



# **Push the boundaries: individual and heat exposure challenges to threshold determination and acute recovery from severe-intensity exercise**

Gil Bourgois

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## PUSH THE BOUNDARIES

Individual and heat exposure challenges to threshold determination  
and acute recovery from severe-intensity exercise

Gil Bourgois

Thesis submitted in fulfillment of the requirements for  
the degree of Doctor of Health Sciences at Ghent University, Belgium  
and  
le grade de Docteur en Sciences et Techniques des Activités Physiques et  
Sportives à l'Université de Lille, France

GHENT, 2023

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“If physiology can aid in the development of athletics as a science and an art, I think it will deserve well of mankind.”

*Archibald V. Hill, 1925*



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## PREFACE

This work represents the culmination of four years of dedicated research and study. Undertaking this doctoral thesis has been an enriching and rewarding experience, allowing for deep exploration within the realm of exercise physiology.

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This work is dedicated to my cherished stars in the sky.

It is my hope that this thesis contributes meaningfully to the field of sports science and serves as a foundation for further exploration and advancement in this area.

Off to new adventures!

Gil Bourgois

December, 2023



## ENGLISH SUMMARY

Exercise is a crucial aspect of maintaining overall health and well-being. Participating in recreational or professional sports involves a range of metabolic demands, and a proper understanding of physiological processes taking place at different intensities is vital for optimizing training and performance. Generally, exercise intensity can be distributed into three domains, i.e., moderate, heavy and severe. To set realistic goals, prescribe training and monitor (long term) performance, the determination of the heavy-severe intensity boundary is a key feature. This threshold marks the boundary between sustainable and non-sustainable exercise, hence, it can be seen as the highest work rate at which a metabolic equilibrium is possible, i.e., maximal metabolic steady state (MMSS). Critical power (CP), as part of a mathematical framework with underlying physiological mechanisms is frequently put forward as the 'gold standard' methodology to determine the MMSS. This CP is the asymptote to the hyperbolic relationship between power output and time to exhaustion. Together with  $W'$ , the curvature constant (or work capacity that can be spent above CP), it forms the CP concept. Yet, there are also threshold concepts that serve as an indirect estimation of the MMSS, i.e. second lactate threshold and the respiratory compensation point determined from STEP or RAMP protocols using capillary blood samples or pulmonary gas exchange, respectively. The popularity of these exercise tests is based on the time efficient and feasible manner to determine exercise thresholds.

In the first part of this PhD thesis, the effect of acute heat exposure on the determination of the heavy-severe intensity boundary was investigated. As climate change is affecting global temperature, heat exposure is becoming an increasing challenge for individuals participating in physical activity, recreational or competitive sports events. Furthermore, heat stress is also used during the training process (i.e., heat acclimation/acclimatization) to achieve beneficial training effects and physiological/hematological adaptations to optimize performance in hot as well as temperate environments. The physiological response to exercise in the heat is complex, as the development and extent of these adjustments are dependent on many factors such as the severity of the heat stress, prior whole body heating, individual characteristics, the intensity distribution of the exercise and the duration of the exposure. The developed heat strain results in alterations in the cardiovascular system, central nervous system and skeletal muscle function. We showed that the interaction between exercise intensity and exercise duration within exercise test protocols defines the magnitude of decrement in power output corresponding to the threshold. No sex differences were found in the effect of acute heat exposure on performance and the determination of the heavy-severe exercise intensity boundary, at least for the CP test methodology. The large interindividual variation in response to heat exposure in *study 1* and *study 2* supports the fact that even a short incremental exercise test with acute heat exposure can give valuable insight into the acute heat response of an athlete. This could provide

additional support for performance diagnostics, optimizing prescription and steering of the training process, and monitoring exercise intensity in hot environments.

Since physical activity and many sports involve frequent changes in exercise intensity, the CP concept has also been applied to intermittent exercise, in which exercise above CP ( $W'$  depletion) is alternated with exercise below CP ( $W'$  reconstitution). Therefore, the second part of this PhD thesis, is focused on the parameter  $W'$ . Since the beginning of the 21<sup>st</sup> century, the evolution of  $W'$  reconstitution research progresses exponentially, possibly due to feasible implementation by technical innovations (e.g., development of power meters in cycling) and its capabilities towards monitoring of an individual's energetic balance during intermittent exercise. Several years ago a model was developed by exercise physiologists that provided insight into the energy balance during intermittent exercise, or in other words the available  $W'$  at any moment in time (i.e.,  $W'_{\text{BAL}}$  model). This model, consisting of a mono-exponential  $W'$  recovery kinetics, could be used for performance prediction and optimizing pacing strategies. However, the question was raised very fast after the development of the model whether the  $W'_{\text{BAL}}$  model is a universal model for everyone and every exercise protocol. Therefore, we have performed two experimental studies (*study 3* and *study 4*) to gain a better understanding of the underlying mechanisms of  $W'$  recovery. We found that a two phase exponential model is superior to predict  $W'$  reconstitution, instead of a mono-exponential fit, following exercise fully depleting  $W'$ . Furthermore, characteristics of the exercise protocol, i.e., repeated bouts to exhaustion, can slow the  $W'$  reconstitution, and the rate of  $W'$  recovery is strongly influenced by individual factors, such as aerobic fitness and sex. This has important implications for training prescription in men and women and indicates the importance of including sex as a biological variable in sports science research. Based on previous work done by colleagues of the same research group and the work done during this PhD project, it allowed us to initiate the development a new model to optimize the prediction of  $W'$  recovery. As a result, a multifactorial approach to accurately estimate  $W'$  reconstitution is required, considering individual characteristics and exercise modalities. Moreover, an optimum between mathematical fine-tuning and integrating physiological processes is required to establish a solid  $W'$  reconstitution model.

## NEDERLANDSTALIGE SAMENVATTING

Lichaamsbeweging is een cruciaal aspect in het behouden van de algemene gezondheid en het welzijn. Deelname aan recreatieve of professionele sporten brengt een scala aan metabolische eisen met zich mee en een goed begrip van fysiologische processen die plaatsvinden bij verschillende intensiteiten is van vitaal belang voor het optimaliseren van training en prestaties. Over het algemeen kan trainingsintensiteit worden onderverdeeld in drie domeinen: matig, zwaar en zeer zwaar. Om realistische doelen te stellen, training voor te schrijven en prestaties (op lange termijn) te monitoren, is het bepalen van de grens tussen zware en zeer zware intensiteit een belangrijk aspect. Deze drempel markeert de grens tussen inspanning die kan worden volgehouden of moet worden stopgezet, en kan daarom worden gezien als de hoogste belasting waarbij een metabool evenwicht mogelijk is, oftewel maximale metabole steady state (MMSS). Critical power (CP), als onderdeel van een wiskundig concept met onderliggende fysiologische mechanismen, wordt vaak naar voren geschoven als de 'gouden standaard' methodologie om de MMSS te bepalen. CP is de asymptoot van de hyperbolische relatie tussen het geleverde vermogen en de tijd tot uitputting. Samen met  $W'$ , de krommingsconstante (of de arbeid die kan worden geleverd boven de CP), vormt dit het CP-concept. Er zijn echter ook drempelconcepten die dienen als indirecte bepaling voor de MMSS, namelijk lactaatrempels en het respiratoire compensatiepunt bepaald uit STEP- of RAMP-protocollen waarbij respectievelijk capillaire bloedstalen of pulmonale gasuitwisseling worden gebruikt. De populariteit van deze inspanningstesten is gebaseerd op de tijdsefficiënte en haalbare manier om inspanningsdrempels te bepalen.

In het eerste deel van dit proefschrift werd het effect van acute blootstelling aan hitte op de bepaling van de zwaar-zeer zware intensiteitsgrens onderzocht. Omdat de klimaatverandering de globale temperatuur beïnvloedt, wordt blootstelling aan hitte een steeds grotere uitdaging voor individuen die aan lichaamsbeweging doen, of deelnemen aan recreatieve of competitieve sportwedstrijden. Bovendien wordt hittestress ook gebruikt tijdens het trainingsproces (warmteacclimatie/ acclimatisatie) om gunstige trainingseffecten en fysiologische/hematologische aanpassingen te bereiken om prestaties in zowel warme als gematigde omgevingen te optimaliseren. De fysiologische respons op inspanning in de hitte is complex, omdat de ontwikkeling en omvang van deze aanpassingen afhankelijk zijn van veel factoren, zoals de ernst van de hittestress, voorafgaande verwarming van het hele lichaam, individuele kenmerken, de intensiteitsdistributie van de inspanning en de duur van de blootstelling. De ontwikkelde hittebelasting resulteert in veranderingen in het cardiovasculaire systeem, het centrale zenuwstelsel en de skeletspierfunctie. We toonden aan dat de interactie tussen inspanningsintensiteit en inspanningsduur binnen inspanningstestprotocollen de grootte bepaalt van de afname in het vermogen dat overeenkomt met de inspanningsdrempel. Er werden geen geslachtsverschillen gevonden in het effect van acute blootstelling aan hitte op prestatie en de bepaling van de zwaar-zeer zware

intensiteitsgrens, althans voor de CP-testmethodologie. De grote interindividuele variatie in respons op hitteblootstelling in *studie 1* en *studie 2* ondersteunt het feit dat zelfs een korte incrementele inspanningstest met acute hitteblootstelling waardevol inzicht kan geven in de acute hitte respons van een atleet. Dit zou extra ondersteuning kunnen bieden voor prestatiediagnostiek, het optimaliseren van het voorschrijven en sturen van het trainingsproces en het monitoren van de trainingsintensiteit in warme omgevingen.

Aangezien fysieke activiteit en veel sporten gepaard gaan met frequente veranderingen in inspanningsintensiteit, kan het CP-concept ook toegepast worden in intermittente sporten, waarbij inspanning boven CP ( $W'$  depletie) wordt afgewisseld met inspanning onder CP ( $W'$  reconstitutie). Daarom is het tweede deel van dit proefschrift gericht op de parameter  $W'$ . Sinds het begin van de 21<sup>e</sup> eeuw heeft het onderzoek naar  $W'$  reconstitutie een exponentiële ontwikkeling doorgemaakt, mogelijk door de implementatie van technische innovaties (bv. de ontwikkeling van vermogensmeters in de wielersport), en de mogelijkheden ervan om de energiebalans van een individu tijdens intermittente inspanning te monitoren. Enkele jaren geleden werd door inspanningsfysiologen een model ontwikkeld dat inzicht gaf in de energiebalans tijdens intermitterende inspanning, oftewel de beschikbare  $W'$  op elk moment in de tijd (het  $W'_{\text{BAL}}$  model). Dit model, bestaande uit een mono-exponentiële  $W'$  herstelkinetiek, zou kunnen worden gebruikt voor het voorspellen van prestaties en het optimaliseren van pacing-strategieën. Na de ontwikkeling van het model rees echter al snel de vraag of het  $W'_{\text{BAL}}$  model een universeel model is voor iedereen en voor elk inspanningsprotocol. Daarom hebben we twee experimentele studies uitgevoerd (*studie 3* en *studie 4*) om een beter inzicht te krijgen in de onderliggende mechanismen van  $W'$  herstel. We ontdekten dat een exponentieel model met twee fasen superieur is om het herstel van  $W'$  te voorspellen, in plaats van een mono-exponentiële fit, na een training waarbij  $W'$  volledig wordt uitgeput. Bovendien kunnen kenmerken van het trainingsprotocol, zoals herhaalde blokken tot uitputting, de  $W'$  reconstitutie vertragen, en wordt de snelheid van  $W'$ -herstel sterk beïnvloed door individuele factoren, zoals aerobe conditie en geslacht. Dit heeft belangrijke implicaties voor het voorschrijven van training bij mannen en vrouwen en duidt op het belang om geslacht als een biologische variabele te includeren in sportwetenschappelijk onderzoek. Op basis van eerder werk van collega's uit dezelfde onderzoeksgroep en het werk dat is gedaan tijdens dit doctoraatsproject, zijn we begonnen met de ontwikkeling van een nieuw model om de voorspelling van  $W'$  herstel te optimaliseren. Daarom is een multifactoriële benadering nodig om een nauwkeurige inschatting te maken van de  $W'$  reconstitutie, rekening houdend met individuele kenmerken en trainingsmodaliteiten. Bovendien is er een optimum nodig tussen wiskundige finetuning en de integratie van fysiologische processen om een solide  $W'$  reconstitutiemodel op te stellen.

## RÉSUMÉ EN FRANÇAIS

L'exercice physique est un aspect crucial dans le maintien de la santé et du bien-être. La participation à des sports récréatifs ou professionnels implique différents niveaux de demande métaboliques. Dans ce contexte, une bonne compréhension des processus physiologiques qui se déroulent aux différentes intensités d'exercice est vitale pour optimiser l'entraînement et la performance. En général, l'intensité de l'exercice peut être répartie en trois domaines : modérée, intense et sévère. Pour fixer des objectifs réalistes, prescrire un entraînement et surveiller les performances (à long terme), il est essentiel de déterminer la limite entre le domaine intense et sévère. Ce seuil marque la limite entre un exercice durable et un exercice non durable ; il peut donc être considéré comme le travail le plus élevé auquel un équilibre métabolique est possible, c'est-à-dire l'état d'équilibre métabolique maximal (EEMM). La puissance critique ou critical power (CP), qui s'inscrit dans un cadre mathématique avec des mécanismes physiologiques sous-jacents, est souvent présentée comme la méthodologie de référence pour déterminer l'état d'équilibre métabolique maximal. Cette CP est l'asymptote de la relation hyperbolique entre la puissance et le temps d'épuisement. Avec  $W'$ , la constante de courbure (ou la capacité de travail qui peut être dépensée au-dessus de la CP), elle forme le concept de CP. Cependant, il existe également des concepts de seuil qui servent d'estimation indirecte d'EEMM, à savoir les seuils de lactate et le point de compensation respiratoire déterminés à partir des protocoles STEP ou RAMP en utilisant respectivement des échantillons de sang capillaire ou une mesure des échanges gazeux pulmonaires. La popularité de ces tests d'exercice est due au fait qu'ils permettent de déterminer les seuils d'exercice de manière rapide et pratique.

Dans la première partie de cette thèse de doctorat, l'effet d'une exposition aiguë à la chaleur sur la détermination du seuil d'intensité intense-sévère a été étudiée. Le changement climatique affectant la température globale, l'exposition à la chaleur devient un défi de plus en plus important pour les personnes pratiquant une activité physique, un sport de loisir ou de compétition. Par ailleurs, le stress thermique est également utilisé pendant le processus d'entraînement (l'acclimation/acclimatation à la chaleur) pour obtenir des effets bénéfiques et des adaptations physiologiques/hématologiques afin d'optimiser les performances dans les environnements chauds ou tempérés. La réponse physiologique à l'exercice dans la chaleur est complexe, car l'évolution et l'étendue de ces ajustements dépendent de nombreux facteurs tels que la sévérité du stress thermique, le réchauffement préalable du corps, les caractéristiques individuelles, l'intensité de l'exercice et la durée de l'exposition. La contrainte thermique générée entraîne des altérations du système cardiovasculaire, du système nerveux central et de la fonction des muscles squelettiques. Nous avons montré que l'interaction entre l'intensité et la durée de l'exercice dans les protocoles de test d'exercice définit l'ampleur de la diminution de la puissance de pédalage correspondant au seuil. Aucune différence entre les sexes n'a été constatée en

ce qui concerne l'effet de l'exposition aiguë à la chaleur sur la performance et la détermination du seuil lourd-sévère, du moins pour la méthodologie de test CP. L'importante variation interindividuelle de la réponse à l'exposition à la chaleur dans *l'étude 1* et *2* confirme le fait que même un test d'exercice incrémental de courte durée avec exposition aiguë à la chaleur peut donner des indications précieuses sur la réponse aiguë à la chaleur d'un athlète. En retour, cela fournit des informations utiles pour optimiser la prescription de l'entraînement et le suivi de l'intensité de l'exercice.

Comme de nombreuses activités physiques et sports impliquent des changements fréquents dans l'intensité de l'exercice, le concept de CP a également été appliqué à l'exercice intermittent, dans lequel l'exercice au-dessus de la CP (épuisement de  $W'$ ) est alterné avec l'exercice en dessous de la CP (reconstitution de  $W'$ ). Par conséquent, la deuxième partie de cette thèse de doctorat se concentre sur le paramètre  $W'$ . Depuis le début du 21<sup>ème</sup> siècle, l'évolution de la recherche sur la reconstitution du  $W'$  progresse de manière exponentielle, probablement en raison de sa détermination facilitée par des innovations techniques (par ex., le développement des wattmètres dans le cyclisme) et une progression dans les capacités à surveiller l'équilibre énergétique d'un individu au cours d'un exercice intermittent. Il y a plusieurs années, des physiologistes de l'exercice ont mis au point un modèle qui donnait une idée de l'équilibre énergétique pendant un exercice intermittent, ou en d'autres termes du  $W'$  disponible à tout moment : le modèle  $W'_{BAL}$ . Ce modèle, qui consiste en une cinétique de récupération mono-exponentielle de  $W'$ , pourrait être utilisé pour la prédiction des performances et l'optimisation des stratégies d'allure. Cependant, une question a été très rapidement soulevée après le développement du modèle, à savoir si le modèle  $W'_{BAL}$  est un modèle universel pour tout le monde et tous les protocoles d'exercice. Nous avons donc réalisé deux études expérimentales (*étude 3 et 4*) pour mieux comprendre les mécanismes sous-jacents de la récupération du  $W'$ . Nous avons constaté qu'un modèle exponentiel à deux phases est meilleur pour prédire la reconstitution du  $W'$ , au lieu d'un ajustement mono-exponentiel, après un exercice épuisant complètement le  $W'$ . De plus, les caractéristiques du protocole d'exercice, comme des séances répétées jusqu'à l'épuisement, peuvent ralentir la reconstitution du  $W'$ , et la vitesse de récupération du  $W'$  est fortement influencée par des facteurs individuels, tels que l'aptitude aérobie et le sexe. Cela a des implications importantes pour la prescription d'entraînement chez les hommes et les femmes et indique l'importance d'inclure le sexe comme variable biologique dans la recherche en sciences du sport. Sur la base de travaux antérieurs réalisés par des collègues du même groupe de recherche et des travaux réalisés dans le cadre de ce projet de doctorat, nous avons pu initier le développement d'un nouveau modèle pour optimiser la prédiction de la récupération du  $W'$ . Une approche multifactorielle est nécessaire pour estimer avec précision la reconstitution du  $W'$ , considérant des caractéristiques individuelles et des modalités d'exercice. Afin d'avoir un modèle solide de la reconstitution du  $W'$ , un équilibre est nécessaire entre la modélisation mathématique et l'intégration des processus physiologiques.

## LIST OF ABBREVIATIONS

|          |   |
|----------|---|
| ANOVA    | Analysis of variance                            |
| ATP      | Adenosine triphosphate                          |
| BF       | Body fat  |
| CI       | Confidence interval                             |
| CNS      | Central nervous system                          |
| CP       | Critical power                                  |
| CWR      | Constant work rate                              |
| $D_{CP}$ | Difference between recovery power output and CP |
| GET      | Gas exchange threshold                          |
| HOT      | Hot environmental conditions                    |
| HR       | Heart rate                                      |
| $[La^-]$ | Lactate concentration                           |
| LBM      | Lean body mass                                  |
| LT       | Lactate threshold                               |
| MFT      | Muscle fiber type                               |
| MLSS     | Maximal lactate steady state                    |
| MMSS     | Maximal metabolic steady state                  |
| MRT      | Mean response time                              |
| OBLA     | Onset of blood lactate accumulation             |
| PO       | Power output                                    |
| Q        | Cardiac output                                  |
| RAMP     | Ramp incremental exercise                       |
| RCP      | Respiratory compensation point                  |
| RER      | Respiratory exchange ratio                      |
| RH       | Relative humidity                               |
| RMSE     | Root mean square error                          |
| RPE      | Rate of perceived exertion                      |
| RT       | Respiratory threshold                           |
| SE       | Standard error                                  |
| STEP     | Step incremental exercise                       |
| SV       | Stroke volume                                   |
| TEMP     | Temperate environmental conditions              |
| TTE      | Time to exhaustion                              |
| TTF      | Time to task failure                            |

|                   |                                    |
|-------------------|------------------------------------|
| $\tau_{W'}$       | $W'$ recovery time constant tau    |
| $\dot{V}E$        | Ventilation                        |
| $\dot{V}CO_2$     | Carbon dioxide production          |
| $\dot{V}O_2$      | Oxygen uptake                      |
| $\dot{V}O_{2max}$ | Maximal oxygen uptake              |
| $W'$              | $W$ prime (work capacity above CP) |
| $W'_{BAL}$        | $W$ prime balance                  |
| $W'_{REC}$        | $W$ prime recovery                 |
| 3MT               | 3 minute all-out test              |

# *Chapter 1*

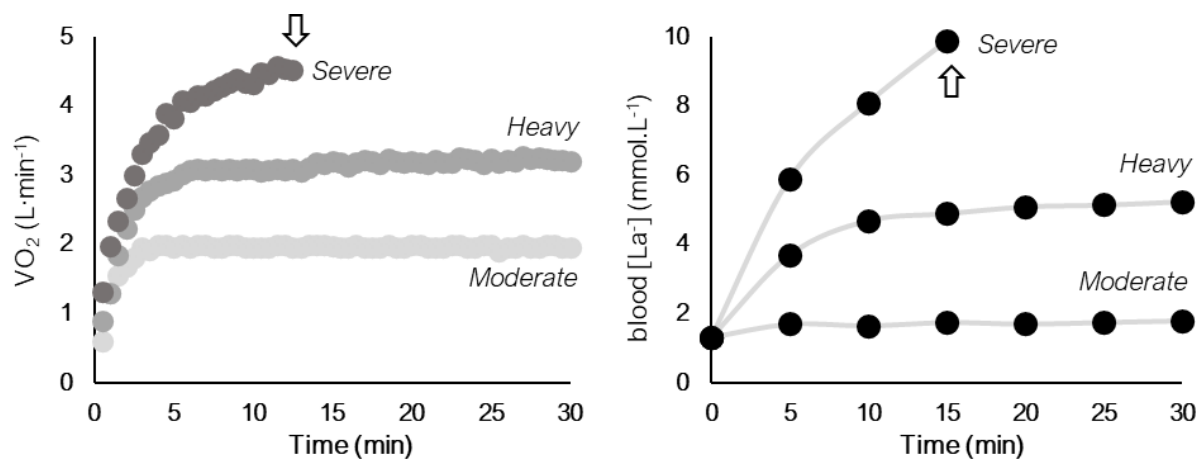
## *General introduction*



## 1.1 The fundamentals

Exercise is a crucial aspect of maintaining overall health and well-being. Humans engage in various activities, where they often shift between different metabolic rates, such as transitioning from a seated position to climbing stairs, running to catch transportation or engaging in physical labor. Participating in recreational or professional sports involves a range of metabolic demands, and a proper understanding of physiological processes taking place at different intensities is vital for optimizing training and performance.

Exercise intensity refers to the level of effort required to perform physical activity and quantifies the metabolic response to exercise, which encompasses the physiological changes that occur to meet the adenosine triphosphate (ATP) requirement for energy supply. The exercise intensity domain schema proposed by Whipp & Mahler (1980) has been a fundamental model in explaining metabolic responses and exercise tolerance. This schema identifies specific ranges of intensities, known as the moderate, heavy and severe intensity domain, in which oxygen uptake ( $\dot{V}O_2$ ) and blood lactate concentration ( $[La^-]$ ) have common characteristics (Poole & Jones, 2012; see Figure 1). This framework provides a direct advantage by establishing a set of domain-specific mechanisms that determine metabolic responses and tolerable duration of exercise (Black et al., 2017).



**Figure 1** Visual representation of oxygen uptake ( $\dot{V}O_2$ ) (left panel) and blood lactate concentration (blood  $[La^-]$ ) (right panel) response to exercise in the moderate, heavy and severe intensity domain. Point of task failure or exhaustion in the severe intensity domain is denoted by an arrow (reproduced from Poole & Jones, 2012).

Moderate exercise intensity is characterized by a gradual increase in  $\dot{V}O_2$  as the body adjusts to the increased energy demands of the activity. A steady state  $\dot{V}O_2$  is reached after approximately 90 to 180 seconds (Poole & Jones, 2012). The upper limit ranges between 50-80% of an individual's maximal

oxygen uptake ( $\dot{V}O_{2\max}$ ), determined mainly by the oxidative capacity of the skeletal muscle (Joyner & Coyle, 2008; van der Zwaard et al., 2021). Blood  $[La^-]$  remains relatively low and stable, as the body is able to maintain a balance between lactate production and clearance through oxidative metabolism.  $\dot{V}O_2$  reaches a steady state proportionally to work rate, indicating that ATP production is being met predominantly via oxidative phosphorylation of fat and carbohydrates. Exercise in this domain can be sustained for a prolonged period of time, as the body is able to maintain a steady state level of aerobic energy expenditure, notwithstanding that central fatigue (i.e., decreased neural drive and a loss in motivation) can influence the exercise duration (Barstow et al., 1994; Black et al., 2017; Burnley & Jones, 2007; Poole et al., 2016; Poole & Jones, 2012).

During heavy exercise intensity, the body experiences a delayed steady state of  $\dot{V}O_2$  due to the increased energy demand, after approximately 15 minutes (Gaesser & Poole, 1996; Whipp & Wasserman, 1972). It has been suggested that the recruitment of less efficient type II motor units and metabolic instability (i.e., accumulation of metabolites and depletion of substrates) within the working fibers are the main physiological factors contributing to the muscular component of the delayed steady state of  $\dot{V}O_2$  during exercise, together with the extra cost of ventilatory and cardiac work (Colosio et al., 2021; Gaesser & Poole, 1996; Jones et al., 2011). Oxidative phosphorylation of carbohydrates is still the main source of ATP production, although anaerobic respiration, with the formation of lactic acid (further dissociated in lactate and  $H^+$ ), contributes to the energy supply to meet the additional demand. During the initial 5 to 10 minutes of heavy intensity exercise, blood  $[La^-]$  increases, however, it tends to stabilize thereafter as the production and elimination reach an equilibrium (Black et al., 2017; Poole et al., 1988). Even though muscle and capillary lactate concentrations increase during exercise in the heavy intensity zone, the anaerobic glycolytic contribution to the total energy supply remains minor, compared to aerobic energy delivery.

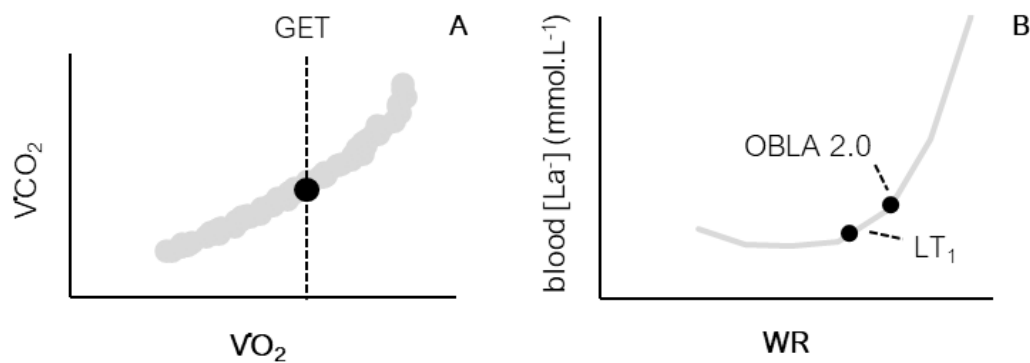
In the severe intensity domain, the body is unable to achieve a steady state, resulting in a continuous increase in both  $\dot{V}O_2$  and blood  $[La^-]$  until the point of exhaustion (Poole & Jones, 2012). Maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) will be reached together with a high, although not necessarily the highest, blood  $[La^-]$ . The oxidative energy system alone is insufficient to sustain the required metabolic rate within the severe intensity domain, resulting in the involvement of anaerobic energy sources. Factors such as the attainment of critical levels of metabolites and possible partial or full substrate depletion, depending on exercise intensity and duration, within the exercising muscles contribute to peripheral fatigue in this domain (Black et al., 2017; Chidnok et al., 2013; Jones et al., 2011). This phenomenon occurs along with increased signaling from group III/IV muscle afferents, altered motor unit recruitment patterns, and increased muscle temperature (Amann et al., 2011; Grassi et al., 2015). These mechanisms decrease mechanical efficiency and impair exercise tolerance. Due to the high metabolic load during severe exercise, exhaustion occurs relatively quickly with time to exhaustion varying between a couple of

minutes up to 30-45 minutes, depending on work intensity. It is noteworthy that the extent of peripheral fatigue observed during time-to-exhaustion trials in the severe domain remains consistent regardless of the task duration (Black et al., 2017; Burnley et al., 2012).

Boundaries of these different exercise intensity domains, often referred to as exercise thresholds, represent the points of transition where the physiological processes change significantly. The determination of such exercise thresholds has gained considerable importance in exercise science in the past few decades, as they allow to evaluate an individual's fitness level and estimate performance. Furthermore, they are also commonly used to prescribe and monitor training and/or rehabilitation programs.

Numerous methods have been proposed for determining a threshold in exercise metabolism with ensuing debate about which is best. Two scientific schools are responsible for the advancements in the physiological understanding and determination of exercise thresholds since the 1960 of the last century. In the Anglo-Saxon world, the group of Karlman Wasserman (e.g., Wasserman & McIlroy, 1964; Wasserman et al., 1973) was recognized for major breakthroughs using novel non-invasive breath-by-breath gas exchange technology. Similar ideas were developed by the Cologne-based group of Wildor Hollmann, Alois Mader and Hermann Heck (e.g., Mader et al., 1976; Heck et al., 1985) based on blood  $[La^-]$  determination measurements. However, at that time, they did not receive recognition equivalent to Wasserman's efforts, perhaps because of its invasive methodology or even ignorance, as most of the research was published in German (Wackerhage et al., 2022). This changed from the 1980s onwards, as it nowadays a frequently used manner to determine exercise thresholds.

It is widely accepted that the boundary between moderate and heavy exercise can be defined by means of pulmonary gas exchange or blood  $[La^-]$  (Jamnick et al., 2020). For example, a visible upwards inflection of carbon dioxide production ( $\dot{V}CO_2$ ) vs.  $\dot{V}O_2$  during ramp incremental exercise (RAMP) determines the gas exchange threshold (GET) (Beaver et al., 1986) (see Figure 2A). The first lactate threshold or turn point ( $LT_1$ ) is stipulated by the first significant increase in blood  $[La^-]$  above baseline values during step incremental exercise (STEP) (Binder et al., 2008). A fixed blood  $[La^-]$  value of  $2 \text{ mmol}\cdot\text{L}^{-1}$  (i.e., onset of blood  $La^-$  accumulation or OBLA 2.0) is also often used to indicate the first lactate threshold (Skinner & McLellan, 1980), however work rate corresponding to these lactate thresholds can differ significantly (see Figure 2B). The occurrence of both thresholds (i.e., GET and first lactate threshold) is reflective of changes in the energy supply during the transition from moderate to heavy exercise as they mark the upper limit of nearly exclusive aerobic metabolism. Despite the scientific value of the moderate-to-heavy boundary and its significance for training practice, the current dissertation will only focus on the boundary between heavy and severe exercise and exercise above this threshold.



**Figure 2** Threshold determination of the moderate-heavy intensity boundary, marked by (A) the gas exchange threshold (GET) determined through pulmonary gas exchange, and (B) lactate turn point (LT<sub>1</sub>) or onset of blood lactate accumulation (OBLA 2.0).  $\dot{V}O_2$ , oxygen uptake;  $\dot{V}CO_2$ , carbon dioxide production; WR, work rate; blood  $[La^-]$ , blood lactate concentration.

The heavy-severe exercise intensity boundary denotes the highest metabolic rate at which homeostasis (i.e., equilibrium of the internal milieu) can be maintained, i.e., the maximal metabolic steady state (MMSS) (Iannetta et al., 2022; Jones et al., 2019). It is worth noting that the use of the term MMSS in this context is independent of the determination method used (such as gas exchange or blood  $[La^-]$ ) and solely pertains to the boundary between heavy and severe exercise. Debate consists on which threshold concept and underlying mechanism truly corresponds to this MMSS (Jones et al., 2019). However, this discussion will not be held within the scope of this manuscript, as we will not compare them directly. Two threshold concepts are widely considered as a "gold standard" for identifying this intensity. The first method for determining MMSS is by analyzing the kinetics of blood  $La^-$  during prolonged constant work rate (CWR) exercise, known as the maximal lactate steady state (MLSS). The MLSS is defined as the highest intensity where there is an equilibrium between blood  $La^-$  production and maximal elimination (*chapter 1.2.1*) (Billat et al., 2003; Faude et al., 2009; Heck, 1990). The second is the critical power (CP), which corresponds to the power-asymptote of the hyperbolic relationship between power output (PO) and exercise duration (Hill, 1993; Jones et al., 2010; Poole et al., 2016). This concept and its practical implications will be the main focus of this dissertation (see *chapter 1.2.2* and original *studies 1, 3 and 4*).

Given the extensive test protocol required for the determination of MLSS and CP, numerous other threshold concepts have been proposed to define the upper boundary of sustainable energy from a single incremental exercise test. The determination of these thresholds are based on pulmonary gas exchange or blood  $[La^-]$ , likewise the boundary between the moderate and heavy intensity domain (i.e., GET and LT<sub>1</sub>). During RAMP or short STEP (< 1 min stages) the respiratory compensation point (RCP) defines the metabolic intensity at which hyperventilation starts, by an inflection of ventilation ( $\dot{V}E$ ) vs.

$\dot{V}\text{CO}_2$  response (Binder et al., 2008; Keir et al., 2022; Wasserman, 1984). Several points on the blood  $[\text{La}^-]$  curve established during STEP, using different methodologies, give an estimation of the MLSS (Faude et al., 2009; Jamnick et al., 2018). These indirect estimations or surrogates of the MMSS will be discussed more in depth in *chapter 1.2.3* and original *study 2*.

Since climate change is affecting global temperature (Haines et al., 2006), heat waves becoming more frequent and intense in the 21<sup>st</sup> century (Meehl & Tebaldi, 2004). Next to that, there is increasing popularity of (mass participation) sporting events under extreme hot (and humid) environmental conditions (e.g., Ironman Triathlon in Hawaii, United States of America, Marathon des Sables in the Moroccan part of the Sahara desert, etc.). It has been stated that recreational and elite athletes are likely to face performance challenges and potential health risks due to extreme heat exposure (Racinais et al., 2015). Heat stress is also used during the training process (i.e., heat acclimation/acclimatization) to achieve beneficial training effects and physiological/hematological adaptations to perform in hot as well as temperate environments (Baranauskas et al., 2021; Lundby et al., 2023; Maunder et al., 2021; Mikkelsen et al., 2019; Oberholzer et al., 2019; Rønnestad et al., 2021; Rønnestad et al., 2022a; Rønnestad et al., 2022b). Therefore, the determination of exercise thresholds in hot environments is necessary as these are employed for performance diagnostics, prescription and steering of the training process, and monitoring exercise intensity. It will help to understand the physiological responses to exercise in these conditions and provide information on performance parameters and the design of training programs for individuals who need to perform in hot environments (see *chapter 1.3* and original *studies 1 and 2*).

## 1.2 The heavy-severe exercise intensity boundary

Debate consists in the sports science and exercise physiology community which threshold concept “truly” reflects the boundary between the heavy and severe intensity domain and thus is favorable to be considered as the “real” MMSS (Jones et al., 2019). Moreover, recently the abrupt distinction between heavy and severe intensity, marked by the MMSS, has been questioned, as it has been demonstrated that there is a transition phase or ‘grey zone’ from heavy to severe, rather than a sudden threshold (Ozkaya et al., 2022; Pethick et al., 2020). This implies that each threshold marker has a band of uncertainty around them (Jones et al., 2019), taking into account biological and methodological variation (Caen et al., 2022; Poole et al., 1988). Although these studies provide valuable insights into the mechanisms underpinning exercise thresholds, the debate on the “true” reflection of the MMSS falls outside the scope of this manuscript .

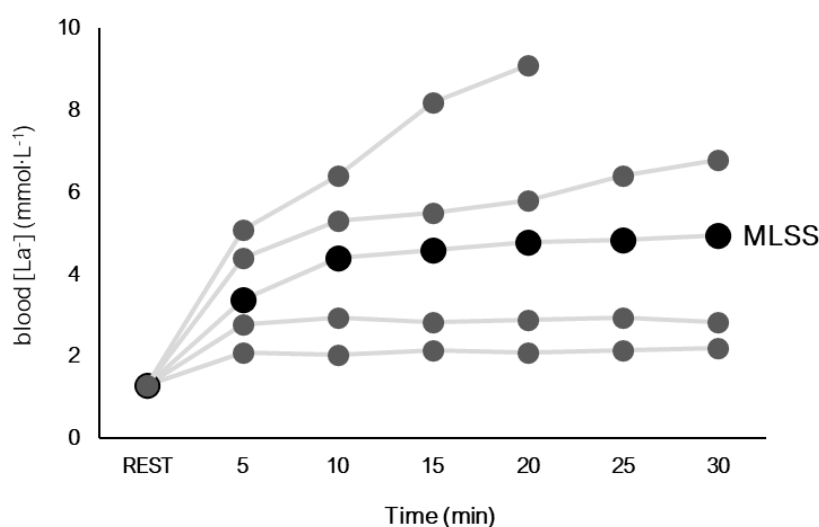
The MLSS (*chapter 1.2.1*) with the direct measurement (i.e., blood  $\text{La}^-$ ) of the physiological response to exercise, and the CP (*chapter 1.2.2*), as part of a mathematical framework with underlying physiological mechanisms, are both determined using multiple CWR trials and put forward as the gold standard methodology to determine MMSS (Iannetta et al., 2022; Jones et al., 2019). The RCP and several lactate thresholds during non-steady state incremental exercise, respectively RAMP and STEP (*chapter 1.2.3*), are considered as surrogates or estimates of the MMSS (Jamnick et al., 2018; Keir et al., 2022). All of these threshold concepts have a common characteristic, which is the identification of a metabolic “boundary” that causes changes in muscle metabolism, leading to alterations in cellular and physiological homeostasis. The variability in the outcome could stem from issues related to the methodology and approaches used for their determination.

This dissertation will not discuss the equivalence of these thresholds, nor the validity to determine the MMSS. For this, the reader is referred to other studies (Caen et al., 2022; Caen et al., 2018; Iannetta et al., 2022; Mattioni Maturana et al., 2016). Nevertheless, all concepts will be described with the CP concept as the main focus of this dissertation. It does not only determine the MMSS, but additionally also provides insight on the work capacity that can be performed above CP.

### 1.2.1 Maximal Lactate Steady State

The maximal lactate steady state (MLSS) is the highest exercise intensity that can be sustained without a significant rise in blood  $[\text{La}^-]$  over time, as it represents the highest level of equilibrium between lactate production in the muscles and maximal elimination or clearance from the bloodstream (Billat et al., 2003; Faude et al., 2009; Heck, 1990).

The denomination of the MLSS was first used by Heck et al. (1985) during successive 25 min CWR treadmill tests at different intensities. The blood  $[La^-]$  was measured at rest and after every 5 min of exercise. MLSS was defined as the highest speed that did not result in a rise of blood  $[La^-]$  greater than 1  $mmol \cdot L^{-1}$  in the last 20 min. While the general methodology for MLSS determination has remained largely unchanged over the years, minor modifications have been made by researchers to the test protocol and determination criteria (Beneke, 2003). These adjustments have led to some degree of methodological variation in the existing literature, primarily concerning the trial duration, the magnitude of the power output increments or decrements between successive tests, and the criteria for the maximum permissible increase in blood  $[La^-]$ . Since the beginning of the 21<sup>st</sup> century, it has been suggested that the protocol to establish the MLSS contains a series (3-5) of 30 minutes CWR bouts (see Figure 3), where the rise in blood  $[La^-]$  is < 1.0  $mmol \cdot L^{-1}$  from the 10th to the 30th minute (Beneke, 2003). A second optional criterion sometimes is added, i.e., a rise in blood  $[La^-]$  < 0.5  $mmol \cdot L^{-1}$  from the 20th to the 30<sup>th</sup> min, due to possible delayed steady state after 20 minutes instead of 10 minutes (Bourgois & Vrijens, 1998). It has been shown that the blood  $[La^-]$  at MLSS is not equal in all individuals and can vary considerably, as values from 2 up to 10  $mmol \cdot L^{-1}$  were reported in several studies (Beneke et al., 2000; Bourgois & Vrijens, 1998; MacIntosh et al., 2003; Van Schuylenbergh et al., 2004).



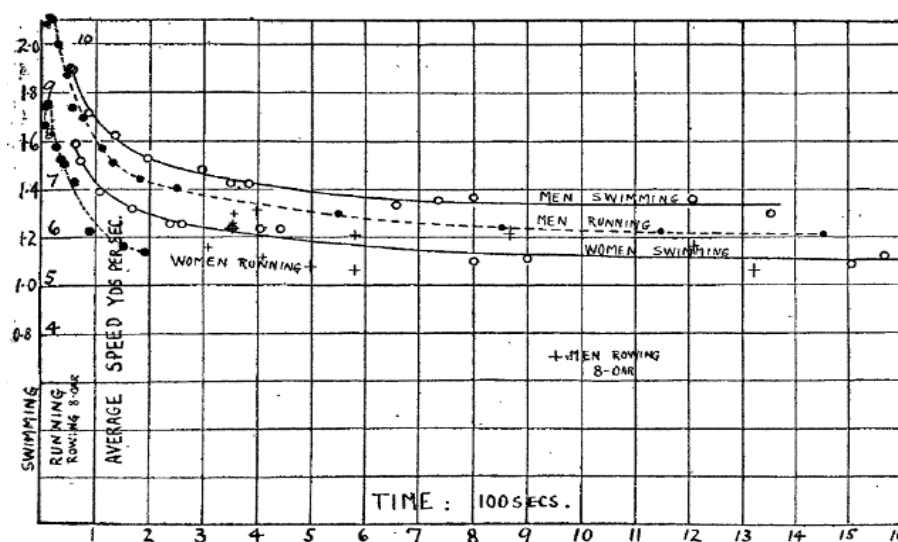
**Figure 3** Blood  $[La^-]$  response during CWR trials at different intensities to determine the maximal lactate steady state (MLSS) (based on figure of Beneke et al., 2000).

The reliance on a direct measurement of the physiological response during CWR exercise is stated to be the main reason why the MLSS was preferred as the gold standard to determine the MMSS. However, the concept is not without its limitations, as there are the practical feasibility (invasive measurement and time-consuming protocol) and, although also mentioned as a strength, the reliance on a single parameter (i.e., blood  $[La^-]$ ). Absolute values of blood  $[La^-]$  can be influenced by several factors, such as

sampling site (ear lobe or fingertip), blood specimen type (venous, arterial or capillary blood), analytical techniques (whole blood, plasma or hemolyzed blood), ambient air temperature, prior exercise, changes in glycogen reserves and dehydration (Jacobs, 1986). Furthermore, the accuracy of measuring blood  $[La^-]$  also relies on the reliability and accuracy of the analytical device used, with reported measurement error between 0.2 to 0.5 mmol·L<sup>-1</sup> (Bonaventura et al., 2015). Moreover, whether MLSS and its dependency on blood  $[La^-]$  truly reflect the intramuscular processes is questioned, given the dynamic interplay between muscle and blood  $[La^-]$  (Ferguson et al., 2018). Regardless of its limitations, consistency in exercise test procedures and interpretation might still be the most crucial factor to take into account. Although the MLSS concept has its value and usefulness, this methodology will not be further discussed in this dissertation. Exercise thresholds as part of a broader application (i.e., CP) or defined via less time consuming incremental protocols (i.e., RCP and lactate thresholds) were preferred to determine the heavy-severe intensity boundary and were investigated more in detail.

### 1.2.2 Critical Power concept

The foundations of today's critical power (CP) concept can be found in the beginning of the 20<sup>th</sup> century. In 1925, the renowned physiologist Archibald V. Hill established speed-time curves, based on world records at various distances in runners, swimmers and rowers. This resulted in a hyperbolic relationship between the intensity of the maximal effort and time to exhaustion (Hill, 1925; Figure 4).



**Figure 4** The hyperbolic relationship of world records for men and women (running, swimming and rowing) as observed in 1925 (original graph copied from Hill, 1925).

Importantly, such a hyperbolic relationship remains valid not only when current world records are considered, but also when an individual athlete performs exercise at different intensities until

exhaustion or task failure, for both single-limb exercise (Monod & Scherrer, 1965) as well as whole-body exercise (Moritani et al., 1981). The consistency of these observations indicates that the power-duration relationship, and the bioenergetics underpinning it, are an integral feature of exercise performance. This power-duration relationship has been quantified within the so-called CP concept. This concept does not only provide insight in the performance capacity of human beings, as it has been shown to be an integral reflection of evolutionary developments towards the physiological understanding of survival and reproduction in the animal kingdom (Burnley, 2023). As such, its unique as well as broad application is demonstrated.

