ORIGINAL ARTICLE

Influence of fitness level and technique on Wingate test result in different positions

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ABSTRACT

The level of anaerobic performance primarily influences the result of the 30-second Wingate anaerobic test (WAnT). This study aims to determine the influence of position on the overall performance achieved in different phases of the WAnT in participants with different fitness levels. Sixty participants (21.00 ± 2.24 years): 20 race cyclists, 20 competitive runners, and 20 non-athletes performed three WAnTs (sitting, standing, and combined position) in one week. For the analysis, we used a random mixed effect model with type and position as a fixed effect. We studied the meaning of interactions and the main effects of fixed variables ($\rho \leq 0,05$). Technically advanced individuals perform significantly better in standing than sitting from the 8th second until the end of the test. Technically and physically advanced individuals achieve significantly higher performance levels in the first half of the standing position test than those who are fitness-ready but without the necessary level of technique. Fit individuals without of technique achieve high performance in the second half of the WAnT in the standing position. The main benefit of the work is the finding that the level of fitness and technique of pedalling have a different influence on performance in different phases of the WAnT.
Keywords: Performance analysis, Anaerobic test, Cycling position, Pedalling technique, Test phase.

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INTRODUCTION

In many sports, one-off or repeated maximum effort is used in short-run sprints. The quantification of anaerobic performance is required for its evaluation (Baron, 2001; Bringhurst et al., 2020; Delextrat and Cohen, 2008; Dorel et al., 2005). The gold standard for anaerobic evaluation is the 30-second Wingate anaerobic test (WAnT). The WAnT is one of the most widely used tests of anaerobic assumptions in athletes, especially cyclists, where movement is specific, but is also used by speed skaters, hockey players, and other athletes, but also by non-athletic populations (Bahenský et al., 2020, 2020a; Jaafar et al., 2014; Krishnan et al., 2017; Ramírez-Vélez et al., 2016). The main parameters that can be measured with the WAnT are 1-s peak performance (PP), 30-s average performance (AP), and percentage of performance decline from peak to minimum (fatigue index = FI) (Vandewalle et al., 1987). Other parameters that can be evaluated include average cadence (AC) peak heart rate (PHR) and relative values: relative 1-s peak power (RPP), relative 30-s average power (RAP) and relative 5-s peak power (R5PP). Although the standard 30-second WAnT is classified as an anaerobic test, it is clear that a certain amount of aerobic work is also performed during its completion (de Poli et al., 2021; Wilson et al., 2009).

In both cycling and cross-country competitions, overall performance is determined by both aerobic and anaerobic capacity levels, the ratio of which depends on the length of the racetrack. Short bursts of anaerobic performance occur during and at the end of the races. It is common for cyclists to lift from the saddle and move to a standing position during sprints. Given the frequency of using of this position in cycling races, there is great interest in its effectiveness. The pedal slope, the direction of force, and the position of the centre of gravity change in different positions (Caldwell et al., 1998). The standing position also increases the involvement of the upper limbs (Duc et al., 2008). Previous research suggests that the standing position may be the most effective for maximum effort (Bouillod and Grappe, 2018; Kadlec et al., 2022; Li and Caldwell, 1998; Rohsler et al., 2020). However, the standing position is preferable to the sitting position only at high power intensities (Turpin et al., 2017).

Without a doubt, the specificity of the test is intricately connected to the obtained results (Kadlec et al., 2022; Marko et al., 2021). In the WAnT, the most commonly used position is seated, but some athletes move to a standing position at the end of the WAnT when great muscle fatigue has already occurred. Performance cyclists have been confirmed to achieve higher performance in the standing and combined positions (first half of the test sitting and second half standing) than in the sitting position, which is probably influenced by their excellent cycling technique (Jaafar et al., 2014). The WAnT is an ideal test for comparing anaerobic performance in different riding positions.

There is evidence that during standing pedalling, there is an increase in torque in the ankle and knee joints, whereas torque in the hip joint decreases (Li, 2004). In the standing position, there was greater activation of the rectus femoris, gluteus maximus, and tibialis anterior throughout the pedal stroke cycle. No changes in activity between standing and sitting positions were observed in the gastrocnemius and biceps femoris (Li, 2004). There was an 8% reduction in cadence for standing rides (Bouillod and Grappe, 2018; Li, 2004). Elite cyclists achieve higher performance standing than sitting; the same is true for performance cyclists. But at the same time, elite cyclists achieve significantly better performance standing than recreational cyclists (Bertucci et al., 2008). This shows that excellent technique is not necessary for effective standing rides, but a certain level of technique is sufficient. No changes in speed were observed for elite cyclists during the transition from sitting to standing (Bouillod and Grappe, 2018). When comparing the metabolic cost of sitting and standing riding through O_2 uptake, the higher energy intensity of standing riding has been demonstrated (Ryschon and Stray-Gundersen, 1991).

