4-Second Exercise Test: Reference Values for Ages 18–81 Years

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Abstract

Background: Physiological reflexes modulated primarily by the vagus nerve allow the heart to decelerate and accelerate rapidly after a deep inspiration followed by rapid movement of the limbs. This is the physiological and pharmacologically validated basis for the 4-s exercise test (4sET) used to assess the vagal modulation of cardiac chronotropism.

Objective: To present reference data for 4sET in healthy adults.

Methods: After applying strict clinical inclusion/exclusion criteria, 1,605 healthy adults (61% men) aged between 18 and 81 years subjected to 4sET were evaluated between 1994 and 2014. Using 4sET, the cardiac vagal index (CVI) was obtained by calculating the ratio between the duration of two RR intervals in the electrocardiogram: 1) after a 4-s rapid and deep breath and immediately before pedaling and 2) at the end of a rapid and resistance-free 4-s pedaling exercise.

Results: CVI varied inversely with age ($r = -0.33$, $p < 0.01$), and the intercepts and slopes of the linear regressions between CVI and age were similar for men and women ($p > 0.05$). Considering the heteroscedasticity and the asymmetry of the distribution of the CVI values according to age, we chose to express the reference values in percentiles for eight age groups (years): 18–30, 31–40, 41–45, 46–50, 51–55, 56–60, 61–65, and 66+, obtaining progressively lower median CVI values ranging from 1.63 to 1.24.

Conclusion: The availability of CVI percentiles for different age groups should promote the clinical use of 4sET, which is a simple and safe procedure for the evaluation of vagal modulation of cardiac chronotropism. (Arq Bras Cardiol. 2015; 104(5):366-374)

Keywords: Exercise Test/drug effects; Heart Rate; Adult; Autonomic Nervous System.

Introduction

The existence of vagal modulation of cardiac chronotropism has been known since the late 19th century. Studies conducted between 1900 and 1935[1-3] investigated the mechanisms of this modulation at rest and during exercise and were supplemented independently in 1966 by clinical and physiological tests conducted by Jose[4] and Robinson et al.[5] In these studies, patients underwent consecutive or concurrent infusion of atropine and propranolol, which caused pharmacological denervation and allowed the intrinsic heart rate (HR) of each individual to be determined. However, the correlation between decreased vagal modulation and higher risk of ventricular fibrillation and cardiovascular death[6,7] stimulated the interest in the assessment of cardiac autonomic function. Since then, several strategies and techniques have been proposed to assess autonomic function[8-10], particularly of the parasympathetic component, although assessment of this function has not become routine in clinical practice.

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In 1985, Araújo[11] showed that fast rest-exercise-rest HR transitions were strongly dependent on vagal activity. Later studies[12-14] found that in the first 4 s of a rapid exercise involving large muscle groups, the increase in HR was blocked by atropine but not influenced by propranolol and indicated vagal deactivation that is predominant at rest as the mechanism involved in this physiological response to exercise. These experiments validated the 4-s exercise test (4sET)[13,14] as a simple and non-invasive technique for assessing vagal modulation of chronotropism in the rest-exercise transition, and it has been routinely applied in clinical practice since then[15-19]. The aim of this study was to present the reference values for 4sET which were generated over the last 20 years in a large sample of healthy adults.

Methods

Sample

Data from 7,566 individuals evaluated between January 1994 and June 2014 in a private clinic specializing in exercise and sports medicine were revised. Individuals volunteered for assessment, almost always at the request of their doctors. This evaluation included anamnesis, physical examination, electrocardiography, resting spirometry, evaluation of aerobic and non-aerobic variables of physical ability, a cardiopulmonary exercise test, and 4sET. For the present study, only data from

4sET, anamnesis, and physical examination were included, with emphasis on the clinical conditions and regular or recent use of medications with potential effects on the autonomic nervous system. The inclusion criteria were as follows: a) age ≥ 18 years, b) absence of diseases or clinical abnormalities and/or regular or recent use of medications affecting the cardiopulmonary system and/or capable of interfering with the autonomic nervous system, as assessed by anamnesis and physical examination, c) absence of obesity, characterized by a body mass index < 30 kg/m^2, d) normal blood pressure and normal HR at rest and/or before performing 4sET, e) not being registered as an athlete in any sports federations at the time of evaluation, and f) availability of data and history related to the proper execution of 4sET. After applying the above criteria, a large sample of 1,605 healthy adults (61.2% men) aged between 18 and 81 years was selected. All subjects signed a free and informed consent form prior to evaluation, and the retrospective data analysis was approved by the Research Ethics Committee of the Institution.

