



(RESEARCH ARTICLE)



## Agreement between $VO_2\text{max}$ estimated from six-minute walk test and Chester step test in normal adults

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World Journal of Advanced Research and Reviews, 2022, 15(01), 018–030

Publication history: Received on 19 May 2022; revised on 27 June 2022; accepted on 29 June 2022

Article DOI: <https://doi.org/10.30574/wjarr.2022.15.1.0650>

### Abstract

**Background:** Cardiorespiratory fitness is a health-related component of overall physical fitness assessed as maximal oxygen uptake ( $VO_2\text{max}$ ) during any physical activity. The six-minute walk test (6MWT) and Chester step test (CST) are two submaximal exercise tests routinely administered to estimate the  $VO_2\text{max}$  of an individual. Thus, the aim of this study was to assess whether an agreement exists between the  $VO_2\text{max}$  estimated by 6MWT and CST in normal adults.

**Method:** This crossover study was conducted with 80 healthy adults aged 18-40 years. All participants performed the 6MWT and CST on consecutive days.  $VO_2\text{max}$  from the 6MWT was estimated using a prediction equation whereas from the CST using the graphical plot method.

**Results:** The mean  $VO_2\text{max}$ -6MWT and  $VO_2\text{max}$ -CST were  $43.53 \pm 4.65$  ml/kg/min and  $38.34 \pm 4.94$  ml/kg/min respectively. The Bland and Altman analysis revealed that the mean difference between  $VO_2\text{max}$ -6MWT and  $VO_2\text{max}$ -CST was  $5.19 \pm 5.50$  ml/kg/min which exceeded the maximum acceptable difference of 3 ml/kg/min decided a priori. The line of equality (X axis) did not fall within the confidence interval of the mean difference indicating that  $VO_2\text{max}$  estimated using the two submaximal exercise tests significantly differ from each other. The standard error of mean was 0.61 ml/kg/min and the standard error of limits of agreement was 1.06 ml/kg/min.

**Conclusion:**  $VO_2\text{max}$  estimated from the six-minute walk test and Chester step test show no agreement with each other.

**Keywords:** Cardiorespiratory fitness;  $VO_2\text{max}$ ; Submaximal exercise tests; Six-minute walk test; Chester step test; Bland Altman plot analysis

### 1. Introduction

Physical fitness is defined as the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy leisure time pursuits and meet unforeseen emergencies [1]. Cardiorespiratory fitness (CRF) is one of the “health related” components of overall physical fitness which denotes the capacity to perform large muscle, dynamic, moderate to vigorous physical activity for a prolonged period of time. It depends upon the integrated functional status of the cardiovascular, respiratory, metabolic and musculoskeletal systems [2]. CRF is dependent upon a multitude of factors such as gender, age, education, socioeconomic status, ethnicity, body mass index, body weight, waist circumference, body fat, resting heart rate, C-reactive protein level, smoking, alcohol consumption and multiple measures of leisure-time physical activity [3]. High CRF has a linear relationship with various health benefits such as primary and secondary prevention of cardiac diseases, diabetes, hypertension, obesity and osteoporosis [4]. Hence the

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assessment of CRF is of pivotal importance as it gives us a general idea of the fitness of the individual and is a vital part of any preventive or rehabilitation program.

The criterion used to assess CRF is maximal oxygen uptake ( $VO_{2max}$ ). It is expressed in liters/minute (L/min) as an absolute value or in milliliters/kilogram/minute (ml/kg/min) as relative  $VO_{2max}$  [5].  $VO_{2max}$  is the maximal rate at which one can consume oxygen during physical activity. It is the true physiological limit reached where no further increase in the oxygen uptake will occur on continuing the exercise [6].  $VO_{2max}$  is dependent upon the oxygen transport system (cardiac output, pulmonary diffusion capacity, oxygen-carrying capacity and renal function) and the ability of the end cells (muscle diffusion capacity, mitochondrial enzymes and capillary density) to take up and utilize that oxygen for energy production [7].  $VO_{2max}$  can be assessed using multiple methods such as maximal and submaximal exercise tests, non-exercise prediction equations and non-exercise (pharmacological and nuclear) stress tests [8-13].

The gold standard for measuring  $VO_{2max}$  is graded maximal exercise testing (GxT), performed in a lab using gas analysis. Most commonly used modes of maximal exercise testing are treadmill walking, running and stationary cycling. The attainment of true  $VO_{2max}$  occurs when the oxygen uptake plateaus and does not increase further by more than 150 ml/min with further increase in workload, failure of the heart rate to increase with incremental workload, venous lactate concentration exceeding 8 mmol/L, respiratory exchange ratio greater than 1.15 and rate of perceived exertion (RPE) greater than 17 on the 6-20 Borg scale. Thus, maximal exercise testing protocols require the participant to exercise to the point of volitional fatigue. However if the criteria for  $VO_{2max}$  is not achieved during the maximal exercise testing due to the subject experiencing fatigue or pain first, the maximal  $VO_2$  attained is termed as the  $VO_{2peak}$  [14]. Maximal exercise testing protocols mandate the requirement of an expensive laboratory setup of equipments, monitoring systems and emergency equipments, require trained personnel, are time consuming and labor intensive and risk the precipitation of unwarranted symptoms which may lead to adverse events [15].

When compared to maximal exercise testing, submaximal exercise tests have a greater applicability to physiotherapists in assessment of CRF and exercise prescription due to their low-risk, low-cost, low-supervision nature and objective evaluation of  $VO_{2max}$ . In many cases, maximal exercise testing is not indicated (e.g. in elderly individuals or cardiac patients) due to an increased risk of adverse cardiac events. The most commonly used modes for submaximal exercise testing are cycling, walking, running, and stepping [16]. They include an array of tests involving cycling such as the Astrand and Rhythmic cycle ergometer test; walk tests namely the six-minute walk test, the twelve minute walk test, modified shuttle walk test; brisk running test namely the Cooper 12 min run test and stepping tests such as the Queens college step test and the Chester step test etc. These tests are more functional to perform, require less space and no explicit setup, involve minimum risk, do not provoke maximal fatigue and the results can be extrapolated to determine the  $VO_{2max}$  of the individual. Thus considering the merits of submaximal exercise testing over maximal exercise testing, the former is more useful in a clinical setup or as a screening tool for masses as well as patient populations [17].