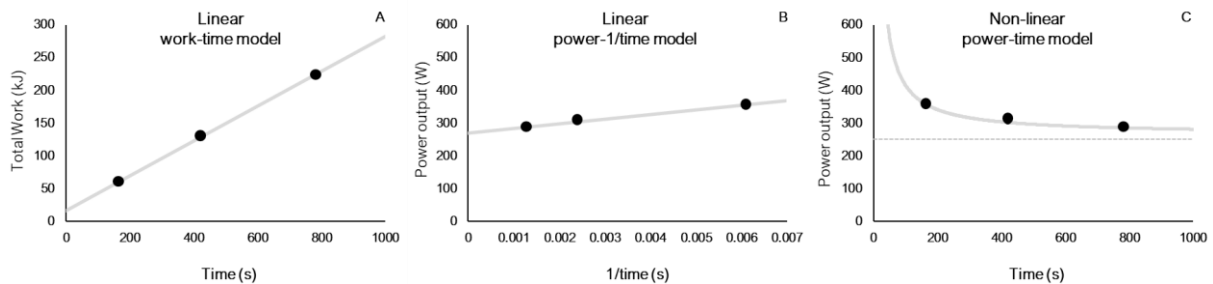
#### 1.2.2.1 Mathematical and physiological framework

It were Monod & Scherrer (1965) who first observed a linear relationship between total work of the muscle, performed during dynamic contractions at different intensities, and the obtained time to exhaustion (TTE; equivalent with the terms time to task failure (TTF) and limit of tolerance) (Equation 1 and Figure 5A). The y-intercept corresponded to the available energy reserve of the muscle, which was then defined as the anaerobic work capacity (AWC) and is nowadays known as the  $W'$  prime ( $W'$ ). The slope represents the maximum work rate that a muscle (group) could keep up for a very long time without fatigue and was called, for the first time, critical power (CP). A second equivalent transformation is the  $1/\text{time}$  model (Equation 2 and Figure 5B), and the most widespread transformation is the hyperbolic model (Equation 3 and Figure 5C), representing the initial finding of Hill (1925). In the equations, the parameter  $W$  is the total work performed,  $P$  is a given PO above CP and  $t$  is the time to task failure.

$$W = W' + CP \times t \quad (1)$$

$$P = W' \times \left(\frac{1}{t}\right) + CP \quad (2)$$

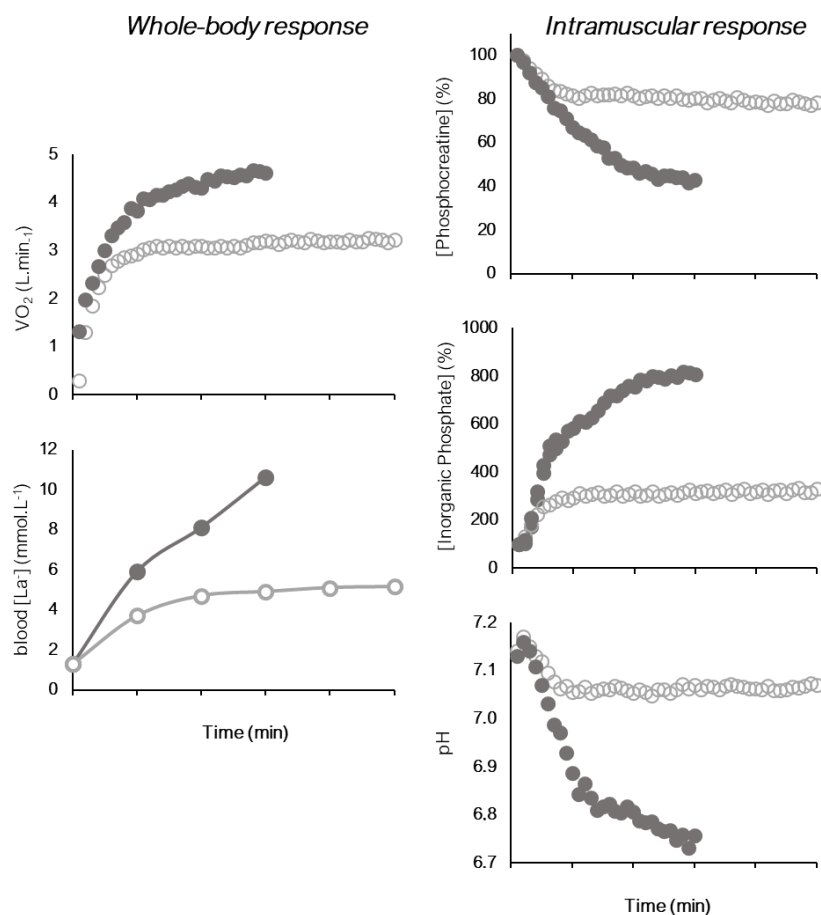
$$t = \frac{W'}{P - CP} \quad (3)$$



**Figure 5** Regression analyses and curve fits for each of the three two-parameter mathematical models that are commonly used to determine CP and  $W'$ : (A) linear work-time model, (B) linear power- $1/\text{time}$  model and (C) non-linear or hyperbolic power-time model.

These versions of the two-parameter CP model have been employed interchangeably in scientific studies for the determination of CP and  $W'$ . Nevertheless, it is crucial to note that these model variants, despite their mathematical similarity, are not statistically equivalent in terms of predicting the CP and  $W'$  parameters. Several investigations have demonstrated that the estimations for both parameters may differ significantly among the used models (Bergstrom et al., 2014; Bull et al., 2000; Gaesser et al., 1995).

From a physiological perspective, CP has been considered as the threshold between the heavy-intensity domain and severe-intensity domain (Jones et al., 2008; Poole et al., 1988). More specifically, for CWR exercise below CP the physiological responses ( $\dot{V}O_2$ , blood  $[La^-]$  and muscle metabolites) will reach a steady state. On the other hand, CWR exercise above CP will result in non-steady state conditions, and whole-body ( $\dot{V}O_2$ , blood  $[La^-]$ ) and intramuscular (phosphocreatine (PCr), inorganic phosphate ( $P_i$ ), pH) responses evolve to maximal or “critical” values (Figure 6). CP is therefore considered as a critical threshold for intramuscular metabolic control (Black et al., 2017; Chidnok et al., 2013; Jones et al., 2008; Poole & Jones, 2012; Poole et al., 2020).



**Figure 6** Whole body (oxygen uptake and blood lactate concentration) and intramuscular (phosphocreatine and inorganic phosphate concentration relative to baseline value, and pH) response to exercise above (dark gray) and below (light gray) CP (reproduced from Poole et al., 2020; Poole & Jones, 2012; Jones et al., 2008).

The mathematical nature of the CP concept has certain consequences on the interpretation of the physiological meaning. The CP concept defines a two-component bioenergetic supply-and-demand system, which are based on certain assumptions (Hill, 1993; Jones et al., 2010; Morton, 2006):

1. There is an “aerobic” energy supply component that is rate- but not capacity-limited, i.e., CP.
2. There is an “anaerobic” energy supply component that is capacity- but not rate-limited, i.e.,  $W'$ .
3. Exercise can continue at any PO as long as supply is adequate to meet demand.

The biochemical pathways of the human energy metabolism are far too complex than just a two-component system, as CP and  $W'$  should not be seen as separate aerobic or anaerobic energy delivery systems, but rather as part of an integrated bioenergetic system (Poole et al., 2016). It has been shown that CP and  $W'$  are not independent from each other and can be influenced by the energy system that covers the other parameter (Jenkins & Quigley, 1992; Jones & Burnley, 2009; Vanhatalo et al., 2010). The initial definition was that exercise at CP intensity can be maintained “for a very long time without fatigue” (Monod & Scherrer, 1965). However, this was a vague interpretation of CP and nowadays this definition has been refuted, as it does not reflect the current understanding and use of the concept (Black et al., 2022; Burnley, 2022). Furthermore, it has been suggested that  $W'$  depletion is associated with low values of pH and PCr, and the accumulation of fatigue-related metabolites (e.g.,  $H^+$ ,  $P_i$ , and ADP) reaching a “critical threshold”, which results in disturbance of the intramuscular homeostasis, irrespective of the PO that is selected. Nevertheless the assumptions upon which the original CP concept is based, the current understanding and practical applications of the concept cannot be overlooked to study the mechanistic bases of fatigue development and exercise tolerance (Jones et al., 2010).

Similar to CP, with equivalent threshold concepts,  $W'$  is often mentioned together with the maximal accumulated oxygen deficit (MAOD) method. This method was first introduced by Krogh & Lindhard (1920) and is put forward as a manner to estimate the anaerobic capacity (Noordhof et al., 2013). MAOD can be calculated by subtracting the  $\dot{V}O_2$  uptake measured during and integrated over the duration of the supramaximal exercise bout (i.e., the accumulated  $\dot{V}O_2$  uptake) from the accumulated  $\dot{V}O_2$  demand (Medbø et al., 1988; Noordhof et al., 2013). However,  $W'$  is supplied by both anaerobic and aerobic energy sources, as part of integrated bio-energetic system for whole-body exercise (Poole et al., 2016). Assuming that  $W'$  is similar to MAOD and thus solely supplied based on anaerobic energy is inaccurate and invalid. Furthermore, the concept of MAOD is not flawless. The validity of MAOD has been criticized for many reasons (Noordhof et al., 2010). Therefore, the MAOD concept will not be further discussed in this PhD thesis.

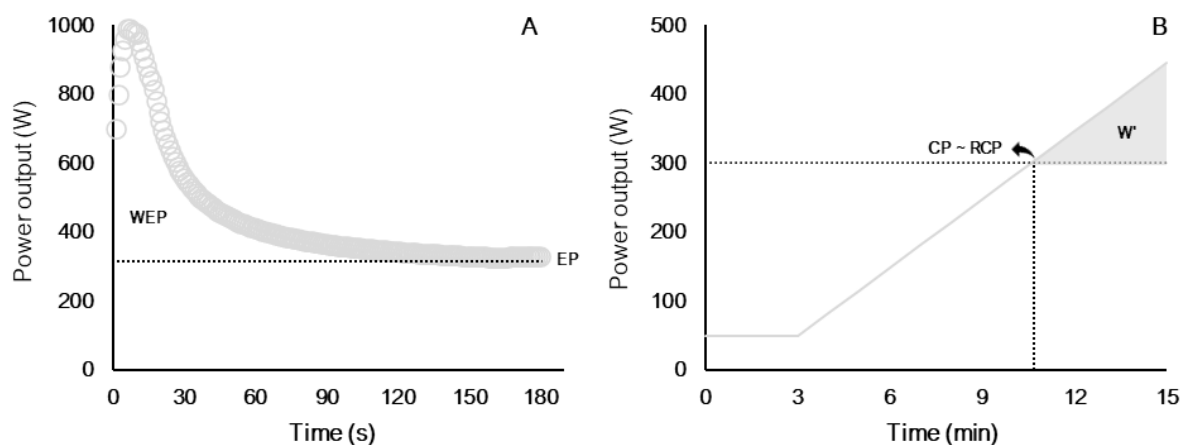
#### 1.2.2.2 CP and $W'$ determination

The parameters of the power-duration relationship can be established in a relatively simple way. It is sufficient to perform CWR efforts (cycling or another exercise modality) with a different workload and record the TTE with a stopwatch in the laboratory. In a field setting, maximal exercise during time trials of fixed duration or distance can serve as an alternative (Karsten et al., 2015).

Typically, to accurately determine CP and  $W'$ , a series of three to five exercise bouts has to be performed on different days (Mattioni Maturana et al., 2018; Vanhatalo et al., 2011). The selection of exercise intensities for these tests is crucial to estimate optimal TTE of these tests. It has been shown that the duration of predictive trials has an impact on the model estimates of CP and  $W'$ , with shorter trials leading to higher CP values and lower  $W'$  values (Bishop et al., 1998). Current recommendations advocate for trial durations distributed proportionally between 2 and 15 minutes (Jones et al., 2010; Poole et al., 2016) or 20 min (Mattioni Maturana et al., 2018), deviating from the earlier suggestion of 1 to 10 minutes (Hill, 1993). Shorter trials (i.e., < 2 minutes) should be avoided, as they being heavily affected by “aerobic inertia” and leading to exhaustion before reaching  $\dot{V}O_{2\max}$  (Hill et al., 2002). Conversely, longer trials should be avoided to minimize the variability, due to the influence of motivation or other psychological factors on TTE (Chorley & Lamb, 2020; Morton, 1994). Other potential influencing factors during longer trials, such as diet and hydration (Bishop et al., 1998), can already be excluded by standardizing the protocol up to 24h prior to the test. After taking into account these guidelines, the obtained data from the predictive trials to task failure can be fitted by linear or nonlinear regression analysis, depending on the mathematical model selected (see Equations 1-3 and Figure 5).

The CP determination method is subject to an inherent “measurement error”, which may arise due to the heavy reliance on exhaustive effort from participants. Between-day variability in TTE of trials has been reported to be around 2-30% (McLellan et al., 1995), which can affect the accuracy of CP and  $W'$  estimation. These limitations can be exacerbated by using fewer prediction trials or alternative methods for CP estimation. However, reducing the number of trials may lead to under- or over-predictions of CP, resulting in larger errors in its estimation. The typical error of CP is approximately 5%, which contributes to the considerable interindividual variability of TTE at CP (Brickley et al., 2002; Housh et al., 1989). It is generally accepted that the standard error of the estimates (SEE), expressed as a coefficient of variation (CV%), should be < 5% for CP and < 10% for  $W'$  (Black et al., 2015; DW Hill, 1993). Furthermore, Black et al. (2015) proposed to select the model that provides the “best individual fit”. The model (i.e., linear work-time, linear power-1/time or hyperbolic power-time model) associated with the lowest total error is selected on an individual basis. However, Hill & Smith (1994) reported that the accuracy of  $W'$  can only be accepted when there is a high similarity of the values obtained using the different model forms and when the SEE are sufficiently small.

Despite its potential benefits, the application of the conventional CP test methodology in practice is contested due to the requirement of conducting multiple CWR tests to the limit of tolerance, which are not only time-consuming but also physically and mentally demanding for athletes. Therefore, researchers have investigated alternative testing protocols such as a 3-minute all-out test (3MT) (Burnley et al., 2006; Vanhatalo et al., 2007) and RAMP test(s) (Caen et al., 2023; Morton, 1994; Pouilly et al., 2005) to address these issues (Figure 7). The design of the 3MT is based on two assumptions; (1) the test has to be long enough to elicit  $\dot{V}O_{2\max}$ , and (2) an all-out effort is required to deplete  $W'$  all at once, and thus if  $W' = 0$ , then  $P = CP$  (see Equation 2). The test itself consists of cycling against a fixed resistance and the aim is to fully deplete  $W'$  within the first 150 seconds. This will result in a plateau of power output in the final 30 seconds of the test. The final power output observed from this test, the end power (EP), and the work above EP (WEP) have been shown to be equivalent to CP and  $W'$ , respectively (Figure 7A).



**Figure 7** Alternative models to determine CP and  $W'$ : (1) The 3 minute all-out test (7A) with end power (EP), corresponding to CP, and work done above end power (WEP), corresponding to  $W'$  (reproduced from Vanhatalo et al., 2007). (2) In a RAMP test (7B), CP corresponds to RCP and  $W'$  is the work done above RCP (reproduced from Caen et al., 2023).

Although it has been demonstrated that the 3MT is a valid protocol for the estimation of CP and  $W'$  (Vanhatalo et al., 2007), some studies question its validity, as an overestimation of CP and underestimation of  $W'$  have been reported (Bergstrom et al., 2014; McClave et al., 2011; Wright et al., 2017). On the other hand, the determination of CP and  $W'$  from a series of RAMP tests was first investigated by Morton (1994), with the development of a formula (equation 4) that linked the specific ramp slope ( $s$ ) with TTE of that test. It was stated that the ramp CP test protocol provides an alternative for estimation of CP and  $W'$ , yet caution is warranted as the SEE of CP ( $\sim 14\%$ ) and  $W'$  ( $\sim 38\%$ ) were

large. Although this theory and formula provided interesting findings, the use of it nowadays is negligible.

$$TTE = \frac{CP}{s} + \sqrt{\frac{2 \cdot W'}{s}} \quad (4)$$

The estimation of CP and  $W'$  from a single RAMP test is based on the equivalency in metabolic rate between CP and RCP (Caen et al., 2022; Keir et al., 2015) (Figure 7B). Pouilly et al. (2005) were the first to estimate CP and  $W'$  from a single RAMP test, showing similar values for CP, yet an underestimation of  $W'$  during the RAMP test. Importantly, it should be mentioned that the authors used GET instead of RCP as a surrogate for CP. Recently, Caen et al. (2023) accurately compared CP and  $W'$  using CWR tests with a single RAMP test in 153 participants, showing an underestimation of CP and  $W'$ , together with a larger variability. They suggest that CP and  $W'$  derived from a single RAMP test vs. CWR tests should not be used interchangeably, as they question the validity of CP and  $W'$  determination out of a single RAMP exercise.

#### 1.2.2.3 $W'$ reconstitution

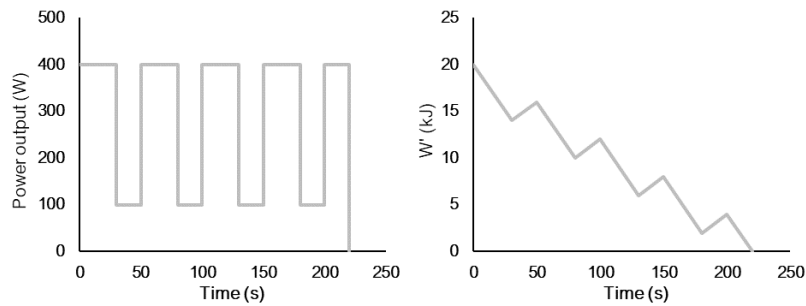
Since many sports involve frequent changes in exercise intensity, the CP concept has also been applied to intermittent exercise, in which exercise above CP ( $W'$  depletion) is alternated with exercise below CP ( $W'$  reconstitution) (Chidnok et al., 2012; Jones et al., 2008). More specific,  $W'$  depletion is the gradual consumption of the work reserve during intense exercise above CP, leading to task failure or exhaustion.  $W'$  reconstitution is the process by which the body replenishes the depleted energy stores and defuses metabolic byproducts during recovery periods, allowing the athlete to recover and perform high-intensity efforts again in subsequent bouts. However, to date, the development of an accurate  $W'$  reconstitution model remains a scientific quest for many researchers. In the following paragraphs a better understanding will be provided on the basis and advancements of such a  $W'$  reconstitution model: (1) which factors can influence the recovery of  $W'$ , and (2) which elements still have to be elucidated.

In their pioneering work, Morton and Billat (2004) delineated intermittent exercise patterns above and below CP, and defined four distinct parameters: (1) work intensity above CP ( $PO_w$ ); (2) work duration above CP ( $t_w$ ); (3) recovery intensity below CP ( $PO_r$ ); and (4) recovery duration below CP ( $t_r$ ). Through systematic manipulation of these parameters, they demonstrated that total work performed above CP can be augmented beyond what is attainable with continuous severe-intensity exercise. Subsequently, they formulated a mathematical model based on the linear depletion and reconstitution of  $W'$  at equal

rates relative to CP (Equation 5), taken into account the amount of complete (work + recovery) cycles (n).

$$TTE = n \cdot (t_w + t_r) + \frac{W' - n \cdot ((PO_w - CP) \cdot t_w - (CP - PO_r) \cdot t_r)}{PO_w - CP} \quad (5)$$

This model allowed for the prediction of the total work that can be sustained above CP and how it depends on the above-mentioned parameters (Figure 8).



**Figure 8** Intermittent exercise and prediction of TTE (figure based on Morton & Billat 2004).

Although, several years after their initial proposal, the concept of linear recovery kinetics of  $W'$  was challenged by Ferguson et al. (2010), who identified a curvilinear reconstitution pattern following exhaustive exercise. This pattern was fitted using three different recovery durations, i.e., 2, 6 and 15 minutes. Consequently, further research was needed, and the development of the  $W'$  balance ( $W'_{BAL}$ ) model (Skiba et al., 2012) was a breakthrough for providing insight into the available  $W'$  at any moment in time during intermittent exercise. In this perspective,  $W'$  can be considered as a battery, which is used and reloaded depending on whether an athlete exercise above or below CP, respectively. A thorough understanding of the  $W'$  reconstitution kinetics, and an accurate and direct quantification of the available  $W'$  (taking into account the already performed exercise) would have important implications for several populations. In athletes, the balance of  $W'$  (i.e.,  $W'_{BAL}$ ) remaining at a given time point during a race determines the frequency, duration and intensity of bouts above CP an athlete might perform, either to escape a competitor or close a gap. Also, it would allow a more individualized and adequate prescription of interval training sessions in competitive and recreational athletes as well as in patient populations, providing immediate insight into the energy balance at a certain moment.

The model of Skiba et al. (2012) was based on three assumptions. The first implies that consumption of  $W'$  starts only when CP is exceeded by the athlete. The second assumption states that reconstitution of  $W'$  can only take place when the athlete drops the intensity of exercise back below CP. Finally, and novel

back then, they state that the reconstitution of  $W'$  has a predictable and mono-exponential time course. Based on these 3 assumptions, they formulated the following formula (Equation 6):

$$W'_{BAL} = W' - \int_0^t (W'_{EXP})(e^{-(t-u)/\tau_{W'}}) \quad (6)$$

The  $W'_{BAL}$  model states that the amount of  $W'$  remaining at any time point ( $t$ ) during exercise ( $W'_{BAL}$ ) equals the difference between the athlete's known  $W'$  and the amount of  $W'$  that has been expended during efforts above CP ( $W'_{EXP}$ ).  $W'$  is exponentially reconstituted with the speed determined by recovery time constant tau ( $\tau_{W'}$ ) during recovery intervals below CP ( $t-u$ ).  $\tau_{W'}$  is a mono-exponential fitting of the  $W'$  reconstitution based on the group data of the respective study (Skiba et al., 2012). It is only dependent on one variable, i.e., the difference between CP and the recovery PO ( $D_{CP}$ ) (Equation 7).

$$\tau_{W'} = 546 \cdot e^{(-0.01 \cdot D_{CP})} + 316 \quad (7)$$

While successfully validated against similar exercise protocols (Skiba et al., 2014a; Skiba et al., 2014b), it was also found that  $\tau_{W'}$  differed between participants. The authors suggested already back then that  $W'$  recovery kinetics should be determined on an individual basis. Furthermore, this equation is modelled for a specific exercise protocol, i.e. short intermittent bouts of work and recovery until exhaustion. This raises the question if the  $W'_{BAL}$  model is a 'one size fits all' model for everyone and every exercise protocol. Recently, Skiba & Clarke (2021) stated clearly that the development of  $W'_{BAL}$  model was a starting point, rather than a final model, and needs further refinement based on current insights. In Table 1, an overview is provided of 32 original studies (reviews excluded) published on  $W'$  reconstitution, since the initial study of Morton & Billat (2004) until August 2023 (search term " $W'$  reconstitution" on Web of Science). Research investigations should focus on the influencing factors of the modalities of the exercise protocol, individual characteristics and environmental conditions. For example, in the original model of Skiba et al. (2012) the  $W'$  recovery kinetics were determined by  $\tau_{W'}$ , which is mono-exponential, assuming the simplest mathematical form for exponential recovery. Possible other curve fitting recovery kinetics, related to recovery kinetics of physiological data, should be looking into. The characteristics of the exercise protocol itself, both work ( $> CP$ ) and recovery ( $< CP$ ), by means of duration, intensity and frequency (i.e., multiple bouts to exhaustion), and the response to partial or full  $W'$  depletion can alter the  $W'$  recovery profile (Caen et al., 2019; Chorley et al., 2019; Lievens et al., 2021). This should be studied in more detail, combining different exercise protocol

modalities. Furthermore, it has been shown that individual characteristics, such as anthropometrics, training status and aerobic fitness (i.e.  $\dot{V}O_{2\max}$ ) can influence the rate of  $W'$  recovery during intermittent exercise (Bartram et al., 2018; Chorley et al., 2019, 2020). It has been shown that there is a strong relationship between type I muscle fiber typology (Lievens et al., 2020), and the faster recovery from repeated-sprint bouts of high intensity. Hence, muscle fiber typology might be worth investigating as well in  $W'$  reconstitution studies. Furthermore, it has been shown that  $W'_{\text{BAL}}$  model can be used in hypoxia with the prerequisite that CP and  $W'$  were also measured at same level of hypoxia (Shearman et al., 2016). However, modification of the parameters is necessary according to the altitude level, i.e., degree of hypoxia (Townsend et al., 2017). Possibly other environmental factors, such as heat, could have an significant impact on  $W'$  reconstitution (Richard & Koehle, 2022) and need further investigation.

It must be noted that the scientific progress on  $W'$  recovery in physically active and trained individuals, as well as in elite athletes, is mainly based on research studies with male participants. However, significant differences in anthropometrics, body composition and physiological characteristics exist between men and women, related to the sex chromosomes and sex hormones, in particular, testosterone (Ansdell et al., 2020; Besson et al., 2022; Hunter, 2016; Hunter et al., 2023; Santisteban et al., 2022). Typically, men are taller and heavier than women, but also body composition differs. More lean body mass and less body fat percentage are inherent to male individuals, as compared to a female counterparts (Fryar et al., 2021). Considering physiological characteristics, men have larger lungs, even when matched for height (Schwartz et al., 1988), and larger airways (Dominelli et al., 2018). As a consequence, women tend to have a higher work of breathing, while efficiency of breathing is lower, especially at higher ventilation (Dominelli et al., 2019; Dominelli et al., 2015). Furthermore, a larger heart size and mass (and thus greater stroke volume) (St. Pierre et al., 2022), and higher blood hemoglobin concentration (Cureton et al., 1986) have been observed, leading toward a higher  $\dot{V}O_{2\max}$  in men compared with women (Joyner & Coyle, 2008). At the muscular level, women have smaller type II muscle fibers (smaller cross sectional area) than men, and are thus likely to produce less force (Miller et al., 1993). Subsequently, the proportional area of type I muscle fibers in the whole muscle is larger in women compared to men (Hunter, 2014; Simoneau & Bouchard, 1989). It has been shown that women have greater capillarization per unit of skeletal muscle (Roepstorff et al., 2006), faster peripheral  $O_2$  extraction (Beltrame et al., 2017), while having lower contractile properties and better fatigue resistance (Wüst et al., 2008). In contrast, men typically have a greater glycolytic capacity in type II muscle fibers which enables a higher production of work during exercise in the severe intensity domain (Hunter, 2016; Komi & Karlson, 1978). These findings indicate that women have a greater ability to utilize  $O_2$  in the periphery (diffuse capacity), while men have a greater ability to transport  $O_2$  at the central level (convective capacity) (Ansdell et al., 2020). Thus, these differences are important to

understand the underlying mechanisms of the CP model (i.e., Critical Power,  $W'$ , and  $W'$  reconstitution) in women and men.

Providing normative data in female athletes and including sex as a biological variable in research studies is of utmost importance (Devries & Jakobi, 2021; Emmonds et al., 2019; Hunter et al., 2023; Mannon et al., 2020; Shannon et al., 2019). Regarding research related to CP and  $W'$  and its reconstitution, this could be beneficial for appropriate monitoring of the training process in sports and rehabilitation in both sexes (Ansdell et al., 2020). Numerous studies contain values of the parameters of the CP concept of their participants (see recent reviews of Chorley & Lamb, 2020; Dotan, 2022; Lipková et al., 2022), however, to our knowledge, only a few studies have provided information regarding CP and  $W'$  in women (Ansdell et al., 2020; Bishop et al., 1998; James et al., 2023; Kantor et al., 2019; Sundberg et al., 2017). These studies showed a lower CP and  $W'$  (absolute and normalized to body mass) for women compared with men, which is reasonable as sex differences in body composition are evident (Kirchengast, 2010). Only seven studies included women, as presented in the overview table on  $W'$  reconstitution studies (Table 1), yet no sex-based comparison was made in any study. It has been speculated that women may reconstitute  $W'$  faster than men, as indicated by better retainment of muscle tissue oxygenation in women during one exercise bout to task failure with subsequent recovery phase (Ansdell et al., 2019). However, Ansdell et al. (2019) could not directly demonstrate a possible difference in  $W'$  reconstitution between women and men, as a second bout to task failure was missing. Scientific data concerning CP,  $W'$  and  $W'$  reconstitution in women is scarce, and therefore the applicability of current  $W'$  reconstitution models in a female population is questionable.

**Table 1** Overview of  $W'$  reconstitution studies.

|    | Year | Authors           | Training status          | Sex            | Exercise mode             | Primary result in relation to $W'$ reconstitution   |
|----|------|-------------------|--------------------------|----------------|---------------------------|---|
| 1  | 2004 | Morton & Billat   | Well trained runners     | 6 men          | Running                   | Extension of CP concept for intermittent exercise; linear $W'$ reconstitution.  |
| 2  | 2009 | Vanhatalo & Jones | Recreationally active    | 7 men          | Cycling                   | 79% and full $W'$ reconstitution following respectively 2 and 15 min rest after 30 s all-out cycling (extent of $W'$ depletion is unknown).   |
| 3  | 2010 | Ferguson et al.   | Recreationally active    | 6 men          | Cycling                   | $W'$ reconstitution found to be curvilinear. Half-time of $W'$ reconstitution was faster than that of blood $[La^-]$ but slower than that of $\dot{V}O_2$ (a proxy for PCr reconstitution). |
| 4  | 2012 | Chidnok et al.    | Recreationally active    | 7 men          | Cycling                   | No recovery in severe domain. Recovery rate of $W'$ slower than expenditure in relation to critical power (CP).   |
| 5  | 2012 | Skiba et al.      | Recreationally active    | 7 men          | Cycling                   | Creation of Skiba $W'_{BAL}$ model based on intermittent exercise to exhaustion, together with generic $W'$ reconstitution tau equation based on CP.  |
| 6  | 2013 | Chidnok et al.    | Recreationally active    | 9 men          | Single leg knee extension | $W'$ reconstitution increases with recovery duration. PCr and pH levels were always the same at exhaustion. PCr recovery correlated to $W'$ reconstitution but was faster.                  |
| 7  | 2014 | Skiba et al. (a)  | Recreationally active    | 5 men, 6 women | Cycling                   | Real time $W'_{BAL}$ prediction possible. Large inter- and intra-individual variations in reconstitution rate observed.   |
| 8  | 2014 | Skiba et al. (b)  | Well trained triathletes | 6 men, 2 women | Cycling                   | Validation of Skiba $W'_{BAL}$ on training and race data. When exhaustion is set at $W'_{BAL} = 1.5$ kJ prediction of exhaustion was 80% appropriately classified as exhausted.             |
| 9  | 2015 | Skiba et al.      | Recreationally active    | 6 men, 4 women | Single leg knee extension | Underestimation of $W'$ reconstitution by Skiba $W'_{BAL}$ model, and recommendation to individualize $W'$ reconstitution tau.  |
| 10 | 2016 | Broxterman et al. | Healthy                  | 6 men          | Dynamic handgrip          | Validation of Skiba $W'_{BAL}$ across the contraction-relaxation cycles comprising severe intensity constant power handgrip exercise.   |
| 11 | 2016 | Shearman et al.   | Well trained cyclists    | 11 men         | Cycling                   | Validation Skiba $W'_{BAL}$ in hypoxia with proviso that CP and $W'$ were also measured at same level of hypoxia.   |

**Table 1** Cont.

|    | Year | Authors                       | Training status                     | Sex             | Exercise mode                                 | Primary result in relation to $W'$ reconstitution  |
|----|------|-------------------------------|-------------------------------------|-----------------|---|--|
| 12 | 2017 | Townsend et al.               | Trained cyclists                    | 9 men           | Cycling                                       | Modified equation for CP based on altitude for use in $W'_{BAL}$ to allow better prediction of $W'$ reconstitution at increasing altitude.   |
| 13 | 2017 | Vinetti et al.                | Recreationally active               | 7 men           | Cycling                                       | Extensive mathematical representation of discontinuous ramp exercise.  |
| 14 | 2018 | Bartram et al.                | Elite cyclists                      | 4 men           | Cycling                                       | Underestimation of $W'$ reconstitution by Skiba model. New $W'$ reconstitution tau formula proposed for elite cyclists.  |
| 15 | 2019 | Caen et al.                   | PE students                         | 11 men          | Cycling                                       | Underestimation of $W'$ reconstitution by Skiba model. Individual variation in $W'$ reconstitution tau. Preceding depletion rate affects $W'$ reconstitution.                          |
| 16 | 2019 | Chorley et al.                | 9 trained cyclists,<br>11 untrained | 19 men, 1 woman | Cycling                                       | $W'$ reconstitution slowed with repeated bouts of exhaustive exercise.   |
| 17 | 2020 | Chorley et al.                | 9 trained cyclists<br>11 untrained  | 20 men          | Cycling                                       | Assessment of anthropometric and physiological relationships with $W'$ reconstitution and its slowing following repeated bouts.  |
| 18 | 2020 | Felippe et al.                | Recreationally active               | 10 men          | Cycling                                       | $W'$ recovery is not associated to the recovery of peripheral or central fatigue alone. Rather, $W'$ seems to be associated to the recovery of the overall capacity to generate force. |
| 19 | 2020 | Sreedhara et al.              | Trained cyclists                    | 7 men           | Cycling                                       | $W'$ reconstitution did not increase from 6 min to 15 min recovery, hence $W'$ reconstitution was not exponential.   |
| 20 | 2020 | Vassallo et al.               | Recreationally active               | 9 men           | Running                                       | Over-ground $W'_{BAL}$ accurately tracked $W'$ kinetics during intermittent running to exhaustion on flat surfaces.  |
| 21 | 2021 | Lievens et al.                | PE students                         | 12 men          | Cycling                                       | $W'$ reconstitution accelerates more with decreasing intensity in the heavy- versus the moderate-intensity domain.   |
| 22 | 2021 | Abdalla et al.                | Recreationally active               | 10 men          | Single leg knee extension and plantar flexion | $W'$ reconstitution tau during intermittent isometric exercise is slower with greater muscle size and maximal strength.  |
| 23 | 2021 | do Nascimento Salvador et al. | Recreationally active               | 13 men          | Cycling                                       | Non-linear $W'$ reconstitution pattern during the 10 min of recovery following prior high-intensity cycling exercise, linked to physiological disturbances.                            |

**Table 1** Cont.

|    | Year | Authors            | Training status  | Sex             | Exercise mode | Primary result in relation to $W'$ reconstitution  |
|----|------|--------------------|--|-----------------|---------------|--|
| 24 | 2022 | Chorley et al.     | Trained cyclists   | 9 men, 1 woman  | Cycling       | $W'$ reconstitution kinetics were best described by a bi-exponential model consisting of distinct fast and slow phases.  |
| 25 | 2022 | Raimundo et al.    | 9 national swimmers<br>4 regional swimmers                             | 13 men          | Swimming      | Skiba $W'_{BAL}$ model was inconsistent to track $W'$ responses for swimming sessions, possibly due to large intra-individual variability.                                       |
| 26 | 2022 | Pugh et al.        | 4 international cyclists<br>4 national cyclists<br>4 regional cyclists | 12 men          | Cycling       | Faster rates of $W'$ recovery in international cyclists than national and regional cyclists at 25 and 50 W below CP.   |
| 27 | 2022 | Bartram et al.     | Elite cyclists   | 6 men           | Cycling       | Adjustment of $W'$ reconstitution tau from Skiba model resulted at exhaustion in end $W'$ values that were not different from zero.  |
| 28 | 2023 | Millour et al.     | High-level cyclists  | 15 men          | Cycling       | Three models of $W'_{BAL}$ developed in the literature provide different results during high-level competitions of long durations, the models are not interchangeable.           |
| 29 | 2023 | Black et al.       | Healthy  | 11 men, 1 woman | Cycling       | Overestimation of $W'$ reconstitution by some $W'_{BAL}$ models, accounting for dynamic changes in the power-duration relationship improves the accuracy of $W'_{BAL}$ modeling. |
| 30 | 2023 | Galán-Rioja et al. | Trained cyclists   | 14 men          | Cycling       | $W'_{BAL}$ model may prove to be a useful tool for coaches to construct interval training programs.  |
| 31 | 2023 | Vaccari et al.     | Recreationally active  | 9 men           | Running       | $W'$ reconstitution has an exponential behavior that can be exploited in HIIT protocols to prolong time to exhaustion and time spent above 90% $\dot{V}O_{2max}$ .               |
| 32 | 2023 | Chorley et al.     | Trained cyclists   | 9 men, 1 woman  | Cycling       | Development of a full dynamic model of $W'$ accounting for power and duration during the expenditure and reconstitution of $W'$ above and below CP.                              |

*Training status is what the authors of these studies specified; exercise mode refers to the experimental protocol of the respective study; PE, physical education; blood  $[La^-]$ , blood lactate concentration;  $\dot{V}O_2$ , oxygen uptake; PCr, phosphocreatine; HIIT, high-intensity interval training (adapted from Chorley and Lamb, 2020).*

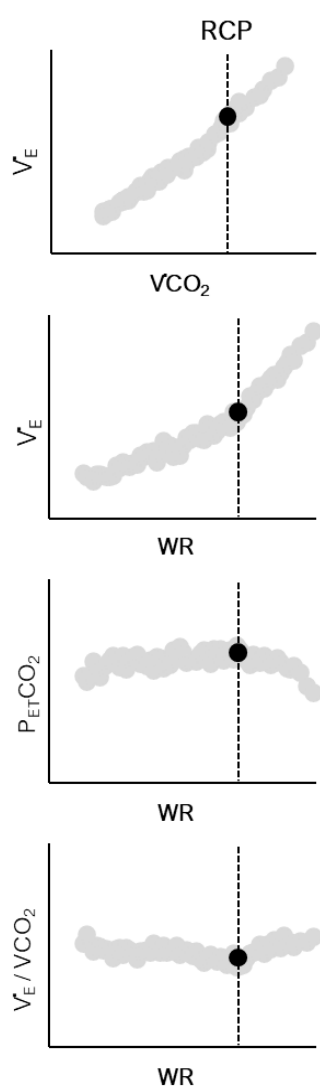
### 1.2.3 Incremental exercise test

The heavy-severe exercise intensity boundary can also be determined through less time-consuming exercise tests, namely ramp incremental (RAMP) and step incremental (STEP) exercise tests.

RAMP tests are characterized with a linear continuous increase in work rate, starting from rest to task failure or volitional exhaustion, while measuring breath-by-breath pulmonary gas exchange. Measuring the rate of  $\dot{V}O_2$  during an exercise protocol, designed to increase metabolic demand through muscle work (i.e., power output), provides valuable insight into the body's ability to supply (pulmonary system), transport (cardiovascular system), and consume (muscular system)  $O_2$  for the resynthesis of ATP. The typical non-steady state character of the RAMP test ensures that an athlete will surpass both thresholds (moderate-heavy and heavy-severe) in a short time frame, as the intensity increases rapidly. Next to the determination of thresholds, this protocol allows to determine the  $\dot{V}O_{2max}$  and work efficiency ( $\dot{V}O_2/PO$  relationship) (Boone & Bourgois, 2012). The benefits of this protocol are the time-efficient and non-invasive manner to evaluate exercise capacity. Furthermore, the protocol can be individualized by adjusting the work rate (i.e., ramp slope), based on the subject's anthropometrics and physical fitness, to reach volitional exhaustion within approximately 8-12 minutes (Buchfuhrer et al., 1983). The non-invasive breath-by-breath measurement of pulmonary gas exchange provides a greater sampling frequency (as compared to blood  $La^-$  measurement), which makes that thresholds can be determined very precise. Often is referred to the thresholds determined in RAMP tests as ventilatory thresholds (i.e. based on ventilation), however, other gas exchange variables might be used as well (Binder et al., 2008). The heavy-severe intensity boundary, or MMSS, can be estimated by means of the respiratory compensation point (RCP). RCP is defined as the point where  $\dot{V}_E$  increased disproportionately to  $\dot{V}CO_2$ , and a frank hyperventilation is perceived. Other criteria are the second departure from linearity in  $\dot{V}_E$ , an increase in both  $\dot{V}_E/\dot{V}O_2$  and  $\dot{V}_E/\dot{V}CO_2$ , and the deflection point of end-tidal carbon dioxide tension ( $P_{ET}CO_2$ ) (see Figure 9).

Given the non-steady state conditions this exercise test relies upon, assigning a given PO to this threshold is difficult. The measured  $\dot{V}O_2$  will lag behind the true metabolic needs for any given intensity. This leads to a situation in which the ramp-identified PO for a given  $\dot{V}O_2$  in reality elicit a metabolic intensity that exceeds what is preferred. Below GET, this discrepancy between  $\dot{V}O_2$  and PO is due to a mean response time (MRT). This MRT incorporates both the time constant  $\tau$  of  $\dot{V}O_2$  kinetics (i.e., the characteristic response of the system) and the pure time delay resulting from the time interval for the local metabolic responses to reach the pulmonary system. MRT can be quantified as the time interval between the onset of the ramp and the intersection of the forward extrapolation of the baseline  $\dot{V}O_2$  and the backwards extrapolation of the linear  $\dot{V}O_2$ /time relationship below the GET (Boone & Bourgois, 2012; Davis et al., 1982). A second manner to determine the MRT is by using a new approach in which

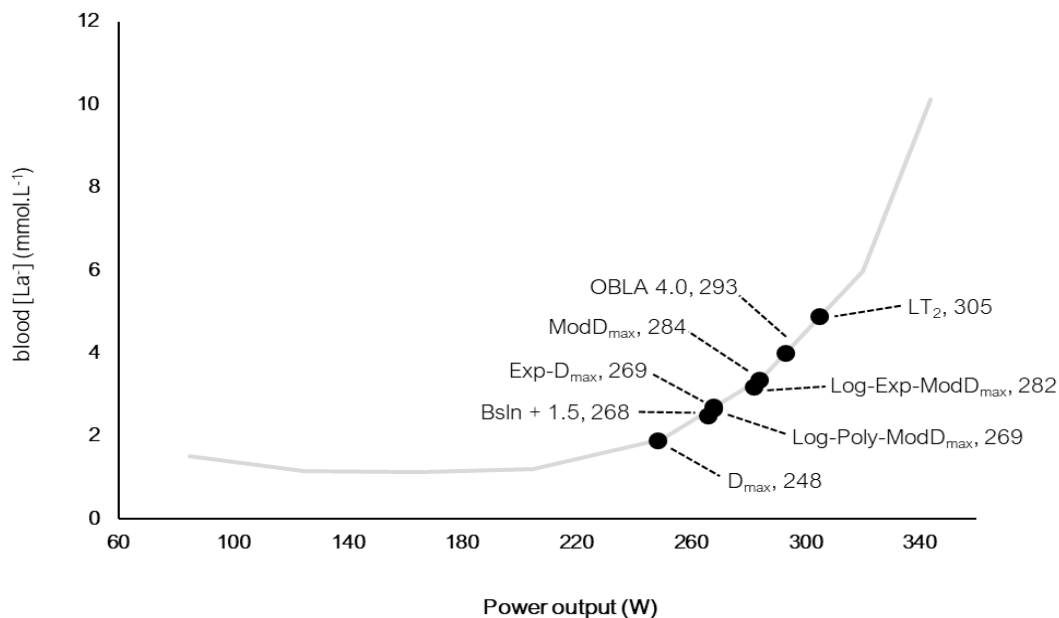
the test is preceded by an additional CWR bout in the moderate intensity domain. The MRT (in this case expressed in PO) is then calculated as the difference in PO between CWR bout and the ramp-identified PO that elicited the same  $\dot{V}O_2$  as obtained during the CWR bout (Iannetta et al., 2019). Above GET, the dissociation of the  $\dot{V}O_2$ /PO relationship between RAMP and CWR become apparent, as the loss in muscle efficiency (cfr. delayed steady state in CWR exercise in heavy intensity domain) was not accounted for in RAMP tests at higher intensities (Iannetta et al., 2019). This would make this type of test less interesting towards exercise prescription, as it fails to provide an accurate external intensity (PO) for a given metabolic intensity ( $\dot{V}O_2$ ). To solve this problem, Iannetta and colleagues (2019), proposed the use of an extremely slow ramp slope (i.e., 5 W·min<sup>-1</sup>), however, the practical feasibility of this test is questioned. The translation strategy proposed by Caen et al. (2020) makes it possible to determine an extra individual (on top of the MRT-correction) adjustment to close the gap between  $\dot{V}O_2$  and PO. This allows to assign a proper PO corresponding the metabolic intensity at RCP.



**Figure 9** Graphical representation of the four physiological events to determine the respiratory compensation point (RCP) using pulmonary gas exchange during a RAMP test. The graph on top is expressed on  $\dot{V}CO_2$ , next three graphs on work rate (WR) (based on figure of Binder et al., 2008).

During a STEP test, blood samples are drawn at the end of each stage to measure blood  $[La^-]$ . When these values are plotted as a function of the increasing PO, a typical blood  $La^-$  curve is obtained. STEP tests involve the gradual increase in exercise intensity in discrete, step-wise increments. The duration of the step can vary between 1-8 min, however a minimum of 3 min is recommended (Bentley et al., 2007). The step increments can be adjusted according to the athlete's anthropometrics and training status, in order to obtain at least 5 blood  $[La^-]$  measurements to fit a proper curve.

The determination of the second lactate threshold (LT) is a widely used method to evaluate endurance performance, as it offers a convenient way to estimate the abovementioned MLSS within a single test session. Over the years, a large variety of LT methods has been suggested and utilized (Faude et al., 2009; Jamnick et al., 2018), each with unique nomenclature and methodology for determination. An overview of the threshold determination methodology of 8 commonly used threshold concepts, as displayed in Figure 10, is provided below:



**Figure 10** Graphical representation of lactate thresholds, as a surrogate of the MLSS, spread across the lactate-performance curve during a 3 min STEP test with 40 W increments (based on figure of Jamnick et al., 2018).

1. Baseline + absolute value(s) (Bsln + mmol·L $^{-1}$ ): The intensity at which blood  $[La^-]$  increased 1.5 (Bsln + 1.5) mmol·L $^{-1}$  above baseline value (Berg et al., 1990; Zoladz et al., 1995).
2.  $D_{max}$ : The point on the third order polynomial regression curve that yielded the maximum perpendicular distance to the straight line formed by the two end points of the blood  $La^-$  curve (Cheng et al., 1992).
3. Modified  $D_{max}$  (Mod $D_{max}$ ): The intensity at the point on the third order polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the point

preceding the first rise in blood  $[La^-]$  of  $> 0.4 \text{ mmol}\cdot\text{L}^{-1}$  lactate and the final blood  $La^-$  point (Bishop et al., 1998).

4. Exponential  $D_{\max}$  (Exp- $D_{\max}$ ): The point on the exponential regression curve that yielded the maximum perpendicular distance to the straight line formed by the two end points of the blood  $La^-$  curve (Hughson et al., 1987; Machado et al., 2012).
5. Log-log Modified  $D_{\max}$  (Log-Poly-Mod $D_{\max}$ ): The intensity at the point on the third order polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final blood  $La^-$  point (Jamnick et al., 2018).
6. Log-log Exponential Modified  $D_{\max}$  method (Log-Exp-Mod $D_{\max}$ ): The intensity at the point on the exponential plus-constant regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final blood  $La^-$  point (Jamnick et al., 2018).
7. Lactate turning point: The blood  $La^-$  curve is divided into three segments. Two double-linear fits are performed, which the second intersection point between the lines (segments) is considered as lactate turn point 2 (LT<sub>2</sub>) (Binder et al., 2008).
8. Fixed lactate thresholds or onset of blood  $La^-$  accumulation (OBLA) value of 4.0 (OBLA 4.0)  $\text{mmol}\cdot\text{L}^{-1}$  (Kindermann et al., 1979; Skinner & McLellan, 1980; Heck et al., 1985).

However, even under standardized conditions, many of these “accepted” methods are influenced by test protocol design and their underlying validity has not been reported (Faude et al., 2009; Jamnick et al., 2018). Some of these methods take into account the individual kinetics of the lactate-performance curve (e.g.,  $D_{\max}$ ), while others rely on fixed blood  $[La^-]$  (e.g., OBLA 4.0). Furthermore, alterations in step duration and increments can influence the determination and interpretation of exercise thresholds (Faude et al., 2009; Jamnick et al., 2018). While the determination methodology of an exercise threshold targets a specific exercise intensity boundary, confusion exists here as well to which boundary (moderate-heavy or heavy-severe) the lactate threshold concept corresponds. Moreover, in the study of Jamnick et al. (2018), it has been shown that the majority of thresholds methods (47 out of 58 unique thresholds) could not make a valid estimation of the MLSS. For example, the better known threshold concepts of  $D_{\max}$  and OBLA of  $4.0 \text{ mmol}\cdot\text{L}^{-1}$  did not provide valid estimates of the MLSS. The best estimation of the MLSS was the Log-Poly-Mod $D_{\max}$  derived from a STEP test with a stage duration of 4 minutes (Jamnick et al., 2018). Lastly, although the blood  $La^-$  response to exercise is highly reproducible when conditions are standardized, concerns on blood  $La^-$  measurements are also applicable in this case (as discussed in *chapter 1.2.1*).

### 1.3 Exercise with acute heat exposure

Exercise in the heat is common for athletes, military personnel, and outdoor enthusiasts. The combination of exercise and heat stress can lead to a range of physiological responses that can impact exercise performance and, in extreme cases, result in serious heat-related illnesses. As the Earth's temperature continues to rise, heat exposure is becoming an increasing challenge for individuals participating in physical activity (Smith et al., 2016). Understanding the physiological responses to exercise in the heat is essential to optimize performance, prevent heat-related illnesses, and promote safety during exercise in hot environments. In this dissertation the focus is on the effects of heat exposure on the determination of exercise thresholds in recreational athletes, as this a crucial step in performance diagnostics, prescription and steering of the training process, and monitoring exercise intensity. First, a brief outline on human thermoregulation is provided, whereafter an overview is presented of what is currently known on the physiological responses to exercise in the heat and the impact of acute heat exposure on performance and exercise tests to determine the heavy-severe intensity boundary. Although the effects of heat acclimation/acclimatization (i.e., chronic heat exposure) is an interesting topic to cover, in this dissertation the focus is solely on the effect of acute heat exposure.

