 4sET performed in the orthostatic position proved to be a valid option for assessing the vagal cardiac tonus in laboratories lacking a cycloergometer, without jeopardizing clinical interpretation. In addition to simplicity and applicability, the procedure also provides low operational costs.

Key words

4-second exercise test, orthostatic position, exercise, vagal, heart rate

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Cardiovascular diseases are the major cause of mortality in Brazil, accounting for 32% of all recorded deaths and 10% of all hospital admissions in 2000 [1]. The risk of several cardiovascular events increases many times due to autonomic dysfunction, frequently caused by a reduction in parasympathetic activity [2], which is a strong and independent indicator of a negative prognosis [3] both in individuals with heart failure [4,5] and after infarction [6], and even in the general population [7].

There are several valid procedures to investigate the autonomic condition of an individual, providing relevant clinical or physiological contributions, or both [8-10], among which the 4-second exercise test (4sET), originally proposed by Araújo et al [11], stands out. The 4sET is based on eliminating the vagal cardiac tonus in the first seconds of rapid exercise involving large muscle groups. The 4sET is a pharmacologically validated autonomic test, whose finality is the isolated analysis of the vagal modulation of heart rate in the initial transient of dynamic exercise [12]. Since it was reported, 4sET has been applied in the clinical area in some sports medicine and cardiology laboratories, in addition to being used as an instrument of scientific investigation in the research developed by our group [11,13-18] and also by other research centers [19].

Despite the simplicity and low cost of the 4sET, and the easy interpretation of its results, the need for a cycloergometer remains a limitation for the broader use of the test according to the original protocol. Considering that a cycloergometer is not frequently found in specialized laboratories and clinics for exercise testing, it is necessary to analyze the possibility of performing 4sET under more appropriate conditions. This study aimed to test the operational viability of the 4sET performed in the orthostatic position without an ergometer and to validate a 4sET protocol.

Methods

The study comprised 47 adults of both sexes (40±17 years, 169±9 cm, 72±14 kg) with different clinical conditions, who randomly underwent 3 consecutive repetitions of 4sET in the 2 protocols suggested as follows: 1) the CYCLO protocol using a cycloergometer, according to the original 4sET [12]; and 2) the alternative ORTHO protocol, with 4sET performed in the orthostatic position. The first repetition in each protocol served to acquaint the individuals with the procedures, and the best result of the last 2 repetitions was considered to represent the vagal cardiac index of the individual. All participants signed the written consent before the tests. The protocol and the study design were approved by the institution.

Protocols

• The 4-Seconds Exercise Teste - (CYCLO)

The objective of the 4sET is to assess in isolation the integrity of the parasympathetic branch of the autonomic nervous system in the initial transient of heart rate (rest-exercise transition). The conventional 4sET consisted of cycling, as fast as possible, an unloaded cycloergometer, from the fourth to the eighth second of a maximum inspiratory apnea of 12 seconds. The patients were commanded to act every 4 seconds as follows: a) to inspire maximally and rapidly, primarily through the mouth; b) to cycle as fast as possible; c) to stop abruptly; and d) to expire.

To minimize occasional anticipatory responses to the commands, the individual should see neither the chronometer nor the electrocardiograph, which provides a continuous tracing of a single electrocardiographic lead (usually CC_5 or CM_5) during 35 seconds, at a velocity of 25 mm/s with a 10-ms resolution, initiated 5 seconds before the maximum inspiration command.

To determine the magnitude of the vagal tonus, the longest RR interval (RRB) – the one immediately before, or the first during exercise – and the shortest RR interval during exercise – usually the last (RRC) – are identified. The ratio between these 2 intervals indicates the cardiac vagal index, an adimensional index, obtained through 4sET.

Previous studies showed that the magnitude CVI does not depend on the presence or absence of resistance to the movement of the pedals [13], on active or passive practice [15,16], or whether the test is performed with the lower or upper limbs [20].

• The Osthostatic 4-Seconds Exercise Test - (ORTHO)

To reproduce the conditions probably encountered in the conventional exercise physiology and ergometry laboratories, the individuals with electrocardiographic monitoring were positioned on a treadmill, which was kept powered off during the entire procedure. This strategy aimed at facilitating the establishment of a routine for the procedure, such that it preceded conventional exercise testing.

To trigger the heart rate response in the ORTHO 4sET, accelerated stationary walking was performed, ie, alternate flexion of the thighs, with bent knees, until the thigh reached an angle of approximately 90º with the trunk. The exercise was performed without a flight phase, ie, the feet did not simultaneously loose contact with the ground at any given time. For greater equilibrium, the individual being tested was asked to keep his hands on the frontal safety bar of the treadmill.