According to the American Thoracic Society guidelines, 2002, the six-minute walk test (6MWT) is a commonly used submaximal exercise test to assess the functional exercise capacity of an individual. It is potentially more representative of the activities of daily living. It is a self-paced, level ground walking test performed across a 100 feet hallway for six-minutes [18]. The reliability and validity of the six-minute walk test have been established across healthy and various patient populations [19-22]. The 6MWT results and the anthropometric variables of the individual are used to estimate the  $VO_{2max}$  using the equation given by Burr et al [23].

The Chester Step Test is a submaximal incremental metronome based multistage step test developed by Dr. K. Sykes at the University College Chester, United Kingdom. The total test time is of 10 minutes comprising of five stages lasting for 2 minutes each. It warrants the individual to step at a rate fixed by metronome beats starting with 15 beats per minute. The step rate is increased by 5 beats for each incremental stage. The step height is adaptable depending upon the age and the physical abilities of the individual. The Chester step test is performed indoors and does not require any explicit setup thus making it a feasible method to assess  $VO_{2max}$  in any clinical or community setting. In literature the Chester step test has shown to correlate well with other exercise testing methods; with  $VO_{2max}$  treadmill test ( $r = 0.92$ ), Astrand cycle test ( $r = 0.94$ ) and multistage shuttle run ( $r = 0.81$ ). The  $VO_{2max}$  is estimated by plotting a graph of the heart rate points achieved at the end of each stage on the specified Chester step test data record sheet. The physiological rationale for the same is that a linear relationship exists between workload and exercise heart rate [24].

Thus the need for this study was to find out whether an agreement exists between the  $VO_{2max}$  estimated by the two above mentioned submaximal exercise tests, namely the six-minute walk test and Chester step test; the results of which can allow extending this study to various patient populations and also to determine if Chester step test can be used as a baseline routine test for recruiting individuals for a fitness training program considering that it can be individualized to the subject being tested and can be performed in limited space in the clinical and community settings.

## 2. Material and methods

This cross over study was conducted at PT School and Center, Seth G.S. Medical College and KEM Hospital, Mumbai, India during February to September 2021. Institutional Ethics Committee approval was obtained. 80 healthy adults (40 males and 40 females) between the age group of 18-40 years were recruited using convenient sampling. Individuals engaged in regular sports or vigorous physical activities and those with BMI greater than  $23\text{kg/m}^2$  were excluded from this study since the criteria for step height varies [25]. Written informed consent was obtained from all participants.

The six-minute walk test was performed on the first day followed by the Chester step test on the next day for all odd numbered participants and vice versa for even numbered participants.

Participants were instructed to wear loose comfortable clothing and appropriate shoes, have a light meal and refrain from any caffeine consumption and exercise at least 2 hours prior to the exercise testing. A practice test and then the final test were performed on the same day with a gap of minimum 1 hour between the two tests [16]. Vital parameters - heart rate, respiratory rate, blood pressure, RPE,  $\text{SpO}_2$  were recorded pre and post test.

### 2.1. Six-minute walk test

The six-minute walk test was conducted as per the ATS Statement: Guidelines for the Six-Minute Walk Test, 2002. The test was performed indoors on a straight, flat, hard surface 30 m long walkway. The turnaround points of the walkway were marked by cones. A floor marking (red tape) was used to mark the starting line. Standardized instructions regarding performing the test were given. The test was terminated at the end of 6 minutes or prior if the participant wished to discontinue or due to the precipitation of unwarranted symptoms such as chest pain, leg cramps, intolerable dyspnea, diaphoresis and pale or ashen appearance etc. The point at which the participant stopped the test was marked with a tape. The number of laps and the additional distance covered were used to calculate the final walk distance [18]. Anthropometric variables and the six-minute walk test results were used to estimate the  $\text{VO}_2\text{max}$  from the equation derived by Burr et al [23].

### 2.2. Chester step test

A stepper of 20 cm in height was chosen as this step height is the criteria for subjects under 40 years of age who take little or no regular physical activity.  $\text{HR}_{\text{max}}$  was calculated using the formula  $\{220 - \text{Age (years)}\}$  from which 80%  $\text{HR}_{\text{max}}$  was derived. The test was performed using the Chester Step Test Audio Tape with prerecorded metronome beats. The pace of the beats increased every 2 minutes. At the end of every 2 minute stage HR and RPE were recorded. The Chester Step test stepping rate (metronome pacing) is as follows:

- Stage 1= 15 steps/min = 60 beats/min
- Stage 2= 20 steps/min = 80 beats/min
- Stage 3 = 25 steps/min = 100 beats/min
- Stage 4= 30 steps/min = 120 beats/min
- Stage 5= 35 steps/min = 140 beats/min

The stepping pattern was up/up/down/down. Holding onto a railing or the wall and use of hands on the thighs for support during the test was prohibited. If the participant deviated from the beat, they were instructed a few times but if they kept slowing down due to fatigue, the test was terminated. The test was also terminated if the subject wished to discontinue, reached 80% of maximal heart rate, reported a RPE of 15 (hard) or experienced symptoms such as overtiredness, breathlessness or dizziness.