This study aimed to determine the influence of position on performance and performance progress at the WAnT in participants with different levels of fitness and pedalling techniques. In contrast to the majority of studies, we conducted measurements for these parameters in three distinct positions: sitting, standing, and a combination of both. Given the absence of studies exploring performance variations during the WAnT, our objective was also to discern potential differences between positions and among groups at various stages of the test. We hypothesise that the preferred position in the Wingate test will be different from group to group depending on the level of fitness and technique of pedalling. Additionally, we hypothesise that the technique of pedalling will have an influence on the Wingate test result. The question is what that influence will be, especially from the point of view of the course of the test.

METHODS

Participants

The study involved 60 males (Table 1), 20 competitive (elite) bikers at a national level, 20 middle- and long-distance runners at the national level, and 20 non-athletes, all of comparable age. Inclusion criteria included age about 20 years, optimal health, no injuries in the last year, for athletes regular training at least six times a week for at least two years. For non-athletes, the criterion was the absence of regular physical activity; in the last year, the Weekly leisure activity score was greater than or equal to 30 (Godin, 2011). The members of the non-athlete group reach values 18–29. All participants or their parents completed a written informed consent. There was no compensation for any of the participants, and all protocols and procedures conformed to the Declaration of Helsinki statements and were approved by The Ethical Committees of the Faculty of Education, University of South Bohemia study on October 19, 2018 (002/2018).

Table 1. Characteristics of participants.

	Cyclists	Runners	Non-athletes
Age (years)	20.77 ± 1.84	20.86 ± 2.78	21.37 ± 1.94
Body Mass (kg)	77.04 ± 6.96	67.52 ± 9.32	79.95 ± 15.66
Height (cm)	183.50 ± 5.08	181.25 ± 6.20	179.00 ± 7.77
Fat Percentage (%)	11.41 ± 3.63	11.43 ± 3.93	21.37 ± 1.94
Weekly training (hrs)	11.13 ± 1.48	10.75 ± 1.50	0.33 ± 0.31

Design and procedures

The current study was a randomized experimental design that examined the differences in anaerobic parameters in different riding positions and groups of participants during anaerobic tests in elite, competitive cyclists, runners, and non-athletes. Sixty participants with different fitness levels and different pedalling techniques visited the Laboratory of Load Diagnostics to complete the WAnTs in three different riding positions (sitting, standing, and combined) on a cycle ergometer in a randomized fashion, with one day of rest between tests. During each WAnT, the following relative anaerobic performance variables were collected: RPP, RAP, FI, R5PP, AC, and PHR. The WAnT tests were performed under the same conditions for all participants. Each participant was instructed to avoid intensive activity the day before each WAnT.

Measures

Participants completed the three 30-second WAnTs over one week, and each test was 48 hours apart. Three variations of the same test were completed. Participants were randomly split (randomizer.org) into six possible orders of the WAnT completion. The purpose of this study was to compare the relative anaerobic performance characteristics (RPP, RAP, FI, R5PP, AC, PHR) in a group of race cyclists, race runners, and a group of non-athletes while undergoing three different WAnTs in three different riding protocols, and to see if there were differences between the groups. All participants completed three variants of the test: a sitting-only test (SIT), a standing-only test (STD), and a combined test (COMB), in which participants started sitting and moved to a standing position halfway through (after 15 seconds) the test (see Table 2).

Before each test, participants were asked to abstain from caffeine for 12 hours and alcohol for 48 hours. Each participant was instructed to avoid intensive activity and train maximally at a low to moderate intensity for less than 1 hour the day before each WAnT. Conditions in the lab were similar across all three visits (20–22 °C). First, height, body composition, and weight were assessed using a digital device called InBody 770 (Cerritos, CA, USA). All WAnTs were completed on a LODE Excalibur Sport (Lode B.V., Groningen, The Netherlands) ergometer, and individualized seat and handlebar positions were determined. The participants were explained the design of the test before the test. All tests were preceded by a 5-minute standardized warm-up (Figure 1), which included two short sprints. Output data were measured and analysed with Lode Ergometry Manager 10 (Lode B.V., Groningen, The Netherlands) software. Heart rate during each test was monitored with a Polar chest strap (model T34, Polar, Finland). All participants completed the WAnT tests with cycling straps, and verbal encouragement was given during all tests.

