Validation of four indirect VO2max laboratory prediction tests in the case of soccer players

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Abstract:
The aim of this study was to validate four commonly used tests (Astrand-Ryhming, Bruce, Balke and PWC150) for indirect maximum oxygen uptake (VO2max) prediction and to assess their usage in the case of soccer players. Sixteen male subjects (age = 27.8 ± 3.8 y, height = 182.8 ± 4.7 cm and body mass = 81.5 ± 8.5 kg) participated in this study. Four observed tests were compared with direct VO2max assessment which was performed as breath-by-breath gas analysis during graded treadmill protocol until exhaustion. The Astrand-Ryhming and PWC150 tests were performed on a stationary bike while the Bruce and Balke tests were conducted on a motorized treadmill. The methods of statistical analysis and comparison used were Bland-Altman plot analysis, regression analysis, the coefficient of variation and one way ANOVA. Our results revealed no agreement among measurement techniques and mean differences in all four protocols. The relationship was modest but statistically significant in the case of Bruce protocol ($R^2 = 32 \%, p \leq 0.05$). We conclude moderate reliability to be sufficiently satisfactory for the observed population and can be used under given circumstances.

Key words: Indirect, VO2max, CPET, Ergometry, Soccer.

Introduction
Cardiopulmonary exercise testing with direct measurement of VO2 (CPET) is widely considered as a golden standard for maximum oxygen uptake (VO2max) and fitness level assessment. However, this method is expensive, requires specialized staff and highly demanding preconditions need to be met (American Thoracic Society, 2003). Regrettably, not all countries meet these requirements, making CPET unavailable. Under such circumstances, especially in studies of large population, indirect VO2max prediction needs to be implemented. For indirect VO2max prediction tests, the linear relation between heart rate (HR) and oxygen output is calculated as well as the relation between maximal power output and oxygen output. With regard to the indirect VO2max measurement, the so-called field tests and laboratory ergometer tests may be used. However, accuracy of field test is not the subject of this paper and we will focus on the laboratory tests.

Over the years, several laboratory tests with different testing methodology were developed. The first submaximal cycle test was designed by Astrand and Ryhming in 1954, called the Astrand-Ryhming test (Astrand, 1954). This test extrapolates to the predicted maximal heart rate (HR) achieved from submaximal power output as its method of prediction. Another well-known submaximal cycle test is the Physical Working Capacity 150 (PWC150) test, which uses power output achieved at a target HR of 150 beats per minute (bpm) as a prediction method (Campbell, 2001). Additionally, several treadmill tests were developed, with Balke (Balke, 1959) and Bruce (Bruce, 1972) being the most preferred. These tests use time to the point of exhaustion as a method of prediction. The aforementioned tests, however, were developed for diagnostics of cardio pulmonary disorders in sedentary population and were not designed for athletes. Therefore, choosing an inappropriate test could lead to inaccurate results, questioning its accuracy for specific population groups (Basset, 2000). Consequently, before choosing a suitable test, its validity should be adequately established. Various studies have attempted to verify indirect VO2max prediction methods in laboratory conditions with the aim of assuring their validity in dissimilar population groups (sedentary population, adolescents and athletes) with constraining final results (Froelicher, 1974; Pollock, 1976, 1976 & 1982; Grant, 1999; Noonan, 2000; Chavda, 2013). Additionally, no study has been performed assessing largest athletic population, male soccer players, making findings on this topic rather scarce.

Therefore, this study was performed in order to compare the four abovementioned tests with CPET to determine whether they could be used for accurate VO2max estimate in the case of male soccer players.
**Materials and Methods**

**Participants**

Sixteen healthy, midfield male soccer players took part in this study. The athletes competed in a national league, with ≥ 13 h of training per week over the last 8 y. They were recruited through a voluntary, open-access, online poll. Prior to the testing, all participants completed a questionnaire regarding their exercise and health history. Furthermore, they were made familiar with the ethical principles, potential health risks, aims and objectives and willingly signed a statement of consent prior to participating in the study. The entire research complies with the policy statement of the Declaration of Helsinki and was approved by the Institutional Ethics Committee. The subjects’ body mass and height were assessed at the time of their first visit to the laboratory using a Seca 763 digital medical scale and stadiometer (SECA, Hamburg, Germany). The athletes morphological characteristics were: age = 27.8 ± 1.8 y, height = 182.8 ± 2.7 cm and body mass = 81.5 ± 4.5 kg.

