



*Original Research*

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## **Effect of Test Sequence on Maximal Anaerobic and Aerobic Power Achievements in Adults**

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### ABSTRACT

*International Journal of Exercise Science 14(4): 657-665, 2021.* The purpose of this study was to examine the effect of test sequence on adults' ability to achieve maximal aerobic and anaerobic power during a single assessment visit. Forty-one adults (24 men, 17 women;  $22.0 \pm 1.8$  years) completed two baseline visits in randomized order consisting of either a maximal oxygen consumption ( $VO_{2max}$ ) or Wingate anaerobic test (WAnT). The subsequent experimental visit consisted of both  $VO_{2max}$  and WAnT in randomized order separated by 20 minutes of rest. Mixed-model ANOVAs compared baseline and experimental performance between and within groups. Chi Squared Goodness of Fit tests determined if test sequence significantly affected  $VO_{2max}$  criteria achievement. Significant interaction effects were observed for relative  $VO_{2max}$  ( $p = 0.005$ ), RER ( $p < 0.001$ ), and exercise time ( $p = 0.022$ ). Within WAnT/ $VO_{2max}$  subjects, these values significantly decreased from baseline to experimental tests. No differences were found for WAnT values. During the experimental session, 50% of subjects who performed WAnT/ $VO_{2max}$  and 81% of subjects who performed  $VO_{2max}$ /WAnT achieved a valid  $VO_{2max}$ . Chi squared analysis found the change to be significant in WAnT/ $VO_{2max}$  subjects only. Therefore, performing the WAnT before  $VO_{2max}$  sequence significantly reduced the percent of subjects who achieved  $VO_{2max}$  criteria. These findings indicate that the sequence of  $VO_{2max}$  testing before a WAnT allowed maximal results similar to expected baseline values.

**KEY WORDS:** Wingate,  $VO_{2max}$ , exercise testing, maximal testing

### INTRODUCTION

Maximal aerobic power ( $VO_{2max}$ ) is a measurement of one's capacity to consume oxygen at maximal workloads (2, 7). With direct measurements of pulmonary ventilation and expired concentrations of oxygen and carbon dioxide,  $VO_{2max}$  is the gold standard measurement of cardiorespiratory fitness (2, 7). Maximal anaerobic power is the capability to perform high intensity bouts of exercise for a short duration that depend on non-oxidative (i.e. anaerobic) metabolic pathways (2). Although a single accepted gold standard assessment of maximal anaerobic power has not been established, the Wingate anaerobic test (WAnT) is the most commonly used. Factors such as peak muscular power, local muscular endurance, and fatigability of the muscle can all be assessed using the WAnT (5, 11).

Exercise testing of maximal aerobic and anaerobic power has many applications, such as assessing the risk of developing cardiovascular disease, prescription of specific exercise programs, and prediction of sport performance (1, 6, 12). However, due to the physical nature of these maximal exertion tests, many researchers and clinicians schedule  $VO_{2max}$  and WAnT tests on separate days to ensure optimal results. This requires participants to have multiple testing visits, which could increase the likelihood of subject attrition. In addition, if subjects were able to perform these tests in sequence with little rest between them (i.e., within a single assessment session of approximately one-hour duration), this would further decrease the time burden on both subject and researcher. To our knowledge, only two studies have included both a WAnT and  $VO_{2max}$  test during the same session (2, 17).

Rivera-Brown et al. (17) studied the attainment of  $VO_2$  plateau following a WAnT and found that only six out of eighteen pre-pubertal children, aged seven to eleven, were able to attain a  $VO_2$  plateau. However, Rivera-Brown et al. (17) only studied the effects of performing a WAnT before a  $VO_{2max}$  and did not compare with the alternate test sequence. Andreacci et al. (2) examined how the sequence of WAnT and  $VO_{2max}$  tests during the same session impacted test performance in children. It was observed that about 50% of subjects who completed a WAnT before a  $VO_{2max}$  did not achieve  $VO_{2max}$  criteria with twenty minutes of rest between tests (2). However, the subjects who completed a  $VO_{2max}$  before a WAnT had no negative effects on WAnT performance (2). Therefore, Andreacci et al. (2) concluded that the optimal testing sequence for children was to administer a  $VO_{2max}$  test before a WAnT with at least twenty minutes of rest between tests. It was theorized that due to the significant aerobic metabolic contribution to the WAnT documented in previous research (8, 10), twenty minutes of rest was not sufficient recovery prior to  $VO_{2max}$  testing. However, the  $VO_{2max}$  did not result in significant anaerobic fatigue to decrease WAnT performance in those children.

