

**Enhancing specificity in proxy-design for the assessment of bioenergetics** (1<sup>st</sup> heading in importance)

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## **Enhancing specificity in proxy-design for the assessment of bioenergetics** (1<sup>st</sup> heading in importance)

### **Abstract**

The purpose of this study was to examine the hypothesis that improved prediction of bioenergetics may be achieved when proxies are designed to closely simulate gold standard laboratory protocols. To accomplish this, a modified 'square' variation (SST) of the classical 20m Multistage Shuttle Run Test (MST) was designed aiming to reduce the stopping, turning, and side-stepping manoeuvres. Within two weeks, 50 male volunteers (age  $21.5 \pm 1.6$ , BMI  $24.4 \pm 2.2$ ) randomly underwent three maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) assessments using a treadmill test (TT), the SST and MST. To assess SST reproducibility, 10 randomly-selected subjects performed the test twice. Validity results showed that mean predicted  $\dot{V}O_{2\max}$  from SST was not significantly different compared to TT  $\dot{V}O_{2\max}$  ( $p > 0.05$ ). In contrast, the equivalent value from MST was significantly higher ( $p < 0.001$ ) than TT. Furthermore, TT  $\dot{V}O_{2\max}$  correlated with SST and MST at  $r = 0.88$  ( $p < 0.001$ ) and  $r = 0.61$  ( $p < 0.05$ ), respectively. The '95% limits of agreement' analysis ( $LIM_{AG}$ ) for SST and MST indicated a range of error equal to  $-0.5 \pm 5.4$  and  $8.1 \pm 8.0$  ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) with a coefficient of variation of  $\pm 6$  and  $\pm 8.2\%$ , respectively. Test-retest results for SST revealed no mean difference in  $\dot{V}O_{2\max}$  ( $p > 0.05$ ) and a correlation coefficient of  $r = 0.98$  ( $p < 0.001$ ), while  $LIM_{AG}$  demonstrated a range of error equal to  $-0.2 \pm 2.6$  ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) with a coefficient of variation of  $\pm 5.6\%$ . It is concluded that, compared to MST, the SST had a higher agreement with TT. The latter may well be explained by the closer simulation in bioenergetics between the two protocols (i.e. the continuous nature of SST provides a closer proxy of TT).

**Abstract word count:** 249

## **Introduction** (2<sup>nd</sup> heading in importance)

Field assessment of bioenergetics [namely maximal oxygen uptake ( $\dot{V}O_{2max}$ )] with minimal equipment and cost presents a continuous interest for many researchers seeking information on cardiorespiratory elements associated with health-related fitness and performance enhancement (1-3). Although voluminous literature has appeared about the attributes of this approach (4, 5), it remains curtailed mainly because the majority of proxies represent field measures designed to predict laboratory bioenergetics which, in turn, are used to provide information on ‘field performance’. It seems, therefore, that minor methodological flaws in proxy-design may have significant impact on assessing cardiorespiratory fitness and/or performance.

The majority of proxies assessing bioenergetics utilize various exercise protocols and powerful statistical tools in order to link specific field-performance indices (e.g. velocity, time, heart rate) with  $\dot{V}O_{2max}$  measured – usually – during laboratory treadmill running (2, 6). However, it seems reasonable to suggest that prediction power may be limited when physiological and/or biomechanical disparity between the proxy and the gold standard laboratory test are considered. Lack of specificity in factors such as intensity, duration, exercise mode, technique and, particularly, musculature employed may account for significant performance differences between the proxy and the gold standard. This may explain the reduced precision frequently reported in relation to field-testing (3, 7, 8).

The 20m multistage shuttle run test (MST) (6), a widely-used proxy-assessment of treadmill  $\dot{V}O_{2max}$ , incorporates stopping, turning and side-stepping at the end of each 20-meter shuttle. However, such manoeuvres may considerably increase net muscle activation compared to steady-state forward running (9). Since energy utilization depends largely on the muscle mass being employed (10), variations between musculature activated during the MST and the treadmill test will probably result in performance discrepancies. Conversely, it seems reasonable to suggest that improved prediction of bioenergetics may be achieved when proxies closely simulate the laboratory protocols. Therefore, the main purpose of this study was to examine the effects of minimized stopping, turning and side-stepping manoeuvres on MST precision. To achieve this, a

modified 'square' version of the MST was devised to incorporate a reduced turning angle – thus resembling more the actions of forward treadmill running.