#### 1.3.1 Human thermoregulation

Human thermoregulation is the physiological process by which the body maintains its core temperature within a narrow range, with an average normal human body temperature of 36.6°C (95% range 35.7-37.3°C; Obermeyer et al., 2017). However, the core body temperature varies between individuals and fluctuates throughout the day ( $\pm 0.4^{\circ}\text{C}$ ), as part of the circadian rhythm (Reilly & Waterhouse, 2009). This process of maintaining homeostasis is essential for the proper functioning of the body's cells and organs. When core temperature rises above this baseline value, it is called hyperthermia (The Commission for Thermal Physiology of the International Union of Physiological Sciences, 2003). In contrast to the broad temperature range in places of human habitation, human body temperature is usually regulated within a narrow range (35-41°C) (Sawka et al., 1996). Women experience varied thermoregulatory responses throughout the menstrual cycle and during menopause, influenced by reproductive hormones like estradiol and progesterone (Brooks et al., 1997; Charkoudian et al., 2017). During the luteal phase of the menstrual cycle or when using oral contraceptives, higher levels of these hormones lead to a shift of 0.3°C to 0.7°C in the core body temperature (Baker et al., 2020; Marsh & Jenkins, 2002).

Several models exist to explain how thermoregulation occurs, i.e., the central governor model (Hammel et al., 1963), the comparator model (Kobayashi, 1989), the null zone model (Bligh, 2006) and the heat

regulation model (Webb, 1995). However, none of them have unanimously been accepted, as each one has inherent advantages and limitations. The most widespread is, perhaps, the central governor (or central integrator) model of thermoregulation proposed by Hammel et al. (1963). This model suggests that thermoreceptors located in the core (central thermoreceptors) and the skin (peripheral thermoreceptors) detect an increase in temperature above a threshold temperature (e.g.) and send a signal to the thermoregulatory center (located in the preoptic area of the anterior hypothalamus) and temperature sensors located in the spinal cord. Thermal-sensitive neurons are activated, and heat loss pathways are initiated in a manner similar to that of a domestic thermostat (i.e., negative feedback mechanism), whereafter thermoregulation is achieved through a combination of autonomic and behavioral mechanisms. Autonomic thermoregulation for heat dissipation involves the activation of the sympathetic nervous system, which causes the activation of eccrine sweat glands and widening of cutaneous blood vessels in the skin. Eccrine sweat glands, found throughout the body, are tubular coiled glands influenced by cholinergic innervation, with an undulating coiled duct leading to a sweat pore on the skin surface (Shibasaki et al., 2006). Adults possess approximately 2 million of these glands (i.e., 100-250 glands/cm<sup>2</sup>) (Cramer et al., 2022). However, their distribution, thermal sensitivity, and secretion rates differ across body segments, leading to regional variations in sweat production (Sato & Dobson, 1970). The evaporation of sweat from the skin surface aids in heat dissipation, effectively cooling the body by releasing energy into the environment. This process plays a crucial role in thermal regulation during exercise in hot environments (Wenger, 1972). Approximately 0.5 L.min<sup>-1</sup> blood (5-10% of the cardiac output) is directed to the circulation of the skin, during rest in a thermoneutral environment. However, when the body experiences heat stress, the skin circulation receives up to 8 L.min<sup>-1</sup> (50-70% of the cardiac output). This is only possible due to a concurrent rise and redistribution of cardiac output—resulting in a substantial decrease in splanchnic and renal blood flow during heat stress (Rowell, 1983). Skin blood vessels manage such profound changes in circulation under the influence of two branches of the autonomic nervous system—one responsible for widening blood vessels (vasodilation), the other for narrowing them (vasoconstriction) (Johnson et al., 2014). Sex differences have been observed in thermoregulatory responses, when the requirement for heat loss is high, related to an increase in metabolic heat production and ambient conditions. However, these are only observed in the sudomotor (i.e., sweat) activity: greater sweat production, higher sudomotor thermo-sensitivity and more sweat gland output is observed in males (Gagnon & Kenny, 2012). Nevertheless, there is currently no evidence that women have an inferior overall thermoregulation than men, when biophysical characteristics are matched (Cramer et al., 2022; Notley et al., 2017; Yanovich et al., 2020). Next to autonomic thermoregulation, behavioral thermoregulation involves voluntary actions such as reduction in work rate, fluid intake or the removal of clothing (Flouris & Schlader, 2015). The primary

objective behind the voluntary reduction in work rate during exercise in hot conditions is to decrease the metabolic heat generation and subsequent heat storage. This is mainly driven by an increase in skin temperature which changes thermal perception, and an increased cardiovascular strain, leading to an increased perceived rate of exertion. (Sawka et al., 2011; Schlader et al., 2010; Tucker et al., 2006).

The biophysics of human heat exchange can best be described in the human heat balance (Equation 8) and refers to the equilibrium between the internal rate of metabolic heat production and rate of heat exchange to the surrounding environment. This equilibrium, expressed as the rate of body heat storage (S), is derived from the First Law of Thermodynamics (Joule, 1850; Ravanelli et al., 2019):

$$S = (M - W) \pm C \pm K \pm R - E \quad (8)$$

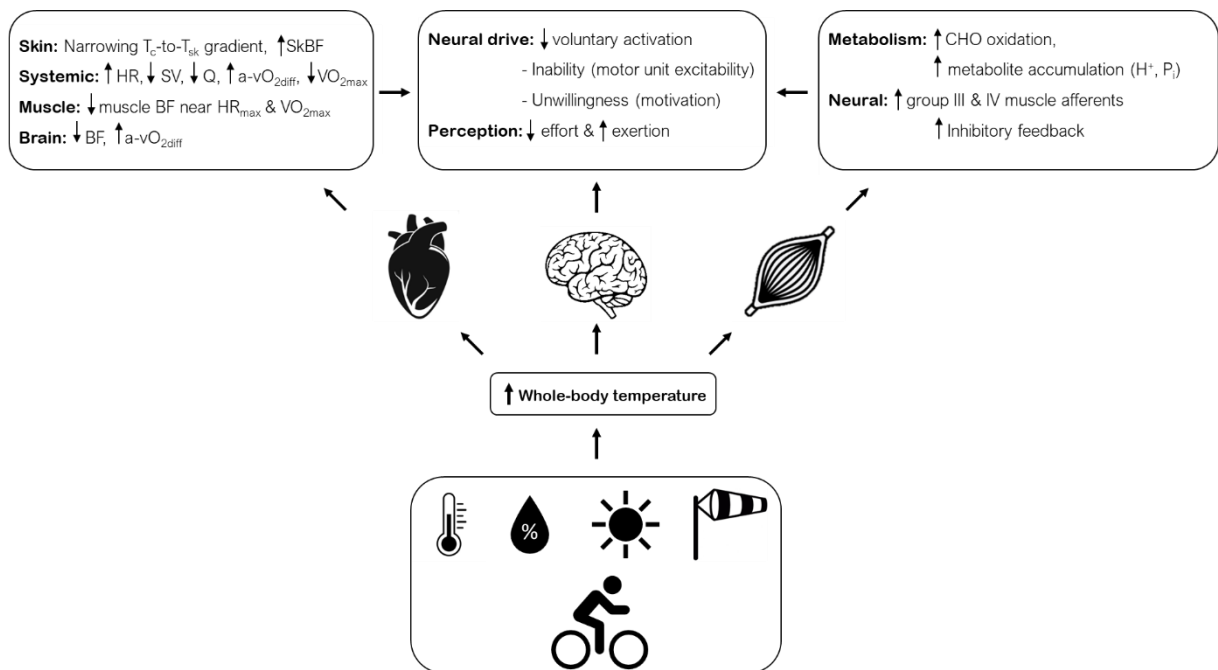
The metabolic rate is represented by M and W is the external work rate. Only ~ 20-25% of metabolic energy is converted into mechanical work, while the majority is heat production (Shephard, 1975). Thus, M-W determines the rate metabolic heat gain. The rate of convection (C) is the transfer of heat from the body to the air due to the movement of fluid or gas. Conduction (K) transfers heat from one object to another through physical contact. Radiation (R) transfers heat via infrared radiation. Evaporation (E) transfers heat as water changes state from a liquid to a gas.  $C \pm K \pm R$  determines the rate of dry heat exchange and E reflects the rate of evaporative heat loss. With increasing temperature evaporative heat loss is more beneficial, as less dry heat exchange is possible due to the narrowing of the skin-ambient air temperature gradient (Ravanelli et al., 2019). A positive value indicates heat gain, a negative value indicates heat loss and 0 indicates thermal balance. Body temperature increases when S is positive, decreases when S is negative and remains constant when S equals zero (Gagge & Gonzalez, 2011). Furthermore, heat exposure can be quantified as compensable or uncompensable, resulting in the attainment or disturbance of the human heat balance, respectively. This is determined by the amount of evaporation required for heat balance ( $E_{req}$ ) and the maximal evaporative capacity of the environment ( $E_{max}$ ). This means that if  $E_{req} \leq E_{max}$ , heat exposure is compensable. On the other hand, if  $E_{req} > E_{max}$ , heat exposure is uncompensable (Ravanelli et al., 2019).

Heat stress refers to environmental conditions that influence temperature regulation and tend to increase body temperature. Four main environmental parameters affect the biophysical properties of human heat balance: ambient air temperature, humidity, air velocity, and solar radiation. The dynamic interplay between those environmental parameters determine the severity of the heat stress. In this dissertation the effect of acute heat exposure solely refers to the ambient air temperature, as the others factors were kept constant.

### 1.3.2 Physiological responses to exercise in the heat

During exercise, heat is generated before any heat loss mechanisms are activated, causing an increase in core body temperature (Nielsen, 1938). As the body detects the rise in heat storage, it initiates heat loss pathways to slow down the rate of core temperature increase. In moderate climates, a balance is achieved where heat loss matches heat production, resulting in a stable but elevated core temperature. In such cases, the increase in core body temperature is primarily influenced by metabolic rate rather than environmental conditions. However, in environments where heat production is higher or heat dissipation is limited due to external factors, core body temperature will continue to rise beyond a steady state (Sawka et al., 2011; Sawka et al., 1993).

The physiological response to exercise in the heat is complex, as the development and extent of these adjustments, i.e., thermal strain, are dependent on many factors such as the severity of the heat stress, individual characteristics, the intensity distribution of the exercise and the duration of the exposure. The rise in whole body (core, skin and muscle) temperature during exercise in the heat is the start of a cascade of physiological responses (Périard et al., 2021; Sawka et al., 2011). This section will focus on the alterations in the cardiovascular system, central nervous system and skeletal muscle function (overview provided in Figure 11).



**Figure 11** Schematic representation of the impact of exercise with heat stress (combination of air temperature, humidity, solar radiation and wind speed) on cardiovascular system, central nervous system (CNS) and skeletal muscle function associated with fatigue development. Abbreviations:  $T_c$ -to- $T_{sk}$ , core-to-skin temperature; SkBF, skin blood flow; HR, heart rate; SV, stroke volume; a- $\dot{V}O_{2diff}$ , arterio-venous oxygen difference;  $\dot{V}O_{2max}$ , maximal oxygen uptake; BF, blood flow; CHO, carbohydrate (reproduced from Périard et al., 2021).

During exercise in the heat, the cardiovascular system experiences increased thermal strain due to the combined demands of thermoregulation and the metabolic requirements of exercise (Crandall & González-Alonso, 2010; Nybo et al., 2014; Rowell, 1974). This strain is characterized by various physiological responses and adaptations. The narrowing of the core-to-skin temperature ( $T_{c-to-Tsk}$ ) gradient and the redistribution of blood flow towards the skin, i.e., increased skin blood flow (SkBF), are the primary contributors to cardiovascular strain (Rowell, 1974). In return, this provides thermal information and feedback signals to the thermoregulatory system (Romanovsky, 2014). One of the primary challenges for the cardiovascular system is to provide sufficient cardiac output to the active skeletal muscle to meet the energetic demands for muscular activity and to the skin to meet the demands of temperature regulation (González-Alonso et al., 2008; Sawka et al., 2011). To facilitate heat dissipation, blood vessels in the skin and periphery dilate, resulting in decreased peripheral resistance and decreased venous return (i.e. postural blood pooling). A redistribution of blood flow requires an augmented cardiac output ( $Q$ ), in the early stages of exercise, to maintain adequate perfusion to the working muscles and other essential organs (Rowell et al., 1965). As a result, there is an increased demand placed on the heart, leading to an elevated heart rate (HR) and increased myocardial work. During exercise near maximal HR,  $Q$  gets compromised, as a consequence of a lower stroke volume (SV). This seems to be a combined effect of reductions in cardiac filling pressure due to reduced central blood volume (Rowell et al., 1966) and decreased diastolic filling time (Fritzsche et al., 1999). Furthermore, blood flow to both the muscles and the brain are compromised as well (Nielsen et al., 1990; Nybo et al., 2002). All these responses result in a reduced exercise capacity, despite an increase in arterio-venous oxygen difference ( $a-vO_2diff$ ) (Périard et al., 2012).

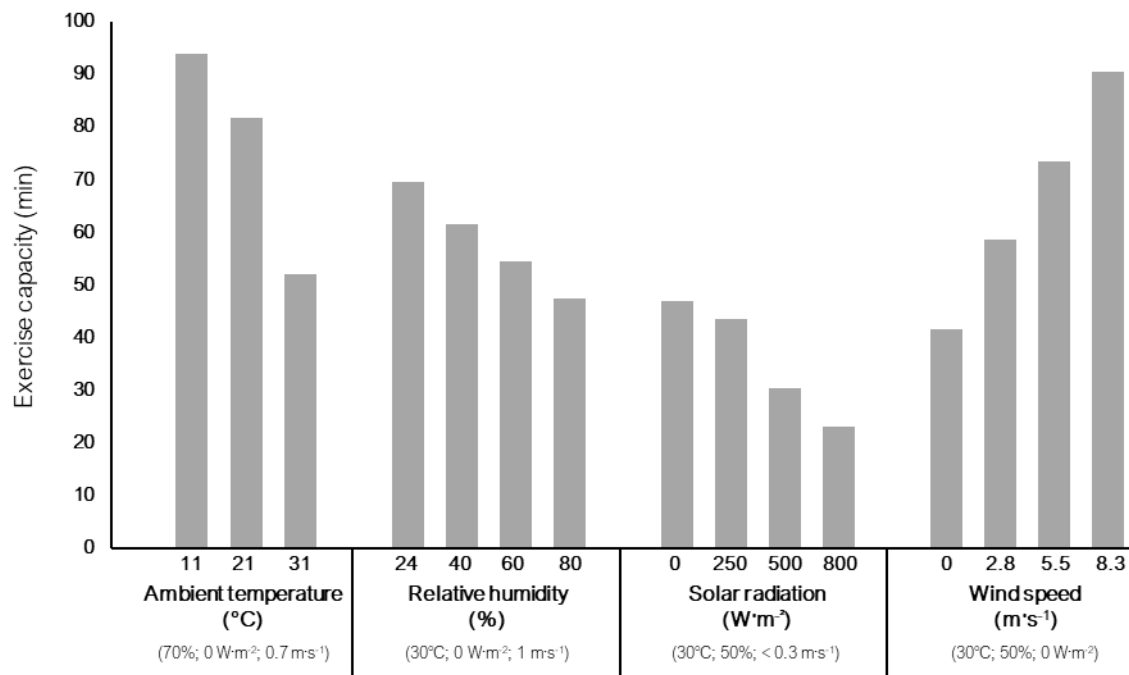
The central nervous system (CNS) regulates exercise performance through a complex interplay of neural drive, afferent feedback sensors and neurotransmitter activity affecting voluntary muscle force production. It has been shown that hyperthermia influences CNS function during maximal voluntary contractions, by suppressing voluntary activation, either through an inability or unwillingness to contract exercising muscles, as an elevated brain temperature might trigger inhibitory signals in temperature-sensitive areas of the hypothalamus (Nielsen & Nybo, 2003; Nybo & Nielsen, 2001). However, the precise mechanisms responsible for the decline in performance under hyperthermic conditions are still not fully elucidated. Among the commonly discussed theories, the “critical core temperature hypothesis” suggests that exercise or work is terminated in hot conditions when the core body temperature reaches a critical level ( $\sim 40^{\circ}C$ ) to protect the body from potential heat-related damage (González-Alonso et al., 1999; Nielsen et al., 1993). An alternative concept, known as the “central governor theory”, proposes rather than reaching a potentially catastrophic endpoint such as critically high core temperature, a down-regulation in work output occurs in advance. This anticipatory

response involves self-regulating exercise intensity in hot conditions, resulting in a reduction in work output before reaching a critical limit (Tatterson et al., 2000; Tucker et al., 2004). The “central fatigue” theory addresses alterations in cerebral neurotransmitter activity and concentrations of serotonin and especially dopamine as the cause of fatigue due to hyperthermia (Meeusen & Roelands, 2010; Newsholme & Blomstrand, 1995). However, it is likely that the etiology of hyperthermia-induced fatigue can be found in an integrated model of multiple physiological and psychological factors, instead of one specific mechanism or system (Noakes et al., 2005; St Clair Gibson et al., 2018).

Lastly, elevations in muscle temperature have notable effects on skeletal muscle function and metabolism. An increase in muscle temperature enhances acute explosive exercises, as metabolic and contractile function are improved and nerve conduction velocity is increased (Girard et al., 2015; Racinais & Oksa, 2010; Sargeant, 1987). On the other hand, during prolonged exercise in hot conditions, there is an increase in muscle glycogen utilization (i.e., carbohydrate (CHO) oxidation) and a greater reliance on the anaerobic glycolytic metabolism, due to the reduction in muscle blood flow limiting oxygen delivery (Febbraio et al., 1996; Febbraio et al., 1994; Fink et al., 1975). This results in a greater accumulation of fatigue-related metabolites ( $H^+$  and  $P_i$ ), which in turn activate group III/IV muscle afferents sending inhibitory neural feedback to the central nervous system, and reducing central motor drive (Amann, 2011; Blain et al., 2016).

### *1.3.3 Performance in hot environments*

The effect of heat stress on performance is well-documented by numerous studies (see below). The quantification of heat stress is dependent of the 4 environmental factors (ambient temperature, relative humidity, solar radiation and wind speed). Ronald Maughan and colleagues conducted research studies investigating these 4 parameters independently. In each study, participants had to cycle to exhaustion at 70% of their  $\dot{V}O_{2max}$ , while one variable was manipulated, i.e. ambient air temperature (Galloway & Maughan, 1997), relative humidity (Maughan et al., 2012), solar radiation (Otani et al., 2016) or wind speed (Otani et al., 2018). A visual representation of the independent effect of these parameters on exercise capacity is provided in Figure 12. Increased ambient temperature, higher relative humidity, more solar radiation or lower wind speed resulted in a diminished exercise capacity. Nevertheless the overall heat stress is a combination of these 4 factors, in most experimental study designs the effect of ambient temperature is the main point of interest. In Table 2, an overview is provided of studies that demonstrated a change in performance during exercise to task failure (i.e., constant work rate) or time trials (i.e., self-paced exercise) due to an increased ambient temperature, reporting performance losses between 6 and 50%.



**Figure 12** The effect of thermal stress on exercise capacity in the heat. In each study participants had to cycle to exhaustion at 70%  $\dot{V}O_{2max}$ , while one environmental factor was manipulated; ambient temperature (Galloway & Maughan, 1997), relative humidity (Maughan et al., 2012), solar radiation (Otani et al., 2016) or wind speed (Otani et al., 2018). All other environmental factors were controlled within the same study.

Furthermore, analysis of data from the International Association of Athletic Federations (IAAF) World Championships (WC) between 1999 and 2011 revealed that endurance performance (5000m, 10.000m and marathon) was decreased when the ambient temperature at the WC was  $> 25^{\circ}\text{C}$  compared with WC's taking place in  $< 25^{\circ}\text{C}$  (Guy et al., 2015). The findings from Guy et al. (2015) on the slower marathon performance time when ambient air temperature is high ( $> 25^{\circ}\text{C}$ ) are in accordance with the studies of El Helou et al. (2012) and Ely et al. (2007). On the other hand, heat stress can also have a positive effect on performance, namely maximal exercise of short duration (i.e. explosive). In the same study of Guy et al. (2015), it has been demonstrated that single sprint events (100m and 200m) were faster in hot environmental conditions ( $> 25^{\circ}\text{C}$ ) compared to temperate conditions ( $< 25^{\circ}\text{C}$ ). The mechanisms underlying the improvement in contractile function and speed/power output in the heat are attributed to faster phosphocreatine utilization (Gray et al., 2006), increased ATP turnover from anaerobic energy sources (Febbraio et al., 1996), and faster muscle fiber conduction velocity (Farina et al., 2005; Gray et al., 2006).

**Table 2** Overview of studies investigating the effect of acute heat exposure on performance.

| Study  | Ambient temperature | Individuals              | Exercise protocol        | Δ performance loss (%) |
|--|---------------------|--------------------------|--------------------------|------------------------|
| <b>Exercise to task failure - constant work rate</b> |                     |                          |                          |                        |
| Saltin et al. (1972)                                 | 10°C vs. 30°C       | 4 endurance trained men  | 91% PO $\dot{V}O_{2max}$ | -19 %                  |
| Galloway & Maughan (1997)                            | 11°C vs. 31°C       | 8 healthy men            | 70% $\dot{V}O_{2max}$    | -45 %                  |
| Parkin et al. (1999)                                 | 20°C vs. 40°C       | 8 endurance trained men  | 70% $\dot{V}O_{2max}$    | -50 %                  |
| Mitchell et al. (2014)                               | 10°C vs. 37°C       | 11 endurance trained men | 80% $\dot{V}O_{2max}$    | -50 %                  |
| Girard & Racinais (2014)                             | 22°C vs. 35°C       | 11 physically active men | 66% PO $\dot{V}O_{2max}$ | -35 %                  |
| <b>Time trial - self-paced exercise</b>              |                     |                          |                          |                        |
| Tatterson et al. (2000)                              | 23°C vs. 32°C       | 11 male cyclists         | 30 min                   | -6.5 %                 |
| Tucker et al. (2004)                                 | 15°C vs. 35°C       | 10 male cyclists         | 20 km                    | -6 %                   |
| Périard et al. (2011)                                | 20°C vs. 35°C       | 9 male cyclists          | 40 km                    | -13 %                  |
| Peiffer & Abbiss (2011)                              | 17°C vs. 32°C       | 9 male cyclists          | 40 km                    | -6 %                   |
| Racinais et al. (2015)                               | 8°C vs. 37°C        | 9 male cyclists          | 43.4 km                  | -16 %                  |

*For the environmental conditions, only ambient air temperature was manipulated in all studies. Characteristics of the individuals and exercise protocol were presented as specified by the authors of the respective study. Δ Performance loss during exercise to task failure refers to a decrease in time to task failure, while during time trials it refers to a reduction in mean power output in hot compared with temperate environments. PO, power output;  $\dot{V}O_{2max}$ , maximal oxygen uptake.*

Fewer studies have investigated the impact of acute heat exposure on the determination of the heavy-severe intensity threshold (gold standard or surrogates). Maunder et al. (2021) reported decreases for OBLA 2.0, OBLA 3.0 and OBLA 4.0, respectively of 16%, 13% and 10% between 18 and 36°C (60 % RH) in 16 competitive endurance-trained males, and a 17 % and 12 % decrease is noticed, respectively at first (i.e., GET) and second (i.e., RCP) RT. de Barros et al. (2011) found that PO corresponding to MLSS and RCP decreased by 18% in 40°C in comparison to 22°C (50% RH) for eight healthy young untrained male participants. Furthermore, Tyka et al. (2009; 2010) reported a 13% and 11% decrease at respectively LT (Exp- $D_{max}$ ) and GET (V-slope method) in 37°C compared to 23°C (55% RH). Lorenzo et al. (2011) found an overall decrease of 12% in power output at several blood- and ventilation-based thresholds in twelve highly trained endurance cyclists (10 men and 2 women) when cycling in 38 °C compared to 13°C (30% RH). More recently, two studies reported estimates of CP and  $W'$  using a 3 min all-out test (3MT) in a hot environment (Kaiser et al., 2021; Kuo et al., 2021). While Kaiser et al. (2021) hypothesized that CP

would decrease and  $W'$  would increase, they demonstrated that mild hyperthermia (core temperature 38.5°C) via preceding hot water immersion (41.5°C) was not sufficient to alter CP and  $W'$  during a 3MT in hot conditions (38°C). Conversely, Kuo et al. (2021) found a reduction in CP, but not in  $W'$ , after a combination of passive (40 min) and active (i.e., 5 min cycling in moderate intensity domain and 3×10 s sprints) heat exposure (35°C) compared with the same protocol in temperate conditions (22°C). Together with these contradictory findings, and notwithstanding the broad application of this CP concept and its additional potential to calculate the  $W'$  reconstitution in intermittent exercise, the impact of external factors such as heat, is very scarcely documented (Richard & Koehle, 2022). Furthermore, to date, limited exercise performance studies are conducted in hot environments investigating potential sex-differences, showing ambiguous results. Observed sex-differences might be related to a difference in relative intensity or secondary to differences in anthropometrics and fitness level (Corbett et al., 2023). Moreover, it has been shown that female reproductive hormones have trivial effects on the thermoregulatory system and exercise performance in the heat (Giersch et al., 2020; Notley et al., 2019).

Finally, a distinction must be made between 'exercise with acute heat exposure' and 'exercise with hyperthermia'. The former refers to the situation where individuals are exercising in a hot environment, experiencing heat stress, and is investigated in this PhD thesis. The latter specifically denotes the physiological state where the body temperature has risen significantly above the individual's baseline value inducing heat strain. This can be achieved by various factors, including prolonged exposure to a hot environment or intense physical activity.

## 1.4 Aims and hypotheses

This dissertation focuses on the determination of the heavy-severe exercise intensity boundary in temperate and hot environments, and the acute recovery from severe-intensity exercise.

The critical power (CP) concept is employed as the gold standard to determine this heavy-severe intensity boundary or maximal metabolic steady state (MMSS), represented by CP itself. The combination of climate change and mass participation events or competitions under extreme environmental conditions, makes that there is a need for specific guidance of individuals exercising in hot environments. As the determination of the heavy-severe intensity boundary is often the first step in the preparation phase to set goals, prescribe training and monitor (long term) performance, it is useful to gather this information in the environmental conditions the individual has to perform. However, numerous threshold concepts with various test methodologies are employed to determine the heavy-severe intensity boundary, which could be affected differently by heat exposure. The effect of the interaction between intensity and duration could play a role in the determination of thresholds. Furthermore, the effect of acute heat exposure would allow to study the underlying mechanisms of exercise thresholds concepts.

Therefore, it was our goals to examine how acute heat exposure affected the determination of exercise thresholds, representing the boundary between heavy and severe exercise intensity, more specifically, in *study 1*, CP (and  $W'$ ) in women and men, and in *study 2*, surrogates of the maximal metabolic steady state, i.e., respiratory compensation point and second lactate threshold.

*We hypothesized that that not all thresholds would be equally affected by heat exposure but that the impact of heat would depend on the design of the exercise test protocol, more specifically the interaction between the intensity and duration. Furthermore, we hypothesized that the effect of acute heat exposure on CP and  $W'$  would not be different between women and men.*

Once the CP is determined as the heavy-severe exercise intensity boundary, together with the second parameter  $W'$ , it could be applied to intermittent exercise and acute recovery from severe-intensity exercise. The CP concept is currently very popular for the prescription of (interval) training and predict performance, due to technical innovations (e.g., development of power meters in cycling). However, despite the increasing number of studies on the CP concept, little is known about  $W'$  reconstitution in women. Furthermore, a broader application during intermittent exercise, i.e.,  $W'_{\text{BAL}}$ , requires further insight into the underlying mechanisms defining  $W'$  recovery.

In *study 3*,  $W'$  reconstitution following exhaustive exercise was assessed within a broad range of recovery durations, i.e., between 30 seconds and 15 minutes, to obtain a better representation of the

$W'$  recovery pattern. Furthermore, we investigated if  $W'$  reconstitution was associated with aerobic fitness and muscle fiber type distribution.

*We hypothesized that  $W'$  reconstitution would be better fitted by a two-phase than a mono-exponential model, and that a higher aerobic fitness and a higher percentage of type I muscle fibers would be positively correlated with  $W'$  reconstitution.*

In study 4, the aim was to observe  $W'$  reconstitution following repeated maximal exercise, and this in women and men.

*We hypothesized that a slowing of  $W'$  would occur, i.e.,  $W'$  reconstitution is less during the second recovery period as compared with the first recovery period, despite similar work and recovery characteristics. Furthermore, fractional  $W'$  reconstitution (i.e.,  $W'$  reconstitution normalized to the individual's maximal value of  $W'$ ) would occur faster in women as compared with men, although absolute  $W'$  reconstitution (i.e., in kJ) would be larger in men.*

## *Chapter 2*

### *Original research*



# ***STUDY 1***

## **Effect of acute heat exposure on the determination of critical power and $W'$ in women and men**

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## ABSTRACT

The goal of this study was to investigate to what extent acute heat exposure would affect the parameters of the power-duration relationship, i.e. CP and  $W'$ , using multiple constant workload tests to exhaustion, in women and men. Twenty four young physically active participants (12 men, 12 women) performed 3 to 5 constant load tests to determine CP and  $W'$ , both in temperate (TEMP; 18°C) and hot (HOT; 36°C) environmental conditions. A repeated-measures ANOVA was executed to find differences between TEMP and HOT, and between women and men. In HOT, CP was reduced by 6.5% ( $227 \pm 50$  vs.  $212 \pm 47$  W), while  $W'$  increased 12.4% ( $16.4 \pm 4.4$  vs.  $18.5 \pm 5.6$  kJ). No significant two-way sex  $\times$  temperature interactions were observed, indicating that the environmental conditions did not have a different effect in men compared with women. The intersection of the average curvatures in TEMP and HOT occurred at 137 s and 280 W in women, and 153 s and 397 W in men. Acute heat exposure had an impact on the parameters CP and  $W'$ , i.e., CP decreased whereas  $W'$  increased. The increase in  $W'$  might be a consequence of the mathematical modelling for the used test methodology, rather than a physiological accurate value of  $W'$  in HOT. No differences induced by heat exposure were observed between women and men.

## KEY WORDS

Constant workload; power-duration relationship; sex; temperature; threshold

## INTRODUCTION

The power-duration relationship is shaped by critical power (CP), the boundary between heavy and severe intensity exercise (i.e., maximal metabolic steady state), and  $W'$ , the total amount of work that can be performed above CP (i.e., finite work capacity) (Jones et al., 2019). These parameters can be used by coaches and athletes to set goals, design training interventions and long term monitoring of performance at an individual level. Moreover, this concept enables the prediction of time to task failure (TTF) at a given intensity above CP. Therefore, by allowing to estimate exercise tolerance, these variables also could provide a framework for strategic management during competition (e.g., pacing strategies) (Jones & Vanhatalo, 2017; Vanhatalo et al., 2011). Despite the broad application of the CP concept, the impact of heat exposure on the concurrently measured parameters of the power-duration relationship *per se*, i.e., CP and  $W'$ , is scarcely documented (Richard & Koehle, 2022). Investigating this matter might be of interest both from a performance and health perspective, as more athletes (recreational as well as elite) have to perform in hot environmental conditions.

Recently, two studies reported estimates of CP and  $W'$  using a 3 min all-out test (3MT) in a hot environment (Kaiser et al., 2021; Kuo et al., 2021). While Kaiser et al. (2021) hypothesized that CP would decrease and  $W'$  would increase, they demonstrated that mild hyperthermia (core temperature 38.5°C) via preceding hot water immersion (41.5°C) was not sufficient to alter CP and  $W'$  during a 3MT in hot conditions (38°C). Conversely, Kuo et al. (2021) found a reduction in CP, but not in  $W'$ , after a combination of passive (40 min) and active (i.e., 5 min cycling in moderate intensity domain and  $3 \times 10$  s sprints) heat exposure (35°C) compared with the same protocol in temperate conditions (22°C). Although it has been demonstrated that the 3MT is a valid protocol for the estimation of CP and  $W'$  (Burnley et al., 2006), some studies question its validity as an overestimation of CP and underestimation of  $W'$  have been reported (Bergstrom et al., 2014). Together with contradictory findings, there is a lack of studies investigating the impact of heat exposure on CP and  $W'$  determined using the conventional methodology, i.e., 3-5 constant workload tests to task failure in the severe intensity domain (Mattioni Maturana et al., 2018).

Previous studies have demonstrated a reduction in absolute power output (PO) at the exercise threshold demarcating the heavy-to-severe boundary, i.e., conceptually similar to CP (Keir et al., 2015), in hot environmental conditions (Bourgois et al., 2023; de Barros et al., 2011; Lorenzo et al., 2011; Maunder et al., 2021), mostly using blood lactate-related determination methodologies. However, the use of blood lactate for determining the heavy-severe boundary is questioned (Jones et al., 2019). Heat exposure, which can affect blood flow dynamics (González-Alonso & Calbet, 2003), may consequently impact these measurements, as they depend on the balance of lactate production and removal from the blood. Underlying to the reduction of PO at the heavy-severe boundary is possibly a disrupted

skeletal muscle oxygen delivery due to a decline in cardiac output and mean arterial pressure, as a result of thermoregulatory adjustments to hyperthermia (González-Alonso & Calbet, 2003). Partially linked to the reduction of the heavy-severe boundary, it has been shown that performance in the severe intensity domain (i.e., maximal exercise up to 30 min), is impaired in heat. Several studies reported a reduction in TTF during constant work rate exercise (González-Alonso & Calbet, 2003; Mitchell et al., 2014; Saltin et al., 1972) or a progressive decrease in PO during self-paced exercise (Ely et al., 2010; Périard & Racinais, 2015; Tattersson et al., 2000; Tucker et al., 2004) ranging from -4% to even -50% with heat exposure. The impact of heat exposure on performance has been described to depend on the severity of the environmental conditions, the initial thermal strain, total exposure time and exercise duration/intensity (Périard et al., 2021). From a mechanistic point of view, it would be interesting to be able to make a clear distinction on the effect of acute heat exposure between CP on the one hand and  $W'$  on the other.

There is an increasing demand for an individualized scientific approach to monitor training and optimize performance. Nonetheless, female data on this topic is lacking as women are generally underrepresented in sport science research (Costello et al., 2014). An understanding of the response to exercise is crucial to optimize training outcomes in both sexes (Ansdell et al., 2020). There is some discussion on the potential lesser thermoregulation pathways of women affecting performance in the heat, although, a recent review by Yanovich et al. (2020) summarized that there is currently no evidence that women have an inherent disadvantage in thermoregulation when exercising in the heat compared with men of similar age and health status.

The goal of this study was to examine the effect of acute heat exposure on CP and  $W'$  in men and women using the conventional methodology (i.e., multiple constant load tests to task failure) in temperate (18°C) and hot (36°C) environmental conditions. This will provide more insight into the response to exercise with acute heat exposure and ensure useful support to enhance the preparation of athletes, employing the CP concept, for training and competition in hot environments. In accordance with the review of Richard & Koehle (2022), we hypothesized that CP would be lower, whereas  $W'$  would not be influenced in hot environmental conditions, with no difference between women and men.

## METHODS

### Participants

Twelve women and twelve men were recruited to take part in this study. Characteristics of the participants are displayed in Table 1. A medical history questionnaire had to be completed and training status of the volunteers was self-reported for type, duration and frequency. Exclusion criteria were competitive cycling, chronic diseases, (lower limb) injury, smokers and a negative advise from a medical doctor related to pulmonary and cardiovascular anomalies. Female participants declared to be not pregnant at the start of the study, nor planning to be pregnant during the study. Two women had a hormonal intrauterine device (IUD), seven were using combined oral contraceptive pills and three did not use any contraceptives. Participants did not train for more than one week in hot environments three months preceding the study to avoid heat acclimation/acclimatization effects. After receiving a description of the procedure of the experiment, all participants gave their written informed consent to volunteer in this study. All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval was received (reference: B6702020000260) of the ethical committee of the Ghent University Hospital (Ghent, Belgium).

**Table 1** Characteristics of the participants.

|  | All         | Women       | Men         |
|--|-------------|-------------|-------------|
| <i>n</i>   | 24          | 12          | 12          |
| <b>Demographics</b>  |             |             |             |
| Age (years)  | 25.8 ± 3.3  | 26.9 ± 3.1  | 25.0 ± 3.4  |
| Training (h·week <sup>-1</sup> )                             | 6.4 ± 1.5   | 5.4 ± 0.8   | 7.5 ± 1.1   |
| <b>Anthropometrics</b>                                       |             |             |             |
| Body height (cm)   | 175 ± 8     | 169 ± 5     | 182 ± 5     |
| Body mass (kg)   | 68.1 ± 9.7  | 61.9 ± 5.5  | 74.3 ± 9.1  |
| BMI  | 22.0 ± 2.0  | 21.6 ± 1.1  | 22.5 ± 2.5  |
| <b>Incremental exercise test</b>                             |             |             |             |
| $\dot{V}O_{2PEAK}$ (L·min <sup>-1</sup> )                    | 3.40 ± 0.94 | 2.53 ± 0.37 | 4.26 ± 0.30 |
| $\dot{V}O_{2PEAK}$ (ml·min <sup>-1</sup> ·kg <sup>-1</sup> ) | 49.4 ± 10.0 | 41.0 ± 5.2  | 57.9 ± 5.2  |
| PO <sub>PEAK</sub> (W)                                       | 341 ± 75    | 277 ± 37    | 406 ± 35    |
| PO <sub>PEAK</sub> (W·kg <sup>-1</sup> )                     | 5.0 ± 0.7   | 4.5 ± 0.5   | 5.5 ± 0.5   |

Group mean ± SD for all participants (*n* = 24), and separated for women (*n* = 12) and men (*n* = 12) of demographical data, anthropometrical measurements and peak values obtained from the incremental exercise test in temperate environmental conditions (18°C) in women and men. BMI, body mass index;  $\dot{V}O_2$ , pulmonary oxygen uptake; PO, power output.

## General study design

All participants visited the lab 7-11 times with a minimum of 48 h between two tests over a period of 6 weeks in the autumn period. The first visit contained an incremental exercise test, for the assessment of peak power output ( $PO_{PEAK}$ ) and peak oxygen uptake ( $\dot{V}O_{2PEAK}$ ), which are presented in Table 1. The following 6-10 tests were to determine CP and  $W'$ , i.e., 3-5 tests in temperate environmental conditions (18°C and 40% RH) and 3-5 tests in hot environmental conditions (36°C and 40% RH), in randomized order to deal with practice effects. Before the start of each exercise test, participants were seated for 15 min to accommodate to the respective environmental conditions. All tests took place on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) in the climatic chamber at the Sport Science Laboratory Jacques Rogge of the Ghent University (Ghent, Belgium, sea level) between 2.00 p.m. and 8.00 p.m. Participants were instructed to abstain from any exhaustive exercise 24 hours leading up to an exercise test and to refrain from consumption of caffeine and alcohol for 24 hours prior to testing. Participants were asked to maintain the same type of meals on the day of an exercise test (including carbohydrates with a low glycemic index) and to drink ~ 500 mL of water over 2 h prior to the beginning of the test. Before every test, participants were asked to empty their bladder and provide a urine sample to check whether urine-specific gravity (USG) was < 1.020 with a refractometer (PAL-10S, ATAGO, Tokyo, Japan) to assure a euhydrated status (Sawka et al., 2007). During the first test participants were instructed to choose their cadence between 70 and 90 revolutions per minute (rpm), and maintain their preferred cadence during all upcoming exercise tests. Strong verbal encouragement was provided throughout all exercise tests to ensure maximum effort. All trials to task failure were terminated when the participant was unable to maintain the preferred cadence minus 10 rpm for more than 5 consecutive seconds.

## Measurements and data analysis

### Incremental exercise test

A ramp incremental exercise test was performed with work rate increasing linearly with 20 W·min<sup>-1</sup> for female participants and 30 W·min<sup>-1</sup> for male participants. Pulmonary gas exchange was registered breath by breath using a metabolic cart (MetaLyzer 3B; Cortex Biophysik, Leipzig, Germany) for the determination of  $\dot{V}O_{2PEAK}$ , which was defined as the highest 30 s average achieved throughout the protocol.

### Constant workload tests

A minimum of three constant workload tests to task failure had to be performed in TEMP and HOT to estimate CP and  $W'$ . The aim was to evoke TTF approximately between 2-5 min for the short trial (T1), 5-10 min for the middle-long trial (T2) and 10-20 min for the longest trial (T3). Based on existing

expertise and pilot testing in our laboratory, it was opted to start with the following intensities relative to  $PO_{PEAK}$ , obtained from the incremental exercise test: 90%, 80% and 75% in TEMP and 90%, 75% and 70% in HOT. If the duration of a trial was too short, relative intensity was lowered for the other trials. When three tests were completed in TEMP and HOT, CP and  $W'$  were determined using three mathematical models, the linear time-work relationship model (1), linear inverse-of-time model (2) and hyperbolic model (3).

$$W = W' + CP \times t \quad (1)$$

$$P = W' \times \left(\frac{1}{t}\right) + CP \quad (2)$$

$$t = \frac{W'}{P - CP} \quad (3)$$

The parameter  $W$  is the total work performed,  $P$  is a given PO above CP and  $t$  is the time to task failure. Using the best individual fit method, the model with the smallest combined sum of the standard error of the estimates (SEE) for CP (< 5%) and  $W'$  (< 10%) was selected for appropriate estimation of CP and  $W'$  (Black et al., 2015). When this criterium was not met with three tests in the respective environmental condition, one or two additional constant load tests to task failure were performed. The intensity for those extra trials was selected in between the already applied individual intensities. Thereafter, the three tests with the smallest combined sum of SEE were selected to determine CP and  $W'$ . The hyperbolic model was used for 7 female and 6 male participants and the linear inverse-of-time model for 5 women and 6 men in TEMP. In HOT 2 women and 6 men had the best fit using the hyperbolic model, while the linear inverse-of-time model was better suited for 10 women and 6 men. A total of  $3.5 \pm 0.7$  and  $3.7 \pm 0.7$  tests were executed, respectively in TEMP and HOT. Overall, PO was selected between 63 and 90%  $PO_{PEAK}$ , determined in the incremental exercise test.

Participants did not receive information on elapsed time and power output during the trials. The test was preceded with 5 min warm-up at 50 W, after which the PO immediately switched to the predetermined work rate. The participants were instructed to increase their cadence with 10 rpm in the final 5 s of the warm-up to facilitate the transition, whereafter they went back to the target cadence in 5-10 s. TTF was marked when the participant was unable to maintain the preferred cadence minus 10 rpm for more than 5 consecutive seconds.  $\dot{V}O_2$  and heart rate (HR) were registered continuously throughout the tests.  $\dot{V}O_2$  obtained during the last 60 s before task failure, as well as the highest HR value during the last 60 s before task failure was used for analysis. Achievement of 95% of  $\dot{V}O_{2PEAK}$

(obtained during the ramp incremental exercise test) was required for all tests (Black et al., 2015). A blood sample was taken within 30 s after the test was finished, to analyze for blood lactate concentration ( $[Bla^-]$ ). The sample consisted of 20  $\mu$ L of blood from the left middle finger, was collected into a capillary tube and analyzed (Biosen C-Line Clinic; EKF-diagnostic GmbH, Magdeburg, Germany).

### **Statistical analysis**

Data are presented as mean  $\pm$  SD for  $n = 24$ , and separately for  $n = 12$  female and  $n = 12$  male participants. Data were normally distributed, as assessed by Shapiro-Wilk's test ( $p > 0.05$ ) and homogeneity of variances was assured by the Levene's test of homogeneity of variance ( $p > 0.05$ ). All statistical analyses were performed with SPSS Statistics 25 (IBM Corp.) Statistical significance was accepted when  $p < 0.05$ . A two-way mixed repeated measures analysis of variance (sex [between-subject factor with two levels: women and men]  $\times$  temperature [within-subject factor with two levels: 18°C (TEMP) and 36°C (HOT)]) was performed to detect differences in CP and  $W'$ . In order to test whether the changes in CP and  $W'$  from TEMP to HOT are related, a Pearson correlation coefficient ( $r$ ) was used. A three-way mixed repeated measures analysis of variance (sex [between-subject factor with two levels: women and men]  $\times$  temperature [within-subject factor with two levels: 18°C (TEMP) and 36°C (HOT)]  $\times$  intensity [within-subject factor with three levels: short trial (T1), middle-long trial (T2) and long trial (T3)]) was performed to detect differences in physiological variables  $\dot{V}O_2$ , HR and  $[Bla^-]$  during the constant workload tests to task failure.

## RESULTS

Values of CP and  $W'$  in TEMP and HOT can be found in Table 2. No significant sex  $\times$  temperature interactions were found for CP and  $W'$ . The main effect of temperature showed that CP was lower in HOT compared with TEMP ( $p < 0.01$ ), whereas  $W'$  was increased in HOT ( $p = 0.002$ ). CP and  $W'$  were significantly lower in women compared with men ( $p < 0.01$ ). However, this was not the case when CP was expressed as a percentage of  $PO_{PEAK}$  ( $p = 0.122$ ). The mean SE for CP and  $W'$  estimates did not differ between men and women nor TEMP and HOT ( $p > 0.05$ ). Changes in CP and  $W'$  from TEMP to HOT were inversely related ( $r = -0.74$ ;  $p < 0.001$ ), indicating that a larger decrease in CP was associated with a larger increase in  $W'$ .

**Table 2** CP and  $W'$  values using best individual fit method in women and men in TEMP (18°C) and HOT (36°C).

| <i>n</i>                   | All        |            | Women      |            | Men        |            |     |
|----------------------------|------------|------------|------------|------------|------------|------------|-----|
|                            | 24         |            | 12         |            | 12         |            |     |
|                            | 18 °C      | 36 °C      | 18 °C      | 36 °C      | 18 °C      | 36 °C      |     |
| CP (W)                     | 227 ± 50   | 212 ± 47   | 187 ± 30   | 176 ± 29   | 267 ± 29   | 249 ± 30   | a,b |
| CP (W·kg <sup>-1</sup> )   | 3.3 ± 0.5  | 3.1 ± 0.5  | 3.0 ± 0.4  | 2.8 ± 0.4  | 3.6 ± 0.4  | 3.4 ± 0.4  | a,b |
| CP (% PO <sub>PEAK</sub> ) | 67 ± 3     | 62 ± 3     | 68 ± 3     | 63 ± 3     | 66 ± 3     | 61 ± 4     | b   |
|                            |            |            |            |            |            |            |     |
| W' (kJ)                    | 16.4 ± 4.4 | 18.5 ± 5.6 | 12.8 ± 1.6 | 14.3 ± 2.5 | 20.0 ± 3.1 | 22.7 ± 4.5 | a,b |
| W' (J·kg <sup>-1</sup> )   | 239 ± 45   | 269 ± 62   | 208 ± 25   | 231 ± 36   | 270 ± 39   | 308 ± 60   | a,b |
|                            |            |            |            |            |            |            |     |
| CP SE (W)                  | 2.0 ± 1.4  | 2.4 ± 1.8  | 1.6 ± 0.9  | 2.3 ± 1.7  | 2.4 ± 1.7  | 2.5 ± 2.1  |     |
| CP SE (%)                  | 0.9 ± 0.6  | 1.1 ± 0.8  | 0.8 ± 0.5  | 1.3 ± 0.8  | 0.9 ± 0.7  | 1.0 ± 0.8  |     |
|                            |            |            |            |            |            |            |     |
| W' SE (kJ)                 | 0.9 ± 0.4  | 0.9 ± 0.7  | 0.8 ± 0.4  | 0.7 ± 0.5  | 1.0 ± 0.4  | 1.1 ± 0.9  |     |
| W' SE (%)                  | 5.9 ± 2.7  | 5.1 ± 3.4  | 6.4 ± 2.7  | 5.3 ± 3.4  | 5.3 ± 2.6  | 4.8 ± 3.5  |     |

Group mean  $\pm$  SD for all participants ( $n = 24$ ), and separated for women ( $n = 12$ ) and men ( $n = 12$ ) in temperate (18°C) and hot (36°C) environmental conditions. Significant differences ( $p < 0.05$ ) are presented in the table as <sup>a</sup> significant difference between women and men; <sup>b</sup> significant difference between TEMP and HOT. %  $PO_{PEAK}$ , peak power output out of the incremental exercise test in TEMP; SE, standard error for the applied model.