4-s exercise test

After monitoring the CM5 or CC5 leads using a conventional electrocardiograph until 2001 (model TEC-7100K, Nihon Khoden, Japan) and a digital device since then (model Elite PC, Micromed, Brazil), each participant was properly seated on a lower-limb cycle ergometer (model Cateye EC-1600, Japan, or Inbrasport CG-04, Brazil), with the saddle and handlebar positions adjusted to increase comfort and efficiency during pedaling. Feet with adequate shoes were placed on pedals containing toe-clip strips for attachment and were positioned so that one foot, chosen by the participant, would be placed in a higher position to ensure better conditions to begin pedaling.

After waiting for the HR to return to a level similar to that measured in the resting 12-lead ECG performed in the supine position, 4sET was performed. The procedure consisted of four steps performed at the verbal command of the examiner and followed clearly defined time intervals: 1) a quick maximal inspiratory apnea was performed and maintained for 12 s, 2) at the end of the 4th second of apnea, the participant would pedal as fast as possible for 4 s (until the end of the 8th second) without any resistance being added to the cycle ergometer (powered off position), 3) at the end of the 8th second, leg movements would be interrupted rapidly and completely, with the patient still in apnea, and 4) at the end of the 12th second, the patient would exhale and breathe freely again. Before the exercise, the evaluator explained the procedure and emphasized two points: a) inspiration should be performed as quickly and deeply as possible through the mouth and b) the patient should attempt to pedal as soon as possible without getting up from the saddle during the 4-s exercise. Because the exercise was performed with virtually free pedals and without adding resistance (cycle ergometer in the powered off position), pedaling quickly was quite simple. At least five pedaling motions (75 rpm or 1.25 Hz) should be performed in 4 s so that the physiological response could be determined correctly without the influence of higher rates of pedaling on the final result. This sequence was repeated until two technically correct exercise cycles had been performed and to ensure that the period between each cycle would be sufficient for the HR to return to a value similar to or equal to that observed before the first cycle, generally less than a minute. To minimize any anticipatory responses to the commands, subjects were positioned so they could not see the timer on the computer screen.

Measurement of Cardiac Vagal Index (CVI)

The 35-s electrocardiographic recording was conducted at 25 mm/s and initiated 5 s before the examiner's first verbal command. The four verbal commands were given at 5, 9, 13, and 17 s after the initiation of the ECG recording, respectively. The measurement of cardiac vagal index (CVI), a dimensionless variable that reflects the loss of vagal tone induced by rapid exercise, corresponds to the ratio between the lengths of two RR intervals as follows: a) RRB interval: immediately before exercise (or the 1st RR interval of the exercise, whichever was longer) and b) RRC interval: the shortest interval during exercise, more typically the last.

To quantify the duration of the RR intervals, the examiner opened the electrocardiogram or the computer screen with the electrocardiographic data and identified and measured the duration of the RRB and RRC intervals, with a resolution of 10 ms, using the electrocardiogram or the resources of the ECG recording software, for each 4sET cycle performed. The identification of RRB and RRC intervals is generally facilitated by the presence of artifacts in the electrocardiographic profile caused by the rapid movement of the legs (Figure 1). The result with the highest CVI among the exercises performed was used for subsequent analysis. In approximately 95% of the cases, the duration of RR intervals and consequently the CVI was identified and measured by only four physicians widely trained in 4sET protocols (five other physicians collected the remaining 5% of the data) over the 20-year study period.

Statistical analysis

Four approaches were adopted: a) global descriptive analysis of the various data obtained, b) analysis of the distribution of CVI data according to age and gender, c) analysis of the correlation between the variables and linear regressions between CVI and age, and d) establishment of reference values with different cutoffs as percentiles for the different age groups. For statistical analysis, we considered only the highest CVI result observed in the exercises performed by each participant.

Despite the availability of a relatively large number of cases for each age group evaluated and considering the biological aspects of the CVI measurement, with the theoretical limitation of a minimum value of 1 and with no limitation on the maximum value, distribution asymmetries tend to occur, and these asymmetries were assessed using specific tests (Shapiro–Wilk). Therefore, we opted to use non-parametric techniques for the inferential analysis; the Kruskal–Wallis test for the comparisons between medians, the Spearman–Rank test to evaluate the associations, and Fisher's exact test or chi-square test to evaluate the frequencies, considering a value of 5% as the criterion for statistical significance. All statistical analyses were performed using Prism 6.02 software (GraphPad, USA).

