Peer Review File

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Reviewer A:

1. With such short exercise times achieved for 3 of the work rates metabolic data collected and subsequent peak work rates hard to compare as the maximum exercise times are very short.

Reply 1:

We thank the reviewer for the question and comments.

In the initial selection of incremental work rates, we thought about the widest possible work rate range that a normal human upper limb might perform, which is 5-50W/min in isometric increments of 15 W/min. The incremental work rate range was not only set to enable upper limb muscle mass to complete CPET at the right exercise time. It was simultaneously designed to validate whether certain key variables of CPET remain similar or differ significantly at peak exercise, when work rate increasing rate levels are either very high or very low.

The maximal exercise times achieved during arm ergometer CPET at 35 and 50 W/min incremental work rate were brief. However, the key variables were as effectively compared as feasible by splitting the data collected for each breath second-by-second and calculating a 10 s average. The final data acquired also supports the inappropriateness of such a high work rate selection for arm CPET.

2. Questions raised about the selection of the work rates being so varied 5 - 50W/min. Consideration needed for selection of these work rates for upper limbs considering muscle mass.

Reply 2:

We thank the reviewer for the question and comments.

We firmly agree that using 10-20 W/min of incremental work rate is preferable for subjects in clinical practice, and that further study is necessary to fully understand the specific differences and similarities. As advised, we have included a paragraph with study limitations in the "Discussion" section explaining the study's shortcomings. (see Page 17, line 1184-1195)

Reviewer B:

General comments

This study examines the effect of different incremental work rates during arm crank ergometry on peak physiological responses in otherwise healthy male and female participants. Furthermore, the data are also compared to data from an additional cycle ergometry trial. The title and abstract need some reworking, mainly from a scientific wording perspective. The introduction focusses upon the Holistic Integrative Physiology and Medicine (HIPM), but his does not frame the design or discussion of the results. It is not clear what HIPM alters or improves, if anything, for this type of study. The introduction needs to be stronger and, specifically for the data presented, should note that a range of increments have been examined previously for arm ergometry protocols (e.g., 35 and 30W Price and Campbell, 1997; 1W.6 sec; Smith et al, 20W per stage, Smith et al, and greater increments in elite paddles e.g. Tesch et al) but hese have not been compared in one study – this would strengthen the rationale and subsequent aim substantially – and add novelty. The authors should also consider what benefit the additional cycle ergometry protocol data set provides. There are frequent suggestions of how the presented work may relate to clinical groups - to which end to the authors cannot extend any findings by using their chosen healthy population, even though they may be clinicians. Additionally, if the authors refer to clinical CPET, increment intensities such as 50W are massive in relation to what such a population would be able to perform at – such a load is generally reserved for upper body trained individuals / elite paddlers. As such this would not fit the rational relating to clinical groups. Having n=9 female participants is a novelty as most arm data sets are from male populations. This may be useful for revising the novelty of the study. Throughout the methods (and rest of manuscript) there is the need for specific details to be presents – e.g. such as calibration details. The respiratory exchange ratio date appears to have a large focus in the results and discussion but there is no rationale for this in the introduction. If RER is a key focus this needs to be added clearly to the study rationale. Results need to be written more scientifically. I suggest consulting other exercise physiology journal or those reporting similar ergometry responses. Finally, the discussion needs considerable work as, in its present form, does not discuss the comparison of different ramp rates in arm ergometry protocol, especially in relation to the underlying physiology. The peak values for each protocol have not been compared to previous studies which is important for this type of work. There is an overreliance of application to clinical groups throughout which cannot be upheld when data was collected from non-symptomatic participants. Focus on the novelty of the study which, in my view, is the first comparison of typical ramp rates reported in arm ergometry.

Specific comments

Comment 1: Title: Wording, '…. in arm ergometry….'. More importantly, the title suggests difference and similarities, please change to a shorter less ambiguous title, e.g. 'The effects of different incremental work rates on cardiopulmonary exercise testing in arm ergometry'.

Reply 1: Thank you for your valuable suggestions. The title does fit my manuscript better.

Changes in the text: We have changed the title to'The effects of different work rate increasing rates on cardiopulmonary exercise testing in arm ergometer' as suggested (see Page 1, line 1-2)

Abstract

Comment 2: L11: Wording, 'The leg ergometer cardiopulmonary exercise test' better worded as 'Lower body ergometry is commonly used for cardiopulmonary exercise testing (CPET)' or 'Cycle ergometry is commonly used…..'.

Reply 2: Thank you for your valuable suggestions. 'Lower body ergometry is commonly used for cardiopulmonary exercise testing (CPET)' is really a better way to phrase the expression.

Changes in the text: We have changed the wording of this sentence as advised.

'Cycle ergometer is commonly used for cardiopulmonary exercise testing (CPET), which is the objective and quantitative golden standard for functional evaluation and training,' (see Page 2, line 40-41)

Comment 3: L13: Wording; 'while arm CPET is rarely investigated' is misleading as it is quite frequently used in a range of applications, try 'arm CPET is less commonly used…'?

Reply 3: Thank you for your valuable suggestions. We have changed the wording of this sentence as suggested.

Changes in the text: We have changed 'while arm CPET is less commonly used to clinically assess a patient's overall functioning.' (see Page 2, line 41-42)

Comment 4: L13-14; Wording; 'To optimize performing protocol', try 'To determine optimal CPET protocols ……'.

Reply 4: Thank you for your valuable suggestions.

Changes in the text: We have changed the wording of this sentence as advised.

'To determine optimal CPET protocols, we studied the effect of different incremental work rates of arm ergometer on CPET key parameters.' (see Page 2, line 42)

Comment 5: L18; 'then followed' change to 'followed by'.
Reply 5: Thank you for your valuable suggestions.
Changes in the text: We have changed 'then followed' to 'followed by' as advised (see Page 2, line 46)

Comment 6: L18/19: Was the order of testing randomized or counterbalanced? Four trials in one week (7 days or 5 day working week?) seems a lot for non-specifically upper body trained individuals. Was any learning / order effect observed?
Reply 6: Thank you very much for reminding us to clarify this point. A repeated measure design was used in our study, where four incremental arm CPET test protocols were performed in random order. We did not make an observational comparison of order effects and do not draw the appropriate conclusions at this time. The four trials were completed within one week (7 days) with a minimum of 24 h between each test day. This test meets exercise physiology standards (ACSM recommends ≥24-48 hours between high-intensity tests).
Changes in the text: We have added explanations to these points that were unclear based on your comments.
'followed by four maximal arm ergometer CPETs using different work rate increasing rates (5, 20, 35, and 50W/min) in random order on various days in one week (7days).' (see Page 2, line 46-48)

Comment 7: L20: Delete 'CPET data were calculated by our standard methods, such as' as is superfluous wording.
Reply 7: Thank you for your valuable suggestions.
Changes in the text: We have deleted that. (see Page 2, line 48)

Comment 8: L22: Exercise time is usually referred to as 'Tlim' (subscript 'lim').
Reply 8: Thank you for your valuable suggestions.
Changes in the text: We have changed 'Ex. Time' to 'Tlim' and replaced the word wherever it appears in the text. (see Page 2, line 50)

Comment 9: L23: Delete 'etc.' as this is probably a complete list of what is presented in the manuscript, if not there can only be a small number not listed.
Reply 9: Thank you for your valuable suggestions.
Changes in the text: We have deleted that. (see Page 2, line 50)

Comment 10: L23: Delete 'and were compared their difference and similarity among

different incremental work rates', and state as appropriate something like 'Statistical analysis between protocols and exercise tie and between peak exercise responses involved ANOVA and paired comparisons, respectively' – although I do not know what the paired t-test would be use to analyze at this point of reading (arm vs leg? Post-hoc?) as four protocols would be compared using ANOVA. Please state.

Reply 10: Thank you for your valuable suggestions.

Changes in the text: We have deleted it and state 'One-way ANOVA with Tukey's post-hoc test compared outcomes across four increasing rate protocols, and paired t-test assessed arm vs. leg differences.' as suggested. (see Page 2, line 50-51)

Comment 11: L26: No need to refer to participants as 'NS', state 'non-symptomatic participants' in the first instance.

Reply 11: Thank you for your valuable suggestions.

Changes in the text: We have changed "young healthy normal subjects" to "non-symptomatic participants" and corrected all occurrences of "NS" in the text. (see Page 2, line 45,53)

Comment 12: L26: '…ergometer[s]…'.

Reply 12: Thank you for your valuable suggestions.

Changes in the text: We have changed 'ergometer' to 'ergometers'. (see Page 2, line 53)

Comment 13: L27: no need to restate 'four ergometer protocols' just reword as 'Each arm ergometer protocol elicited similar *V*O2, ……..'.

Reply 13: Thank you for your valuable suggestions.

Changes in the text: we have changed the wording of this sentence as advised.

'Each arm ergometer protocol elicited similar $\dot{V}O_2$ (1.37±0.31, 1.35±0.32, 1.34±0.31 and 1.33±0.30 L/min, P=0.986), HR (P=0.958), $\dot{V}E$ (P=0.976), VT (P=0.980) and Bf (P=0.812) at peak and AT(P=0.958).' (see Page 2, line 54)

Comment 14: L30-31; Valeus for watts should be reported as whole numbers.

Reply 14: Thank you for your valuable corrections. We have corrected the value of watts as whole numbers to re-report as you suggested. At the same time, we also modified the watts data of the results and tables in the article to integers.

Changes in the text:

Modify the sentence to 'there were significantly different peak WR (58±11, 80±18, 95±22 and 110±22W, P<0.001).' (see Page 2, line 57)

Comment 15: L31: State where the differences exist with regard to the specific protocols rather than showing an upward trend positively depend on incremental work rate' (L33).

Reply 15: Thank you for your valuable guidance. We have carefully considered your suggestion and have removed the sentence 'showing an upward trend positively depend on incremental work rate'; however, it is constrained by the word count of the abstract, which limits our ability to specify the differences between groups in this section. A detailed description of these differences can be found in the results section of the article.

Changes in the text: We have deleted the sentence 'showing an upward trend positively depend on incremental work rate'. (see Page 2, line 59)

Comment 16: L33-35: If there were 'significantly different Ex. Time with negative relationship' them state the R and R squared values, otherwise, if no correlation was undertaken state the results as per specific protocol differences / ANOVA post-hoc etc.

Reply 16: We performed a correlation analysis between Ex. Time and the work rate increasing rate, supplementing the R squared values as suggested. In addition, we also added the R squared values derived from the peak WR, peak RER, and maximal RER during recovery correlation analyses.

Changes in the text:

We have modified the sentence to 'There were significantly different Tlim with negative relationship (11.61±2.29, 4.02±0.91, 2.71±0.64 and 2.19±0.44min, $P<0.001$, $R^2=0.383$).' (see Page 2, line61)

'…which were positively correlated with work rate increasing rate ($R^2=0.985$, 0.823, 0.939, respectively).' (see Page 2, line 59-60)

Comment 17: Conclusion: As you have not compared the same parameters in leg as well as the arm ergometer protocols is it best to delete 'The study indicates that same as leg ergometer CPET', although this is known in the literature.

Reply 17: Thank you for your valuable suggestions. We have already compared the same parameters in the leg and arm ergometer protocols, so this statement is retained.

Changes in the text:

We have added and rewritten sentences 'We preliminarily recommend a work rate increasing rate of about 10-20 W/min in arm ergometer CPET for healthy individuals, which needs further investigation for functional evaluation and training.' (see Page 2, line 64-65)

Comment 18: Key words: Better to state arm ergometry and leg ergometry as separate terms as an 'arm and leg ergometer' involve the arms and legs exercising concurrently.

Also, delete 'difference and similarity' as these do not refer to anything specific and are unlikely to be used a search terms.

Reply 18: Thank you for your valuable suggestions.

Changes in the text: We have changed 'arm and leg ergometer' to separate terms for arm ergometer and leg ergometer. (see Page 3, line 120-121)

We have deleted 'difference and similarity'. In addition, we removed 'respiratory exchange rate' and added 'protocol optimization'. (see Page 3, line 123)

Comment 19: Key findings

Consider rewording to something similar to '……..' optimize [arm ergometry] protocol[s], it's crucial to balance [the increase in] exercise intensity' delete' levels—not too high or too low'.

Further states 'Opting for a moderate intensity allows subjects to sustain exercise for appropriate Ex. Time, ensuring adequate physiological responses without inducing premature fatigue', true, so which protocol provides a moderate intensity based on the data obtained? For examples, where VO2 testing is of importance any of the four protocols yields the same results, whereas for peak power the greater the increment, the greater the peak power value. What do you recommend? There is a large decrease in exercise time from ~11 min to ~2-4 min with the larger increment protocols – which could probably be surmised from the literature (e.g. 'Jump-max' tests, see Lawler, J., Powers, S. K., & Dodd, S. (1987). A time-saving incremental cycle ergometer protocol to determine peak oxygen consumption. British journal of sports medicine, 21(4), 171–173. https://doi.org/10.1136/bjsm.21.4.171).

What is known and what is new? Give cycle tests protocol recommendations for greater context.

What is the implication, and what should change now?