The interval between the repetitions of the same protocol lasted 1 to 2 minutes, and the interval between the 2 protocols lasted 5 to 10 minutes, depending on heart rate behavior at rest, and waiting for heart rate to return to premaneuver levels before repeating the protocol.

For the CYCLO protocol, an EC-1600 cycloergometer (*Cateye, Japan*) was used, while the ORTHO protocol was performed on an ATL 10200 treadmill (*Inbramed, Brazil*), kept powered off during the entire experiment. In both protocols, for the electrocardiographic recording, equipment and specific digital electrocardiography software (ErgoPC Elite, version 3.2.1.5, *Micromed*, Brazil) were used, allowing storage of the tracings and measurement of the RR intervals with 10-ms accuracy. All measurements were taken by a single evaluator with much experience in the technique of identification of the RRB and RRC intervals and in the measurement of their durations with the aid of the software.

In addition to analyzing the sample as a whole, the responses were assessed whether they depended on the magnitude of the CVI or on the clinical condition. The sample was divided into 2 groups, according to the CVI of the CYCLO protocol (reference value), using the median as the cut point. Therefore, the existence of a difference in the CVI between the protocols for the lower and upper values of the distributions was assessed. Finally, to evaluate the possible influence of the clinical condition on occasional differences in the CVI obtained in the 2 protocols, the sample was divided into an asymptomatic cardiorespiratory group and a group with known cardiovascular disease.

Initially the normality (Kolmogorov-Smirnov) and homoscedasticity of the distribution (Hartley test) were tested, validating the use of parametrical statistics. The paired Student *t* test was used to compare the results of the 2 protocols, and the Pearson correlation was used to quantify the association of the RRB and RRC variables (both measured in ms) and the CVI (adimensional) in the ORTHO and CYCLO protocols. The linear regression between the CVI values was also determined in the 2 protocols, and the significance level of $P<0.05$ and 95% confidence interval were adopted. The statistical software *SPSS* version 10.0 (*SPSS*, Chicago, USA) was used.

Results

All variables had homoscedasticity, with ratios between the variances of both protocols, values below 1.69 (cut point for 46 degrees of freedom), and also normal distribution ($P>0.10$), justifying the parametrical analysis of data.

In only 5 (11%) cases, a relevant clinical difference was observed in the results of the CVI in the 2 protocols, according to the original cut points of 4sET for indicating autonomous dysfunction (<1.20) and vagotonia (>1.70). Two of these cases were close to the limits (1.11 vs 1.22 and 1.32 vs 1.18, for CYCLO vs ORTHO, respectively). In only 13 (27.7%) cases, the difference exceeded the standard error of the estimate calculated in the analysis of regression (±0.14). A small difference between the results was observed in the CVI and in RRB, but not in RRC (tab. I). The

Table I - Comparison between the results of the 4-second test in the 2 protocols for each variable

Variable	CYCLO	ORTHO	P	95%CI
CVI	1.48 ± 0.04 (1.00 - 2.40)	1.42 ± 0.04 (1.05 - 2.27)	0.008	0.02 a 0.11
RRB (ms)	917±25 (670 - 1380)	879±24 (600 - 1230)	0.007	11 a 64
RRC (ms)	638 ± 20 (460 - 1000)	636 ± 18 (460 - 1030)	0.741	-13 a 18

The results are shown as mean ± standard error of the mean (minimum and maximum).

correlation coefficients r=0.84; 0.85; and 0.93, were found for the CVI, RRB, and RRC, respectively, measured in the 2 protocols, (P<0.001 for all), as was the linear regression equation $CVI_{CYCLO} = 0.73 \bullet CVI_{ORTHO} + 0.33 \pm 0.14$ (fig. 1, 2, and 3).

When the sample was divided into 2 groups according to the CYCLO CVI (reference value), the differences in the CYCLO and ORTHO CVI between the individuals with lower CVI (1.26±0.03 both for CYCLO and ORTHO; P = 1.00; 95%CI = -0.06 to 0.06) disappeared. In the other half of sample individuals with higher CVI, the results were significantly greater in the CYCLO protocol (1.71±0.04 vs 1.58±0.05; P<0.001; 95%CI = 0.06 to 0.20).

In regard to the influence of the clinical condition on the results, the individuals with known cardiovascular disease, as expected, had a lower CVI as compared with that of the asymptomatic individuals in both protocols (tab. II). A strong association between the results of the CVI in the 2 protocols was observed in the group with cardiovascular disease and the asymptomatic group (r=0.79 vs r=0.84, respectively, P<0.001).