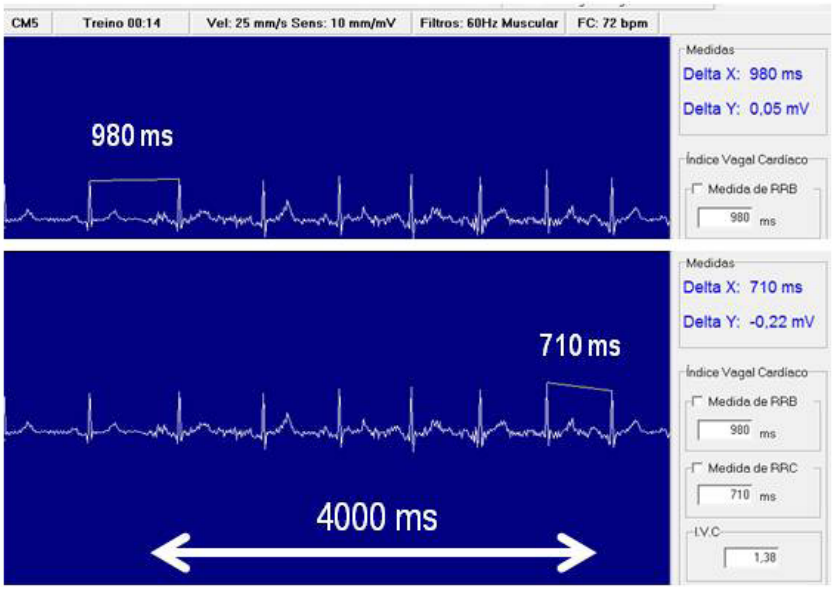

Figure 1 – *Identification and measurement of the length of the RRB and RRC intervals and calculation of CVI (RRB/RRC) in a healthy adult.*

Results

The adults evaluated were aged between 18 and 81 years, with a median of 42 years [5–95 percentiles (P5–P95) between 24 and 64 years]. Of these, 983 were men and 622 were women, and the median height, weight, and body mass index (BMI) were 176.3 cm, 79.2 kg, and 25.36 kg/m^2 for men and 164.0 cm, 60.7 kg, and 22.62 kg/m^2 for women, respectively. The median resting HR for the entire study group was 63 bpm (P5–P95 between 46 and 83 bpm).

The CVI results correlated significantly with several variables evaluated in the study, such as resting HR ($r = –0.310$) and BMI ($r = –0.168$) ($p < 0.01$). Notably, the CVI results correlated significantly but discretely with the duration of the RRC interval ($r = 0.092$), moderately with the duration of the RRB interval ($r = 0.698$), and strongly with the difference between the durations of the RRB and RRC intervals ($r = 0.955$).

For the set of 1,605 healthy adults, CVI varied between 1.03 and 2.82, with a median of 1.52 (P5–P95 between 1.19 and 1.98), and these results showed a tendency to significantly decrease with age ($r = –0.333$; $p < 0.01$). Linear regression analysis between CVI and age indicated a decrease of 0.0068 [95% CI = –0.078 to −0.0059] in CVI for each additional year of age, with a standard error of estimate of 0.2414 considering an intercept of 1.836 [95% CI = 1.793–1.878], and only 10% of the CVI variability was explained by age ($r^2 = 0.10$).

Regression analysis was repeated after classifying the study group by gender. The comparison test between the regressions obtained between CVI and age for male and female adults indicated no differences in the slope ($p = 0.132$) and intercept ($p = 0.068$). Therefore, for subsequent analysis, the sample was regrouped without any distinction for gender.