$PO$ , TTF and physiological data obtained during the three constant load tests to task failure are presented in Table 3. No significant three-way interaction effects were found for  $\dot{V}O_2$ , HR and  $[Bla^-]$ . No significant two-way sex  $\times$  temperature interactions were observed. A significant two-way temperature  $\times$  intensity interaction showed that the change in  $[Bla^-]$  between trials was different across the two temperatures. Post hoc analysis revealed that  $[Bla^-]$  in HOT was higher in T1 ( $p = 0.003$ ) and lower in T3 ( $p = 0.029$ ), while no difference was observed between TEMP and HOT in T2 ( $p = 0.817$ ). A main effect for sex ( $p = 0.002$ ) was found for  $\dot{V}O_2$  and  $[Bla^-]$ , showing higher values in men compared with women. Furthermore, a significant main effect of temperature showed that  $\dot{V}O_2$  was higher in HOT compared

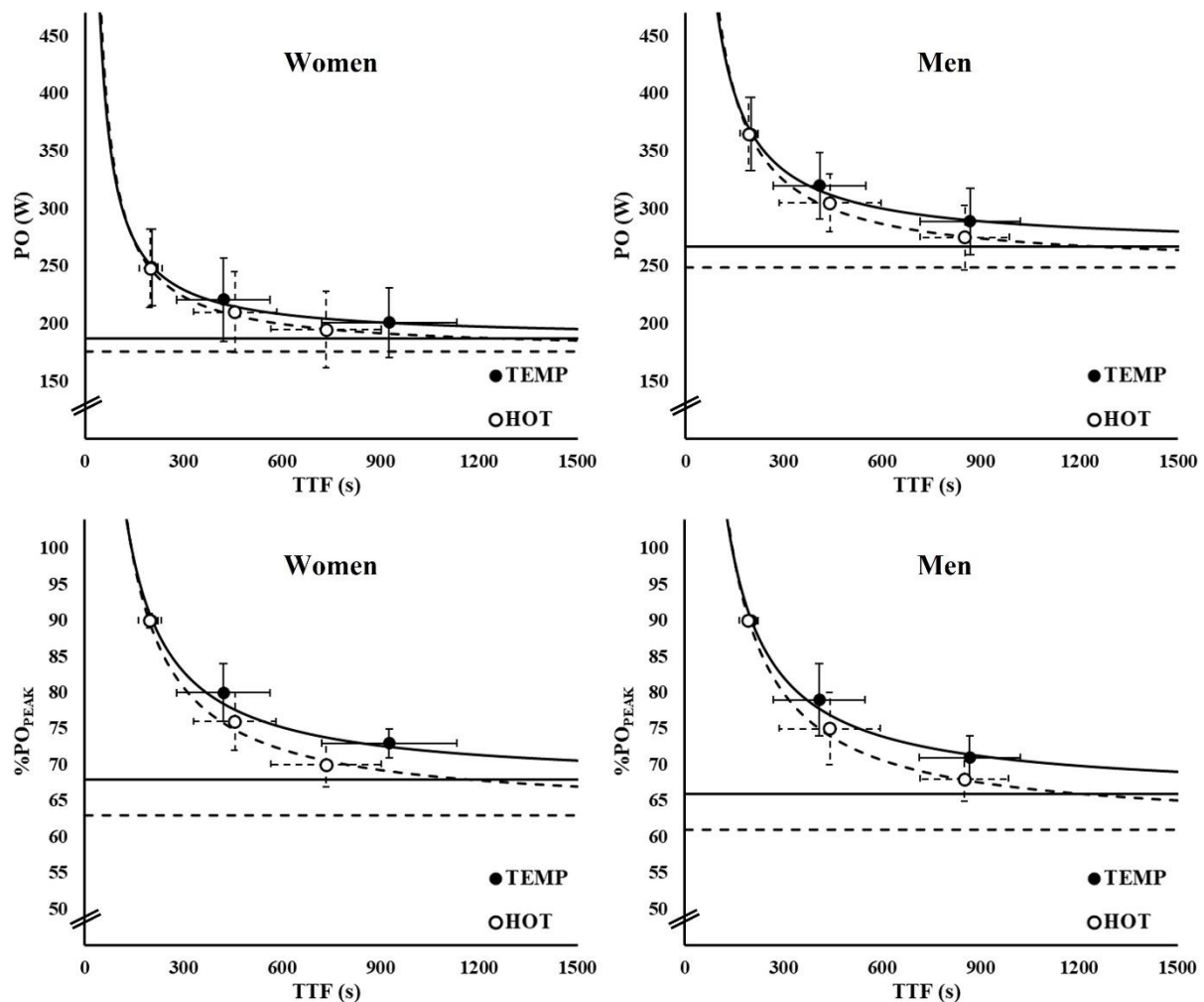
with TEMP ( $p = 0.005$ ). A significant main effect of intensity was found for  $\dot{V}O_2$  and HR. In T1  $\dot{V}O_2$  was lower than in T2 ( $p < 0.001$ ) and T3 ( $p = 0.014$ ), while  $\dot{V}O_2$  in T3 was also lower than in T2 ( $p = 0.033$ ). HR in T1 was lower than T2 ( $p = 0.003$ ) and T3 ( $p < 0.001$ ), and that HR in T2 was also lower than in T3 ( $p = 0.002$ ).

**Table 3** Constant workload tests to exhaustion to determine the power-duration relationship and estimate CP and  $W'$  in women and men in TEMP (18°C) and HOT (36°C).

| <i>n</i>                                    | All          |              | Women        |              | Men          |              |       |
|---|--------------|--------------|--------------|--------------|--------------|--------------|-------|
|   | 24           |              | 12           |              | 12           |              |       |
|   | 18 °C        | 36 °C        | 18 °C        | 36 °C        | 18 °C        | 36 °C        |       |
| Short trial (T1)                            |              |              |              |              |              |              |       |
| PO (W)                                      | 307 ± 67     | 307 ± 68     | 249 ± 33     | 248 ± 29     | 365 ± 32     | 365 ± 32     |       |
| PO (% PO <sub>PEAK</sub> )                  | 90 ± 0       | 90 ± 1       | 90 ± 0       | 90 ± 1       | 90 ± 0       | 90 ± 0       |       |
| TTF (s)                                     | 203 ± 19     | 196 ± 31     | 204 ± 19     | 199 ± 35     | 202 ± 20     | 194 ± 28     |       |
| ṼO <sub>2</sub> (L·min <sup>-1</sup> )      | 3.23 ± 0.91  | 3.31 ± 0.91  | 2.40 ± 0.33  | 2.48 ± 0.38  | 4.07 ± 0.27  | 4.14 ± 0.32  | a,b   |
| HR (bpm)                                    | 180 ± 7      | 180 ± 7      | 181 ± 7      | 179 ± 6      | 178 ± 8      | 181 ± 8      |       |
| [BLa <sup>-</sup> ] (mmol·L <sup>-1</sup> ) | 12.04 ± 1.80 | 13.08 ± 1.65 | 11.59 ± 1.71 | 12.39 ± 1.67 | 12.48 ± 1.86 | 13.77 ± 1.36 | a,b   |
| Middle-long trial (T2)                      |              |              |              |              |              |              |       |
| PO (W)                                      | 270 ± 60     | 258 ± 57     | 221 ± 136    | 210 ± 35     | 320 ± 29     | 305 ± 25     |       |
| PO (% PO <sub>PEAK</sub> )                  | 79 ± 4       | 75 ± 4       | 80 ± 4       | 76 ± 4       | 79 ± 5       | 75 ± 5       |       |
| TTF (s)                                     | 421 ± 138    | 464 ± 138    | 442 ± 142    | 457 ± 126    | 419 ± 140    | 471 ± 154    |       |
| ṼO <sub>2</sub> (L·min <sup>-1</sup> )      | 3.35 ± 0.92  | 3.39 ± 0.92  | 2.50 ± 0.33  | 2.55 ± 0.37  | 4.20 ± 0.25  | 4.23 ± 0.34  | a,b,c |
| HR (bpm)                                    | 182 ± 7      | 183 ± 7      | 182 ± 6      | 183 ± 5      | 181 ± 8      | 183 ± 8      | c     |
| [BLa <sup>-</sup> ] (mmol·L <sup>-1</sup> ) | 12.96 ± 2.32 | 13.05 ± 2.14 | 12.00 ± 2.46 | 11.91 ± 1.94 | 13.92 ± 1.79 | 14.19 ± 1.72 | a     |
| Long trial (T3)                             |              |              |              |              |              |              |       |
| PO (W)                                      | 245 ± 53     | 235 ± 51     | 201 ± 30     | 195 ± 33     | 289 ± 29     | 275 ± 28     |       |
| PO (% PO <sub>PEAK</sub> )                  | 72 ± 3       | 69 ± 3       | 73 ± 2       | 70 ± 3       | 71 ± 3       | 68 ± 3       |       |
| TTF (s)                                     | 896 ± 179    | 793 ± 160    | 926 ± 205    | 735 ± 168    | 867 ± 153    | 851 ± 135    |       |
| ṼO <sub>2</sub> (L·min <sup>-1</sup> )      | 3.32 ± 0.94  | 3.33 ± 0.88  | 2.46 ± 0.37  | 2.55 ± 0.41  | 4.17 ± 0.31  | 4.12 ± 0.34  | a,b,c |
| HR (bpm)                                    | 184 ± 7      | 185 ± 6      | 183 ± 6      | 185 ± 6      | 184 ± 9      | 186 ± 8      | c,d   |
| [BLa <sup>-</sup> ] (mmol·L <sup>-1</sup> ) | 12.41 ± 2.54 | 11.61 ± 2.33 | 10.88 ± 2.35 | 10.37 ± 1.84 | 13.95 ± 1.69 | 12.84 ± 2.17 | a,b   |

Group mean ± SD for all participants ( $n = 24$ ), and separated for women ( $n = 12$ ) and men ( $n = 12$ ) in temperate (18°C) and hot (36°C) environmental conditions. Significant differences ( $p < 0.05$ ) are presented in the table as <sup>a</sup> significant difference between women and men; <sup>b</sup> significant difference between TEMP and HOT; <sup>c</sup> significantly different from T1; <sup>d</sup> significantly different from T2. PO, power output; % PO<sub>PEAK</sub>, peak power output out of the incremental exercise test in TEMP; TTF, time to task failure;  $\dot{V}O_2$ , oxygen uptake obtained during the last 60 s before exhaustion; HR, highest heart rate value during the last 60 s before exhaustion; [BLa<sup>-</sup>], blood lactate concentration obtained in the 30 s after exhaustion.

The relationship between PO (absolute and relative to  $PO_{PEAK}$  out of ramp incremental exercise test) and TTF, established out of the three constant load tests in TEMP and HOT, of women and men is presented in Figure 1. The intersection of the average curvatures in TEMP and HOT occurred at 137 s and 280 W in women, and 153 s and 397 W in men.



**Figure 1** Relationship between absolute power output (PO) and relative power output ( $\% PO_{PEAK}$  out of the incremental exercise test in TEMP), and time to task failure (TTF) using constant workload tests to task failure. Data are displayed for women (left) and men (right). Full lines and black dots are corresponding to the tests performed in TEMP, while dashed lines and white dots are corresponding to the tests performed in HOT. Based on three constant workload tests to task failure a hyperbolic function is established and the asymptote CP is represented by the fixed horizontal line.

## DISCUSSION

The aim of the present study was to investigate to what extent acute heat exposure would affect CP and  $W'$  in individuals of both sexes, using the conventional test methodology. To our knowledge, this study is the first to investigate the determination of the power-duration relationship in hot environmental conditions using multiple constant workload tests to task failure, both in women and men. We demonstrated that CP was reduced, whereas  $W'$  was increased with acute heat exposure. Furthermore, aside from the expected differences in absolute performance between women and men, performance changes due to heat exposure were not different between the two sexes.

In our study, a  $6.5 \pm 3.3\%$  decrease in PO at CP was found in HOT compared with TEMP, which was similar in women and men. This is consistent with the majority of the studies that showed a reduction in the absolute PO corresponding to the maximal metabolic steady state with acute heat exposure (Bourgois et al., 2023; de Barros et al., 2011; Lorenzo et al., 2011; Maunder et al., 2021). It has been noticed that the extent of the reduction, induced by heat, varies between studies, i.e., -4% up to -18%. This might be affected by the severity of the environmental conditions, additional (primary) thermal strain and the test protocol (e.g. constant workload or incremental exercise test) used to determine the exercise thresholds. The duration of the test protocol used in the present study and alternative incremental exercise tests to determine thresholds are predominantly short (< 30 min), whereby the detrimental effects of heat exposure seems rather small as compared with prolonged submaximal or maximal exercise (Nybo & Nielsen, 2001). More specifically related to the CP concept, two studies derived CP from a 3 min all-out test (3MT), in a hot environment. Kuo and colleagues (2021) reported a decrease of 4% in CP (228 vs. 219 W), while Kaiser et al. (2021) did not find a significant reduction in CP (239 vs. 234 W). Although, the decrease in PO at CP is rather small or not observed in these studies, the heat exposure time prior to the test in the study of Kuo et al. (2021) was longer than in our study (45 min vs 15 min). Furthermore, Kaiser et al. (2021) applied a passive heat strategy (warm water immersion) to increase core temperature to 38.5°C prior to 3MT, as it has been shown that hyperthermia by means of an increased core temperature (> 38.5°C) is an important factor leading to a decrease in performance (Nybo, 2008). It is possible that, because of its short duration, a 3MT is not able to detect heat-induced changes in CP. In support of this speculation, no change in performance was observed during the short trial (T1) in our study, which lasted on average between 3 and 4 min.

On the other hand, we found a  $12.4 \pm 18.0\%$  increase in  $W'$  in HOT compared with TEMP. This was a novel and surprising finding, as neither Kuo et al. (2021) nor Kaiser et al. (2021) reported significant changes in  $W'$  with acute heat exposure. Depletion of  $W'$  is associated with the depletion of muscle high-energy phosphates (ATP, PCr) and accumulation of fatigue-inducing metabolites ( $H^+$ , ADP, AMP, Pi) (Black et al., 2017; Chidnok et al., 2013; Jones et al., 2008). In our study, a larger  $W'$  would imply that

the attainment of a critical threshold in muscle metabolites would be at a slower rate or at higher levels in HOT compared with TEMP and/or the slower depletion of energy substrates. This could possibly be mediated by an increased sympathetic nervous system activity and glycolytic/glycogenolytic metabolism. It has been shown that heat exposure affects muscle metabolism by an increased rate of ATP utilization, matched by an increased anaerobic glycolysis and PCr hydrolysis (Febbraio et al., 1996; Febbraio et al., 1994). This is reflected by an increased  $[Bla^-]$ , as a surrogate of  $H^+$ , in the short trial (T1), although  $[Bla^-]$  is similar in T2, and even lower in T3 in HOT. In our opinion, a more plausible explanation for the increase in  $W'$  could be related to the test procedure and determination methodology of CP and  $W'$ . In contrast to 3MT, the conventional methodology (i.e., multiple constant workload tests), as applied in our study, requires also tests of longer duration. We found that these trials (i.e., T2 and T3), lasting 5-15 min, were more affected by the heat compared with the shortest trial (T1), i.e., 2-5 min, therefore impacting the calculations. As a result the hyperbolic curvature had a downwards shift only for a longer duration (see Figure 1), whereby the area below the hyperbolic curvature became larger and  $W'$  increased. Moreover, it has been shown that a larger decrease in CP was associated with a larger increase in  $W'$  ( $r = -0.74$ ). It should be noted that, in the context of the determination of CP and  $W'$ , we used the models with the best fit for each subject in each environmental condition. Therefore, in some subjects CP and  $W'$  were determined from one mathematical model in TEMP and another model in HOT. It is possible that this could slightly have affected CP and  $W'$ . However, a comparison between CP and  $W'$  derived from the same model in TEMP and HOT in each subject, shows similar findings as currently reported in the results.

The established power-duration curvatures, in TEMP and HOT, crossed each other at 137 s and 280 W in women, and 153 s and 397 W in men (see Figure 1), indicating that for maximal exercise up to this intersection a higher PO can be produced. This is in accordance with existing literature, as single high-intensity exercise of short duration is most likely improved in the heat, due to an increased muscle temperature which improves muscle contractibility (Girard et al., 2015; Guy et al., 2015). Although, the application of the CP concept on exercise lasting < 2 min, might not be desirable (Hill et al., 2002). The middle-long trial (5-10 min) and long trial (10-20 min) are situated beyond this crossing point of the two curvatures, where heat exposure has a negative impact on performance. Participants reached similar physiological peak values ( $\dot{V}O_2$ , HR and  $[Bla^-]$ ) in both conditions, however at a lower PO (T2 and T3) in HOT (see Table 3), indicating progressive increase in relative intensity occurring during constant work rate exercise in HOT compared with TEMP, mediated by an increased thermal strain on the cardiovascular system (González-Alonso et al., 2008) and muscle metabolism (Febbraio et al., 1994).

## Limitations

While the aim of the current investigation was to examine the extent to which acute heat exposure would affect CP and  $W'$  in women and men, some limitations should be noted. In the present study no efforts were made to control for menstrual cycle. It has been shown that the power-duration relationship is equally reproducible throughout the different phases of the menstrual cycle in women (James et al., 2023), and currently no unambiguous evidence is provided that women have an inferior thermoregulation during exercise in the heat compared with men (Yanovich et al., 2020). However, we cannot exclude that hormonal cycles affected the thermoregulatory responses to heat exposure, e.g., by means of a thermogenic effect of progesterone. Albeit there is no general consensus on this topic and underrepresentation of women in exercise thermoregulation research requires to be cautious (Hutchins et al., 2021). Future studies should make an effort to address this issue. Second, the absence of core temperature measurement, by rectal thermistor or ingestible pill, did not allow to gain insight if an elevated core temperature *per se* influenced the power-duration relationship, especially during the middle-long and long trial.

## Conclusion

Following our observations, acute heat exposure had an impact on the parameters CP and  $W'$ , i.e., CP decreased whereas  $W'$  increased. However, it is our belief that the increase in  $W'$  might be a consequence of the mathematical modelling for the used test methodology, rather than a physiological accurate value of  $W'$  in HOT. The power-duration relationship provided useful insights, as the intersection of the curvature in TEMP and HOT confirmed that only exercise from a certain duration is negatively impacted with acute heat exposure. Individual testing and monitoring is favored in temperate as well as hot environmental conditions, whereby further research is required applying different modalities of heat exposure, taking into account the limitations of this study. This could lead to a better understanding of the underlying mechanisms of the impact of heat exposure on exercise tolerance and capacity, and optimize the use of the CP concept in women and men.

## ADDITIONAL INFORMATION

**Author contributions:** GB, PM, KC, JGB and JB conceived and designed research. GB, ALC and MK conducted the experiments. GB, PM, KC, ALC, MK, JGB, SP and JB analyzed the data. GB, SP and JB wrote the manuscript. All authors revised and approved the manuscript.

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**Ethics approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethical committee of the Ghent University Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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# ***STUDY 2***

## **The effect of acute heat exposure on the determination of exercise thresholds from ramp and step incremental exercise**

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## ABSTRACT

The aim of this study was to examine how respiratory (RT) and lactate thresholds (LT) are affected by acute heat exposure in the two most commonly used incremental exercise test protocols (RAMP and STEP) for functional evaluation of aerobic fitness, exercise prescription and monitoring training intensities. Eleven physically active male participants performed four incremental exercise tests, two RAMP ( $30 \text{ W} \cdot \text{min}^{-1}$ ) and two STEP ( $40 \text{ W} \cdot 3 \text{ min}^{-1}$ ), both in  $18^\circ\text{C}$  (TEMP) and  $36^\circ\text{C}$  (HOT) with 40 % relative humidity to determine 2 RT and 16 LT, respectively. Distinction was made within LT, taking into account the individual lactate kinetics ( $\text{LT}_{\text{IND}}$ ) and fixed value lactate concentrations ( $\text{LT}_{\text{FIX}}$ ). A decrease in mean power output (PO) was observed in HOT at LT ( $-6.2 \pm 1.9 \%$ ), more specific  $\text{LT}_{\text{IND}}$  ( $-5.4 \pm 1.4 \%$ ) and  $\text{LT}_{\text{FIX}}$  ( $-7.5 \pm 2.4 \%$ ), compared to TEMP, however not at RT ( $-1.0 \pm 2.7 \%$ ). The individual PO difference in HOT compared to TEMP over all threshold methods ranged from  $-53 \text{ W}$  to  $+26 \text{ W}$ . Mean heart rate (HR) did not differ in LT, while it was increased at RT in HOT ( $+10 \pm 8 \text{ bpm}$ ). This study showed that exercise thresholds were affected when ambient air temperature was increased. However, a considerable degree of variability in the sensitivity of the different threshold concepts to acute heat exposure was found and a large individual variation was noticed. Test design and procedures should be taken into account when interpreting exercise test outcomes.

## KEY WORDS

Heat; incremental exercise test; respiratory threshold; lactate threshold

## INTRODUCTION

Incremental exercise tests are widely used to determine exercise thresholds that demarcate the intensity domains of moderate, heavy and severe exercise (Gaesser & Poole, 1996). By use of these thresholds, aerobic fitness can be assessed and exercise prescription can be optimized according to the specific profile of the sports discipline and athlete (Bourgois et al., 2019). It has been shown that incremental exercise tests with continuous linear (RAMP) or stepwise (STEP) increases in intensity can provide valuable insights into these thresholds, although they do not represent the gold standard procedures (i.e., multiple constant load exercise tests) (Keir et al., 2015). Despite a high feasibility (i.e., time-efficiency) of these protocols, appropriate protocol design and careful data analysis by experienced physiologists or coaches are required for accurate determination and interpretation of exercise thresholds (Jamnick et al., 2018; Caen et al., 2021). RAMP tests typically last between 8 and 12 min and are mostly used when thresholds are determined from pulmonary gas exchange variables (Keir et al., 2022), with maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) and respiratory thresholds (RT), i.e., gas exchange threshold (GET) and respiratory compensation point (RCP), as key outcomes. STEP tests are more common when blood lactate measurement is the main parameter for threshold determination, and a minimum stage length (i.e., 3 min) is proposed (Bentley, 2007). Over the years, a large variety of lactate thresholds (LT) methods has been suggested and utilized (Faude et al., 2009; Jamnick et al., 2018). Some of these methods take into account the individual kinetics of the lactate-performance curve (LT<sub>IND</sub>; e.g., D<sub>max</sub> method; Cheng et al., 1992), while others rely on fixed blood lactate concentrations (LT<sub>FIX</sub>; e.g., 4 mmol·L<sup>-1</sup>; Kindermann et al., 1979).

Furthermore, even when the testing procedure and analysis are applied adequately, the occurrence of these exercise thresholds can be influenced by multiple factors, including environmental conditions. In fact, heat can alter the physiological responses to exercise (Périard et al., 2021). A redistribution of blood to the skin for heat dissipation leads to a higher cardiovascular strain (Rowell, 1974). Furthermore, there is a shift toward a greater reliance on the glycolytic metabolism (Febbraio et al., 1994), resulting in a decreased mechanical efficiency (Hettinga et al., 2007). Subsequently, the impact of heat on parameters used for exercise prescription and monitoring training intensity, i.e., power output (PO), heart rate (HR), blood lactate concentration ([BLa<sup>-</sup>]) and oxygen uptake ( $\dot{V}O_2$ ), can result in a modified occurrence of LT from STEP and/or RT from RAMP during incremental exercise.

Several studies reported changes in threshold occurrence when exercise testing is executed in hot environmental conditions (Tyka et al., 2009; 2010; de Barros et al., 2011; Lorenzo et al., 2011; Maunder et al., 2021) and made suggestions on how this additional information could be used for training prescription and monitoring during training camps and tournaments in hot environments. However,

these studies do not compare different incremental exercise tests (RAMP and STEP) and/or only include limited exercise threshold concepts.

Therefore, the purpose of this study is to examine how different exercise thresholds (RT and LT) are affected by acute heat exposure in the two most commonly used incremental exercise test protocols (RAMP and STEP) for functional evaluation of aerobic fitness. This will allow us to gain more insight into the physiological mechanism of exercise threshold determination and provide practical implications for sports scientists and coaches. We hypothesize that short-term heat exposure (i.e., duration of the test) will have an impact on PO and HR at all exercise thresholds, although we expect differences related to the protocol of the test (i.e., time duration-intensity relationship of RAMP vs. STEP) and the methodology of LT determination (i.e., lactate kinetics in LT<sub>IND</sub> vs. LT<sub>FIX</sub>). Furthermore, we hypothesize that thresholds taking place at a higher intensity will be affected more as heat exposure time is increased, accelerating cardiorespiratory and metabolic perturbations.

## METHODS

### Participants

Eleven male participants (age:  $24.9 \pm 1.7$  years, height:  $1.82 \pm 0.06$  m, body mass:  $77.0 \pm 6.6$  kg) volunteered in this study. All participants were physically active, and performed recreational physical exercise on a self-reported basis of  $5.0 \pm 1.4$  hours per week. Participants did not train in hot environments 3 months preceding the study to avoid heat acclimation/acclimatization effects. Participants completed a medical questionnaire and underwent a medical examination. Participants did not report any history of cardiovascular, respiratory or metabolic disease. After receiving a description of the procedure of the experiment, all participants gave their written informed consent. The protocol was in accordance with the Declaration of Helsinki and was approved by the ethical committee of the Ghent University Hospital (Ghent, Belgium).

### Study design

*General procedure.* All participants performed four incremental exercise tests on an electromagnetically braked cycle ergometer (Cyclus 2, RBM Elektronik-Automation, Leipzig, Germany) at the Sport Science Laboratory Jacques Rogge of the Ghent University (Ghent, Belgium, sea level) between 1.00 p.m. and 6.00 p.m. to limit variability due to the circadian rhythm. Each participant performed all tests at the same time of the day ( $\pm 30$  min) with a minimum of 72 hours between two tests. Trials were completed during the spring months. Two ramp and two step incremental exercise tests were executed, one of each in temperate (TEMP:  $18 \pm 1^\circ\text{C}$ ) and one of each in hot environmental conditions (HOT:  $36 \pm 1^\circ\text{C}$ ), with partial counterbalancing to deal with practice effects. Air relative humidity (RH) was kept constant at  $40 \pm 3$  %. All exercise tests took place in a built-in climatic chamber. Before the start of each exercise test, participants were seated for 10 min to accommodate to the environmental conditions. Participants were asked to maintain the same type of meals at the day of an exercise test and to drink 500 mL of water over 2 hours prior to the beginning of the test. Participants were instructed to abstain from any exhaustive exercise 24 hours leading up to an exercise test and to refrain from consumption of caffeine and alcohol for 24 hours prior to testing. During the first test, participants were instructed to choose their cadence between 70 and 90 revolutions per minute (rpm), and maintain their preferred cadence during all upcoming exercise tests. Strong verbal encouragement was provided throughout all exercise tests to ensure maximum effort. The protocol was terminated at volitional exhaustion, which was defined as the inability to maintain a minimal cadence of 70 rpm for more than 5 consecutive seconds. HR was monitored on a continuous basis (H7 Sensor; Polar, Kempele, Finland).

*RAMP test.* Warm-up consisted of 6 min cycling at 120 W, 2 min seated rest and 4 min baseline cycling at 70 W. Subsequently, the work rate increased continuously and linear with  $30 \text{ W}\cdot\text{min}^{-1}$ , as this would

result in a test duration of 8-12 min in our population based on anthropometrics and reported physical exercise. Pulmonary gas exchanges for the determination of  $\dot{V}O_{2peak}$ , GET and RCP were measured on a breath-by-breath basis using a metabolic instrument (Cortex MetaLyzer 3B; Cortex Biophysik, Leipzig, Germany).

*STEP test.* The test started at a work rate of 80 W for 3 min and increased stepwise with 40 W every 3 min to obtain at least five  $[BLa^-]$  measurements. At the end of each stage, 20  $\mu$ L of blood from the right middle finger was collected into a capillary tube and analysed for  $[BLa^-]$  (Biosen C-Line; EKF-diagnostic GmbH, Magdeburg, Germany). Peak  $[BLa^-]$  ( $[BLa^-]_{peak}$ ) was obtained 1 min after cessation of the exercise test.

### Data analysis

For all exercise thresholds, corresponding values of PO and HR were determined and the differences in PO and HR between TEMP and HOT were calculated and expressed as the relative change (%) compared to values of TEMP ( $\Delta$ ). PO at thresholds in TEMP were calculated relative to peak PO ( $\%PO_{peak}$ ) within the respective test protocol, to express the relationship between PO- $\Delta$  and (relative) exercise intensity.

*RAMP test.* Breath-by-breath data were averaged into 10 s intervals.  $\dot{V}O_{2peak}$  was determined as the average of the highest three consecutive 10 s values. Two respiratory thresholds were determined by four independent researchers using four different criteria for each threshold. The mean of the closest three values was used. GET was defined as (1) the point where  $\dot{V}CO_2$  increased disproportionately to  $\dot{V}O_2$  using the V-slope method, (2) the first departure from the linear increase in ventilation ( $\dot{V}_E$ ), (3) an increase in  $\dot{V}_E/\dot{V}O_2$  without a simultaneous increase in  $\dot{V}_E/\dot{V}CO_2$  and (4) the first rise in end-tidal oxygen tension ( $P_{ET}O_2$ ) (Beaver et al., 1986; Binder et al., 2008). RCP corresponded to (1) the point where  $\dot{V}_E$  increased disproportionately to  $\dot{V}CO_2$ , (2) the second departure from linearity in  $\dot{V}_E$ , (3) an increase in both  $\dot{V}_E/\dot{V}O_2$  and  $\dot{V}_E/\dot{V}CO_2$  and (4) the deflection point of end-tidal carbon dioxide tension ( $P_{ET}CO_2$ ) (Wasserman, 1984; Binder et al., 2008). To obtain the precise work rate at which GET occurred, an individual correction was made, i.e., to account for the mean response time (MRT) of the  $\dot{V}O_2$  kinetics. MRT was quantified as the time interval between the onset of the ramp and the intersection of the forward extrapolation of the baseline  $\dot{V}O_2$  and the backwards extrapolation of the linear  $\dot{V}O_2$ /time relationship below the GET (Boone & Bourgois, 2012). For RCP, an additional correction was made to close the gap for the extra dissociation of the  $\dot{V}O_2$ /PO relationship between ramp incremental exercise and constant work rate exercise at higher intensities (Caen et al., 2020). Oxygen pulse ( $O_2$  pulse:  $\dot{V}O_2/HR$ ) was calculated as an indirect indicator of cardiac stroke volume (SV) at peak level and the two RT.

*STEP test.* Sixteen thresholds were calculated based on  $[BLa^-]$ , using nine threshold methods. A distinction was made between individual ( $LT_{IND}$ ) and fixed value ( $LT_{FIX}$ ) lactate thresholds.

#### *Individual lactate thresholds:*

1. Log-log: The lactate curve was divided into two segments and the intersection point of the two lines with the lowest residuals sum of squares was taken as the lactate threshold (Beaver et al., 1985).
2. Baseline + absolute value(s) (Bsl<sub>n</sub> + mmol·L<sup>-1</sup>): The intensity at which [BLa<sup>-</sup>] increased 0.5 (Bsl<sub>n</sub> + 0.5), 1.0 (Bsl<sub>n</sub> + 1.0) or 1.5 (Bsl<sub>n</sub> + 1.5) mmol·L<sup>-1</sup> above baseline value (Berg et al., 1990; Zoladz et al., 1995).
3. D<sub>max</sub>: The point on the third-order polynomial regression curve that yielded the maximum perpendicular distance to the straight line formed by the two end points of the curve (Cheng et al., 1992).
4. Modified D<sub>max</sub> (ModD<sub>max</sub>): The intensity at the point on the third order polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the point preceding the first rise in [BLa<sup>-</sup>] of > 0.4 mmol·L<sup>-1</sup> lactate and the final lactate point (Bishop et al., 1998).
5. Exponential D<sub>max</sub> (Exp-D<sub>max</sub>): The point on the exponential regression curve that yielded the maximum perpendicular distance to the straight line formed by the two end points of the curve (Hughson et al., 1987; Machado et al., 2012).
6. Log-log modified D<sub>max</sub> (Log-Poly-ModD<sub>max</sub>): The intensity at the point on the third order polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final lactate point (Jamnick et al., 2018).
7. Log-log exponential modified D<sub>max</sub> method (Log-Exp-ModD<sub>max</sub>): The intensity at the point on the exponential plus-constant regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final lactate point (Jamnick et al., 2018).
8. First and second lactate turning points: The lactate curvature is divided into three segments. Two double-linear fits are performed, which the intersection points between the lines (segments) are considered as Lactate Threshold 1 (LT<sub>1</sub>) and Lactate Threshold 2 (LT<sub>2</sub>) (Binder et al., 2008).

#### *Fixed value lactate thresholds:*

9. Fixed lactate thresholds or onset of blood lactate accumulation (OBLA) values of 2.0 (OBLA 2.0), 2.5 (OBLA 2.5), 3.0 (OBLA 3.0), 3.5 (OBLA 3.5), or 4.0 (OBLA 4.0) mmol·L<sup>-1</sup> (Kindermann et al., 1979; Skinner & McLellan, 1980; Heck et al., 1985).

#### **Statistical analysis**

All data were expressed as mean values and standard deviations (SD) for  $n = 11$ . A priori sample size calculations have been performed in G\*Power 3.1.9 (University Düsseldorf, Germany) with significance

level 0.05 and power 80 %. An estimated effect size of 0.65 results in a total sample size of  $n=11$ . Participants served as their own controls. SPSS statistics 25 (IBM Corp., Armonk, NY) was used for statistical analysis. The Shapiro-Wilk test was used to confirm normal distribution of the data. Repeated-Measures (RM) ANOVA ( $2 \times 2$ ) was performed to investigate differences in time to exhaustion (TTE) and peak performance parameters (PO and HR) between TEMP and HOT in RAMP and STEP. Paired-samples  $t$  tests were used to compare  $\dot{V}O_2$  in RAMP and  $[BLa^-]$  in STEP between TEMP and HOT.

RM ANOVA was used to observe a difference how thresholds determined in RAMP (i.e., RT) and STEP (i.e., LT) are affected by heat (design: 18 thresholds  $\times$  2 conditions), and this was done for PO and HR corresponding to the threshold. If a significant effect was seen, post hoc paired samples  $t$  tests with Bonferroni correction were executed for comparison between TEMP and HOT for one threshold at a time. Significance was set at  $p < 0.05$  and 95 % Confidence Interval ( $CI_{95\%}$ ) was given. Cohen's  $d$  effect size (ES) was calculated to standardize mean differences. Pearson correlation coefficient ( $r$ ) was used to mark a linear relationship between absolute and relative exercise intensity (i.e., PO and  $\%PO_{peak}$ ) in TEMP and the size of difference in PO ( $\Delta$ ) between TEMP and HOT.

## RESULTS

At peak level, no interaction effect ( $F = 2.078$ ;  $p = 0.180$ ) was found for  $PO_{peak}$  in TEMP and HOT for RAMP ( $361 \pm 29$  W vs.  $353 \pm 40$  W) and STEP ( $306 \pm 31$  W vs.  $288 \pm 29$  W); nonetheless, there was a main effect of temperature ( $F = 24.605$ ;  $p < 0.001$ ), indicating that the  $PO_{peak}$  in HOT was lower compared to TEMP. On the other hand, significant interaction effects were found for TTE ( $F = 9.718$ ;  $p = 0.011$ ) and HR ( $F = 7.111$ ;  $p = 0.024$ ). TTE was reduced in STEP ( $1018 \pm 140$  s vs.  $937 \pm 130$  s;  $p < 0.001$ ) in HOT compared to TEMP, but not in RAMP ( $581 \pm 59$  s vs.  $566 \pm 80$  s;  $p = 0.212$ ) and HR was higher in RAMP ( $182 \pm 10$  bpm vs.  $187 \pm 9$  bpm;  $p = 0.017$ ) in HOT compared to TEMP, but not in STEP ( $187 \pm 8$  bpm vs.  $185 \pm 7$  bpm;  $p = 0.346$ ).  $[BLa^-]_{peak}$  did not differ in STEP between TEMP and HOT ( $12.14 \pm 1.65$  mmol·L<sup>-1</sup> vs.  $11.48 \pm 2.17$  mmol·L<sup>-1</sup>;  $p = 0.198$ ). Furthermore,  $\dot{V}O_{2peak}$  was higher in HOT in RAMP ( $3.93 \pm 0.46$  L·min<sup>-1</sup> vs.  $4.19 \pm 0.40$  L·min<sup>-1</sup>;  $p < 0.001$ ) and  $O_2$  pulse at peak level did not differ in RAMP ( $21.7 \pm 3.0$  ml·bpm<sup>-1</sup> vs.  $22.5 \pm 2.4$  ml·bpm<sup>-1</sup>;  $p = 0.053$ ).

Table 1 gives an overview of all threshold methods (expressed as PO and HR) in TEMP and HOT within their respective exercise test protocol. When expressed as PO, a significant interaction effect ( $F = 2.038$ ;  $p = 0.012$ ) was seen for the effect of heat on thresholds, determined in RAMP and STEP, meaning that there is a difference in the way thresholds are impacted by heat exposure. Post hoc analysis shows a significant decrease in PO in HOT for all thresholds, except GET, RCP and the log-log method. When expressed as HR, a significant interaction effect ( $F = 4.040$ ;  $p < 0.001$ ) was found for the effect of heat on thresholds, determined in RAMP and STEP. Post hoc analysis shows a significant increase in HR in HOT at RT, but not LT. Figure 1 provides the representation of individual PO difference in HOT compared to TEMP for all threshold methods, with a range from -53 W to +26 W.  $\dot{V}O_2$  was higher at GET ( $2.76 \pm 0.30$  L·min<sup>-1</sup> vs.  $2.95 \pm 0.32$  L·min<sup>-1</sup>;  $p = 0.006$ ) and RCP ( $3.50 \pm 0.44$  L·min<sup>-1</sup> vs.  $3.74 \pm 0.40$  L·min<sup>-1</sup>;  $p = 0.013$ ) in HOT.

A negative correlation was observed between the exercise intensity at which the thresholds occurred and the change in PO between TEMP and HOT, both for absolute PO (Fig. 2A;  $r = -0.34$ ;  $p < 0.001$ ) as relative to  $PO_{peak}$  (Fig. 2B;  $r = -0.31$ ;  $p < 0.001$ ).

**Table 1** Power output (PO) and heart rate (HR) at which the exercise thresholds occurred in RAMP and STEP for TEMP and HOT in order of appearance

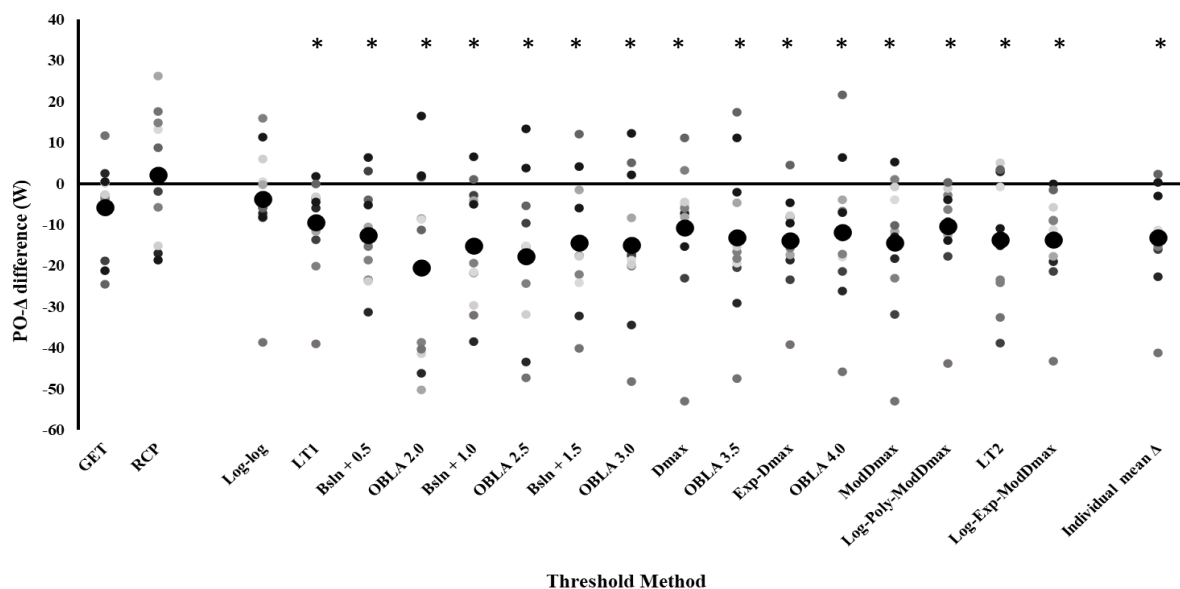
| Threshold method                                  | Power output |            |       |      | Heart rate |           |      |      |
|---|--------------|------------|-------|------|------------|-----------|------|------|
|   | 18°C         | 36°C       | Δ     | ES   | 18°C       | 36°C      | Δ    | ES   |
|   | PO (W)       | PO (W)     | %     |      | HR (bpm)   | HR (bpm)  | %    |      |
| <b>RAMP</b>                                       |              |            |       |      |            |           |      |      |
| <i>GET</i>  | 198 ± 26     | 193 ± 27   | -2.9  | 0.31 | 142 ± 9    | 154 ± 9 * | 8.6  | 1.95 |
| <i>RCP</i>  | 240 ± 33     | 242 ± 27   | 0.9   | 0.10 | 163 ± 10   | 171 ± 8 * | 5.1  | 1.26 |
| <b>STEP</b>                                       |              |            |       |      |            |           |      |      |
| <i>Log-log</i> <sup>IND</sup>                     | 159 ± 20     | 155 ± 19   | -2.3  | 0.27 | 130 ± 9    | 136 ± 7   | 4.7  | 1.04 |
| <i>LT<sub>1</sub></i> <sup>IND</sup>              | 166 ± 22     | 156 ± 21 * | -5.7  | 0.62 | 132 ± 8    | 136 ± 8   | 2.8  | 0.66 |
| <i>Bsln + 0.5</i> <sup>IND</sup>                  | 179 ± 24     | 166 ± 21 * | -7.0  | 0.79 | 137 ± 8    | 139 ± 8   | 1.6  | 0.38 |
| <i>OBLA 2.0</i> <sup>FIX</sup>                    | 185 ± 38     | 164 ± 33 * | -11.1 | 0.81 | 139 ± 11   | 139 ± 11  | -0.4 | 0.07 |
| <i>Bsln + 1.0</i> <sup>IND</sup>                  | 201 ± 31     | 186 ± 25 * | -7.6  | 0.76 | 146 ± 7    | 147 ± 8   | 0.8  | 0.22 |
| <i>OBLA 2.5</i> <sup>FIX</sup>                    | 204 ± 40     | 186 ± 30 * | -8.7  | 0.71 | 147 ± 10   | 147 ± 9   | 0.2  | 0.04 |
| <i>Bsln + 1.5</i> <sup>IND</sup>                  | 216 ± 33     | 202 ± 29 * | -6.7  | 0.66 | 152 ± 10   | 153 ± 9   | 0.8  | 0.19 |
| <i>OBLA 3.0</i> <sup>FIX</sup>                    | 218 ± 40     | 203 ± 32 * | -6.9  | 0.59 | 152 ± 8    | 153 ± 9   | 0.9  | 0.24 |
| <i>D<sub>max</sub></i> <sup>IND</sup>             | 218 ± 23     | 207 ± 23 * | -4.9  | 0.66 | 152 ± 9    | 155 ± 7   | 2.0  | 0.54 |
| <i>OBLA 3.5</i> <sup>FIX</sup>                    | 229 ± 39     | 215 ± 32 * | -5.8  | 0.53 | 156 ± 9    | 158 ± 8   | 1.3  | 0.32 |
| <i>Exp-D<sub>max</sub></i> <sup>IND</sup>         | 231 ± 25     | 217 ± 23 * | -6.0  | 0.81 | 157 ± 8    | 159 ± 7   | 1.0  | 0.30 |
| <i>OBLA 4.0</i> <sup>FIX</sup>                    | 238 ± 37     | 226 ± 30 * | -5.0  | 0.50 | 160 ± 9    | 162 ± 8   | 1.4  | 0.37 |
| <i>ModD<sub>max</sub></i> <sup>IND</sup>          | 240 ± 30     | 226 ± 28 * | -6.0  | 0.71 | 161 ± 9    | 162 ± 7   | 0.8  | 0.23 |
| <i>Log-Poly-ModD<sub>max</sub></i> <sup>IND</sup> | 241 ± 26     | 231 ± 25 * | -4.3  | 0.57 | 161 ± 9    | 164 ± 7   | 1.9  | 0.51 |
| <i>LT<sub>2</sub></i> <sup>IND</sup>              | 246 ± 31     | 232 ± 33 * | -5.5  | 0.60 | 162 ± 8    | 164 ± 7   | 0.7  | 0.21 |
| <i>Log-Exp-ModD<sub>max</sub></i> <sup>IND</sup>  | 250 ± 28     | 236 ± 26 * | -5.5  | 0.73 | 164 ± 9    | 166 ± 7   | 1.1  | 0.30 |

Values are mean ± standard deviation (SD) for n = 11 participants. Effect size (ES) is calculated and significant differences are marked (\*) for p < 0.05. In STEP, a distinction has been made between individual (<sup>IND</sup>) and fixed value (<sup>FIX</sup>) lactate threshold methods.

## DISCUSSION

In this study, we made a comparison between 18 exercise thresholds determined from two commonly used incremental test protocols (i.e., RAMP and STEP) in temperate (TEMP: 18°C) and hot (HOT: 36°C) environments with the same relative humidity (RH: 40 %). To our knowledge, this study is the first to comprehensively examine the effect of acute heat exposure on RT and LT in non-acclimatized physically active individuals.

Our first hypothesis was that short-term heat exposure (i.e., only the time duration of an incremental exercise test) would have a negative impact on all exercise thresholds. We found a significant interaction effect of ambient air temperature on the occurrence of the thresholds for PO, meaning that not all thresholds were impacted in the same way. This points at a considerable degree of variability in the sensitivity of the different threshold concepts to acute heat exposure (Figure 1).