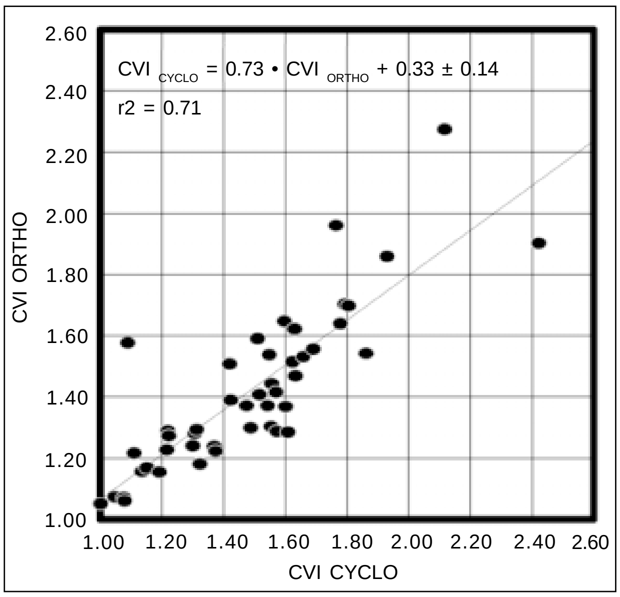

Fig. 1 - Individual results of CVI in the ORTHO and CYCLO protocols.

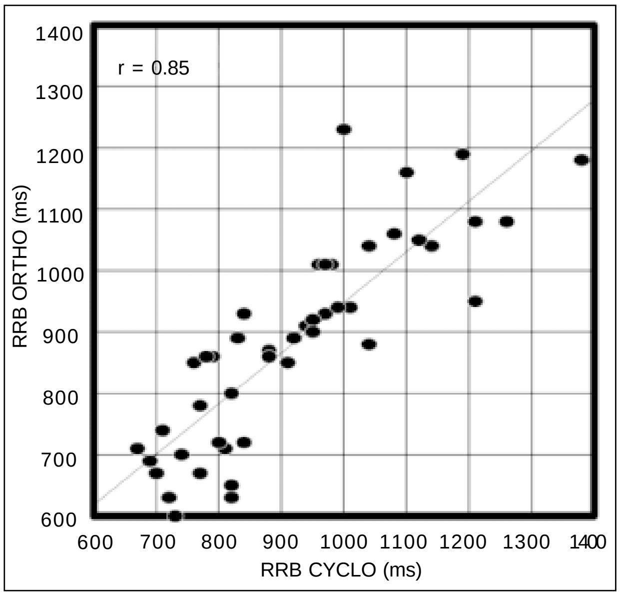

Fig. 2 - Individual results of RRB in the ORTHO and CYCLO protocols.

Discussion

This study aimed at testing the operational viability of and validating the 4-second test protocol performed without a cycloergometer, facilitating, therefore, its use in conventional ergometry laboratories. A simple explanation, followed by a single attempt, was sufficient for the individuals to successfully follow the ORTHO protocol with no problem or accident, even considering that some were older than 50 years. Validating a measuring instrument requires comparing the results of the protocol proposed with those of the reference protocol (gold standard).

Despite the difference found between the CVI in the 2 protocols, a trend towards the results physiologically expressing the same phenomenon was observed, with CVI values minimally greater in the CYCLO protocol (fig. 1), clearly due to a longer RRB (fig. 2). The posture adopted in the ORTHO protocol is the most probable reason for the difference found in the RR[3] intervals in the premaneuver inspiration phase. Sustaining the body in the erect position induces lower vagal stimulation [21], possibly as a consequence of the more important action of gravity, impairing venous return, as compared with the sitting position in the cycloergometer [22,23]. However, it is worth emphasizing that the RRC measures were equal in both protocols (fig. 3), indicating that, even without using the ergometer, the movement proposed in the ORTHO protocol (accelerated stationary walking) was sufficient to trigger the same magnitude of vagal inhibition in heart rate modulation, probably due to the action of the mechanoreceptors located in the articulations [24].

The clinical condition of the individuals apparently did not interfere with the results of the ORTHO protocol, because the group with known cardiovascular disease had similar CVI values in the 2 protocols. Such situation was not observed in the asymptomatic group, although these differences were not physiologically expressed or were not clinically relevant, because the difference between the means again did not exceed the standard error of the estimate. The correlation coefficients of both clinical conditions were high for the CVI, showing a strong association between the protocols.