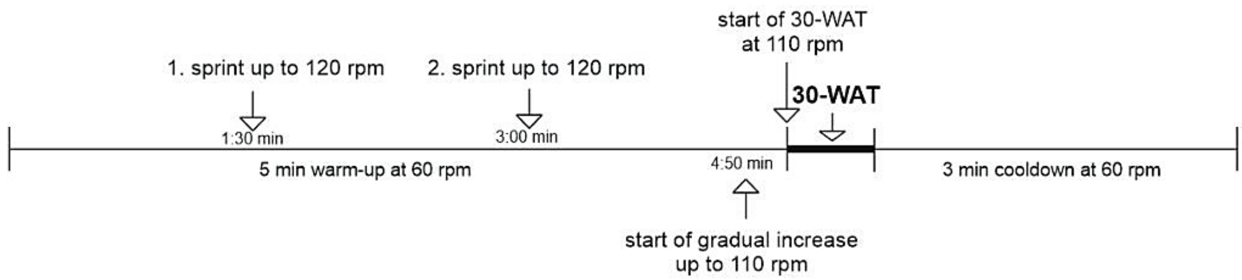


Figure 1. The design of the WAnT protocol.

Statistical analysis
The sample size calculations were done using the software G*Power (G*Power 3.1.7.). Repeated-measures analysis between factors was used to calculate the power analysis, indicating a total sample size of 42, with an assumed type I error of 0.05 and a power of 0.80. The analysed data are attached by fixed effect (type) and repeated measurements (position). The position is repeated measurement since it was measured on the same people. Therefore, we used the analysis of the random mixed effect model (Zuur et al., 2009) with type and position as a fixed effect; the dependence among different positions was solved by adding the random effect, the individual. Inside this model, we studied the significance of interactions and the main effects of fixed variables. Since the results are difficult to interpret, we also analysed the data using functional methods in order to find the times when the differences between types of sportsmen and positions are. First, we did the functional one-way ANOVA for three different data sets, i.e., stand data, site data, and combined data. The grouping factor in this functional model was always the type of sportsman, i.e., the fixed effect. For this analysis, we chose the false discovery rate envelope method (Mrkvička and Myllymäki, 2023) since it allows for graphical interpretation and is suitable for finding all differences in the null model due to the usage of false discovery control of multiple testing problems. Another advantage of this method which we use here is a possibility of using any test statistics, due to the permutation nature of the method.

The output figures show the two different test statistics (the mean group function: Figure 2 and the difference between two mean group functions: Figure 3) together with the 95% false discovery rate envelope (grey zone), which is the area where the test statistic should lie under the H0. Since the null hypothesis is the equality of mean group functions for all three categories, the deviance of the first test function from the grey zone shows the significant difference between the particular group and the overall group. The deviance of the second test function from the grey zone shows the significant difference between the two groups. This deviation, shown by bold dots outside the grey zone, specifies where and at what times the functional test is significant, i.e., for which time the group mean differs from the overall mean or the two groups differ.

As a next step, we want to explore the differences between different positions, but since it is a repeated measurement factor, we analysed only the interaction effect (the main effect was, anyway, not significant in random mixed effect analysis). For that reason, we used as a test statistic the difference of the group mean (type) between different positions, e.g., mean function for cyclists in the sit position minus mean function for cyclists in the stand position, and we applied again functional ANOVA with the group factor the type of sportsman. Since here we apply the comparison for three different test statistics (sit-stand, sit-comb, stand-comb), we performed the tests with a significance level equal to 0.05/3 in order to account for multiple testing problems. The division comes from Bonferroni's multiple testing adjustment (Dunn, 1961). This analysis shows for which times the difference between two positions for certain groups of sportsmen differ from the mean differences computed over all types of sportsmen. This is equivalent to the study of the interaction effect between the two factors but in the functional style.

RESULTS

Table 2 presents the performance parameters in the individual participant groups and in the individual bike positions. During the sitting position, all performance parameters except for RAP, the cyclists dominate in front of the runners. The elite cyclists group achieves the best RPP, R5PP, and AC in the standing position. The combined position yields the best RAP and the highest PHR. The seated position results in the highest FI. Consequently, for cyclists during the WAnT, the standing position proves most advantageous, while the combi position serves as an alternative for RAP. Runners show optimal performance and the highest cadence when seated. In the standing position, they achieve the highest FI, and in the combi position, the highest PHR. Hence, the WAnT position in a seated stance is most favourable for runners. The non-athletes group attains the best R5PP and RPP in the standing position, where the highest PHR and FI are also achieved. In the seated position, they reach the highest RAP and AC. Therefore, for non-athletes, the WAnT position in a seated stance emerges as the most suitable choice.