Visual inspection of the dispersion and percentile ranges for the CVI and age suggested a significant degree of heteroscedasticity in the distribution, with a more pronounced variability in CVI among older individuals. Therefore, the study group was divided in age groups starting at 18–25 years and thereafter for every 5 years until age 66, ending with the age group 71–75 years, which was the group with the lowest number of cases. The descriptive results for each age group were analyzed with emphasis on the values of the main percentiles (P5, P25, P50, P75, and P95), considering the abovementioned heteroscedasticity and the slightly asymmetric nature of the distribution of the CVI results, particularly among older patients. The analysis of the results indicated great similarity between the results of some contiguous age groups, which allowed us to group some of these age groups into wider ranges, including the groups 18–30 years and 31–40 years. To verify whether this new approach would generate artifacts or significant errors, the slopes of the linear regressions of the CVI values were tested for each age group. It was noted that the results included the value zero within the 95% confidence interval, indicating that the decreased modulation with age was not significant within that age range. After a new classification by age group, the differences between the CVI values for the different age groups were significant ($p < 0.01$).

Therefore, a total of eight age groups were used for the presentation of the CVI reference values obtained with 4sET according to age (Figure 2). A detailed presentation of these results with various percentiles for the total study group and for each age group is shown in Table 1, including the traditional parametric descriptive values. Figure 3 shows the CVI data according to age for all 1,605 adults evaluated.

Considering the frequent use of adjectives in medical jargon, we suggest the following standard terms to express

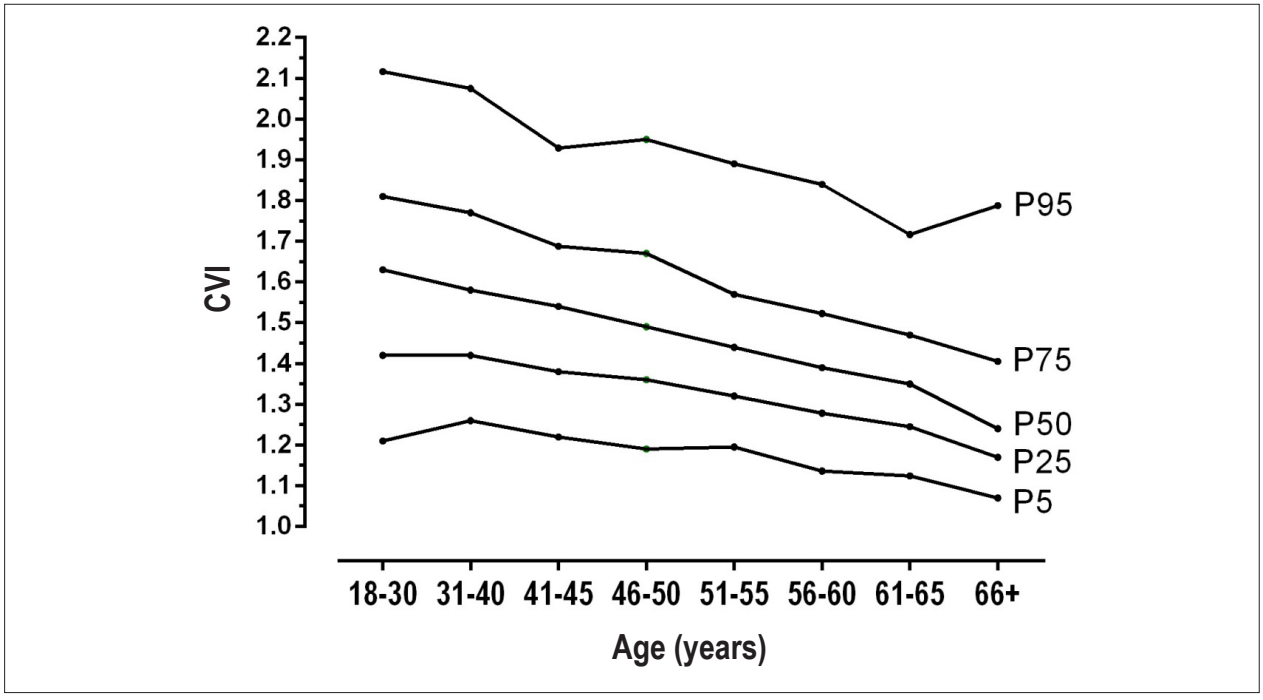

Figure 2 – *ECG tracing (lead CM5) of 4sET with a 35-s duration, allowing visualization and identification of post-pedaling vagal rebound (RRF = 1180 ms) approximately 8 s after the end of the exercise.*

