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"Mind the drift" of HR for accurate exercise intensity implementation in prolonged aerobic exercise

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"Mind the drift" of HR for Accurate Exercise Intensity Implementation in Prolonged Aerobic Exercise –Massimo Teso
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SOMMARIO

L'esercizio fisico è considerato una medicina in grado di mantenere e promuovere la salute cardiorespiratoria nell'arco della vita di un individuo. La somministrazione dell'esercizio fisico si basa su questi quattro ingredienti: frequenza, intensità, tempo e tipo di esercizio. Tra questi, l'intensità rimane il termine più elusivo di ogni prescrizione di esercizio fisico. In una prescrizione di esercizio aerobico essa viene stabilita tipicamente mediante l'individuazione di un carico esterno (ad esempio: velocità, passo al chilometro o potenza) che possa suscitare l'intensità metabolica desiderata (ossia, una frazione di consumo di ossigeno massimale o di riserva). Qual ora l'utilizzo di un carico esterno sia impossibile o infattibile, l'indice usato più comune rimane quello della frequenza cardiaca (FC) usata sia per monitorare che per prescrivere l'intensità sia in contesti sportivi che clinici. La prescrizione dell'esercizio mediante l'utilizzo della FC si basa sull'esistenza e consistenza nel tempo di una relazione lineare con l'intensità metabolica. Tuttavia, se tale relazione esiste per esercizi di breve durata, durante esercizi prolungati di durata superiore ai 10 minuti, la frequenza cardiaca ed il consumo di ossigeno divergono nel tempo come risultato di un aumento lento della FC indipendente dal metabolismo. Questo fenomeno è stato studiato per anni ed è chiamato a seconda di durata e meccanismi fisiologici sottostanti *cardiovascular drift* o *HR slow component*. Zuccarelli L. et al. (2018) hanno evidenziato per primi le implicazioni pratiche di questo fenomeno nell'ambito della prescrizione dell'esercizio aerobico. Nello specifico hanno mostrato come l'uso di Target fissi di FC, per prescrivere un'intensità di esercizio, porti ad una diminuzione nel tempo sia del carico esterno che del carico metabolico durante una sessione di esercizio aerobico finalizzata ad un allenamento d'intensità costante. Questo fenomeno è stato consistentemente mostrato in soggetti uomini, sia sani che obesi tuttavia la sua quantificazione, la relazione con il consumo di ossigeno in diverse intensità di esercizio ed in diverse popolazioni mancano assieme ai meccanismi fisiologici sottostanti.

L'obiettivo di questa tesi è quindi quello di analizzare le origini della componente lenta, e proporre una soluzione per consentire un'accurata prescrizione dell'intensità dell'esercizio aerobico usando target di FC. Nel capitolo uno viene

fornita una breve spiegazione della risposta della FC durante l'esercizio e delle attuali teorie esplicative per la componente lenta della frequenza cardiaca congiuntamente alle implicazioni pratiche nella prescrizione dell'esercizio fisico. Nel capitolo due, sono spiegati gli scopi di ricerca di questa tesi. Il capitolo tre, quattro, cinque e sei illustrano i risultati di quattro diversi studi sperimentali. Infine, il capitolo sette riassume i risultati della tesi.

ABSTRACT

An appropriate and individualized dose of regular aerobic exercise is recommended to promote and preserve cardiorespiratory health throughout life. The exercise dose is defined by four ingredients: frequency, intensity, time, and type of exercise. Among these, intensity is the most elusive term of the exercise prescription dose. Exercise intensity is typically determined from an external load such as speed, watt, or pace that elicits a desired metabolic intensity (e.g., a fraction of maximal or reserve oxygen consumption). Whenever this approach is not feasible, heart rate (HR) remains the most commonly used intensity index in both clinical practice and sports. The prescription of exercise using HR targets relies on the existence and constancy of a linear relationship with metabolic intensity over time. However, during constant-load exercise lasting more than 10 min, a mismatch between HR and oxygen consumption emerges over time due to a slow rise in HR independent of metabolism. This phenomenon has been known for years as cardiovascular drift and, more recently, HR slow component. In 2018, Zuccarelli L. et al. suggested a practical implication of this phenomenon. They showed that prescribing exercise intensity using fixed HR targets could lead to an unanticipated and undesired reduction in work rate and metabolic activation during a prolonged session rather than the desired constant training intensity. This phenomenon has been consistently observed in healthy males and those with obesity. However, its physiological quantification and relationship with oxygen consumption across exercise intensities and in different populations are missing, along with its physiological underpinnings.