States an implication for clinical groups, but such a group was not tested. Better th sat, 'Although a clinical population was not studied …….' or 'Although a non-symptomatic healthy population was studied ….'.

Reply 19: Thank you for your kind guidance and valuable suggestions. We have carefully revised the Highlight box section line by line based on the suggestions.

Changes in the text:

- Change the wording 'Different work rate increasing rates in the arm ergometer CPET affect several key parameters, primarily peak WR, Tlim, and RER. To optimize arm ergometry protocols, it's crucial to balance the increase in exercise

intensity.'

- Indicate the preliminary recommendations of our study 'A work rate increasing rate of 10-20 W/min is recommended for arm CPET in healthy populations, balancing test duration (Tlim ≈4-8 min) and physiological validity.'
- Give cycle tests protocol recommendations 'The recommended duration of exercise is between 8 to 12 minutes.'
- Rewrite content 'First systematic comparison of four incremental rates (5-50 W/min) in a unified arm CPET framework. Demonstrated RER and Tlim sensitivity to protocol selection, despite stable traditional indices (peak $\dot{V}O_2$, AT). Arm CPET protocol optimization via incremental work rate parameter analysis.
- Supplement 'Protocols are flexible in healthy populations and need to be consistent for longitudinal monitoring.'
- Rewrite 'Although a non-symptomatic healthy population was studied, tailoring the work rate increasing rate to each individual's fitness level and physiological capacity allows us to optimize the sensitivity of the arm ergometer CPET in evaluating functional capacity, thereby enhancing its effectiveness in guiding exercise rehabilitation training.' as suggested. (see Page 3, line 124)

Introduction

Comment 20: L45-50; These lines do not add to the specific study background of exercise testing and can be deleted. Start with 'the cardiopulmonary exercise test ….'. Relating to the HIPM theory does not affect the interest of exercise testing per se.

Reply 20: Thank you very much for your valuable comment. We have been acutely aware of this important issue of the reader being puzzled by the unclear presentation in this section of the relevance of the HIPM theory to CPET and the purpose of this experimental study. As a result, we revised the introduction's organization and substance.

Changes in the text: We have rewritten the content 'In the new theory of Holistic Integrative Physiology and Medicine (HIPM) (7-10), it is posited that oxygen metabolism needs the mutual cooperation among respiratory system, blood circulation system and metabolic system, and the coupling between internal and external respiration can be accomplished under the regulation of nerves and body fluids, etc.; while at the same time, energy substances need the digestive system and urinary system to maintain the stability of the internal circulation and the internal environment, and the participation of other physiological systems is also needed.' (see Page 5, line 186-192)

References:

7. Sun X G. New theory of holistic integrative physiology and medicine. I: New

insight of mechanism of control and regulation of breathing. Chinese Journal of Applied Physiolog 2015;31:295-301+87.
8. Sun X G. New theory of holistic integrative physiology and medicine. II: New insight of the control and regulation of circulation. Chinese Journal of Applied Physiology 2015;31:302-7.
9. Sun X G. New theory of holistic integrative physiology and medicine. III: New insight of neurohumoral mechanism and pattern of control and regulation for core axe of respiration, circulation and metabolism. Chinese Journal of Applied Physiology 2015;31:308-15.
10. Sun X G. Establishing the idea of holistic integrative medicine, optimizing the quality of health care service in prevention and treatment. Chinese Journal of Applied Physiology 2015;31:289-94.

Comment 21: Likewise L52-55 'It reflects the….'.

Reply 21: Thank you for your valuable comment. We have made more profound and detailed additions to the relevant parts of HIPM and CPET.

Changes in the text: We've revised the sentence 'The CPET embodies the core concept of overall regulation, which is realized under the combination of respiration, blood circulation, nerves, metabolism and other systems, and all the peak indexes obtained have important clinical significance. The HIPM theory, articulated by Professor Xing-guo Sun, provides a theoretical framework for comprehending the physiological responses observed in human CPET.' (see Page 5, line 192-196)

Comment 22: L59: 'In recent years' makes it sound as if the two exercise modes stated are novel, which they are not.

Refer to 'lower body power bicycle' simply as 'cycle ergometer'. Key factor here to state is that 'Lower body exercise modes have traditionally been used to assess ……'.

L60: Then change wording to 'The latter has become the preferred choice'.

L68: Reword; 'This dynamic assessment under gradually increasing [exercise intensity]'.

Reply 22: Thank you very much for your professional suggestions. We added the key factor of "what Lower body exercise modes have traditionally been used to assess" as suggested. We have also carefully revised the wording of this section.

Changes in the text: We have deleted "In recent years" and shortened 'lower body power bicycle' to 'cycle ergometer'. (see Page 4, line 140)

We have changed the wording from 'The latter has increasingly become the preferred choice' to 'The latter has become the preferred choice'. (see Page 4, line 141)

We've added the key factors here 'Traditional CPET employs cycle ergometer as a

standardized assessment tool. Its core parameters, including peak oxygen uptake (peak $\dot{V}O_2$) and anaerobic threshold (AT), etc., are obtained based on the exercise patterns of large muscle groups in the lower limbs and have become the gold standard for evaluating cardiorespiratory fitness. Peak $\dot{V}O_2$ is recognized as the primary indicator of cardiorespiratory fitness, reflecting the body's maximum aerobic metabolism and cardiorespiratory reserve capacity; it serves as the gold standard for assessing an individual's exercise endurance. The AT is the critical point when the skeletal muscle energy supply mode shifts from aerobic metabolism to anaerobic metabolism to compensate for aerobic deficiency during incremental loading exercise, and individualized exercise prescription is often formulated based on the corresponding heart rate (HR), $\dot{V}O_2$, and METs at the time of the anaerobic threshold. The respiratory exchange ratio (RER) is a crucial parameter that reflects substrate metabolism and ventilatory compensation. It indicates the percentage of carbohydrate oxidation at sub-extreme intensities and is frequently utilized as a reference criterion for determining maximal effort during the exhaustion phase.' (see Page 4, line 144-156)

3. Lehtonen E, Gagnon D, Eklund D, et al. Hierarchical framework to improve individualized exercise prescription in adults: A critical review. BMJ open sport & exercise medicine 2022;8(2).

The 'load resistance' has been rewritten as 'exercise intensity' (see Page 5, line 185)

Comment 23: L71-72: What is stated regarding CPET seems standard exercise testing and rehabilitation considerations, I don't see how HIPM would change this? Similarly to the above, this paragraph would start better from L73.

Reply 23: Thank you for your valuable feedback. The HIPM does not make a change here and we have deleted it as suggested.

Changes in the text: We have deleted the sentence 'Based on the HIPM, the objective is to facilitate the healthy return of individuals with chronic diseases through individualized and precise upper and lower extremity exercise, while prioritizing safety.' (see Page 5, line 209)

Comment 24: L74: I would disagree that there is a paucity of information regarding arm crank ergometry protocols (in non-symptomatic, healthy participants). Protocol considerations have been examined since the 1980's (see work of Sawka et al's group) and more recently in the 2000s onwards in the development of exercise testing guidelines (e.g. Price and Campbell, Smith et al., Price et al. , Weissland and Marais, etc.).; e.g. continuous / discontinuous protocols, cadence / crank rate, mechanical efficiency, intensity increments, ramps rates. The authors would be correct though in

considering this work has not been as extensively covered in clinical groups where protocols are simply adjusted according to functional capacity – to which end to the authors cannot really extend any findings by using their chosen healthy population. Additionally, if the authors refer to clinical CPET, workloads such as 50W are massive in relation to what such a population would be able to perform at – such as load is generally reserved for upper body trained individuals / elite paddlers. As such this would not fit the rational relating to clinical groups.

Reply 24: We sincerely appreciate the valuable comments. We apologize for the misrepresentation of 'there remains a paucity of studies...' and have corrected it. We have read this literature carefully and added more references on arm ergometry protocols into the INTRODUCTION part in the revised manuscript. It is indeed true that CPET in clinical patient populations needs to be personalized according to the individual's functional status, which fundamentally differs from standard protocols used for healthy populations. The 50 W/min work rate increasing rate protocol employed in our study exceeds the functional capacity of most clinical patients, thereby complicating the direct extrapolation of our findings to clinical populations. However, our research was able to analyze the underlying physiological mechanisms and make an initial optimization of the work rate increasing rate protocol, and future studies will consider designing controlled trials that include clinical subgroups.

Changes in the text: We have modified our text as advised.

'Notably, existing studies have shown that test parameters such as crank rate type of incremental protocol and work rate increasing rate significantly modulate the sensitivity and specificity of CPET-evoked physiological responses. The work rate increasing rate acts as a temporal gradient controller for load intensity, directly influencing the timing of the AT trigger and the stability of the peak $\dot{V}O_2$. There have been more studies on leg CPET work rate increasing rate protocols, and research concerning arm ergometer work rate increasing rates remains limited. The arm CPET based on hand-cranked arm ergometer can accurately quantify the gas metabolic response and hemodynamic changes of the shoulder girdle muscles during exercise by establishing a standardized upper limb incremental work rate protocol.' (see Page 5, line 197-205)

References:

11. Smith P M, Doherty M, Price M J. The effect of crank rate strategy on peak aerobic power and peak physiological responses during arm crank ergometry. Journal of Sports Sciences 2007;25:711-18.
12. Smith P M, McCrindle E, Doherty M, et al. Influence of crank rate on the slow component of pulmonary O2 uptake during heavy arm-crank exercise. Applied

physiology, nutrition, and metabolism 2006;31:292-301.
13. Smith P M, Doherty M, Drake D, et al. The influence of step and ramp type protocols on the attainment of peak physiological responses during arm crank ergometry. International Journal of Sports Medicine 2004;25:616-621.

Modified limitations section 'First, the sample size of the current study was limited and predominantly comprised healthy individuals; therefore, future research should expand the sample to consider the inclusion of different age, gender, and disease groups. Second, the 20 W/min work rate increasing rate protocol demonstrated better overall performance in healthy individuals. However, the incremental gradients set in this study (5, 20, 35, and 50 W/min, with 15 W/min intervals between adjacent groups) may restrict the sensitivity required to accurately identify the optimal protocol. For instance, the difference from 5 W/min to 20 W/min is considerable (Δ15 W/min), whereas the physiological response of upper limb muscles to load variations may be more nuanced. Thus, it is necessary to implement finer incremental gradients (e.g., 5, 10, 15, and 20 W/min) within the 5-20 W/min range in future studies to elucidate the effects of different work rate increasing rates on key peak variables, Tlim, and other differential impacts, thereby enhancing the optimization of arm ergometer CPET protocols.' (see Page 17, line 1189-1200)

Comment 25: L77-80: Probably not that new information.

Reply 25: Thank you for your kind feedback. We have added information for authoritative reference to support our idea as suggested.

Changes in the text: We cited the earliest studies 'At the earliest Smith et al. compared two different ramp rates (6 and 12 W/min) during arm crank ergometry (ACE) to investigate the effect of ramp rate on the physiological response to peak physiological responses in subjects. They found that the difference in ramp rate significantly affected peak work rate (WR), carbon dioxide emission ($\dot{V}CO_2$), and RER, but did not influence $\dot{V}O_2$ and HR during ACE.' (see Page 5, line 205-209)

Reference:

14. Smith P M, Amaral I, Doherty M, et al. The influence of ramp rate on VO2peak and "excess" VO2 during arm crank ergometry. International Journal of Sports Medicine 2006;27:610-616.

Comment 26: L82-82: Need to note that a range of work rates have been examined for arm ergometry, but (likely) not compared in one study, that may be more novel.

Reply 26: We sincerely thank you for your insightful and constructive comment regarding the manuscript. We have revised the purpose of the study in the introduction

section and, as suggested, pointed out the novelty of arm ergometry with a range of work rates being compared in one study.

Changes in the text:

We've rewritten the content 'While current arm CPET research has explored arm ergometer protocols with different work rate increasing rates, these protocols have been limited to two-by-two comparisons of limited combinations of incremental rates, and systematic comparisons of cross-gradient protocols have not yet been achieved in a unified experimental framework (14,15).' (see Page 5, line 209-236)

References:

14. Smith P M, Amaral I, Doherty M, et al. The influence of ramp rate on VO2peak and "excess" VO2 during arm crank ergometry. International Journal of Sports Medicine 2006;27:610-616.
15. Hutchinson M J, Paulson T A W, Eston R, et al. Assessment of peak oxygen uptake during handcycling: Test-retest reliability and comparison of a ramp-incremented and perceptually-regulated exercise test. PLOS One 2017;12:e0181008.

Comment 27: L84-85: I am not sure what is meant by 'obtain more objective assessment data'. Do the authors mean additional variables to the standard maximal / peak responses – that are usually tabulated in previous studies, rather than acting as a research aim per se.

I feel that the novelty of this study is that it encompasses the standard ramp rates / increment intensities that have been used in previous studies but not necessarily all compared in one data set. In my opinion, the research aim should reflect this.

Reply 27: Thank you very much for your professional suggestions. We have removed the ambiguous expression and modified the research aim to reflect their novelty.