## **Methods and procedures** (2<sup>nd</sup> heading in importance)

### **Subjects** (3<sup>rd</sup> heading in importance)

Fifty adult males volunteered to participate in the study. The subjects were recreational athletes, not specialized in a particular sport. For the purpose of data analysis subjects were randomly assigned to either the model (n=40) or the validation (n=10) group. Anthropometrical data appear in Table 1. Exclusion criteria included smoking and any benign medical history. Written informed consent was obtained from all subjects after full explanation of the procedures involved. This study received approval from the Research Ethics Board of the University of Thessaly.

Each participant visited the data collection sites on three different occasions within a 14-day period. One visit was reserved for the laboratory assessment of  $\dot{V}O_{2max}$ , while field-testing [i.e., the 'square' variation (SST) and the classic MST] was conducted in the same rubber-floored gymnasium during the two remaining occasions. To assess whether the SST was reproducible, the validation group performed this test twice, seven days apart. Prior to data collection visits, subjects were familiarised with all assessment protocols. They were also advised to avoid stressful activities 36-48 hours prior to data collection visits. Tests were conducted in a random order by the same investigators and at approximately the same time of the day (late mornings or early afternoons).

\*\*\* Table 1 near here \*\*\*

### *Incremental treadmill test (TT)* (4<sup>th</sup> heading in importance)

A modified Bruce treadmill test (TT) to exhaustion was used to elicit  $\dot{V}O_{2max}$  (11). The test commenced at 9 km·h<sup>-1</sup> with 2 min speed increments of 1 km·h<sup>-1</sup> until exhaustion. Treadmill inclination throughout testing remained at 0° while  $\dot{V}O_{2max}$  was confirmed when at least two of the following criteria were met: 1) maximal heart rate greater than 185 bpm, 2) respiratory quotient greater than 1.1, and 3) detection of plateau in  $\dot{V}O_2$  curve. Oxygen uptake was measured via open circuit spirometry using an automated gas analyser (Vmax 29, SensorMedics, USA). Respiratory parameters were recorded every

20 seconds during testing while subjects inspired room air through a low-resistance two-way Rudolph valve. The gas analysers were calibrated with standard gases previously checked by microtechniques. Spot checks were made on the calibration of the pneumotachograph for volume flows up to 200 l·min<sup>-1</sup>.

Unlike the inclined treadmill running adopted by Léger and Gadoury (6), the horizontal treadmill protocol used herein has a closer agreement with field running (12). Nevertheless, since the MST has been designed to predict  $\dot{V}O_{2max}$  of a specific treadmill test, this protocol-diversity was addressed by introducing a new prediction model based on the current data (see Statistical Analysis section).

#### *20m square shuttle test (SST) (4<sup>th</sup> heading in importance)*

This test involves running on the four 20m-long sides of a square marked on the floor of a gymnasium (fig.1) with the choice of performing the test running either clockwise or counter-clockwise. Four pairs of cones are placed at the corners of the square to ensure adherence. One to four subjects can perform the test simultaneously. Each subject should start the test at one of the cone stations and follow the prescribed pace for as long as he/she is able to be at the cone stations in synchrony (i.e.  $\pm 1$ sec) with the sound signals emitted from the classical MST pre-recorded audiotape. Individuals should be advised to perform wide turns, thus avoiding disturbances in their running technique. The test is terminated when subjects are unable to maintain the prescribed pace for three consecutive signals. In the present study, subjects performed the test individually to eliminate competition bias.

\*\*\* Figure 1 near here \*\*\*

#### *20m multistage shuttle run test (MST) (4<sup>th</sup> heading in importance)*

This test was conducted according to published procedures (6). Subjects performed the test individually and were instructed to run between two lines 20m apart in synchrony with a sound signal emitted from an audiocassette. The test was terminated when subjects were unable to maintain the prescribed pace for three consecutive signals.

### ***Statistical analysis*** (3<sup>rd</sup> heading in importance)

Stepwise linear regression analysis was used to develop a  $\dot{V}O_{2max}$  prediction equation for the SST (EQ<sub>SST</sub>) using data from the model group. A  $\dot{V}O_{2max}$  prediction equation for the MST (EQ<sub>MST</sub>) was also developed, using the same model group data, to cater for the fact that a different treadmill protocol was originally utilized (6). Correlation coefficients and analysis of variance (ANOVA) were used to detect possible bias between the actual and the predicted values from the two models. Thereafter, data from the validation group were used to cross-validate EQ<sub>SST</sub>, EQ<sub>MST</sub>, as well as the original equation reported by Léger and Gadoury (6) (EQ<sub>LÉG</sub>). Correlation coefficients, ANOVA, 95% limits of agreement analyses (LIM<sub>AG</sub>) and percent coefficients of variation (CV%) were adopted for both validity and reproducibility assessments according to known procedures (13). The level of significance for all statistical analyses was set at  $p < 0.05$ .