To our knowledge, no known investigations have assessed whether testing sequence affects an adult's ability to achieve maximal aerobic and anaerobic power during a single session. Therefore, the primary purpose of this study was to examine the influence of testing sequence on an adult's ability to achieve maximal aerobic and anaerobic power during a single session. A secondary purpose of this study was to examine if testing sequence influenced the achievement of  $VO_{2max}$  criteria in adults. It was hypothesized that the performance of a  $VO_{2max}$  test prior to a WAnT would be the optimal sequence for maximal performance during both assessments. This includes adequate achievement of  $VO_{2max}$  criteria in a greater proportion of subjects, similar to previous research.

## METHODS

### *Participants*

A total of 62 healthy college-aged adults were recruited for this study. Prior to participation, all subjects' written informed consent was acquired in accordance to the Bloomsburg University Institutional Review Board guidelines. In addition, all procedures were conducted according to the ethical policies established by the International Journal of Exercise Science Editorial Board (16). Each subject completed the Physical Activity Readiness Questionnaire screening for

potential cardiovascular or metabolic risk. There were no specific requirements for athletic competence. Based on  $\text{VO}_{2\text{max}}$  results, a heterogeneous sample of subjects regarding aerobic fitness participated in this investigation. Any subject who did not achieve the criteria for a valid  $\text{VO}_{2\text{max}}$  during the baseline visit was excluded. After exclusion of subjects, a total of forty-one subjects (24 men, 17 women) between the ages of 18 and 28 (mean  $22.0 \pm 1.8$  years old) participated in this study.

**Table 1.** Represents subject characteristics.

	N (M/F)	Age (years)	Height (cm)	Weight (kg)	Body Fat (%)
Group A (WAnT/ $\text{VO}_{2\text{max}}$ )	20 (11/9)	22.3 $\pm$ 1.9	170.4 $\pm$ 10.4	77.1 $\pm$ 19.5	25.9 $\pm$ 9.3
Group B ( $\text{VO}_{2\text{max}}$ /WAnT)	21 (13/8)	21.6 $\pm$ 1.7	173.0 $\pm$ 10.4	73.3 $\pm$ 17.1	22.2 $\pm$ 9.5

All values are shown as mean  $\pm$  SD.

### Protocol

Each subject made four separate visits to the Human Performance Laboratory at the same time of day ( $\pm 1$  hour). Although a previous investigation indicated that time of day may impact aerobic contribution to the WAnT, subjects were allowed to choose the time of day that they participated based on their individual schedules (19). The first visit consisted of signing an informed consent, passing a pre-screening questionnaire, orientation of the investigation, and a review of pre-testing guidelines. Pre-testing guidelines were followed as previously described by Masteller et al. (13). The WAnT and  $\text{VO}_{2\text{max}}$  tests were randomly assigned to be performed individually during the second and third visits. During the fourth visit (experimental session), subjects completed a  $\text{VO}_{2\text{max}}$  and WAnT in a randomized order with a twenty-minute rest period between assessments. Participants were provided two - five days of recovery between visits two - four. Height and mass were measured before each session. Height was measured using a stadiometer (SECA model 240, Hamburg, Germany) to the nearest 0.1 cm. Body mass was assessed from a weighing scale (Tanita model BWB-800, Tokyo, Japan). Body composition was assessed using a Dual Energy X-ray Absorptiometer (DEXA) (General Electric, DEXA model Lunar Prodigy Advance - Boston, Massachusetts, USA) during the baseline WAnT session to optimize session durations. DEXA calibration and procedures were followed as previously described by Masteller et al. (13). These procedures were approved by the Bloomsburg University Institutional Review Board.