This thesis aims to deal with this gap, study the origins of the HR slow component, and propose a solution to accurately prescribe exercise intensity using HR targets. In chapter one, a brief explanation of the HR response during exercise and the current explanatory theories for the HR slow component are provided. In chapter two, the experimental aims of the thesis are explained. Then, the results of four different studies are presented in chapters three, four, five, and six. Finally, chapter seven summarizes the main findings of this research work.

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CHAPTER

1

Heart Rate Exercise Prescription and Response During Constant Work Rate Exercise

Exercise prescription dose:

Physical inactivity has been recognized as one of the biggest health problems worldwide, being associated with impaired cognitive functions, higher risk of neurodegenerative, cardiovascular, muscular function disease, and all-cause mortality (Earnest et al., 2013; Farì et al., 2021; Herold et al., 2019; Riebe et al., 2018). On the contrary, regular physical activity is able to promote and preserve health throughout a person's life in a dose-response manner (Earnest et al., 2013; Herold et al., 2019; Riebe et al., 2018). The optimal dose of exercise can be quantified through the elements of frequency, intensity, time, and type according to the "FITT" scheme (Riebe et al., 2018). Methods for prescribing the frequency, duration, or volume of training are relatively simple, as these factors can be altered by manipulating the number of exercise sessions per week, the duration of each session, or the total volume of training in a given time frame (e.g., per week). On the contrary, exercise intensity is arguably the most critical and elusive component of an exercise prescription dose (Iannetta, Inglis, Mattu, et al., 2020; Jamnick et al., 2020). Failure to meet minimal threshold values may result in a lack of a training effect. At the same time, too high exercise intensity could lead to over-training and negatively impact adherence to an exercise program (Iannetta, Inglis, Mattu, et al., 2020; Jamnick et al., 2020). Controversy exists regarding the most appropriate methods to normalize exercise intensity between individuals. This likely contributes to sub-optimal exercise prescription and complicates the ability to compare the outcomes of different research studies and training programs (Iannetta, Inglis, Mattu, et al., 2020; Jamnick et al., 2020).

To date, a popular model for prescribing exercise is the *"relative percent method,"* which expresses the intensity of exercise in terms of the percentage of maximal or reserve heart rate (%HR_{max} or %HRR) or oxygen uptake (%$\dot{V}O_{2max}$ or %$\dot{V}O_2R$). However, this concept includes a considerable heterogeneous variation in the metabolic strain and individual variations in training adaptations, thus resulting in positive responders and non-responders to chronic exercise training (Iannetta, Inglis, Mattu, et al., 2020; Jamnick et al., 2020). Alternatively, the individual identification of the exercise intensity boundaries permits an exercise *threshold-based* prescription

and allows the attainment of a more homogeneous body's homeostasis disturbances across individuals.

Whatever the approach is used, the actual implementation of a given absolute or relative exercise intensity target requires the appropriate translation of the desired metabolic intensity into an external load (e.g., power output, speed, or pace) (Caen et al., 2020; Iannetta, Inglis, Pogliaghi, et al., 2020). Whenever the quantification or implementation of workload is impossible or impractical (e.g., unavailable individual $\dot{V}O_2$/workload relationship, workload resulting from combinations of speed and inclination, or a complex movement task in real-life context), heart rate remains the most common, easy and low-cost used method to prescribe intensity in sport and clinical settings (Achten & Jeukendrup, 2003; Colosio et al., 2018; Colosio, Lievens, et al., 2020a; Pettitt et al., 2008; Weltman et al., 1989).

Thanks to technological development in the last 40 years, HR measurement is an accessible method for a wide audience and can be easily taken and recorded continuously both in laboratory and field conditions (Achten & Jeukendrup, 2003). The prescription of exercise intensity using HR targets requires the continuous monitoring of HR and relies on the existence and constancy over time of a linear relationship with $\dot{V}O_2$ (absolute, relative to max/reserve and metabolic equivalents) (Åstrand & Ryhming, 1954; Legge & Banister, 1986; Riebe et al., 2018).