### **Results** (2<sup>nd</sup> heading in importance)

#### ***Prediction of $\dot{V}O_{2max}$*** (3<sup>rd</sup> heading in importance)

Stepwise linear regression analyses revealed that the maximal attained speed (MAS) ( $\text{km}\cdot\text{h}^{-1}$ ) was the best predictor of  $\dot{V}O_{2max}$  ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) for both SST and MST. Examination of residuals scatterplots detected no violation of normality, linearity, and homoscedasticity between predicted  $\dot{V}O_{2max}$  scores and errors of prediction, while Mahalanobis distance of each case to the centroid of all cases detected no multivariate outliers for  $\chi^2 < 0.001$ . Relevant statistics from the calculated  $\dot{V}O_{2max}$  prediction models for SST [1] and MST [2] appear in Table 2.

$$[1] \text{ SST } \dot{V}O_{2max} = \text{MAS} \times 3.679 - 7.185$$

$$[2] \text{ MST } \dot{V}O_{2max} = \text{MAS} \times 3.56 + 2.584$$

\*\*\* Table 2 near here \*\*\*

#### ***Validity assessments*** (3<sup>rd</sup> heading in importance)

Means ( $\pm$ SD) and correlation coefficients of various performance indices from all three  $\dot{V}O_{2max}$  protocols appear in Table 3. Preliminary analyses for LIM<sub>AG</sub> revealed no positive

relationship between the differences/errors [either ( $EQ_{SST} - TT$ ) or ( $EQ_{MST} - TT$ ) or ( $EQ_{LÉG} - TT$ )] and the size of measurements [given by either (the mean of  $EQ_{SST}$  and  $TT$ ) or (mean of  $EQ_{MST}$  and  $TT$ ) or (mean of  $EQ_{LÉG}$  and  $TT$ )], respectively. Thus, the  $LIM_{AG}$  can be reported as absolute measurements (14). Finally, unlike  $EQ_{SST}$  and  $TT$  ( $t = -0.1$ ,  $p > 0.05$ ), the mean difference (error) between estimates from  $EQ_{MST}$  and  $TT$  ( $t = -2.4$ ,  $p < 0.05$ ) as well as  $EQ_{LÉG}$  and  $TT$  was biased ( $t = -8.1$ ,  $p < 0.001$ ). Indices for  $LIM_{AG}$  and  $CV\%$  appear in Table 3.

\*\*\* Table 3 near here \*\*\*

### ***Reproducibility assessment*** (3<sup>rd</sup> heading in importance)

Table 4 demonstrates no significant differences ( $p > 0.05$ ) between the mean values from the first ( $SST_1$ ) and the second ( $SST_2$ ) trial in the studied performance parameters. The correlation coefficient between trials for all parameters was  $r = 0.98$  ( $p < 0.001$ ). Preliminary investigation for the  $LIM_{AG}$  analysis revealed no positive relationship between the  $\dot{V}O_{2max}$  differences/errors [ $SST_1 - SST_2$ ] and the size of measurements [given by the mean of  $SST_1$  and  $SST_2$ ]. The mean difference between  $\dot{V}O_{2max}$  estimates on the first and second trial was not biased ( $t = -1.7$ ,  $p > 0.05$ ). Results for  $LIM_{AG}$  and  $CV\%$  appear in Table 4.

\*\*\* Table 4 near here \*\*\*

### **Discussion** (2<sup>nd</sup> heading in importance)

The main purpose of this study was to examine the hypothesis that improved prediction of bioenergetics may be achieved when proxies are designed to closely simulate gold standard laboratory protocols. To fulfil this, we investigated the validity of the widely-used MST against the SST. The latter test was designed to minimize stopping, turning and side-stepping manoeuvres – thus closely resembling the gold standard forward treadmill running. The main finding was that, compared to the classic MST, the SST had a higher agreement with the gold standard laboratory test in predicting  $\dot{V}O_{2max}$  and assessing relevant performance parameters. Furthermore, the SST preserved the high reproducibility previously reported for the classical version of the test (i.e. MST) (1).