Table 1 – Main cardiac vagal index results for each one of the nine age-groups

Age group (years)	18–81	18–30	31–40	41–45	46–50	51–55	56–60	61–65	≥ 66
Number of individuals	1605	282	449	260	239	128	110	76	61
Minimum	1.030	1.130	1.110	1.080	1.030	1.160	1.090	1.060	1.050
P5	1.190	1.210	1.260	1.220	1.190	1.195	1.136	1.124	1.070
P10	1.240	1.293	1.300	1.270	1.240	1.229	1.191	1.147	1.110
P25	1.350	1.420	1.420	1.380	1.360	1.320	1.278	1.245	1.170
P50 (median)	1.520	1.630	1.580	1.540	1.490	1.440	1.390	1.350	1.240
P75	1.700	1.810	1.770	1.688	1.670	1.570	1.523	1.470	1.405
P90	1.888	1.960	1.940	1.839	1.880	1.740	1.708	1.640	1.652
P95	1.980	2.117	2.075	1.929	1.950	1.890	1.840	1.717	1.788
Maximum	2.820	2.710	2.480	2.820	2.460	2.210	2.050	1.990	2.150
Average	1.545	1.637	1.608	1.547	1.524	1.468	1.416	1.379	1.314
Standard deviation	0.2556	0.2804	0.2484	0.2298	0.2389	0.2033	0.2013	0.1884	0.2308

P: percentile.

CVI results for a given age group, even though these terms do not have an objective clinical connotation and use only the tendency in the distribution of the results: for CVI values <minimum: extremely decreased, CVI between minimum and P5: strongly decreased, CVI between P5 and P10: moderately decreased, CVI between P10 and P25: slightly decreased, CVI between P25 and P75 (50% of the mid-range cases): within normal limits, CVI between P75 and P90: slightly increased, CVI between P90 and P95: moderately increased, CVI between P95 and maximum: strongly increased, and CVI >maximum: extremely increased.

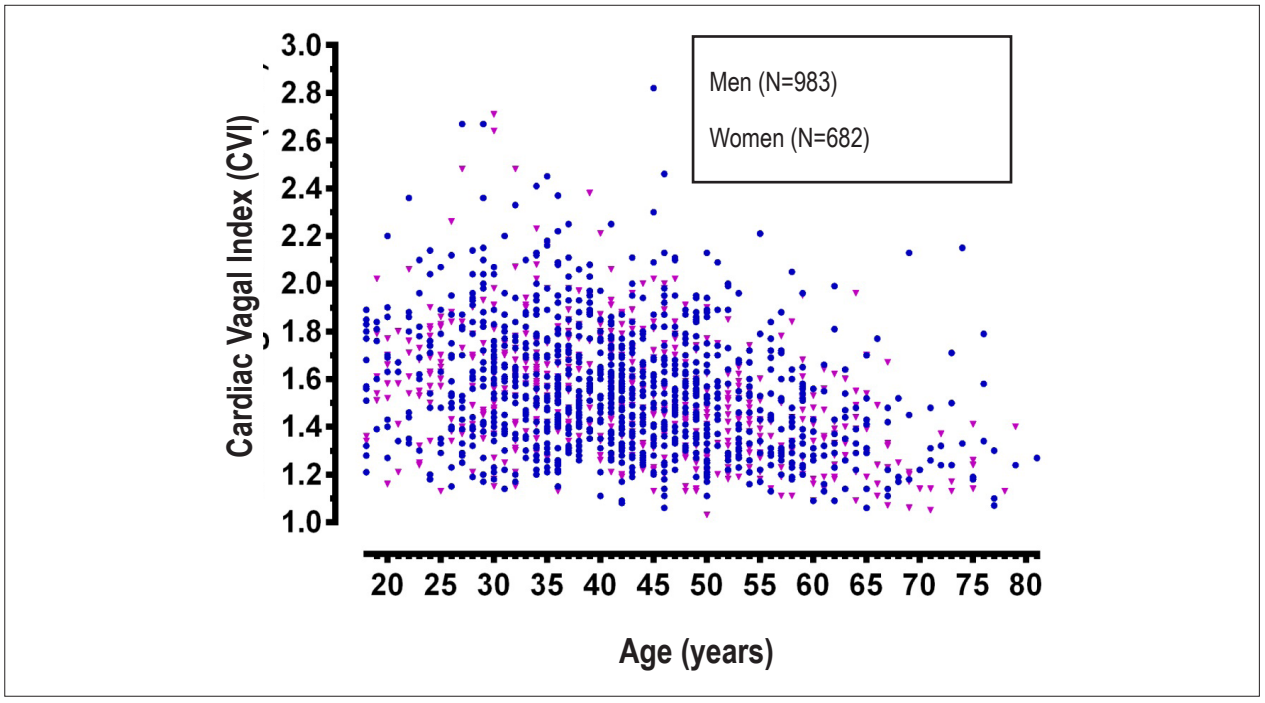

Figure 3 – *Reference data of the 4-s exercise test: percentile curves of the cardiac vagal index for men and women aged between 18 and 81 years (n = 1605).*

Discussion

Considerations about 4sET

Since the late 1980s, 4sET has been used in > 10,000 individuals using diverse clinical conditions and age groups, without any recorded relevant clinical complication, demonstrating the safety of the procedure.