$\text{VO}_2\text{max}$  can be predicted using two exercise heart rate points however, for the accuracy of the estimation it is important that the participant completed three stages. Heart rate points below 50% and above 85% of the maximal heart rate were excluded. The exercise heart rate points were then plotted on the Chester step test data record sheet. The visual line of best fit was drawn for the heart rate points (X axis) which was extrapolated to coincide with the maximal heart rate line drawn parallel to the Y axis indicating the  $\text{VO}_2\text{max}$ . From the point of where the lines coincide, a perpendicular was dropped on the Y axis to estimate the  $\text{VO}_2\text{max}$  of the participant [24].

Maximum acceptable difference in the estimated  $\text{VO}_2\text{max}$  by six-minute walk test and Chester step test of  $3\text{ml/kg/min}$  was decided a priori [26].

### 2.3. Statistical analysis

The Bland Altman plot analysis is used to assess and quantify the agreement between two quantitative methods of measurement by studying the mean difference and constructing limits of agreement. It evaluates the bias between the mean differences and estimates an agreement interval which falls within 95% of the difference of the second method when compared to the first method. Acceptable limits of agreement must be defined a priori based on clinical necessity and judgment.

The Bland Altman analysis results in a scatter plot in which X axis represents the average of the two measurements (Mean of VO<sub>2</sub>max-6MWT and VO<sub>2</sub>max-CST) and the Y axis represents the difference between the two measurements (Difference between VO<sub>2</sub>max-6MWT and VO<sub>2</sub>max-CST). The lack of agreement is indicated by calculating the bias which is estimated by the mean difference ( $\bar{d}$ ) and the standard deviation of the differences (s). After verifying the normal distribution of the differences, it is expected that 95 % of differences will lie between  $\bar{d} + 1.96s$  and  $\bar{d} - 1.96s$  defining the upper and lower limits of agreement respectively. With a significant bias, the line of equality (X axis) does not fall within the confidence interval of the mean difference [27]

### 3. Results

The data was analyzed using the SPSS version 16 software and Microsoft Excel 2016.

Descriptive statistics of the study participants namely the gender distribution, age and BMI are shown in table 1.

**Table 1** Descriptive Statistics of the study participants (Mean  $\pm$  SD)

Variable	Male	Female	Total
n	40	40	80
Age (years)	25 $\pm$ 4.87	24.1 $\pm$ 2.20	24.55 $\pm$ 3.79
BMI (kg/m <sup>2</sup> )	21.97 $\pm$ 1.34	21.08 $\pm$ 1.65	21.52 $\pm$ 1.56

Table 2 and 3 indicate the change in the vital parameters (mean  $\pm$  SD) namely heart rate, respiratory rate, blood pressure (systolic and diastolic), oxygen saturation and rate of perceived exertion prior to and after performing the six-minute walk test and Chester step test respectively.

**Table 2** Change in vital parameters with six-minute walk test (Mean  $\pm$  SD)

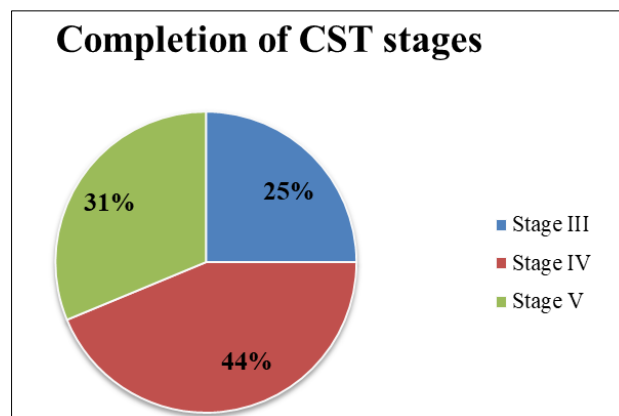
Parameter	Male		Female		Total	
	Pre	Post	Pre	Post	Pre	Post
Heart Rate (beats/min)	81.68 $\pm$ 9.85	112.13 $\pm$ 16.86	81.78 $\pm$ 10.39	123.73 $\pm$ 17.95	81.73 $\pm$ 10.06	117.93 $\pm$ 18.26
Respiratory Rate (breaths/min)	17.83 $\pm$ 2.44	24.90 $\pm$ 3.73	16.68 $\pm$ 2.52	25.80 $\pm$ 4.12	17.25 $\pm$ 2.53	25.35 $\pm$ 3.93
Systolic Blood Pressure (mmHg)	120.28 $\pm$ 10.07	133.85 $\pm$ 12.70	109.23 $\pm$ 11.26	123.78 $\pm$ 12.93	114.75 $\pm$ 11.98	128.81 $\pm$ 13.71
Diastolic Blood Pressure (mmHg)	78.70 $\pm$ 8.41	82.55 $\pm$ 9.32	71.33 $\pm$ 12.35	79.18 $\pm$ 8.39	75.01 $\pm$ 11.14	80.86 $\pm$ 8.97
Oxygen Saturation (%)	98.23 $\pm$ 0.73	98.48 $\pm$ 0.85	98.85 $\pm$ 0.70	98.53 $\pm$ 1.11	98.54 $\pm$ 0.78	98.50 $\pm$ 0.98
Rate of Perceived Exertion	6 $\pm$ 0	7.80 $\pm$ 1.68	6 $\pm$ 0	8.75 $\pm$ 2.05	6 $\pm$ 0	8.28 $\pm$ 1.92

**Table 3** Change in vital parameters with Chester step test (Mean ± SD)

Parameter	Male		Female		Total	
	Pre	Post	Pre	Post	Pre	Post
Heart Rate (beats/min)	80.13 ± 10.61	150.15 ± 11.12	81.08 ± 9.63	156.20 ± 12.50	80.60 ± 10.08	153.18 ± 12.14
Respiratory Rate (breaths/min)	18.05 ± 2.62	29.98 ± 3.53	16.73 ± 2.72	29.50 ± 3.00	17.39 ± 2.74	29.74 ± 3.26
Systolic Blood Pressure (mmHg)	119.85 ± 10.62	137.23 ± 12.21	107.98 ± 10.69	127.93 ± 10.49	113.91 ± 12.16	132.58 ± 12.24
Diastolic Blood Pressure (mmHg)	78.08 ± 9.08	82.75 ± 8.64	74.53 ± 9.40	78.63 ± 7.72	76.30 ± 9.35	80.69 ± 8.40
Oxygen Saturation (%)	98.55 ± 0.78	98.35 ± 0.95	98.93 ± 0.86	98.53 ± 0.75	98.74 ± 0.84	98.44 ± 0.85
Rate of Perceived Exertion	6.03 ± 0.16	11.68 ± 2.48	6 ± 0	11.68 ± 2.96	6.01 ± 0.11	11.68 ± 2.71