The main reason for assessing  $\dot{V}O_{2\max}$  is to provide relevant data that will allow a more precise planning of training and, ultimately, enhance field performance. Previous studies examining different laboratory tests have stressed the importance of specificity when assessing bioenergetics (15). For instance, the quantitative effects of training cardiovascular and respiratory functions are optimally evaluated only by adopting tests that primarily activate muscles used for this training (16). Despite the suggestion that predicted  $\dot{V}O_{2\max}$  is significantly influenced by the test utilized (17), according to our knowledge, specificity of proxies has not been scrutinized hitherto. Application of the specificity principle in proxies predicting  $\dot{V}O_{2\max}$  would suggest similar exercise mode, intensity, duration, technique and muscular action between the laboratory protocol used as gold standard and the proxy. Results from the present study support the latter notion demonstrating that proxies should be designed to assess bioenergetics should mimic the intensity, duration, exercise mode, technique, and muscular action of the gold standard laboratory test in order to achieve the highest accuracy and precision.

The MST utilizes information from shuttle running to predict  $\dot{V}O_{2\max}$  which has been derived from forward treadmill running. However, published reports suggest that manoeuvres incorporated in shuttle running may increase net muscle activation compared to forward running (9). In contrast, the SST  $\dot{V}O_{2\max}$  prediction has been based on fairly similar running modes (i.e. continuous ‘elliptic’ field-running and forward treadmill-running). Since energy utilization depends largely on the muscle mass being employed (10), variations in the mechanics – and, therefore, musculature activated – between the two field tests and the gold standard contribute significantly to the observed variations in  $\dot{V}O_{2\max}$ , maximal velocity, and test duration. Furthermore, these differences allude to the notion that intensity in the MST is markedly increased compared to the gold standard test. These results are also in line with previous reports questioning metabolic (8, 18, 19) and performance-based (20) aspects of the classical MST.

In addition, it seems tenable that the aforementioned manoeuvres incorporated in MST represent biomechanical complexities which are dealt by each subject according to individual skills. Although agility, strength, and sport-specific skills are very important in sport performance, these factors should be evaluated individually by element- and sport-specific tests. The presence of these factors in a cardiorespiratory fitness field test constitutes a significant source of inter-individual variation that is not present during the



gold standard test. As illustrated by the present CV% indices, the  $\dot{V}O_{2max}$  prediction of  $EQ_{MST}$  and  $EQ_{LÉG}$  can be up to 1.4 times as ‘unreliable’ as the prediction of  $EQ_{SST}$ . Although the limits of agreement in  $EQ_{SST}$  are still relatively wide, this range is more likely to be acceptable by exercise scientists and coaches compared to  $EQ_{MST}$  and  $EQ_{LÉG}$ . Due to the increased agreement and precision with the gold standard  $\dot{V}O_{2max}$ , results from the SST can be used as parsimonious means for cross-sectional as well as longitudinal evaluation of training prescription and analysis of training adaptation. Further, due to the elimination of factors unrelated to cardiorespiratory fitness, it seems reasonable that results from SST can be employed in diverse sporting disciplines. Ergo, the SST may represent a valid and cost-effective tool in circumstances where, although laboratory testing is not feasible, an accurate and precise evaluation of bioenergetics is required.

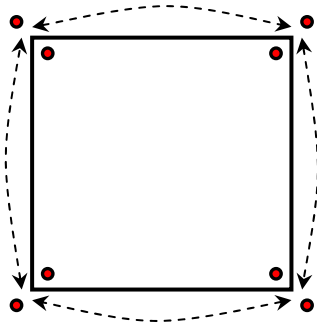
The present study is limited by the relatively small sample spectrum and by the lack of examining the effect of diverse sporting backgrounds on SST and MST performance. Within these limits, it is concluded that improved prediction of bioenergetics may be achieved when proxies closely simulate laboratory protocols. Although the rapid screening of large groups of individuals by practical proxies such as the MST is acknowledged, scientists should appreciate the validity and precision required to accurately assess cardiorespiratory fitness levels and advise the individual.