All aerobic power tests were conducted on a Woodway (PRO XL 27, DESMO - Waukesha, WI) motorized treadmill and began with a standardized baseline and warmup.  $\text{VO}_{2\text{max}}$  was expressed relative to total body mass (i.e., mL/kg/min) and assessed using the Bruce incremental exercise test. The Bruce protocol was used due to being found appropriate in individuals who are moderately active (1).  $\text{VO}_{2\text{max}}$  was found by averaging three data points from fifteen second averaging by hand calculation and subsequent review by a second laboratory technician.

The criteria for attainment of  $\text{VO}_{2\text{max}}$  was based on the achievement of four of the five following criteria: (a) a change in  $\text{VO}_2$  of  $\leq 2.1$  mL/kg/min with increasing exercise intensity, (b) a respiratory exchange ratio (RER) of  $\geq 1.10$ , (c) a heart rate (HR) within  $\pm 10$  beats per minute of

the age-predicted maximum at the end of the  $VO_{2max}$ , (d) a rating of perceived exertion (RPE) of  $\geq 9$  using the OMNI Walk/Run scale of perceived exertion, and (e) volitional termination due to exhaustion (14, 20). HR was monitored throughout the assessment using a HR monitor (Polar model RS100 - Bethpage, NY, USA). A Parvo Medics TrueOne 2400 metabolic measurement system (Murray, Utah, USA) was used to measure expired  $O_2$  and  $CO_2$  concentrations. Verbal encouragement was provided throughout all tests to provoke maximal effort. Undifferentiated (overall) OMNI-RPE was randomly assessed fifteen seconds prior to the end of each stage throughout the graded exercise test as well as maximal OMNI-RPE immediately following the termination of the test (20).

Subjects completed a standardized warm-up on a cycle ergometer (Monark, Ergonomic 828E - Varberg, Sweden) as previously described (9, 11). Subjects were given two - five minutes of rest prior to the start of the WAnT. All WAnTs were completed on a mechanically braked cycle ergometer (Monark, Ergonomic 894E - Varberg, Sweden) interfaced to a computer. Seat height and handlebars were adjusted for each subject. Subjects were instructed to pedal as fast as they could while no resistance was applied to the flywheel. Once the subject reached the maximum RPM achieved during the acceleration phase, the basket loaded with the predetermined resistance automatically dropped and the test began. Verbal encouragement was given throughout the entirety of the test. All WAnTs lasted 30-seconds with a resistance of 0.075 kg/kg body mass. This resistance was selected due to being commonly used for recreationally active college-aged participants (15). HR was measured throughout the assessment using a Polar HR monitor as in the aerobic power test.

Prior to the fourth visit, all participants were randomly assigned to one of two experimental groups based on test sequence: WAnT/ $VO_{2max}$  or  $VO_{2max}$ /WAnT. Both groups performed the assessments as described above and were allotted a 20-minute passive rest period between tests.

#### *Statistical Analysis*

Statistical analyses were performed using SPSS Statistics 25 for Windows (IBM, IBM SPSS Software - Armonk, New York, USA). All values are expressed as mean  $\pm$  standard deviation. Statistical significance was established a priori at  $p < 0.05$ .

An independent t-test was used to compare subject characteristics. Comparisons of maximal anaerobic and aerobic variables were compared within groups (from baseline to experimental) and between groups (test sequence) using mixed model ANOVAs and Bonferroni post hoc analyses. A Chi Squared Goodness of Fit test was used to detect if the order of test sequence made a significant difference in achievement of  $VO_{2max}$  criteria between expected and observed results. The baseline for all forty-one subjects served as the expected results, while the experimental visit served as our observed results.

## RESULTS

Subject characteristics between WAnT/ $\text{VO}_{2\text{max}}$  and  $\text{VO}_{2\text{max}}$ /WAnT groups can be seen in Table 1. No significant differences were observed between groups for age, height, mass, or body fat percentage.

