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VALIDITY AND TEST-RETEST RELIABILITY OF A SPEED-BASED MAXIMAL OXYGEN UPTAKE ($\dot{V}O_{2MAX}$) PROTOCOL IN MILITARY PERSONNEL

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ABSTRACT

Maximal oxygen uptake ($\dot{V}O_{2max}$) is routinely assessed via a graded treadmill protocol and can be confirmed using a supramaximal verification test. Speed-based running tests may be better suited to assess $\dot{V}O_{2max}$ in populations less familiar with inclined running testing. Fourteen healthy adults (11 males, 3 females; 12 U.S. Army Soldiers, 2 civilians; mean ± SD; age, 24 ± 6 y; body mass, 73.2 ± 15.7 kg; height, 171 ± 8 cm) completed a speed-based incremental running test and a verification test (110% maximal incremental test speed) during two laboratory visits. The starting treadmill speed and speed increments were individualized based on each participant's two-mile run time from the Army Combat Fitness Test (ACFT) or civilian equivalent. The incremental and supramaximal verification phase were performed until volitional exhaustion and were separated by 15 min of passive rest, while each laboratory visit was separated by ≥ 48 h of recovery. $\dot{V}O_2$ plateau was observed during incremental tests in 84.6% and 83.3% of participants during Visit 1 and 2, respectively. Peak $\dot{V}O_2$ was statistically equivalent between the incremental and verification tests during Visit 1 (mean difference, -0.08 mL·kg^{-1}·min^{-1}; 90% CI, [-0.86, 0.70]) and Visit 2 (-0.95 mL·kg^{-1}·min^{-1} [-1.87, -0.04]). Peak $\dot{V}O_2$ obtained during the incremental test was not statistically equivalent between visits (1.35 mL·kg^{-1}·min^{-1} [0.38, 2.33]). However, the test-retest reliability was excellent between the speed-based incremental running test (ICC=0.936; 95% CI, [0.786, 0.979]) and the verification tests (ICC=0.951; 95% CI, [0.836, 0.985]). The speed-based incremental running test investigated in the current study is a valid, reliable method for assessing $\dot{V}O_{2max}$ in military personnel.

Keywords: maximal oxygen uptake, cardiopulmonary exercise test, verification phase

INTRODUCTION

The rate of maximal oxygen uptake ($\dot{V}O_{2max}$) is the gold standard for determining cardiorespiratory fitness and reflects an individual's capacity to perform large muscle, dynamic high-intensity exercise for prolonged periods of time (1-3). The achievement of $\dot{V}O_{2max}$ during a graded treadmill protocol can be confirmed by the presence of a plateau in $\dot{V}O_2$ despite a continued increase in workload (4). In instances where $\dot{V}O_2$ plateau does not occur, researchers and practitioners may utilize a supramaximal verification test whereby, after a rest period (e.g., 10-15 min), where a biphasic or square-wave bout of supramaximal exercise is performed until volitional exhaustion (5-10). Alternatively, $\dot{V}O_{2max}$ can be assessed using an incremental speed-based treadmill protocol (2), but whether a verification test is required to confirm the achievement of $\dot{V}O_{2max}$ during such tests has not yet been determined. Furthermore, current literature lacks a universal method for determining the appropriate starting running speed and magnitude of speed increases for a speed-based $\dot{V}O_{2max}$ protocol. Selecting the correct starting speed and increments is critical, otherwise a test may be invalid due to the duration being too short or too long given the intensity of the exercise (4) which would impair the ability to observe $\dot{V}O_2$ plateau and assess $\dot{V}O_{2max}$ (11).