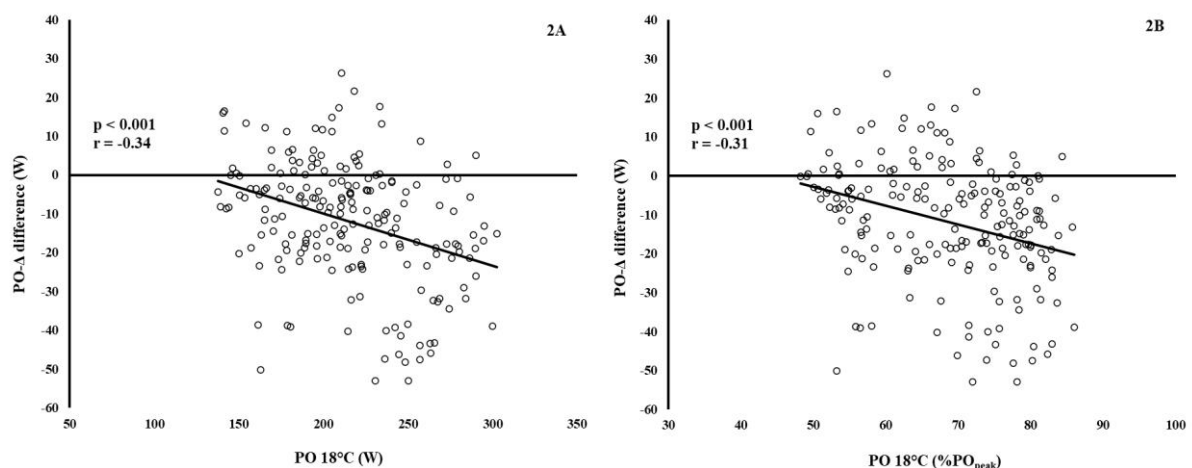


**Figure 1** Mean (big dots) and individual (small dots) difference in PO between 18°C and 36°C for all determined exercise thresholds; two respiratory thresholds, GET and RCP, in RAMP and 16 lactate thresholds in STEP. Mean difference of all exercise thresholds within the individual is displayed as Individual mean Δ. \* denotes significant changes ( $p < 0.05$ ) between TEMP and HOT.

The PO at some thresholds was highly impacted (e.g., OBLA 2.0) as shown in the medium to large effect sizes (0.50-0.81), whereas in others, PO remained unchanged (e.g., GET). The mean decrease of PO at the different thresholds in our study was less pronounced (RT:  $-1.0 \pm 2.7$  % and LT:  $-6.2 \pm 1.9$  %), as compared to other studies investigating performance decrements in heat. Maunder et al. (2021) reported decreases for OBLA 2.0, OBLA 3.0 and OBLA 4.0, respectively, of 16 %, 13 % and 10 % between 18 and 36°C (60 % RH) in 16 competitive endurance-trained males, and a 17 % and 12 % decrease is noticed, respectively, at first (i.e., GET) and second (i.e., RCP) RT. de Barros et al. (2011) found that PO corresponding to RCP decreased by 18 % in 40°C in comparison to 22°C (50 % RH) for eight healthy

young untrained male participants. Furthermore, Tyka et al. (2009; 2010) reported a 13 % and 11 % decrease at, respectively, LT (Exp-D<sub>max</sub>) and GET (V-slope method) in 37°C compared to 23°C (55 % RH). Lorenzo et al. (2011) found an overall decrease of 12 % in power output at several blood- and ventilation-based thresholds in 12 highly trained endurance cyclists (10 men and 2 women) when cycling in 38°C compared to 13°C (30 % RH).

The above variation in results coming from different studies can be explained by differences in test designs and procedures. First, exercise tests took place in various environmental conditions (i.e., ambient air temperature and RH) using different test populations, so that a direct comparison is difficult. Second, in the present study, participants were exposed to hot environmental conditions for ~30 min (i.e., 10 min rest + exercise test). This exposure time is shorter than in the study of Maunder et al. (2021) and Tyka et al. (2010), where participants rested passively for 20 min and 30 min, respectively. Other studies reported the use of immersion in a hot bath (41°C) for 30 min to induce whole-body hyperthermia before start of incremental exercise (Lorenzo et al., 2011) or do not report the time of exposure before start of the test (de Barros et al., 2011). Furthermore, we speculate that the impact of heat exposure on exercise thresholds, using a protocol that prolongs the duration of the incremental exercise test (e.g., RAMP with a smaller ramp slope or STEP with longer stages), would be more fierce. This could also be the reason why our hypothesis, that thresholds taking place at a higher intensity will be affected more as heat exposure time is increased, was only partially supported by a weak correlation (see Fig. 2).



**Figure 2** Scatterplots showing the relationship between all 198 (11 participants x 18 exercise thresholds) unique thresholds determined in 18°C for (A) absolute and (B) relative PO and the PO difference between 18°C and 36°C for the determined threshold.

The total duration of our protocol was too short to induce severe cardiorespiratory and metabolic perturbations, even in the thresholds occurring at higher intensities. Finally, it should be pointed out that the exercise test protocol is different. A single RAMP protocol with continuous increase in PO is

preferred to determine RT as it is the most appropriate way to detect break point in the slope of the gas exchange and ventilatory response patterns (Keir et al., 2022). This is in contrast to other studies, where they used various STEP protocols to determine RT (Tyka et al., 2009; Tyka et al., 2010; de Barros et al., 2011) or merged STEP and RAMP into one exercise test protocol (Mauder et al., 2021), which will affect threshold determination.

The determination of LT in STEP is based on different underlying methodologies, i.e.,  $LT_{IND}$  or  $LT_{FIX}$ . We observed an alteration in lactate kinetics in HOT ( $LT_{IND}$ :  $-5.4 \pm 1.4$  % and  $LT_{FIX}$ :  $-7.5 \pm 2.4$  %), meaning that all LT methods (except log-log method) are sensitive to heat exposure. Higher  $[BLa^-]$  values were observed at the same absolute intensities in HOT compared to TEMP, resulting in lower PO at OBLA 2.0 – 4.0. This could indicate that there is a more pronounced production rate of  $La^-$  due to a greater reliance on the glycolytic metabolism, possible mediated by a higher thermal strain (i.e., elevated muscle temperature) and an increased sympathoadrenal response (i.e., increased circulating epinephrine) for the same absolute PO (Febbraio et al., 1994, 1996). Although, it should be emphasized that  $BLa^-$  accumulation is the result of a balance between production by the muscles and clearance from the blood by active and inactive muscle mass, heart, brain, liver and kidneys. A redistribution of the blood flow toward the skin (i.e., vasodilatation for heat dissipation) occurs with heat exposure, suggesting a lower  $La^-$  elimination rate (Brooks, 2018; Rowell et al., 1968). The determination of LT by means of the  $D_{max}$  method ( $D_{max}$ ,  $ModD_{max}$ ,  $Exp-D_{max}$ ,  $Log-Poly-ModD_{max}$ ,  $Log-Exp-ModD_{max}$ ) depends on baseline  $[BLa^-]$ , lactate kinetics and  $[BLa^-]_{peak}$ . An equal mean  $[BLa^-]_{peak}$  was obtained at  $PO_{peak}$  ( $12.14 \pm 1.65$  mmol·L<sup>-1</sup> vs.  $11.48 \pm 2.17$  mmol·L<sup>-1</sup>), although  $PO_{peak}$  was lower in HOT ( $306 \pm 31$  W vs.  $288 \pm 29$  W). As a consequence, the course of the  $[BLa^-]$ -PO curve shifted to the left thus also resulting in a lower PO for the  $LT_{FIX}$  thresholds.

We found a higher HR for a given submaximal absolute PO, which can be attributed to the direct temperature effect on intrinsic HR at the sinoatrial node (Jose et al., 1970) and/or indirect effect by a reduced venous return due to increased skin blood flow (Rowell, 1974). In the context of exercise prescription, Mauder et al. (2021) proposed to rely on the HR instead of PO in the early phase of a heat acclimation/acclimatization camp, as HR at the thresholds did not differ between TEMP and HOT. This is in line with what we found in our study in STEP (i.e., equal HR and reduced PO). However, it must be pointed out that they do not take into account the negative heat effects during prolonged exercise at submaximal intensity. Deterioration of cardiac function and more specific reduction cardiac output will be more pronounced with prolonged (intense) exercise in heat as core temperature increases. When prescribing exercise based on HR, it is important that this is in accordance with the correct metabolic intensity (Teso et al., 2022), however, this is complicated in heat as both absolute and relative intensity will change over time.

We found that RT, with PO adjustment for GET (Boone & Bourgois, 2012) and RCP (Caen et al., 2020), in RAMP were less susceptible to heat. This might be due to a difference in duration-intensity ratio

between RAMP and STEP where time spent above 50 % $\dot{V}O_{2peak}$  was almost double in STEP compared to RAMP ( $669 \pm 67$  s vs.  $357 \pm 34$  s). As such, the heat strain, which is a function of absolute intensity and time, could be less pronounced in RAMP vs. STEP. We found that GET and RCP were identified at a higher  $\dot{V}O_2$ , and thus a higher metabolic intensity or internal load. However, converted to PO or external load, the RT did not change. It has been suggested that extra myocardial  $\dot{V}O_2$  in HOT is the reason, at least to a certain degree, for the higher  $\dot{V}O_2$  at a given submaximal power output. Gross efficiency during cycling is impacted by sustaining muscle blood flow in combination with a higher skin blood flow for heat dissipation (Hettinga et al., 2007; Nielsen et al., 1990). The relative intensity (i.e., % $\dot{V}O_{2peak}$ ), however, did not change at GET ( $70 \pm 3$  %) and RCP ( $89 \pm 4$  %) between TEMP and HOT, as also  $\dot{V}O_{2peak}$  reached during RAMP in HOT was higher compared to TEMP. This might be surprising as several other studies (Arngrímsson et al., 2004; James et al., 2017; Lorenzo et al., 2011) found that  $\dot{V}O_{2peak}$  is impaired in the heat, attributed to a lower cardiac output (González-Alonso & Calbet, 2003) and increases in core temperature limiting  $\dot{V}O_{2peak}$  (Arngrímsson et al., 2004). Others, however, observed no reduction in  $\dot{V}O_{2peak}$  (Rowell et al., 1965; Schlader et al., 2011; Tyka et al., 2010) or even an increase (Kuo et al., 2021; Lafrenz et al., 2008), possibly related to the short duration of the test (Rowell, 1974). The characteristics of the participants, who are physically active but not habituated to cycling exercise, must also be taken into account. In this context, it is possible that in TEMP, the exercise tests were terminated as a consequence of fatigue in the locomotor muscles instead of cardiopulmonary exhaustion. In our study, we observed, not only submaximal, but also at peak level a higher HR, so that the  $O_2$  pulse (oxygen consumption per heart beat) is equal at GET, RCP and peak in both environmental conditions. Clearly, further investigation on the effects of acute heat exposure on limitations of exercise performance is required.

In conclusion, acute short-term heat exposure, by means of increased ambient air temperature, does impact RT and LT expressed in PO or HR, determined from a ramp ( $30 \text{ W} \cdot \text{min}^{-1}$ ) or step ( $40 \text{ W} \cdot 3 \text{ min}^{-1}$ ) incremental exercise test. Results regarding the outcomes of exercise tests in heat are still diverse and cannot be generalized without taking into account the underlying components, as consistency in methodology of exercise testing, threshold determination and specific environmental conditions are key. Based on PO and/or physiological values (HR,  $BLa^-$  and  $\dot{V}O_2$ ) obtained from one exercise test in heat, translation to practice remains complicated. However, given the large variation in response to heat exposure (see Fig.1), even a short incremental exercise test with acute heat exposure can give valuable insight on the acute heat response of an athlete. Yet, performing exercise tests in a broad range of environmental conditions provide the opportunity to gather useful information for sports scientists and coaches to optimize exercise prescription and monitoring exercise intensity in moderate physically active individuals. Therefore, every case has to be (re)viewed individually by an experienced staff, so that optimal training outcomes can be achieved.

## ADDITIONAL INFORMATION

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# *STUDY 3*

## *W'* recovery kinetics after exhaustion: a two-phase exponential process influenced by aerobic fitness

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## ABSTRACT

The aims of this study were (1) to model the temporal profile of  $W'$  recovery following exhaustion, (2) to estimate the contribution of changing  $\dot{V}O_2$  kinetics to this recovery, and [3] to examine associations with aerobic fitness and muscle fiber type (MFT) distribution. 21 men (age:  $25 \pm 2$  yr,  $\dot{V}O_{2peak}$ :  $54.4 \pm 5.3$  mL·min<sup>-1</sup>·kg<sup>-1</sup>) performed several constant load tests to determine CP and  $W'$  and eight trials to quantify  $W'$  recovery. Each test consisted of two identical exhaustive work bouts (WB1 and WB2), separated by a variable recovery interval of 30, 60, 120, 180, 240, 300, 600 or 900 s. Gas exchange was measured and muscle biopsies were collected to determine MFT distribution.  $W'$  recovery was quantified as [1] observed  $W'$  recovery ( $W'_{OBS}$ ), model-predicted  $W'$  recovery ( $W'_{BAL}$ ), and  $W'$  recovery corrected for changing  $\dot{V}O_2$  kinetics ( $W'_{ADJ}$ ).  $W'_{OBS}$  and  $W'_{ADJ}$  were modelled using mono- and bi-exponential fitting. Root mean square error (RMSE) and Akaike Information Criterion ( $\Delta AIC_C$ ) were used to evaluate the models' accuracy. The  $W'_{BAL}$  model ( $\tau$ :  $524 \pm 41$  s) was associated with RMSE of 18.6 % in fitting  $W'_{OBS}$  and underestimated  $W'$  recovery for all durations below 5 min ( $P < 0.002$ ). Mono-exponential modelling of  $W'_{OBS}$  resulted in:  $\tau = 104$  s with RMSE = 6.4 %. Bi-exponential modelling of  $W'_{OBS}$  resulted in:  $\tau_1 = 11$  s and  $\tau_2 = 256$  s with RMSE = 1.7%.  $W'_{ADJ}$  was  $11 \pm 1.5$  % lower than  $W'_{OBS}$  ( $P < 0.001$ ).  $\Delta AIC_C$  scores favoured the bi-exponential model for  $W'_{OBS}$ , but not for  $W'_{ADJ}$ .  $\dot{V}O_{2peak}$  ( $P = 0.009$ ) but not MFT distribution ( $P = 0.303$ ) was associated with  $W'_{OBS}$ . We showed that  $W'$  recovery from exhaustion follows a two-phase exponential time course that is dependent on aerobic fitness. The appearance of a fast initial recovery phase was attributed to an enhanced aerobic energy provision resulting from changes in the  $\dot{V}O_2$  kinetics.

## KEY WORDS

$W'$ ; recovery; modeling; aerobic fitness; muscle fiber type

## INTRODUCTION

The critical power (CP) model defines the hyperbolic relationship between power output (PO) and time to exhaustion (TTE) by two parameters: CP (i.e., an estimate of the maximal metabolic steady state intensity) and  $W'$  (i.e., a finite work capacity that is available above CP). Although the two-parameter CP model has been extensively used as a framework to study whole-body fatigue, the applicability to real sport situations is rather limited as the model's predictive capabilities are restrained to constant intensity performance. This means that the CP model can only be used for estimating TTE for a single and gradual depletion of  $W'$ , while most sport disciplines are characterized by intermittent efforts above and below CP (Jones & Vanhatalo, 2017). In this context, the introduction of the  $W'$  balance ( $W'_{\text{BAL}}$ ) model by Skiba et al. (2012) offered a solution by providing an equation that estimates the amount of  $W'$  that is available at any time point during intermittent exercise (i.e.,  $W'_{\text{BAL}}$ ):

$$W'_{\text{BAL}} = W' - \int_0^t (W'_{\text{EXP}}) (e^{-\frac{t-u}{\tau_{W'}}}) du \quad (1)$$

The  $W'_{\text{BAL}}$  model assumes that  $W'$  is linearly expended during exercise above CP and exponentially recovered during exercise below CP. The speed of this exponential recovery depends on two variables: the recovery duration ( $t-u$ ) and the time constant  $\tau_{W'}$ . Calculation of the latter is solely based on the difference between CP and the recovery PO ( $D_{\text{CP}}$ ), predicting a faster  $W'$  recovery when  $D_{\text{CP}}$  increases:

$$\tau_{W'} = 546e^{(-0.01D_{\text{CP}})} + 316 \quad (2)$$

The ability to monitor the dynamic state of  $W'$  in real time may offer new possibilities to quantify residual performance capacity, and could therefore facilitate athletes in optimizing their pacing strategies during races or assist coaches in prescribing more individualized training programs (Jones & Vanhatalo, 2017).

Although Skiba et al. (2014a) demonstrated that the  $W'_{\text{BAL}}$  model may be a robust method to predict exhaustion, they also found that the model tends to underpredict TTE for intermittent exercise with variable work and recovery intervals (Skiba et al., 2014b). Likewise, in our previous work (Caen et al., 2019), we discovered that the  $W'_{\text{BAL}}$  model underestimated  $W'$  recovery up to 30 % following exhaustive exercise. As this underprediction was most pronounced during shorter recovery durations, the recovery of  $W'$  seems to occur faster than expected particularly during the initial phase of the recovery. Unfortunately, the small number of time points ( $n = 3$ ) and the rather limited time frame of recovery

durations (i.e., 2 to 6 min) did not allow for a formal kinetic characterization of the  $W'$  response at the time. Nevertheless, the observation of a fast initial recovery component led us to question whether the  $W'$  recovery from exhaustive exercise follows a simple exponential time course or may be better described by a more complex two-phase exponential model.

It has been repeatedly shown that the depletion of  $W'$  is associated with the attainment of a critical level in muscle metabolic perturbation, which is reflected by consistently low values of [ATP], [PCr] and pH, and consistently high values of [ADP],  $[P_i]$  and  $[La^-]$  at the point of exhaustion (Black et al., 2017; Burnley et al., 2010; Vanhatalo et al., 2010). By extension, Johnson et al. (2014) found that the accumulation of metabolites in the blood induced by prior upper body exercise reduced the magnitude of  $W'$  during cycling. These results suggest that the restoration of the overall metabolic milieu, both inside and outside the muscle, may play a significant role in the recovery of  $W'$  (Chidnok et al., 2013). It has been previously demonstrated that the recovery of intramuscular [PCr] and the recovery of  $\dot{V}O_2$ , which can serve as a proxy for [PCr] (Rossiter et al., 2002), are considerably faster than the recovery of  $W'$  (Ferguson et al., 2010; Skiba et al., 2015). However, given the fact that the PCr and  $W'$  recovery kinetics are related (Skiba et al., 2015), the faster recovery of [PCr] might be partially reflected in the appearance of a faster than expected  $W'$  recovery component during the initial recovery phase. In addition, changes in the  $\dot{V}O_2$  kinetics originating from varying recovery durations and/or intensities could enhance the aerobic energy contribution during subsequent exercise and therefore, may also contribute to the  $W'$  recovery.

Apart from general factors defining the  $W'$  recovery kinetics, previous studies have consistently pointed to a large degree of individual variability in the recovery rate of  $W'$ . Although the precise determinants of the  $W'$  recovery remain complex, it appears that the level of aerobic fitness may play an important role (Skiba et al., 2012; Tomlin & Wenger, 2001). Bartram et al. (2018) found that the  $W'_{BAL}$  model underestimated  $W'$  recovery in a group of elite cyclists but indicated that this was not a surprise since the calculation of the model's  $\tau_{W'}$  is based solely on data of recreational athletes. In a recent study, Chorley et al. (2020) observed that the size and the maintenance of  $W'$  recovery during repeated maximal ramp tests was related to aerobic fitness. However, the exact effects of aerobic fitness on the temporal profile of the  $W'$  recovery requires further investigation.

As an important determinant of oxidative capacity (Essén et al., 1975), muscle fiber type (MFT) distribution may affect the speed of  $W'$  recovery. When introducing the  $W'_{BAL}$  model, Skiba et al. (2012) suggested a relationship between  $W'$  and the recruitment and relative fatigue state of type II muscle fibers, and they reasoned that separate compartments of type I and type II fibers differently contribute to the total  $W'$  recovery. Several studies demonstrated that differences in metabolic and contractile characteristics between type I and type II muscle fibers affect fatigue development and recovery during

high-intensity isolated muscle work (Colliander et al., 1988; Tesch et al., 1978; Thorstensson & Karlsson, 1976). The common finding of these studies is that a higher relative amount of type I fibers is associated with less force decline and faster recovery. A recent study of Lievens et al. (2020) demonstrated that MFT substantially influences the time to recover from three repeated Wingate tests by assessing maximal isometric knee exercise. While individuals with predominantly type I fibers were fully recovered after 20 min, maximal torque in the group with a dominant fast typology was still impaired after 5 h. Although these findings suggest a link between MFT distribution and  $W'$  recovery, there are currently no studies that investigated this by directly assessing whole-body performance instead of isolated muscular work.

The purpose of the present study was threefold. The first aim was to model the  $W'$  recovery from exhaustive exercise across an extensive range of recovery durations between (i.e., between 30 s and 15 min). We hypothesized that the  $W'$  recovery kinetics would be better fitted by a two-phase than a one-phase exponential model. Second, we studied the  $\dot{V}O_2$  on- and off-kinetics in order to examine the extent to which changes in the  $O_2$  deficit contribute to the  $W'$  recovery, and to quantify the recovery pattern of  $\dot{V}O_2$  as a proxy for intramuscular [PCr]. We expected that changes in the  $O_2$  deficit at the onset of exercise would enhance the  $W'$  recovery and that the recovery of  $\dot{V}O_2$  would be faster than the recovery of  $W'$ . Third, we investigated if the  $W'$  recovery is associated with the level of aerobic fitness and/or MFT distribution. It was hypothesized that a higher aerobic fitness and a higher % type I fibers would be associated with a faster  $W'$  recovery.

## METHODS

### Participants

Twenty-one young men ( $25 \pm 2$  yr,  $1.82 \pm 0.06$  m,  $74.6 \pm 5.8$  kg) volunteered for participation in the study. All participants indicated to have a physically active lifestyle and to engage in one or more weekly sports activities. A medical examination prior to the start of the study did not reveal any health issues or risk factors that would hinder further participation. Informed consent was received from all participants. The study was approved by the Ethical Committee of the Ghent University Hospital (Ghent, Belgium).

### Experimental procedure and protocols

All exercise tests were performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, the Netherlands) in the Sport Science Laboratory - Jacques Rogge (Ghent, Belgium). Ambient temperature was set at 18°C with a relative humidity of 50%. In total, participants attended the laboratory on 13 or 14 occasions. Each visit was planned at a fixed time of the day and separated by at least 48 h of recovery. We asked the participants to standardize their food and drink intake on test days. Also, participants were instructed to avoid strenuous exercise in the last 24 h before testing. During all tests, participants wore a nose clip and a mouthpiece with a TripleV sensor to measure breath-by-breath gas exchange ( $\dot{V}O_2$ ,  $\dot{V}CO_2$ ,  $\dot{V}E$ ) (Jaeger Oxycon Pro, Viasys Healthcare, Höchberg, Germany). The metabolic system was calibrated before each test according to the manufacturer's recommendations, including an automatic flow meter calibration and an automatic gas analyzer calibration using room air and a known reference gas (16%  $O_2$  and 5%  $CO_2$ ). Heart rate was registered continuously using a chest strap (H7, Polar, Kempele, Finland). During exercise, participants were blinded to all feedback, except for their pedal cadence.

#### Ramp incremental test

During the first lab visit, participants completed 3 min of baseline cycling at 50 W, followed by a  $30 \text{ W} \cdot \text{min}^{-1}$  ramp incremental test to exhaustion. Participants had to select a cadence between 70 and 90 rpm and were instructed to keep it constant throughout the test. The same cadence was also imposed during all subsequent tests. The ramp incremental test ended at voluntary exhaustion or when participants could not maintain their self-selected cadence for more than 5 s despite strong verbal encouragement. This criterion was also used for all other exhaustive tests.

#### Constant load tests

Each participant completed a minimum of three constant load tests to exhaustion. Each trial started with 3 min cycling at 50 W, immediately followed by an abrupt increase to the appropriate PO between

70% and 100%  $PO_{peak}$ . These PO were chosen to elicit TTE that were proportionally distributed between 2 and 20 min, with a difference of >5 min between the longest and the shortest trial. Participants were verbally encouraged to give full effort. If the determination criteria for CP and  $W'$  were not met (see data analysis), participants had to perform one or more additional trials.

#### Experimental tests

The experimental test protocol was characterized by two identical exhaustive work bouts (i.e., WB1 and WB2), interspersed with an active recovery period that was varied in duration. After 3 min of cycling at 50 W, the PO abruptly increased to  $PO_{4min}$  (i.e., PO predicted to result in exhaustion after 4 min) at which the participants had to cycle until exhaustion (i.e., WB1). At the point of exhaustion, participants were instructed to immediately start cycling at a recovery PO that corresponded to 90% of their gas exchange threshold (GET). The duration of this recovery bout was different in each test with a total of eight conditions which were randomly assigned (i.e., 30 s, 1, 2, 3, 4, 5, 10 and 15 min). Following the recovery period, a second and identical work bout at  $PO_{4min}$  was performed to exhaustion (i.e., WB2). TTE at both work bouts was manually recorded.

#### Muscle biopsies

During the final lab visit, muscle biopsies at rest were taken from the distal section of the left M. Vastus Lateralis. Under local anesthesia (Xylocaine 1%), a skin incision was made with a disposable scalpel (Swann-Morton, Sheffield, UK). In each participant, two muscle samples were taken using a 12-gauge × 13 cm true-cut biopsy needle (Bard Magnum biopsy instrument, Bard, Inc., New Jersey, USA). After collection, samples were mounted with Tissue-Tek (O.C.T. Compound), submerged in nitrogen-cooled isopentane and stored at  $-80^{\circ}\text{C}$  for subsequent immunohistochemical analysis.

### Data analysis

#### Ramp incremental test

$PO_{peak}$  was defined as the final PO that was reached at the end of the test. Raw  $\dot{V}O_2$  data were inspected to exclude aberrant data points and then expressed as 10-s averages.  $\dot{V}O_{2peak}$  was calculated as the highest 30-s value throughout. GET was determined using the V-slope method  $\dot{V}CO_2/\dot{V}O_2$  (Beaver et al., 1986), and verified by identifying the first breakpoint in the  $\dot{V}E$ ,  $\dot{V}E/\dot{V}O_2$  and  $P_{ET}O_2$  response (Binder et al., 2008). To retrieve the PO that was associated with 90% GET, the  $\dot{V}O_2/PO$  relationship of each test was left-shifted in order to account for the mean response time (Boone et al., 2008; Caen et al., 2020).

#### Constant load tests

CP and  $W'$  were determined by means of a 'best individual fit'-approach using the nonlinear power-time model, the linear work-time model and the linear power-1/time model. Trials of which the TTE fell

outside the predefined 2-to-20 min range, or trials of which the end- $\dot{V}O_2$  was < 95% of the ramp  $\dot{V}O_{2peak}$  were excluded from the modelling (Jones et al., 2019; Mattioni Maturana et al., 2018). For each participant, the regression model that was associated with the smallest total error (i.e., the sum of the standard errors associated with CP and  $W'$ ) was selected. To ensure sufficient accuracy in estimating both parameters, the coefficient of variation (CV%) for CP and  $W'$  was set *a priori* at < 5% and < 10%, respectively (Black et al., 2015). If the aforementioned criteria were not met after three tests, one or more additional trials were performed. In that case, the best model from all possible trial combinations including a minimum of three trials was selected.

#### Experimental tests

Assuming that  $W' = 0$  at the end of WB1, the relative (%) observed  $W'$  recovery ( $W'_{OBS}$ ) was calculated as TTE at WB2 expressed relative to TTE at WB1 (Caen et al., 2019). Since the experimental test protocol included a single exhaustive work bout and one recovery interval at an individually fixed PO, the model-predicted  $W'$  recovery ( $W'_{BAL}$ ) for each condition could be calculated using a simple exponential function:

$$W'_{BAL} = W' \times (1 - e^{-x/\tau_{W'}}) \quad (3)$$

where  $W'$  is the amount of work done above CP during WB1,  $x$  is the recovery duration and  $\tau_{W'}$  is the recovery time constant calculated using Eq. (2). In this way,  $W'_{OBS}$  and  $W'_{BAL}$  were individually calculated for all recovery conditions. One-phase (Eq. 4) and two-phase (Eq. 5) exponential modelling of  $W'_{OBS}$  was performed by means of nonlinear regression analyses with sequential quadratic programming (SPSS Statistics 24, IBM Corp., Armonk, NY, USA) using the following equations:

$$W'_{OBS} = A \times (1 - e^{-\frac{x}{\tau}}) \quad (4)$$

$$W'_{OBS} = (A \times \alpha_1) \times (1 - e^{-\frac{x}{\tau_1}}) + (A \times \alpha_2) \times (1 - e^{-\frac{x}{\tau_2}}) \quad (5)$$

where  $A$  is the amplitude of the response,  $x$  is the recovery duration and  $\tau$  represents the time constant(s). In Eq. (5),  $\tau_1$  and  $\tau_2$  correspond to, respectively, the fast and the slow time constant of the regression. Variables  $\alpha_1$  and  $\alpha_2$  represent the fraction of the total amplitude that is accounted for by each of these two time constants. Modelling of  $W'_{OBS}$  was performed twice, once by using a variable

amplitude ( $A$ ) and once by using a predefined theoretical amplitude ( $A = 100\%$ ). The same model fitting procedure, with  $A$  allowed to vary, was applied to the adjusted  $W'$  recovery ( $W'_{ADJ}$ : see further). For each regression model, the root mean square error (RMSE) was calculated to quantify the model's predictive error.

The corrected Akaike Information Criterion ( $AIC_C$ ) (Burnham & Anderson, 2004) was calculated to determine which model provided a relative superior fit for  $W'_{OBS}$  and  $W'_{ADJ}$ :

$$AIC_C = N \times \ln(RSS/N) + 2K + [(2K \times (K + 1))/(N - K - 1)] \quad (6)$$

where  $N$  is the number of data points,  $RSS$  is the residual sum of squares from each model, and  $K$  is the number of parameters in the fitted model ( $K = 2$  for the mono-exponential model and  $K = 4$  for the bi-exponential model). The difference score ( $\Delta$ ) of the corrected AICC was calculated by subtracting AICC of the bi-exponential model from AICC of the mono-exponential model. A negative  $\Delta AIC_C$  suggests that the mono-exponential model provides a better fit, whereas a positive  $\Delta AIC_C$  favors the bi-exponential model.

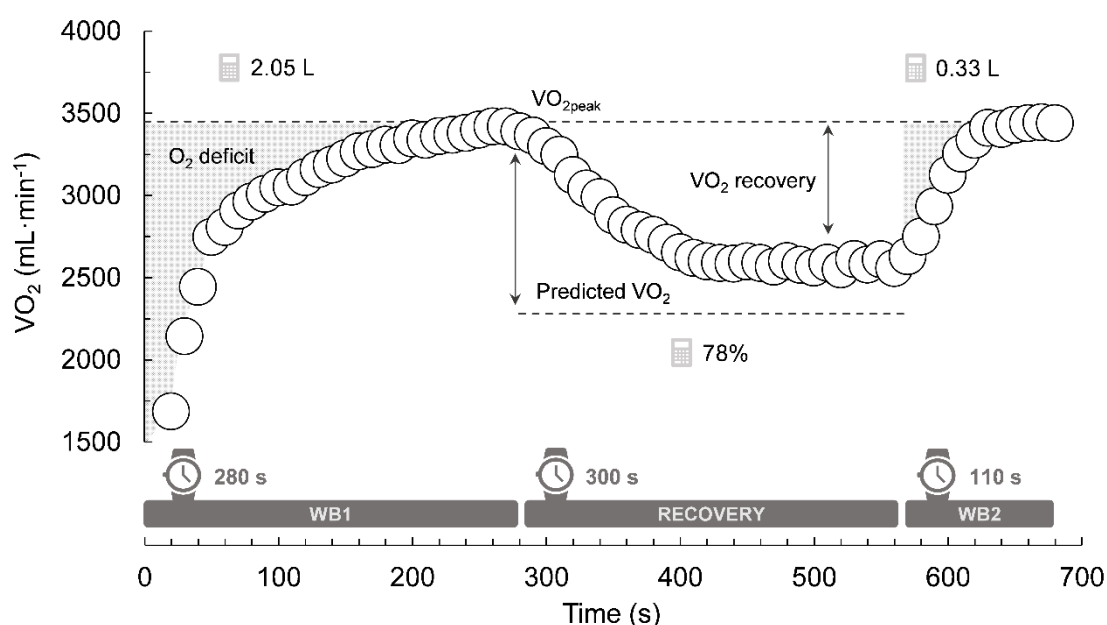
$\dot{V}O_2$  kinetics at the onset of WB1 and WB2 were examined by calculating the magnitude of the  $O_2$  deficit relative to the end- $\dot{V}O_2$ . For each recovery condition, the  $O_2$  deficits of WB1 and WB2 were calculated by subtracting the measured  $\dot{V}O_2$  from the end- $\dot{V}O_2$ , integrated over time (Fig. 1). In order to assess the potential effects of differences in the initial  $\dot{V}O_2$  response on  $W'_{OBS}$ , the recovery was recalculated by correcting for these differences. This estimated adjusted  $W'$  recovery ( $W'_{ADJ}$ ) would then describe the  $W'$  recovery in the hypothetical situation that the  $O_2$  deficit of WB2 would be identical to WB1. To do this, the following step-by-step calculations were performed for each recovery duration:

- The  $O_2$  deficit of WB2 was subtracted from the  $O_2$  deficit of WB1, yielding the absolute difference between both work bouts
- The difference in  $O_2$  deficit (expressed in L) was converted into an energy measure (expressed in kJ) by using the energy equivalent for  $O_2$  (1 L  $O_2 = 21.1$  kJ) (Scott, 2014).
- The amount of energy was translated into an estimate of mechanical work by assuming a gross mechanical efficiency of 22% (value derived from (Ettema & Lorås (2009))).

The outcome of the above calculations was considered as the amount of additional work (kJ) that participants could perform during WB2 vs. WB1 due to differences in the onset  $\dot{V}O_2$  kinetics between both work bouts. This value was also expressed in seconds as the time gain during WB2 by dividing the additional work by  $PO_{4min}$ . Finally, the estimated additional work was subtracted from the total work of

WB2 and the adjusted total work of WB2 was expressed relative to the total work of WB1 to calculate  $W'_{ADJ}$ . By comparing  $W'_{OBS}$  and  $W'_{ADJ}$ , it could be evaluated whether the observed  $W'_{OBS}$  recovery kinetics are an artefact of differences in the onset  $\dot{V}O_2$  kinetics.

The recovery of  $\dot{V}O_2$  between WB1 and WB2 was calculated as the measured  $\dot{V}O_2$  at the end of each recovery interval relative to the difference between the end- $\dot{V}O_2$  of WB1 and the predicted  $\dot{V}O_2$  demand associated with the recovery PO (Fig. 1).  $\dot{V}O_2$  responses were time aligned to the start of the recovery interval and ensemble averaged across conditions to obtain the  $\dot{V}O_2$  at the end of the recovery. Because the recovery PO was set below GET (i.e., 90% GET), the  $\dot{V}O_2$  demand could be calculated individually by means of the mean response time-corrected  $\dot{V}O_2$ /PO relationship derived from the ramp incremental test.



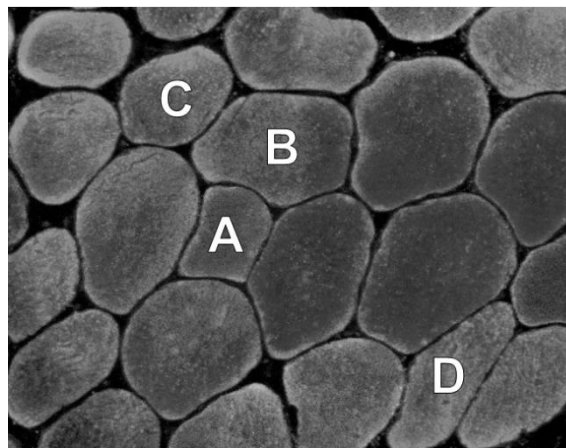
**Figure 1** Representative example showing the  $\dot{V}O_2$  response of a participant who performed the 5-min recovery condition. The figure illustrates the calculation of the O<sub>2</sub> deficit of WB1 and WB2, and the quantification of the  $\dot{V}O_2$  recovery between both work bouts.

## Muscle biopsies

Muscle samples were cut into 4- $\mu$ m cross-sections (Microm HM 550 OMVP, Microm GmbH, Neuss, Germany) at a temperature of  $-15^{\circ}\text{C}$  and mounted on glass slides. Per participant, four sections were stained for myosin heavy chain (MHC) isoforms based on the protocols of Bloemberg & Quadrilatero, 2012 and De Meulemeester et al. (2017). In short, the following antibodies were used in PBS: BA-F8 for MHC I at 1:16, SC-71 for MHC IIA at 1:26, 6H1 for MHC IIX at 1:10, and Laminin at 1:100 to visualize the basement membranes. Incubation with primary antibodies was done for two hours, followed by a one hour incubation with the secondary antibodies in PBS. The secondary cocktail consisted of Alexa Fluor

633 to BA-F8 at 1:500, Alexa Fluor 488 to SC-71 at 1:500, Alexa Fluor 555 to 6H1 at 1:500, and Alexa Fluor 405 to Laminin at 1:10.

The slides were imaged with an Axio Scan.Z1 (Zeiss, Jena, Germany) using a 10X Plan-Apochromat 0.45 NA (0.650  $\mu\text{m}/\text{pixel}$ ) and a Hamamatsu Orca Flash camera. With an HXP illumination source, the following filters were used in the acquisition: DAPI (BP 445/50), HE GFP (BP 525/50), HE DsRed (BP 605/70), and Cy5 (BP 690/50). Visualization of the tissue was optimized using ZEN 3.1 (ZEISS, Oberkochen, Germany). After exporting the images, an average of  $368 \pm 132$  muscle fibers were manually counted per participant ( $n = 19$ ) using ImageJ 1.52p (Wayne Rasband, NIH, USA). Based on the staining, muscle fibers were classified into four categories: type I (BA-F8), type IIA (SC-71), type IIX (6H-1) and type IIA/X (SC-71 and 6H-1) (Fig. 2). The amount of fibers assigned to each category was then expressed relative (%) to the total fiber count.



**Figure 2** Microscopic image of a muscle cryosection after immunohistochemical staining. A = type I fiber, B = type IIA fiber, C = type IIA/X fiber, D = type IIX fiber.

### Statistical analysis

Data are presented as mean  $\pm$  SD for  $n = 21$  participants, unless otherwise specified. Normal distribution of the variables was confirmed by the Shapiro-Wilk test ( $P > 0.05$ ) and by analysis of skewness and kurtosis (cut-off:  $\pm 1$ ).

The observed and model-predicted TTE of WB1 was compared using a one sample t-test. One-way repeated-measures (RM) ANOVA's were conducted to (1) find differences in TTE of WB1 between recovery conditions, (2) to detect differences between  $\dot{V}O_{2\text{peak}}$  and the end- $\dot{V}O_2$  of WB1 and WB2, and (3) to check for possible order effects in TTE and the end- $\dot{V}O_2$  of WB1.

A full factorial two-way RM ANOVA ( $W'$  quantification [3 levels:  $W'_{\text{OBS}}$ ,  $W'_{\text{BAL}}$  and  $W'_{\text{ADJ}}$ ]  $\times$  recovery duration [8 levels: 30 s, 1, 2, 3, 4, 5, 10 and 15 min]) was performed to compare the time course of

$W'_{OBS}$ ,  $W'_{BAL}$ ,  $W'_{ADJ}$ . When Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, a Greenhouse-Geisser correction was applied. Post hoc tests with LSD correction were chosen for multiple comparisons. A one-way RM ANOVA with  $\dot{V}O_{2peak}$  and MFT distribution included as covariates was conducted to investigate their relationship with  $W'_{OBS}$  over time ( $n = 19$ ). A multiple regression was run to predict  $W'_{OBS}$  from recovery duration,  $\dot{V}O_{2peak}$  and MFT distribution. The Pearson correlation coefficient ( $r$ ) was applied to examine the overall relationship between  $W'_{OBS}$  and individual characteristics.

A two-way RM ANOVA was used to compare the  $O_2$  deficit between WB1 and WB2 across recovery conditions. The estimated performance benefit derived from possible differences in the  $O_2$  deficit was then compared using a one-way RM ANOVA. A two-way RM ANOVA was performed to compare the recovery of  $W'_{OBS}$  and  $\dot{V}O_2$  over time.

All statistical analyses were performed with SPSS Statistics 24 (IBM Corp., Armonk, NY, USA). Statistical significance was accepted when  $P < 0.05$ .

## RESULTS

An overview of the performance and cardiorespiratory response during the ramp incremental exercise test is shown in Table 1.

**Table 1** Group mean performance and cardiorespiratory response during the ramp incremental exercise test.

|                     |  | 30 W·min <sup>-1</sup> |
|---------------------|--|------------------------|
| PO <sub>peak</sub>  | W                                      | 392 ± 36               |
|                     | L·min <sup>-1</sup>                    | 4.05 ± 0.44            |
| VO <sub>2peak</sub> | mL·min <sup>-1</sup> ·kg <sup>-1</sup> | 54.4 ± 5.3             |
| HR <sub>peak</sub>  | bpm                                    | 192 ± 8                |
| RER <sub>peak</sub> |  | 1.25 ± 0.11            |
|                     | L·min <sup>-1</sup>                    | 2.57 ± 0.33            |
| GET                 | W                                      | 179 ± 33               |
|                     | s                                      | 51 ± 21                |
| MRT                 | W                                      | 25 ± 10                |

PO, power output; VO<sub>2</sub>, pulmonary oxygen uptake; HR, heart rate; RER, respiratory exchange ratio; GET, gas exchange threshold; MRT, mean response time.

To calculate CP and  $W'$ , the nonlinear power-time model and the linear power-1/time model were selected for, respectively, 11 and 10 participants. The linear work-time model was never associated with the lowest total error. Mean CP and  $W'$  were 269 ± 31 W (CV% = 1.3 ± 0.9%) and 19.2 ± 5.1 kJ (CV% = 5.6 ± 3.3%), respectively. The average TTE at PO4min (348 ± 34 W) during WB1 was 228 ± 38 s with no significant differences between recovery conditions ( $P = 0.377$ ). TTE at WB1 and WB2 for each recovery condition is presented in Table 2.

**Table 2** Time to exhaustion (TTE) at work bout 1 (WB1) and 2 (WB2) and comparison between the observed ( $W'_{OBS}$ ), model-predicted ( $W'_{BAL}$ ) and adjusted ( $W'_{ADJ}$ )  $W'$  recovery at each recovery duration.

| Recovery duration | TTE      |          | $W'_{OBS}$  | $W'_{BAL}$  | $W'_{ADJ}$                |
|-------------------|----------|----------|-------------|-------------|---------------------------|
|                   | WB1 (s)  | WB2 (s)  | %           | %           | %                         |
| 30 s              | 227 ± 45 | 63 ± 18  | 28.6 ± 8.2  | 5.6 ± 0.4*  | 15.8 ± 9.0* <sup>†</sup>  |
| 60 s              | 230 ± 46 | 77 ± 22  | 34.8 ± 11.1 | 10.9 ± 0.8* | 22.7 ± 12.0* <sup>†</sup> |
| 120 s             | 234 ± 39 | 102 ± 24 | 44.2 ± 9.7  | 20.6 ± 1.5* | 32.0 ± 10.5* <sup>†</sup> |
| 180 s             | 226 ± 46 | 112 ± 26 | 50.5 ± 12.1 | 29.2 ± 1.9* | 38.4 ± 12.3* <sup>†</sup> |
| 240 s             | 230 ± 54 | 123 ± 29 | 55.1 ± 13.3 | 36.9 ± 2.3* | 45.5 ± 13.5* <sup>†</sup> |
| 300 s             | 235 ± 48 | 130 ± 34 | 56.8 ± 16.4 | 43.7 ± 2.6* | 47.3 ± 17.5*              |
| 600 s             | 217 ± 42 | 155 ± 31 | 73.7 ± 19.3 | 68.3 ± 2.9  | 64.9 ± 19.8*              |
| 900 s             | 225 ± 42 | 159 ± 51 | 71.3 ± 20.8 | 82.1 ± 2.4* | 60.3 ± 23.4* <sup>†</sup> |

\*significantly different from  $W'_{OBS}$  ( $P < 0.05$ )

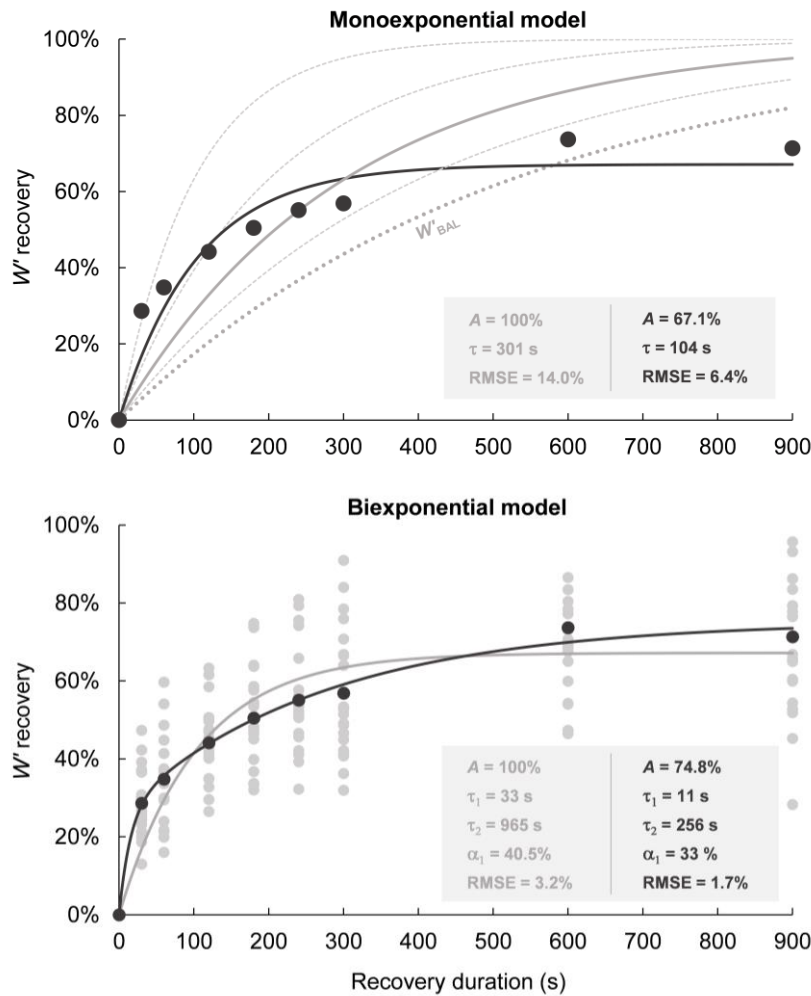
<sup>†</sup> significantly different from  $W'_{BAL}$  ( $P < 0.05$ )

Mean TTE at WB1 was not significantly different from the model-predicted TTE of 240 s ( $P = 0.171$ ). The end- $\dot{V}O_2$  of WB1 ( $4.09 \pm 0.46$  L·min<sup>-1</sup>) and WB2 ( $4.05 \pm 0.47$  L·min<sup>-1</sup>) were not different from each other and did also not differ from  $\dot{V}O_{2peak}$  derived from the ramp incremental test ( $4.05 \pm 0.44$  L·min<sup>-1</sup>) ( $P = 0.575$ ). These results support the validity of our experimental protocol. There was no order effect for TTE at WB1 ( $P = 0.613$ ), nor for the end- $\dot{V}O_2$  of WB1 ( $P = 0.502$ ), suggesting that participants did not improve their physical fitness throughout the study period.

