Introduction
Numerous exercise protocols have been employed to assess levels of physical power (i.e., fitness) in children [1, 5, 6, 9–11, 17, 19, 26, 27, 29, 33–35]. Maximal aerobic power or maximal oxygen consumption (V˙O2max) is a measurement of the highest rate at which energy can be derived from oxidative processes during exercise [3]. Low levels of aerobic fitness have been linked to obesity and other metabolic disorders afflicting children [4, 11, 17, 22]. Anaerobic power is the ability to perform short-bursts of high-intensity activities that depend on anaerobic metabolism. One of the most commonly performed methods of assessing anaerobic power is the Wingate Anaerobic Test (WAnT) [20]. The WAnT measures peak muscle power, local muscle endurance and fatigability of the muscle [5, 20]. Exercise tests allow researchers and clinicians the ability to accurately assess physical fitness levels and design specific exercise prescriptions. However, because maximal physical exertion is a requirement during aerobic and anaerobic power tests, the tests are often performed on separate days. This requires multiple visits to the testing facility which can be burdensome to the participant as well as the parent or guardian of the child. Rivera-Brown et al. [27] conducted an investigation in which prepubertal boys performed a WAnT followed by a V˙O2max test using the McMaster cycle ergometer protocol. The subjects were provided a 30-minute rest period between the two assessments. It was found that 12 of their 18 subjects did not achieve a V˙O2 plateau when the exercise tests were performed in the previously stated order [27]. Whether the testing sequence influenced the subjects’ ability to reach V˙O2 plateau was not examined in their investigation. To our knowledge, no investigations have examined the influence that testing sequence has on a child’s ability to achieve maximal anaerobic and aerobic power during a single assessment session. Therefore, the purpose of this investigation was to examine the order of testing sequence on a child’s ability to achieve maximal anaerobic and aerobic power during a single assessment session.

Abstract
The aim of the study was to examine the order of testing sequence on a child’s ability to achieve maximal anaerobic and aerobic power. Thirty-two children (20 females, 12 males) between 7–11 years of age participated in this study. All subjects were tested on three separate occasions as follows: anaerobic power session – Wingate Anaerobic Test (WAnT) only; aerobic power session – maximal oxygen consumption (V˙O2max) test only; and experimental session – WAnT followed by a V˙O2max test (WAnT/V˙O2max) or a V˙O2max test followed by a WAnT (V˙O2max/WAnT), each with 20 minutes of rest between the assessments. No significant differences were observed between the baseline WAnT or V˙O2max between the two groups. No significant differences were observed for WAnT power values in either group regardless of testing sequence. Children in the WAnT/V˙O2max group had significantly lower experimental V˙O2max (38.6 ± 7.6 vs. 40.6 ± 7.4 mL·kg⁻¹·min⁻¹; p < 0.05), RER (1.10 ± 0.08 vs. 1.13 ± 0.07; p < 0.05), and exercise time (472 ± 87 vs. 511 ± 79 s; p < 0.01) values when compared to the baseline V˙O2max test. The results of this study indicate that when assessing a child’s anaerobic and aerobic power during the same testing session, the testing sequence is of importance. However, it appears that a V˙O2max test can be performed 20 minutes prior to the WAnT without affecting anaerobic power in children.
Methods

Subjects
A total of thirty-two healthy children (20 girls and 12 boys) between 7 and 11 years of age (mean 9.8 ± 1.4 years) were recruited to participate in this study. Subjects were moderately active, nonathletes and had no prior experience with maximal exercise testing. Parents/guardian’s written consent and subjects’ written assent were obtained prior to participation according to the Bloomsburg University Institutional Review Board guidelines.

Procedures
All tests were performed at the same time of day, and the laboratory temperature was maintained at a constant 72 °F. Each subject made three separate visits to the Human Performance Laboratory. The first visit (orientation/anaerobic power session) consisted of body composition assessment and a WAnT. During the second visit (aerobic power session), a V˙O2max test was performed on a motorized treadmill. During the third visit (experimental session), participants performed both a WAnT and a V˙O2max test with a 20-minute rest period between each assessment.