The maximal aerobic power data are presented as a function of testing session and group in Table 2. Significant interaction effects were observed for relative  $\text{VO}_{2\text{max}}$  ( $F_{1,39} = 9.082$ ,  $p = 0.005$ , partial  $\eta^2 = 0.189$ ), RER ( $F_{1,39} = 14.733$ ,  $p < 0.001$ , partial  $\eta^2 = 0.274$ ), and exercise time ( $F_{1,39} = 5.730$ ,  $p = 0.022$ , partial  $\eta^2 = 0.128$ ). No significant interaction effects were observed for  $\text{HR}_{\text{max}}$  or RPE. Significant main effects of session were observed for RER ( $F_{1,39} = 12.102$ ,  $p = 0.001$ , partial  $\eta^2 = 0.237$ ), and exercise time ( $F_{1,39} = 8.576$ ,  $p = 0.006$ , partial  $\eta^2 = 0.180$ ). No significant main effects of session were observed for relative  $\text{VO}_{2\text{max}}$ ,  $\text{HR}_{\text{max}}$ , or RPE. A significant main effect of group was observed for RPE ( $F_{1,39} = 4.138$ ,  $p = 0.049$ , partial  $\eta^2 = 0.096$ ). No significant main effects of group were observed for relative  $\text{VO}_{2\text{max}}$ , RER, exercise time, or  $\text{HR}_{\text{max}}$ . Post hoc analyses revealed WAnT/ $\text{VO}_{2\text{max}}$  had significantly decreased experimental relative  $\text{VO}_{2\text{max}}$  ( $p = 0.027$ ), RER ( $p < 0.001$ ), and exercise time ( $p = 0.001$ ) compared to their baseline values, respectively. WAnT/ $\text{VO}_{2\text{max}}$  had a significantly lower RER ( $p = 0.002$ ) than  $\text{VO}_{2\text{max}}$ /WAnT during the experimental session. Lastly,  $\text{VO}_{2\text{max}}$ /WAnT had a significantly greater RPE ( $p = 0.041$ ) during the baseline session compared to WAnT/ $\text{VO}_{2\text{max}}$ .

**Table 2.** Represents maximal aerobic values during baseline and experimental tests.

	Baseline (WAnT/ $\text{VO}_{2\text{max}}$ )	Experimental (WAnT/ $\text{VO}_{2\text{max}}$ )	Baseline ( $\text{VO}_{2\text{max}}$ /WAnT)	Experimental ( $\text{VO}_{2\text{max}}$ /WAnT)
$\text{VO}_{2\text{max}}$ (mL/kg/min)	42.3 ± 7.6	41.4 ± 7.2*	42.3 ± 6.9	43.3 ± 6.2
RER	1.15 ± 0.06	1.07 ± 0.07*	1.14 ± 0.07	1.14 ± 0.07**
Exercise Time (s)	694 ± 120	668 ± 114	717 ± 72	715 ± 78
Heart Rate (bpm)	194 ± 8	192 ± 10	195 ± 7	194 ± 7
OMNI- RPE (Overall)	8.2 ± 1.6	8.2 ± 1.5	9.0 ± 0.9†	8.9 ± 1.2

All values are shown as mean ± SD. \* indicates significantly ( $p < 0.05$ ) decreased from baseline within Group A. \*\*indicates significantly ( $p < 0.05$ ) greater during experimental compared to Group A. † indicates significantly ( $p < 0.05$ ) greater during baseline compared to Group A.

The maximal anaerobic power data are presented as a function of testing session and group in Table 3. No significant interaction effects were observed for absolute peak power, mean power, minimum power, fatigue index, or maximum HR. No significant main effects for session were observed for absolute peak power, mean power, minimum power, fatigue index, or maximum HR. No significant main effects for group were observed for absolute peak power, mean power, minimum power, fatigue index, or maximum HR.