Heart rate and $\dot{V}O_2$ relationship

Both HR and $\dot{V}O_2$ increase linearly with subsequent increments in exercise demand until the maximum intensity (Achten & Jeukendrup, 2003; Åstrand & Ryhming, 1954; Legge & Banister, 1986; Panton et al., 1996). As such, the relationship between HR and $\dot{V}O_2$ is used to predict, estimate, and monitor individual fitness levels (Achten & Jeukendrup, 2003; Åstrand & Ryhming, 1954; Legge & Banister, 1986). Medical doctors, coaches, and athletes take advantage of this method to plan training intensity and adjust HR during the same session if necessary (Achten & Jeukendrup, 2003; Gormley et al., 2008; Ivey et al., 2007; Macko et al., 2005; Nybo et al., 2010; Pettitt et al., 2008; Piepoli et al., 2016; Weltman et al., 1989).

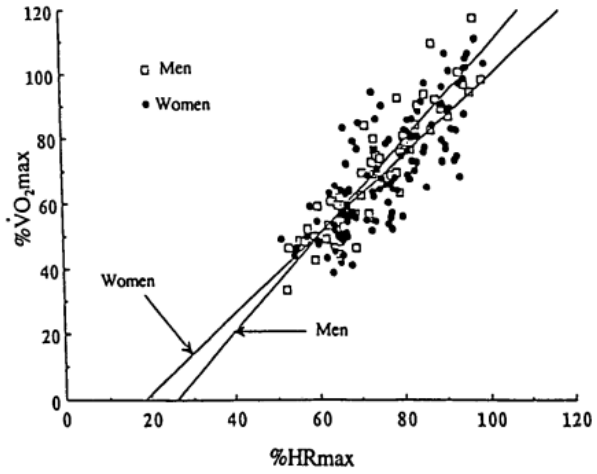

Figure 1. Maximal oxygen uptake (%$\dot{V}O2_{max}$) and percent maximal heart rate (%HR_{max}) relationship in older men and women (N = 55) (Panton et al., 1996)

Despite the easy use of HR as a tool for exercise prescription and monitoring, the validity of HR as an index of metabolic intensity has been questioned; an increase of HR over time totally or partially dissociated from the slow component of $\dot{V}O_2$ has been observed during prolonged constant exercise (Iannetta et al., 2023; Iannetta, Inglis, Mattu, et al., 2020; Wingo, Ganio, et al., 2012; Zuccarelli et al., 2018, 2021).

In particular, HR seems to display a positive slope as a function of time at all exercise intensities (*i.e.,* below the gas exchange threshold (GET), moderate exercise domain), while $\dot{V}O_2$ shows this behavior only at intensities exceeding GET (*i.e.,* heavy and severe exercise intensity domains) (Zuccarelli et al., 2018). A slow increase of HR over time during exercises lasting over 10-20 min has been classically described under the name of "cardiovascular drift" in association with a decrease in stroke volume (SV) and a parallel increase in body temperature due to dehydration and hyperthermia (Coyle & González-Alonso, 2001; Mostardi et al., 1974; Trinity et al., 2010; Watso & Farquhar, 2019). More recently, it was suggested that an increase in HR over time (*i.e.,* a "true slow component") occurs even before 15-20 min of exercise, unrelated to the dehydration, hyperthermia, or a decrease in SV that characterize the traditionally described "cardiovascular drift" (Zuccarelli et al., 2018). However, the physiological underpinnings of this phenomenon remain to be fully elucidated (Zuccarelli et al., 2018).