**Exercise protocols**

Experimental data collection was managed between 09:00 and 14:00 h during the preparatory autumn period. All tests were conducted in a controlled laboratory environment (steady 21 °C aired conditioned room, 43 % air humidity). Between each test, a period of 48 h was to elapse in order to ensure adequate physiological recovery. The Astrand and PWC\(_{150}\) test were performed on a CX1 stationary bike (KETTLER\(_{Ense-Parsit, Germany}\)\), while the Balke, Bruce and CPET test were carried out on a T170 motorized treadmill (COSMED\(_{Rome, Italy}\)\). HR was collected continuously during the tests using a telemetric heart rate monitor (POLAR\(_{Kempele, Finland}\)\) for all tests. The participants visited the laboratory on five separate occasions to perform test sessions in a predetermined order, at the same time of the day, to avoid circadian variance. During the experimental period, they were required to refrain from any systematic training activity 24 h prior to the each test and to consume their last meal, free of caffeine or nutritional supplements, 2 h before every test. All participants’ had previous experience in ergometry testing.

**Direct measurement**

The preliminary CPET was assessed using a Quark PFT Ergo (COSMED\(_{Rome, Italy}\)) system by way of real breath-by-breath exchange data analysis during graded exercise testing (GXT) performed to the point of voluntary cancellation due exhaustion. The primary parameter measured was \(\text{VO}_{2\max}\) expressed in milliliters per kilogram per minute (ml·kg\(^{-1}\)·min\(^{-1}\)). Gas analyzers for oxygen (\(\text{O}_2\)) and carbon dioxide (\(\text{CO}_2\)) were calibrated for every test with a specific gas concentration (\(\text{O}_2\) 16.10 % and \(\text{CO}_2\) 5.20 % and \(\text{NO}_2\) test) according to the manufacturer’s recommendation. Flow meter turbine calibration (3 L syringe) was performed on a test basis. The GXT protocol was comprised of three stages: inaction, exercise and recovery. The inaction stage lasted for 2 min to achieve a baseline HR. The exercise stage started at speed of 6 km·h\(^{-1}\) followed by speed rise of 1 km·h\(^{-1}\) every 2 min. The incline remained constant at 1%. When person had reached exhaustion and submits, the active recovery stage began lasting for 3 min at starting values. \(\text{VO}_{2\max}\) was determined by the following parameters: a respiratory quotient (RQ) ≥ 1.15 or a plateau of \(\text{VO}_2\) in spite of a load increase (Howley, 1995). Quark PFT Suite monitoring software (COSMED\(_{Rome, Italy}\)) was used to display data numerically and graphically in real time. Fluctuations of breath-by-breath data were minimized using six breaths smoothing and 30 sec averaging.

**Indirect measurements**

The indirect \(\text{VO}_{2\max}\) tests were performed in the following order: Astrand, Bruce, PWC\(_{150}\) and Balke test. Test protocols and stage durations used were implemented to the nearest as earlier described (Astrand, 1954; Balke, 1959; Bruce, 1972; Campbell, 2001). The equations for \(\text{VO}_{2\max}\) calculation associated with the study tests, were designed for different population groups, scientifically validated and commonly cited. Accordingly, they were considered to be appropriate references for the observed population.

- Bruce test (Foster, 1984): \(\text{VO}_{2\max} = 14.8 \times (1.379 \times T) + (0.451 \times T^2) - (0.012 \times T^3)\),
- Balke test (Pollock, 1976): \(\text{VO}_{2\max} = 1.444 \times T + 14.99\),
- Astrand-Ryhming test (Buono, 1989): \(\text{VO}_{2\max} = (0.00212 \times W + 0.299) / (0.769 \times \text{HR}_{\text{max}} - 48.5) \times 100\),
- PWC test (Rost, 1982): \(\text{VO}_{2\max} = 3.5 + 12 \times W_{\text{end}}\).