Incremental speed-based $\dot{V}O_{2max}$ tests increase treadmill speed only, until volitional exhaustion, while the treadmill grade remains constant at 0% (12). By eliminating the grade element, speed-based protocols may reduce injury potential and localized muscular fatigue (13), provide superior task-specificity (14), and may be better suited for populations less familiar with graded maximal running (e.g., track athletes, military personnel) (15). For example, military personnel are far less likely to be familiar with $\dot{V}O_{2max}$ assessment methods compared to athletes, despite being frequently used as human research volunteers (15). Eliminating the complexity of incline variation during cardiorespiratory fitness evaluation may assist in the assessment of $\dot{V}O_{2max}$ in these populations (16), while also simplifying the calculations for determination of the verification test protocol. It is important that the procedures for selecting a starting running speed and magnitude of speed increase are personalized for an individual's estimated cardiorespiratory fitness or there is risk of the test becoming invalid due to an inappropriate test intensity and duration (4). However, to our knowledge, the current literature lacks a data-supported method for determining these starting running speeds and increments for speed-based $\dot{V}O_{2max}$ protocols. In this context, the purpose of the present study was to investigate the concurrent validity and test-retest reliability of a novel speed-based protocol (based on self-reported two mile run times) in eliciting $\dot{V}O_{2max}$ in military personnel and to establish the efficacy of validating the novel protocol via a supramaximal verification test. We hypothesized that 1) the current protocol would elicit $\dot{V}O_2$ plateau in ≥80% of participants; 2) the $\dot{V}O_{2max}$ measured during the speed-based protocol and a subsequent supramaximal effort verification test would be statistically equivalent, indicating true $\dot{V}O_{2max}$ achievement; and 3) the speed-based protocol would display excellent test-retest reliability in achieving $\dot{V}O_{2max}$.

METHODS

Experimental Approach to the Problem.

A within-subject study was employed to assess the validity and reliability of a speed-based $\dot{V}O_{2max}$ protocol in military personnel. Participants completed two laboratory visits (Visit 1 and 2) that involved completing a speed-based incremental running test to determine $\dot{V}O_{2max}$. Each test was performed until volitional exhaustion, followed by a supramaximal verification test (110% of the highest 2 min average speed recorded during the incremental test). Test speeds were individualized based on each participant's self-reported two-mile run time using a customized spreadsheet (Supplemental Digital Content 1). Incremental and verification tests were separated by 15 min of passive rest, while each laboratory visit was separated by ≥ 48 h. $\dot{V}O_{2max}$ values obtained during the incremental and verification tests were assessed for statistical equivalence within and between Visits 1 and 2. Test-retest reliability was assessed for both the speed-based incremental protocol and the verification test between visits.

Participants.

Fourteen healthy individuals (11 males, 3 females; mean ± SD; age, 24 ± 6 y; body mass, 73.2 ± 15.7 kg; height, 171 ± 8 cm) participated in this study. Twelve participants (nine males and three females) were active-duty U.S. Army Soldiers and two (both male) were U.S. Department of Defense civilians. The inclusion criteria required participants to be between 18-44 years old, engaged in aerobic or resistance exercise for at least thirty minutes two days per week. Exclusion criteria were 1) injuries, illness, or conditions that would compromise the ability to exercise; 2) difficulty breathing into a mouthpiece or claustrophobia; 3) pregnant; 4) or had any history of gastrointestinal disease or surgery. This study was approved by the institute's scientific review committee and by the institutional review board at the U.S. Army Medical Research and Development Command (USAMRDC; Ft. Detrick, MD). Each participant was briefed on the on the purpose, risks, and benefits of the study prior to providing written informed consent.

Procedures.

Each participant completed two morning laboratory visits with ≥ 48 hours of recovery between visits. Before each visit, participants were required to abstain from high-intensity exercise for ≥ 48 hours, alcohol for ≥ 24 hours, and caffeine, nicotine, and food for ≥ 10 hours. Participants were instructed to drink at least 500 mL of water the night before the visit as well as the morning of the visit. Each participant wore typical physical training attire (t-shirt, shorts, socks, and running shoes). Participants provided a spot urine sample and urine specific gravity (< 1.030) was measured via refractometry (Goldberg TS Meter, Reichert, Buffalo, NY). Then, body mass was measured by a standard laboratory scale (Model 876, Seca, Hamburg, Germany). Participants then donned a physiological status monitor which provided real-time 2-lead ECG observation, (Equivital™ eq02+ LifeMonitor, Hidalgo Ltd. Cambridge, UK). Each participant was fitted with a respirometer mask connected to an open circuit laboratory spirometry unit (ParvoMedics TrueOne 2400; ParvoMedics; Salt Lake City, UT), which was calibrated per the manufacturer's instructions to ensure < 1% error in expired gas measurements.