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**Figure 1.**



**Legend**

**Figure 1.** The 20 meter square shuttle test.

**Table 1.** Anthropometrical data and dynamometry results for all subjects and groups [mean(SD)].

Parameters	Model Group (n=40)	Validation Group (n=10)	Entire Sample (n=50)
Age (years)	21.6(1.6)	21.3(1.7)	21.5(1.6)
Height (cm)	177.2(6.3)	178.1(9.6)	177.5(7.3)
Weight (kg)	78.6(10.6)	75.3(11.9)	77.7(11)
BMI (kg/m <sup>2</sup> )	24.7(2.3)	23.6(2.3)	24.4(2.2)

Note: ANOVA detected no significant differences between the two sub-groups in any of the parameters presented.

Key: BMI = body mass index.

**Table 2.** Stepwise multiple regression for predicting  $\dot{V}O_{2\max}$  using maximal attained speed in the model group (n=40).

	MAS	R <sup>2</sup>	adjR <sup>2</sup>	Intercept	B	β	SEE	$\dot{V}O_{2\max}$	r
EQ <sub>SST</sub>	14.5(1.3)	0.77	0.76	-7.185	3.679**	0.88	2.55	46.2(4.6)	0.88**
EQ <sub>MST</sub>	12.3(0.1)	0.38	0.35	2.584	3.56*	0.61	4.23	46.4(3.2)	0.61*

Note: \* p<0.05; \*\* p<0.001.

Key: MAS = maximal attained speed [mean(SD)]; R<sup>2</sup> = coefficient of determination; adjR<sup>2</sup> = adjusted coefficient of determination; Intercept & B = unstandardized coefficients; β = standardized coefficient; SEE = standard error of the estimate;  $\dot{V}O_{2\max}$  = predicted values using the calculated models [mean(SD)]; r = correlation coefficient between actual and predicted values; EQ<sub>SST, MST</sub> = prediction models for each test developed from the model group.

**Table 3.** Comparison between all three tests [means(SD)] in the validation group (n=10).

Test	$\dot{V}O_{2max}$	LIM <sub>AG</sub>	CV%	r	MAS	Time
TT	47.2(6.0)	---	---	---	15.4±1.2	14:30±2:25
SST	46.9(4.9) <sub>SST</sub>	-0.3±5.4 <sub>SST</sub>	6.0 <sub>SST</sub>	0.79**	14.7±1.4†**	12:38±2:36**
MST	50.1(2.7) <sub>MST</sub> †	2.9±7.6 <sub>MST</sub>	7.9 <sub>MST</sub>	0.58**	13.4±0.7‡*	9:36±1:41‡*
	55.3(4.9) <sub>LÉG</sub> ‡	8.1±8.0 <sub>LÉG</sub>	8.2 <sub>LÉG</sub>	0.50*		

Note: ANOVA against TT: † different at p<0.05; ‡ different at p<0.001.

Correlation coefficient against TT: \* significant at p<0.05; \*\* significant at p<0.001.

Key:  $\dot{V}O_{2max}$  = maximal oxygen uptake ( $ml \cdot kg^{-1} \cdot min^{-1}$ ); LIM<sub>AG</sub> = calculated limits of agreement for  $\dot{V}O_{2max}$ ; CV% = percent coefficient of variation for  $\dot{V}O_{2max}$ ; r = correlation coefficient against TT for  $\dot{V}O_{2max}$ ; MAS = maximal attained speed ( $km \cdot h^{-1}$ ); Time = exercise time to exhaustion (min); EQ<sub>SST, MST</sub> = prediction models for each test developed from the model group; LÉG = prediction model for MST reported by Leger and Gadoury (1989).

**Table 4.** Reproducibility results [means(SD)] from the two trials in the validation group (n=10).

Test	$\dot{V}O_{2max}$	MAS	Time
SST <sub>1</sub>	46.9(4.9)	14.7 (1.3)	12:38(2:36)
SST <sub>2</sub>	47.1(5.0)	14.8(1.4)	12:40(2:37)
LIM <sub>AG</sub> : -0.21±2.6      CV% : 5.6      r = 0.98 (p<0.001)			

Note: ANOVA detected no significant differences in any of the parameters presented.

Key: SST<sub>1</sub> = first trial; SST<sub>2</sub> = second trial;  $\dot{V}O_{2max}$  = maximal oxygen intake (ml·kg<sup>-1</sup>·min<sup>-1</sup>); MAS = maximal attained speed (km·h<sup>-1</sup>); Time = exercise time to exhaustion (min); LIM<sub>AG</sub> = calculated limits of agreement; CV% = percent coefficient of variation r = correlation coefficient between trials for all parameters presented.