Table 2. Power output outcomes for three different WAnT protocols.

	Cyclists		
	Sit	**Stand**	**Middle**
Relative 30-s Average Power – RAP (W·kg^{-1})	9.40 ± 0.67	9.69 ± 0.66	9.70 ± 0.57
Relative 5-s Peak Power – R5PP (W·kg^{-1})	11.95 ± 1.12	12.33 ± 1.45	11.85 ± 1.28
Relative 1-s Peak Power – RPP (W·kg^{-1})	14.83 ± 1.66	15.35 ± 1.87	14.63 ± 1.38
Fatigue Index – FI	53.10 ± 9.39	51.91 ± 7.31	52.50 ± 11.69
Average Cadence – AC (rpm)	133.0 ± 8.1	136.2 ± 7.3	135.6 ± 7.3
Peak Heart Rate – PHR (bpm)	180.9 ± 11.7	180.5 ± 8.3	181.9 ± 10.4
	Runners		
	Sit	**Stand**	**Middle**
Relative 30-s Average Power – RAP (W·kg^{-1})	9.49 ± 0.48	9.13 ± 0.57	9.26 ± 0.56
Relative 5-s Peak Power – R5PP (W·kg^{-1})	11.35 ± 1.09	10.74 ± 0.91	11.24 ± 1.29
Relative 1-s Peak Power – RPP (W·kg^{-1})	13.47 ± 1.44	12.93 ± 1.17	13.31 ± 1.43
Fatigue Index – FI	46.63 ± 8.11	48.58 ± 8.58	47.37 ± 6.22
Average Cadence – AC (rpm)	136.5 ± 9.2	130.9 ± 7.6	132.6 ± 7.4
Peak Heart Rate – PHR (bpm)	181.7 ± 11.7	182.8 ± 8.3	184.1 ± 13.2

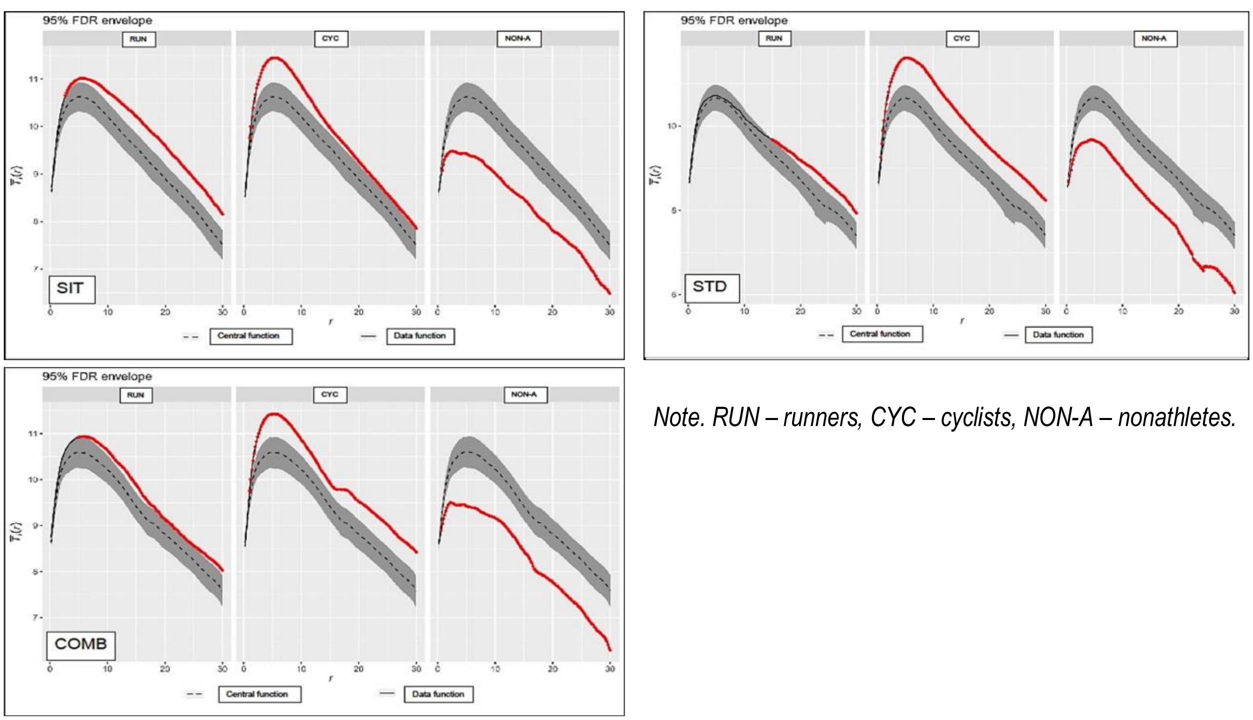
	Non-athletes		
	Sit	Stand	Middle
Relative 30-s Average Power – RAP (W·kg-1)	7.77 ± 1.12	7.43 ± 1.15	7.68 ± 1.16
Relative 5-s Peak Power – R5PP (W·kg-1)	8.26 ± 1.92	8.67 ± 2.17	8.23 ± 2.19
Relative 1-s Peak Power – RPP (W·kg-1)	10.66 ± 1.71	11.16 ± 2.46	10.99 ± 2.05
Fatigue Index – FI	47.12 ± 9.40	55.81 ± 15.81	53.55 ± 12.83
Average Cadence – AC (rpm)	112.9 ± 13.9	109.7 ± 15.0	112.4 ± 15.4
Peak Heart Rate – PHR (bpm)	184.5 ± 11.8	189.2 ± 11.2	186.8 ± 14.1