**Table 4** Test completion statistics of Chester step test

	Reasons For termination of test:	Male	Female	Total
1.	Completion	16	9	25
2.	80% HR max reached	18	24	42
3.	RPE -15 reached	4	7	11
4.	Leg Fatigue	2	0	2
<b>Completion of stages</b>				
1.	Stage III	8	12	20
2.	Stage IV	16	19	35
3.	Stage V	16	9	25



**Figure 1** Completion of Chester step test stages by study participants

All the study participants completed the six-minute walk test. The mean six-minute walk distance was  $581.18 \pm 60.09$  m which was  $99.90 \pm 10.43\%$  of age, height and weight predicted distance. The mean six-minute walk distance for males was  $588.32 \pm 48.92$  m ( $100.22 \pm 8.00\%$  of age, height and weight predicted distance) and for females was  $574.03 \pm 69.40$  m ( $99.59 \pm 12.50\%$  of age, height and weight predicted distance). However, only 25 of the 80 participants completed the Chester step test. The test completion statistics for Chester step test are shown in table 4 and figure 1.

The mean  $VO_2\max$  estimated from the six-minute walk test ( $VO_2\max$ -6MWT) and Chester step test ( $VO_2\max$ -CST) are shown in table 5.

**Table 5**  $VO_2\max$  estimated from the 6 minute walk test and Chester step test

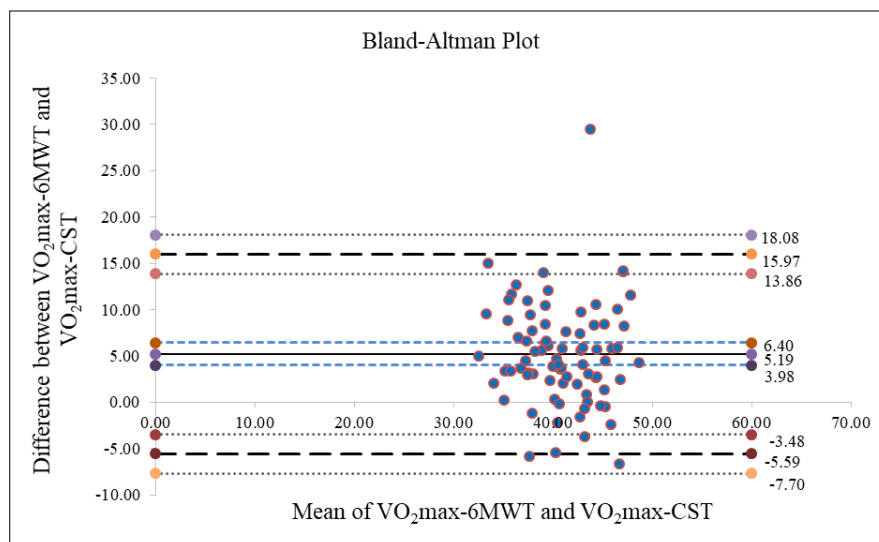
$VO_2\max$	Male	Female	Total
$VO_2\max$ - 6MWT (ml/kg/min)	$45.76 \pm 4.51$	$41.30 \pm 3.65$	$43.53 \pm 4.65$
$VO_2\max$ - CST (ml/kg/min)	$39.60 \pm 4.55$	$37.08 \pm 5.03$	$38.34 \pm 4.94$

The mean difference ( $\bar{d}$ ) between the  $VO_2\max$  estimated from the six-minute walk test ( $VO_2\max$ -6MWT) and Chester step test ( $VO_2\max$ -CST) was  $5.19 \pm 5.50$  ml/kg/min which exceeded the maximum acceptable difference of 3 ml/kg/min decided a priori. The standard error of mean (SE) was 0.61 ml/kg/min and the standard error of limits of agreement was 1.06 ml/kg/min. Table 6 shows the Bland Altman analysis parameters for the total study population (n = 80).

**Table 6** Bland Altman analysis parameters for total study population (n = 80)

Parameter	Value	Standard Error formula	Standard Error (SE)	t value	Confidence (SE*t)	Confidence Interval (CI)	
						From	To
Mean difference ( $\bar{d}$ )	5.19	$\sqrt{s^2/n}$	0.61	1.99	1.21	3.98	6.40
Upper limit of Agreement ( $\bar{d} + 1.96s$ )	15.97	$\sqrt{3s^2/n}$	1.06	1.99	2.11	13.86	18.08
Lower limit of Agreement ( $\bar{d} - 1.96s$ )	-5.59	$\sqrt{3s^2/n}$	1.06	1.99	2.11	-7.70	-3.48

n=80, degrees of freedom (df) =79, Standard Deviation (s)=5.50 ml/kg/min



**Figure 2** Bland Altman plot for total study population (n = 80), shows a solid horizontal line = Mean Difference ( $\bar{d}$ ) = 5.19 ml/kg/min

