

## Accuracy of Graded Exercise Test and Alternative Protocols in Predicting O<sub>2</sub>Pmax

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We were very interested in the recent research focused on evaluating cardiovascular performance in young populations. The maximal oxygen pulse ( $O_2P_{max}$ ), calculated as the ratio of peak oxygen uptake ( $VO_{2peak}$ ) to peak heart rate ( $HR_{peak}$ ), has become an important, non-invasive marker for assessing cardiopulmonary function (Wasserman, K., et al., 2012). Nevertheless, direct measurement through cardiopulmonary exercise testing (CPET) is often impractical for large-scale studies (Fletcher, G. F., et al., 2013), highlighting the need for reliable estimation methods. Although several such protocols are available for adults (Lang, J. J., et al., 2018), their accuracy in adolescents—who experience significant physiological changes—has not been thoroughly validated. Our study sought to address this by comparing  $O_2P_{max}$  estimates derived from one laboratory-based and three field-based exercise tests against a standard reference in adolescent males. We conducted a cross-sectional study involving 60 healthy adolescent boys (mean age  $16.05 \pm 0.81$  years). Participants underwent four exercise tests in a randomized order: a Graded Exercise Test (GXT) on a treadmill, two cycle ergometer tests (PWC195 and PWC212) (McMurray, R. G., et al., 2014), and a 1-mile run (Table 1). The Cooper protocol served as the criterion measure for comparison.  $O_2P_{max}$  was calculated for each test as the ratio of estimated  $VO_{2peak}$  (using validated, protocol-specific regression equations (George, J. D., et al., 1993; Cooper, K. H., 1968) to measured  $HR_{peak}$ . We analyzed the absolute mean deviations of each protocol's  $O_2P_{max}$  estimate from the Cooper criterion and used binomial tests to identify systematic bias (over- or underestimation).

**Table 1.** Absolute mean deviations of maximal oxygen pulse estimates from the Cooper criterion protocol

Protocol	Mean $\pm$ SD
Cooper-GXT (ml/beat)	1.59 $\pm$ 0.90
Cooper-PWC 212 (ml/beat)	3.80 $\pm$ 1.82
Cooper-PWC 195 (ml/beat)	3.12 $\pm$ 1.67
Cooper-1 mile (ml/beat)	2.49 $\pm$ 1.42

Our results revealed significant differences in the accuracy of the protocols (Table 2). The GXT protocol demonstrated the closest agreement with the Cooper criterion, showing the smallest mean absolute deviation (1.59  $\pm$  0.90 ml/beat) and, crucially, no significant systematic bias ( $p=0.519$ ). In stark contrast, the cycle ergometer protocols showed substantial underestimation. The PWC212 protocol had the largest mean deviation (3.80  $\pm$  1.18 ml/beat), followed by the PWC195 protocol (3.12  $\pm$  1.67 ml/beat); both significantly underestimated  $O_2P_{max}$  ( $p<0.001$  for both). The 1-mile run field test also significantly underestimated  $O_2P_{max}$  (mean deviation: 2.49  $\pm$  1.42 ml/beat,  $p=0.003$ ). We suggest that the GXT protocol's superiority stems from its progressive, individualized treadmill design, which promotes a more natural running pattern and allows adolescents to fully express their cardiopulmonary capacity.

**Table 2.** Analysis of systematic bias (overestimation/underestimation) in exercise protocols compared to the Cooper criterion.

Protocol	Underestimation (n)	Overestimation (n)	p-value
1-mile run	42	18	0.003
PWC195	54	6	<0.001
PWC212	54	6	<0.001
GXT	33	27	0.519

n: number of subjects; GXT: graded exercise test; PWC: physical work capacity 212 and or 195 test

Its regression model, which includes variables such as body weight and cardiac output, seems particularly appropriate for this age group (George, J. D., et al., 1993). A key observation is the consistent underestimation by the PWC protocols, which we mainly attribute to the very high target heart rates (195 and 212 bpm) they require. As noted by Wasserman et al., stroke volume in adolescents reaches a plateau at submaximal exercise intensities (Wasserman, K., et al., 2012). To reach these elevated heart rates, increases in cardiac output rely primarily on tachycardia. Because  $O_2P_{max}$  is calculated as  $VO_{2peak}$  divided by  $HR_{peak}$ , an unusually high  $HR_{peak}$  relative to  $VO_{2peak}$  mathematically leads to a lower, underestimated  $O_2P$  value. Additionally, the substantial mechanical workloads (~180 Watts) necessary to attain these heart rates on a cycle ergometer likely cause localized muscle fatigue, which may impair peripheral oxygen extraction (arteriovenous oxygen difference) and further contribute to the underestimation (Rowland, T. W., 2018; Castro-Piñero, J., et al., 2020). The underestimation by the 1-mile run, a validated test for

predicting  $\text{VO}_{2\text{peak}}$  (Cooper, K. H., 1968), suggests that it may often be a submaximal effort for estimating a true maximal parameter like  $\text{O}_{2\text{Pmax}}$ . The cardiovascular stress elicited may not consistently reach peak levels in all individuals, leading to an underestimation of the peak oxygen pulse (Armstrong, N., & Barker, A. R., 2011; Stringer, W. W., et al., 2018). Our findings have direct practical implications. For researchers and clinicians seeking the most accurate estimate of  $\text{O}_{2\text{Pmax}}$  in adolescent males without direct CPET, a progressive treadmill test like the GXT protocol is strongly recommended over high-intensity cycle tests or submaximal field runs. The systematic error introduced by the latter protocols could lead to misinterpretations of cardiovascular health and function in this population.

A limitation of our study is the use of predictive equations rather than direct gas analysis to determine  $\text{VO}_{2\text{peak}}$ . However, this methodology is standard for comparative validation studies of field tests and does not detract from the relative differences we observed between protocols. Our sample was also limited to healthy boys, and further research is needed to confirm these findings in females and clinical populations.

In conclusion, this study provides novel evidence that the exercise protocol chosen significantly impacts the estimation of maximal oxygen pulse in adolescents. The treadmill-based GXT protocol offers a more valid and unbiased estimate compared to high-intensity cycle ergometer tests or a 1-mile run. This underscores the importance of careful test selection for accurately evaluating cardiovascular efficiency in adolescent males.

Sincerely,

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