Intra and interdays reliability of the 4-second exercise test

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ABSTRACT

The 4-second exercise test (T4s) is pharmacologically validated to assess cardiac vagal tone, and consists in pedaling, as fast as possible, a cycle-ergometer unloaded, from the 4th to the 8th second of a 12-second maximum inspiratory apnea. An dimensionless cardiac vagal index (CVI) is calculated from the ratio of the duration of the cardiac cycles (RR intervals at the electrocardiogram) from immediately before the exercise and the shorter of the exercise. Our objective was to determine T4s intra and interdays reliability, and the actual need for two trials, as described on the original protocol. In study 1, the interday reliability of the results was assessed prospectively from 15 asymptomatic subjects (28 ± 6 years) submitted to T4s for five consecutive days, being two trials carried out at each day. To determine CVI intraday reliability, in one of the five days, randomly selected, nine T4s consecutive trials were made. In study 2, the CVI intraday reliability was calculated from 1699 subjects (47 ± 17 years) in two trials. CVI presented high intraday and interday reliability (r_i = 0.92; 95%CI = 0.84 to 0.97 and r_i = 0.77; 95%CI = 0.49 to 0.92, respectively) for study 1 and for study 2 (r_i = 0.89; 95%CI = 0.88 to 0.90). In spite of high reliability, there were some minor differences between the means (mean ± SEM = 1.32 ± 0.01 vs 1.37 ± 0.01; $p < 0.001$), and in only 15% of the cases this difference was higher than 0.20, thus not representing major clinical meaning. It was also observed that in 65% of the cases, the second trial was considered the best and, with only one trial, clinical misinterpretation could occur in 27% of the data. In summary, this study evidenced high CVI reliability assessed by T4s, and confirmed the need for two consecutive trials, as prescribed in its protocol.

Key words: 4-second exercise test. Physical exercise. Heart rate. Cardiac vagal tone. Reliability.

INTRODUCTION

The behavior of heart rate (HR) in exercises transient has been investigated in a number of studies over the past few years[1-4], which shows the importance of its clinical and physiological investigations. HR modulation is mediated by the sympathetic and parasympathetic branches of the autonomous nervous system (ANS), and its integrity is associated to a decrease in mortality risk from cardiac events[5,6].

In a study carried out by Nolan *et al.*[7], it was seen that patients with heart failure (moderate and severe), with autonomic dysfunction, had a mortality rate 10-fold higher than their peers whose parasympathetic activity was closer to normal ranges. These results confirm the impression that cardiac vagal activity is a powerful and independent prognostic marker[5].

A number of physiological and clinical procedures have been proposed to assess autonomic condition[8-11]. Among them, one can mention the 4-second exercise test (T4s), originally proposed by Araújo *et al.*[12]. The T4s is intended to indirectly assess cardiac vagal tone through the initial heart rate transient of a dynamic short term exercise performed in apnea[13]. This test was pharmacologically validated and has been applied in clinical trials and sports medicine, proving to be quite useful both as a diagnostic tool[14,15], and longitudinal follow-up of cardiac parasympathetic activity[16].

Thus, in order to consolidate this procedure, it is important to determine T4s consistency and reliability in assessing cardiac vagal autonomic activity of a heterogeneous sample. Therefore, the purposes of this study were to determine intra and interdays T4s reliability in measuring cardiac vagal tone, and the need for two measurements, as described in the original protocol.

MATERIALS AND METHODS

To meet the intended purposes, the results from two different labs, presented separately, were assessed, looking

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Received in 12/7/03

Approved in 22/9/03

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for better understanding of the experimental procedures used. Furthermore, all tests and their measurements and interpretations were performed by experienced evaluators in the T4s protocol, and strictly followed its standards. The subjects submitted to the T4s read and signed an informed consent form before the procedures.

The 4-second Exercise Test – T4s

The purpose of the T4s is to assess exclusively the integrity of the parasympathetic branch of the autonomic nervous system through the initial HR transient at rest-exercise transition. The T4s consists in pedaling as quickly as possible a unloaded cycleergometer, from the 4th to the 8th second of a maximum inspiratory apnea of 12 seconds. Four consecutive commands are given at each four seconds: (a) a maximum and swift inhale through the mouth and then sustain apnea; (b) to pedal as fast as possible; (c) suddenly stop pedaling, and (d) normally exhale.