Performances achieved in individual positions are different from group to group. The effect of sport is significant in all endpoints. The effect of position is not significant for any summarizing characteristics. However, differences between positions vary between different types of athletes (see Table 3).

Table 3. Significance of individual factors in random mixed effect models.

	Interaction between position and sport	Effect of sport	Position
RAP (W·kg-1)	0.0019 **	2.2e-16 ***	0.1617
R5PP (W·kg-1)	0.0578	2.2e-16 ***	0.7389
RPP (W·kg-1)	0.0679	8.03e-16 ***	0.6244
FI	0.0973	0.0449 *	0.1216
AC (rpm)	0.0066 **	2.2e-16 ***	0.2058

*Note: *p < .05, **p < .01, ***p < .001***.*

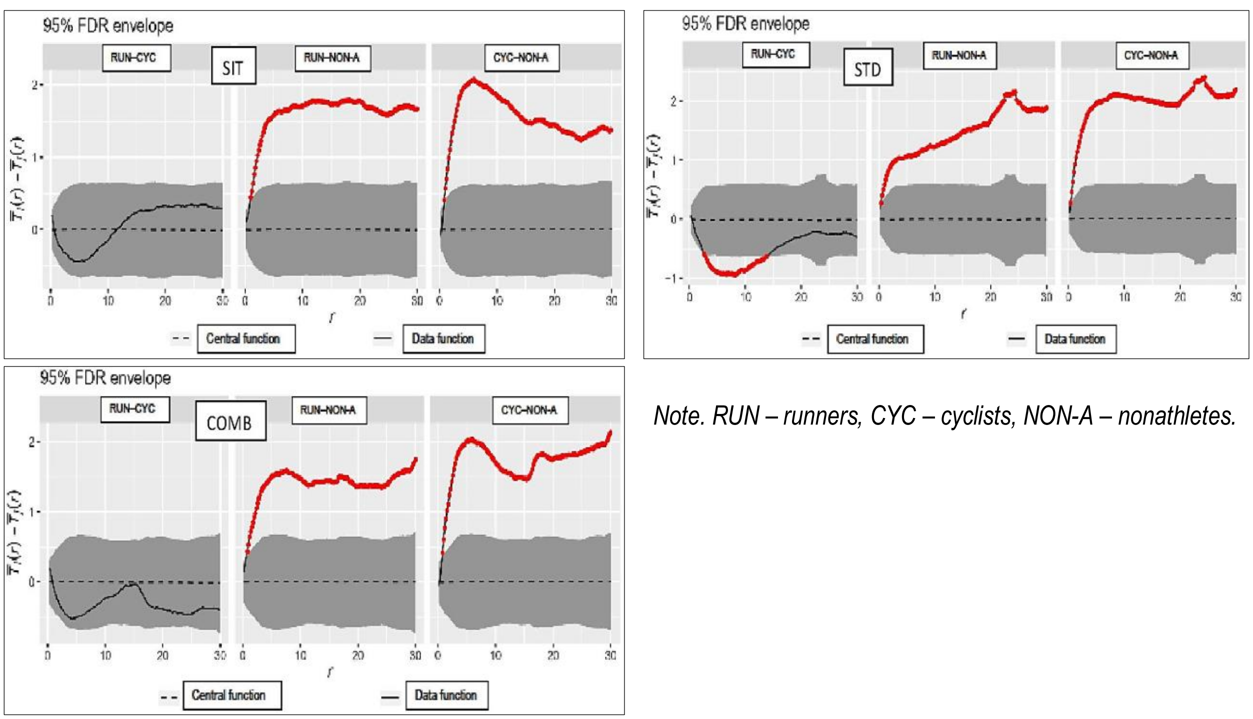


Note. RUN – runners, CYC – cyclists, NON-A – nonathletes.

Figure 2. WAnT protocols in the sitting (SIT), standing (STD) and combined (COMB) position.

The quality of the difference between the positions in the different stages of the test for each group is presented in Figures 2–4. These show the WAnT performance pattern recorded every 0.2 s in the individual positions for a group of runners, cyclists, and non-athletes. The Figure 2 show the performance of each group in the individual positions compared to the entire set of participants. The bold line outside the grey zone shows a significantly different result. The grey zone represents an area in which the null hypothesis is not rejected. A group of runners achieve significantly better performance in the sitting position from the 3rd to the 30th second. Cyclists achieve significantly better results throughout the test, achieving the largest difference from the other groups in the first 13 seconds of the test. A group of non-athletes achieved significantly worse results in the sitting position compared to the other groups.

In the standing position shown in Figure 2, runners achieve significantly better results than other groups from the 15 second until the end of the test. Cyclists achieve significantly better results throughout the WAnT; again, in the first 10 seconds, the difference is greatest compared to other groups. A group of non-athletes is recorded with significantly the smallest result throughout the WAnT. In the combined position (the first 15 s in the sitting position and the second 15 s in the standing position), runners achieved a significantly better result than others tested from 5th to 30th, with the WAnT runners achieving a significantly better result than others tested from 5th to 30th with the WAnT. For cyclists, a significantly better result is recorded throughout the WAnT, with the largest difference to other groups being recorded during the first 10 s of the test and then after 15 seconds when the position change occurred. For the non-athlete group, significantly the lowest performance is recorded throughout the WAnT. For all groups, there was an optical improvement in performance due to the position change at 15s.