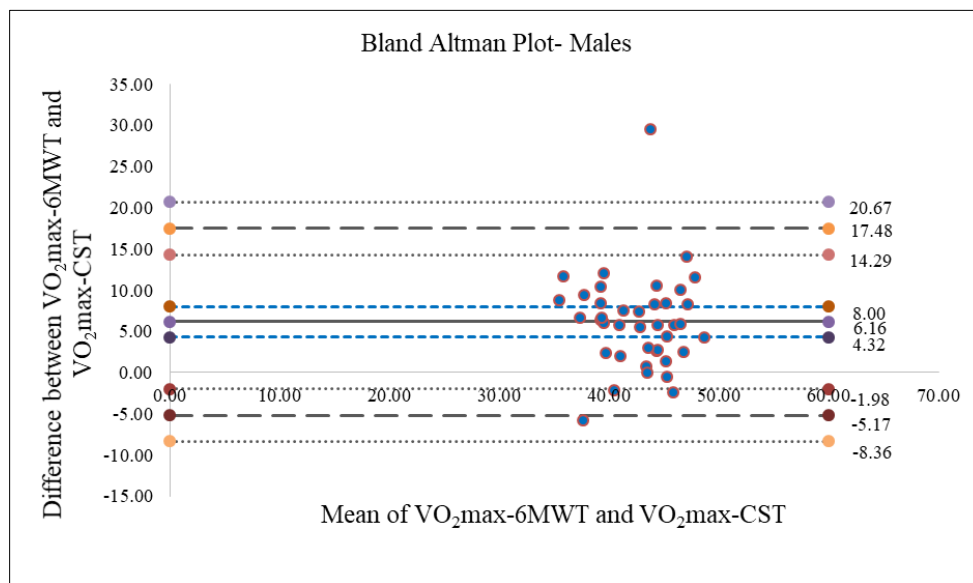
In Figure 2, the solid horizontal line indicates the mean difference ( $\bar{d}$ ) which is 5.19 ml/kg/min. The dotted blue lines above and below the solid black line are the upper and lower 95% confidence intervals of the mean difference ( $\bar{d}$ ) at 6.40 ml/kg/min and 3.98 ml/kg/min respectively. The line of equality (X axis) does not fall within the confidence interval of the mean difference indicating significant bias. The dotted black line (above the mean difference) denotes the upper limit of agreement at 15.97 ml/kg/min. It is bound by two fine dotted grey lines which are the upper and lower 95% confidence intervals at 18.08 ml/kg/min and 13.86 ml/kg/min respectively. Similarly, the dotted line (below the mean difference) denotes the lower limit of agreement at -5.59 ml/kg/min. It is bound by two fine dotted grey lines which are the upper and lower 95% confidence intervals at -3.48 ml/kg/min and -7.70 ml/kg/min respectively.

The mean difference ( $\bar{d}$ ) between the VO<sub>2</sub>max estimated from the six-minute walk test (VO<sub>2</sub>max-6MWT) and Chester step test (VO<sub>2</sub>max-CST) for males was 6.16 ± 5.78 ml/kg/min which exceeded the maximum acceptable difference of 3 ml/kg/min decided a priori. The standard error of mean (SE) was 0.91 ml/kg/min and the standard error of limits of agreement was 1.58 ml/kg/min. Table 7 shows the Bland Altman analysis parameters for the male population (n = 40).

**Table 7** Bland Altman analysis parameters for males (n = 40)

Parameter	Value	Standard Error formula	Standard Error (SE)	t value	Confidence (SE*t)	Confidence Interval (CI)	
						From	To
Mean difference ( $\bar{d}$ )	6.16	$\sqrt{s^2/n}$	0.91	2.02	1.84	4.32	8.00
Upper limit of Agreement ( $\bar{d} + 1.96s$ )	17.48	$\sqrt{3s^2/n}$	1.58	2.02	3.19	14.29	20.67
Lower limit of Agreement ( $\bar{d} - 1.96s$ )	-5.17	$\sqrt{3s^2/n}$	1.58	2.02	3.19	-8.36	-1.98

n=40, degrees of freedom (df)=39, Standard Deviation (s)=5.78ml/kg/min



**Figure 3** Bland Altman plot for males (n = 40), shows a solid horizontal line = Mean Difference ( $\bar{d}$ ) = 6.16 ml/kg/min

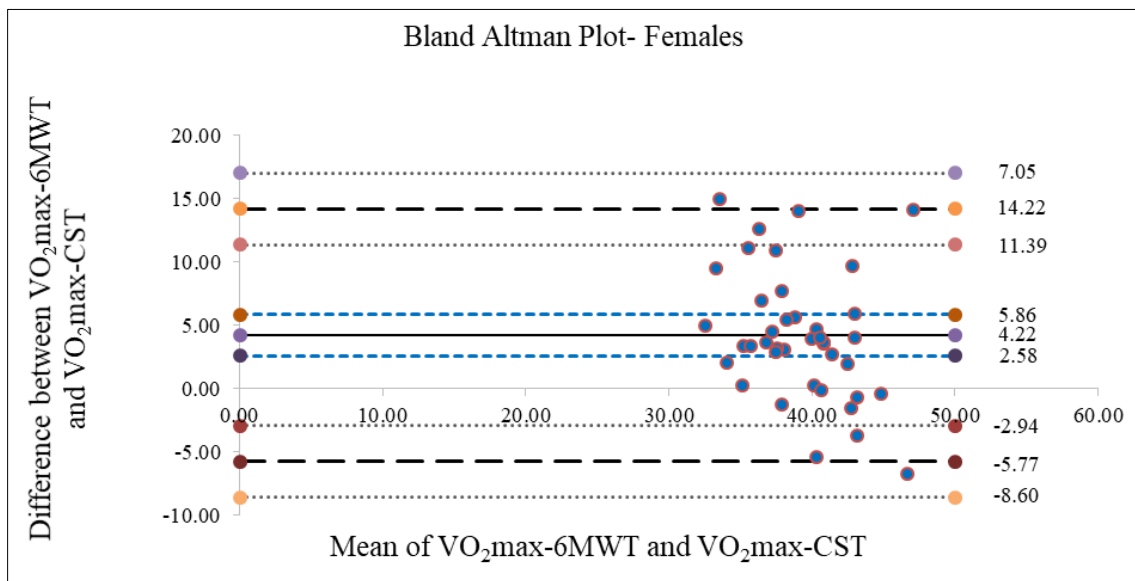
In Figure 3, the solid horizontal line indicates the mean difference ( $\bar{d}$ ) which is 6.16 ml/kg/min. The dotted blue lines above and below the solid black line are the upper and lower 95% confidence intervals of the mean difference ( $\bar{d}$ ) at 8.00 ml/kg/min and 4.32 ml/kg/min respectively. The line of equality (X axis) does not fall within the confidence interval of the mean difference indicating significant bias. The dotted black line (above the mean difference) denotes the upper limit of agreement at 17.48 ml/kg/min. It is bound by two fine dotted grey lines which are the upper and lower 95% confidence intervals at 20.67 ml/kg/min and 14.29 ml/kg/min respectively. Similarly, the dotted line (below the mean difference) denotes the lower limit of agreement at -5.17 ml/kg/min. It is bound by two fine dotted grey lines which are the upper and lower 95% confidence intervals at -1.98 ml/kg/min and -8.36 ml/kg/min respectively.