\(T\) stands for the total time of the test expressed in minutes and fractions of a minute, \(W\) is maximal workload achieved during test expressed in Watt’s, \(\text{HR}_{\text{max}}\) stands for maximal heart rate achieved and \(W_{\text{end}}\) is maximal workload achieved during test divided per kilogram of body mass (Watt·kg\(^{-1}\)). In order to be awarded with the T, \(W\) or \(W_{\text{end}}\), subjects had to complete the commenced test stage in its entirety. All testing results achieved were manually logged in a data journal and evaluated in correspondence with the equations and methods discussed in the previous sections.

**Statistical analysis**

All data obtained were analyzed using MedCalc 12 (MEDCALC\(_{Belgium}\)) statistical software and presented with a mean ± standard deviation (SD) and a 95 % confidence interval (CI). The Shapiro–Wilk test was used to observe normality of all measured data with normal distribution accepted. The one way ANOVA with
repeated measures was used to assess differences in mean VO_{2\text{max}} between tests. Regression analysis with the coefficient of determination ($R^2$) was used to estimate the relationships between tests and the possibility of prediction of one result over another with CPET being dependent variable (Y) and other tests being the X variable. The Bland-Altman plot was used to compare measurement techniques and the possibility of replacement. The coefficient of variation (CV) was used to measure the relative variability of the data sets with CPET considered as the true value. The standard error of the estimate (SEE) was used to reflect the random error within the tests. Alpha levels for all hypothesis testing were set at $p \leq 0.05$ as the level of significance for statistical tests unless otherwise stated.

**Results**

An analysis of the data has found inconsistency of the VO_{2\text{max}} values in the observed tests. The within-subjects characteristics with CPET values shown for comparison can be seen in Table 1. We observed highest differences related to measure in Balke test, followed by PWC_{150} and Astrand-Rhyming test. When compared with CPET, highest similarity was present in Bruce test.

The pairwise comparison of one way ANOVA revealed highest VO_{2\text{max}} mean difference between CPET and PWC_{150} test (22.37 ml·kg·min^{-1} · CI 17.49 to 27.25 and SEE = 1.49). The Astrand-Rhyming test (19.75, 95 % CI 15.04 to 24.45 and SEE = 1.43) and Balke test (11.75, 95 % CI 6.83 to 16.72 and SEE = 1.51) followed. The lowest difference and error of estimate was observed in Bruce test (6.26, 95 % CI 1.61 to 10.90 and SEE = 1.41).

Table 1. Physiological characteristics of participants ($n = 16$) VO_{2\text{max}} (ml·kg^{-1}·min^{-1}) in all five tests

<table>
<thead>
<tr>
<th>Protocols</th>
<th>PWC</th>
<th>Astrand</th>
<th>Balke</th>
<th>Bruce</th>
<th>CPET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arithmetic mean *</td>
<td>31.25</td>
<td>33.87</td>
<td>41.84</td>
<td>47.36</td>
<td>53.62</td>
</tr>
<tr>
<td>$SD$</td>
<td>2.53</td>
<td>2.07</td>
<td>1.69</td>
<td>5.61</td>
<td>6.41</td>
</tr>
<tr>
<td>$SEE$</td>
<td>0.63</td>
<td>0.52</td>
<td>0.42</td>
<td>1.40</td>
<td>1.60</td>
</tr>
<tr>
<td>$CV$</td>
<td>8.10</td>
<td>6.11</td>
<td>4.04</td>
<td>11.85</td>
<td>11.95</td>
</tr>
<tr>
<td>95 % CI</td>
<td>29.90 to 32.60</td>
<td>32.77 to 34.97</td>
<td>40.94 to 42.74</td>
<td>44.37 to 50.35</td>
<td>50.20 to 57.0</td>
</tr>
</tbody>
</table>

* $p \leq 0.05$, $n =$ number of participants, $SD =$ standard deviation, $SEE =$ standard error, $CV =$ Coefficient of variation (SD*100/mean)

The regression equations, derived from the collected data can be observed in Table 2. The correlation was found to be significant ($p \leq 0.05$) only in the case of the Bruce test.