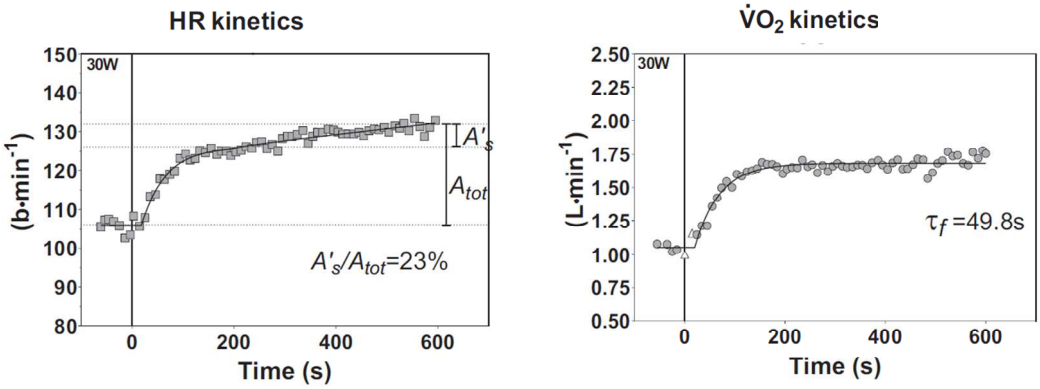

Figure 2. Individual examples of Heart Rate and pulmonary O2 uptake time course during a moderate intensity constant-work rate exercise. Horizontal dashed lines indicate the amplitudes of the total response (Atot) and the actual amplitude of the slow component (A's). Revisited from (Baldassarre et al., 2022)

Whatever the physiological cause of the slow component of HR may be, the practical implication of this phenomenon is that prescribing exercise intensity based on HR targets leads to an unanticipated, undesired, and domain-specific reduction in work rate/metabolic intensity during a prolonged exercise session that is intended to provide a constant training load (Zuccarelli et al., 2018, 2021). The results of the above studies performed on adult males, healthy and with obesity, have raised the awareness of the scientific community on this perhaps overlooked problem in exercise implementation (Baldassarre et al., 2022, 2023; Gallus et al., 2006; Zuccarelli et al., 2018, 2021).

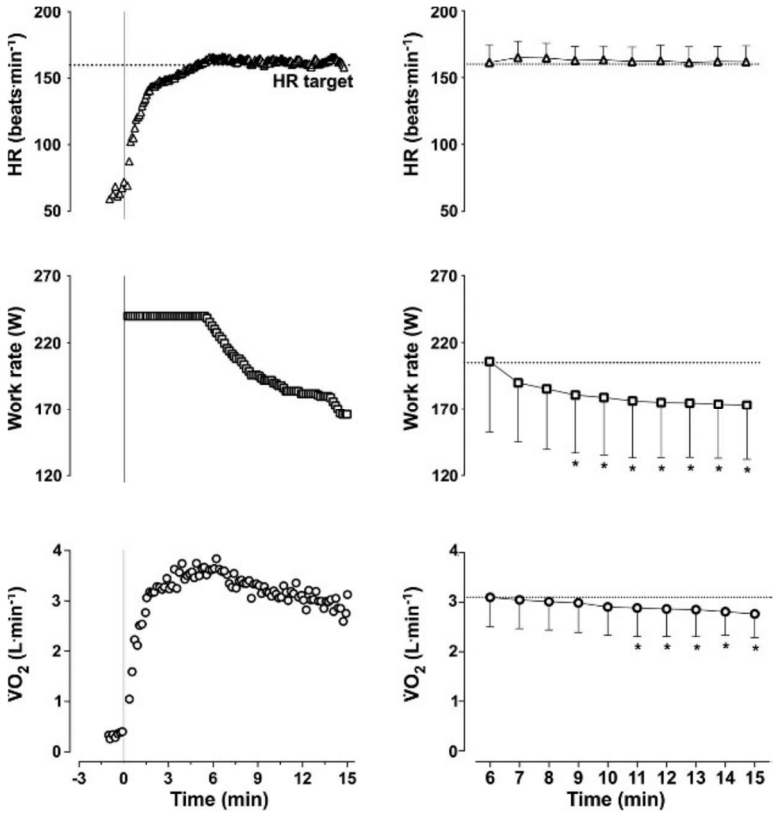

Figure 3. In the left panels, HR, work rate (W), and pulmonary $\dot{V}O_2$, for a representative subject during HR-fixed exercise (HR clamped). In the right panels, the mean ± SD of HR, work rate (W), and pulmonary $\dot{V}O_2$ for the whole group (n. 17) (Zuccarelli et al., 2018a).