Changes in the text: We have changed the content to 'In this study, we achieve a direct comparison of multi-gradient loading protocols for the first time in a uniform subject population by innovatively integrating four incremental rate datasets: slow (5 W/min), medium (20 W/min), fast (35 W/min), and high (50 W/min). To this end, the study includes 14 healthy participants who first complete a traditional leg CPET, followed by sequential arm ergometer tests at rates of 5, 20, 35, and 50 W/min. We observe both similarities and differences in the peak exercise-related indices of arm CPET across different work rate increasing rates, analyzing their effects on the physiological responses of upper limb exercise in healthy participants.' (see Page 6, line 236-243,)

'Furthermore, we will optimize protocol selective strategies to provide high-confidence evidence for determining the optimal intensity-time gradient for upper limb exercise

testing. Additionally, since arm exercise predicts clinical outcomes , the core indicators of arm and leg CPET were also compared between the 14 participants in this study to explore differences in metabolic mechanisms between limb movements, which offers potential benefits for the precise guidance of exercise rehabilitation.' (see Page 6, line 252-256)

Reference:

16. Ilias N A, Xian H, Inman C, et al. Arm exercise testing predicts clinical outcome. American Heart Journal 2009;157:69-76.

Comment 28: L87: The term normal subject should be changed to health participants / non-trained / sedentary but otherwise healthy etc., as 'normal' begs the question as to what is 'normal' [average, with 1 SD of 'average'?], and the term 'subjects' has been surpassed with 'participants' over the last ten years or so in most journals, i.e. volunteers participate of their own free will rather than a being subjected to testing.

Reply 28: Thank you for your professional suggestions. The word "normal," which was used in the text to refer to a healthy individual free of clinical illness, has been identified as problematic and changed. The word "subjects" has also been changed.

Changes in the text: We have corrected the word "normal subject" to "health participants" in the text. (see Page 6, line 239)

Comment 29: L88: States 'The foundation for a safe and effective arm ergometer CPET relies on establishing appropriate and precise incremental work rates' – I would delete this as CPET safety is not just the protocol, validity of data maybe – and that is the importance of the current study.

Reply 29: Thank you for your professional suggestions. We agree that the safety of CPET is not only dependent on the testing protocol, but also on a number of aspects such as the state of health of the participants, the monitoring process, and the validity of the data. We have removed this inappropriate expression as suggested.

Changes in the text: We've deleted 'The foundation for a safe and effective arm ergometer CPET relies on establishing appropriate and precise incremental work rates.'

Comment 30: L89: States 'The findings from this investigation are expected to contribute to the development of tailored training protocols and rehabilitation programs, enhancing patient care and outcomes in clinical practice' but, in line with the previous sentence, most of the protocols proffered in this study would not be appropriate for clinical groups. It would be more effective to have introduced the fundamental physiology of increment intensity, that as intensity increases, participants would likely

reach their exercise capacity in a shorter time, but at a greater power output. Shorter tests though limit the ability for submaximal measures to be obtained such as lactate threshold (or ventilatory threshold if more clinical groups are of interest) etc.

Reply 30: Thank you for your kind feedback. We have removed statements about clinical patients that were not consistent with this study population as suggested.

Changes in the text: We've deleted 'The findings from this investigation are expected to contribute to the development of tailored training protocols and rehabilitation programs, enhancing patient care and outcomes in clinical practice.'

Comment 31: L91: I am not sure of specific journal guidelines or preferences, but would the STROBE statement be better placed in the methods?

Reply 31: Given the guidelines and preferences of this journal and the layout of previous articles, the STROBE statement is placed at the end of the introduction, so we will not change it here, and thank you for the reminder.

Changes in the text: None.

Methods

Comment 32: L94: Change 'subjects' to 'Participants'.

Reply 32: Thank you for your professional suggestions.

Changes in the text: We have changed 'Subjects' to 'Participants' as advised. (see Page 6, line 259)

Comment 33: L95: 'From August to November 2023' is not needed.

Reply 33: Thank you for your professional suggestions.

Changes in the text: We have deleted 'From August to November 2023' as advised. We have changed to 'A total of 14 healthy participants (5 males and 9 females) who were working at xxx Hospital and did not have diagnosed illness were recruited for the study in 2023.' (see Page 6, line 260-261)

Comment 34: L97: Change 'weight' to 'mass'.

Having n=9 female participants is a novelty as most arm data sets are from male populations. This may be useful for revising the novelty of the study.

Reply 34: Thank you for your professional suggestions and constructive guidance on the manuscript. We have changed 'weight' to 'mass' as advised. Indeed, we have also noted this situation and plan to explore the effect of gender on arm CPET in the future after further expanding the sample size of the female population, so we do not analyze the gender factor in this article for the time being and add explanations in the limitations

section.

Changes in the text:

We have changed 'weight' to 'mass' as advised. (see Page 6, line 262)

'First, the sample size of the current study was limited and consisted primarily of a mixed population of healthy men and women; therefore, future research should expand the sample to consider the inclusion of different age, gender, and disease groups.' (see Page 17, line 1192-1195)

Comment 35: L103: The authors state the equipment was calibrated daily, but was it calibrated prior to each test? If not was the calibration checked before each test to ensure there was no drift in calibration?

Reply 35: Thank you very much for your valuable comments. The equipment is calibrated before each daily test, and calibrating it once a day is sufficient to ensure that multiple tests conducted on the same day will proceed normally. Before each test, we checked the calibration to ensure that there has been no drift before conducting the test. We have added to this point in the text.

Changes in the text: We have rewritten the sentences 'Before each test, the equipment underwent comprehensive calibration procedures, including gas volume calibration, high, medium, and low flow rate calibration, as well as air, oxygen, and carbon dioxide gas calibration.' (see Page 7, line 288)

Comment 36: L104: What does the 'a metabolic simulator calibration' refer to? Using calibration gasses and gas flow rates that are physiological in nature rather than set calibration values?

Similarly, what does the integrity of the system refer – this would seem more structural or mechanical in nature.

Reply 36: Thank you for your valuable comments. The metabolic simulator uses a nitrogen balance gas containing CO_2 at a precision of up to 0.01% with a concentration of approximately 20.93% as the titration standard gas, and indoor air (with 20.93% O_2 and 0.04% CO_2 under good ventilation conditions at sea level and 1 atmosphere of pressure) as the reference gas. By using a cylindrical piston pump driven by a high-precision motor, a precise amount of air is generated at a predetermined operating rate (breathing frequency, BF) and amplitude, which is then injected into the CPET system a known quantity of CO_2 and N_2 flowing through a precision flowmeter. The tidal volume produced by the simulator is adjusted by adjusting the piston operation rate and operation amplitude to simulate the respiratory ventilation state of the human body at different metabolic levels, in order to replace the human body's input to the CPET setup

for gas exchange at rest, exercise, and extreme exercise. The $\dot{V}O_2$ and $\dot{V}CO_2$ measured by the device at this point were compared to the actual $\dot{V}O_2$ and $\dot{V}CO_2$ produced by the metabolic simulator to calculate the CPET system measurement error.

The integrity of the system was intended to convey a complete calibration process for the device, but due to the vagueness of this wording we have removed it.

Changes in the text: We rewrote the sentences 'Additionally, the metabolic simulator's low, medium, and high metabolic rates were utilized daily to validate the CPET for measuring errors in $\dot{V}O_2$ and $\dot{V}CO_2$. Validation was deemed successful when the absolute values of all three titers—low, medium, and high—were ≥10%, thereby enhancing and sustaining data accuracy and reliability within the cardiopulmonary exercise laboratory.' (see Page 7, line 292-296)

Comment 37: L104: What O2 and CO2 gas fraction (or percentages) were used in calibration?

Reply 37: Thank you for your valuable comments. The calibration of gas concentration is performed using a two-point standard gas method, which involves a reference gas and a calibration gas. The reference gas consists of a nitrogen equilibrium gas containing 0.00% CO_2 and 21.00% O_2, while the calibration gas comprises a nitrogen equilibrium gas with 5.00% CO_2 and an O_2 concentration ranging from 10.00% to 15.00%.

Changes in the text: We added the sentences 'The calibration of gas concentration is performed using a two-point standard gas method, which involves a reference gas (0.00% CO_2 and 21.00% O_2) and a calibration gas (5% CO_2 and 10% ~ 15% O_2).' (see Page 7, line 290-292)

Comment 38: L109: What did the resting pulmonary data involve – static and dynamic lung volumes? If data is not reported here it is acceptable to state 'data is not reported here as a non-patient population was examined'.

Reply 38: Thank you for your valuable comments. The resting pulmonary data involved lung volumes, lung ventilation function and diffusing capacity of the lungs for carbon monoxide. Indicators include forced vital capacity (FVC), first second forced expiratory volume (FEV1), slow vital capacity (SVC), maximal voluntary ventilation (MVV), etc.

Changes in the text: We added the sentences 'The resting pulmonary data involved lung volumes, lung ventilation function and diffusing capacity of the lungs for carbon monoxide. Indicators include forced vital capacity (FVC), first second forced expiratory volume (FEV1), slow vital capacity (SVC), maximal voluntary ventilation

(MVV), etc.' (see Page 7, line 301-305)

Comment 39: L110-111: Wording: Wearing the mouthpiece throughout the test does not prevent leakage (if you are referring to an actual mouthpiece inserted into the mouth rather than a face mask, there is the possibility leaks through the mouth). Wearing a face mask continuously does, however, help this.

Reply 39: Thank you for your professional suggestions. We have changed the wording of this sentence as advised.

Changes in the text: We have changed the wording to 'Participants were fitted with a mouthpiece and a nose clip (or a mask, as an alternative) to ensure no air leaks' (see Page 7, line 305-306)

Comment 40: L112: What did the functional evaluation provide for the cycle ergometry test other than for reference to peak / maximal values from a traditional cycle ergometry test?

Reply 40: Thank you for your professional comments. In addition to the reference peak/maximum value, the sub-extreme exercise indicators offered by the cycle ergometry test, such as AT, OUEP, Lowest $\dot{V}E/\dot{V}CO_2$, and $\dot{V}E/\dot{V}CO_2$ Slope, can also effectively evaluate the functional status of the subjects.

Changes in the text: None.

Comment 41: L116: The sentence 'Max test (13) confirms whether CPET qualifies as an extreme exercise' does not make sense, in addition, the supporting reference title (ref 13) does not make sense. Are the authors referring to whether a maximal effort was achieved? If so, this is usually considered in terms of heart rate, respiratory exchange ratio, blood lactate, ratings of perceived exertion and volitional exhaustion. See also line where it is states symptom limited – as healthy individuals were tested the exercise capacity limitations would not be symptomatic per se.

Reply 41: Thank you for your professional suggestions. We have been deeply aware of the multifaceted aspects of considering whether volunteers are doing their best to complete the ultimate CPET, and have rewritten this section.

The Max test is conducted at a constant intensity of 130% of an individual's peak WR. Under this regimen, the VO_2 should theoretically approximate or slightly exceed the peak VO_2 observed during the incremental CPET, provided the subject exerts maximal effort and encounters no performance issues. The calculated and analyzed values are as follows: Difference = Max - Peak; Percentage difference = (Max - Peak) / Max × 100%. Results Evaluation: (1) A percentage difference in HR and VO_2 of ≤ -

10% indicates that the Max value is lower than the Peak value, thereby classifying the Max test as a failure; otherwise, it is deemed a success. (2) If the percentage differences in HR and VO_2 fall within the range of -10% to 10%, the Max test is considered successful, confirming that the CPET data reflects the exercise limit and that the Peak value accurately assesses the patient's capacity. (3) A percentage difference of ≥ 10% indicates a successful Max test, suggesting that the CPET data is not representative of extreme exercise and that the subject did not exert maximal effort.

Changes in the text: We have rewritten the sentence as 'Indicators such as peak HR, peak RER, and subjective fatigue and exhaustion as assessed by the Borg Rating Scale were integrated with the Max test (19) to ascertain that the participant had performed the CPET to the best of their ability.' (see Page 7, line 313-316)

Also, we have deleted the word "symptom limited".

Reference:

19. Zhang Y, Sun X G, Liu F, et al. Max test verify further clinical research for whether individualized symptom-limited cardiopulmonary exercise testing is the maximum extreme exercise. Chinese Journal of Applied Physiology 2021;37:147-53.

Comment 42: L117: Stating 'many kinds of data were continuously monitored' is vague (delete the word 'including' as this implies an incomplete list). Just state exactly what was recorded. i.e. 12-lead ECG, oxygen saturation (SaO2), non-invasive cuff blood pressure measurements and [various – again sounds vague, state them specifically] gas exchange parameters.

Likewise, state '(note: blood pressure and SaO2 were not measured in the arm ergometer CPET),' after the list of variables noted and state why. NB: SaO2 is usually measured at the ear for arm work.

Reply 42: Thank you for your professional suggestions. We have modified the vague statement to accurately state only what was recorded, as suggested. And added the reason for not measuring blood pressure and SaO_2.

Changes in the text:

We have changed the wording to 'Throughout the CPET procedure, many kinds of data were continuously monitored and recorded, i.e. 12-lead ECG, oxygen saturation (SaO_2), non-invasive cuff blood pressure measurements, and gas exchange parameters. Blood pressure and SaO_2 were not measured in the arm ergometer CPET due to interference.' (see Page 7, line 316-333)

Comment 43: L122-123: No need to re-state the standard procedure used (these have

been stated earlier so just repeats information). Start section at 'The raw data were ……'.