The present study was part of a larger protocol investigating physical performance in austere environments (17). Consequently, the warm-up for the present study consisted of performing the activities in the larger protocol in a temperate environment (20.2 ± 0.8 °C, 49.6 ± 4.6 % RH). Briefly, participants completed one cycle of a performance battery circuit that included: a) three squat jumps separated by 30 s rest, b) three maximal effort isometric mid-thigh pulls separated by 30 s rest, c) a seated dexterity task for ~ 60-70 seconds (Complete Minnesota Dexterity Test, Lafayette Instruments, Lafayette, IN), and d) three maximal effort isometric handgrip attempts with each hand using a handgrip dynamometer (Jamar Plus Digital Hand Dynamometer, Jamar, Duluth, Minnesota). Then, participants performed a twenty-minute incremental treadmill walk using various speed (0.54-1.07 $m \cdot s^{-1}$) and grade (0-24%) combinations. Finally, participants completed an additional cycle of the performance battery. Participants were then allowed to drink ad libitum prior to instrumentation for the $\dot{V}O_{2max}$ testing procedures.

The speed-based incremental $\dot{V}O_{2max}$ test consisted of an initial 4 min warmup stage followed by 2 min stages at progressively higher speeds until the participant reached volitional exhaustion. The treadmill grade remained at 0% for the entirety of the test. Speeds for the incremental test stages were calculated using a customized Microsoft Excel spreadsheet (Supplemental Digital Content 1) based on the participant's self-reported two-mile run pace (3.56 ± 0.64 $m \cdot s^{-1}$) from their most recent Army Combat Fitness Test (ACFT) or civilian equivalent. Speeds were set so that the participants reached their two-mile run pace during the fourth stage with a constant speed increment (7.5% of two-mile run pace) between stages. This constant speed increment was selected since it results in an approximately equal predicted $\dot{V}O_2$ increase as an 2.5% increase in treadmill grade (18): the work rate increment used by the previously validated Modified Astrand Running Test (15). The speed increment was < 0.4 $m \cdot s^{-1}$ per stage for all participants. The test would be stopped immediately if the participant reported angina-like symptoms, showed signs of exertional syncope or poor perfusion (e.g., light-headedness, confusion, ataxia, cyanosis, nausea), or if there was a technical issue with testing equipment (e.g., poor mask fitting, noisy signal). None of the trials in the present study needed to end before completion. Each participant was expected to reach $\dot{V}O_{2max}$ within 8-15 min of beginning the test. Participants were provided 15 min of passive rest following test completion to ensure reproducibility of $\dot{V}O_{2peak}$ responses (19), during which they were unmasked, free to move leisurely about the testing space, and drink water ad libitum. The verification test consisted of ≤ 6 min of running at 110% of the highest 2-min average speed attained during the incremental test with the same cessation criteria. All testing was administered by personnel credentialed in cardiorespiratory exercise testing.

Statistical Analyses.

Data were analyzed using R (Version 3.3.1; R Foundation for Statistical Computing; Vienna, Austria) and are reported as mean difference and 90% confidence intervals (90% CI) unless otherwise noted. Peak $\dot{V}O_2$ was considered to be the highest 30 s average for each participant (20). We utilized the approach outlined in Midgley et al. (21) for $\dot{V}O_2$ plateau identification definitive of $\dot{V}O_{2max}$ achievement by fitting a linear regression model to the $\dot{V}O_2$ data between minutes -6 and -2 from the time of stopping. The extrapolated $\dot{V}O_2$ value at minute 0 was considered to be the model's prediction of peak $\dot{V}O_2$. $\dot{V}O_2$ plateau was attained if the measured peak $\dot{V}O_2$ value was ≥ 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ lower than the predicted $\dot{V}O_{2max}$ value (22). Planned contrasts were made within visits (incremental vs. verification) and between visits for incremental and verification tests. $\dot{V}O_{2max}$ was statistically equivalent between tests if the 90% CI was within ± 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ (22). Mean differences and 90% CI were determined using mixed effects models with random effects of participant on intercepts. Test-retest reliability for the incremental and verification tests was assessed using intraclass correlation coefficients (ICC) (23). Reasons for missing data and exclusion from analysis are a) two participants withdrew from the study after completing Visit 1 for reasons unrelated to the present study; b) one participant elected not to complete the Visit 2 verification test; and 3)

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one participant did not comply with protocol requirements during Visit 1 (i.e., participant was not fasted for ≥ 10 h).