HR response at the onset of exercise:

All activities of daily living create an increased need to supply oxygen to the working muscle to increase the generation of adenosine triphosphate (ATP) to support muscle contraction (Brooks A. et al., 2005).

As described in the previous paragraph, HR is closely related to the rate of change of $\dot{V}O_2$; as exercise intensity increases, so does the rate of change of oxygen uptake. This rate of increase is determined by the rate at which oxygen is transported to the tissues, the blood's oxygen-carrying capacity, and the amount of oxygen extracted from the blood (Brooks A. et al., 2005). Mathematically, the relationship between $\dot{V}O_2$ and HR can be expressed using the Fick equation:

$$\dot{V}O_2 = HR \cdot SV\ (a\text{-}v)o_2$$

Where $(a\text{-}v)o_2$ is the difference in oxygen content between the coronary arteries and the coronary venus sinus.

At the same time, there is also the need to expel the increased carbon dioxide produced as a result of increased cellular respiration (Brooks A. et al., 2005). To achieve these goals: i) HR and force of contraction increase, leading to a large increase in cardiac output, and ii) blood vessels in different regions of the body adjust their diameter so that more blood is supplied to the working muscle and less to inactive areas (Brooks A. et al., 2005).

These adjustments occur due to changes in myocardial cells and the smooth muscle cells surrounding blood vessels (Brooks A. et al., 2005). In turn, myocardial cells and muscle cells may be responding to different stimuli: i) neural (sympathetic nervous system) (Coote & Bothams, 2001; Fu & Levine, 2013; ii) hormonal (such as noradrenaline and epinephrine) (Orizio et al., 1988a); iii) local chemical (such as nitric oxide) (Zanzinger, 1999), and mechanical (such as the degree of stretch) (Rowell, 1974; Souissi et al., 2021).

At the onset of exercise, an immediate withdrawal of parasympathetic outflow to the heart is followed by increased sympathetic activation of the heart and blood vessels (Maciel et al., 1986). Mechanoreceptors and chemoreceptors stimulated by muscle contraction contribute to increased HR (McCloskey & Mitchell, 1972; Rotto & Kaufman, 1988). Mechanoreceptors are situated within the muscle and are sensitive to the length and tension; similar chemoreceptors are exposed to the metabolic by-products as exercise progresses (Joyner, 1992; McCloskey & Mitchell, 1972; Rotto & Kaufman, 1988). Both receptors relay this information to the cardiovascular control centers, further increasing sympathetic outflow (Joyner, 1992; Rotto & Kaufman, 1988). Moreover, catecholamines (epinephrine and norepinephrine) released from the adrenal medulla reinforce the direct effect of sympathetic nervous systems and have a cardioaccelerator effect (Davies et al., 1974; Orizio et al., 1988a).

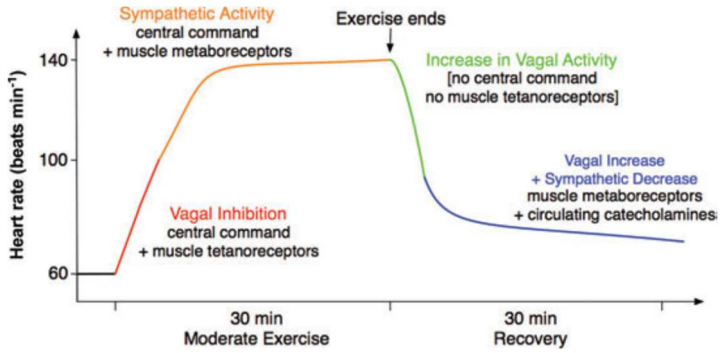

Figure 4. Changes in heart rate during and following exercise The graph illustrates the change in heart rate from rest in a subject undergoing a 30 min period of moderate dynamic exercise followed by a 30 min recovery period. The timing of the contribution from changes in cardiac vagal and cardiac sympathetic activity and their relation to central command and inputs from exercising muscle (Coote, 2010)

In addition to adjustments due to oxygen delivery and waste removal, the cardiovascular system adjusts to exercise in ways that maintain homeostasis (Brooks A. et al., 2005). As such, to prevent hyperthermia, heat dissipation is increased (due to increased sweating and cutaneous blood flow), and to compensate for a decrease in blood volume (due to sweating and blood flow redistribution), heart rate increases (Ganio et al., 2006; Laginestra et al., 2023; Trinity et al., 2010).