Reply 43: Thank you for your professional suggestions. We have deleted the standard procedure used as advised.

Changes in the text: We have deleted the sentence 'According to the standardized principles of FDA clinical trial CPET data analysis and interpretation at Harbor-UCLA Medical Center' (See Page 8, line 335)

Comment 44: L134: No need to state 'to produce a new 9 plots and data analysis (14, 15)' unless the plots are new to CPET analysis and specifically explaining your data – more importantly your research question. Therefore, what did the VO2/VCO2 plot provide? Only cite what needs to be explained - and if important state it in the methods. For the data present in this manuscript there are only standard variables reported.

Reply 44: Thank you for your professional suggestions. We have revised the methods section to retain only those methodological statements that contribute new insights to the research question. The creation of the new 9 plots is an essential step for the standardized analysis of data following the collection of CPET data. The 9 plots effectively visualize the dynamic processes of each indicator, facilitating the analysis of similarities and differences in CPET data concerning the arm ergometer across varying work rate increasing rates. By segmenting the movement into brief 10-second intervals, we were able to analyze the collected data more effectively. The $\dot{V}O_2/\dot{V}CO_2$ plot provides information on the collection of $\dot{V}O_2$ and $\dot{V}CO_2$ to facilitate the selection of AT points.

Changes in the text: None.

Comment 45: L128: State what the key variables were with units.

Reply 45: Thank you for your valuable comments. This part refers to how the data of each indicator is obtained in different time periods, and does not involve unit issues.

Changes in the text: None.

Comment 46: L130-131: Reword sentence 'All graphs and data processing was undertaken using Sigma Plot professional mapping software (version xxx)'.

How was peak power determined? Was this the final incremental stage reached or (more correctly) the final 'peak minute power' (i.e. where the proportion of each of the last and previous stage are taken into account (e.g. if last stage reached was 100W but for only 20 sec and the previous stage was 50W, peak minute power would be the resultant of ((100/60)*20))+((50/60)*40))/60 = (33) + (33) = 67W (rounded up)

What were the warm-up and initial power outputs exercised at?

What was the cadence implemented (e.g. 70 rev.min-1)?

Reply 46: Thank you for your professional suggestions.

We have rewritten the sentence as suggested and noted that the software version used was version 14.

In our test, the motion stage utilized a continuous incremental ramp protocol, in which the increases almost linearly per second (non-stepped segmentation). Taking 50W/min as an example, let us assume that the test is terminated after 2 minutes (120 seconds). Given the linear increase in power, the average power over the last 30 seconds can be calculated as the mean of the start and end power: (75W + 100W)/2 = 87.5W. Thus, the peak power recorded is 87.5W, rather than 100W at the conclusion of the test. This approach mitigates bias introduced by short sprints or transient fatigue, thereby more accurately reflecting the true sustained output capacity.

During the warm-up phase the legs begin to move and overcome the resistance of the cycle ergometer's flywheel equivalent to a 20W load, while the upper body warm-up phase equates to a 10W load.

The implemented cadence was 60 rpm.

Changes in the text:

We have added content 'followed by a 3-minute no-load warm-up period' and 'The CPET cadence was maintained at approximately 60 rpm.' (See Page 7, line 309-311)

'The 3-minute warm-up phase was set at 0W resistance (60 rpm), generating baseline power outputs of 10W (arm ergometer) and 20W (cycle ergometer) through system inertia and limb movement.' (See Page 7, line 311-313)

'All graphs and data processing were undertaken using Sigma Plot professional mapping software (version 14).' (See Page 8, line 342-343)

Comment 47: L135: Is the 'oxygen uptake ventilation efficiency platform' the slope of the VO2 power output slope? This has not been defined in the method.

Reply 47: Thank you for your valuable comments. OUEP expresses the highest platform value of oxygen (ml) that can be taken up by the circulatory system per liter of pulmonary ventilation during exercise, reflecting the matching status of pulmonary blood flow/ventilation. We have added the OUEP definition in the method as advised.

Changes in the text:

We add to the definition of observation indicator terms in our methodology: 'The peak oxygen uptake ventilation efficiency platform (OUEP) is a reliable indicator of oxygen uptake efficiency, The minimum value of carbon dioxide emission ventilation efficiency (Lowest $\dot{V}$ E/ $\dot{V}$ CO_2)and slope of ventilatory equivalent for carbon

dioxide($\dot{V}E/\dot{V}CO_2$ Slope) are excellent indicator for assessing the effectiveness of ventilation, The oxygen uptake per unit work rate ($\Delta\dot{V}O_2/\Delta WR$) indicates the oxygen delivery capacity of peripheral exercising muscles.' (See Page 8, line 351-356)

Comment 48: L139: Delete 'in the country'. Predictive equations have been developed. Otherwise, values need to be referred to previous literature for 'interpretation and comparison' (a recent review may help here comparing arm crank and cycle ergometry values in relation to age and sex; Price, M. J., Smith, P. M., Bottoms, L. M., & Hill, M. W. (2024). The effect of age and sex on peak oxygen uptake during upper and lower body exercise: A systematic review. Experimental gerontology, 190, 112427. https://doi.org/10.1016/j.exger.2024.112427).
What was the purpose of the cycle ergometer test – for comparison to norm values?
Reply 48: Thank you for your professional suggestions. We have carefully read the literature and deleted that as advised. The purpose of the cycle ergometer test is to show that the overall cardiorespiratory fitness of the participants meets normal standards through various indicators. Also, comparisons with arm and leg CPET data were made to assess the feasibility and safety of arm ergometers in CPET.
Changes in the text: I have deleted the reference to "in the country" in the text as suggested. (See Page 8, line 359)

Comment 49: L145; 'Parameter' refers to values that don't change or will differ between conditions (e.g. height), 'variable' refers to those that do (i.e. your CPET measures).
Reply 49: Thank you for your valuable guidance. We have learned the difference between 'Parameter' and 'variable' and corrected it in the text.
Changes in the text: The word "parameter" that appears in the text to indicate a CPET variable has been changed.

Comment 50: L146: Replace 'groups' with 'tests' as 'groups' suggests different populations.
Reply 50: Thank you for your professional suggestions.
Changes in the text: We have replaced 'groups' with 'tests' as advised (see Page 8, line 360)

Comment 51: L147: What are the two – by two comparisons – the t-tests referred to in the abstract? Consistent terminology is key.
Reply 51: Thank you for your professional suggestions. We have changed the

terminology to be consistent with the abstract as suggested.

Changes in the text:

We've rewritten sentence 'One-way ANOVA with Tukey's post hoc test were performed to compare each variable among the four groups with different work rate increasing rates. A paired t-test assessed arm vs. leg differences.' (see Page 8, line 359-495)

Comment 52: L147-148: For the 'relationships between arm ergometer CPET key parameters and incremental work rate' what was correlated? Was it the variable and its associated power output? Please state clearly.

Reply 52: Thank you for your valuable comments. Correlation refers to the linear regression analysis between the variables and incremental work rate, which we have modified to be more explicit as suggested.

Changes in the text: We rewrote the sentences 'the linear regression analysis between arm ergometer CPET key variables and incremental work rate of arm ergometer CPET were analyzed via Pearson's correlation coefficient.' (see Page 9, line 495-497)

Results

Comment 53: L152-154: Participant information should be presented in the methods section.

Reply 53: Thank you for your professional suggestions. We have added the participant information in the methods section as advised.

Changes in the text: We added the sentence 'Basic information of the participants was recorded (age 34.36±7.65 years, body mass 65.93± 11.03 kg, height 1.67±0.06 cm and BMI 23.66±3.20 kg/m^2).' (see Page 6, line 262-263)

Comment 54: L156: Grammar, replace 'all of them' with 'all participants completed all trials' or something similar.

Reply 54: Thank you for your professional suggestions. We have changed the grammar of this sentence as advised.

Changes in the text: We revised the sentence to 'All participants completed all trials, and the maximal leg ergometer CPET results indicated that their physiological function was essentially normal, with a peak $\dot{V}O_2$ of 1.96 ± 0.43 L/min, 30.22 ± 6.06 ml/(min· kg), and 92.89 ± 18.37% of the predicted value, as well as an AT of 1.01 ± 0.12 L/min, 15.82 ± 2.88 ml/(min· kg), and 88.86 ± 18.83% of the predicted value.' (see Page 9, line 500-503)

Comment 55: L156-159: Text can be much more concise by simply referring to Table 2 and that the data was as expected for the population.

Reply 55: Thank you for your valuable suggestions. This sentence does need to be simplified to make the text more concise, and we have modified it as suggested.

Changes in the text: We revised the sentence to 'As shown in **Table 2**, the data was consistent with our expectations for this population.' (see Page 9, line 503-504)

Comment 56: L160: Section heading can be simpler e.g. 'Comparison of peak physiological responses for each work rate protocols'

Reply 56: Thank you for your valuable suggestions. We have changed the section heading to be simpler as suggested.

Changes in the text: The section heading has been changed to 'Comparison of peak physiological responses for each work rate protocols' (see Page 9, line 505)

Comment 57: L163: Delete sentence a sit is not required.

Reply 57: Thank you for your valuable suggestions. We have deleted that as suggested.

Changes in the text: We have deleted 'WR was zero during the resting stage, while $\dot{V}O_2$, $\dot{V}CO_2$, and RER all changed smoothly.' (see Page 9, line 508)

Comment 58: L164: States the WR was zero, this is not technically correct as the participants are overcoming the resistance of the ergometer flywheel (or equivalent). Better the state the HR and VO2 etc. increased during the warm-up, but state in the methods that the warm-up was undertaken on the unloaded ergometer.

Reply 58: Thank you for your valuable guidance. There is error regarding our notation of WR being zero, as you pointed out. We recognize that participants must overcome the resistance of the ergometer flywheel during the warm-up phase. We have rewritten this section and stated it in the Methods section.

Changes in the text:

Methods section 'The 3-minute warm-up phase was set at 0W resistance (60 rpm), generating baseline power outputs of 10W (arm ergometer) and 20W (cycle ergometer) through system inertia and limb movement.' (See Page 7, line 311-313)

Comment 59: L164-165: Reword, as 'the arm started to spend some energy while exercising' is not scientifically written – would be discussion anyway, avoid explaining any results in the results section, just report what happened.

Reply 59: Thank you for your valuable guidance. We have realized that the results section only needs to report what happened and should not explain the results, so we

have rewritten this section as suggested.

Changes in the text:

We only report '$\dot{V}O_2$ and $\dot{V}CO_2$ rose during the warm-up phase, but the RER barely changed.' (See Page 9, line 508-509)

Comment 60: L165-166: Again, this is already stated in the methods and has no part in the results.

Reply 60: Thank you for your valuable guidance. We have deleted this duplication with the method section.

Changes in the text: We have deleted the phrase 'At 6 minutes, exercise was done at incremental work rates of 5, 20, 35, and 50 W/min.' (see Page 9, line 509)

Comment 61: L165: 'at 60 r/min' is methods information. Why was 70 rev.min-1 or above not used?

Reply 61: Thank you for your valuable comment. We selected a rotation speed of 60 rpm, in accordance with the standards established by the Harbor-UCLA Center Cardiopulmonary Exercise Laboratory in the United States for CPET. Previous research, along with the practical experiences observed in clinical CPET assessments at hospitals, indicates that the desired physiological response can be effectively observed at this rotation speed.

Reference:

20. Sun X G. Standardizing clinical performance, data analysis, graphics display, interpretation and report for cardiopulmonary exercise testing. Chinese Journal of Applied Physiology 2015;3:361-5.

Changes in the text: We have added the information "at 60 r/min" to the method and deleted it here. (See Page 7, line 311)

Comment 62: L166-167: Report if a difference or otherwise existed, not just a lose description.

Reply 62: Thank you for your valuable guidance. We reported the between-group differences in key metrics in detail in result 3.2.2, and only described the dynamic change process of variables here.