The mean difference ( $\bar{d}$ ) between the VO<sub>2</sub>max estimated from the six-minute walk test (VO<sub>2</sub>max-6MWT) and Chester step test (VO<sub>2</sub>max-CST) for females was 4.22 ± 5.10 ml/kg/min which exceeded the maximum acceptable difference of 3 ml/kg/min decided a priori. The standard error of mean (SE) was 0.81 ml/kg/min and the standard error of limits of agreement was 1.40 ml/kg/min. Table 8 shows the Bland Altman analysis parameters for the male population (n = 40).

**Table 8** Bland Altman analysis parameters for females (n = 40)

Parameter	Value	Standard Error formula	Standard Error (SE)	t value	Confidence (SE*t)	Confidence Interval (CI)	
						From	To
Mean difference ( $\bar{d}$ )	4.22	$\sqrt{(s^2/n)}$	0.81	2.02	1.64	2.58	5.86
Upper limit of Agreement ( $\bar{d} + 1.96s$ )	14.22	$\sqrt{(3s^2/n)}$	1.40	2.02	2.83	11.39	17.05
Lower limit of Agreement ( $\bar{d} - 1.96s$ )	-5.77	$\sqrt{(3s^2/n)}$	1.40	2.02	2.83	-8.60	-2.94

n=40, degrees of freedom (df)=39, Standard Deviation (s)=5.10ml/kg/min



**Figure 4** Bland Altman plot for females (n = 40), shows a solid horizontal line = Mean Difference ( $\bar{d}$ ) = 4.22 ml/kg/min

In Figure 4, the solid horizontal line indicates the mean difference ( $\bar{d}$ ) which is 4.22 ml/kg/min. The dotted blue lines above and below the solid black line are the upper and lower 95% confidence intervals of the mean difference ( $\bar{d}$ ) at 5.86 ml/kg/min and 2.58 ml/kg/min respectively. The line of equality (X axis) does not fall within the confidence interval of the mean difference indicating significant bias. The dotted black line (above the mean difference) denotes the upper limit of agreement at 14.22 ml/kg/min. It is bound by two fine dotted grey lines which are the upper and lower 95% confidence intervals at 17.05 ml/kg/min and 11.39 ml/kg/min respectively. Similarly, the dotted line (below the mean difference) denotes the lower limit of agreement at -5.77 ml/kg/min. It is bound by two fine dotted grey lines which are the upper and lower 95% confidence intervals at -2.94 ml/kg/min and -8.60 ml/kg/min respectively.

#### 4. Discussion

The purpose of this study was to assess whether an agreement exists between VO<sub>2</sub>max-6MWT and VO<sub>2</sub>max-CST. The mean VO<sub>2</sub>max-6MWT and VO<sub>2</sub>max-CST were 43.53 ± 4.65 ml/kg/min and 38.34 ± 4.94 ml/kg/min respectively. The Bland and Altman analysis revealed that the mean difference between VO<sub>2</sub>max-6MWT and VO<sub>2</sub>max-CST was 5.19 ± 5.50 ml/kg/min which exceeded the maximum acceptable difference of 3 ml/kg/min decided a priori. The line of equality (X



axis) did not fall within the confidence interval of the mean difference indicating that the  $VO_2\text{max}$  estimated using the two submaximal exercise tests significantly differ from each other. The standard error of mean was 0.61 ml/kg/min and the standard error of limits of agreement was 1.06 ml/kg/min. Therefore, it was concluded that an agreement did not exist between  $VO_2\text{max}$ -6MWT and  $VO_2\text{max}$ -CST in the given study population.

Bland and Altman analysis for agreement was also done for both the genders separately. Similar to the findings of the total study population, both genders did not show an agreement between  $VO_2\text{max}$ -6MWT and  $VO_2\text{max}$ -CST. The mean difference between  $VO_2\text{max}$ -6MWT and  $VO_2\text{max}$ -CST was  $6.16 \pm 5.78$  ml/kg/min for males and  $4.22 \pm 5.10$  ml/kg/min for females, both exceeding the maximum acceptable difference of 3 ml/kg/min decided a priori. The standard error of mean was 0.91 ml/kg/min and 0.81 ml/kg/min whereas the standard error of limits of agreement was 1.40 ml/kg/min and 1.58 ml/kg/min for males and females respectively thus concluding that no agreement exists between  $VO_2\text{max}$ -6MWT and  $VO_2\text{max}$ -CST in both the genders.