### General recovery kinetics

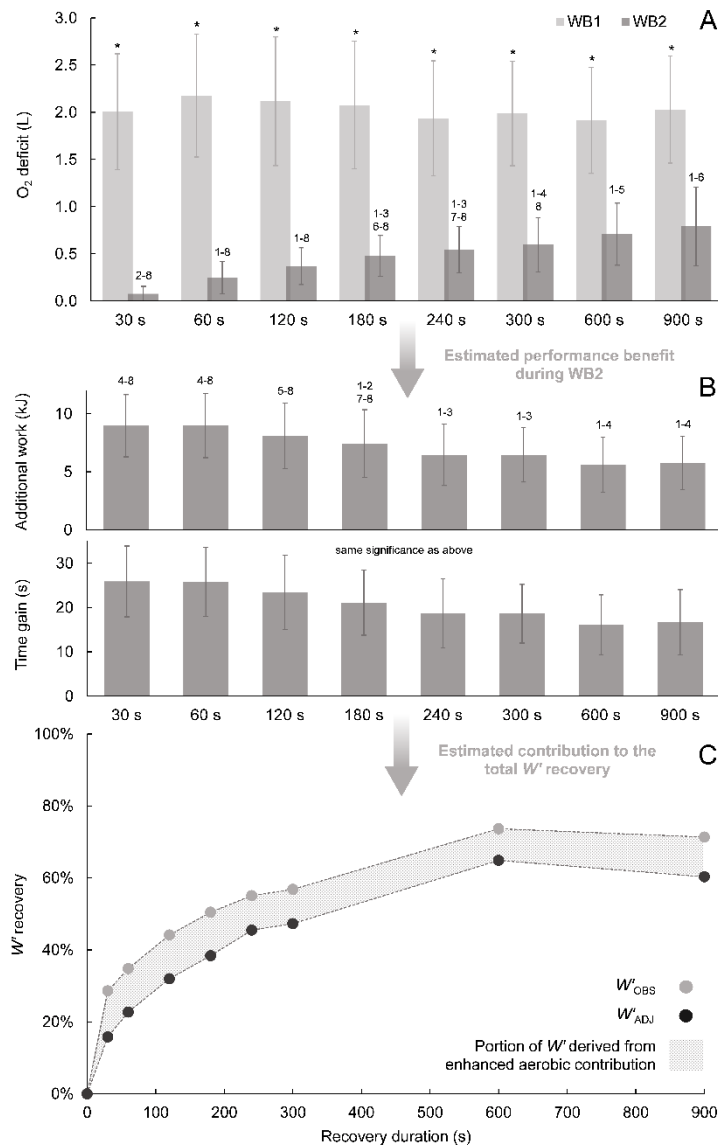
A significant interaction effect ( $W'$  quantification  $\times$  recovery duration) showed differences in the time course of  $W'_{OBS}$ ,  $W'_{BAL}$  and  $W'_{ADJ}$  ( $P < 0.001$ ) (Table 2). Differences between  $W'_{OBS}$  and  $W'_{BAL}$  and between  $W'_{ADJ}$  and  $W'_{BAL}$  were dependent on the recovery condition ( $P < 0.001$ ). Specifically,  $W'_{OBS}$  was higher than  $W'_{BAL}$  at all recovery durations between 30 s and 5 min ( $P < 0.05$ ), but lower than  $W'_{BAL}$  in the 15-min recovery condition ( $P = 0.019$ ). At the 10th min of recovery, there was no significant difference between  $W'_{OBS}$  and  $W'_{BAL}$  ( $P = 0.205$ ).  $W'_{ADJ}$  was higher than  $W'_{BAL}$  at all recovery durations ( $P < 0.05$ ), except for the 5-min ( $P = 0.344$ ) and 10-min ( $P = 0.423$ ) recovery condition.

In general,  $W'_{OBS}$  increased with longer recovery durations ( $P < 0.001$ ) but exhibited a plateau between the 10th and 15th min of recovery ( $P = 0.558$ ), yielding the highest  $W'_{OBS}$  ( $73.7 \pm 19.3\%$ ) after 10 min (Fig. 3). The  $W'_{BAL}$  model generated a  $\tau_{W'}$  of  $524 \pm 41$  s and was associated with a RMSE of 18.6% in predicting  $W'_{OBS}$ . Figure 3 visualizes the temporal profile of  $W'_{OBS}$  with mono- and bi-exponential model fitting. Model parameters derived from the mono-exponential fitting of  $W'_{OBS}$  were:  $\tau_{W'} = 104 \pm 22$  s,  $A = 67.1 \pm 4.3\%$  and RMSE = 6.4%. When  $A$  was set at 100%, model parameters were:  $\tau_{W'} = 301 \pm 55$  s and RMSE = 14%. Bi-exponential fitting of  $W'_{OBS}$  resulted in:  $\tau_1 = 11 \pm 10$  s,  $\tau_2 = 256 \pm 51$  s,  $A = 74.8 \pm 2.7\%$ ,  $\alpha_1 = 33 \pm 5.4\%$  and RMSE = 1.7%. When  $A$  was set at 100%, parameter estimates were:  $\tau_1 = 33 \pm 9$  s,  $\tau_2 = 965 \pm 175$  s,  $\alpha_1 = 40.5 \pm 4.1\%$  and RMSE = 3.2%.  $\Delta AIC_C$  calculated for  $W'_{OBS}$  was positive (11.51 for  $A$  = variable and 14.65 for  $A$  = 100%), suggesting that the bi-exponential model provided a better fit compared to the mono-exponential model. Model parameters derived from the mono-exponential fitting of  $W'_{ADJ}$  were:  $\tau_{W'} = 168 \pm 21$  s,  $A = 61.8 \pm 2.9\%$  and RMSE = 3.5%. Bi-exponential fitting of  $W'_{ADJ}$  resulted in:  $\tau_1 = 10 \pm 30$  s,  $\tau_2 = 233 \pm 49$  s,  $A = 64.4 \pm 2.9\%$ ,  $\alpha_1 = 15.2 \pm 8.1\%$  and RMSE = 2.1%.  $\Delta AIC_C$  calculated for  $W'_{ADJ}$  was negative  $\Delta AIC_C$  (-2.86), suggesting that the bi-exponential model was no longer favored over the mono-exponential model.



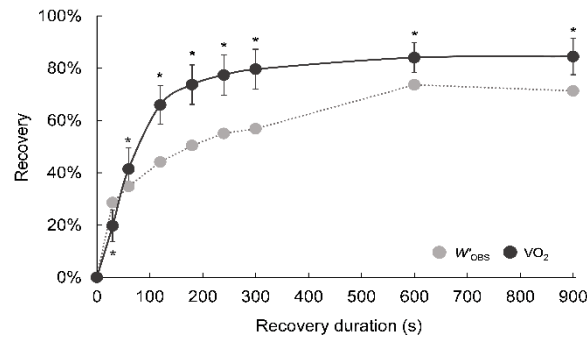
**Figure 3** Mathematical modelling of the group mean  $W'$  recovery (black dots) by means of a mono-exponential and a bi-exponential fitting procedure. Model fits were obtained twice, once using a variable amplitude (black lines) and once using a predefined theoretical amplitude of 100% (grey lines). The dotted line shows the  $W'$  recovery with a time constant ( $\tau_{W'}$ ) of 524 s, as predicted by the  $W'_{BAL}$  model. Dashed lines visualize randomly chosen model fits in order to demonstrate that no single  $\tau_{W'}$  can accurately model the entire  $W'$  recovery time course. Individual data points ( $n = 21$ ) at each recovery duration are presented in the lower panel (grey dots).  $A$  = amplitude,  $\alpha_1$  = fraction of the total response that is accounted for by  $\tau_1$ , RMSE = root mean square error.

There were no differences in the  $O_2$  deficit of WB1 across recovery conditions ( $P = 0.226$ ) (Fig. 4A). The  $O_2$  deficit of WB2 was consistently lower compared to WB1 ( $P < 0.001$ ) with differences that were dependent on the recovery duration ( $P < 0.001$ ) (Fig. 4A). Specifically, the  $O_2$  deficit of WB2 increased with longer recovery durations ( $P < 0.001$ ) (Fig. 4A). The performance benefit associated with an increased aerobic contribution during WB2 was estimated in a range from  $9.0 \pm 2.7$  kJ ( $26 \pm 8$  s) to  $5.6 \pm 2.4$  kJ ( $16 \pm 7$  s), showing an overall decline with increasing recovery durations ( $P = 0.001$ ) (Fig. 4B). On average,  $W'_{OBS}$  was  $11.0 \pm 1.5\%$  higher than  $W'_{ADJ}$  ( $P < 0.001$ ), showing no differences across recovery conditions ( $P = 0.075$ ) (Fig. 4C).



**Figure 4** Overview of the differences in aerobic energy contribution, as reflected by changing  $\dot{V}O_2$  kinetics, at the onset of work bout 1 (WB1) vs. work bout 2 (WB2) and their subsequent effect on the  $W'$  recovery. Panel A compares of the  $O_2$  deficit between WB1 and WB2. \* denotes significant differences from WB2. Numeric labels above the bars indicate from which recovery conditions (1-8) each bar differs. Panel B shows the estimated performance benefit during WB2 resulting from the differences in  $O_2$  deficit at each recovery duration. This performance benefit was expressed as additional work (kJ) and the time gain (s). Numeric labels above the bars indicate from which recovery condition (1-8) each bar differs. Panel C illustrates the estimated effect of the aerobic advantage during WB2 vs. WB1 by comparing the temporal profile of the originally determined  $W'$  recovery ( $W'_{OBS}$ ) to the  $W'$  recovery that has been adjusted for this advantage ( $W'_{ADJ}$ ). The shaded area corresponds to the estimated proportion of  $W'$  recovery that derives from an enhanced aerobic contribution.

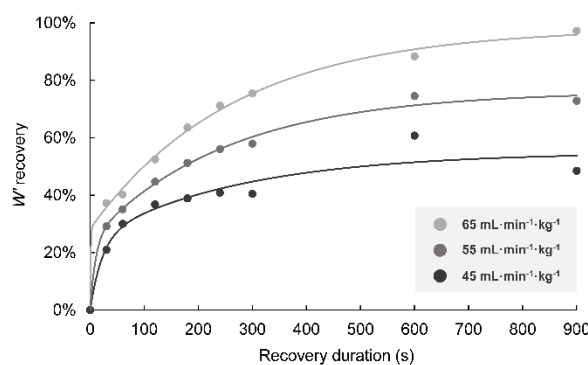
The temporal recovery profile of  $\dot{V}O_2$  is presented in Figure 5. Differences between  $W'_{OBS}$  and  $\dot{V}O_2$  recovery were dependent on the recovery duration ( $P < 0.001$ ). More specifically,  $\dot{V}O_2$  recovery was higher than  $W'_{OBS}$  at all time points, except for the recovery at 30 s, where it was lower ( $P < 0.001$ ) (Fig. 5). Mean  $\dot{V}O_2$  recovery was positively associated with mean  $W'_{OBS}$  ( $r = 0.47$ ,  $P = 0.033$ ).



**Figure 5** Comparison between the group mean  $\dot{V}O_2$  recovery and the observed  $W'$  recovery ( $W'_{OBS}$ ). \* Different from  $W'_{OBS}$  ( $P < 0.05$ ).

### Individual characteristics

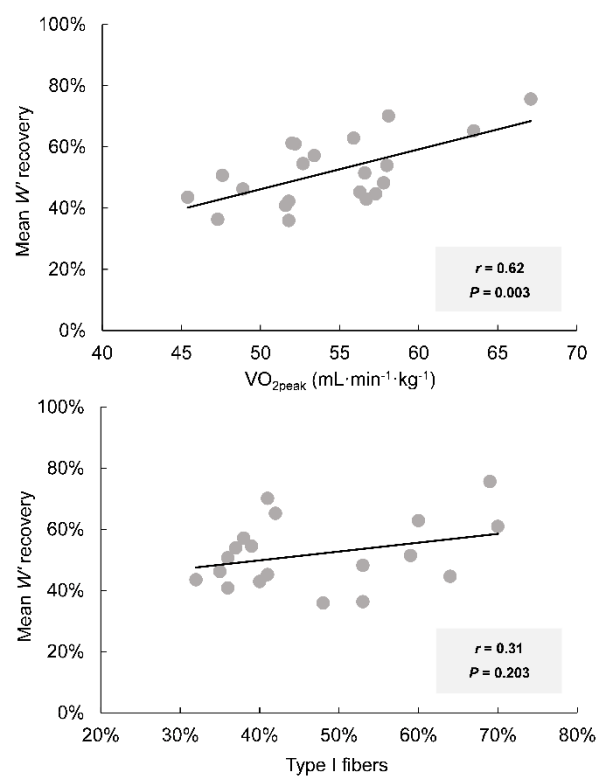
On average,  $368 \pm 132$  fibers were counted per subject ( $n = 19$ ). The group mean MFT composition consisted of  $46.8 \pm 12.2\%$  type I,  $26.3 \pm 8.7\%$  type IIA,  $20.7 \pm 8.7\%$  type IIA/X and  $6.2 \pm 4.6\%$  type IIX fibers. A significant interaction between recovery duration and  $\dot{V}O_{2peak}$  ( $P = 0.013$ ) showed that  $W'_{OBS}$  was affected by  $\dot{V}O_{2peak}$  and indicated a larger effect with increasing recovery durations (Fig. 6). On the contrary, there was no significant interaction between recovery duration and MFT distribution ( $P = 0.196$ ), nor a significant main effect of MFT ( $P = 0.816$ ), indicating that  $W'_{OBS}$  was not influenced by MFT distribution. The multiple regression model provided a significant prediction of  $W'_{OBS}$  (adjusted  $R^2 = 0.53$ ,  $P < 0.001$ ). Recovery duration ( $P < 0.001$ ) and  $\dot{V}O_{2peak}$  ( $P < 0.001$ ) significantly contributed to the prediction, while MFT distribution did not ( $P = 0.678$ ).



**Figure 6** Model-predicted  $W'$  recovery for a hypothetical athlete with either a low ( $45 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ), medium ( $55 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) or high ( $65 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )  $\dot{V}O_{2peak}$ .

Mean  $W'_{OBS}$  was significantly correlated with  $\dot{V}O_{2peak}$  ( $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) ( $r = 0.62$ ,  $P = 0.003$ ) (Fig. 7) and with CP ( $\text{W}\cdot\text{kg}^{-1}$ ) ( $r = 0.57$ ,  $P = 0.003$ ), but was not associated with  $PO_{peak}$  ( $\text{W}\cdot\text{kg}^{-1}$ ) ( $r = 0.37$ ,  $P = 0.095$ ) or GET ( $\text{W}\cdot\text{kg}^{-1}$ ) ( $r = 0.39$ ,  $P = 0.079$ ). Mean  $W'_{OBS}$  was not associated with % type I ( $r = 0.31$ ,  $P = 0.203$ ) (Fig. 7), % type IIA ( $r = -0.05$ ,  $P = 0.844$ ), % type IIA/X ( $r = -0.15$ ,  $P = 0.533$ ) or % type IIX ( $r = -0.43$ ,  $P = 0.065$ ) fibers.

There were no significant correlations between % type I fibers and CP or  $W'$  (CP:  $r = 0.45$ ,  $P = 0.053$ ,  $W'$ :  $r = -0.18$ ,  $P = 0.465$ ), neither when these parameters were expressed relative to body mass (CP:  $r = 0.35$ ,  $P = 0.145$ ,  $W'$ :  $r = -0.23$ ,  $P = 0.35$ ). Also none of the other fiber types (i.e., IIA, IIA/X, IIX) were related to CP or  $W'$ .



**Figure 7** Scatterplots showing the relationship between mean  $W'$  recovery and  $\dot{V}O_{2peak}$  ( $n = 21$ ) (upper panel) and between mean  $W'$  recovery and % type I fibers ( $n = 19$ ) (lower panel).

## DISCUSSION

This study is the first to investigate the  $W'$  recovery profile following exhaustive exercise across a broad range of recovery durations (i.e., eight time points between 30 s and 15 min). Contrary to what was previously thought, we demonstrated that the temporal course of the  $W'$  recovery was better described by a two-phase than by a one-phase exponential model, thereby exhibiting a fast initial and a slow second recovery phase. Our results indicated that the bi-exponential character of the  $W'$  recovery could be attributed to an enhanced aerobic energy contribution originating from differences in the  $O_2$  deficit between WB1 and WB2. It was also hypothesized that the recovery of  $W'$  would be dependent on aerobic fitness and MFT distribution. Although we found that the recovery was highly influenced by aerobic fitness, a clear association with MFT distribution was not demonstrated.

### General recovery kinetics

To the best of our knowledge, only three studies to date have investigated the effects of different recovery durations on the  $W'$  recovery following exhaustive whole-body exercise (Caen et al., 2019; Felipe et al., 2020; Ferguson et al., 2010). However, none of these studies performed a kinetic characterization of the  $W'$  recovery due to the limited amount of time points and the small number of participants included. Based on the mathematical model of Skiba et al. (2012) (i.e., the  $W'_{BAL}$  model), it is generally assumed that this recovery follows a mono-exponential time course. However, the predictive capabilities of this model have been questioned (Bartram et al., 2018; Caen et al., 2019; Sreedhara et al., 2019). While Bartram et al. (2018) showed that the  $W'_{BAL}$  model underpredicts  $W'$  recovery in elite cyclists, results from our own laboratory revealed a similar finding in a group of physically active men (Caen et al., 2019). We observed that this underprediction was especially evident after shorter recovery durations. This finding was confirmed in the present study as we showed that  $W'_{OBS}$  was higher than  $W'_{BAL}$  at all recovery durations below 10 min with differences being more pronounced during the shortest recovery durations. It is interesting to see that, on average, participants were capable of restoring almost 30% of their initial performance capacity after only 30 s of recovery. This reflects the ability of the human body to recover very rapidly following exhaustion. Mathematical modelling demonstrated that due to this fast initial component, the  $W'$  kinetics could not be properly fitted by a simple exponential model, regardless of the  $\tau_W$ , that is used (Fig. 3). Instead, it was demonstrated that the  $W'$  recovery following exhaustive exercise is better fitted by a two-phase exponential model that accounts for a fast and a slow recovery phase. The superiority of a bi-exponential over a mono-exponential model was reflected by differences in the RMSE (1.7% vs. 6.4%) and by the positive  $\Delta AIC_c$  score. As we described in the data analysis, one-phase and two-phase exponential modelling was performed twice, once where the amplitude was left free to be fitted by the model and once where the amplitude was fixed to the theoretically assumed  $W'$  recovery of 100%. A better model

fit was acquired when the amplitude was not defined beforehand due to the fact that  $W'_{\text{OBS}}$  exhibited a plateau-like response around ~70 to 75% between the 10th and 15th min. The manifestation of such flattening in the recovery profile might be attributed to an overall higher accumulated fatigue, especially within the type II fibers as they take longer to recover the intracellular pH and regain their initial contractile potential (Achten et al., 1990; Lievens et al., 2020). For comparison, 15 min after exhaustive  $\text{PO}_{6\text{min}}$  exercise, Ferguson et al. (2010) and Felipe et al. (2020) reported, respectively ~86% and 66% of  $W'$  recovery. Whereas the higher recovery in the study of Ferguson et al. (2010) can result from a lower recovery intensity (i.e., 20 W), the low values of Felipe et al. (2020) following passive recovery are surprising. As suggested by the authors, this shortfall could be attributed to a lower level of physical fitness. Our findings support this hypothesis by demonstrating a strong association between  $\dot{V}\text{O}_{2\text{peak}}$  and  $W'$  recovery, especially at longer recovery durations (Fig. 6). Although a flattening in the  $W'$  response was visible at the group mean level, our statistical model suggests that participants with a high  $\dot{V}\text{O}_{2\text{peak}}$  fully recovered within 15 min, while participants with a low  $\dot{V}\text{O}_{2\text{peak}}$  recovered only half within the same duration (Fig. 6). In the latter case, model estimates even showed a decline in  $W'$  from the 10th minute of recovery, suggesting that some participants would not have recovered any further if they had to cycle beyond 15 min.

In theory, the total energy supply during a  $\text{PO}_{4\text{min}}$  work bout until exhaustion (i.e., WB1) consists of 21% anaerobic and  $79 \pm 10\%$  aerobic energy contribution (Gastin, 2001). According to the CP model, the anaerobic energy contribution during WB1 in the present study was  $23.1 \pm 5.3\%$  and thus, matched the theoretical expectation. Because the proportionality of the work performed above CP and the total work done is fixed, the CP model presumes that the anaerobic contribution of 23.1% in WB1 is retained in WB2. However, it is likely that differences in the  $\dot{V}\text{O}_2$  kinetics between WB1 and WB2 will have induced changes in the energetic contribution at the onset of exercise. Quantification of the  $\text{O}_2$  deficit relative to the end- $\dot{V}\text{O}_2$  for both work bouts confirmed this by showing that the  $\text{O}_2$  deficit of WB2 was consistently lower than WB1, exhibiting the lowest values for the shortest recovery conditions (Fig. 4A). These findings indicate that at the beginning of WB2, the fractional aerobic contribution to the total energy turnover was higher compared to WB1. To investigate whether this aerobic advantage would have potentially influenced the  $W'$  recovery kinetics, we corrected the total work of WB2 for the estimated additional mechanical work resulting from this lower  $\text{O}_2$  deficit, and we used the adjusted total work to recalculate the  $W'$  recovery ( $W'_{\text{ADJ}}$ ). We found that the aerobic advantage during WB2 ranged between ~5 and 9 kJ and was more pronounced at shorter recovery durations (Fig. 4B). Although the percentual difference between  $W'_{\text{OBS}}$  and  $W'_{\text{ADJ}}$  was fairly consistent across conditions (~11%), in relative terms, the enhanced aerobic contribution counted for almost 50% of the performance during WB2 in the 30-s recovery condition (Fig. 4C). It has to be noted that the quantification of  $W'_{\text{ADJ}}$  in the

present study is an estimation and that changes in the aerobic energy provision could slightly deviate from our calculations. In any case, contrary to its theoretical definition, it is important to understand that  $W'$  always contains a specific portion of energy that is aerobic in origin. Given the dynamic balance of aerobic and anaerobic energy provision during intermittent exercise, it is however difficult to distinguish the extent to which each contributes to  $W'$ . Considering the complex nature of  $W'$ , it remains therefore important, at least from a practical perspective, to appreciate  $W'$  as the resultant of all physiological processes that contribute to the work capacity above CP ( $\sim W'_{\text{OBS}}$ ).

Although  $\Delta\text{AIC}_c$  did no longer retain the bi-exponential model as the preferred model for  $W'_{\text{ADJ}}$ , RMSE associated with the bi-exponential model was slightly lower compared to the mono-exponential model (RMSE = 2.1% vs. 3.5%). A closer look at the data revealed that especially at the shortest recovery durations (i.e., 30 and 60 s) the mono-exponential model tends to underestimate the recovery ( $\Delta = \sim 4$  to 6%), even when the changes in the  $\dot{V}\text{O}_2$  kinetics have been accounted for (Fig. 4C). These deviations suggest that the bi-exponential response of  $W'_{\text{OBS}}$  is not necessarily an artefact of the changing  $\dot{V}\text{O}_2$  kinetics, but might also be linked to the restoration of  $W'$  itself. There is longstanding evidence that the recovery of intracellular PCr following high-intensity exercise is biphasic, consisting of an early fast and a second slow component (Harris et al., 1976; Nevill, Jones, McIntyre, Bogdanis, & Nevill, 1997). In accordance with Ferguson et al. (13), we found that the recovery of  $\dot{V}\text{O}_2$ , which was used as a proxy for intramuscular [PCr], recovered overall faster than  $W'_{\text{OBS}}$ . Figure 5 shows that most of the recovery of  $\dot{V}\text{O}_2$ , and thus the recovery of PCr, is completed within the first 2 to 3 min. Therefore, it can be suggested that the fast recovery of PCr is reflected in the first phase of the bi-exponential profile of the total  $W'$  recovery. In summary, the rapid regeneration of the ATP-PCr energy system together with an increased aerobic contribution may provide an explanation for the high  $W'_{\text{OBS}}$  values that were found after only 30 s of recovery. However, since the  $W'$  recovery was lower than the recovery of  $\dot{V}\text{O}_2$  for all durations beyond 30 s (Fig. 5), other and more slowly evolving processes such as the restoration of the acid-base homeostasis (Stringer et al., 1992) will also contribute to the total  $W'$  recovery. In addition, also neuromuscular fatigue will play a role in the ability to repeat high-intensity efforts (Black et al., 2017; Felipe et al., 2020; Schäfer et al., 2019). It is known, for example, that complete recovery of muscle function may take several hours due to prolonged impairment of  $\text{Ca}^{2+}$  release and/or sensitivity, and depending on the intensity and duration of the exercise, more central fatigue will occur as well (Carroll et al., 2017). Therefore, as highlighted by Felipe et al. (2020), it is important to understand that the  $W'$  recovery does not depend exclusively on peripheral or central factors, but rather, on the interplay between both. In the present study, we have examined the recovery of  $W'$  by means of whole-body cycling exercise following exhaustion. Although we approached this recovery from a theoretical perspective (i.e., the CP model), we must understand that the current modelling of  $W'_{\text{OBS}}$  integrates all

physiological processes, both at the central or peripheral level, that may have contributed to the overall performance recovery that we found. In this context, our study is the first to comprehensively model the recovery of whole-body cycling performance after exhaustion.

Although this study demonstrates that the  $W'$  recovery occurs in a bi-exponential way after complete exhaustion, it is still unknown if these kinetics would be similar in case of a partial and not a complete  $W'$  depletion. Finding an answer to this question is highly relevant since  $W'$  is rarely fully depleted during training and/or races in practice. In our opinion, it is doubtful that a partial  $W'$  depletion would evoke an equally large and steep fast recovery component. Instead, it seems more plausible that the expression of the fast recovery phase is dependent on the degree of  $W'$  depletion, as it was suggested before by Skiba et al. (2015). This would of course further complicate the final modelling of the  $W'$  recovery and advocates the use of a variable time constant based on the instantaneous size of  $W'$ .

### Individual characteristics

Besides the need for general modifications to the model (e.g., taking into account work bout characteristics and accumulated fatigue), the ability to recover may be very much dependent on individual characteristics of the athlete. This is clearly demonstrated by the appearance of a large interindividual variability in the  $W'$  recovery profile in the current study (i.e., CV% = 26.9%). Although it is difficult to unravel all of the underlying factors explaining this variability, we hypothesized that aerobic fitness and MFT distribution would markedly influence the rate of  $W'$  recovery. As expected, participants with a higher  $\dot{V}O_{2peak}$  demonstrated a higher overall  $W'_{OBS}$  ( $r = 0.62$ ) and also CP was positively associated with  $W'_{OBS}$  ( $r = 0.57$ ). As visualized in Fig. 6, differences in  $\dot{V}O_{2peak}$  could lead to changes in the  $W'$  recovery up to 50% at the longest recovery durations. The observed relationship between  $W'$  recovery and key parameters of aerobic fitness such as  $\dot{V}O_{2peak}$  and CP is in agreement with recent findings of Chorley et al. (2020). However, while these authors only found a significant association in trained cyclists, we now confirmed such a relationship in a group of non-cyclists. The mechanisms underlying the association of aerobic fitness level and acute recovery from high-intensity exercise may be related to an increased aerobic response and an enhanced PCr recovery (Tomlin & Wenger, 2001).

Since the muscle's oxidative capacity is determined by the MFT composition, the amount of type I or type II fibers may directly impact these factors and thus, may be a key determinant of the  $W'$  recovery. Skiba et al. (2012) reasoned that both type I and type II fiber pools might have different time constants that contribute to the total  $W'$  recovery. However, MFT distribution was not found to be a significant predictor of the  $W'$  recovery in the present study. Also, we did not find a significant relationship between MFT distribution and CP or  $W'$ , although the association between % type I fibers and CP just missed statistical significance ( $r = 0.45$ ,  $P = 0.053$ ). This association has already been demonstrated by Vanhatalo

et al. (2016) and Mitchell et al. (2018). Potential effects of MFT distribution may have been overshadowed by the large variability in  $\dot{V}O_{2\text{peak}}$  among our participants (ranging from ~45 to 67 mL·min<sup>-1</sup>·kg<sup>-1</sup>), which in turn already exerted a strong influence on the  $W'$  recovery, or by insufficient heterogeneity in MFT composition within our subject sample. Although not reported in the result section, the presence of some significant associations at distinct recovery durations between % type I fibers and  $W'_{\text{OBS}}$  ( $r = 0.46$  to  $0.58$ ), and between % type IIX fibers and  $W'_{\text{OBS}}$  ( $r = -0.48$  to  $-0.58$ ) still suggests the possible influence of MFT distribution on the rate of  $W'$  recovery. This is further supported by evidence showing that  $\dot{V}O_{2\text{peak}}$  is closely related to muscle fiber oxidative capacity in a wide variety of populations, from patients to professional cyclists (van der Zwaard et al., 2016). As we found a close relationship between  $\dot{V}O_{2\text{peak}}$  and  $W'$  recovery in the present study, these data make it sound contradictory to state that the  $W'$  recovery would not depend on MFT distribution.

Although the current study showed that individual characteristics can have an important impact on the  $W'$  recovery, studies that would control and manipulate each of these variables separately are required to gain more insight into their specific contribution to the total  $W'$  recovery. In this context, future studies should also account for other related factors (e.g., training status and anthropometrics).

## Conclusion

This study is the first to model the  $W'$  recovery following exhaustive exercise over a broad range of recovery durations. It was found that the  $W'$  recovery is best described by a two-phase rather than a one-phase exponential time course, exhibiting a fast initial and a second slow recovery component. Our results suggested that the higher than expected recovery at short durations is attributable to both a higher aerobic energy provision resulting from changes in the onset  $\dot{V}O_2$  kinetics during intermittent exercise, and the fast regeneration of the ATP-PCr energy system, as reflected by the  $\dot{V}O_2$  recovery. Furthermore, it was demonstrated that the  $W'$  recovery is highly influenced by aerobic fitness.

## ADDITIONAL INFORMATION

**Author contributions:** KC, GB, JGB and JB conceived and designed research. KC, GB, LB, KV, EL and LP conducted the experiments. KC, GB, CD, EL, JV, WD, JGB and JB analyzed the data. KC, GB and JB wrote the manuscript. All authors revised and approved the manuscript.

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**Ethics approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethical committee of the Ghent University Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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# ***STUDY 4***

## **Critical power, $W'$ and $W'$ reconstitution in women and men**

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## ABSTRACT

The aim of this study was to compare critical power (CP) and work capacity  $W'$ , and  $W'$  reconstitution ( $W'_{\text{REC}}$ ) following repeated maximal exercise between women and men. Twelve women ( $\dot{V}O_{2\text{PEAK}}$ :  $2.53 \pm 0.37 \text{ L}\cdot\text{min}^{-1}$ ) and twelve men ( $\dot{V}O_{2\text{PEAK}}$ :  $4.26 \pm 0.30 \text{ L}\cdot\text{min}^{-1}$ ) performed a minimum of 3 constant workload tests, to determine CP and  $W'$ , and 1 maximal exercise repetition test with three work bouts (WB) to failure, to quantify  $W'_{\text{REC}}$  during 2 recovery periods, i.e.,  $W'_{\text{REC1}}$  and  $W'_{\text{REC2}}$ . An independent samples t test was used to compare CP and  $W'$  values between women and men, and a repeated-measures ANOVA was used to compare  $W'_{\text{REC}}$  as fraction of  $W'$  expended during the first WB, absolute  $W'_{\text{REC}}$ , and normalized to lean body mass (LBM). CP normalized to LBM was not different between women and men, respectively  $3.7 \pm 0.5$  vs.  $4.1 \pm 0.4 \text{ W}\cdot\text{kg}_{\text{LBM}}^{-1}$ , while  $W'$  normalized to LBM was lower in women  $256 \pm 29$  vs.  $305 \pm 45 \text{ J}\cdot\text{kg}_{\text{LBM}}^{-1}$ . Fractional  $W'_{\text{REC1}}$  was higher in women than in men, respectively  $74.0 \pm 12.0\%$  vs.  $56.8 \pm 9.5\%$ . Women reconstituted less  $W'$  than men in absolute terms ( $8.7 \pm 1.2$  vs.  $10.9 \pm 2.0 \text{ kJ}$ ) during  $W'_{\text{REC1}}$ , while normalized to LBM no difference was observed between women and men ( $174 \pm 23$  vs.  $167 \pm 31 \text{ J}\cdot\text{kg}_{\text{LBM}}^{-1}$ ).  $W'_{\text{REC2}}$  was lower than  $W'_{\text{REC1}}$  both in women and men. Sex differences in  $W'_{\text{REC}}$  (absolute women < men; fractional women > men) are eliminated when LBM is accounted for. Prediction models of  $W'_{\text{REC}}$  might benefit from including LBM as a biological variable in the equation. This study confirms the occurrence of a slowing of  $W'_{\text{REC}}$  during repeated maximal exercise.

## KEY WORDS

Body composition; maximal exercise; high-intensity; recovery; sex

## INTRODUCTION

In the past few decades the critical power (CP) concept has frequently been used in a sport scientific context in function of performance diagnostics, exercise prescription, training guidance and performance prediction (Vanhatalo et al., 2011). This concept quantifies, following a set of exhaustive efforts, the hyperbolic relationship between power output (PO) and time to task failure (TTF) by means of two parameters: (1) CP, the asymptote to this relationship, which from a physiological point of view is seen as the maximal metabolic steady state intensity, and (2) the curvature constant of this relationship ( $W'$ ), which corresponds to a finite energy reserve or work capacity above CP (Burnley & Jones, 2018; Jones et al., 2019; Poole et al., 2016).

Since many sports involve frequent changes in exercise intensity, the CP concept has also been applied to intermittent exercise (Morton & Billat, 2004), in which exercise above CP ( $W'$  depletion) is alternated with exercise below CP ( $W'$  reconstitution) (Chidnok et al., 2012). The development of the  $W'$  balance ( $W'_{BAL}$ ) model (Skiba et al., 2012) was a breakthrough for providing insight into the available  $W'$  at any moment in time during intermittent exercise. As recently suggested by Skiba & Clarke (2021), the  $W'_{BAL}$  model is not final and needs further refinement based on current insights; i.e., individualization (Bartram et al., 2018; Caen et al., 2021; Chorley et al., 2020), adequate  $W'$  recovery kinetics (Bartram et al., 2018; Caen et al., 2021; Chorley et al., 2022) and exercise characteristics (i.e., duration and intensity) of both work ( $> CP$ ) and recovery ( $< CP$ ) bouts (Caen et al., 2021; 2019; Chorley et al., 2019; 2022; Lievens et al., 2021).

Scientific progress on this topic in physically active and trained individuals, as well as elite athletes, is mainly based on research studies with male participants. Despite the significant differences in anthropometrics, body composition and physiological characteristics between men and women (Ansdell et al., 2020; Besson et al., 2022; Hunter, 2016; Santisteban et al., 2022), the use of the CP concept in a female population is under-studied and has yet to be comprehensively examined. Providing normative data in female athletes and including sex as a biological variable in research studies concerning CP and  $W'$  and its reconstitution could be beneficial for monitoring the training process in sports and rehabilitation (Ansdell et al., 2020). Numerous studies have already described the CP concept (see reviews of Chorley & Lamb, 2020; Dotan, 2022; Lipková et al., 2022), however, to our knowledge, only a few studies have provided information regarding CP and  $W'$  in women (Ansdell et al., 2020; Bishop et al., 1998; James et al., 2022; Sundberg et al., 2017), showing a lower CP and  $W'$  (absolute and normalized to body mass) compared with men. In some other studies, women constitute only a small part of the sample groups. Out of the 19 studies of  $W'$  reconstitution ( $W'_{REC}$ ) in the review of Chorley & Lamb (2020), only four studies included women (13 out of 49 were female participants), yet no sex-based comparison was made. It has been speculated that women may reconstitute  $W'$  faster than men, as indicated by

better retainment of muscle tissue oxygenation in women during one exercise bout to task failure with subsequent recovery phase (Ansdell et al., 2019). However, Ansdell et al. (2019) could not directly demonstrate a difference in  $W'$  reconstitution between women and men, as a second bout to task failure was missing. Scientific data concerning CP,  $W'$  and  $W'_{\text{REC}}$  in women is scarce, and therefore the applicability of current  $W'_{\text{REC}}$  models in a female population is questionable.

The aim of this study was to compare CP,  $W'$  and  $W'_{\text{REC}}$  following repeated maximal exercise between women and men, and to explore their relationship with anthropometrics, body composition and physiological characteristics. We hypothesized that CP and  $W'$  would be lower in women (absolute and normalized to body mass and lean body mass). Furthermore, we hypothesized that fractional  $W'_{\text{REC}}$  (i.e.,  $W'$  reconstitution normalized to the individual's maximal value of  $W'$ ) would occur faster in women as compared with men, although absolute  $W'_{\text{REC}}$  (i.e., in kJ) would be larger in men.

## METHODS

### Ethical approval

All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards, apart from registration in a database. Ethical approval was obtained from the ethical committee of the Ghent University Hospital (Ghent, Belgium) (reference: B6702020000260). After receiving a description of the experimental procedure, all participants provided written informed consent to participate in this study as volunteers.

### Participants

Volunteers of both sexes were recruited through local advertisement. Twelve women and 12 men were selected to take part in this study. A medical history questionnaire was completed and training status of the volunteers was self-reported, including the type, duration and frequency of training. To obtain a representative sample of a physically active population, inclusion criterion were set for age (18-36 years), training status (i.e.,  $\geq 4$  hours/week,  $\geq 2$  times/week and  $\geq 2$  years) and physical fitness level (i.e., during ramp incremental exercise test achieve a peak oxygen uptake ( $\dot{V}O_{2PEAK}$ )  $\geq 50^{\text{th}}$  percentile according to the guidelines for exercise testing and prescription by the American College of Sports Medicine (American College of Sports Medicine, 2021). Exclusion criteria were competitive cycling, chronic diseases, (lower limb) injury, smokers and a negative advise from a medical doctor related to pulmonary and cardiovascular anomalies. Female participants declared that they were not pregnant at the start of the study, and were not planning to become pregnant during the study. Two of them had a hormonal intrauterine device (IUD), seven were using combined oral contraceptive pills and three were not using any contraceptives.

### General study design

All participants visited the lab five to seven times over a period of 4 weeks, with a minimum of 48 h between two tests. The first visit involved the measurement of anthropometric characteristics and a ramp incremental exercise test to assess peak oxygen uptake ( $\dot{V}O_{2PEAK}$ ), peak power output ( $PO_{PEAK}$ ) and the gas exchange threshold (GET). The following three to five tests were performed to determine CP and  $W'$ , and on the last visit, participants performed three maximal exercise repetitions to assess  $W'$  reconstitution. All tests were conducted on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) at the Sport Science Laboratory Jacques Rogge of Ghent University (Ghent, Belgium), at sea level, 18°C and 40% relative humidity, between 2.00 p.m. and 8.00 p.m. Each participant performed all tests at the same time of the day ( $\pm 1$  h) to limit variability due to the circadian rhythm (Winget et al., 1985). Participants were asked to maintain the same type of meals on the day of an exercise test (including carbohydrates with a low glycemic index) and to drink  $\sim 500$  mL

of water over 2 h prior to the beginning of the test. Participants were instructed to refrain from any exhaustive exercise 24 h leading up to an exercise test and to abstain from consuming caffeine and alcohol 24 h prior to testing. During the first test participants were instructed to choose their cadence between 70 and 90 revolutions per minute (rpm), and to maintain their preferred cadence during all upcoming exercise tests. Strong verbal encouragement was provided throughout all exercise tests to ensure maximum effort. All trials to task failure were terminated when the participant was unable to maintain the preferred cadence minus 10 rpm for more than 5 consecutive seconds.

## Measurements and data analysis

### Anthropometric measurement

Body height, body mass, and skinfold measurements were taken following the standard procedures set by the International Society for the Advanced Kinanthropometry (ISAK) (Marfell-Jones et al., 2006). An ISAK level 1 anthropometrist collected the data. Duplicate measurements were obtained from each site, and a third measurement was taken if the technical error of measurement advised by ISAK was exceeded. Eight skinfold thicknesses (triceps, biceps, subscapular, iliac crest, supraspinale, abdominal, anterior thigh and medial calf) were marked and measured using a Harpenden skinfold calliper (Baty International, West Sussex, UK). Body mass index (BMI) was calculated (Quetelet, 1835) and body fat (BF) was estimated by the formulas of Withers and Siri for women and men (Siri, 1961; Withers et al., 1987; Withers et al., 1987). Lean body mass (LBM) was calculated from body mass and fat mass.

### Incremental exercise test

The warm-up consisted of 6 min of cycling at 100 W (men) or 80 W (women), followed by 2 min seated rest and 4 min baseline cycling at 50 W. Subsequently, the work rate increased linearly with  $30 \text{ W} \cdot \text{min}^{-1}$  for male participants and  $20 \text{ W} \cdot \text{min}^{-1}$  for female participants (Bourgois et al., 2022; Caen et al., 2022). These load increments were chosen to obtain a test duration of 8-12 min in our population based on anthropometrics and reported physical exercise (Yoon et al., 2007). Pulmonary gas exchange was registered breath-by-breath using a metabolic cart (Cortex MetaLyzer 3B; Cortex Biophysik, Leipzig, Germany) to determine  $\dot{V}O_{2\text{PEAK}}$  and GET.  $\dot{V}O_{2\text{PEAK}}$  was defined as the highest 30 s average achieved throughout the protocol. Predicted  $\dot{V}O_{2\text{PEAK}}$  was calculated using a reference equation based sex, age and body mass (Myers et al., 2017). Heart rate (HR) was monitored continuously throughout the test (H7 Sensor; Polar, Kempele, Finland).  $PO_{\text{PEAK}}$  and  $HR_{\text{PEAK}}$  were defined as the highest values obtained during the test. GET was determined by three independent researchers based on the V-slope method (Beaver et al., 1986) and secondary gas exchange parameters (Binder et al., 2008). For a more detailed description of the methodology, see the corresponding references. PO at GET was corrected to account for the mean response time (Caen et al., 2020).

### Constant workload tests

To estimate CP and  $W'$ , a minimum of three constant load tests to task failure had to be performed. The tests were preceded by a 5 min warm-up at 50 W, after which PO was immediately switched to the predetermined work rate. The PO for these trials was selected between 65 and 90%  $PO_{PEAK}$ , as obtained from the incremental exercise test, to evoke TTF approximately between 2-5 min for the short trial, 5-10 min for the middle-long trial and 10-20 min for the longest trial. Tests were executed on separate days in a randomized order, and participants did not receive information on elapsed time or PO during the trials. The participants were instructed to increase their cadence with 10 rpm in the final 5 s of the warm-up to facilitate the transition, subsequently going back to the target cadence in 5-10 s. TTF was marked when the participant was unable to maintain the preferred cadence minus 10 rpm for more than 5 consecutive seconds. CP and  $W'$  were determined using three mathematical models: the linear work-time relationship model (1), the linear inverse-of-time model (2) and the hyperbolic model (3).

$$W = W' + CP * t \quad (1)$$

$$P = W' * \left(\frac{1}{t}\right) + CP \quad (2)$$

$$t = \frac{W'}{P - CP} \quad (3)$$

The parameter  $W$  represents the total work performed,  $P$  is a given PO above CP and  $t$  is the time to task failure. Using the best individual fit method, the model with the smallest combined sum of the standard error of the estimates (SEE) for CP (< 5%) and  $W'$  (< 10%) was selected to estimate CP and  $W'$ , in line with the limits proposed by Black et al. (2015). The hyperbolic model was used for 7 female and 6 male participants, the linear inverse-of-time model for 5 women and 6 men. The linear work-time model was never retained as the best fit model. A total of  $3.5 \pm 0.7$  and  $3.4 \pm 0.7$  constant workload tests were executed by women and men, respectively.

### Maximal exercise repetition test

The test began with 5 min seated rest on the bicycle. This was followed by 5 min cycling at 50 W and 5 min at a PO corresponding to 90% of the corrected GET ( $PO_{90\%GET}$ ), to ensure recovery within the same intensity domain for every participant (Lievens et al., 2021). Thereafter, work rate increased to the PO corresponding to a calculated theoretical time to task failure of 4 min (P4). Based on the individual estimation of CP and  $W'$ , P4 was determined using Eqs. (1), (2) and (3), with  $t = 240$  s. This was executed three times to task failure followed by an active recovery of 5 min at  $PO_{90\%GET}$ , respectively three work

bouts (WB1, WB2 and WB3) and three recovery bouts. The test ended with 5 min at 50 W and 5 min seated rest on the bicycle. Transition from rest to start of cycling and an increase in PO were announced 30 s, 10 s prior to the next step and by a 5 s countdown. Prior to WB1, WB2 and WB3 participants were instructed to increase their cadence by 10 rpm to facilitate the transition, subsequently going back to the target cadence in 5-10 s. When the participant was unable to maintain the preferred cadence minus 10 rpm for more than 5 consecutive seconds, TTF was marked and PO was transferred to the recovery intensity. The expended  $W'$  in WB1 and reconstituted  $W'$  in WB2 ( $W'_{\text{REC1}}$ ) and WB3 ( $W'_{\text{REC2}}$ ) were calculated as the work completed above CP.  $W'_{\text{REC}}$  was expressed both in absolute units (kJ) and relative to LBM ( $\text{J} \cdot \text{kg}_{\text{LBM}}^{-1}$ ), as well as a fraction (%) of the expended  $W'$  during WB1. The difference between  $W'_{\text{REC1}}$  and  $W'_{\text{REC2}}$  was defined as  $\Delta W'_{\text{REC}}$ .

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD) for  $n = 12$  female and  $n = 12$  male participants, unless otherwise specified. Data were normally distributed, as assessed by Shapiro-Wilk's test ( $p > 0.05$ ) and homogeneity of variances was assured by the Levene's test of homogeneity of variance ( $p > 0.05$ ). Demographic and anthropometric characteristics, and maximal and submaximal results from the ramp incremental exercise tests were compared between women and men through an independent samples t test. The same analysis was used to find differences between women and men for TTF of the constant workload trials and the estimates of CP and  $W'$  and P4. Cohen's  $d$  effect size was calculated to standardize mean differences. TTF and expended  $W'$  during WB1 were compared with model predicted TTF (i.e., 240 s) and  $W'$  determined from the constant workload tests using a one sample t test. A two-way repeated measures analysis of variance (sex [between-subject factor with two levels: women and man]  $\times$   $W'_{\text{REC}}$  [within-subject factor with two levels:  $W'_{\text{REC1}}$  and  $W'_{\text{REC2}}$ ]) was performed to detect differences in absolute  $W'_{\text{REC}}$ ,  $W'_{\text{REC}}$  normalized to LBM and fractional  $W'_{\text{REC}}$ . A Greenhouse-Geisser correction was applied when the assumption of sphericity had been violated. A one-sample t test was used to compare  $\Delta W'_{\text{REC}}$  to a 0 J  $W'$  reconstitution difference. The Pearson correlation coefficient ( $r$ ) was applied to examine the relationship between CP,  $W'$  and  $W'_{\text{REC}}$  with  $\dot{V}\text{O}_{2\text{PEAK}}$ , GET, body fat and lean body mass. All statistical analyses were performed with SPSS Statistics 25 (IBM Corp., Armonk, NY) Statistical significance was accepted when  $p < 0.05$ .