Changes in the text: We modified the dynamic change process 'The data of the third healthy male participant is provided as an example to demonstrate the dynamic change process (see **Figure 1**). $\dot{V}O_2$ and $\dot{V}CO_2$ rose during the warm-up phase, but the RER barely changed. During this time, there was an increase in $\dot{V}O_2$ and $\dot{V}CO_2$, a slightly reduced RER, and a continuous rise in RER after the AT. Peak WR, $\dot{V}O_2$, and $\dot{V}CO_2$

achieved their maximums when the peak movement was reached, but peak RER did not. WR was at zero when the recovery stage began, and both $\dot{V}O2$ and $\dot{V}CO_2$ decreased. In contrast, the RER showed a rapid increase, reaching its maximum in approximately two minutes before gradually decreasing.' (see Page 9, line 507-513)

Comment 63: L166-169: From 'During this time, there was an increase' just state 'On the initiation of each protocol there was an increase in HR, VO2, RER ….. [as appropriate] up to volitional exhaustion'. Here you should report the main effect for time (as appropriate) before noting any differences in maximal responses.
Although it is interesting physiologically, be sure to explain (in the discussion) why you have reported RER during recovery (add the reason to the method first) – other than reflecting the buffering of hydrogen ions from the associated lactic acidosis, what does it tell us and how can it be used?
This paragraph has not presented any statistical results in relation to the four protocols (and thus the research question). Please add this if it occurred. L171 is the first mention of a specific protocol.
Reply 63: Thank you for your valuable suggestions. We have adjusted this section in the revision to ensure that the main effects of time are highlighted first, and then the differences in maximum response are discussed later. This paragraph has also been supplemented with the changes of the key variables under the influence of the four work rate increasing rates.
Changes in the text:
We have changed the content 'Tlim decreases significantly as the work rate increasing rate increases. In the 5 W/min protocol, $\dot{V}O_2$ and $\dot{V}CO_2$ increases slowly, the peak occurs at a later point in time. The RER value is more stable without significant fluctuations. $\dot{V}O_2$ increases rapidly and peaks in the shortest Tlim. $\dot{V}CO_2$ reaches its maximum peak swiftly, accompanied by a sharp rise in RER, which subsequently declines rapidly back to baseline during the recovery phase.' (See Page 9, line 513-517)

Comment 64: L172: What is a 'suitable time frame'? If there are preferred exercise duration (typically 9-16 min or so for a max test, shorter for 'jump-max tests') please give a rationale in the introduction – your ramp rates will have a massive affect such data and should be considered as part of the rationale for the study.
Reply 64: Thank you for your valuable comments. The duration of exercise at this rate is slightly shorter than the guidelines set forth by the American Thoracic Society/ American College of Chest Physicians, recommending 8~12 min as the ideal duration for incline exercise protocols, which is based on data generated from exercise tests

performed in a treadmill and bicycle. Whereas arm ergometer CPET peak $\dot{V}O_2$ is approximately 70% of leg CPET, we speculated that the appropriate arm CPET exercise duration would be approximately 4-8 min. We have removed the ambiguity of "suitable time frame" and described the results with clear data.

Reference:

1. ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med 2003;167:211-77.
2. Buchfuhrer M J, Hansen J E, Robinson T E, et al. Optimizing the exercise protocol for cardiopulmonary assessment. Journal of applied physiology 1983;55:1558-64.

Changes in the text:

We have changed the content 'The group with a work rate increasing rate of 20 W/min was able to complete the work rate increase stage in approximately 5 min, while the lower and higher work rate increasing rate groups lengthened and decreased the Tlim, respectively.' (see Page 9, line 517-520)

Comment 65: L180: What does the 'Lowest *V* E/*V* CO2' etc. refer to? Please clarify. Headings for sections 3.3.1, 3.3.2, 3.3.3 essentially separate out the significant and non-significant findings, but the headings do not help the flow of information. Better to separate out into submaximal and maximal responses.

Reply 65: Thanks for kindly reminding us to clarify this variable. Lowest $\dot{V}E/\dot{V}CO_2$, which is the smallest 30-s average of carbon dioxide ventilation equivalents measured during CPET, provides a better assessment of a subject's ventilatory effectiveness than $\dot{V}E/\dot{V}CO_2$ Slope. Definitions have been clarified in the Methods section. I have reorganized this section by dividing the results into two categories, "submaximal responses" and "maximal responses", to present the differences in importance and responses more clearly.

Changes in the text:

Methodology section 2.5 'The minimum value of carbon dioxide emission ventilation efficiency (Lowest $\dot{V}E/\dot{V}CO_2$) and slope of ventilatory equivalent for carbon dioxide ($\dot{V}E/\dot{V}CO_2$ Slope) are excellent indicator for assessing the effectiveness of ventilation.' (see Page 8, line 347-349)

We have categorized the Results 3.2 section sub-headings into 3.2.1 submaximal responses and 3.2.2 maximal responses. (see Page 9, line 506,521)

Comment 66: L184-185: Watts are whole numbers (and heart rates, see text and tables throughout).

Reply 66: Thank you for your valuable suggestions. We completely concur with your

reference to watts and heart rate as integer values. In my complete article and the accompanying tables, we have treated the measurements of watts and heart rate as integer values to ensure accuracy and consistency of the data.

Changes in the text: The heart rates and watts values in the tables and complete text have been changed to whole numbers.

Comment 67: L184-185: Convention is to state the results but with an indication of which trial each mean is associated with i.e. '…… (58 ±11, 80 ±18, 95 ±22 and 110 ±22W, for the 5, 20, 35 and 50W.min-1 protocols, respectively, P<0.001).'. However, all data is presented in Table 3 so there is no real need to replicate the values.

Reply 67: Thank you for your valuable suggestions. We will remove duplicates and ask readers to see Table 3 for details.

Changes in the text: We have modified the results section and no longer replicate specific values.

'There was a significant difference in peak WR among the four protocols of the arm ergometer CPET (P < 0.001). The 50 W/min protocol demonstrated the highest peak WR, followed by the 35 W/min and 20 W/min protocols, while the 5 W/min protocol exhibited the lowest peak WR. All comparison between work rate increasing rate protocol were significantly different (P = 0.001 ~0.048).' (See Page 10, line 683-686)

Comment 68: L184-185: This data would be better shown as a block graph (also a key finding) with post-hoc comparisons indicated. Reword 'also significant differences for all six paired comparisons' as 'all comparison between ramp rate protocol were significantly different (P<……'. The six comparisons have not been stated in the methods. This comment is the same for L193-194 for exercise time and protocol.

Reply 68: Thank you for your valuable suggestions. We visualized these key variables using error bar plots (**Figure 2**) instead of block graph, which also clearly demonstrated stage-specific variations in key variables across protocols and their statistically significant differences.

Changes in the text:

We have changed the content 'There was a significant difference in peak WR among the four protocols of the arm ergometer CPET (P < 0.001). The 50 W/min protocol demonstrated the highest peak WR, followed by the 35 W/min and 20 W/min protocols, while the 5 W/min protocol exhibited the lowest peak WR. All comparison between work rate increasing rate protocol were significantly different (P = 0.001 ~0.048).' (See Page 10, line 683-686)

For details on L193-194 movement times and protocols, see Reply 71

Comment 69: L186-187: Delete the sentence 'The peak WR was significantly positively correlated with incremental work rate (R2 187 =0.985)' as this is better indicated with ANOVA results.

Reply 69: Thank you for your valuable suggestions. While we agree that ANOVA effectively highlights group-level differences in peak workload (WR) across protocols, we propose retaining the correlation analysis between peak WR and incremental work rate. First, the correlation (R^2 = 0.985) specifically quantifies the strength of the linear relationship between incremental work rate and peak WR within each protocol. Second, including both analyses provides readers with a complete understanding of how protocols mechanistically influence outcomes, beyond group comparisons.

Changes in the text: We have deleted that here and added sentences 'In arm ergometer CPET, peak WR, peak RER, and maximal RER during recovery were positively correlated with work rate increasing rate (R^2=0.985, 0.823, 0.939, respectively), while Tlim was negatively correlated with it (R^2=0.383). (**Figure 3**).' (see Page 10, line 695-697)

Comment 70: L189-190: Delete 'which showing an upward trend positively depend on incremental work rate' has to have correctly given the specific pairwise comparisons in the subsequent sentence (i.e. L190-193).

Reply 70: Thank you for your valuable suggestions. We propose retaining the correlation analysis.

Changes in the text: We have deleted that here and given the specific pairwise comparisons in the subsequent sentence. (see Page 10, line 686-691)

We added sentences 'In arm ergometer CPET, peak WR, peak RER, and maximal RER during recovery were positively correlated with work rate increasing rate (R^2=0.985, 0.823, 0.939, respectively), while Tlim was negatively correlated with it (R^2=0.383). (**Figure 3**).' (see Page 10, line 695-697)

Comment 71: L193-194 for exercise time and protocol

Table 1: Please provide means for male and female as well as the whole group (Table can show male and female data grouped together rather than by participant number which essentially is not required - unless specifically referring to them in any subsequent text).

Table 2: Differentiate between bpm for heart rate (beats per min) and breathing frequency (breathes per min).

Reply 71: Thank you very much for your excellent guidance and valuable suggestions.

We have recreated **Table 1** as suggested, which groups men and women according to gender and provides means for both men and women and for the group as a whole. In the **Table 2** we have made a distinction between the units of heart rate and respiratory rate (bpm).

Changes in the text:

We have modified that 'Tlim for the four tested protocols were significantly different ($P < 0.001$). The high incremental rate (50 W/min) protocol resulted in the shortest Tlim, whereas the low incremental rate (5 W/min) protocol led to the longest Tlim. However, no statistically significant difference in Tlim was observed between the 35 W/min and 50 W/min test protocols ($P = 0.291$). (**Tables 3 and 4, Figure 2**).' (See Page 10, line 691-695)

Table 1. General information of normal participants

	Male (n=5)	Female (n=9)	Total (n=14)	p
Age (years)	33±8.4	35.11±7.62	34.36±7.65	0.636
Weight (kg)	75.6±6.8*	60.56±9.14	65.93±11.03	0.013
Height (m)	1.73±0.05**	1.63±0.04	1.67±0.06	0.007
BMI (kg/m^2)	25.39±2.96	21.26±3.06	23.66±3.2	0.076

BMI: body mass index; $p < 0.05$, ** $p < 0.01$ compared to female.

Table 1(see Page 22, line 1413)

Peak HR is in beats/min and Peak Bf is in br/min. (see Page 23, line 1500)

Comment 72: L524: Respiratory exchange 'ratio' not 'rate'.

Reply 72: We were really sorry for our careless mistakes. Thank you for your reminder.

Changes in the text: We have changed 'rate' to 'ratio'. (see Page 28, line 1649)

Comment 73: L524-525: States 'CPET at different incremental work rates in normal subjects. The third subject was used as an example…..', reword to 'CPET at different incremental work rates in participant 3 as a typical example' as figure is for one example participant not the whole group.

Reply 73: Thank you for your valuable comments. Your suggestions are very valid and I have revised the text accordingly to ensure clarity and to highlight the representative nature of this example.

Changes in the text: We have rewritten the sentence to 'Dynamic change of oxygen uptake (A), carbon dioxide excretion (B), respiratory exchange ratio (C), and work rate

(D) of arm ergometer CPET at different incremental work rates in participant 3 as a typical example, with arm ergometer CPET finished at incremental work rates of 5 (orange), 20 (red), 35 (blue), and 50 W/min (green), respectively.' (see Page 28, line 1648-1651)

Comment 74: L525: State 'finished at incremental work rates' but this just list the legend not the end point of the tests.
Figure 1D may be useful to add to the methods in demonstrating the different protocols.
Figure 3. Linear correlation analysis: Pael B is not linear, did the authors considered a curvilinear fit to increase the R squared value?
I am not sure this is the best analysis, figure essentially shows the range of peak values for each protocol – which was analysed using ANOVA. For linear regression / correlation it is usual to determine the slope / intercept etc, for each participant and then calculate the mean and SD for the group. It is appropriate to plot the peak RER against peak WR for each protocol in for a group responses / mean as there is a physiological rationale to do so. This would help identify any systematic differences between protocols.
Reply 74: Thank you for your valuable comments. Thank you for raising these important methodological points, and we agree with you about curvilinear fit. However, the linear model simplifies the transformation of the actual exercise prescription threshold, and the correlation between individual and population level is also shown in this figure. We agree that the individual level regression (slope/intercept) is valuable, so we keep **Figure 3** for now, and we will redo the fitting curve diagram if necessary.
Changes in the text:
We have changed the sentence '…with arm ergometer CPET at work rate increasing rates…' (see Page 28, line 1649)
We have added **Figure 1D** to method 2.3 section. (see Page 7, line 308)

Comment 75: L199-200: What statistical test was undertaken to compare VO2 in arm vs cycling – all four ramp protocol and cycling together, or a t-test for cycling vs. the mean of all ramp rates (which is fine to do as there was no difference between ramp rates)?
Reply 75: Thank you for your valuable comments. We employed the paired t-test as the statistical method to compare the core variables of the leg ergometer CPET with those of the arm ergometer CPET at four different work rate increasing rates, obtaining a set of p-value ranges.
Changes in the text: None

Comment 76: L202-206: What is the rationale for comparing leg and arm data at the midway point of each CPET? I don't see what it will inform us about other that arm values will [generally] be lower?

Reply 75: Thanks for your valuable comment giving me the opportunity to answer this question. While pure comparisons provide limited information, particularly regarding lower arm values, comparisons between upper and lower extremities at the midway of CPET can yield insights into subtle indicators. For instance, a higher Lowest VE/VCO_2 in CPET of the upper limbs than the lower limbs, suggesting early activation of respiratory compensation. This midway data is particularly beneficial for individualized exercise prescriptions, as it more accurately reflects the intensity of a subject's daily activities than peak values.

Changes in the text: None.

Comment 77: Likewise L211-213: What is the rationale for comparing leg and arm data 2 min into recovery?

Reply 77: We appreciate the opportunity to clarify this point. Wasserman, recognized as the father of cardiopulmonary exercise, in his work "Principles of Exercise Testing and Interpretation," clearly advocates for monitoring at least 2 minutes of data during the recovery period. This recommendation is grounded in classical physiological principles and the necessity for comparability across studies. While we adhered to the traditional two-minute data collection for comparison purposes, we stood to benefit the cardiovascular circulation by having all participants in this trial have a recovery period of five minutes and above.