According to the data from the Cooper Institute for Aerobics research, 2005, the mean  $VO_2\text{max}$ -6MWT for males (45.76 ml/kg/min) falls into 'good' category whereas the mean  $VO_2\text{max}$ -CST (39.60 ml/kg/min) is categorized as 'poor' depending upon the age of the subjects. Similarly, for the female population the mean  $VO_2\text{max}$ -6MWT (41.30 ml/kg/min) falls into 'good' category whereas the mean  $VO_2\text{max}$ -CST (37.08 ml/kg/min) lies between the 'fair-good' categories [28]. It was consistently noted that  $VO_2\text{max}$ -CST was lower than  $VO_2\text{max}$ -6MWT as shown in table 5.

The completion rate of an exercise test is an important criterion for test selection. In the present study, all the study participants completed the six-minute walk test, however, only 25 (31%) of the 80 participants completed the Chester step test. The test was prematurely terminated for 42 participants as they reached 80% of age predicted maximum heart rate, 11 participants reached RPE- 15 (hard) and 2 complained of leg fatigue. Thus, the test had to be terminated for 35 participants (44%) at stage four and 20 participants (25%) at stage three as shown in table 4.

Taking into account the post exercise test hemodynamic parameters, there was a difference between the heart rate, respiratory rate and RPE at the end of the six-minute walk test and Chester step test. The heart rate at the end of six-minute walk test was  $117.93 \pm 18.26$  beats/minute and Chester step test was  $153.18 \pm 12.14$  beats/minute. The respiratory rate at the end of the six-minute walk test was  $25.35 \pm 3.93$  breaths/ minute and Chester step test was  $29.74 \pm 3.26$  breaths/ minute. The inclusion of RPE in regulating the submaximal exercise intensity leads to more accurate and reliable estimation of  $VO_2\text{max}$  [29]. The RPE at the end of six-minute walk test was  $8.28 \pm 1.92$  whereas for Chester step test was  $11.68 \pm 2.71$ . 42 individuals (52.5%) reached their 80% HRmax during the test and thus the test needs to be administered with caution for older individuals and for those with cardiopulmonary involvement. This variability in the post test parameters indicated that the six-minute walk test was less physiologically stressful as opposed to Chester step test. In a study done by Bohannon et al, six-minute walk test and three minute step test were compared as two practical measures of functional endurance. Similar to the present study, all the one hundred and eighty nine individuals, aged 14 to 85 years, completed the six-minute walk test. However, fifty one participants could not complete the three minute step test. The reasons for stopping the test were fatigue, task difficulty, inability to maintain cadence, balance problems, joint or muscle discomfort and failure to stand up completely on the step. The participants completing the three minute step test also walked significantly further in the six-minute walk test. The average heart rate and perceived exertion were significantly higher after the three minute step test than the six-minute walk test, which correlated significantly but not strongly. The authors inferred that individuals who completed the three minute step test were more likely males, healthier by self-report, were of younger age and had lower BMI [30].

Accurate estimation of  $VO_2\text{max}$  from exercise testing is of pivotal importance as it is used for diagnostic, prognostic and functional evaluation, to study the response profile of specific conditions and defining the response to intervention [31]. The estimation of  $VO_2\text{max}$  from the six-minute walk test uses a prediction equation developed by Burr et al accounting for the six-minute walk distance, weight, gender, resting heart rate and age as variables. The authors found that the intensity of the six-minute walk test was 70-75% of the actual  $VO_2\text{max}$  with 8.2 METs (range 4.7-11.2 METs), which can be classified as a moderate-vigorous level activity. There was also a moderately strong correlation between the six-minute walk distance and  $VO_2\text{max}$ . Stepwise multiple linear regressions accounted for 72.4% of the variance in  $VO_2\text{max}$  by refining the six-minute walk test distance by body weight, sex, resting heart rate and age. When using the six-minute walk test distance as the outcome variable, the predictors of height and body weight accounted for 41% of the variance in the walk distance. The coefficients of variation (unexplained variance) for  $VO_2\text{max}$  and six-minute walk test distance regression equations were 0.11 and 0.08, respectively, which were within the accepted standard. Thus the inclusion of the multiple variables provides a reasonable estimation of the predicted  $VO_2\text{max}$  from the six-minute walk test [23].

The Chester step test estimates  $VO_2\text{max}$  by establishing absolute intensity estimate of  $VO_2$  for a given step rate at the given step height and then extrapolating  $VO_2\text{max}$ , relative to the exercise intensity using percentage of maximum heart

rate based upon the age predicted HRmax [24]. This is based upon the assumption that heart rate and  $VO_2$  have a linear relationship. In healthy subjects, the heart rate increases linearly with increasing  $VO_2$ . This increase in heart rate is initially mediated by a reduction in the parasympathetic activity and subsequently and also exclusively with an increase in the sympathetic activity [32]. However, Buckley et al has shown that a rather curvilinear relationship exists between the two variables. This curvilinear relationship is more evident in stage I and during near maximal effort. This can be negated by excluding the heart rate points from stage I and those beyond 80% of HRmax. Incorrect and unsteady stepping rate and technique will affect the mechanical efficiency and the physiological response of heart rate and oxygen consumption which can also be a potential source of error [33]. Using the visual line of best fit and the HRmax equation can also introduce a potential source of error in the accuracy of the estimated  $VO_{2max}$ . The HRmax equation used (Fox-Haskell formula:  $220 - \text{age}$ ) introduces a potential source of error of  $\pm 17$  beats in the estimation of maximum heart rate which can lead to underestimation of  $VO_{2max}$  by 19% or an overestimation by 11%. However, Izquierdo et al found that the  $VO_{2max}$  estimated from the Chester step test by using the Fox-Haskell formula for HRmax determination had the strongest correlation ( $r = 0.989$ ,  $p < 0.001$ ) with the  $VO_{2max}$  measured during the cardiopulmonary exercise testing when compared to the Nes ( $220 - 0.64 \times \text{age}$ ) and Tanaka ( $220 - 0.7 \times \text{age}$ ) formulae [34].