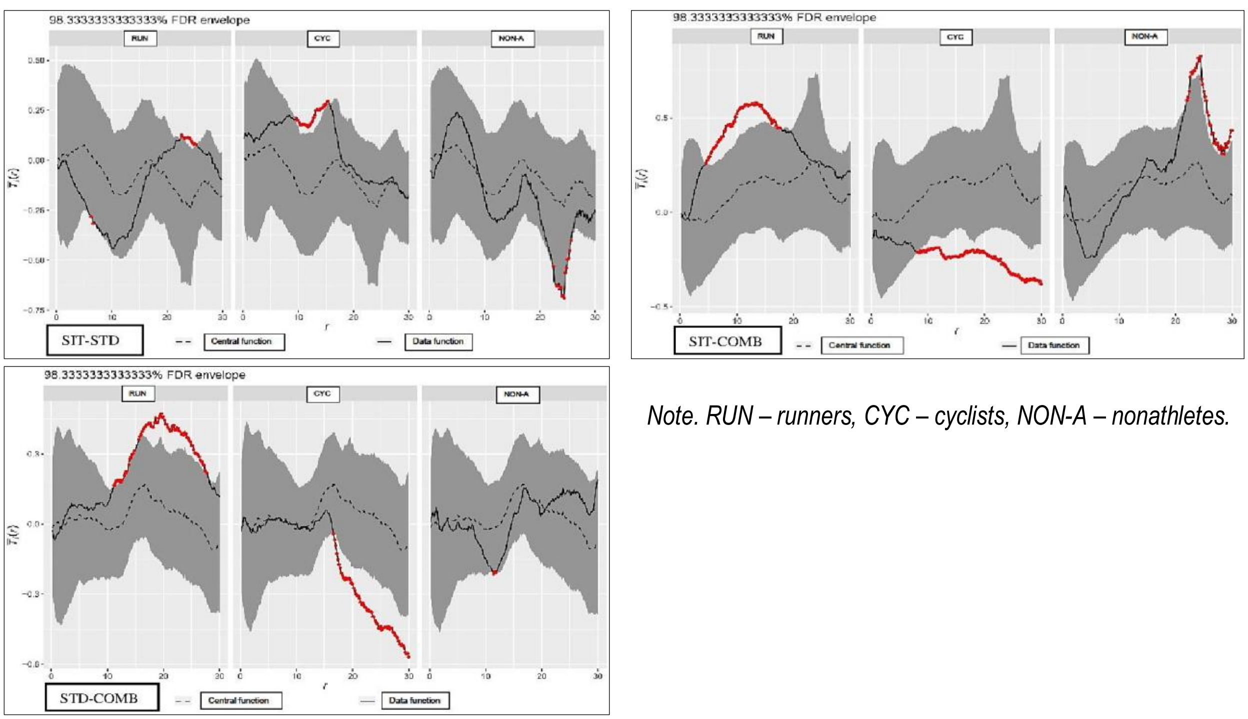


Note. RUN – runners, CYC – cyclists, NON-A – nonathletes.

Figure 3. Performance differences in WAnTs in the sitting (SIT), standing (STD) and combined (COMB) position between different groups of participants.

The Figure 3 show the performance difference between groups in each position. Cyclists achieve higher performance in sitting position than runners for the first 12 seconds of the test, then runners dominate. Performance differences between groups are not significant at any stage of the test. Non-athletes achieve significantly lower performance in the sitting position throughout the WAnT compared to both runners and cyclists. Cyclists achieve a higher level of performance during the whole standing test than runners; this difference is significant between the 2nd and 14th seconds. Non-athletes achieve significantly lower performance than both runners and cyclists, even in standing positions. In the combined position, cyclists perform better than runners throughout the test, but the difference is insignificant. Halfway through the test, there is a clear improvement in the performance of cyclists over runners. Non-athletes achieve significantly less performance than runners and cyclists in the combined position.

The Figure 4 show the difference in performance between individual positions in individual groups of participants. For a group of runners, there is a significant difference in favour of the sitting position in 5–17 sec, between the sitting and standing positions. For a group of cyclists, there is a significantly better standing result from 8–30 sec. For a group of non-athletes, there is a significantly better sitting performance in 22–30 sec. For runners, when comparing sitting and combined performance, significantly better sitting performance is recorded in 11th-14th s and 16th-28th s. For cyclists, significantly better performance is seen in the combined position from the 17th to 30th s test. For non-athletes, significantly better performance is seen only in the 13th-14th s test. The difference in standing and combined performance is described in the Figure 4 as well. For runners, the combined performance is significantly higher in the 6th and 7th sec; in the standing position, they achieve significantly better performance in the 13th-16th sec. A group of cyclists achieved significantly better performance in the standing position in the 9th-16th seconds. For non-athletes, significantly better performance was achieved in the combined position in the 22nd-26th sec.