RESULTS

$\dot{V}O_2$ plateaus were identified in 11 of 13 (84.6%) participants during the Visit 1 incremental test and 10 of 12 (83.3%) participants during the Visit 2 incremental test. Peak $\dot{V}O_2$ was statistically equivalent between incremental and verification trials for both Visit 1 (mean difference, −0.08 mL·kg^{-1}·min^{-1}; 90% CI, [−0.86, 0.70]) and Visit 2 (−0.95 mL·kg^{-1}·min^{-1} [−1.87, −0.04]). Conversely, peak $\dot{V}O_2$ obtained during the incremental test was not statistically equivalent between visits (1.35 mL·kg^{-1}·min^{-1} [0.38, 2.33]). However, peak $\dot{V}O_2$ measured during the verification tests were statistically equivalent between visits (−0.25 mL·kg^{-1}·min^{-1} [−1.24, 0.74]). **Figure 1** displays Bland-Altman plots of agreement between the speed-based incremental and verification tests within and between the two test visits. Excellent test-retest reliability was observed across visits for both the incremental (ICC=0.936; 95% CI, [0.786, 0.979]) and verification tests (ICC=0.951; 95% CI, [0.836, 0.985]). **Figure 2** presents the test-retest reliability of the speed-based incremental protocol and verification trial between visits.

DISCUSSION

The ability to monitor, measure, and predict energy costs of military service members continues to be a complex but important issue (24-28). Studies like these, provide clear scientific steps towards improving the methods and technologies for athletes, civilians, and military service members. Improvements allow for optimal prediction of activity costs (26, 28-30), enable improved pacing strategies (31, 32), and progress towards advanced methods for individual aerobic health assessments (33-35).

The present study investigated the concurrent validity and test-retest reliability of a novel speed-based protocol (based on self-reported two mile run times) in eliciting $\dot{V}O_{2max}$ in military personnel and established the efficacy of validating the novel protocol using a supramaximal verification test. We identified the occurrence of $\dot{V}O_2$ plateau during almost all tests (84%), indicating that many but not all military personnel can achieve $\dot{V}O_{2max}$ during the speed-based incremental protocol. The incremental protocol displayed excellent agreement when compared against the supramaximal verification test. Additionally, our findings indicate that a verification test is not required to confirm $\dot{V}O_{2max}$ attainment for military personnel completing the incremental protocol.

To our knowledge, the current study provides novel findings regarding the efficacy of a $\dot{V}O_{2max}$ protocol that manipulates only the treadmill speed based on the participant's two-mile run pace. Specifically, the procedures reported in the present study produce a valid protocol for attaining $\dot{V}O_{2max}$ in military personnel with excellent test-rest reliability. Similar studies have also assessed the efficacy of speed-based $\dot{V}O_{2max}$ protocols, including Sperlich et al. (36), which evaluated the $\dot{V}O_{2max}$ of fourteen well trained runners using a protocol consisting of constant 0% grade and speed increases of 0.5 km·h^{-1} every 30 seconds, and Davies et al. (37), in which 10 participants ran a flat $\dot{V}O_{2max}$ test with speed increases of 1.0 km·h^{-1} every minute. Additionally, a number of other studies have tested either self-paced $\dot{V}O_{2max}$ protocols (i.e., the participant manipulates the speed element based on their perceived exertion) (38-41) or only changed the speed while the treadmill grade remained at a constant slight incline (0.4-3.0%) (42-45).

None of the studies presented have individualized the protocol to a prior measurement of the participant's fitness, yet recent literature has theorized that individualized protocols would account for inter-individual variability in physiological responses to maximal exercise and yield higher $\dot{V}O_{2max}$ values (36, 46). Furthermore, individualized $\dot{V}O_{2max}$ protocols that are simplified to contain only the speed element may be better suited to populations not familiar with graded maximal running, such as military personnel (15). Thus, a protocol developed from the individual's previously measured two-mile run time provides a feasible option for cardiorespiratory fitness assessment in this population.