To minimize anticipatory responses to the commands, the subject should not see the chronometer nor the electrocardiograph, while a continuous trace is recorded from only one ECG lead (typically CC_5 or CM_5) for 35 seconds at a speed of 25 mm/s, started five seconds before the command for maximum inhale.

To determine the magnitude of the cardiac vagal tone, one must identify the immediately before or the first RR interval of the exercise (which is the longest) (RRB), and the shortest one during the exercise, usually the last (RRC). The quotient or ratio between these two intervals shows the cardiac vagal index (CVI), a T4s dimensionless index.

Two consecutive trials are made with a one-to-two-minute interval between them, which is typically enough for heart rate to return to resting level, being selected the highest of the two CVI values as representative of the subject's cardiac vagal tone.

Prior methodological studies with T4s showed that CVI value independs on the presence or absence of resistance opposing pedal movement[17], if the exercise is performed actively or passively[18], or if performed by lower or upper limbs[19].

Study 1

To assess T4s reliability, we made a prospective analysis of the results from 15 subjects (eight women), and 13 subjects (six women), for intra and interdays, respectively. The subjects were considered asymptomatic, aged 28 ± 6 (21 to 42) years, who volunteered for the study carried out in a lab.

Data were collected from a 380B electrocardiograph (*Siemens*, Germany), from a single lead (CC_5), at a speed of 25 mm/s, and T4s was performed in a mechanic-breaking cycle-ergometer *Monark*. Measurement of the RR intervals on the recordings was made by a single evaluator, experienced in the test, with a 10 ms resolution.

The subjects were asked to rest in a room for about five minutes prior to the beginning of the test protocol. T4s was repeated for five days, always at the same time of the day, in order to measure interdays reliability. Intraday reliability was investigated in nine consecutive trials carried out in one of those five days, randomly selected for each subject.

Study 2

In a second assessment, intraday reliability was retrospectively determined from the results of 1,699 subjects (613 women), aged 47 ± 17 (8 to 85) years, including subjects of different clinical and fitness conditions (even athletes), who spontaneously went for a detailed medical-functional assessment in one of the labs between 1994 and 2003.

For this analysis, a TEC 7100 electrocardiograph (*Nihon-Kohden*, Japan) was used in the tests carried out until 2001, and a digital electrocardiograph with a specific software (ErgoPC Elite version 3.2.1.5. *Micromed*, Brazil), in the tests from 2001 on, both in single lead (CC_5 or CM_5), recorded at a speed of 25 mm/s, and an electro-magnetic-break cycle-ergometer Cateye model Ergociser EC 1600 (*CatEye*, Japan). The measurement of the duration of the RR intervals on the electrocardiograph recordings were made by experienced evaluators, using a 10 ms resolution.

In order to assess T4s reliability in subjects with different magnitudes of vagal tone, we sorted the values as a function of the CVI from the second trial, and then used cut-off points arbitrarily defined in our database, of less than 1.20, between 1.20 and 1.70, and higher than 1.70 for vagal hypotonic, normal, and hypertonic (vagotonic), respectively (unpublished data).

Statistical analysis

Intraclass correlation coefficient was used in the studies to measure the degree of association between the trials. Furthermore, to compare the means, ANOVA for repeated measures, and paired t-test (for study 1 and study 2, respectively) were employed. Significance level of 5% and 95% confidence interval was used for all results.

RESULTS

Study 1 – The CVI obtained in the T4s showed high intra and interdays reliability, as one can see from the intraclass correlation coefficients (r_i = 0.92; 95%CI = 0.84 to 0.97 and r_i = 0.77; 95%CI = 0.49 to 0.92, respectively). These results were confirmed by ANOVA, and no differences between the trials were found ($p > 0.10$) (figures 1 and 2).