## RESULTS

### Anthropometrics and incremental exercise test

An overview of demographic data and anthropometric measurements can be found in Table 1, along with the variables of the incremental exercise test. Men had more weekly training hours than women. BMI of women and men was within normal range, stated by the World Health Organization (WHO).  $\dot{V}O_{2PEAK}$  and  $PO_{PEAK}$  were, both expressed in absolute and relative values, higher in men than in women.  $\dot{V}O_2$  and  $PO$  at GET were also higher in men than in women, but when expressed as a percentage of  $\dot{V}O_{2PEAK}$  and  $PO_{PEAK}$  no differences were found between women and men. The duration of the test (i.e., TTF) did not significantly differ between women and men.

**Table 1** Demographics, anthropometrics and results of the incremental exercise test

|  | Women |   |      | Men  |   |      | <i>p</i> -value | <i>ES</i> |
|--|-------|---|------|------|---|------|-----------------|-----------|
| <i>n</i>   | 12    |   |      | 12   |   |      | n/a             | n/a       |
| Demographics   |       |   |      |      |   |      |                 |           |
| Age (years)  | 26.9  | ± | 3.1  | 25.0 | ± | 3.4  | 0.389           | 0.8       |
| Training (h·week <sup>-1</sup> )                               | 5.4   | ± | 0.8  | 7.5  | ± | 1.1  | < 0.001         | 3.1       |
| Anthropometrics  |       |   |      |      |   |      |                 |           |
| Body height (cm)   | 169   | ± | 5    | 182  | ± | 5    | < 0.001         | 3.7       |
| Body mass (kg)   | 61.9  | ± | 5.5  | 74.3 | ± | 9.1  | 0.001           | 2.3       |
| BMI (kg·m <sup>-2</sup> )                                      | 21.6  | ± | 1.1  | 22.5 | ± | 2.5  | n/a             | n/a       |
| BF (%)   | 19.2  | ± | 3.7  | 11.9 | ± | 4.5  | < 0.001         | 2.5       |
| LBM (kg)   | 49.9  | ± | 3.8  | 65.3 | ± | 7.9  | < 0.001         | 3.5       |
| Incremental exercise test                                      |       |   |      |      |   |      |                 |           |
| ̇VO <sub>2PEAK</sub> (L·min <sup>-1</sup> )                    | 2.53  | ± | 0.37 | 4.26 | ± | 0.30 | < 0.001         | 7.3       |
| ̇VO <sub>2PEAK</sub> (ml·min <sup>-1</sup> ·kg <sup>-1</sup> ) | 41.0  | ± | 5.2  | 57.9 | ± | 5.2  | < 0.001         | 4.6       |
| % predicted ̇VO <sub>2PEAK</sub> (%)                           | 106   | ± | 14   | 117  | ± | 7    | 0.022           | 1.4       |
| ̇VE <sub>PEAK</sub> (L·min <sup>-1</sup> )                     | 105   | ± | 13   | 161  | ± | 26   | < 0.001         | 3.9       |
| RER  | 1.31  | ± | 0.04 | 1.22 | ± | 0.07 | 0.001           | 2.2       |
| PO <sub>PEAK</sub> (W)   | 277   | ± | 37   | 406  | ± | 35   | < 0.001         | 5.1       |
| PO <sub>PEAK</sub> (W·kg <sup>-1</sup> )                       | 4.5   | ± | 0.5  | 5.5  | ± | 0.5  | < 0.001         | 2.8       |
| PO <sub>PEAK</sub> (W·kg <sub>LBM</sub> <sup>-1</sup> )        | 5.6   | ± | 0.5  | 6.2  | ± | 0.5  | 0.011           | 1.7       |
| HR <sub>PEAK</sub> (bpm)                                       | 187   | ± | 6    | 190  | ± | 7    | 0.278           | 0.7       |
| RPE  | 20    | ± | 1    | 19   | ± | 1    | 0.813           | 1.4       |
| TTF (s)  | 680   | ± | 110  | 711  | ± | 71   | 0.425           | 0.5       |
| ̇VO <sub>2GET</sub> (L·min <sup>-1</sup> )                     | 1.66  | ± | 0.20 | 2.82 | ± | 0.28 | < 0.001         | 6.7       |
| ̇VO <sub>2GET</sub> (%̇VO <sub>2PEAK</sub> )                   | 65.8  | ± | 5.7  | 66.0 | ± | 4.5  | 0.913           | 0.1       |
| PO <sub>GET</sub> (W)  | 124   | ± | 19   | 193  | ± | 27   | < 0.001         | 4.2       |
| PO <sub>GET</sub> (%PO <sub>PEAK</sub> )                       | 44.9  | ± | 2.9  | 47.3 | ± | 3.5  | 0.080           | 1.1       |

Group mean ± SD of demographical data, anthropometric measurements and variables of the incremental exercise test in women and men are presented along with the result of the independent samples t test comparison and Cohen's *d* effect size. Significance was accepted when *p* < 0.05. *ES*, effect size; BMI, body mass index; BF, body fat; LBM, lean body mass;  $\dot{V}O_2$ , pulmonary oxygen uptake;  $\dot{V}E$ , pulmonary ventilation; RER, respiratory exchange ratio;  $PO$ , power output; HR, heart rate;  $[BLa^-]$ , blood lactate concentration; RPE, rate of perceived exertion; TTF, time to task failure; GET, gas exchange threshold.

## Critical power concept

Individually determined values of CP and  $W'$  (absolute and normalized to body mass) were lower in women compared with men (Table 2). Normalized to LBM, CP was not different, while  $W'$  was larger in men. CP was located at the same % $PO_{PEAK}$ , determined in the incremental exercise test, in women and men. The mean standard error (SE) for CP and  $W'$  estimates did not differ between men and women. Duration of the constant workload tests (i.e., time to task failure) did not differ between women and men. The short trial lasted  $204 \pm 19$  s ( $249 \pm 33$  W) for women and  $202 \pm 20$  s ( $365 \pm 32$  W) for men, the middle-long trial was  $442 \pm 142$  s ( $221 \pm 36$  W) for women and  $419 \pm 140$  s ( $320 \pm 29$  W) for men, and the long trial  $926 \pm 205$  s ( $201 \pm 30$  W) for women and  $867 \pm 153$  s ( $289 \pm 29$  W) for men. CP (in W) was correlated with  $\dot{V}O_{2PEAK}$  (absolute and relative to body mass) and LBM in women. In men, CP (in W) was only correlated with absolute  $\dot{V}O_{2PEAK}$ .  $W'$  did not have significant correlations with aerobic fitness or body composition parameters in women and men (see correlation matrix Table 4).

**Table 2** Critical power and  $W'$  values using best individual fit method

|   | Women          | Men            | <i>p</i> -value | <i>ES</i> |
|---|----------------|----------------|-----------------|-----------|
| <i>n</i>                                  | 12             | 12             | n/a             | n/a       |
| CP (W)                                    | 187 $\pm$ 30   | 267 $\pm$ 29   | < 0.001         | 3.8       |
| CP (W·kg <sup>-1</sup> )                  | 3.0 $\pm$ 0.4  | 3.6 $\pm$ 0.4  | 0.002           | 2.1       |
| CP (W·kg <sub>LBM</sub> <sup>-1</sup> )   | 3.7 $\pm$ 0.5  | 4.1 $\pm$ 0.4  | 0.062           | 1.2       |
| CP (% $PO_{PEAK}$ )                       | 68 $\pm$ 3     | 66 $\pm$ 3     | 0.217           | 0.9       |
| <br>                                      |                |                |                 |           |
| $W'$ (kJ)                                 | 12.8 $\pm$ 1.6 | 20.0 $\pm$ 3.1 | < 0.001         | 4.1       |
| $W'$ (J·kg <sup>-1</sup> )                | 208 $\pm$ 25   | 270 $\pm$ 39   | < 0.001         | 2.7       |
| $W'$ (J·kg <sub>LBM</sub> <sup>-1</sup> ) | 256 $\pm$ 29   | 305 $\pm$ 45   | 0.004           | 1.8       |
| <br>                                      |                |                |                 |           |
| CP SE (%)                                 | 0.8 $\pm$ 0.5  | 0.9 $\pm$ 0.7  | 0.834           | 0.2       |
| $W'$ SE (%)                               | 6.4 $\pm$ 2.7  | 5.3 $\pm$ 2.6  | 0.337           | 0.6       |

Group mean  $\pm$  SD in women and men are presented along with the result of the independent samples *t* test comparison and Cohen's *d* effect size. Significance was accepted when  $p < 0.05$ . *ES*, effect size; *SE*, standard error for the applied model.

## W' reconstitution

There was a significant difference in P4 between women and men ( $p < 0.001$ ), respectively  $240 \pm 32$  W and  $350 \pm 32$  W. The intensity of P4 relative to  $PO_{PEAK}$  did not differ between women and men ( $87 \pm 1\%$  vs.  $86 \pm 1\%$ ;  $p = 0.268$ ), nor relative to CP ( $129 \pm 6\%$  vs.  $132 \pm 6\%$ ;  $p = 0.333$ ).

The experimentally obtained TTF of WB1 did not differ from the prediction based on the constant load tests calculations (i.e., TTF = 240 s) for women ( $226 \pm 32$  s;  $p = 0.165$ ) and men ( $235 \pm 38$  s;  $p = 0.651$ ). Accordingly, the available W' upon the start of WB1 did not differ from W' determined during prior constant load tests in women ( $11.9 \pm 2.3$  kJ vs.  $12.8 \pm 1.6$  kJ;  $p = 0.201$ ) and men ( $19.3 \pm 2.8$  kJ vs.  $20.0 \pm 3.1$  kJ;  $p = 0.433$ ).

No significant sex  $\times$  absolute  $W'_{REC}$  interaction effect was identified ( $p = 0.520$ ). The main effect of sex showed a statistically significant higher  $W'_{REC}$  in men ( $W'_{REC1}$ :  $10.9 \pm 2.0$  kJ;  $W'_{REC2}$ :  $9.8 \pm 1.7$  kJ) compared with women ( $W'_{REC1}$ :  $8.7 \pm 1.2$  kJ;  $W'_{REC2}$ :  $7.2 \pm 1.0$  kJ) ( $p < 0.001$ ). Absolute  $W'_{REC2}$  was lower than  $W'_{REC1}$  in women and men ( $p < 0.001$ ). Furthermore,  $\Delta W'_{REC}$  was not equal to 0 J for women and men ( $p < 0.05$ ), and was not different between women and men ( $p = 0.521$ ) (Table 3).

No significant sex  $\times$   $W'_{REC}$  relative to LBM interaction effect was identified ( $p = 0.155$ ). The main effect of sex showed no difference in  $W'_{REC}$  in women ( $W'_{REC1}$ :  $174 \pm 23$  J $\cdot$ kg $_{LBM}^{-1}$ ;  $W'_{REC2}$ :  $145 \pm 22$  J $\cdot$ kg $_{LBM}^{-1}$ ) compared with men ( $W'_{REC1}$ :  $167 \pm 31$  J $\cdot$ kg $_{LBM}^{-1}$ ;  $W'_{REC2}$ :  $151 \pm 28$  J $\cdot$ kg $_{LBM}^{-1}$ ) ( $p < 0.001$ ).  $W'_{REC2}$  relative to LBM was lower than  $W'_{REC1}$  in women and men ( $p < 0.001$ ) (Table 3).

**Table 3** W' reconstitution following the first ( $W'_{REC1}$ ) and second ( $W'_{REC2}$ ) recovery periods in women and men. Measurements are expressed in absolute values, normalized to lean body mass and as a fraction of W' expended during WB1.

|       | $W'_{REC1}$<br>(kJ) | $W'_{REC1}$<br>(J $\cdot$ kg $_{LBM}^{-1}$ ) | $W'_{REC1}$<br>(%) | $W'_{REC2}$<br>(kJ) | $W'_{REC2}$<br>(J $\cdot$ kg $_{LBM}^{-1}$ ) | $W'_{REC2}$<br>(%) | $\Delta W'_{REC}$<br>(kJ) |
|-------|---------------------|--|--------------------|---------------------|--|--------------------|---------------------------|
| Women | $8.7 \pm 1.2$       | $174 \pm 23$                                 | $74.0 \pm 12.0$    | $7.2 \pm 1.0$ †     | $145 \pm 22$ †                               | $62.0 \pm 12.3$ †  | $1.5 \pm 1.0$ \$          |
| Men   | $10.9 \pm 2.0$ *    | $167 \pm 31$                                 | $56.8 \pm 9.5$ *   | $9.8 \pm 1.7$ *†    | $151 \pm 28$ †                               | $51.3 \pm 8.8$ *†  | $1.1 \pm 1.6$ \$          |

W' expended during WB1 ( $11.9 \pm 2.3$  kJ and  $239 \pm 44$  J $\cdot$ kg $_{LBM}^{-1}$  for women, and  $19.3 \pm 2.8$  kJ and  $297 \pm 43$  J $\cdot$ kg $_{LBM}^{-1}$  for men) was equated with 100%. \* Significantly different from women; † Significantly different from  $W'_{REC1}$ ; \$ Significantly different from 0 kJ ( $p < 0.05$ ).

A significant sex  $\times$  fractional  $W'_{REC}$  interaction effect was found ( $p = 0.047$ ). Post hoc analysis showed that  $W'_{REC1}$  ( $p = 0.001$ ) and  $W'_{REC2}$  ( $p = 0.022$ ) were lower in men than in women, respectively  $56.8 \pm 9.5$  % vs.  $74.0 \pm 12.0$  % , and  $51.3 \pm 8.8$  % vs.  $62.0 \pm 12.3$  %. Furthermore,  $W'_{REC2}$  was lower than  $W'_{REC1}$  for women ( $p < 0.001$ ) and for men ( $p = 0.019$ ).

$W'_{REC1}$  (fractionally and absolute) was not correlated body composition parameters. The only significant relationship was found in men for  $W'_{REC1}$  and  $\Delta W'_{REC}$  with absolute  $W'$  as determined with constant workload trials (see correlation matrix Table 4).

**Table 4** Correlation matrix of physical performance parameters and body composition characteristics with CP,  $W'$ ,  $W'_{REC}$  and  $\Delta W'_{REC}$  for women and men.

|  | CP<br>(W) | $W'$<br>(J) | $W'_{REC1}$<br>(kJ) | $W'_{REC1}$<br>(%) | $\Delta W'_{REC}$<br>(kJ) |
|--|-----------|-------------|---------------------|--------------------|---------------------------|
| <b>Women</b>   |           |             |                     |                    |                           |
| $\dot{V}O_{2PEAK}$ (L·min <sup>-1</sup> )                    | .966 **   | .157        | .377                | .192               | -.081                     |
| $\dot{V}O_{2PEAK}$ (ml·min <sup>-1</sup> ·kg <sup>-1</sup> ) | .706 *    | -.111       | .378                | .505               | -.278                     |
| GET (% $\dot{V}O_{2PEAK}$ )                                  | -.486     | -.288       | -.335               | -.293              | -.158                     |
| CP (W)   |           | .156        | .342                | .063               | -.033                     |
| CP (W·kg <sup>-1</sup> )                                     | .836 **   | -.110       | .364                | .364               | -.232                     |
| $W'$ (J)   | .156      |             | .272                | -.447              | .210                      |
| $W'$ (J·kg <sup>-1</sup> )                                   | -.286     | .741 **     | .203                | -.171              | .052                      |
| BF (kg)  | .103      | .366        | -.170               | -.535              | .213                      |
| LBM (kg)   | .779 **   | .334        | .215                | -.149              | .135                      |
| <b>Men</b>   |           |             |                     |                    |                           |
| $\dot{V}O_{2PEAK}$ (L·min <sup>-1</sup> )                    | .881 **   | .385        | .298                | .021               | .170                      |
| $\dot{V}O_{2PEAK}$ (ml·min <sup>-1</sup> ·kg <sup>-1</sup> ) | .078      | -.416       | -.253               | .188               | -.377                     |
| GET (% $\dot{V}O_{2PEAK}$ )                                  | .168      | .105        | .177                | -.176              | -.138                     |
| CP (W)   |           | .062        | .134                | .097               | -.069                     |
| CP (W·kg <sup>-1</sup> )                                     | .452      | -.502       | -.247               | .222               | -.433                     |
| $W'$ (J)   | .062      |             | .613 *              | .576               | .592 *                    |
| $W'$ (J·kg <sup>-1</sup> )                                   | -.346     | .677 *      | .335                | .290               | .072                      |
| BF (kg)  | -.020     | .167        | .121                | -.103              | .389                      |
| LBM (kg)   | .510      | .516        | .365                | -.092              | .328                      |

Correlation matrix with the Pearson correlation coefficient ( $r$ ). Correlation is significant at  $p < 0.05$  (\*) and  $p < 0.001$  (\*\*).  $\dot{V}O_2$ , pulmonary oxygen uptake; *GET*, gas exchange threshold; *LBM*, lean body mass; *BF*, body fat;  $W'_{REC1}$ ;  $W'$  reconstitution following the first recovery period;  $\Delta W'_{REC}$ ; difference between  $W'_{REC1}$  and  $W'_{REC2}$ .

## DISCUSSION

The aim of this study was to compare CP,  $W'$  and  $W'_{\text{REC}}$  following repeated maximal exercise at the same relative intensity normalized to CP between women and men. We found that CP and  $W'$  were lower in women than in men, for absolute values and normalized to body mass.  $W'$ , normalized to lean body mass, was lower in women compared with men. To our knowledge, this study is the first to demonstrate that the fractional reconstitution of  $W'$  (i.e., normalized to the expended  $W'$  during WB1) occurs faster in women, although absolute  $W'_{\text{REC}}$  (i.e., in kJ) was larger in men (Table 3).

### Critical power concept

Only a limited number of studies already examined CP and  $W'$  in women (Ansdell et al., 2020; Bishop et al., 1998; James et al., 2022; Sundberg et al., 2017). In absolute terms, a difference between women and men was observed. The observation of a higher CP and  $W'$  normalized to body mass is reasonable and similar to the findings of Ansdell et al. (2020) and James et al. (2022), as sex differences in body composition are evident (Kirchengast, 2010). This was marked by the higher percentage of body fat in women compared with men in the present study ( $19.2 \pm 3.7$  vs.  $11.9 \pm 4.5$  %). However, even when normalized to lean body mass,  $W'$  was still higher in men. Our results are in accordance with the study of James et al. (2022), where  $W'$  was normalized to lean leg mass using DXA. Furthermore, it has been shown that  $W'$  is related to thigh muscle size (Miura et al., 2002), thigh volume (Kordi et al., 2018) and thigh lean mass (Byrd et al., 2018). A possible explanation for the higher values in men could be found in muscle characteristics. Typically, men have a greater glycolytic capacity which enables a higher production of work during exercise in the severe intensity domain (Hunter, 2016; Komi & Karlson, 1978). However, it is also possible that differences in training status and/or fitness level between our male and female test group could attribute, at least in part, to the higher CP and  $W'$  normalized to (lean) body mass. Men had more weekly training hours and percentage of predicted  $\dot{V}O_{2\text{PEAK}}$ , according to the equation of Myers et al. (2017), was higher compared with women. According to the guidelines for exercise testing and prescription by the American College of Sports Medicine (American College of Sports Medicine, 2021), our male sample group was in the 80<sup>th</sup> percentile for  $\dot{V}O_{2\text{PEAK}}$  and 70<sup>th</sup> percentile for % BF, while the female group was in the 60<sup>th</sup> percentile for both.

### $W'$ reconstitution

To our knowledge, we are the first to describe the reconstitution of  $W'$  in women compared with men. We demonstrated that fractional  $W'_{\text{REC}}$  following repeated bouts of maximal exercise occurs faster in women than in men. In our study fractional  $W'_{\text{REC}}$  was approximately 17% ( $W'_{\text{REC1}}$ ) and 11% ( $W'_{\text{REC2}}$ ) higher in women than in men. On the contrary, men reconstituted more  $W'$  when expressed in absolute values, respectively 2.2 kJ ( $W'_{\text{REC1}}$ ) and 2.6 kJ ( $W'_{\text{REC2}}$ ) (Figure 1).  $W'_{\text{REC}}$  as a fraction of total  $W'$  is a

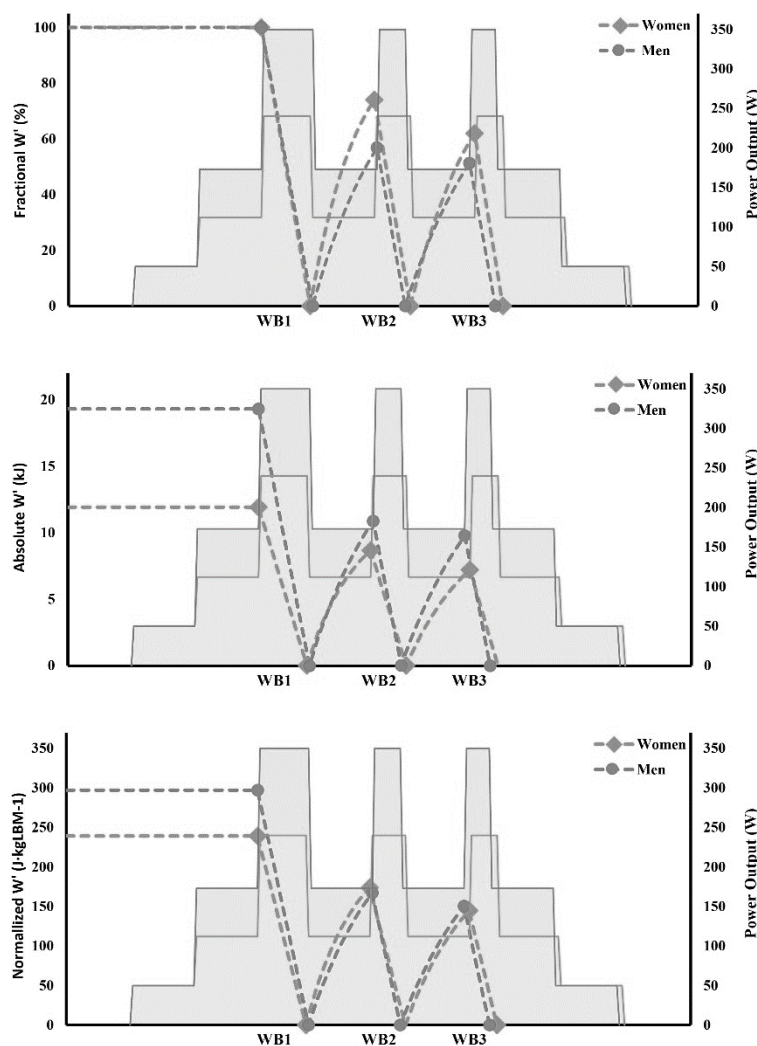
common way in research studies on  $W'$  reconstitution to express the recovery of the individual performance capacity. Although the fractional recovery was higher in women compared with men, the intensity of the bouts of maximal exercise relative to the individual CP was not different between women and men ( $129 \pm 6\%$  vs.  $132 \pm 6\%$ ), nor was the recovery intensity different (i.e., 90% of the individual GET). This recovery intensity was expressed relative to GET, and not CP (as in the model of Skiba et al., 2012), as it has been shown that the reconstitution rate is intensity domain-specific (Lievens et al., 2021).

A possible explanation for the higher fractional  $W'_{\text{REC}}$  in women might be related to the larger proportional area of muscle fiber type I in the vastus lateralis muscles in women (Hunter, 2016). A greater oxidative capacity in this type of muscle, especially the primary locomotor muscle during cycling, i.e., vastus lateralis, implies that the female muscle is more appropriate for resynthesizing ATP from oxidative phosphorylation (Ansdell, et al., 2020). On the contrary, the greater glycolytic capacity in men can result in higher metabolic muscle acidosis during exercise in the severe intensity domain, which might affect  $W'_{\text{REC}}$  (Hunter, 2016). Furthermore, it has been speculated, based on a greater maintenance of  $O_2$  availability within the working muscle, that women would reconstitute  $W'$  faster than men (Ansdell et al., 2019). The faster recovery of  $W'$  in women is consistent with the findings of Hottenrott et al. (2021), who showed that women have a smaller decline in average PO between the first and last 30 s high-intensity all-out cycling exercise and a faster metabolic recovery as compared with men. Our findings are interesting since none of the existing prediction models to quantify  $W'_{\text{REC}}$  account for the difference in recovery between women and men. According to the  $W'_{\text{BAL}}$  model of Skiba et al. (2012),  $W'$  recovery in our subject group would be  $40.7 \pm 2.5\%$  and  $43.1 \pm 1.9\%$ , respectively in women and men. These values are far below the observed reconstitution in our study, in women ( $W'_{\text{REC1}}$ :  $74 \pm 12\%$ ) and men ( $W'_{\text{REC1}}$ :  $57 \pm 10\%$ ).

It should be pointed out that absolute  $W'_{\text{REC}}$  was higher in men compared with women. When the reconstituted  $W'$  was normalized to LBM, this difference disappeared (see Table 3), suggesting a prominent role of LBM in both  $W'$  expenditure and reconstitution. This could be well understood by envisioning the human bioenergetic system as a hydraulic model (Morton, 2006). In such a model, the anaerobic energy system is presented by a vessel of limited capacity (i.e.,  $W'$ ) that gets emptied when PO exceeds CP. During recovery (when  $PO < CP$ ), in this study at the point of task failure, this vessel is refilled by a limited flow (i.e., CP) coming from a second, aerobic energy vessel. Imagine two different athletes, one of whom has a anaerobic vessel that is twice the size of the other. If both athletes have a similar CP (normalized to LBM), and thus a similar flow rate to refill the anaerobic vessel, the same amount of fluid in one athlete will be relatively expressed only half as much as in the other. However, such a model with a clear distinction between aerobic and anaerobic metabolic pathways might be too

simplistic, as  $W'$  cannot be considered purely as an anaerobic work capacity. It has been shown that  $W'$  is related to the oxidative metabolism, more specifically, oxygen availability (Vanhatalo et al., 2010), speed of  $\dot{V}O_2$  kinetics and  $\dot{V}O_2$  slow component (Murgatroyd et al., 2011).

The protocol design in this study, including three successive bouts of  $W'$  depletion and recovery, enabled us to study the apparent slowing effect of  $W'$  recovery over time. We found that  $\Delta W'_{\text{REC}}$  was not equal to 0 Joules (i.e.,  $W'_{\text{REC}2}$  was lower than  $W'_{\text{REC}1}$ ) and not different between men and women, denoting the decreased recovery ability following repeated maximal exercise (visual noticeable in Figure 1).



**Figure 1** Visual representation of the maximal exercise repetition test with the estimated reconstitution of  $W'$  in women (orange) and men (blue). Note that this graph does account for the difference in TTF in WB1-3 between women and men, resulting in a varying width of the WBs in women and men (x-axis). Estimated values for  $W'$  (dashed lines) on the left y-axis and power output (full lines) on the right y-axis are the means of the groups. Fractional  $W'_{\text{REC}}$  (%  $W'$  expended during WB1) is displayed in the upper panel, absolute  $W'_{\text{REC}}$  (kJ) in the middle panel and absolute  $W'_{\text{REC}}$  normalized to lean body mass ( $\text{J} \cdot \text{kgLBM}^{-1}$ ) in the lower panel.

Furthermore, we did not find an inverse relationship between  $\Delta W'_{\text{REC}}$  and  $\dot{V}O_{2\text{PEAK}}$ , which is in line with what previously has been shown by Chorley et al. (2020) in an untrained group. Although, such a correlation was found in a trained endurance group (Chorley et al., 2020). This slowing of  $W'_{\text{REC}}$  was first investigated by Chorley et al. (2019) in trained and untrained male subjects, although using a different protocol than in our study to deplete (i.e., ramp increase to task failure above CP) and reconstitute (i.e.,

recovery intensity of 50 W)  $W'$ . This slowing of  $W'_{\text{REC}}$  has also not been taken into account in the  $W'_{\text{BAL}}$  model, as this model predicts a similar reconstitution rates during repeated maximal exercise with a similar recovery intensity and duration. It has been suggested that the reduced  $W'_{\text{REC}}$  during repeated maximal exercise is related to the incomplete resynthesis of phosphocreatine and, even more, the accumulation of fatigue-related metabolites, which results in disturbance of the intramuscular homeostasis and might diminish the capacity to tolerate repeated high-intensity exercise (Chidnok et al., 2013). Additionally, also neuromuscular fatigue will play a role in the (in)ability to repeat high-intensity efforts (Black et al., 2017). The importance of the aerobic component in  $W'$  reconstitution has been demonstrated by good correlations between indices of aerobic fitness (i.e.,  $\dot{V}O_{2\text{PEAK}}$  or CP) and  $W'_{\text{REC}}$  (Caen et al., 2021) in a male population. Furthermore, Chorley et al. (2020) reported similar findings in endurance-trained men, but not in untrained counterparts. Likewise in our study with physically active, but not endurance-trained participants, we did not find a relationship between  $\dot{V}O_{2\text{PEAK}}$  with  $W'_{\text{REC}}$ . Remarkably, despite our women having lower absolute and predicted  $\dot{V}O_{2\text{PEAK}}$  than men, fractional  $W'_{\text{REC}}$  was higher.

## Limitations

While the aim of the current investigation was to compare CP,  $W'$  and  $W'_{\text{REC}}$  between women and men, some limitations should be noted, so that future research should be able to address these issues. First, menstrual cycle phase was not accounted for in the planning of the experimental protocol in our study. Although the physiological responses to exercise performance can slightly differ across the menstrual cycle (McNulty et al., 2020), it has been shown that the power-duration relationship did not vary across the menstrual cycle in eumenorrheic women (James et al., 2023). However, in this latter study the effect on  $W'_{\text{REC}}$  was not addressed. Second, skinfolds were used to estimate LBM as a whole-body parameter, instead of dual energy X-ray absorptiometry (DXA) to determine (leg) lean mass (Shepherd et al., 2017) for the normalization of CP,  $W'$  and  $W'_{\text{REC}}$  in women and men. Also leg isokinetic dynamometry could be used to express CP,  $W'$  and  $W'_{\text{REC}}$  relative to leg peak power. Third, the training and fitness status of the participants in our study was somewhat higher in men compared with women, as seen in the training hours, percentage of predicted  $\dot{V}O_{2\text{PEAK}}$  and group percentiles of  $\dot{V}O_{2\text{PEAK}}$  and % body fat, resulting in groups that were not perfectly matched. Furthermore, it might be that body composition and physical fitness characteristics of the participants within our female and male test group were too homogeneous and the sample size too small to make appropriate correlations. Lastly, related to the estimation of CP and  $W'$ , we found that, in line with the proposed cut-off criteria of SEE for CP (< 5%) and  $W'$  (< 10%) by Black et al. (2015), CP SE% was 0.9% and 0.8%, respectively in women and men, and  $W'$  SE% was 5.3% in women and 6.4% in men. However, it should be noted that the SEE, especially regarding  $W'$ , is arbitrary and large, and thus caution is warranted concerning accuracy of the model. Subsequently, the

inclusion of a non-oxidative capacity parameter would be interesting to compare with the measured  $W'$  and relate with the  $W'_{\text{REC}}$ . As we provided and analyzed a measure of oxidative capacity ( $\dot{V}O_{2\text{PEAK}}$ ), next to CP, with  $W'_{\text{REC}}$ .

## Conclusion

In this study sex differences were evident in the absolute values of CP and  $W'$ . However,  $W'$ , but not CP, normalized to LBM was found to be greater in men than in women. This study demonstrated that, although fractional  $W'_{\text{REC}}$  was higher in women than in men and absolute  $W'_{\text{REC}}$  was greater in men than in women,  $W'_{\text{REC}}$  normalized to LBM was similar between the sexes. Thus, prediction models of  $W'_{\text{REC}}$  might benefit from including LBM as a biological variable in the equation. Conducting sex-based research is needed and might help to further elucidate the underpinning mechanisms of  $W'_{\text{REC}}$ . Additionally, this study provides confirmation of a decrease in  $W'_{\text{REC}}$  during repeated maximal constant load exercise. These findings have implications for sports and rehabilitation programs, given the importance of individual-level performance diagnostics, exercise prescription, training guidance, and performance prediction.

## ADDITIONAL INFORMATION

**Author contributions:** GB, PM, JB, JGB and KC conceived and designed research. GB and ALC conducted the experiments. GB, PM, ALC, SP and KC analyzed the data. GB, JB, SP and KC wrote the manuscript. All the authors revised and approved the manuscript.

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**Ethics approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethical committee of the Ghent University Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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## *Chapter 3*

### *General discussion*



### 3.1 Overview

This dissertation focused on two issues: 1) the boundary between the heavy and severe intensity domain, and more specifically how performing exercise tests in the heat affected the assessment of this boundary; and, once this threshold was determined, 2) the pattern of acute recovery from exercise in the severe intensity domain.

If you delve into the sport science literature, there are several “accepted” methods to determine the heavy-severe exercise intensity boundary. This threshold is characterized as the highest load at which a metabolic steady state occurs, i.e. maximal metabolic steady state (MMSS), and separates sustainable from non-sustainable exercise. Two methods are considered as gold standard methodologies to indicate this MMSS, the maximal lactate steady state (MLSS) and critical power (CP). Irrespective of which methodology is believed to be the “true” golden standard, the focus of this PhD project was on the application of the CP during cycling exercise (*study 1*, *study 3* and *study 4*), as we believe that this concept offers a major advantage compared with MLSS: the application towards intermittent exercise with alternating  $W'$  depletion and  $W'$  reconstitution (*study 3* and *study 4*). Yet, there are also threshold concepts that serve as an indirect estimation of the MMSS, i.e. second lactate threshold and the respiratory compensation point determined from STEP or RAMP protocols using capillary blood samples or pulmonary gas exchange, respectively. Considering the popularity of these exercise tests, because of its time efficient and feasible manner, we believed it was appropriate to investigate these threshold concepts as well (*study 2*). We combined the interest in exercise thresholds with the determination in hot environmental conditions (*study 1* and *study 2*), as little is known on the acute effect of heat exposure on these thresholds, and which can be useful for performance diagnostics, prescription and steering of the training process, and monitoring exercise intensity in these circumstances. Furthermore, two studies (*study 1* and *study 4*) also include a female population, as an attempt to close the gap of a structural underrepresentation of women in exercise (thermoregulation) research.

The main results regarding these two research interests will be presented and critically discussed. Practical recommendations for exercise scientists and sports enthusiasts, and future directions of this research domain will be integrated within the respective parts. Finally, a concise overall conclusion of the given work will be formed.

### 3.2 Determination of the heavy-severe exercise intensity boundary in temperate and hot environments

We examined how acute heat exposure affected the determination of exercise thresholds, representing the boundary between heavy and severe exercise intensity, more specifically in *study 1* CP (and *W'*) in men and women, and in *study 2* the respiratory compensation point and second lactate threshold, determined in incremental exercise tests. Several studies already investigated the effect of heat exposure on exercise thresholds and performance. It has extensively been shown that performance in the severe intensity domain (i.e., similar to the test methodology to determine CP) is impaired by means of a reduction in time to task failure (TTF) during constant work rate exercise (Galloway & Maughan, 1997; Girard & Racinais, 2014; Mitchell et al., 2014; Parkin et al., 1999; Saltin et al., 1972), ranging from -19 to -50%, or a progressive decrease in PO during self-paced exercise (Peiffer & Abbiss, 2011; Périard et al., 2011; Racinais et al., 2015; Tatterson et al., 2000; Tucker et al., 2004) ranging from -6% to -16% with heat exposure. Due to the open-loop nature of capacity tests (i.e., CWR test to task failure), such tests are typically more variable than closed-loop tests (i.e., performance test) with a fixed time or distance, and the magnitude of changes observed tends to be larger (Tyler et al., 2016). Furthermore, the reduction in PO induced by heat exposure on the occurrence of the heavy-severe intensity threshold was up to -18% (de Barros et al., 2011; Kuo, Cheng, & Kuo, 2021; Lorenzo, Minson, Babb, & Halliwill, 2011; Maunder, Plews, Merien, & Kilding, 2021; Tyka, Pałka, Tyka, Cisoń, & Szyguła, 2009), although sometimes no effect was found (Kaiser et al., 2021; RCP in our own study 2). An overview of these studies supplemented by our own results is provided in Table 3.

**Table 3** Overview of studies investigating the effect of acute heat exposure on the determination of exercise thresholds.

| Study                   | Ambient temperature | Individuals  | Preheating                              | Exercise protocol    | Threshold  | $\Delta$ threshold occurrence (%) |
|-------------------------|---------------------|--|---|----------------------|--|-----------------------------------|
| Smolander et al. (1986) | 25°C vs. 40°C       | 8 physically active men                              | 30-40 min rest                          | STEP<br>25 W / 4 min | GET<br>OBLA 2.5  | No effect<br>-8.5 %               |
| Tyka et al. (2009)      | 23°C vs. 37°C       | 15 healthy men                                       | Not reported                            | STEP<br>30 W / 3 min | ExpD <sub>max</sub>  | -13 %                             |
| Tyka et al. (2010)      | 23°C vs. 37°C       | 12 physically active men                             | 30 min rest                             | STEP<br>30 W / 3 min | GET  | -11 %                             |
| Lorenzo et al. (2011)   | 13°C vs. 38°C       | 16 men, 3 women<br>Highly trained endurance cyclists | 30 min water immersion<br>34°C vs. 41°C | STEP<br><br>RAMP     | Lactate thresholds i.a.<br>OBLA 4.0<br>Ventilatory thresholds i.a. GET | -12 %<br><br>-17 %                |

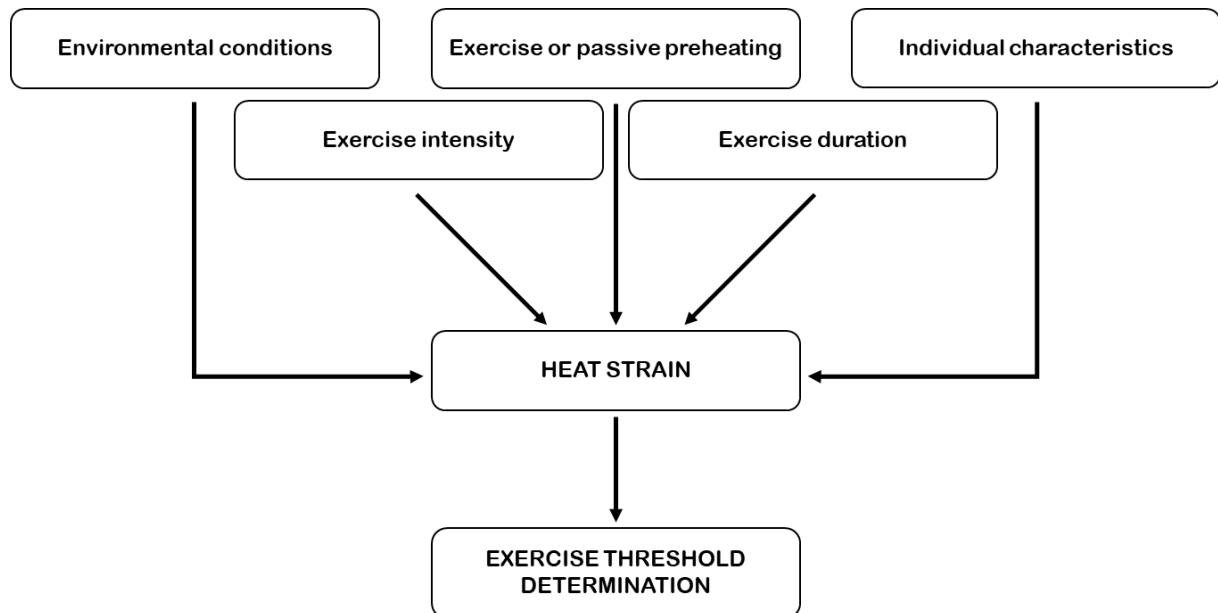
**Table 3** Cont.

| Study                             | Ambient temperature | Individuals                   | Preheating                                   | Exercise protocol                  | Threshold                    | $\Delta$ threshold occurrence (%) |
|-----------------------------------|---------------------|-------------------------------|--|------------------------------------|------------------------------|-----------------------------------|
| de Barros et al. (2011)           | 22°C vs. 40°C       | 8 healthy men                 | Not reported                                 | STEP<br>15 W / 3 min               | RCP                          | -18 %                             |
|                                   |                     |                               |  | CWR<br>30 min                      | MLSS                         | -18 %                             |
| Maunder et al. (2021)             | 18°C vs. 35°C       | 16 endurance trained men      | 20 min rest                                  | First part<br>STEP<br>35 W / 3 min | OBLA 4.0                     | -10 %                             |
|                                   |                     |                               |  | Second part<br>RAMP<br>35 W / min  | RCP                          | -12 %                             |
| Kuo et al. (2021)                 | 22°C vs. 35°C       | 12 male recreational cyclists | 20 min rest + warm-up                        | 3 min all-out test                 | CP                           | -4 %                              |
| Kaiser et al. (2021)              | 18°C vs. 38°C       | 4 men, 4 women                | 30 min<br>Water immersion<br>36°C vs. 40.5°C | 3 min all-out test                 | CP                           | No effect                         |
| Study 1<br>Bourgois et al. (2023) | 18°C vs. 36°C       | 12 men, 12 women              | 15 min rest                                  | CWR trials between 2-20 min        | CP                           | -6.5%                             |
| Study 2<br>Bourgois et al. (2023) | 18°C vs. 36°C       | 11 men                        | 15 min rest                                  | RAMP<br>30 W / min                 | RCP                          | No effect                         |
|                                   |                     |                               |  | STEP<br>40 W / 3 min               | Bsln + 1.5                   | -6.7 %                            |
|                                   |                     |                               |  |                                    | D <sub>max</sub>             | -4.9 %                            |
|                                   |                     |                               |  |                                    | Exp-D <sub>max</sub>         | -6.0 %                            |
|                                   |                     |                               |  |                                    | OBLA 4.0                     | -5.0 %                            |
|                                   |                     |                               |  |                                    | ModD <sub>max</sub>          | -6.0 %                            |
|                                   |                     |                               |  |                                    | Log-Poly-ModD <sub>max</sub> | -4.3 %                            |
|                                   |                     |                               |  |                                    | LT <sub>2</sub>              | -5.5 %                            |
|                                   |                     |                               |  |                                    | Log-Exp-ModD <sub>max</sub>  | -5.5 %                            |

*For the environmental conditions, only ambient air temperature was manipulated in all studies. Characteristics of the individuals, preheating, exercise protocol and exercise threshold determination methodology were presented as specified by the authors of the respective study.  $\Delta$  threshold occurrence refers to the change in PO at the respective threshold in hot compared with temperate environments.*

Although direct comparison of the results is difficult, as a lot of variation exist in test methodology in the first place. Sometimes the test protocol to determine an exercise threshold is not appropriate (e.g., RCP in STEP protocol; de Barros et al., 2011), not all specific information of one threshold is presented

(e.g., combination of 3 lactate thresholds and 3 thresholds based on pulmonary gas exchange; Lorenzo et al., 2011), or the moderate-heavy boundary is determined, instead of the heavy-severe intensity boundary as indicated by the authors (Smolander et al., 1986; Tyka et al., 2010). Second and more interestingly, the variation in how and to which extent acute heat exposure develops heat strain and affects the occurrence of thresholds and performance is a combination of five factors (Figure 13).



**Figure 13** The interaction of five factors influencing exercise threshold determination in hot environmental conditions by inducing heat strain on the human body.

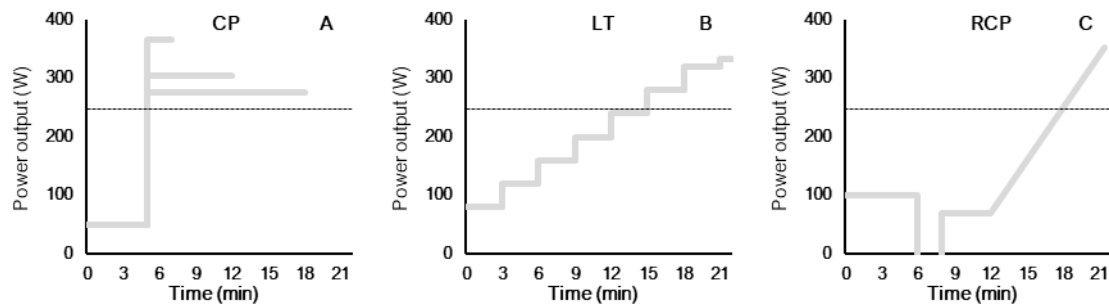
In the next section, these five factors will be discussed in the following order: (1) environmental conditions, (2) exercise or passive preheating, (3) individual characteristics, (4) exercise intensity and (5) exercise duration.

1. The severity of the environmental conditions is an interaction between 4 environmental parameters, i.e., ambient temperature, air relative humidity (RH), solar radiation and wind speed. For example, high temperature (31°C) (Galloway & Maughan, 1997), in combination with high relative humidity (80% RH) (Maughan, Otani, & Watson, 2012), high solar radiation (800 W·m<sup>-2</sup>) (Otani, Kaya, Tamaki, Watson, & Maughan, 2016) and no wind speed (0 m·s<sup>-1</sup>) (Otani, Kaya, Tamaki, Watson, & Maughan, 2018) would create the most severe condition (see Figure 12 in General Introduction). In *study 1* and *study 2*, the environmental conditions were similar. Ambient temperature was the sole environmental factor that was manipulated within each study. The temperature in the temperate condition was 18°C, while in the hot condition it was 36°C. We controlled for relative humidity (40%), wind speed (2 m·s<sup>-1</sup>) and exercise testing took place in a built-in climatic chamber where solar radiation was absent.

2. Exercise or passive preheating prior to the exercise test can result in early onset of the development of heat strain. Exercise actively contributes to a rise in core temperature, as metabolic heat is produced during all types of physical activity. Hot water immersion or wearing a water-perfused suit are examples of passive preheating, and can be applied to the whole body or focusing on the working muscles. It has been shown that hyperthermia by means of an increased core temperature ( $> 38.5^{\circ}\text{C}$ ) is an important factor leading to a decrease in performance (Nybo, 2008). We did not include such procedure in the study design of *study 1* and *study 2*, although, participants were seated 10-15 min prior to the test in the respective environment to accommodate to the environmental conditions. Conversely, some studies use warm water immersion to increase core and skin temperature before the start of the test ((Kaiser et al., 2021; Lorenzo et al., 2011), or do not report any preheating procedure, unfortunately (de Barros et al., 2011; Tyka et al., 2009). On the other side of the spectrum, cooling strategies (e.g., cold water immersion) could be applied to lower core and skin temperature prior to the exercise test, to study the underlying mechanisms (Douzi et al., 2019).
3. Individual factors (e.g., age, sex, morphology, physiological profile, status of health, training and acclimation/acclimatization, etc.) also influence the response to acute heat exposure. Although, we made an effort to standardize the characteristics of our test population, we cannot be aware of all underlying (e.g., genetics) factors possibly interacting with heat exposure. The subjects participated in the studies were young (between 20 and 35 years old), physically active men and women. They were not competitive cyclists, and did not train in hot environments for more than one week 3 months prior to the study to avoid heat acclimation/acclimatization effects. In *study 1*, we focused on the factor sex, as this might have an influence on the response to acute heat exposure. CP and  $W'$  were determined in women and men, although we did not find an interaction effect between heat exposure and sex on the overall performance parameter, reflected by CP. Currently no unambiguous evidence is provided that women have an inferior thermoregulation during exercise in the heat compared with men (Yanovich et al., 2020). However, we cannot exclude that hormonal cycles affect the thermoregulatory responses to heat exposure. One of the most important mechanism is induced by the hormone progesterone, which has higher levels in the luteal phase of the menstrual cycle. This hormone inhibits warm-sensitive neuron activity, thus inhibiting heat-loss mechanisms and increasing body temperature (Nakayama et al., 1975). However, recent studies investigating the heat response of women across the menstrual cycle did not report differences in whole body dry and evaporative heat exchange, and core temperature during exercise (Notley et al., 2019), nor affecting performance in the heat (Lei et al., 2017). At this moment, there is no general consensus on this topic and underrepresentation of women in exercise thermoregulation research requires to be cautious on reported findings in single studies (Hutchins et al., 2021). It must be

mentioned that in *study 2* the participants were only men and a large interindividual variation in response to heat exposure in *study 1* and *study 2* was found. This supports the fact that even a short incremental exercise test with acute heat exposure can give valuable insight on the acute heat response of an athlete. Therefore, every case has to be (re)viewed individually by an experienced staff, so that optimal training outcomes can be achieved.