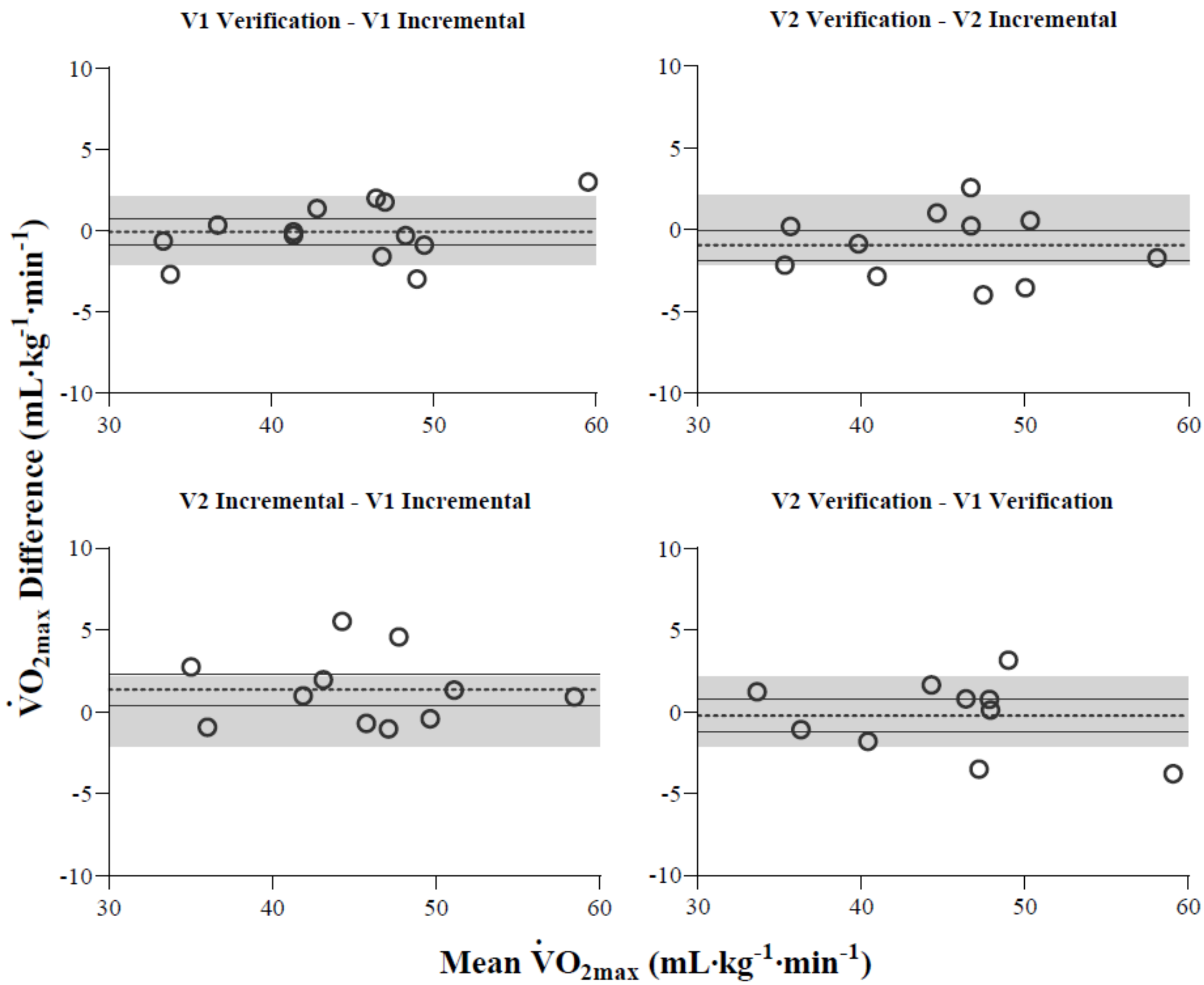
The classical criterion for confirmation of $\dot{V}O_{2max}$ achievement is the occurrence of $\dot{V}O_2$ plateau despite the further increase of work rate (47). However, a number of studies have shown that $\dot{V}O_2$ plateau does not always occur as the individual is approaching volitional fatigue (48, 49). In the present study, $\dot{V}O_2$ plateau occurred in 21 of 25 (84%) incremental tests as confirmed by the modified Midgley approach (21). This indicates that the speed-only incremental test protocol used in the current study is a useful method for eliciting $\dot{V}O_{2max}$ achievement in healthy military-age women and men.