**Table 3.** Represents maximal anaerobic values during baseline and experimental tests.

	Baseline (Group A)	Experimental (Group A)	Baseline (Group B)	Experimental (Group B)
Peak Power (W)	771 ± 251	778 ± 250	754 ± 235	746 ± 207
Mean Power (W)	541 ± 164	543 ± 158	527 ± 141	523 ± 141
Minimum Power (W)	303 ± 96	309 ± 81	305 ± 83	297 ± 74
Fatigue Index (%)	59.8 ± 7.9	58.8 ± 7.7	57.8 ± 10.4	58.9 ± 8.7
Heart Rate (bpm)	182.5 ± 10.6	180.7 ± 11.3	182.2 ± 11.7	180.6 ± 8.5

All values are shown as mean ± SD. \*

The  $VO_{2max}$  criteria data are presented as a function of testing session and group in Table 4. All forty-one subjects achieved a valid  $VO_{2max}$  during the baseline visit based on criteria specified above. During the experimental session, ten out of the twenty (50%) subjects achieved a valid  $VO_{2max}$  in WAnT/ $VO_{2max}$ , while seventeen out of the twenty-one (81%) subjects achieved a valid  $VO_{2max}$  in  $VO_{2max}$ /WAnT. Significant differences were found between expected and observed RER ( $p < 0.001$ ), and HR ( $p < 0.001$ ) for WAnT/ $VO_{2max}$ . No significant differences were found between expected and observed RPE for WAnT/ $VO_{2max}$ . No significant differences were found between expected and observed RER, HR, or RPE for  $VO_{2max}$ /WAnT.

**Table 4.** Represents achievement of  $VO_{2max}$  criteria during baseline and experimental tests.

	Baseline (Expected, n=41)	Group A (WAnT/ $VO_{2max}$ ) Experimental (n=20)	Group B ( $VO_{2max}$ /WAnT) Experimental (n=21)
Plateau	39 (95%)	18 (90%)	21 (100%)
RER	34 (83%)	7* (35%)	16 (76%)
Heart Rate	37 (90%)	13* (65%)	18 (86%)
RPE-OMNI	25 (61%)	8 (40%)	15 (71%)
Volitional Fatigue	41 (100%)	20 (100%)	21 (100%)
Valid $VO_{2max}$	41 (100%)	10 (50%)	17 (81%)

\* Indicates significant differences were found between expected and observed RER, and HR for Group A.

## DISCUSSION

The primary findings of this investigation were that performing a WAnT before a  $VO_{2max}$  during the same session led to significantly reduced maximal aerobic power in healthy college aged subjects. In addition, participants who completed a WAnT before a  $VO_{2max}$  test had a significantly decreased relative  $VO_{2max}$  during the experimental session compared to their respected baseline values. These findings agree with the hypothesis based on previous research. Participants who completed a  $VO_{2max}$  before a WAnT were able to achieve maximal aerobic and anaerobic power during the experimental session with only twenty minutes of rest between tests.

Based on results from the Chi Squared Goodness of Fit test, the secondary finding of this investigation was that testing sequence had a significant influence on the achievement of  $VO_{2max}$  criteria. Specifically, when a WAnT was performed twenty minutes before a  $VO_{2max}$ , only 50% of the participants in that group (10 of 20) were able to achieve established criteria for a valid  $VO_{2max}$  test. However, approximately 81% of the participants completing a  $VO_{2max}$  test first (17 of 21 subjects) were able to achieve established criteria for a valid  $VO_{2max}$  during the experimental session. These results also agree with the hypothesis and are consistent with previous research. It is postulated that performance of a WAnT, as it involves a significant aerobic metabolic contribution, results in fatigue that hinders maximal aerobic power achievement soon after testing.