Note. RUN – runners, CYC – cyclists, NON-A – nonathletes.

Figure 4. Performance differences between WAnTs in sitting and standing, sitting and combined, standing and combined position.

DISCUSSION

The main aim of this study was to assess how performance during the WAnT is influenced by position (sitting, standing, and combined) across diverse participant groups, taking into account variations in fitness levels and pedalling techniques among cyclists, runners, and non-athletes. This was demonstrated by comparing anaerobic power output characteristics (RPP, RAP, R5PP, FI, PHR, AC) in elite cyclists, runners, and non-athletes. At the same time, they completed three distinct WAnTs in 3 different position protocols. Since the results could be more transparent, we also looked at performance differences in different positions during the tests.

We hypothesise that the preferred position in the Wingate test will be different from group to group depending on the level of fitness and technique of pedalling. Additionally, we hypothesise that the technique of pedalling will have an influence on the Wingate test result. This was correct. Although we found no general significant influence of the position on the values of individual parameters measured at the WAnT. However, there is a general relationship between the level of fitness and the results of selected parameters at the WAnT (RAP and AC). Our findings also confirm a significant relationship between the level of performance and the selected position at the test for individual groups according to the level of fitness and technique of pedalling. We found that for cyclists who represent technically and fitness-worthy individuals, the optimal position for the WAnT is the standing or combined position, which partly confirms the already published conclusions (Kadlec et al., 2022, Merkes et al., 2020, Reiser et al., 2002). In evaluating speed skaters who are representatives of fitness-ready individuals and athletes technically at the intermediate level, Wilson et al. (2009) did not find significant differences between the results of the sitting and standing tests. Individuals with high anaerobic fitness levels but without sufficient technique pedalling (runners) and non-athletic individuals achieve the best performance in the WAnT in the sitting position. This position appears to be the best for the WAnT for individuals prepared in fitness but without the necessary pedalling technique and for individuals without fitness and technique (McLester et al., 2004).