Changes in the text: None

Comment 78: L208-210: Add these results after L199-201 as it is confirmatory data for the cycle ergometry protocol.

Reply 78: Thank you for your valuable suggestions. Additionally, we integrated the comparisons of arm and leg CPET results for each of the other phases into a single paragraph. This approach eliminates the need for distinct subheadings, thereby enhancing the focus on the overall trends in dynamic physiological responses.

Changes in the text: We have added these results to follow the warm-up period results as suggested.

'During warm-up, there was only $\dot{V}O_2$ using arm ergometer was slightly lower than that using leg ergometer (all $P<0.05$, $P=0.019\sim0.033$). However, there were no statistically significant differences in other variables, such as HR, $\dot{V}E$, VT, and Bf (all

P>0.05). At midway of exercise, the AT (P<0.001) and OUEP(P=0.024~0.039) using arm ergometer were significantly lower than those using leg ergometer, while Lowest $\dot{V}E/\dot{V}CO_2$ (P=0.027~0.035) showed a slightly higher value. Additionally, there was no statistically significant difference in $\dot{V}E/\dot{V}CO_2$ Slope (P=0.119~0.331). At peak exercise, the $\dot{V}O_2$ (P=0.001~0.005), HR (P<0.001), $\dot{V}E$ (P=0.001~0.003), VT (P=0.011~0.020) and WR (P<0.01) values of arm ergometer CPETs were all significantly lower than those of leg ergometer CPET, while the difference in Bf was not statistically significant (P=0.209~0.728). At resting and 2 minutes of recovery, there were no differences in variables between arm and leg ergometer CPET (all P>0.05).' (see Page 10, line 696-771)

Discussion

Comment 79: L215-228: The first paragraph of the discussion should provide the reader with an overview of the key findings, L230-233 is a good starting sentence. L215-223 is essentially background information and can be deleted, likewise L226-228 is methods information.

Reply 79: Thank you for your valuable suggestions. We have removed the redundant background and methods information and outlined the key findings in the first paragraph of the discussion as suggested.

Changes in the text: We used 'The 5, 20, 35 and 50W/min work rate increasing rates for different arm ergometer CPETs on the same healthy participant obtained similar peak $\dot{V}O_2$, peak HR, peak $\dot{V}E$, peak VT, peak Bf, OUEP, Lowest $\dot{V}E/\dot{V}CO_2$, $\dot{V}E/\dot{V}CO_2$ Slope and AT, respectively.' as the starting sentence. Key findings have been outlined 'The data suggest that variations in the work rate increasing rate (ranging from 5 to 50 W/min) do not significantly influence variables such as peak VO_2, AT, etc., achieved during arm ergometer CPET. However, differing work rate increasing rates resulted in significant variations in peak WR and Tlim. Peak WR was significantly greater in test protocols characterized by shorter Tlim (50 W/min and 35 W/min) compared to those with medium (20 W/min) and longer Tlim (5 W/min). To the best of our knowledge, this is the first study to extend the arm ergometer CPET work rate increasing rate to 50 W/min and systematically observe the effects of varying work rate levels on peak key metrics through the implementation of multiple loading protocols (slow, medium, and fast). Previous studies have reported that work rate increasing rate schemes do not affect peak HR or peak $\dot{V}O_2$, but they do influence peak WR and peak RER (14-15,25-27). The changes in key arm ergometer CPET metrics observed in healthy participants in this study are consistent with those found in previous studies.' (see Page 11, line776-787)

References:

14. Smith P M, Amaral I, Doherty M, et al. The influence of ramp rate on VO2peak and "excess" VO2 during arm crank ergometry. International Journal of Sports Medicine 2006;27:610-616.
15. Hutchinson M J, Paulson T A W, Eston R, et al. Assessment of peak oxygen uptake during handcycling: Test-retest reliability and comparison of a ramp-incremented and perceptually-regulated exercise test. PLOS One 2017;12:e0181008.
25. Bentley D J, McNaughton L R. Comparison of Wpeak, VO2peak and the ventilation threshold from two different incremental exercise tests: relationship to endurance performance. Journal of Science and Medicine in Sport 2003;6:422-35.
26. Gläser S, Lodziewski S, Koch B, et al. Influence of the incremental step size in work rate on exercise response and gas exchange in patients with pulmonary hypertension. BMC pulmonary medicine 2008;8:1-6.
27. Castro R R T, Pedrosa S, Chabalgoity F, et al. The influence of a fast ramp rate on peak cardiopulmonary parameters during arm crank ergometry. Clinical physiology and functional imaging 2010;30: 420-5.

Comment 80: L233-234: 'The degree of similarity is primarily based on the statistical analysis 234 results of the average values of 14 NS, which do not exhibit significant differences' is an interesting point and suggests individual variability, this should be in the results. Can the authors note the frequency of which ramp rate elicited greatest / lowest VO2 (for example)? NB: authors would need to first consider what is a meaningful difference in VO2 (and any other variable considered).

Reply 80: We sincerely appreciate the valuable comments. We have added this point in the results section and explained it in the discussion section as suggested. We note that the greatest $\dot{V}O_2$ in this study was elicited by 5 W/min, while the lowest $\dot{V}O_2$ was elicited by 50 W/min. We also deeply considered meaningful differences between the variables and eventually made significant changes to this paragraph.

Changes in the text:

We have added 'For each individual, the parameters of four arm ergometer CPETs may not exhibit significant similarities. The degree of similarity is primarily based on the statistical analysis results of the average values of 14 healthy participants, which do not exhibit significant differences.' to the results section as suggested. (see Page 10, line 676-679)

We have modified the content 'Although the four groups of participants exhibited high intergroup similarity in key metrics such as peak $\dot{V}O_2$, this finding primarily stemmed

from a statistical analysis of the group mean derived from 14 healthy participants, where none of the intergroup differences reached statistical significance. This suggests that individuals may be characterized by physiological variability. In the present study, we observed that a slightly higher peak $\dot{V}O_2$ was achieved during arm CPET at an increasing rate of 5 W/min, while a slightly lower peak $\dot{V}O_2$ was obtained at a rate of 50 W/min. This discrepancy may be attributed to the rapid onset of muscle fatigue resulting from excessively high work rate increasing rates , which may hinder the achievement of the highest possible peak $\dot{V}O_2$ within a short duration of force exhaustion.' (see Page 11, line 797-847)

Reference:

28. Iannetta D, de Almeida Azevedo R, Keir D A, et al. Establishing the VO2 versus constant-work-rate relationship from ramp-incremental exercise: simple strategies for an unsolved problem. Journal of Applied Physiology 2019;127: 1519-27.

Comment 81: L236: The authors are better stating what percentage of leg values the arm values were – which it typically ~70% (see reviews by Sawka 1986, Larsen et al. 2016, or Price et al 2024).

Reply 81: Thank you for your valuable guidance. We carefully read the literature, which was very helpful for us to compare the results between arm and leg CPET, and we added the information of arm value as a percentage of leg value in the discussion section as suggested.

Changes in the text: We have changed the content 'All participants in this study successfully completed CPET using cycle ergometer and arm ergometer without any adverse events. A comparison of key metrics between the two modes of exercise revealed that $\dot{V}O_2$ during warm-up and the values of $\dot{V}O_2$, HR, $\dot{V}E$, VT, and WR at peak exercise in the arm ergometer group were significantly lower than that in the cycle ergometer group, with a mean peak $\dot{V}O_2$ value of approximately 69% of the leg values and an AT of approximately 72% of the leg values. This finding aligns with previous studies which typically report arm values as a percentage of leg values at around 70%.' (see Page 14, line 920-926)

References：

35. Price M J, Smith P M, Bottoms L M, et al. The effect of age and sex on peak oxygen uptake during upper and lower body exercise: A systematic review. Experimentai Gerontology 2024;190:112427.
36. Larsen R T, Christensen J, Tang L H, et al. A systematic review and meta-analysis comparing cardiopulmonary exercise test values obtained from the arm cycle and the leg cycle respectively in healthy adults. International journal of sports physical

therapy 2016;11:1006.

Comment 82: L236: Why is the warm-up VO2 important? Was it to be used as economy values? However, these would all need to be at the same power output and this was not stated in the method.

Reply 82:

The $\dot{V}O_2$ values during warm-up were primarily used to confirm participants' physiological stabilization before incremental testing, not as direct economy indicators. However, we acknowledge that true economy comparisons would indeed require identical power outputs across subjects.

Although the warm-up was programmed at 0W resistance, the leg CPET warm-up phase is equivalent to a load of 20W and the arm ergometer is 10W, which is due to: inertial resistance of the ergometer flywheel, biomechanical work against limb segment mass (lower limbs: ~18% body mass vs. upper limbs: ~5% body mass) and frictional losses in the drive system.

Changes in the text:

We have modified method section 2.3: 'The 3-minute warm-up phase was set at 0W resistance (60 rpm), generating baseline power outputs of 10W (arm ergometer) and 20W (cycle ergometer) through system inertia and limb movement.' (see Page 7, line 311-313)

The discussion section was reworded as 'A comparison of key metrics between the two modes of exercise revealed that $\dot{V}O_2$ during warm-up and the values of $\dot{V}O_2$, HR, $\dot{V}E$, VT, and WR at peak exercise in the arm ergometer group were significantly lower than that in the cycle ergometer group,' (see Page 14, line 921-923)

Comment 83: L237-239: I am not sure you can state this with confidence as your population was non-symptomatic. Valeus may be different in clinical populations.

Reply 83: Thank you for your valuable comments. We have recognized that conclusions not derived from this study cannot be expressed casually, so we have deleted this sentence and rephrased.

Changes in the text: We have changed and added to the discussion 4.2 section.

'Muscle work during exercise necessitates a complex integration of cardiac, pulmonary, vascular, and peripheral mechanisms. The lower $\dot{V}O2$ observed during arm movements can be attributed to the specificity of the muscle groups involved in this movement pattern. The primary muscles engaged during arm exercise—namely, the biceps, triceps, and deltoids—are smaller and less conditioned compared to the leg muscles. These arm muscles possess a higher proportion of type II muscle fibers relative to the leg muscles,

resulting in a greater O_2 cost compared to the slower type I fibers . The elevated HR observed during cycling exercise is due to the lower limbs, which represent the largest muscle group in the body, generating a greater overall metabolic demand during exercise and imposing a more substantial load on the cardiovascular system. In contrast, the activity of smaller muscle groups during upper limb exercise exerts relatively limited stress on the circulatory system . It suggests that we need to lower the standard of judgment if we use CPET with an arm ergometer to assess the overall functional status of the subject. Schrieks et al. compared a treadmill with an arm crank ergometer and proposed a regression equation to predict $\dot{V}O_2$ on a treadmill based on physiological variables of the ACE. Additionally, it has been demonstrated that the arm ergometer CPET can serve as an alternative mode of exercise for sedentary adults and holds potential for application in clinical populations to assess cardiorespiratory fitness in individuals with lower extremity mobility limitations . Currently, under the guidance of CPET, there are numerous successful cases of chronic disease diagnosis and treatment centered around individualized exercise training utilizing lower limb cycle ergometer . Upper limb exercise training effectively engages the muscles of the upper body, enhances blood circulation in this region, and contributes positively to the overall blood flow to the heart and cerebrovascular system. The use of the arm ergometer CPET for cardiopulmonary function testing and rehabilitation training demonstrates a high degree of safety and feasibility, warranting further investigation.' (see Page 14, line 930-964)

37. Drescher U, Koschate J, Hoffmann U. Oxygen uptake and heart rate kinetics during dynamic upper and lower body exercise: an investigation by time-series analysis. European journal of applied physiology 2015;115:1665-72.
38. Green D J, Hopman M T E, Padilla J, et al. Vascular adaptation to exercise in humans: role of hemodynamic stimuli. Physiological reviews 2017;97:495-528.
39. Schrieks I C, Barnes M J, Hodges L D. Comparison study of treadmill versus arm ergometry. Clinical Physiology and Functional Imaging 2011;31:326-31.
40. Mitropoulos A, Gumber A, Crank H, et al. Validation of an arm crank ergometer test for use in sedentary adults. Journal of sports science & medicine 2017;16:558.
41. D'Ascenzi F, Cavigli L, Pagliaro A, et al. Clinician approach to cardiopulmonary exercise testing for exercise prescription in patients at risk of and with cardiovascular disease. British journal of sports medicine 2022;56:1180-7.

Comment 84: L239-241: 'The OUEP is a reliable indicator of oxygen uptake efficiency, and Lowest *V*E/*V*CO2 is superior to the *V*E/*V*CO2 Slope for better assessment of ventilatory effectiveness in subjects (25, 26,)' should really be in the

methods.

Reply 84: Thank you for your valuable comments. We have added it to the methods section.