4. The exercise intensity will determine how much metabolic heat the body produces during exercise. The higher the exercise intensity, the more metabolic heat is produced. To determine the exercise thresholds in our studies, all tests were performed until task failure or the limit of tolerance was reached. However, differences can be perceived for the intensity distribution of the exercise test protocols (Figure 14).



**Figure 14** Exercise protocols to determine heavy-severe intensity boundary: (A) constant work rate prediction trials to determine critical power (CP), (B) step incremental exercise test to determine second lactate threshold (LT) and (C) ramp incremental exercise test to determine the respiratory compensation point (RCP). The dashed line in each graph represent a hypothetical heavy-severe intensity boundary denoted by the respective threshold concept.

There is an immediate switch in PO to the predetermined constant work rate (CWR) during the CP prediction trials (Figure 14A). These trials take place in the severe intensity domain, and thus are performed above the threshold they determine. The effect of heat exposure in these trials is observed from performance data (i.e., PO and TTE) (*study 1*). This is in contrast to incremental exercise protocols (*study 2*), where the threshold is surpassed at submaximal level during the test, whilst the effect of heat exposure must be detectable in the according physiological data (i.e., gas exchange or blood [La<sup>-</sup>]). Although, differences also exist between incremental exercise protocols. During STEP exercise the stage increments make sure that a substantial part is executed at higher work rates (Figure 14B). On the other hand, during RAMP exercise, the continuous increase in PO leads to rapid attainment of the limit of tolerance (Figure 14C), and thus lesser time is spent at high

work rates compared to STEP. This is illustrated by the time spent above 50% $\dot{V}O_{2peak}$ , which was almost double in STEP compared to RAMP ( $669 \pm 67$  s vs.  $357 \pm 34$  s). Furthermore, in *study 2*, a negative correlation was observed between the exercise intensity at which the thresholds occurred and the change in  $\dot{V}O_2$  between temperate and hot environmental conditions.

5. This brings us seamlessly to the next influencing factor, exercise duration, as there is a dynamic interplay between intensity and duration. Exercise intensity relates to the exercise duration during incremental exercise tests with an identical protocol, as a higher exercise intensity implies a longer heat exposure time until the occurrence of the threshold. To illustrate this with an example: a threshold at 280 W during the STEP test (i.e., 18 minutes) was impacted more than a threshold determined at 200 W (i.e., 12 minutes). The longer an individual exercises in the heat, the more susceptible the individual is to develop heat strain (i.e., time-effect), and thus exercise duration is a key factor. In the two studies we can clearly conclude that exercise duration plays a major role on how (much) the threshold is affected. The longest prediction trial to establish CP in *study 1* lasted approximately 18 minutes. This is a similar test duration as compared with the total length of the RAMP and STEP protocol (20 minutes). Following the explanation on intensity distribution, it might be surprising that the reduction in CP, in *study 1*, was less pronounced and only similarly affected by acute heat exposure as the lactate thresholds (i.e. -6%) in *study 2*. However, this can be explained by the different effect of acute heat exposure on the three prediction trials, which were performed on separate days. The shortest trial, which lasted between 3 and 4 minutes was not affected by acute heat exposure, while the middle-long (5-10 minutes) and especially the longest (~15 minutes) trial were affected when performed in the hot environment. This is, for example, in contrast to exercise under acute hypoxic (Simpson et al., 2015; Vanhatalo et al., 2010) or hyperoxic (Dekerle et al., 2012; Goulding et al. 2020) conditions. In these studies, an immediate effect on exercise performance during the prediction trials is observed due to alterations in partial pressure of oxygen ( $pO_2$ ) in the air which influences the oxygen cascade (pulmonary ventilation and diffusion, cardiovascular oxygen transport, and gas exchange at the muscles). Interestingly, acute heat exposure had also an impact on the determination of the work capacity  $W'$ . As a result of the downwards shift of the hyperbolic curvature, the area below the hyperbolic curvature became larger and  $W'$  increased. However, it is our belief that the increase in  $W'$  might be a consequence of the mathematical modelling for the used test methodology, rather than a physiological accurate value of  $W'$  in hot environments. In addition, similar to what has been demonstrated under hypoxia (Simpson et al., 2015; Vanhatalo et al., 2010) and hyperoxia conditions (Dekerle et al., 2012; Goulding et al., 2020), the change in CP is associated with the change in  $W'$ , meaning that these two factors are mechanistically linked to each other.

Practical applications regarding exercise testing in hot environments could focus on certain topics. With regard to performance diagnostics, assessment of the acute heat stress response of an athlete might give valuable insight into his/her capacity for heat tolerance and performance in the heat. Translation toward prolonged exercise is difficult based on the research in this PhD thesis, and therefore should be investigated more in depth. Second, when training sessions are executed in the summer months of the respective hemisphere, athletes are commonly exposed to heat. Training content can be adapted based on the results of the individual exercise test. Furthermore, specific heat training sessions (i.e., heat acclimation) can be included in the training process to gain heat adaptations (e.g., increase in plasma volume and sweat rate, reductions in heart rate, core and skin temperature, and better thermal comfort; Périard et al., 2015; Périard et al., 2021) and possible additional benefits (e.g., increase in hemoglobin mass; Rønnestad et al., 2022). An exercise test in the heat can serve as the initial step to structure the training program during the first days of the heat training camp (Maunder et al., 2021). On the following days, modification of the exercise intensity parameters, based on current knowledge, will be needed as a consequence of partial or total heat acclimation (Périard et al., 2015). Subsequently, an exercise test in the heat can also be performed as the last step to assess changes in submaximal and maximal performance after heat acclimation/acclimatization. Consistency is key when using a specific exercise threshold, especially to monitor exercise intensity and permanent follow-up is required for optimal training outcomes. The population investigated in this PhD thesis were physically active men and women, participating in recreational sports. However, regardless of the specific research outcomes, the basic concepts can be translated toward other populations (i.e., trained or elite athletes, outdoor workers, patients, etc.).

Future research should focus on the limitations discussed in the respective studies. A first limitation of the present PhD thesis is related to the pre-exercise heat exposure duration, which was limited to 15 min. This might be too short to investigate genuine physiological heat stress responses during exercise. Together with the (lack of) core temperature measurement through a rectal thermistor or ingestible pill, as a manner to quantify the heat strain, this should be more properly addressed in future research. Therefore, it would be interesting to conduct the same research protocol, only prolonging the pre-exercise heat exposure duration or adding a pre-heating protocol (e.g., warm water immersion). In this way, a same level of hyperthermia (e.g., core temperature of 38.5°C) can be induced at the start of the exercise test. The effect of acute heat exposure on the power output corresponding to the determined thresholds probably does not reflect the performance decrements during prolonged (>20 min) exercise. A prolonged exercise test on the work rate corresponding these thresholds could have shed light on the potential use of these exercise thresholds in hot environments and for the estimation of the maximal metabolic steady state (MMSS). Moreover, the effect of intensity and duration of the exercise tests

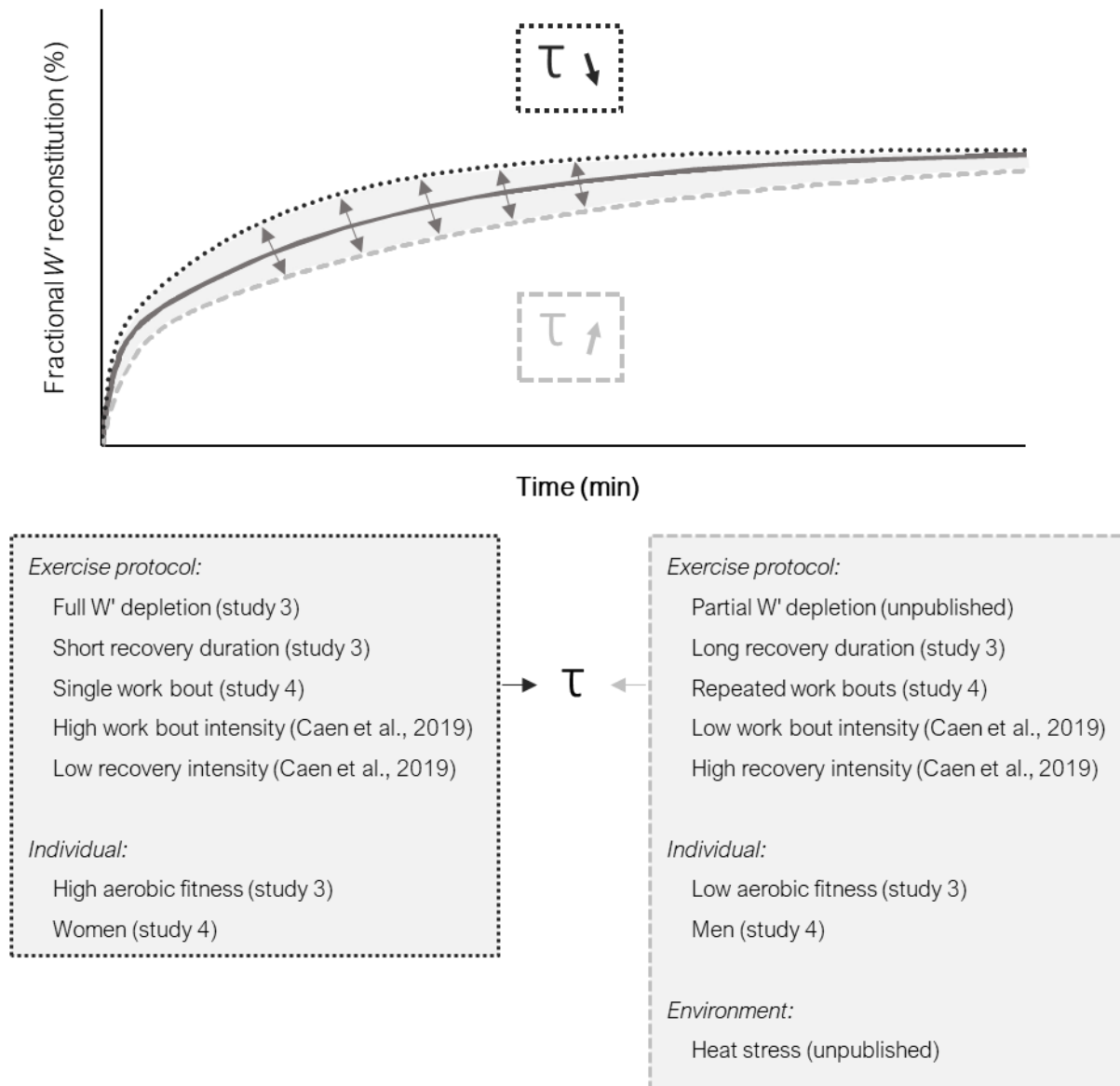
protocol could be explored more in detail, providing additional insights. We speculate that the impact of heat exposure on RCP and second lactate threshold, using a protocol that prolongs the duration of the incremental exercise test (e.g., RAMP with a smaller ramp slope or STEP with longer stages), would be more fierce.

Second, as women are half of the World's population, more research is needed in female populations (Hutchins et al., 2021), while correctly controlling for the menstrual orientation (contraceptives or not) and/or assessment of the menstrual phases in female participants. This would be essential to improve the internal validity of research study designs and interpretation of study results. Given the extensive research protocol (*study 1*) and that performance is the main outcome of this research, it was our belief that variability in this parameter over the months would be larger than potential variability due to the menstrual cycle phase. Moreover, we acknowledge that mechanisms of the menstrual cycle could affect the thermoregulatory responses during exercise in the heat (see discussion in point 3 of chapter 3.2). Nonetheless, at this moment, there is no general consensus on this topic (Yanovich et al., 2020). Therefore, a more structured research methodology is required in a female population regarding exercise performance and thermoregulation, taking into account the menstrual cycle and/or the use of contraceptives (Elliott-Sale et al., 2020; Hutchins et al., 2021; McNulty et al., 2020).

In addition, future work could integrate changes in climatic conditions influencing exercise threshold determination (see Figure 12 of the General introduction). More specifically, performing exercise tests in different climatic environments, with varying temperature, humidity, solar radiation and wind speed, could lead to a better understanding of the heat stress response. Furthermore, as we have reported a considerable inter-individual variation, individual characteristics (age, sex, race, fitness status, physiological characteristics etc.) should be studied more in detail, to provide a tailored approach for everyone.

### 3.3 Acute recovery from severe-intensity exercise

The hyperbolic relationship between power output (PO) and time to task failure (TTF) was established nearly one century ago (Hill, 1925). Since this pioneering work of Morton & Billat (2004), the evolution of  $W'$  reconstitution research progresses exponentially, possibly due to feasible implementation by technical innovations (e.g., development of power meters in cycling) (see Table 1 in the General Introduction for more information). The  $W'_{\text{BAL}}$  of Skiba and colleagues (2012) was a breakthrough for providing insight into the energy balance during intermittent exercise, or in other words the available  $W'$  at any moment in time. Although, from the beginning the authors were well aware that this model was not final and needs further improvements based on current and new insights on the determinants of  $W'$  recovery. As such the application towards performance diagnostics, prescription and steering of the training process, and performance prediction can be further optimized and individualized. Therefore, we have performed two experimental studies (*study 3* and *study 4*) to gain a better understanding of the underlying mechanisms of  $W'$  recovery. In Figure 15 an overview is provided of factors influencing the recovery rate (i.e., the recovery speed constant  $\tau$ ) that were investigated in *study 3* and *study 4*. The results of other studies conducted in our laboratory, of which some are unpublished data, are added as well.



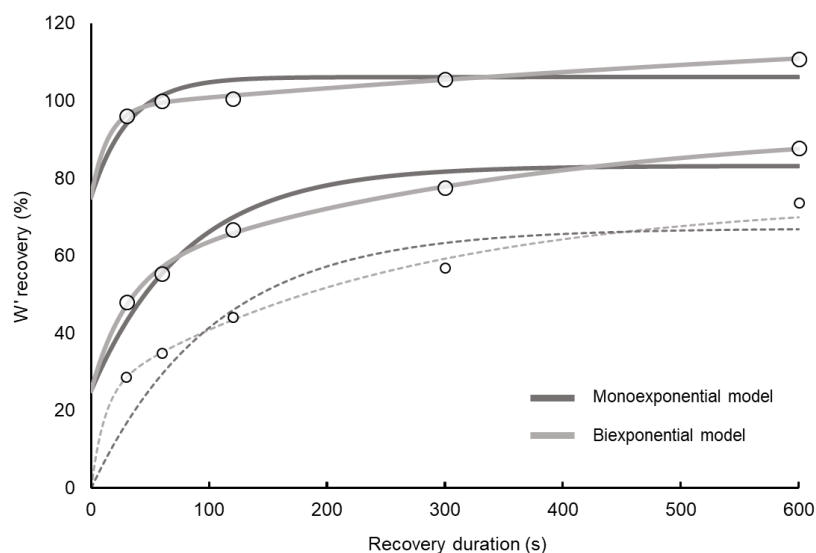
**Figure 15** Characteristics related to the exercise protocol, individual and environment, influencing  $W'$  recovery speed constant  $\tau$  ( $\tau$ ). In left box (black dashed lines) factors resulting in a smaller  $\tau$ , and thus faster  $W'$  recovery. In the right box (grey dashed lines) factors resulting in a larger  $\tau$ , and thus slower  $W'$  recovery.

In the next section, the discussion on the influencing factors of the  $W'$  recovery speed constant  $\tau$  is subdivided in (1) the modalities of the exercise protocol, (2) individual characteristics, and (3) environmental conditions.

1. In *study 3*, we were able to plot the temporal course of  $W'$  reconstitution at eight time points over a period between 30 seconds and 15 minutes. We found that  $W'_{BAL}$  underestimates  $W'$  reconstitution following recovery bouts in the moderate intensity domain of short duration ( $< 5$  min) and overpredicts  $W'$  reconstitution during longer recovery bouts ( $> 10$  min). Other studies reported an underestimation of  $W'$  recovery by the  $W'_{BAL}$  model as well (Bartram et al., 2018; Caen et al., 2019; Chorley et al., 2019; Skiba et al., 2014). This divergence in  $W'$  recovery between short and long

recovery durations points out that the mono-exponential modelling (as applied by  $W'_{\text{BAL}}$  model) is not appropriate to predict  $W'$  reconstitution, when  $W'$  is fully depleted. Using the temporal profile of the measured  $W'$  recovery we were able to make a model fitting for the  $W'$  reconstitution. We found that a bi-exponential fit was better suited to predict  $W'$  reconstitution. This two phase exponential model has its specific characteristics of a fast initial component and a slower second component. This bi-exponential approach of  $W'$  recovery was also investigated by Chorley et al. (2022) in a trained population, finding similar results. The two-component  $W'$  reconstitution model, as presented in *study 3*, is the resultant of all physiological processes taking place during the recovery phase form severe intensity exercise. The complex interaction of the underlying physiology contains (1) the replenishment of high energy stores such as phosphocreatine (PCr) (Chidnok et al., 2013a), (2) elimination of fatiguing metabolites (Chidnok et al., 2013a; Chidnok et al., 2013b; Jones et al., 2008), and (3) recovery from peripheral and central fatigue (Black et al., 2017; Felipe et al., 2020; Hureau, Romer, & Amann, 2018).

Although, in *study 3*, it was demonstrated that the  $W'$  recovery occurs in a bi-exponential way after complete exhaustion, the question raises if these kinetics would be similar in case of a partial and not a complete  $W'$  depletion, as this is highly relevant since  $W'$  is rarely fully depleted during training and/or races in practice. Unpublished data from our laboratory on partial  $W'$  depletion (i.e., 25% or 75%  $W'$  depletion followed by a recovery bout and subsequently a work bout until task failure) show that the use of a bi-exponential model is not superior to a simple exponential model fit (Figure 16).



**Figure 16** Mathematical modelling of the observed  $W'$  recovery (white dots) with a mono-exponential model (dark grey line) and a bi-exponential model (light grey line) for 25%  $W'$  depletion (i.e., start from 75%  $W'$  recovery on y-axis) and 75%  $W'$  depletion (i.e., start from 25%  $W'$  recovery on y-axis). The temporal profiles of  $W'$  recovery following exhaustion (dotted lines) show data reproduced from our own study 3 (reproduced from Lievens et al.; unpublished).

Moreover, we found that the speed of the fast recovery phase (i.e., first 30 seconds of recovery) was not dependent on the severity of  $W'$  depletion, in contrast to what was suggested before by Skiba et al. (2015). This complicates of course the final modelling of the  $W'$  recovery and advocates the use of a dynamic time constant based on the instantaneous size of  $W'$ .

The  $W'_{\text{BAL}}$  model assumes that the recovery is not influenced by changes in the exercise modalities of preceding work intervals. The recovery time constant  $\tau$  in the  $W'_{\text{BAL}}$  model is defined only by the recovery intensity relative to CP (i.e.,  $D_{\text{CP}}$ ). The protocol design of *study 4*, including three successive CWR bouts of  $W'$  depletion (and subsequent recovery), enabled us to study the apparent slowing effect of  $W'$  recovery over time. We discovered that  $W'$  reconstitution was less following the second bout of  $W'$  depletion, although the recovery characteristics were not different. This slowing of  $W'$  recovery has not been taken into account in current  $W'$  reconstitution models, as, for example, the  $W'_{\text{BAL}}$  model predicts a similar reconstitution rate during repeated maximal exercise with a similar recovery intensity and duration. It has been suggested that the reduced  $W'$  recovery during repeated maximal exercise is related to the incomplete resynthesis of PCr and, even more, the accumulation of fatigue-related metabolites (e.g.,  $H^+$ ,  $P_i$ , and ADP). The attainment of a “critical threshold” results in a disturbance of the intramuscular homeostasis and diminishes the capacity to tolerate repeated high intensity exercise (Chidnok et al., 2013a). This slowing of  $W'$  recovery was first investigated by Chorley et al. (2019) in male subjects, although using a different protocol to deplete (i.e., ramp increase to task failure above CP) and reconstitute (i.e., recovery intensity of 50 W), as compared to our *study 4*.

Next to the research presented in this dissertation, other research in our laboratory has shown that the characteristics of (preceding) work bout have an influence on the  $W'$  recovery (Caen et al., 2019). A slower  $W'$  depletion rate generated a slower  $W'$  recovery rate, and vice versa. This finding indicated that  $W'$  recovery depends on the exercise intensity within the severe-intensity domain, which has not been considered in the  $W'_{\text{BAL}}$  model. Moreover, regarding the recovery intensity, the  $W'_{\text{BAL}}$  model assumes that  $W'$  recovery solely depends on the absolute PO difference from CP (i.e.,  $D_{\text{CP}}$ ). Although, it was confirmed by Caen et al. (2019) that a higher  $D_{\text{CP}}$  is associated with a faster  $W'$  recovery, it does not take into account the exercise intensity domains, i.e., moderate vs. heavy intensity domain. Lievens and colleagues (2021) demonstrated that  $W'$  reconstitution accelerates more with decreasing intensity in the heavy versus the moderate intensity domain. Thus, the  $D_{\text{CP}}$  must be placed in a broader perspective, taking into account the physiological mechanisms underpinning the recovery intensity domain instead of the absolute PO difference from CP.

2. We illustrated, in *study 3*, that the  $W'$  recovery rate is significantly impacted by an individual's aerobic fitness level, revealing variations of up to 50% between individuals with low and high  $\dot{V}O_{2\text{peak}}$  values.

A superior aerobic fitness, and thus higher  $\dot{V}O_{2peak}$ , has already been linked to faster recovery from high-intensity intermittent exercise (Tomlin & Wenger, 2001). However, the term high-intensity intermittent exercise in this review refers to repeated sprint exercise, i.e., 5 to 30 seconds work bouts with a varied recovery interval. Although we find a significant positive correlation ( $r = 0.62$ ) in *study 3* between  $\dot{V}O_{2peak}$  and  $W'$  recovery, this is not the case in *study 4*. This may be due to the smaller test population in *study 4*, as well as the composition of the group (more homogeneous), which makes finding a reliable correlation more difficult. Also, in the study of Chorley (2020), a correlation was found between  $\dot{V}O_{2peak}$  and  $W'$  recovery in the trained group but not in the untrained group. These authors linked aerobic fitness with the attenuation of  $W'$  reconstitution as well, i.e., the slowing of  $W'$  recovery following repeated maximal exercise. They found that the higher the aerobic fitness, the smaller the decay was. However, we did not find such correlation in *study 4*. Furthermore, Bartram et al. (2018) adjusted the  $W'$  reconstitution tau of Skiba et al. (2012) with the aim to be better suited for an elite cyclist population. With this study, Bartram et al. (2018) pointed out the importance of training status on the  $W'$  reconstitution as well.

Because the muscle's oxidative capacity, a crucial contributor to whole-body aerobic fitness, is determined by the muscle fiber type (MFT) composition, the amount of type I or type II fibers may directly affect these factors and, thus, may be a key determinant of the  $W'$  recovery. Surprisingly, we did not find a clear relationship between MFT distribution and  $W'$  recovery. Nonetheless, Lievens and colleagues (2020) found a relation between muscle fiber type orientation and recovery from high-intensity exercise ( $3 \times 30$  seconds all out Wingate tests), i.e. people who were type I oriented individuals had a better recovery than type II oriented people. However, it should be noted that the methodology to determine MFT was different between the study of Lievens et al. (2020) and our own *study 3*. In the study of Lievens et al. (2020), a non-invasive measurement of muscle carnosine concentration by  $^1H$ -MRS in the m. gastrocnemius was used to estimate muscle fiber typology (Baguet et al., 2011), whereas we, in *study 3*, conducted muscle biopsies in the m. vastus lateralis. Furthermore, the study conducted by Lievens et al., (2020) examined a difference between 2 groups (type I vs. type II) as opposed to a correlation analysis in our participant population. It might be that the effect of MFT distribution might have been blunted by the larger amount of type II oriented participants in our study.

In all research studies published on  $W'$  reconstitution, the test population was almost exclusively existing out of men. Yet, one of the most interesting findings in *study 4* was the difference in  $W'$  recovery between men and women. This was the first study investigating  $W'$  recovery in a female population, and immediately demonstrating the importance of the inclusion of women in sport science research. We demonstrated that fractional  $W'$  recovery following repeated bouts of maximal

exercise occurs faster in women than in men.  $W'$  recovery as a fraction of total  $W'$  is a common way in research studies on  $W'$  reconstitution, as it expresses the recovery of the individual performance capacity. On the contrary, men reconstituted more  $W'$  when expressed in absolute values. When the reconstituted  $W'$  was normalized to lean body mass (LBM), this difference disappeared. Moreover, we found a lower CP and  $W'$  (absolute and normalized to body mass) for women compared with men, which is reasonable as sex differences in body composition are evident (Kirchengast, 2010). However, normalized to LBM, the difference in CP disappeared, while  $W'$  was still higher in men. These findings support the importance of body composition (ratio body fat vs. lean body mass) and muscle characteristics (oxidative vs. glycolytic capacity) both in  $W'$  expenditure and reconstitution in women and men. However, significant correlations between  $W'$  recovery and LBM were absent in *study 4*, as well as in the study of Chorley et al. (2020).

3. The  $W'_{BAL}$  model assumes that CP and  $W'$  are fixed values prior to the exercise protocol and remain constant throughout exercise, although it has been documented that both parameters can exhibit a certain 'plasticity'. For example, in *study 1*, we demonstrated that acute heat exposure alters these parameters, while other environmental factors are likely to affect CP and  $W'$  as well (Richard & Koehle, 2022). Likewise the determination of exercise thresholds in hot environmental conditions, which were investigated in *study 1* and *study 2*, we assumed that intermittent exercise will also be affected by heat stress. Unpublished data from our laboratory support this hypothesis, however, physiological mechanisms underlying to this results are yet to be unraveled. We found that acute heat exposure diminishes  $W'$  reconstitution and this effect was magnified the longer the exercise protocol lasted (i.e., repeated bouts to deplete and reconstitute  $W'$ ) (Table 4).

**Table 4**  $W'$  reconstitution in temperate and hot environments.

|      | $W'_{REC1}$ (%) | $W'_{REC2}$ (%)   |
|------|-----------------|-------------------|
| TEMP | $65 \pm 13$     | $57 \pm 12^b$     |
| HOT1 | $61 \pm 11^a$   | $46 \pm 11^{a,b}$ |
| HOT2 | $58 \pm 12^a$   | $43 \pm 13^{a,b}$ |

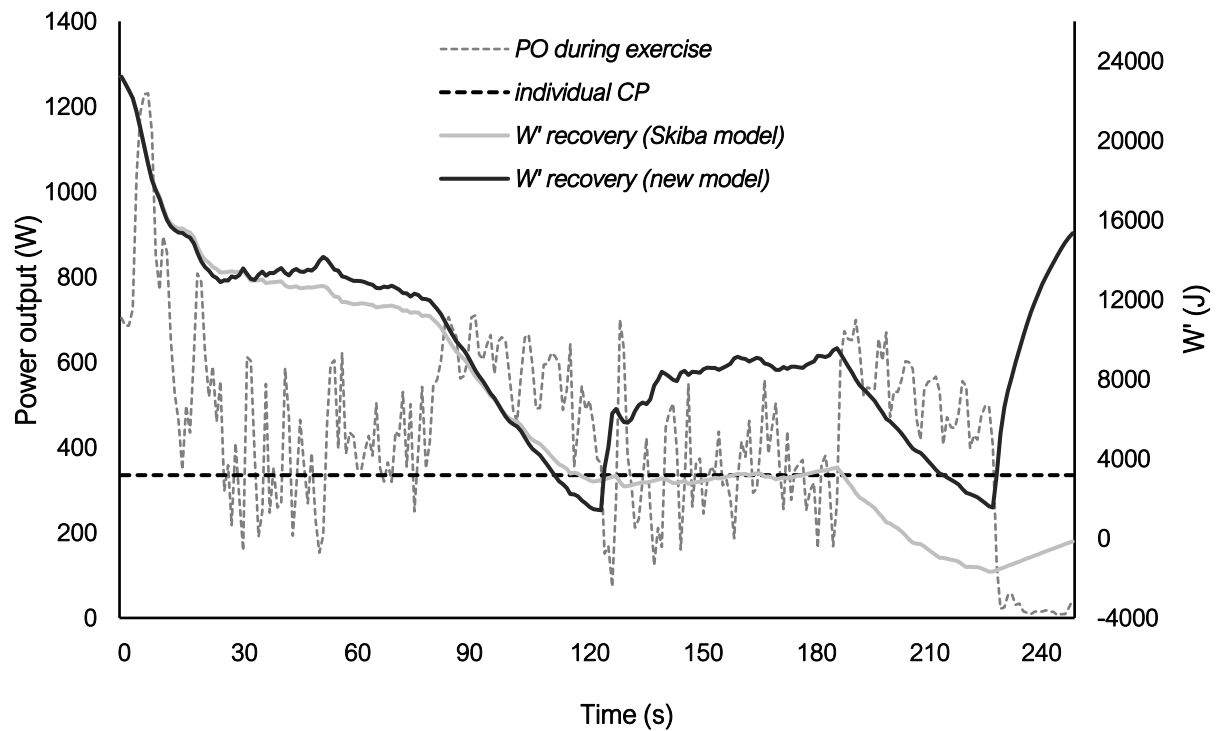
*The protocol to study  $W'$  reconstitution was the same as used in our own study 4, with  $W'_{REC1}$  as the  $W'$  recovery after the first recovery bout, and  $W'_{REC2}$  after the second recovery bout. TEMP was executed in 18°C, where the intensity of the work bouts to exhaustion were based on parameters CP and  $W'$  determined in 18°C. HOT1 was performed in 36°C, where the intensity of the work bouts to exhaustion were based on parameters CP and  $W'$  determined in 18°C. HOT2 was performed in 36°C, where the intensity of the work bouts to exhaustion were based on parameters CP and  $W'$  determined in 36°C. Significant differences ( $p < 0.05$ ) are presented in the table as <sup>a</sup> significant different from TEMP; <sup>b</sup> significant different from  $W'_{REC1}$ .*

Furthermore, determination of CP and  $W'$  in the same hot environmental condition did not produce similar results of  $W'$  reconstitution (HOT2) as found under temperate conditions (TEMP). This is in contrast to what Shearman et al. (2016) found for  $W'$  reconstitution in hypoxia. They validated the  $W'_{\text{BAL}}$  model of Skiba et al. (2012) in hypoxia with the prerequisite that CP and  $W'$  were also measured at same level of hypoxia (Shearman et al., 2016).

The research conducted as part of this thesis focused on work bouts until task failure (i.e., full  $W'$  depletion), starting from a rested metabolic status, and covered maximal two  $W'$  reconstitution phases. However, it has been shown that priming heavy intensity exercise affected the determination of CP and/or  $W'$  (Burnley et al., 2011; Jones et al., 2003; Miura et al., 2009), as well as substrate availability (Clark et al., 2018, 2019; Miura et al., 2000; Miura et al., 1999), and thus, interfere with the modeling of  $W'$  recovery kinetics as well. Furthermore, we assume that over time the  $W'$  reconstitution would reach similar levels irrespective of the characteristics of the individual or exercise protocol, i.e. the amplitude of the curve is equal. The point at which the attainment of optimal recovery is reached is currently indefinite in time and will depend on all factors influencing CP,  $W'$  and recovery kinetics. This advocates for a multifactorial approach toward a comprehensive model for  $W'$  depletion and reconstitution. Based on previous work done by colleagues of the same research group and the work done during this PhD project, it allowed us to initiate the development a new model to optimize the prediction of  $W'$  recovery. The best predicting variables of  $W'$  recovery were integrated in our newly developed model. Currently, these factors are standard modalities of the exercise protocol (i.e., duration and intensity of the recovery bout (relative  $D_{\text{CP}}$  (in %)), and duration and intensity of the work bout), individual characteristics (i.e., CP/kg and  $\dot{V}O_{2\text{max}}$  and a variable time constant tau based on the instantaneous size of  $W'$ ). This is an improvement over the original  $W'_{\text{BAL}}$  model of Skiba et al. (2012), which contained a fixed tau and only took recovery duration and recovery intensity (absolute  $D_{\text{CP}}$  (in watts)) into account.

In Figure 17, a preview of the capabilities of our newly developed model is shown. It can be perceived that the new  $W'$  recovery model corresponds better to the power profile of the intermittent exercise than the Skiba model (Skiba et al., 2012). However, follow-up is required and appropriate adjustments should be made based on current and new insights. Altogether, this is an important step toward a validated prediction model of  $W'$  recovery that could be integrated in sports wearables (e.g. sports watches or bike computers) to track the energetic balance of an athlete on a real time basis. During training periods the use of a  $W'$  recovery model can aid to structure high-intensity interval training. It has been demonstrated that the optimization of the work time at a high fraction of  $\dot{V}O_{2\text{max}}$  during this type of training results in greater gains in performance (Odden et al., 2023). During competition, one of the primary targets for the application of the  $W'$  reconstitution model lies in the stable and standardized environment of track cycling (e.g., team pursuit or Madison). The team pursuit is a discipline in which 4

cyclists complete 4 km as fast as possible. The cyclist in front will deplete his  $W'$  way faster than the cyclists riding in second, third or fourth position. The Madison is a relay event, where two riders alternate during the competition and the replacement rider has to be touched before taking over. As only one rider is racing at any time (i.e.,  $W'$  depletion),  $W'$  reconstitution is possible for the other rider. Taking into account the individual characteristics of the riders into the model, the team tactics can be adjusted adequately, striving toward the best performance outcome.



**Figure 17** Power profile during intermittent exercise in one individual. The horizontal dashed black line represents the individual critical power (CP), the fluctuating dashed grey line is the real-time power output (PO) during exercise. The full gray line is the predictive  $W'_{BAL}$  from Skiba et al. (2012) and the full black line is the available  $W'$  during exercise as calculated by our new model.

Furthermore, more than fifty-five thousand people are registered as competitive or recreational cyclists with the official federation in Flanders. The integration of such a model in a bike computer can aid competitive cyclists to adjust their pacing strategy and race tactics (e.g., duration and intensity at the front of a breakaway or team time trial/team pursuit), based on their individual and real-time  $W'$ . Even for recreational cyclists, not every sports experience is positive. Many of them fail to perform as they envisaged or even do not finish during big cycling “mass” events (e.g., Tour of Flanders, La Marmotte Grandfondo Alpes, etc.). The further development of our model gives the opportunity to provide for each athlete an optimal pacing strategy, taking into account individual characteristics, race features and environmental conditions, so that the energy battery (i.e.,  $W'$ ) is never empty when needed.

### 3.4 Overall conclusion

This PhD dissertation focused on the effects of acute heat exposure on the determination of the heavy-severe intensity boundary (*study 1* and *study 2*), and acute recovery from severe-intensity exercise (*study 3* and *study 4*). Each research study had its own limitations, which were addressed in the respective study, and could taking into account to optimize and strengthen future research.

The research studies on threshold determination in temperate and hot environments showed that:

- The interaction between exercise intensity and exercise duration within exercise test protocols defines the magnitude of decrement in PO corresponding to the threshold.
- No sex-difference was found on the effect of acute heat exposure on the heavy-severe intensity boundary, at least for the CP test methodology.

An exercise test with heat exposure could serve as a manner to assess the heat tolerance of an individual. Furthermore, it could provide additional support for performance diagnostics, optimizing prescription and steering of the training process, and monitoring exercise intensity in hot environments.

In our research studies on  $W'$  recovery we clearly demonstrated that:

- A two phase exponential model is superior to predict  $W'$  reconstitution, instead of a mono-exponential fit following exercise fully depleting  $W'$ .
- Characteristics of the exercise protocol, i.e., repeated bouts to exhaustion, can slow the  $W'$  reconstitution.
- The rate of  $W'$  recovery is strongly influenced by individual factors, such as aerobic fitness and sex.

As a result, a multifactorial approach to accurately estimate  $W'$  reconstitution is required, considering individual characteristics and exercise modalities. Moreover, an optimum between mathematical fine-tuning and integrating physiological processes is required to establish a solid  $W'$  reconstitution model.

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## SCIENTIFIC CONTRIBUTION

### *A1 journal articles as part of this thesis*

1. **Bourgois G**, Mucci P, Caen K, Colosio AL, Kerckhove M, Bourgois JG, Pogliaghi S, Boone J (2023). Effect of acute heat exposure on the determination of Critical Power and  $W'$  in women and men. *European Journal of Sport Science* 23(12), 2425-2434. doi: 10.1080/17461391.2023.2240748
2. **Bourgois G**, Colosio AL, Caen K, Bourgois JG, Mucci P, Boone J (2023). The effect of acute heat exposure on the determination of exercise thresholds from ramp and step incremental exercise. *European Journal of Applied Physiology* 123(4), 847-856. doi: 10.1007/s00421-022-05106-y
3. Caen K, **Bourgois G**, Dauwe C, Blancquaert L, Vermeire K, Lievens E, Van Dorpe J, Derave W, Bourgois JG, Pringels L, Boone J (2021).  $W'$  recovery kinetics following exhaustion : a two-phase exponential process influenced by aerobic fitness. *Medicine & Science in Sports & Exercise* 53(9), 1911-1921. doi: 10.1249/MSS.0000000000002673
4. **Bourgois G**, Mucci P, Boone J, Colosio AL, Bourgois JG, Pogliaghi S, Caen K (2023). Critical Power,  $W'$  and  $W'$  reconstitution in women and men. *European Journal of Applied Physiology* 123(12), 2791-2801. doi: 10.1007/s00421-023-05268-3

### *Other A1 journal articles*

5. Boone J, Caen K, Lievens M, **Bourgois G**, Colosio AL, Bourgois JG (2022). Physical preparation of a world-class lightweight men's double sculls team for the Tokyo 2020 Olympics. *International Journal of Sports Physiology and Performance* 17(12), 1741-1747. doi: 10.1123/ijsp.2022-0056
6. Bouten J, De Bock S, **Bourgois G**, de Jager S, Dumortier J, Boone J, Bourgois JG (2021). Heart rate and muscle oxygenation kinetics during dynamic constant load intermittent breath-holds. *Frontiers in Physiology* 12, 712629. doi: 10.3389/fphys.2021.712629
7. Bouten J, Colosio AL, **Bourgois G**, Lootens L, Van Eenoo P, Bourgois JG, Boone J (2020). Acute apnea does not improve 3km cycling time trial performance. *Medicine & Science in Sports & Exercise* 52(5), 1116-1125. doi: 10.1249/mss.0000000000002236
8. Bourgois JG, **Bourgois G**, Boone J (2019). Perspectives and determinants for training-intensity distribution in elite endurance athletes. *International Journal of Sports Physiology and Performance* 14(8), 1151-1156. doi: 10.1123/ijsp.2018-0722
9. Caen K, Bourgois JG, **Bourgois G**, Van der Stede T, Vermeire K, Boone J (2019). The reconstitution of  $W'$  depends on both work and recovery characteristics. *Medicine & Science in Sports & Exercise* 51(8), 1745–1751. doi: 10.1249/MSS.0000000000001968
10. Boone J, Caen K, Vermeire K, **Bourgois G**, Bourgois JG (2019). The question should be: Is HHbBP equivalent to RCP? *Medicine & Science in Sports & Exercise* 51(4) 829-829. doi: 10.1249/MSS.0000000000001850

### C3 – Conference proceedings

1. **Bourgeois G**, Mucci P, Bourgeois JG, Boone J, Colosio AL (2023). Evaluation of two metabolic carts for cardiopulmonary exercise testing (Cortex Metalyzer 3B and Jaeger Oxycon Pro) in different environmental conditions. *European College of Sport Science (ECSS) – Book of Abstracts* p.952. 28<sup>th</sup> ECSS Annual Congress, Paris, France, 4 July – 7 July 2023.
2. **Bourgeois G**, Kerckhove M, Caen K, Colosio AL, Bourgeois JG, Mucci P, Boone J (2022). Impact of acute heat exposure on the Critical Power Concept and time to exhaustion during a high-intensity interval test in men and women. *European College of Sport Science (ECSS) – Book of Abstracts* p.523. 27<sup>th</sup> ECSS Annual Congress, Sevilla, Spain, 30 August – 2 September 2022.
3. Colosio AL, Caen K, Lievens M, **Bourgeois G**, Bourgeois JG, Boone J (2022). Physical preparation of a world-class lightweight men double sculls team for the Tokyo 2020 Olympics. *European College of Sport Science (ECSS) – Book of Abstracts* p.145-146. 27<sup>th</sup> ECSS Annual Congress, Sevilla, Spain, 30 August – 2 September 2022.
4. **Bourgeois G**, Kerckhove M, Caen K, Colosio AL, Bourgeois JG, Mucci P, Boone J (2022). Impact of acute heat exposure on repeated high-intensity cycling exercise to exhaustion in men and women. *Department of Nutrition, Exercise and Sports (NEXS), University of Copenhagen - Book of Abstracts* p.3. NEXS Grand Depart Symposium, Copenhagen, Denmark, 28-30 June 2022.
5. Kerckhove M, **Bourgeois G**, Boone J, Bourgeois JG (2021). Evaluating tolerance to heat in 3 Olympic athletes: A case study. *VBSW Proceedings* p.27. 26<sup>ste</sup> Symposium Vereniging voor Bewegings- en Sportwetenschappen, Virtual, 10 December 2021.
6. **Bourgeois G**, Middelbos L, Caen K, Bourgeois JG, Van Thienen R, Boone J (2020). The effect of acute heat exposure on exercise capacity and recovery during a high-intensity interval test. *VBSW Proceedings* p.6. 25<sup>ste</sup> Symposium Vereniging voor Bewegings- en Sportwetenschappen, Virtual, 11 December 2020.
7. **Bourgeois G**, Caen K, Bourgeois JG, Boone J (2020). Effect of acute heat exposure on determination of exercise thresholds using different exercise test protocols. *European College of Sport Science (ECSS) – Book of Abstracts* p.405. 25<sup>th</sup> ECSS Annual Congress, Virtual, 28-30 October 2020.
8. **Bourgeois G**, Caen K, Bourgeois JG, Boone J (2019). Exercise thresholds in hot environmental conditions: is there a shift? *VBSW Proceedings*. 24<sup>ste</sup> Symposium Vereniging voor Bewegings- en Sportwetenschappen, Leuven, Belgium, 6 December 2019.
9. Bouten J, **Bourgeois G**, Bourgeois JG, Boone J. Acute apnea does not improve 3 km cycling time trial performance. *VBSW Proceedings*. 24<sup>ste</sup> Symposium Vereniging voor Bewegings- en Sportwetenschappen, Leuven, Belgium, 6 December 2019.

10. **Bourgeois G**, Bourgeois JG, Boone J (2019). Exercise thresholds in hot environmental conditions: is there a shift? *The Physiological Society – Book of Abstracts*. p.40-41. Extreme Environmental Physiology – Life at the Limits, Portsmouth, United Kingdom, 2-4 September 2019.
11. Bouten J, **Bourgeois G**, Bourgeois JG, Boone J. Acute apnea does not improve 3 km cycling time trial performance. *Journal of Science and Cycling* 8(2) p.37. Science & Cycling Congress, Brussels, Belgium, 3-4 July 2019.
12. **Bourgeois G**, Caen K, Vermeire K, Bourgeois JG, Boone J. The effect of a varied recovery interval on the reconstitution of W'. *German Journal of Exercise & Sport Research* 49 (suppl. 1) p.S4-S4. German Exercise Science & Training Conference, Würzburg, Germany, 22-24 February 2019.
13. Bouten J, **Bourgeois G**, Bourgeois JG, Boone J. Acute apnea does not improve 3 km time trial performance. *German Journal of Exercise & Sport Research* 49 (suppl. 1) p.S4-S4. German Exercise Science & Training Conference, Würzburg, Germany, 22-24 February 2019.
14. De Bock S, de Jager S, Bouten J, Dumortier J, **Bourgeois G**, Derom E, Boone J, Herregods L, Bourgeois JG. Diving response : an argument for the aquatic ape theory in human evolution. *Vlaams Instituut voor de Zee (VLIZ) - Book of abstracts* p. 18-19. VLIZ Marine Science Day, Brugge, Belgium, 3 March, 2017.
15. de Jager S, De Bock S, Dumortier J, Bouten J, **Bourgeois G**, Boone J, Herregods L, Bourgeois JG. Apnea trained athletes : rather marine mammals than humans? *Vlaams Instituut voor de Zee (VLIZ) - Book of abstracts* p. 21-21. VLIZ Marine Science Day, Brugge, Belgium, 3 March, 2017.



## CURRICULUM VITAE

Gil Bourgois was born on January 14, 1995, in Ostend, Belgium. In 2018, he graduated magna cum laude as Master of Science in Movement and Sports Sciences from Ghent University, Belgium. During his studies, he also obtained a trainer's degree in athletics (combined events) and alpine skiing. From July 2018, he combined the job of liaison between Ghent University and the Flemish Trainer School (Sport Vlaanderen) with the position of scientific co-worker at Ghent University. In December 2019, he transitioned to a role as a doctoral researcher in exercise physiology. Gil's joint PhD project was a collaboration between Ghent University (Department of Movement and Sports Sciences) and Université de Lille, France (Unité de Recherche Pluridisciplinaire Sport, Santé et Société) under supervision of Prof. Dr. Jan Boone (Ghent) and Prof. Dr. Patrick Mucci (Lille). In the run-up to the Tokyo Olympics (2021), he was the person in charge, as a sports physiologist at PACE (Physiological Academy for Coaching & Education), for the testing of heat tolerance in athletes, applying cooling strategies, and planning heat acclimation protocols in different sports, including athletics, (track) cycling, rowing, and sailing. Gil was awarded a professional development grant as a member from the Physiological Society (United Kingdom), to attend a specialist course on 'exercise performance and training of the top-class athlete' at the University of Copenhagen, Denmark (June 2022). During the summer school of the University of Antwerp (organized by IDLab-imec) on sports data analytics (September 2022), together with his team, Gil took third place in the hackathon competition. In the final year of his PhD, he conducted a research stay at the Università degli studi di Verona, Italy, collaborating with Prof. Dr. Silvia Pogliaghi (October 2022 until January 2023). Throughout his doctoral studies, he completed the doctoral training program from the Faculty of Medicine & Health Sciences at Ghent University. Besides his academic pursuits, Gil coaches youth athletes focusing on combined events at Athletics Club Meetjesland (ACME), and serves as a proud board member of Carotis vzw, organizing biomedical symposia.