HR response during prolonged exercise:

Cardiovascular drift:

During prolonged aerobic exercise (>10-20 minutes), cardiovascular drift appears and is characterized by an increment in HR and a parallel decrement in SV. The traditional theory suggests that during prolonged exercise, body temperature rises, and to dissipate body heat, cutaneous blood flow increases (Coyle & González-Alonso, 2001; Rowell, 1974). An increase in cutaneous blood flow causes a rise in skin venous volume that results in a reduction in ventricular filling pressure and diastolic volume (Coyle & González-Alonso, 2001; Rowell LB, 1986). Additionally, plasma volume is reduced due to progressive dehydration due to perspiration (Ganio et al., 2006) as results HR drifts upward to maintain cardiac output to compensate for the decreasing SV (Coyle & González-Alonso, 2001; Trinity et al., 2010).

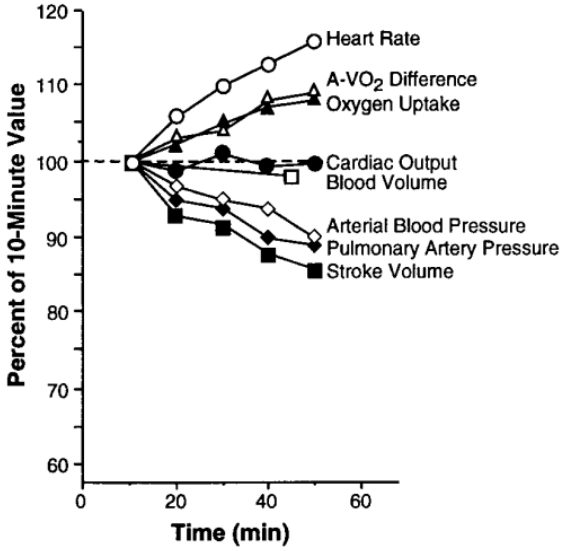

Figure 5. A classic cardiovascular drift response without dehydration (*Redrawn by* Coyle & González-Alonso, 2001; Ekelund, 1967).

However, recently, this theory has been challenged. Data suggest that increased HR with prolonged, moderate exercise reduced the ventricular filling time, thus reducing preload and stroke volume (Trinity et al., 2010). Moreover, data suggest that, after 20min of exercise, SV decreases despite a plateau in skin blood flow (Fritzsche et al., 1999). This indicates no effect of the skin blood flow on the cardiovascular drift. Another hypothesis suggests that an elevation in core temperature directly impacts intrinsic HR, indirectly affects sympathetic nervous system activity, and may be responsible for the progressive rise in HR during prolonged exercise (Trinity et al., 2010)

Heart rate slow component:

More recently, it was suggested that an increase in HR over time occurs even before 10-20 min of exercise (i.e., already from the 3rd min), usually described as the onset of the "cardiovascular drift" (Coyle & González-Alonso, 2001; Trinity et al., 2010; Zuccarelli et al., 2018). This early adjustment has been termed the "slow component of heart rate" ($_{sc}$HR).

$_{sc}$HR seems not related to dehydration, reduced stroke volume, or hyperthermia, characteristics that belong to cardiovascular drift (Zuccarelli et al., 2018). Notably, it has been observed that the $_{sc}$HR occurs even at low intensities (moderate domain), and for the higher (above the gas exchange threshold) it is more pronounced than the

$\dot{V}O_2$ slow component (Zuccarelli et al., 2018). The possible cause for the slow component of HR has been identified in the increased blood catecholamine level and/or hyperthermia (Baldassarre et al., 2023). However, there has been a lack of research on the HR slow component compared to the cardiovascular drift, and the physiological underpinnings remain to be fully elucidated (Zuccarelli et al., 2018).

From a practical point of view, if exercise is prescribed using a target HR, the presence of the $_{sc}$HR, if ignored, will reduce training intensity to keep the heart rate constant (figure 3) (Baldassarre et al., 2022).