Changes in the text: We have supplemented the methods section 2.5 'The peak oxygen uptake ventilation efficiency platform (OUEP) is a reliable indicator of oxygen uptake efficiency, The minimum value of carbon dioxide emission ventilation efficiency (Lowest $\dot{V}E/\dot{V}CO_2$)and slope of ventilatory equivalent for carbon dioxide($\dot{V}E/\dot{V}CO_2$ Slope) are excellent indicator for assessing the effectiveness of ventilation, The oxygen uptake per unit work rate ($\Delta\dot{V}O_2/\Delta WR$) indicates the oxygen delivery capacity of peripheral exercising muscles.' (See Page 8, line 346-351)

Comment 85: L241: Similarly, the statement regarding heart transplantation is completely unrelated to this work.

This paragraph starts by stating no differences in a number of variables and as such should explain why, i.e. similar end points were reached (but with different exercise durations), so similar exercise limitations?

Reply 85: Thank you very much for your valuable suggestions. We have deleted statements about heart transplantation that are not relevant to this study. At the same time, we have made significant changes to this paragraph as suggested, first explaining which variables are not different and then explaining the physiological mechanism.

Changes in the text: We have changed the content 'Peak $\dot{V}O_2$ was not influenced by the work rate increasing rate, indicating that the maximal functional reserve of the cardiorespiratory system may be primarily determined by individual physiological limits rather than the incremental pattern of exercise loads. Peak $\dot{V}O_2$ serves as the most reliable indicator of aerobic metabolism, representing the upper limit of aerobic capacity of the large muscle groups during exercise in the human body. The core determinants of this capacity include maximum cardiac output, the arteriovenous oxygen difference, and the mitochondrial oxidative capacity of skeletal muscle. During extreme exercise in CPET, regardless of the work rate increasing rate, subjects ultimately reach the maximal oxygen supply capacity of the cardiorespiratory system, resulting in stabilization of peak $\dot{V}O_2$. Similarly, peak HR is constrained by the upper limits of autonomic regulation, demonstrating independence from the work rate increasing rate. Although the four groups of participants exhibited high intergroup similarity in key metrics such as peak $\dot{V}O_2$, this finding primarily stemmed from a statistical analysis of the group mean derived from 14 healthy participants, where none of the intergroup differences reached statistical significance. This suggests that individuals may be characterized by physiological variability. In the present study, we

observed that a slightly higher peak $\dot{V}O_2$ was achieved during arm CPET at an increasing rate of 5 W/min, while a slightly lower peak $\dot{V}O_2$ was obtained at a rate of 50 W/min. This discrepancy may be attributed to the rapid onset of muscle fatigue resulting from excessively high work rate increasing rates , which may hinder the achievement of the highest possible peak $\dot{V}O_2$ within a short duration of force exhaustion. Our analysis indicates that the AT, the point where oxygen delivery cannot keep up with metabolic demand, remains relatively constant regardless of work rate increasing rate. This stability is attributed to the threshold's strong correlation with daily exercise and cardiovascular function . Essentially, the AT primarily serves as a reflection of an individual's exercise endurance, an aspect that remains unaffected by variations in effort level or work rate increasing rate. Some researchers noted that increasing rates and crank rate simultaneously does not enhance incremental exercise performance but facilitates valid measurements of peak physiological variables within a shorter test duration . This suggests that the choice of work rate increasing rate is less critical for these parameters of interest; if rapid data acquisition is desired, a higher work rate increasing rate may be selected. To quickly determine peak $\dot{V}O_2$ or AT values in the arm ergometer CPET, we can increase the work rate increasing rate accordingly.' (see Page 11, line 788-857)

References:

28. Iannetta D, de Almeida Azevedo R, Keir D A, et al. Establishing the VO2 versus constant-work-rate relationship from ramp-incremental exercise: simple strategies for an unsolved problem. Journal of Applied Physiology 2019;127: 1519-27.
29. Wasserman K. The anaerobic threshold measurement to evaluate exercise performance. American Review of Respiratory Disease 1984;129:S35-40.
30. Price M J, Bottoms L, Smith P M, et al. The effects of an increasing versus constant crank rate on peak physiological responses during incremental arm crank ergometry. Journal of sports sciences 2011;29:263-9.

Comment 86: L251-259: AT should be noted in the introduction – and that different ramp rates may potentially affect its identification? However, (L257-259) shorter test duration means far fewer test stages are undertaken to determine AT, so this may affect validity and ability to do this. Such aspect needs to be considered here.

Reply 86: Thank you for your professional suggestions. We have considered the identification of AT in the introduction as suggested.

Changes in the text:

Revision of the introductory section. 'The AT is the critical point when the skeletal muscle energy supply mode shifts from aerobic metabolism to anaerobic metabolism

to compensate for aerobic deficiency during incremental loading exercise, and individualized exercise prescription is often formulated based on the corresponding heart rate (HR), $\dot{V}O_2$, and METs at the time of the anaerobic threshold .' (see Page 4, line 149-153)

'Faster work rate increasing rate can reduce test duration, thereby limiting the number of metabolic steady-state phases available for robust AT identification. Confirmation of the AT of the rapid protocol test in this study deserves further attention.' (see Page 6, line 243-245)

Comment 87: L260: Based on earlier analysis comments, the authors should state the that peak WR and RER were affect by ramp rate, but only after giving the specific pairwise differences, and re-stating them her for context.

Reply 87: Thank you for your professional suggestions. We will provide specific pairwise differences between the 4 groups and clearly illustrate the effect of work rate increasing rate on peak WR. The discussion of RER being affected by work rate increasing rate has been revised in the text with additional changes immediately after the peak WR. Also refer to expert comments 91-94 for a point-by-point response.

Changes in the text: We have changed the content 'Castro et al. found that a rapid 20 W/min protocol was effective in healthy young adults performing arm CPET, achieving the same peak $\dot{V}O_2$ at a higher peak WR compared to a slower protocol. The present study corroborates these findings by incorporating faster protocols of 35 W/min and 50 W/min, both of which reached similar endpoints at higher peak WR, but with varying Tlim. There was a significant difference in peak WR obtained with arm ergometer CPET at four work rate increasing rates and all comparison between increasing rate protocol were significantly different. The differences in Peak WR between groups may arise from an increased activation of the anaerobic energy metabolism system. The shorter test protocol prompted subjects to achieve higher peak WR more rapidly, which led the neuromuscular system to recruit higher-order motor units earlier, thereby facilitating an earlier transition to anaerobic metabolic pathways . Higher work rate increasing rates result in a greater increase in load per unit of time, which leads to earlier activation of the muscle anaerobic metabolic system and a rapid accumulation of lactic acid, thereby shortening Tlim. Additionally, high incremental rates may cause subjects to reach subjective exhaustion earlier than cardiorespiratory failure by accelerating peripheral fatigue. This phenomenon aligns with the theory in HIPM that exercise tolerance is limited by the synergistic effects of multiple systems rather than the function of a single organ. Although peak WR increased significantly with the incremental rate (about 27% increase in peak WR in the 50 W/min group

compared with the 20 W/min group), peak $\dot{V}O_2$ remained stable, and peak WR was positively correlated with the work rate increasing rate, which was consistent with the kinetic inertia of $\dot{V}O_2$. The significant differences observed in Peak WR further reinforce the notion that a consistent protocol should be adhered to when making longitudinal comparisons.' (see Page 12, line 860-880)

References:

27. Castro R R T, Pedrosa S, Chabalgoity F, et al. The influence of a fast ramp rate on peak cardiopulmonary parameters during arm crank ergometry. Clinical physiology and functional imaging 2010;30: 420-5.
31. Brurok B, Mellema M, Sandbakk Ø, et al. Effects of different increments in workload and duration on peak physiological responses during seated upper-body poling. European Journal of Applied Physiology 2019;119:2025-31.
32. Gurd B J, Scheuermann B W, Paterson D H, et al. Prior heavy-intensity exercise speeds VO2 kinetics during moderate-intensity exercise in young adults. Journal of Applied Physiology 2005;98:1371-8.

Comment 88: L262 (and L337): No first name initials should be given in study citations in the main text. Was this reference pertinent to arm exercise protocols or lower body protocols?

Reply 88: Thank you for your professional suggestions. We have learned this point and deleted the first name initials from the text. In addition, this irrelevant literature and ambiguous content was also removed.

Changes in the text: We have changed 'Guo Z. Y. et al.' to 'Guo et al.' (see Page 16, line 1146)

Comment 89: L263-269: This does not relate to your work (or research question) so should be deleted.

Reply 89: Thank you for your valuable comments.

Changes in the text: We have deleted the irrelevant content of 'In health management based on the HIPM, the precise formulation of individualized training protocol is the core content of training rehabilitation, and the training intensity is the core of the formulation of an individualized training protocol, which is directly related to the safety and efficacy of the chronic disease diagnosis and treatment . Cardiorespiratory fitness is improved and the risk of cardiovascular disease is decreased when patients with metabolic syndrome follow a well-formulated training prescription that is based on objective and quantitative CPET and involves training with a training intensity of Δ50% WR .'

Comment 90: L271: From 'According to the findings' consider what protocol you would consider for the population you have studied. If you are going to refer to clinical groups it is most likely that the lowest ramp rate would be used (certainly initially) but I the knowledge that all of the ones used in the present study elicit similar values for certain peak responses in non-symptomatic participants – that is what is key.

Reply 90: Thank you very much for your professional guidance. The exploration of what options we would consider for the population we are studying is in Discussion 4.3 section.

Changes in the text:

We have briefly outlined it as 'The results of the study indicate that the work rate increasing rate is a significant factor affecting CPET variables. Selecting the appropriate protocol for arm ergometer CPET is crucial for accurately evaluating exercise capacity and individualized precision training.' (see Page 15, line 969-971)

Comment 91: L278-285: This paragraph does not provide any new insights into the use of RER and can be deleted.

Reply 91: Thank you for your valuable comments. As suggested, we have deleted the content without new insights in the revision.

Changes in the text: We have deleted that.

Comment 92: L288-299: The RER argument would be strengthened if analyzed as RER vs power output for each ramp rate and slopes compared using ANOVA. AS for other factors, if focusing on RER this must be clear why. This section does not provide novel insights in its current form.

Reply 92: Thank you for the constructive feedback. The slopes of RER-power relationships across ramp rates were statistically compared using ANOVA, as recommended. Results are detailed in Table 4 ($P<0.05$ indicates significant differences). We have revised the discussion sections accordingly.

Changes in the text: We have revised the discussion section. 'Although no statistical differences were observed between the peak $\dot{V}O_2$ and peak $\dot{V}CO_2$ groups across the four tests in this experiment, higher work rate increasing rates exhibited a slight upward trend in peak $\dot{V}CO_2$ and a slight downward trend in peak $\dot{V}O_2$. Consequently, peak RER was positively correlated with the work rate increasing rate, and the group with a work rate increasing rate of 5 W/min demonstrated a significantly lower peak RER compared to the other three groups. Furthermore, the recovery RER also varied according to the work rate increasing rate. The RER serves as a secondary characteristic

in CPET to ascertain whether a maximal physiological endpoint has been achieved. Muscle lactate production experiences a surge when exercise intensity surpasses the AT. The neutralization reaction of lactate with bicarbonate ($H^+ + HCO_3^- \rightarrow CO_2 + H_2O$) results in the rapid release of stored CO_2, accompanied by a compensatory enhancement of respiration to expel excess CO_2, leading to a significant increase in RER. Research indicates that the peak RER value is not solely determined by the real-time CO_2 produced during exercise; it is also significantly influenced by the volume of CO_2 reserves expelled from the body. The total CO_2 released through metabolic activity comprises both a generation component and a reserve release component. Consequently, the peak RER is elevated when there is an abundance of CO_2 stored in tissues, a process accelerated by the circulatory system. Furthermore, RER tends to continue to rise rapidly during the initial phase of the transition from exercise to recovery, as oxygen uptake decreases more swiftly than carbon dioxide excretion following the cessation of exercise. CO_2 undergoes several physiological processes from cellular mitochondrial production to eventual excretion via the lungs, including intercellular fluid diffusion, venous transport, and alveolar exchange. This intricate metabolic clearance pathway produces a spatial and temporal delay effect, resulting in a rapid increase in RER during the initial recovery phase. The low peak RER observed during the low-rate incremental protocol may be attributed to the extended duration of exercise, which facilitates the complete mobilization of the aerobic metabolic system and enhances fat oxidation for energy supply. Conversely, higher work rate increasing rates result in shorter Tlim, compelling the body to depend on anaerobic glycolysis for rapid energy provision. This indicates that as the incremental work rate increases, the rate of CO_2 expulsion from the body near peak exercise also increases, leading to a rapid elevation of the RER. However, no significant differences in peak RER values were found among the 20, 35, and 50 W/min tests. This lack of variation may be due to the limited sample size, which predominantly consisted of females, suggesting that further research is necessary to increase the sample size or to account for age and gender differences.' (see Page 13, line 881-918)

Comment 93: L300: RER is not used to assess 'effort', in the contest of CPET it us used as a secondary characteristic to determine whether a maximal physiological end point has been reached. Although conventional end points would not be reached in a below maximal effort, RER does not measure effort per se. Please reword.

Reply 93: Thank you for your valuable advice. We have reworded this sentence as advised.