To better reflect the vagal modulation over cardiac chronotropism, 4sET was based on well-established physiological mechanisms[1,5,14,20,21]: a) respiratory-heart interaction: deep and rapid inspiration stimulates afferent pathways, which act on structures in the central nervous system and trigger a rapid increase in vagal activity on the sinus node, resulting in an abrupt decrease in HR, which is typically observed in a few seconds; b) movement-heart interaction: rapid movement of the extremities stimulates afferent pathways that act on the central nervous system, triggering a strong and fast (latency period probably < 1 s) inhibition of acetylcholine release in vagal efferent fibers of the sinus node, thereby causing an abrupt increase in HR; c) although the movement of large joints and the contraction of skeletal muscles also promotes sympathetic stimulation, this response has a latency > 4 s before cardiac stimulation can be observed.

Therefore, although recent data suggest that the vagus is not completely inhibited even at maximal effort[20], deep and rapid inspiration followed by a 4-s rapid and resistance-free cycling activity during 4sET physiologically causes a sudden and significant inactivation of the vagus, stimulated by the deep breath that precedes pedaling, without sympathetic influence in this short period of evaluation of the HR[14]. By quantifying the ratio between the lengths of RR intervals immediately before pedaling (RRB, the vagal activation point) and at the end of pedaling (RRC, the vagal inactivation point without sympathetic influence), the CVI allows isolated study of the vagal modulation.

HR behavior in the first 4 s of the rest-exercise transition has been the subject of several studies aimed at standardizing 4sET, including the characteristics of the pre-pedal inspiration[22,23]. We observed that the CVI results were similar when 4sET was performed with or without the addition of pedal resistance, when the legs or arms moved quickly[24], or when performed in a standing position (alternating a 90-degree flexion and extension of the hip, simulating a race)[25]. Similar CVI results were obtained when 4sET was performed actively or passively[26] and CVI results were not affected even when a Valsalva exercise was performed concurrently with 4sET. Moreover, in addition to their validity, the CVI results proved reliable when nine 4sET exercise cycles were performed in 1 day or in 5 consecutive days, thereby generating intraclass correlation coefficients of 0.92 and 0.77, respectively[28].

In addition to the abovementioned primarily methodological studies, several other physiological and clinical studies have been published using 4sET both by our group[29-34] and by other groups in Brazil[35-37] and abroad[38-40]. This study will help consolidate 4sET as a protocol for assessing cardiac vagal modulation by providing reference values for the Brazilian adult population.

4sET reference values

It is good scientific practice to publish reference values for evaluation protocols[41]. A detailed listing of the main

methods and their reference values until 1992 is available in two review studies published in the Brazilian Archives of Cardiology[42,43]. Table 2 shows some of the main and/or more recently published studies[44-51] providing reference values for HR variability and shows that the study groups tended to include approximately 200 cases (range: 120–657), which is significantly lower than the 1,605 cases evaluated in the present study. Our study group was significantly larger than those commonly presented in other related studies for the establishment of reference values for 4sET and was the only study group subjected to strict inclusion/exclusion criteria. Therefore, we attempted to reduce the likelihood that latent clinical abnormalities which may affect results were present, thereby stimulating the inclusion of healthy individuals in the study group. More importantly, all data were collected over the 20-year period by a small number of physicians at a single laboratory.

The results of the present study corroborate results from previous studies[10,45,50], indicating that cardiac vagal modulation tends to decrease with age, at least until the 8th decade of life. However, while vagal modulation tends to be negatively influenced by age, this correlation was not mathematically strong, as evidenced by the low coefficient of explanation (r^2), indicating the large variability among individuals of the same age or age group. In this respect, Zulfiqar et al.[51] found an apparent reversal of the decreased modulation of vagal function with age in the analysis of HR variability starting at the age of 80 years and suggested that this could be related to longevity in this age group. Our study group was limited to individuals aged 81 years and therefore did not allow the analysis of this phenomenon. The study results add to the body of knowledge in this subject and indicate that the decreased vagal modulation in adults is very gradual and only seems to start from the age of 30.