Table 1. Peak physiological variables measured for incremental and verification tests during Visits 1 and 2.

Visit	*Test*	*Duration (s)*	*Peak HR (bpm)*	*Peak RPE*	*Peak RER*	*Peak $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)*
1	Incremental	870 [800, 940]	188 [182, 194]	18 [17, 19]	1.07 [1.06, 1.09]	44.3 [41.3, 47.4]
	Verification	220 [208, 231]	176 [171, 182]	17 [17, 18]	1.03 [0.99, 1.07]	44.3 [40.9, 47.6]
2	Incremental	826 [769, 882]	190 [186, 194]	19 [18, 19]	1.07 [1.04, 1.10]	45.7 [42.8, 48.6]
	Verification	213 [200, 227]	174 [164, 185]	18 [18, 19]	1.03 [0.99, 1.06]	44.6 [41.6, 47.6]

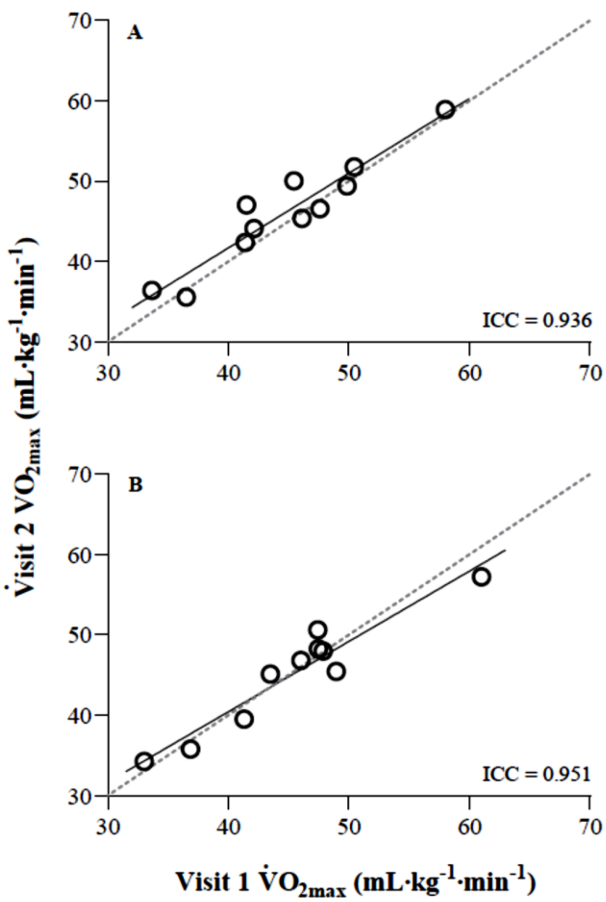
Table legend: vales as mean [95% confidence interval]; s, seconds; HR, heart rate; RPE, Rating of Perceived Exertion; RER, respiratory exchange ratio; $\dot{V}O_2$, oxygen uptake.

Figure 1. Bland-Altman plots of agreement between speed-based incremental and verification tests within and between two test visits (V1 and V2). Grey shading, equivalence limits (± 2.1 mL·kg^{-1}·min^{-1}); solid black line, mean difference; dashed black lines, 90% confidence intervals.



The duration for the incremental tests was 870 ± 159 seconds (mean ± SD) on Visit 1 and 826 ± 129 seconds on Visit 2. Previous research indicates that tests lasting between 8-15 minutes are ideal for assessing $\dot{V}O_{2max}$ (50), which the duration of our test falls well within. Prolonged tests may result in lower peak $\dot{V}O_2$ values given the onset of localized muscle fatigue resulting in reduced muscular efficiency (51), requiring a greater need for oxygen per unit of adenosine triphosphate (ATP) turnover and/or increased ATP turnover per unit of power output (52). Thus, the procedures of the present study appear to have eliminated the concern of longer test durations by allowing personalized prescription of the protocol based on an individual's two mile run time.