Changes in the text: We have rewritten the sentence as 'The RER serves as a

secondary characteristic in CPET to ascertain whether a maximal physiological endpoint has been achieved.' (see Page 13, line 886-887)

Comment 94: L300-312: As above, there is no new information presented here, and no evidence to suggest the RER criteria for a maximal effort should be different or tests should be stopped at an RER of >=1.20.

Reply 94: Thank you for your valuable comments. We have removed the non-new insights section and rewritten the content to express only the concise points that can be drawn from the study.

Changes in the text: 'Additionally, the peak RER achievable by an individual is not constant and can be significantly influenced by varying work rate increasing rates. This finding supports previous research (34), indicating that RER should not be utilized as a criterion for terminating a trial.' (see Page 14, line 918-921)

Reference:

34. Tan X Y, Sun X G. From Clinical Application of Cardiopulmonary Exercise Testing to view the Requirement for Holistic Integrative Physiology and Medicine. Medicine and Philosophy 2013;34:28-31.

Comment 95: L313: This paragraph (section 4.3) is the most important relating to the current data set.

Reply 95: We sincerely appreciate the encouraging comments. We carefully corrected this section based on your extensive professional comments. Some irrelevant content has been removed and additional discussion on protocol optimization has been added.

Changes in the text:

'Too high a work rate increasing rate can impede the attainment of steady-state VO_2 kinetics due to the delayed response of the cardiopulmonary system, potentially leading to an underestimation of the true peak. And the sudden load increase at high work rate may induce a burning sensation in the upper limb muscles or joint discomfort, prompting premature subjective termination by the subject and compromising objectivity. In the tests conducted in this study with all healthy participants, key variables at the 35 and 50 W/min protocols were recorded as feasible by splitting the data collected for each breath second-by-second and calculating a 10 s average. However, due to time constraints, the ultra-rapid testing process impedes effective data collection and analysis. This limitation is particularly concerning for the average healthy participant, which is precisely why ultra-rapid testing is not commonly utilized in clinical practice. High work rate increasing rates are more appropriate for athletes assessing their instantaneous WR capacity. In comparison to the more rapid increase in

resistance observed in the 50 W/min protocol, the 5 W/min test demonstrated a significantly more favorable match between ventilation and perfusion, with an exercise duration ranging from 8 to 15 minutes. This duration is conducive to capturing $\dot{V}O_2$ kinetic homeostasis, including the oxygen deficit recovery curve and the ventilatory threshold. During work rate increments, smaller increases in workload are less easily detected. Consequently, smaller workload increments, such as 1 W per 10 seconds, exert less psychological and physiological impact, thereby delaying fatigue and enabling participants to sustain exercise for extended periods. However, protocols with too low a work rate increasing rate may result in an excessively long Tlim, potentially leading to premature termination due to factors unrelated to cardiorespiratory capacity. These factors may include subjects experiencing dry mouth, localized muscular fatigue, psychological fatigue, or distractions that affect endpoint judgments. Low work rate increasing rate regimens are more suitable for the refined assessment of patients with impaired cardiopulmonary function. If the work rate increasing rate increases too rapidly or insufficiently, or if the peak exercise is reached too soon or too late, the results may miscalculate the patient's actual maximal exercise capacity. The test duration of the 20 W/min protocol was approximately 4 ~ 6 min, effectively reflecting muscle anaerobic reserve with an increase of up to 27% in peak WR, while ensuring the stability of peak $\dot{V}O_2$ data. The duration of exercise at this rate is slightly shorter than the guidelines set forth by the American Thoracic Society/American College of Chest Physicians (1), recommending 8 ~ 12 min as the ideal duration for incline exercise protocols, which is based on data generated from exercise tests performed in a treadmill and bicycle. In contrast, arm exercises that engage smaller muscle masses may lead to premature fatigue before reaching peak $\dot{V}O_2$. Given practical considerations, shorter tests are preferable, provided they can sufficiently induce the physiological load necessary to maximize the subject's $\dot{V}O_2$. Thus, the 20 W/min protocol is suitable for routine upper limb cardiorespiratory fitness assessments and the development of exercise prescriptions.' (see Page 15, line 978-1145)

Reference:

44. ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med 2003;167:211-77.

Comment 96: L316-317: What was 'effectiveness' based on in the Davies study – valid end point criteria?

How do the peak values in the current study (manly VO2, HR and power output) relate to previous studies? This has not been covered and is usually one of the first discussion points to demonstrate (essentially) validity of findings / population tested.

Reply 96: The 'effectiveness' was defined by two main criteria: First, Higher ramp rates ensured participants reached true peak $\dot{V}O_2$ and VT with minimal test duration, reducing fatigue-induced bias. Second, these rates optimized the linearity of VO_2-power relationships, enhancing the accuracy of aerobic parameter estimation compared to slower ramps. The 20-50 W/min range was used as a reference in the conclusions of Davies' study, and the arm CPET work rate increasing rate protocol should not exceed that of the leg CPET.

Changes in the text: None.

Comment 97: L320: 'An excessively high incremental work rate can lead to a rapid rise in resistance' is very true and is a function of the magnitude of the increment, better to state as 'A [greater] incremental work rate [will] lead to a rapid rise in resistance…..'.

Reply 97: Thank you for your professional advice.

Changes in the text: We have changed the wording of this sentence as advised.

'A greater incremental work rate will lead to a rapid rise in resistance, causing the subject's arm to feel strained and resulting in premature fatigue.' (see Page 15, line 976)

Comment 98: L321-322: States 'the 50 W/min group may lead to a shorter Ex. Time to exhaustion due to faster muscle fatigue', which it did, so reword as such and is in agreement with ref 39.

Reply 98: Thank you for your encouraging comment. We have reworded and aligned with the references.

Changes in the text: 'A greater work rate increasing rate will lead to a rapid rise in resistance, causing the subject's muscles to feel strained and resulting in premature fatigue . For instance, the 50 W/min test in this study may lead to a shorter Tlim to exhaustion due to faster muscle fatigue.' (see Page 15, line 976-978)

Reference:

43. Black M I, Jones A M, Blackwell J R, et al. Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains. Journal of Applied Physiology 2017;122:446-59.

Comment 99: L322-324: Was this the case in your female participants? If such context is not given then this statement is very general and does not help explain your data.

Reply 99: Thank you for your valuable comments. We have deleted this generalized statement. We supplemented the data on female participants, added context, and rewrote the discussion section. We have added a note on the gender factor in the limitations section.

Changes in the text: We have deleted the sentence 'The incremental work rate of 50 W/min is too high for females because, generally speaking, old age, female gender, and a state of little exercise are all likely to be relevant variables for limited exercise capacity .'

Limitation:

'First, the sample size of the current study was limited and consisted primarily of a mixed population of healthy men and women; therefore, future research should expand the sample to consider the inclusion of different age, gender, and disease groups.' (see Page 17, line 1184-1187)

Comment 100: L324-325: 'Furthermore, rapid tests with 325 limited time hindered the collection and analysis of data, which is even less desirable for clinical patients', exactly, which is why they are not undertaken.

Reply 100: Thank you for your valuable comments. We have reworded the sentence as suggested to make it more precise.

Changes in the text: We have rewritten the sentence as 'However, due to time constraints, the ultra-rapid testing process impedes effective data collection and analysis. This limitation is particularly concerning for the average healthy participant, which is precisely why rapid testing is not commonly utilized in clinical practice.' (see Page 15, line 985-1121)

Comment 101: L328: What is considered the appropriate range for exercise time? How many of your participants for each protocol were within this desired range?

Reply 101: We thank the reviewer for the opportunity to further clarify this important point. In the 50W/min protocol, only one participant exercised for 8 minutes, while the remaining participants exceeded this duration. In the 20W/min protocol, six volunteers exercised for a duration ranging from 4 to 8 minutes, while the others exercised for less than 4 minutes. All participants did not reach 4 minutes of exercise during the 35Wmin and 50W/min protocols.

Changes in the text:

'In this study, we expanded the upper and lower limits of the duration of test protocols (ranging from 1 min 70 s to 15 min 30 s) to evaluate the effects of shorter and longer duration incremental arm ergometer CPET protocols on peak variables. The data from this study showed that the Tlim of participants in the 5W/min protocol varied between 8 and 15.30 minutes. Specifically, 9 participants exercised for durations ranging from 8 to 12 minutes, while 5 participants engaged in exercise for more than 12 minutes. In the 20W/min protocol, participants' Tlim ranged from 2.75 to 5.10 minutes, with all

male participants exercising for more than 4 minutes, whereas only two females exceeded 4 minutes. Notably, the Tlim in the 35W/min and 50W/min protocols did not exceed 4 minutes.' (see Page 16, line 1147-1177)

Comment 102: L331-336: This does relate to your data set. I am not sure 4 min would be enough if AT data I required though.
Reply 102: We would like to thank the reviewer for pointing out this issue.
Changes in the text:
We've reworded 'We suggest a more appropriate duration for incremental arm ergometer CPET is between 4 and 8 minutes. This window of time ensures that the subject's physical strength is not overexerted for too long and allows for the collection and analysis of respiratory, circulatory, and other relevant data. Consequently, our preliminary investigation suggests a suitable work rate increasing rate protocol for arm ergometer CPET would be between 10 and 20 W/min, with an increase of another 5 W/min if the subject is a strong male.' (see Page 17, line 1177-1182)

Comment 103: L337-338: OK, but presumably over time / a number of testing sessions.
Reply 103: Thank you for your valuable comments. Indeed, this protocol does a number of tests. This is accomplished by integrating multidimensional physiological parameters, such as heart rate variability, metabolic equivalents, and gas exchange efficiency, obtained from multiple consecutive tests on the same subject.
Changes in the text: None

Comment 104: L338-348: Does not relate to you data and you can't evident this.
Reply 104: Thank you for your valuable comments.
Changes in the text: We have deleted the irrelevant content.

Comment 105: L349: Limitations – it is important not just to list these, but to give some accompanying explanation.
Sample size – what was determined a priory? What is required for any suggested differences of importance
Fixed rate protocols rather than individualized ones – this wasn't this the point of the study. The authors (as noted above) can determine which protocol produced the greatest number of 'best' performances for the population studied and would answer this suggested limitation
Blood pressure: Would this be of much surprise in non-symptomatic participants?
Clinical utility – see numerous comments above, your data would suggest that lower

WR (such as for clinical groups) should provide just as valid peak data as observed in the literature for non-symptomatic groups.

Also, the mixed population with regards to males and females – there is enough data to examine the n=9 female group which would help with novelty of the study.

Reply 105: We sincerely appreciate your detailed comments and professional advice. Limitations regarding blood pressure and individualized protocols are not the point of this study, so we have removed this statement and rewritten the rest of the limitations in response to the data.

Changes in the text: We have modified the limitation section 'First, the sample size of the current study was limited and consisted primarily of a mixed population of healthy men and women; therefore, future research should expand the sample to consider the inclusion of different age, gender, and disease groups. Second, the 20 W/min work rate increasing rate protocol demonstrated better overall performance in healthy individuals. However, the incremental gradients set in this study (5, 20, 35, and 50 W/min, with 15 W/min intervals between adjacent groups) may restrict the sensitivity required to accurately identify the optimal protocol. For instance, the difference from 5 W/min to 20 W/min is considerable (Δ15 W/min), whereas the physiological response of upper limb muscles to load variations may be more nuanced. Thus, it is necessary to implement finer incremental gradients (e.g., 5, 10, 15, and 20 W/min) within the 5-20 W/min range in future studies to elucidate the effects of different work rate increasing rates on key peak variables, Tlim, and other differential impacts, thereby enhancing the optimization of arm ergometer CPET protocols.' (see Page 17, line 1184-1195)

Comment 106: L359-362: Conclusion: Here state which protocols are best based o your results when peak power or VO2 etc., for example, are required.

Reword rest of conclusion so it reflects what you have found regarding the first comparison of typical ramp rate protocol for arm ergometry.

Reply 106: We thank the reviewer for this insightful comment. We have rewritten the conclusion section as suggested.

Changes in the text: We have rewritten the conclusion, 'In the range of 5-50 W/min, different work rate increasing rates had no significant effect on core functional indices such as peak $\dot{V}O_2$, peak HR, peak $\dot{V}E$, AT, and $\dot{V}E/\dot{V}CO_2$ slope of arm ergometer CPET in healthy participants. Therefore, when considering these variables, the choice of work rate increasing rates is not critical. However, the high load protocol resulted in higher peak WR, peak RER, and shorter Tlim compared to the low load protocol. Thus, higher work rate increasing rate protocols may be preferred when subjects possess

strong upper extremity muscles and when peak WR is a primary index of exercise capacity. It is also important to note that the work rate increasing rate protocol should not be altered arbitrarily when assessing changes in cardiorespiratory fitness or monitoring adaptations to the upper limb training program for the same subject. Considering the appropriateness of the Tlim (4-8 min), a work rate increasing rate in the range of 10-20 W/min is generally advisable for arm CPET in healthy subjects. This rate can be clinically adjusted by 5 W/min based on the severity of the disease, as well as factors such as gender, age, and baseline fitness level. This work enhanced researchers' understanding of how variations in work rate increasing rate influenced physiological responses and preliminarily optimized the selection of work rate increasing rates for arm ergometer CPET, warranting further investigation.' (see Page 17, line 1197-1265)