Conclusive remarks:

The study of the dynamic adjustments of the HR responses is of interest to clarify how the cardiocirculatory system adapts to these continuous variations and to gain an overall insight into the body's capacity to adapt to them. In this context, the heart rate slow component has received relatively less attention than the cardiovascular drift and still needs to be investigated since there is no common consensus. Moreover, prescribing a given intensity of exercise based on a fixed heart rate value without adequate correction produces an undesired reduction in the metabolic stimulus. From a practical perspective, practitioners and training specialists should be mindful of this phenomenon, and a counter major is required.

CHAPTER

2

Experimental Aims

Purposes and Research Questions:

Heart rate slow component dynamics across different intensity domains, age, and sex

Developing a research-informed framework for optimal exercise prescription requires collecting population-specific data. Such information is essential to develop strategies aiming to maintain the desired stimulus throughout the exercise sessions. In this context, the delayed increase in HR during prolonged exercise has been widely investigated only in young males. Thus, the possible sex and age differences have not been formally analyzed. In addition, the amplitude and its possible relationship with exercise intensity domains have not been clarified, as almost all studies only examined the moderate exercise domain.

In the first two studies, the $_{sc}$HR during prolonged exercise across intensities will be analyzed in a wide age range of populations of both sexes. In particular in:

- **Study 1,** the dynamics of the $_{sc}$HR, in postmenopausal women, will be quantified across several exercise intensities and domains and establish the relationship with it.
- **Study 2,** we will test the effect of sex and age on the $_{sc}$HR. To this aim, young, middle-aged, and elderly individuals will perform two constant work exercises per each domain.

The underlying hypothesis of both studies is that $_{sc}$HR will be present in both sexes and across ages with intensity-dependent dynamics. We hypothesize that male and younger individuals will display a higher slope of HR increments. Moreover, sex and age differences will be partially due to the higher absolute oxygen uptake.

Physiological underpinning of the HR slow component:

Heart rate increments during prolonged exercise are typically associated with decreased stroke volume and increased body temperature (i.e., cardiovascular drift). However, a previous study hypothesized that the mechanism related to the $_{sc}$HR should differ from the cardiovascular drift phenomenon occurring after 10-20 min.

Understanding its mechanisms could represent the starting point through the development of better training and intervention strategies.

By a simultaneous analysis of the HR, SV, body temperature (T°), and $\dot{V}O_2$ kinetics during constant work exercise performed at different relative intensities domains:

- **Study 1** will establish the relationship between the slow component of HR and that of $\dot{V}O_2$ across domains and quantify the relationship between the slow component of HR and other physiological variables (i.e., kinetics of $\dot{V}O_2$, SV, T°, and relative exercise intensity).

The underlying hypothesis of this study is that increments in heart rate will be related to the increments in body temperature and partially associated with the slow component of $\dot{V}O_2$. All of these phenomena will display intensity-dependent dynamics.

Model-predicted heart rate slow component:

The HR associated with a desired metabolic target ($\dot{V}O_2$) would decrease the metabolic stimulus over time due to the emergence of the $_{sc}$HR during prolonged exercise. In this context, estimating the increments in heart rate a priori, would help to adjust the HR target over time and grant that the desired metabolic stimulus is maintained throughout the exercise session in a given individual.

With this aim in:

- **Study 1,** a population-specific predictive model will be developed for postmenopausal women by testing the relationship between the $_{sc}$HR and other physiological variables (*i.e.,* kinetics of $\dot{V}O_2$, SV, T°, and relative exercise intensity);
- **Study 2,** a mathematical model will be developed and validated for $_{sc}$HR prediction across different intensities, sexes, ages, and cardiorespiratory fitness for exercise duration up to 15min;
- **Study 3 & 4,** any time-dependent change in the $_{sc}$HR kinetics will be tested by a linear and double linear fitting model during prolonged exercise.

- **Study 4,** aerobic exercise will be prescribed using our predictive model (adjusting the heart rate over time) versus a typically constant HR (clamped) protocol. The maintenance of the metabolic stimulus ($\dot{V}O_2$) will be tested across intensity domains and protocols.