Figure 2. Test-retest reliability of the speed-based incremental protocol (A) and verification phase (B) between Visit 1 and Visit 2. Grey dashed line, identity; solid grey line, linear fit.



The present study also found that peak $\dot{V}O_2$ was statistically equivalent between the incremental and verification tests during both visits. Additionally, test-retest reliability was excellent for both tests (Figure 2). The duration of the verification test was 220 ± 26 seconds (mean ± SD) for Visit 1 and 213 ± 31 seconds for Visit 2, which falls well within the range suggested by Poole and Jones (53) for 110% workload verification tests (180-540 seconds). The verification tests in the current study were performed at speeds of 4.2 ± 0.7 m·s^{-1} for Visit 1 and 4.3 ± 0.7 m·s^{-1} for Visit 2. While these are severe-intensity workloads, the duration of the tests was likely long enough to allow for the building of the slow component of $\dot{V}O_2$ kinetics (53). Furthermore, the equivalency of the $\dot{V}O_{2max}$ measurements between tests indicate that a verification test is an appropriate method to confirm $\dot{V}O_{2max}$ attainment during the speed-based incremental protocol (19), yet it may not be necessary given the achievement of both primary and secondary criteria during the majority of incremental tests.

Our study is not without limitations. Of the eleven subjects included in the agreement analysis between the Visit 1 and Visit 2 incremental tests, seven achieved a higher $\dot{V}O_{2max}$ during Visit 2. (Figure 1). This may be explained by a systematic bias (i.e., learning or practice effect), which indicates the need for a familiarization trial prior to assessment of $\dot{V}O_{2max}$ using the incremental protocol (54, 55). Additionally, the current study did not exclude participants based off their familiarity with $\dot{V}O_{2max}$ testing or treadmill running, which may have resulted in inexperienced runners greatly improving their performance during the second trial. Another limitation is the potential for changes in cardiorespiratory fitness between the participant's start date and their most recent ACFT. Indeed, of the 12 participants that were Soldiers, the difference in time between their start date and most recent ACFT ranged from one to seven months (4.3 ± 2.0 months). The civilian participants assessed their two-mile run time within one month of their start date in the study. During this time, there was a potential for the participant's cardiorespiratory fitness status to change and may have caused their reported two-mile run time to differ from their current run time. However, given the high prevalence of achieving $\dot{V}O_{2max}$ in the incremental test, this potential limitation appears to not have a significant influence on the results. In fact, our findings support that the protocol of the present study for determining starting speed and speed increments is valid and efficacious even when prolonged gaps in two-mile run testing occur (i.e., several months). Therefore, it is likely that this protocol can be confidently employed in military personnel or other populations maintaining their physical fitness. Finally, another limitation is that of the fourteen participants in the present study, only three were female (i.e., ~21% of the sample size). Notably, however, this is similar to the most recent demographics profile of the total U.S. Department of Defense Military Force reporting that as of 2022, 19.1% of the force was comprised of female members (56) and a higher number of female participants than previous studies mentioned previously.

In conclusion, the speed-based incremental $\dot{V}O_{2max}$ protocol in the present study was found to be a valid and reliable method of assessing cardiorespiratory fitness in military personnel. The present study supports the inclusion of a familiarization trial on a separate day prior to the experimental trial so that the highest $\dot{V}O_2$ uptake can be measured. A supramaximal verification test is not necessary for confirming $\dot{V}O_{2max}$ achievement for military personnel completing the speed-based protocol.

Practical Applications

Military personnel engaging in the speed-based incremental $\dot{V}O_{2max}$ protocol are expected to achieve $\dot{V}O_2$ plateau. A speed-based incremental protocol based on the participant's two-mile run time is a valid and reliable method of assessing cardiorespiratory fitness in this population. A supramaximal verification test may not be necessary to confirm $\dot{V}O_{2max}$ attainment for military personnel completing the incremental protocol.

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Conflicts of interest:

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