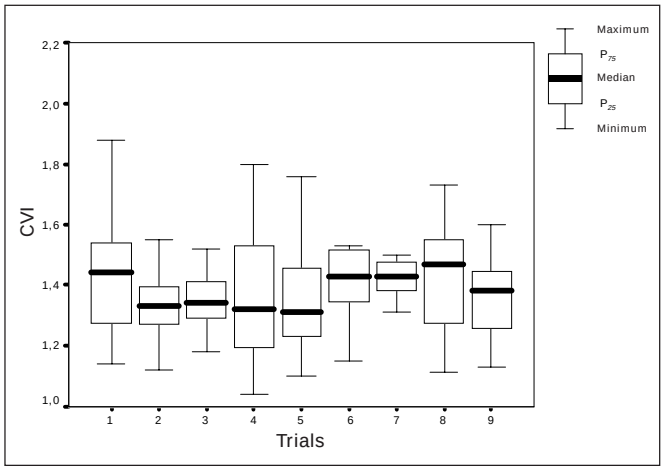

***Fig. 1** – Study 1 intraday reliability*

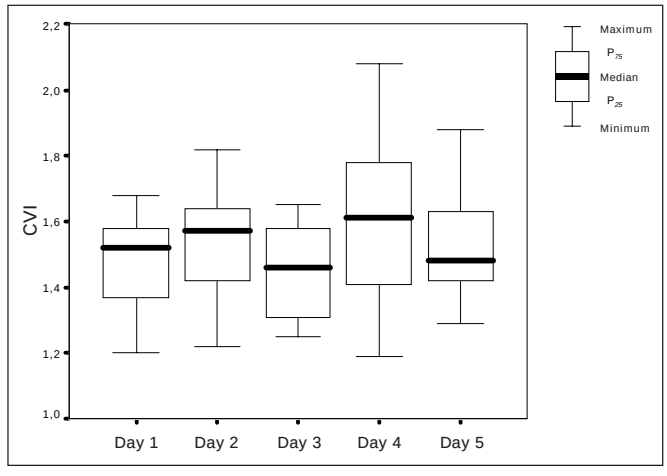

***Fig. 2** – Study 1 interdays reliability*

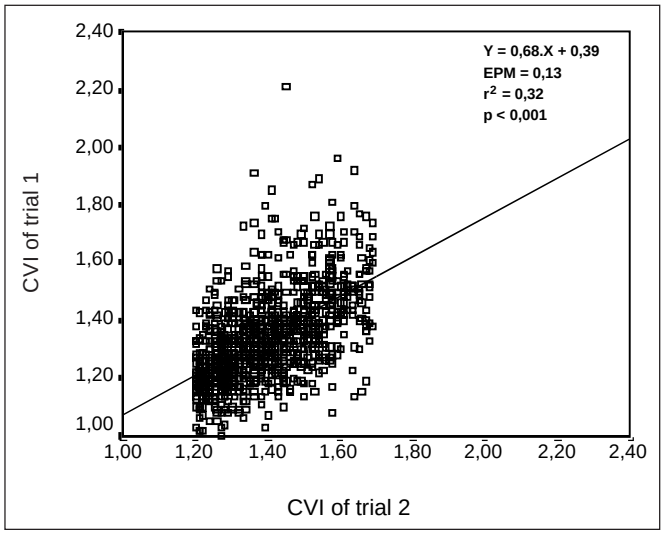

***Fig. 3** – Association between two consecutive T4s trials for values of subjects classified as normal – 1.20 to 1.70 – in the second trial (study 2)*

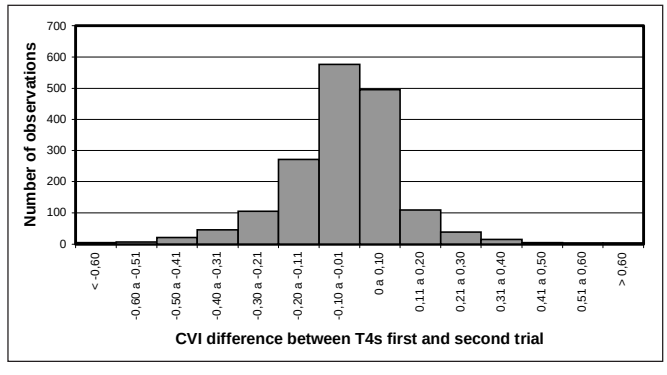

***Fig. 4** – Differences of CVI between trials (study 2)*

Study 2 – In a larger and more heterogeneous sample, the CVI between the two T4s trials was considerably associated (r_i = 0.89; 95%CI = 0.88 to 0.90), attesting once more its reliability (figure 3). However, the t-test showed differences between the means (mean ± SEM = 1.32 ± 0.01 vs 1.37 ± 0.01; p < 0.001), and in only 15% this difference was higher than 0.20 (figure 4). We also noticed that in 65% of the observations, the second trial had higher CVI results.