The results of the baseline and experimental tests are consistent with previous reports in healthy college-aged subjects for both anaerobic and aerobic power (3, 15, 18). These findings allow researchers to administer a  $VO_{2max}$  and WAnT during the same session (~1 hour) with a minimum of twenty minutes of rest between tests while also ensuring that maximal anaerobic and aerobic power is achieved. Having the ability to schedule both tests within an hour could improve the efficiency of the laboratory. To achieve both maximal anaerobic and aerobic power during the same session, a  $VO_{2max}$  test should be performed before a WAnT with at least twenty minutes of rest between assessments. Prior to this investigation, researchers had to schedule WAnT and  $VO_{2max}$  tests on separate days to ensure the achievement of maximal anaerobic and aerobic power. Having separate days could lead to decreased laboratory efficiency, and increased subject attrition rates. To our knowledge, no investigations have assessed administering a WAnT and  $VO_{2max}$  tests during the same session.

Formerly, Rivera-Brown et al. (17) investigated a WAnT and a  $VO_{2max}$  test via the McMaster protocol (4) on a cycle ergometer during the same exercise session. Rivera-Brown et al. (17) investigated the attainment of a  $VO_2$  plateau in children following a WAnT with thirty minutes of rest between tests. Rivera-Brown et al. (17) reported that only six of eighteen (33%) subjects attained a  $VO_2$  plateau. Rivera-Brown et al. (17) observed no significant differences ( $p > 0.05$ ) in peak or mean power between pre-pubertal boys who achieved a  $VO_2$  plateau and those who did not. Additionally, during the  $VO_{2max}$  test;  $VO_2$ , HR, and RER were similar between subjects who achieved a  $VO_2$  plateau and those who did not (17).

Andreacci et al. (2) examined the influence of testing sequence on a child's ability to achieve maximal anaerobic and aerobic power during a single testing session. Similar to this current investigation, Andreacci et al. (2) separated subjects into two groups (WAnT/ $VO_{2max}$ ,  $VO_{2max}$ /WAnT), and provided 20-minutes of rest between tests during the experimental session (2). In addition, Andreacci et al. (2) assessed  $VO_{2max}$  using a Bruce protocol. The primary finding of this investigation was that a significant difference ( $p < 0.05$ ) was observed for the number of subjects achieving  $VO_{2max}$  criteria, indicating that performing the  $VO_{2max}$ /WAnT sequence led to a higher number of subjects achieving criteria of  $VO_{2max}$  testing. Our findings align with the previous investigation indicating that in adults, a  $VO_{2max}$  test needed to be administered before a WAnT to ensure achievement of both maximal anaerobic and aerobic power, as well as achievement of  $VO_{2max}$  criteria.

It has been reported that children have limited anaerobic capacity compared to adults, and therefore may expend more energy through aerobic metabolic pathways during a WAnT which may have led to diminished  $VO_{2max}$  values (2, 8). Contributions from aerobic metabolic pathways during a WAnT in adults have been documented (10, 19). Compared to children, adults have a greater anaerobic capacity, although the reliance on aerobic metabolism during a WAnT is still about 22% according to Hill et al. (10), although it may be as high as 44% in some subjects and can be greater in the afternoon compared to the morning (19). This inter-individual variability in aerobic contribution to the WAnT could be a potential reason that testing sequence was influential. Using a significant proportion of energy through aerobic metabolism during the WAnT could lead to diminished maximal aerobic power and the inability to achieve  $VO_{2max}$  criteria. The present investigation did not calculate aerobic contribution to the WAnT. However, it is clear based on the present results that  $VO_{2max}$  performance was diminished 20 minutes following the WAnT. Therefore, greater rest time must be needed for performance of these tests in that order, which would increase both subject and laboratory burden as opposed to the alternative.

In conclusion, based upon the results from this investigation, researchers and clinicians will be able to administer both a  $VO_{2max}$  and WAnT in the same testing session with only 20-minutes of rest between tests. Performing the  $VO_{2max}$  before the WAnT allows for both tests to be administered during the same session without compromising maximal anaerobic and aerobic power, including achievement of  $VO_{2max}$  criteria. This research has implications that could improve laboratory efficiency and subject attrition rates since both of these important physical performance tests can be performed in the same session lasting approximately one hour. Compared to typical past practice, this allows more subjects to be tested in the same amount of time, or the same number of subjects to be tested in less time. This may also lead to improved overall cost of such research-related exercise testing in young, apparently healthy and recreationally active adults.

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