Table 2. Regression analysis results ($n = 16$)

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Astrand</th>
<th>Balke</th>
<th>Bruce</th>
<th>PWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression Equation</td>
<td>$y = 3.84 + 1.47 x$</td>
<td>$y = -2.48 + 1.34 x$</td>
<td>$y = 23.07 + 0.65 x$</td>
<td>$y = 23.73 + 0.96 x$</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.23</td>
<td>0.13</td>
<td>0.32</td>
<td>0.14</td>
</tr>
<tr>
<td>Standard error for coefficients $a$ and $b$</td>
<td>$a = 24.71$</td>
<td>$a = 39.68$</td>
<td>$a = 12.01$</td>
<td>$a = 19.64$</td>
</tr>
<tr>
<td>$b = 0.73$</td>
<td>$b = 0.95$</td>
<td>$b = 0.25$</td>
<td>$b = 0.63$</td>
<td></td>
</tr>
<tr>
<td>$F$ ratio</td>
<td>4.07</td>
<td>2.00</td>
<td>6.55</td>
<td>2.33</td>
</tr>
<tr>
<td>$p$</td>
<td>$= 0.06$</td>
<td>$= 0.18$</td>
<td>$= 0.02$</td>
<td>$= 0.15$</td>
</tr>
<tr>
<td>$SEE$</td>
<td>5.84</td>
<td>6.21</td>
<td>5.48</td>
<td>6.15</td>
</tr>
</tbody>
</table>

$n =$ number of participants, $SEE =$ standard error of the estimate

The results of the Bland-Altman plot analysis can be seen in Figure 1. The highest divergence was between the PWC_{150} test and CPET (Figure 1a), with 22.37 ml·kg^{-1}·min^{-1} of VO_{2} (CI 95 % 19.21 to 25.54, $p < 0.01$). The limit of agreement was 23.27 ml·kg^{-1}·min^{-1} of VO_{2}. The discrepancy between Astrand-Rhyming test and CPET (Figure 1b) was 19.75 ml·kg^{-1}·min^{-1} of VO_{2} (CI 95 % 16.70 to 22.80, $p < 0.01$). The range of limit of agreement was 22.45 ml·kg^{-1}·min^{-1} of VO_{2}. For Balke test (Figure 1c), mean difference was 11.76 ml·kg^{-1}·min^{-1} of VO_{2} (CI 95 % 8.56 to 14.99, $p < 0.01$). The limit of agreement was 23.59 ml·kg^{-1}·min^{-1} of VO_{2}. The Bruce test revealed lowest (Figure 1d) dissimilarity of the mean with 6.26 ml·kg^{-1}·min^{-1} of VO_{2} (CI 95 % 3.24 to 9.27, $p < 0.01$). The lower and upper limit of agreement was 22.17 ml·kg^{-1}·min^{-1} of VO_{2}.
Fig. 1. Plotting the VO$_{2\text{max}}$ mean differences between indirect methods and CPET with Bland and Altman method of approach. a) PWC$_{150}$ vs CPET; b) Astrand-Ryhming vs CPET; c) Balke vs CPET; d) Bruce vs CPET.

Discussion

This study is the first to examine the validity and existence of a connection between the observed four indirect VO$_{2\text{max}}$ prediction tests and direct VO$_{2\text{max}}$ assessment in the case of male soccer players. The strength of relationship was low, but nevertheless significantly correlated ($R^2 = 32 \%$, $p < 0.05$) only in the case of Bruce test. Bruce test furthermore, demonstrated biggest similarity of the measurement dispersion ($CV = 11.85$), lowest standard error of the estimate ($SEE = 1.40$) and lowest mean difference (6.26 ml·kg$^{-1}$·min$^{-1}$) compared with true CPET values. The highest differences were detected in the PWC$_{150}$ test followed by Astrand-Ryhming and Balke tests, making them a non-valid method of VO$_{2\text{max}}$ prediction in observed population. We consider a direct comparison of our study with any previous studies to be impeded due to the fact how no study was ever performed on male soccer players. However, a comparison with similar studies is possible.