Likewise, by splitting the sample in three groups according to reference values from the second trial (selected for including most of CVI best results), we observed differences between the two trials for all groups (table 1). However, the number of cases in which the difference between trials was higher than 0.20 was lower than 1% for hypotonic, 9% for normals, and 5% for hypertonic subjects, being worth to mention that intraclass correlation coefficient had a significant association between the trials, in each group (p < 0.001). Moreover, 27% of the subjects would have been mistakenly classified if only one trial was to be made.

DISCUSSION

HR behavior is an important marker of the cardiac vagal activity, which, if decreased, is strongly associated to mortality risk[2,3,6,20-22], showing an increase of cardiac vulnerability due to a potential and lethal risk of ventricular arrythmia[5,23,24].

The clinical contributions of autonomic assessment in stratifying mortality risk from cardiovascular events and from all causes have been broadly used by the scientific community[25-28], particularly because vagal activity is the main autonomic dysfunction marker[29-32]. Thus, it is inter-

TABLE 1
Intraclass reliability for each group, according to cardiac vagal index (CVI) of the second trial

Groups	n	Trial 1 Mean ± SEM (min-max)	Trial 2 Mean ± SEM (min-max)	Intraclass correlation r_i (CI 95%)	t-test *p* (CI 95%)	CVI difference higher than 0.20
Hypotonic	502	1.14 ± 0.004 (1.00-1.82)	1.12 ± 0.002 (1.00-1.19)	0.51 (0.41 a 0.58)	0.001 (0.02 a 0.03)	1
Normal	1,004	1.34 ± 0.01 (1.00-2.21)	1.39 ± 0.004 (1.20-1.69)	0.72 (0.68 a 0.75)	0.001 (0.06 a 0.04)	9
Hypertonic	193	1.71 ± 0.02 (1.16-2.97)	1.89 ± 0.01 (1.70-2.65)	0.67 (0.56 a 0.75)	0.001 (–0.22 a –0.15)	5

esting to stress the importance of a valid and reliable test to assess cardiac vagal tone.

As we could see from study 1, the measure of the vagal cardiac tone by T4s showed high intra and interdays reliability, evidencing T4s consistency to assess vagal cardiac function, expressed by CVI. It is also important to mention that ANOVA confirmed these results, with no differences found among the sample means.

Study 2 was based on a very large sample, and again CVI obtained in T4s was highly reliable, in spite of the diverse clinical conditions and populations (children, adolescents, adults, elders, athletes, even of Olympic levels, and asymptomatic non-athletes).

By confronting our results with those of other reliability studies on cardiovascular autonomic tests, we observed some important aspects that favored our T4s studies, such as: the size of the sample, as studies that investigate such topic have a significantly smaller number of subjects in their sample[33,34]; the characteristics of the sample (age group and clinical conditions)[35,36]; and the magnitude of intraclass correlation coefficients, similar to some and higher than others[37,38].

One should also add that, regarding study 2, we observed differences between the first and second trial (t-test), for the whole sample and for the subgroups, even though these results do not indicate clinical or physiological relevance. This fact may be observed from the number of cases in which discrepancy between trials was higher than 0.20, particularly among subjects referred as vagal hypotonic, in whom a possible association with cardiovascular conditions and complex arrhythmias due to a lower vagal cardiac protection draws attention[28,39-41].

In practice, the physician, when supervises the procedure, frequently advises the subject to correct minor distortions or errors from the first trial, so that better results from the second trial of study 2 are to be expected. In fact, about 2/3 of the subjects achieve higher CVI values in the second trial, probably because they are more familiar with the procedure, and thus perform it more appropriately. Furthermore, the second trial prevented more then 1/4 of the subjects to have their CVI wrongly classified or interpreted. These results prove the need for two trials, as described in the original protocol.

T4s seems to comply with scientific authenticity criteria (reliability and validity), considering the clinical conditions and the significant diversity of the sample, as we could see in this and in prior studies[13,15]. Moreover, the simplicity and applicability of this test should be stressed, in addition to its low operational cost.

Thus, incorporating T4s in the routine of pre-exercise test – either conventional or with exhaled gases measurements[42] –, has the potential ability to wide open the clinically relevant information to be obtained with the use of physical exercise in health or unhealthy subjects.

In summary, this study evidenced T4s reliability in assessing cardiac vagal tone, expressed by the CVI, and confirmed the need for two consecutive trials, as recommended in its protocol.

ACKNOWLEDGMENTS

The authors thank Prof. Flavia Dias de Oliveira for her role in the data collection of study 1.

All the authors declared there is not any potential conflict of interests regarding this article.

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