Orientation/anaerobic power session
Following the parental consent/assent to participate, subjects’ age, height and weight were recorded. Height was determined using the attached stadiometer of a Detecto physician’s scale and measured to the nearest 0.5 cm. Body weight and percent body fat was assessed by a leg-to-leg bioelectrical impedance analysis analyzer (Tanita model #TBF-300A, Irlington Heights, IL, USA). During this visit, subjects also completed a WAnT and were familiarized with the respiratory/metabolic apparatus, treadmill and OMNI-RPE scale.

Anaerobic power was estimated using the WAnT protocol [20] and conducted on a friction-loaded cycle ergometer (Monark 894E, Vansboro, Sweden) which was interfaced to a computer. The same ergometer was used for all tests. The seat height and handlebars were adjusted appropriately for each subject, and the resistance was set at 0.065 kg kg⁻¹ body mass. This resistance was selected because it has been previously shown to elicit peak power in children ranging from 6 to 12 years of age [8]. Following a standardized 3-minute warm-up involving pedaling at 60 rpm interspersed with three 5- to 7-seconds all out sprints, the subject rested for 5 to 6 minutes on the ergometer. After resting, subjects were instructed to pedal as fast as possible with no resistance applied to the flywheel. After 3 to 4 seconds, the predetermined load was applied based on body mass. The subject was verbally encouraged to continue to pedal as rapidly as possible for the entire 30 seconds. After the WAnT, the child was instructed to pedal slowly against a light resistance for 3 to 4 minutes to cool down. Pedal rate and power output were continuously monitored throughout the testing session. Peak power (PP) was calculated as the highest mechanical power sustained throughout the first 5 sec of the exercise test. Mean power (MP) was the average power sustained throughout the 30-sec period. Fatigue index was calculated as the difference between the PP and the lowest power multiplied by 100 and divided by the PP. Throughout the exercise test, the child’s heart rate (HR) was continuously monitored by a Polar heart rate monitor (Port Washington, NY, USA).

Aerobic power session
Maximal oxygen consumption (V˙O2max) was indexed to total body mass (i.e., mL·kg⁻¹·min⁻¹) and measured using the Bruce multistage treadmill protocol. This protocol has been found suitable for the assessment of maximal oxygen consumption in children aged 4 years and older [1,10]. All tests were conducted on a Quinton (model Q-50) motor-driven treadmill. The attainment of VO2max was accepted when the participants demonstrated any four of the following five criteria: (a) a change in VO2 of ≥2.1 mL·kg⁻¹·min⁻¹ with increasing exercise intensity; (b) a respiratory exchange ratio (RER) of ≥1.05; (c) HR > 90% of the age-predicted maximum at the end of the exercise test; (d) a OMNI-RPE of ≥9; and (e) volitional termination due to exhaustion [1]. Heart rate was measured continuously throughout the exercise test using a Polar heart rate monitor (Port Washington, NY, USA). Expired concentrations of O2 and CO2 were collected and analyzed by open circuit spirometry in 15-s intervals using a Parvo Medics TrueMax 2400 metabolic measurement system (Salt Lake City, UT, USA). Verbal encouragement was given to all subjects to elicit a maximal effort. The Children’s OMNI Scale of Perceived Exertion [28] was used to measure ratings of perceived exertion (RPE). OMNI-RPE was assessed every 3 minutes during the aerobic exercise test and at the immediate termination of exercise.

Experimental session
Prior to the third visit, all participants were randomly assigned to one of two experimental groups. Group A (WAnT/V˙O2max) performed a WAnT followed by a V˙O2max test with a 20-minute rest period between the first and second assessment. Group B (V˙O2max/WAnT) performed a V˙O2max test followed by a WAnT with a 20-minute rest period between the two assessments.

Statistical analysis
Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). All values are expressed as mean ± standard deviation (SD). Statistical significance was established a priori at p < 0.05. Baseline comparison of subject characteristics, maximal anaerobic and maximal aerobic variables between both groups of children were made using an unpaired two-tailed t-test. Paired-sample t-tests were used to examine differences between baseline and experimental power values for each group. A Fisher’s exact test was applied to detect if the order of the sequence of treatments made a difference.