In study by Grant et al. (1999), when observing Astrand-Ryhming test, $R^2 = 0.16$, $p < 0.05$ and a $CV = 9.19 \%$ was reported when sedentary population (age 18 - 35 y) was tested, with tendency of the Astrand-Ryhming test to over-predict the real VO$_{2\text{max}}$. According to Kasch et al. (1984), the Astrand-Ryhming test is an inappropriate substitute for direct measurement of maximal oxygen intake in sedentary population (age range 30 - 66 y) due to high HR$_{\text{max}}$ calculation inaccuracies (reported $R^2=0.34$ and $CV = 30$). The reasons for such a high error present can be linked to Astrand-Ryhming methodology. Original test recommends cycling at 50 revolutions per minute (rpm), hence McKay et al. (1976) suggests using higher values (80 – 100 rpm) for optimal VO$_{2\text{max}}$. Furthermore, extrapolation of the submaximal HR vs work rate regression line to predict the HR$_{\text{max}}$, which is then used to predict W$_{\text{max}}$, and finally to predict VO$_{2\text{max}}$ is highly age, body size and fitness level sensitive and open for errors in submaximal tests (Froelicher, 1974; Kasch, 1984). Hence, we can conclude how Astrand-Ryhming test is inappropriate method of VO$_{2\text{max}}$ estimation in case of athletes with tendency to under-predict actual oxygen consumption. We believe how this occurrence can be additionally explained by the higher fitness level, strong lower extremities and cycling adaptation of the athletes (Poole, 1996). Grant et al. (1999) tested a sedentary population (age 18 to 35 y) and observed a low connection between the Bruce test and CPET ($R^2=0.24$, $p < 0.10$ and $CV = 8.82 \%$). Furthermore, this study used a different equation for indirect estimation compared to our research. Koultianos et al. (2013) while testing a large group of athletes, reported $R^2=0.41$, $p < 0.05$ and a $SEE = 6.11 \%$ for Bruce test, with overestimation of VO$_{2\text{max}}$. However, this study tested athletes from different sport disciplines and used the ACSM running equation to which the high validity coefficient and higher inter-subjects variation could be attributed. Froelicher et al. (1974) tested sedentary population and reported an
\[ R^2 = 0.76 \text{ and a } SEE = 4.71 \text{ in Bruce test. Same study reported modest degree of relationship between actual and predicted } \text{VO}_{2\text{max}} \text{ in Balke test (} R^2 = 0.64 \text{ and } SEE = 3.95 \text{). They explained their results by means of existence of high metabolic increments between the test stages. Pollock et al. (1976) reported } R^2 = 0.85, p \leq 0.05 \text{ and } CV = 0.25 \% \text{ for a sedentary elderly population in Balke test, using a self-developed nomogram. Although Pollock’s nomogram was used in our study as well, we obtained no similar results in athletes. We consider subjects differences in age and fitness level as main inter-study bias (Poole, 1996). Our results were only consistent with Pollock to confirm under predictions of } \text{VO}_{2\text{max}}. \text{ No study was found comparing the PWC}_{150} \text{ test with CPET. Moreover, our results showed no statistical significance for the PWC}_{150} \text{ test so we concluded that mentioned test is not a valid predictor of actual } \text{VO}_{2\text{max}} \text{ in our observed group. The PWC}_{150} \text{ test demonstrated highest under predictions of } \text{VO}_{2\text{max}} \text{ values and error associated with it. Our observations can be contributed to the testing methodology (submaximal cycle test). An analysis of the data obtained by the Bland and Altman method of approach for limits of agreement between the CPET and predictive tests suggest ambiguous results in all four tests, with lowest mean difference and lowest CI 95 \% observed in the Bruce test with statistical significance accepted. Accounting for our statistical goals, we consider the agreement interval sufficiently narrow for our purpose. Hamlin et al. (2012) revealed increases in limits of agreement caused by group homogeneity and higher participants’ aerobic power, providing the basis for our obtained results.}