The six-minute walk test closely reflects the everyday form of locomotion that is, walking whereas the Chester step test involves stepping which is associated with more severe general fatigue, leg fatigue and dyspnea [35]. Another major fundamental difference is that the six-minute walk test is a self-paced exercise test whereas the Chester step test is an externally paced incremental test. Externally paced exercise tests prove to be of greater physiological challenge to body homeostasis and cause greater increase in the core body temperature and post exercise test blood lactate level when compared to intensity matched self-paced exercise tests. Self-paced exercise tests allow the individual to modify their effort in response to the increasing challenge to homeostasis which can reduce the physiological and metabolic challenge posed when compared to intensity matched externally paced submaximal exercise tests [36]. Apart from the continuous performance in both the tests, the comparison of the tests being equally demanding and physiologically challenging is questionable. Since running is not permitted in the six-minute walk test, prolonged walking even at maximum speed is far from maximal for fit individuals. The walking speed is directly proportional to the  $VO_{2max}$  when other explanatory variables like age, height, weight and body fat are constant [37]. Increased walking speed leads to increased muscle activity and vertical ground reaction force during the loading response phase and increase in the magnitude of heel strike and loading of the lower extremity musculoskeletal system which has a significant influence on the RPE [38]. However, the self-selected effort based performance and comparatively lesser antigravity muscle work involved in six-minute walk test make it less physiologically challenging when compared to the greater muscle work against gravity and a fixed degree of effort which is affected by the body mass and stature in the Chester step test. Physical attributes such as shorter height, increased weight, higher BMI, older age can affect the six-minute walk test performance leading to reduced  $VO_{2max}$  estimation [39].

When using a fixed step height, the difference in the lower limb length of individuals will cause a variance in the energy required to perform each step. A step height that is too high for an individual will cause mechanical disadvantage as there is more dependence on the musculoskeletal system endurance rather than cardiorespiratory endurance whereas, a low step height has proven to be of inadequate resistance to stimulate required cardiorespiratory response. The fixed cadence can be of higher exercise intensity for individuals with higher body mass index, lower body height and reduced exercise capacity possibly leading to vigorous exercise effort and eliminating the benefits of submaximal exercise testing. Thus appropriate individualization of the protocol will yield valid estimation of  $VO_{2max}$ . Altering the step height to individual parameters and limiting the step rate ensure work performance of appropriate intensity, limiting muscular fatigue and ensuring adequate work is done by the cardiopulmonary system. However, reducing the step height or the stepping cadence will reduce the overall work performed during the test and will improve the completion rate but will render the test less challenging to able individuals [40]. Body composition is an important attribute that limits the accuracy of the estimation of  $VO_{2max}$  using step test protocols. At any given submaximal workload, individuals with a higher BMI will have to work at a greater percentage of  $VO_{2max}$ . For example, two individuals with the same absolute  $VO_{2max}$  but different body masses when perform the same step test; the individual with the higher BMI will have a higher exercise heart rate leading to a reduced  $VO_{2max}$  estimation [41]. The vertical excursion during the step test is influenced by the mass of the individual. Since  $\text{Work} = \text{Force} \times \text{Distance}$  and  $\text{Force} = \text{Mass} \times \text{Acceleration}$ , with fixed distance (step height) and fixed acceleration (cadence), mass is the chief determinant of the work performed during the step test [30].

The option of a treadmill six-minute walk test seems like a lucrative option when compared to the traditional six-minute walk test as it combats the need of the uninterrupted 100 feet hallway and allows better monitoring and is less space demanding. However Stevens et al found a statistically significant difference of  $168 \pm 280$  feet (range -326 to 743 feet) between the hallway six-minute walk test versus the treadmill six-minute walk test. The factors contributing to this significant difference were the possible unfamiliarity of the subjects with the treadmill and additional voluntary effort

required for speed manipulations on the treadmill. On the contrary, walking on level ground is a more familiar skill to individuals and requires less effort to make speed manipulations [42]. The six-minute walk test has also been studied across different walkway lengths (50 m, 30 m, 15 m, 18 m, and 10 m) and circular pathways (40 feet) to accommodate for the space constraints and feasibility. The six-minute walk distance and the number of turns differ significantly with the walkway length ( $p < 0.05$ ). The greatest six-minute walk distance and lowest number of turns were recorded in the 30 m walkway. Longer walkways allow for greater room for acceleration and high top speed if the subject is able. It is speculated that shorter walkways require more time and effort to make frequent turns and result in shorter distance covered during the test. The turning bias, that is, the tendency to turn in a given direction has been identified in adults. There is significant preference to turn towards the non-dominant side. However the six-minute walk distance is not significantly influenced by this bias [43].

The use of simple, practical and valid methods of submaximally assessing  $VO_2\text{max}$  is necessary. The use of six-minute walk test and Chester step test is advocated as they are acceptable means of assessing  $VO_2\text{max}$  in healthy adult population and have high test-retest reliability. However there is no agreement between  $VO_2\text{max}$  estimated from the six-minute walk test and Chester step test and thus they cannot be used interchangeably. Also, the tests are not equally likely to be completed. The six-minute walk test is a less stressful, self-paced performance based measure which can be easily performed but mandates the requirement of an unobstructed corridor which may be difficult to procure. Thus less space occupying tests such as the Chester step test would be convenient measures for estimation of  $VO_2\text{max}$ . Chester step test requires less space but is more physiologically demanding due to its incremental nature and more antigravity muscle work required during the stepping activity which affects the completion rate of the test. Thus both the tests should be used independently taking into account the need for testing, specificity of testing, individual variability and space availability.

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## 5. Conclusion

$VO_2\text{max}$  estimated from the six-minute walk test and Chester step test show no agreement with each other.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest.

### *Statement of ethical approval*

The study was approved by the Institutional Ethics Committee, Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India.

### *Statement of informed consent*

Informed consent was obtained from all participants included in the study

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