From the practical point of view, considering the magnitude of

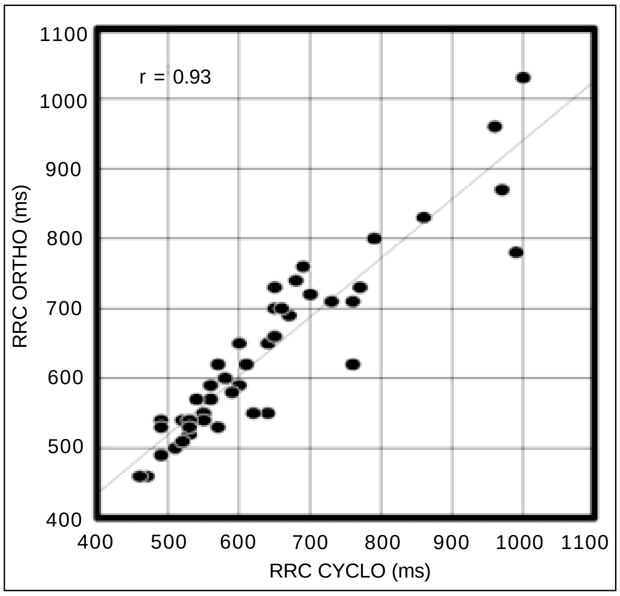

Fig. 3 - Individual results of RRC in the ORTHO and CYCLO protocols.

Table II - Cardiac vagal index measured in the 4-second test in 2 different protocols in individuals with known cardiovascular disease and in asymptomatic individuals

Clinical condition	CYCLO	ORTHO	P	95%CI
No cardiorespiratory symptoms	1.65 ± 0.06 (1.22 - 2.42)	1.54 ± 0.06 (1.18 - 2.27)	0.006	0.03 a 0,18
Cardiovascular disease	1.35 ± 0.05 (1.00 - 1.80)	1.32 ± 0.04 (1.05 - 1.70)	0.313	-0.03 a 0,01

The results are shown as mean ± standard error of the mean (minimum and maximum).

the association between the RRB, RRC, and CVI variables in the 2 protocols and the occurrence of only a reduced number of cases with relevant differences in assessing the vagal cardiac tonus, we suggest that the ORTHO protocol is clinically valid. It uses the same cut points of the initial proposal of the CYCLO protocol, which are especially true for the cases where a relative or abnormally low CVI occurs. These cases are of greater clinical interest, as the CVI values are virtually identical. On the other hand, the individuals with greater CVI had a discrepancy, although the difference between the means remained within the standard error of the estimate determined by the regression equation. In reality, the occasional discrepancies found in the CVI values between the ORTHO and CYCLO protocols were almost fully limited to individuals who could be characterized as having vagotonia, with extremely high CVI values (fig. 1). In addition, the regression equation showed a high predictive value for measuring the CVI in the 2 protocols, therefore, confirming the consistency of the instrument proposed for assessing the CVI.

Two cases with extremely discrepant results when the CVI values were compared between the protocols deserve to be commented on (fig. 1). One was a 72-year-old male who did not respond to the CYCLO protocol in the same magnitude as to the ORTHO protocol (1.09 vs 1.58, respectively). This could have been due to poor adaptation to the cycloergometer, with difficulty in cycling at the minimum necessary frequency to trigger the vagal inhibition expected in 4sET. In reality, in our laboratory, we have occasionally helped in the movement of the legs during cycling in the elderly or sarcopenic individuals, who have difficulty in performing the test. The other case was a 29-year-old female, who, although diagnosed as extremely vagotonic in both protocols (result > 99th percentile in our laboratory's database), had a better response in the CYCLO protocol with an expressive numeric difference between the results (2.42 vs 1.90, in the CYCLO and ORTHO protocols, respectively), but with no clinical significance. This individual finding is in accordance with our experience, indicating a smaller reliability of the CVI values in vagotonic individuals[25]. Therefore, within a more generic context, the results of CVI found in our study confirm the validity of the ORTHO protocol, without the need for using a correcting equation.

In conclusion, 4sET in the orthostatic position proved to be viable from the operational point of view and valid for assessing the vagal cardiac tonus in the exercise physiology and ergometry laboratories that lack a cycloergometer, without jeopardizing the clinical interpretation of the asymptomatic individuals or those with a cardiovascular disease. It is also worth emphasizing the simplicity and applicability of the procedure, in addition to its low operational cost, and we also suggest that the test should be performed on a treadmill powered off prior to exercise testing to facilitate the procedure and its performance in the routine of the laboratories.

Recent guidelines of the Brazilian Society of Cardiology [26,27] have emphasized the use of maximum exertion as an instrument of clinical investigation. The routine incorporation of the 4sET before exercise testing [28] – conventional or with measurement of the expired gases – has the potential to increase the clinically relevant information to be obtained with the use of physical exercise in healthy or unhealthy individuals [29-32].

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