For predictive test to be statistically significant, it is required to have a minimal } R^2 = 0.70, p \leq 0.05 \text{ (Hamlin, 2012). Since } R^2 \text{ depends on the variance and range of a group of scores, less variance will lead to low validity. In our study, the variance in all four tests was very low but only statistically significant in the case of Bruce test (} F – \text{ ratio} = 6.55, p < 0.05 \text{) where a real effect difference was observed. A study with a small sample size, such as ours, has a reduced chance of detecting a real effect and random error, thus being one of the possible reasons for our particular observations. However, our target population were midfield soccer players. Hence, if the target population is narrow, then the sample can be smaller but still be representative. Grant et al. (1999) explained that a low validity coefficient is influenced by high group homogeneity and that this could be the reason for a lower correlation rather than lack of connection. The same study reported that the validity of the test should, instead, be rather observed by the greater importance of the error present with an acceptance in the 10 \% margin. Given that we tested athletes from one sport and an identical playing position, we observed a very high instance of homogeneity which could have affected the final results. Furthermore, lowest } SEE \text{ and error of coefficients } a \text{ and } b \text{ was observed in the Bruce test. Ultimately, we observed that the previous studies tested heterogeneous groups (sedentary population with high age and fitness level range or athletes from different sport disciplines), directly affecting the validity coefficient. We believe, when a homogenous group of top athletes is tested, an underestimation of } \text{VO}_{2\text{max}} \text{ values and a low validity coefficient should be expected when observed indirect prediction methods are used.}

Furthermore, during direct } \text{VO}_{2\text{max}} \text{ assessment, we employed GXT protocol, designed to reproduce real running conditions and objective } \text{VO}_{2\text{max}} \text{ measurement (Jones, 1996). Previous studies, however, used the original Bruce, Balke or Astrand-Ryhming protocol during the direct assessment, affecting final results due to the displacement of actual } \text{VO}_{2\text{max}} \text{ values. Additionally, the observed predictive tests use a different methodology for } \text{VO}_{2\text{max}} \text{ estimation (cycle or treadmill ergometer) were higher } \text{VO}_{2\text{max}} \text{ in treadmills then cycle ergometry tests is to be expected, additionally influencing the final outcome (Storer, 1990). Further reasons behind such inter-study differences could be linked to the equations associated with indirect } \text{VO}_{2\text{max}} \text{ calculations (Bassett, 2000). The observed studies used different equations, making inter-study comparison hindered. We observed how only } T, \text{ HR and load were taken into consideration when calculating the } \text{VO}_{2\text{max}}. \text{ Foster et al. (1984) suggested age and gender as significant impact factors and reported a lack of forecast significance in treadmill tests when only exercise time as a predictor of } \text{VO}_{2\text{max}} \text{ was used due to large metabolic increments. Furthermore, he reported increase in } R^2 > 0.20 \text{ when gender, age and activity variables were added to standard equations. Koultianos et al. (2013) demonstrated a connection between these “traditional” types of equations and inaccurate final results causing underestimation of } \text{VO}_{2\text{max}} \text{ in athletes. As a result, we recommend modifications of the currently used equations for athletes.}

Conclusion

Findings of this study enhance our understanding of indirect } \text{VO}_{2\text{max}} \text{ prediction and may be applied to other similar studies worldwide. We do not consider these new findings trivial, as we do not support recommendations that one could actually substitute the direct } \text{VO}_{2\text{max}} \text{ measurement. By observing the data obtained, we can conclude that statistically acceptable validity is present in the case of Bruce test and that the results obtained are sufficient for the studied population.}

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References