Results
Subject characteristics for both groups of participants are presented in Table 1. Children in Group A had significantly lower height and weight values when compared with the children in
Children in Group A had significantly lower experimental VO₂max, exercise time, heart rate or OMNI-RPE between groups (differences were observed for VO₂max, RER, exercise time, heart rate or OMNI-RPE).

The maximal anaerobic power data are presented as a function of the group and testing session in Table 2. At baseline, no significant differences were observed for relative PP, relative MP, fatigue index or heart rate between groups. Children in Group A had significantly lower baseline absolute PP and MP values when compared with the children in Group B (211.4 ± 66.0 vs. 271.9 ± 75.6 W, p < 0.05; 105.8 ± 35.7 vs. 143.1 ± 47.3 W, p < 0.05, respectively; Table 2).

The maximal aerobic power data are presented as a function of the group and testing session in Table 3. At baseline, no significant differences were observed for VO₂max (mL·kg⁻¹·min⁻¹), RER, exercise time, heart rate or OMNI-RPE between groups (Table 3). Children in Group A had significantly lower experimental VO₂max values when compared to the baseline VO₂max test (1.3 ± 0.2 vs. 1.4 ± 0.3 L·min⁻¹, p < 0.05; 38.6 ± 7.6 vs. 40.6 ± 7.4 mL·kg⁻¹·min⁻¹, p < 0.05, respectively; Table 3). Maximal RER and exercise time were also significantly lower after the experimental test when compared to the baseline VO₂max test for children in Group A (1.10 ± 0.08 vs. 1.13 ± 0.07, p < 0.05; 472 ± 87.3 vs. 511 ± 79.4 seconds, p < 0.01, respectively; Table 3). No differences were observed for VO₂max, RER, exercise time, heart rate or OMNI-RPE between the baseline and experimental session for children in Group B (Table 3).

Due to small sample sizes (15 in Group A and 17 in Group B), a one-sided Fisher’s exact test was used to determine if the proportion of respondents who met the VO₂max criteria in Group B exceeded that of Group A during the experimental session. A significant (p < 0.05) difference was observed indicating that the VO₂max/WAnT testing sequence (i.e., Group B) led to a higher number of subjects achieving the criteria of VO₂max.

### Discussion

The primary finding of this investigation was that testing sequence is of importance when assessing a child’s anaerobic and aerobic power during the same testing session. Specifically, when a VO₂max test was performed 20 minutes after a WAnT, approximately one-half of the participants were unable to attain our criteria for maximal aerobic power. In the present investigation, all subjects performed both a baseline and an experimental exercise test. The baseline and experimental WAnT and VO₂max tests were confirmed by using previously established criteria. The results of the baseline and experimental exercise tests are very consistent with previous reports in children regarding anaerobic and aerobic power [1, 8, 9, 19, 27, 34].

Previously, Rivera-Brown et al. [27] conducted an investigation incorporating both a WAnT and a VO₂max test during the same testing session. In their study, subjects were provided a 30-minute rest period following a WAnT and then completed the McMaster aerobic protocol on a cycle ergometer [27]. They reported that 12 of their 18 subjects (67%) failed to show a plateau in VO₂ with an increase in power output at maximal exercise. They also reported that no differences were observed in peak anaerobic power or mean anaerobic power between VO₂ plateau achievers and non-achievers [27]. In the current investigation, for the group of subjects who performed the WAnT followed by a VO₂max test, 8 of the 15 subjects (53%) failed to meet our criteria for VO₂max. Whereas only 2 of the 17 subjects (12%) in Group B (i.e., VO₂max/WAnT) failed to meet our criteria for VO₂max. As in the Rivera-Brown et al. [27] investigation, no differences were observed between relative peak anaerobic power or mean anaerobic power between our groups of subjects.
It has been previously reported that prepubertal children have significantly lower anaerobic power values than adolescents and young adults [2,15,19]. Factors that could account for this age-dependent difference in anaerobic power have been linked to muscular enzymatic activity and rates of substrate utilization during exercise. Children have shown lower concentrations of the anaerobic enzymes phosphofructokinase [12,16], adenylate kinase [23], lactate dehydrogenase [12,13,23], as well as lower muscle blood lactate concentrations [6,26,29,36] when compared with adolescents and young adults. In addition, it has been reported that children have lower resting muscle concentrations of creatine phosphate and glycogen when compared to adolescents or adults. Rates of creatine phosphate utilization during exercise have been found to be the same or less in children, while the rates of glycogen utilization are much less in children when compared to adolescents and adults [13,14,19,24]. The comparatively lower muscle concentrations of anaerobic enzymes, creatine phosphate and glycogen, as well as the rates at which these anaerobic substrates are utilized, indicate that children may require a greater aerobic contribution to meet the energy demands needed during the WAnT.