This study provides valuable insights into the specific phases of the WAnT, where distinctions exist among various athlete groups in each position. For elite cyclists, the optimum level of technique and fitness allows a higher level of performance to be achieved during the whole standing test than for individuals who possess a high level of fitness but without the necessary pedalling technique (group of runners). While in the first half of the test, the difference is significant. In the sitting position, cyclists achieve higher performance than runners for the first 12 seconds of the test, after which the runners achieve better performance. In the combined position, cyclists perform better than runners throughout the test, but the difference is insignificant. For cyclists, there is also significantly better performance in the standing position than in the sitting position in the 8th-30th sec. Cyclists also perform significantly better in the standing position in the 9th-16th sec than in the combined position. It indicates a better pedalling efficiency in the standing position in this test phase than in the sitting position.

A comparison of the performances of runners and cyclists is shown above. However, since the 15th-second test, runners have performed significantly better than the whole test set. In the combined position, runners have performed significantly better in the 5th-30sec. Runners have achieved significantly better performance in the 4th to 18th seconds in the sitting position compared to the standing position. From the 15th to the 28th seconds, runners performed significantly better in the standing position than in the combined position. This is even though since the 15th sec, the combined position represents the standing position. The change of position in the 15th second allows for a temporary improvement of performance in the second half of the test, even though runners do not have the necessary technique of pedalling standing.

For a non-athlete group, we can see significantly the lowest performance in all positions, significantly lower than that of cyclists and runners. This is the expected result (Bar-Or, 1987, Ramírez-Vélez et al., 2016). There are no significant comprehensive differences between the performances in individual positions, which is confirmed by the already published conclusions (Costa et al., 2022). Non-athletes perform significantly better in the 22nd-30th sec sitting position than in the standing position. They perform significantly better in the combined position than in the standing position between the 22nd and 26th. It indicates that the change of position has a temporary positive effect on their performance.

To generalize the results, obtaining the results of athletes of a more diversified fitness level would be necessary. Thus, the limits of the work include the absence of multiple groups of athletes of different levels. Our results show that in the absence of a pedalling technique, a high level of speed-strength disposition (fitness) can significantly influence performance in the second half of the WAnT in the standing position. A sufficient level of fitness and the absence of technique results in a significantly smaller decrease in performance in the second half of the WAnT in the sitting position. It also allows significantly better performance in the sitting position than in the standing position. In technically advanced individuals, the best results are achieved in the combined and standing position.

CONCLUSION

The level of anaerobic fitness determines the result of the WAnT. An important finding is the influence of pedalling technique on performance during the WAnT. Technique and fitness have a significant influence on the results of the WAnT. Individuals without a rational riding technique achieve individually the best results in the WAnT in the sitting position. For technically advanced individuals with a high level of fitness, the best results are achieved in the combined and standing positions. The level of fitness and technique of pedalling have different influences on performance in the various phases of the WAnT; this is the main benefit of this work. Technically advanced individuals perform significantly better in standing than sitting from the 8th second until the end of the test. The optimum level of technique and fitness will allow a significantly higher level of performance in the first half of the test in the standing position than only for individuals with fitness but without the necessary level of technique. Without a good pedalling technique, a high-speed force disposition can significantly influence performance in the second half of the WAnT in the standing position, also causing a smaller decrease in performance in the second half of the WAnT in the sitting position. These conclusions may be helpful in the realization of the WAnT and its evaluation. They show the influence of technique on performance and the influence of fitness.

AUTHOR CONTRIBUTIONS

Conceptualization, P.B., D.M., M.K., P.B.J. and D.B. ; methodology, P.B., M.K., P.B.J., D.B. and T.M.; software, R.M. and T.M.; validation, P.B., M.K., P.B.J. and D.M.; formal analysis, P.B., D.B., R.M. and T.M.; investigation, P.B. and D.B. ; resources, P.B. and R.M.; data curation, P.B., P.B.J. and D.B.; writing—original draft preparation, P.B., D.M., M.K., P.B.J., D.B. and T.M.; writing—review and editing, P.B., D.M., M.K., P.B.J., D.B., R.M. and T.M.; visualization, P.B., D.B.; supervision, P.B., D.B. and T.M. All authors have read and agreed to the published version of the manuscript.

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