Analysis of the CVI results indicate that decreased vagal modulation with age was similar among men and women, as found by some other authors[10] but not by others[44,47]. At present, it is not possible to explain whether these agreements or disagreements are due to sampling issues or distinct methods of autonomic assessment; however, at least for those who would clinically use 4sET, there is no need to include the gender variable when interpreting the results.

The option of expressing vagal modulation during 4sET as CVI, i.e., as the ratio between the length of RR intervals, as performed in other autonomic tests, including expiration/inspiration, Valsalva, and orthostatism (rather than calculating the difference between the two intervals), did not influence the results, considering that the correlation between these two measures was extremely high ($r = 0.955$; $p < 0.01$).

Study limitations

The large sample size and the care taken to characterize the participants as healthy and to ensure they were not using any medications that could affect the results are considered positive factors. On the other hand, the distinct demographic characteristics of the study group need to be addressed. Although athletes associated in various sports were purposely excluded, it was not feasible to precisely control the pattern of exercise and/or sports participation during the evaluation, and considerable variability was observed in this regard in the study group. However, this issue may not be so important, as suggested by the results from a previous study involving 4sET[12], which indicated very small differences and significant overlap between the distribution of CVI results for elite competitive athletes and young non-athletes. Another potential limitation of the study relates to the demographic characteristics of the study group, particularly the socio-cultural and ethnic profiles, which were typical of a population receiving care in a private clinic. These aspects should be considered when reference values are applied to populations with characteristics distinct from those of the present study.

Implications

Recent data from the literature suggest that the assessment of cardiac vagal modulation has important clinical potential not only in terms of longevity[51] but also for understanding the pathophysiology of several diseases in other medical areas other than cardiology[52], including gastroenterology[48] and clinical oncology[53]. For example, Belgian researchers[53] recently found that the prognosis of patients diagnosed with cancer at late clinical stages depended on vagal modulation, and prognosis was better among those with high levels of the

Table 2 – Selection of eight studies with reference values for autonomic tests

Authors (Year of publication)	Autonomic evaluation	Sample	Age group	Clinical Condition
Ziegler et al.[50] (1992)	HRV	120	15–67 years	Healthy
Umetani et al.[49] (1998)	HRV 24 h	260 (112 M)	10–99 years	Healthy
Fagard[47] (2001)	HRV	614	25–89 years	-
Bonnemeier et al.[45] (2003)	HRV 24 h	166 (85 M)	20–70 years	Healthy
Zulfiqar et al.[51] (2010)	HRV	344	10–99 years	Healthy
Abhishekh et al.[44] (2013)	HRV	189 (114 M)	-	Healthy
De Couck et al.[46] (2013)	HRV	657	-	Cancer (five types)
Farmer et al.[48] (2014)	HRV	200	18–59 years	Healthy

HRV: heart rate variability; M: male.

vagal component of HR variability. Therefore, these authors proposed that this type of autonomic evaluation should be included in the clinical management of patients with different types of cancer. This creates an interesting perspective for professionals involved with exercise testing and/or evaluation of autonomic activity.

In summary, the present study provides reference values for the statistical interpretation of the CVI results, as assessed using 4sET in non-athletic men and women aged between 18 and 81 years. Considering the simplicity and safety of 4sET and the increasing clinical relevance of assessing vagal modulation over cardiac chronotropism not only for cardiovascular diseases but also for several other prevalent diseases, the availability of such data could facilitate the incorporation of 4sET in the clinical setting.

Author contributions

Conception and design of the research: Araújo CG, Castro CLB, João Felipe Franca JF, Ramos PS. Acquisition of data: Araújo CG, Castro CLB, Franca JF. Analysis and interpretation of the data: Araújo CG, Castro CLB, Franca JF, Ramos PS. Statistical analysis: Araújo CG, Ramos PS. Obtaining financing: Araújo CG, Castro CLB. Writing of the manuscript: Araújo CG, Castro CLB, Franca JF, Ramos PS. Critical revision of the manuscript for intellectual content: Araújo CG, Franca JF, Ramos PS. Supervision / as the major investigador: Araújo CG.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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