Although anaerobic pathways have been documented as the main contributors of energy supply for the WAnT, numerous investigations have reported that the aerobic pathway is also a significant contributor of the energy requirements during the test [7,18,21,25,31]. Several investigations have explored the specific contributions of the glycolytic, phosphagenic, and oxidative pathways in young adult males [7,18,21,25,31] and females [21]. These investigations have reported that the aerobic contribution during the WAnT ranges from 13% to 34%, depending on various assumptions regarding mechanical efficiency, time delay, and O2 store utilization [7,18,21,25,31]. Inbar et al. [21] found that the aerobic component of the WAnT contributed only 13% of the total energy supply. Serresse et al. [31] found that the glycolytic and phosphagenic pathways contributed 49% and 23%, respectively, of the total ATP, while the oxidative contribution was 28%. Hill and Smith [18] calculated that the contribution of ATP from the oxidative pathway could range from 14.4% to 28.6% during a WAnT. Similarly, Bogdanis et al. [7] reported that the glycolytic and phosphagenic pathways contributed 45% and 21%, respectively, of the total ATP necessary for the WAnT, while the oxidative pathway contributed 34% of the energy supply.

Due to the limited anaerobic capacity in children, one may suspect that they may require an even greater aerobic energy contribution during the WAnT. Chia et al. [9] examined boys and girls (mean age 9.7 ± 0.3 years) and determined that the aerobic contribution to the WAnT ranged from 19.2% to 44.3% in boys and from 17.7% to 40.7% in girls. In our current study, the extent of the utilization of the aerobic pathway during the performance of a WAnT could explain the degree of muscular fatigue, subsequent reduction in exercise time and failure to meet maximal aerobic power criteria when performing the VO2max test. Limitations of the present investigation include that our subject sample was primarily Caucasian and representative of rural Pennsylvania. However, we would not expect a deviation from the current results given a change in demographic location. We are aware that differences in aerobic fitness between African-American and Caucasian children have been observed [1,17,34]. Future investigations should explore racial differences in anaerobic and aerobic power in children as it applies to testing sequence.

Practical application

A barrier associated with research, especially that involving children, is subject drop-out. Few investigations report drop-out rates, although it is a common problem with research that includes traveling to a clinic/laboratory on more than one occasion. Drop-out rates in pediatric exercise studies have ranged from 11% to 20% [30,32,35]. The implications of the present investigation may provide researchers and clinicians with a better understanding of and evidence supporting the inclusion of both maximal exercise tests in the same clinical/laboratory visit without affecting a child’s physical abilities or affecting subject drop-out. As our findings indicate the specific order of testing should be a VO2max test conducted at least 20 minutes prior to a WAnT.

Conclusion

In conclusion, the oxidative energy requirements utilized during the WAnT vary in children and are of importance when performing both a WAnT and VO2max test in the same testing session. The extent of the utilization of the aerobic pathway during the performance of a WAnT could explain the degree of muscular fatigue and reduction in exercise time when performing a subsequent VO2max test. Based upon the results of this investigation, it appears that maximal power values can be obtained when a VO2max test is conducted 20 minutes prior to a WAnT. The ability to maximize laboratory time (i.e., about 1 hr) is important to researchers, clinicians and their subjects or patients. It allows more subjects or patients to be examined in less time and eliminates the necessity of returning to the hospital or laboratory for another exercise test. Further investigations should examine the relationship between anaerobic and aerobic testing sequence in adolescents and older adults.

References