The underlying hypothesis of these studies is that the $_{sc}$HR rate will be a predictable function of relative exercise intensity, sex, and age. Moreover, a time-dependent change in the $_{sc}$HR would suggest the presence of a step/continuous change in some cardiovascular responses to exercise. Lastly, our predictive model will effectively maintain the metabolic stimulus for up to 30 min of prolonged exercise.

CHAPTER

3

An Intensity-dependent Slow Component of HR Interferes with Accurate Exercise Implementation in Postmenopausal Women

Based on the article published in Medicine and Science in Sports and Exercise 54(4) (2022), doi: 10.1249/mss.0000000000002835

Massimo Teso, Alessandro L Colosio, Silvia Pogliaghi

Abstract

Heart rate (HR) targets are commonly used to administer exercise intensity in sport and clinical practice. Yet, as exercise protracts, a time-dependent dissociation between HR and metabolism can lead to a mis-prescription of the intensity ingredient of the exercise dose. Purpose: we tested the hypothesis that a slow component of HR (i.e. scHR) occurs in all intensity domains, greater than the slow component of oxygen uptake ($sc\dot{V}O_2$), and we developed an equation to predict it across exercise intensities. Method: 18 healthy, postmenopausal women (54 ± 4 years) performed on a cycle-ergometer: *i)* a ramp incremental test for thresholds and $\dot{V}O_{2max}$ detection; *ii)* 30-min constant-work exercise at 40, 50, 60, 70, and 80 $\%\dot{V}O_{2max}$ for the measurement of scHR, $sc\dot{V}O_2$, stroke volume (SV) and body temperature (T°). scHR and $sc\dot{V}O_2$ were compared by two-way RM-ANOVA (intensity and variable); Pearson correlation was calculated between the slow component of all variables, relative intensity, and domain; scHR ($b \cdot min^{-2}$) was predicted with a linear model based on exercise intensity relative to the respiratory compensation point (RCP). Results: A positive scHR was present in all domains, twice the size of $sc\dot{V}O_2$ ($p<0.001$) and significantly correlated with the slow components of $\dot{V}O_2$ ($r^2=0.46$), T° ($r^2=0.52$) and with relative intensity ($r^2=0.66$). A linear equation accurately predicts scHR based on %RCP ($r^2=0.66$, SEE=0.15). Discussion: A mismatch exists between the slow components of HR and metabolic intensity. Whenever exercise is prescribed based on HR, target values should be adjusted over time to grant that the desired metabolic stimulus is maintained throughout the exercise session.

Introduction:

"Exercise is medicine" and induces benefits in both performance and health in a dose-response manner (Borde et al., 2015; McLaughlin & Jacobs, 2017). The exercise dose can be quantified through the elements of frequency, intensity, time, and type, as described in the FITT scheme (Garber et al., 2011). Among these, intensity is the most elusive term of the "exercise prescription" and can be classified as "absolute" or "relative" (Herold et al., 2019). Absolute intensity is typically quantified through the direct measurement of speed/power or its oxygen consumption ($\dot{V}O_2$) equivalent and defines, for example, the energy cost of a given activity (Jamnick et al., 2020). Relative intensity is commonly quantified based on fixed fractions of maximal or "reserve" oxygen consumption ($\%\dot{V}O_{2max}$ or $\%\dot{V}O_2R$) or, more accurately, based on individually determined exercise intensity boundaries (Jamnick et al., 2020), and dictates the extent of the disturbance of the body's homeostasis (Flück, 2006; Jamnick et al., 2020; Jones et al., 2008; Perry et al., 2010; Vanhatalo et al., 2016). The actual implementation of a given absolute or relative exercise intensity target requires the appropriate translation of the desired metabolic intensity into a load or speed (Caen et al., 2020). Whenever the quantification or implementation of workload is impossible or impractical (*e.g.,* unavailable individual $\dot{V}O_2$/workload relationship, workload resulting from combinations of speed and inclination, or a complex movement task in a real-life context), HR remains one of the most commonly used methods to prescribe intensity in sport and clinical practice. The prescription of exercise intensity using HR targets requires the continuous monitoring of HR and relies on the existence and constancy over time of a linear relationship with $\dot{V}O_2$ (absolute, relative to max/reserve and metabolic equivalents) (Colosio et al., 2018; Colosio, Lievens, et al., 2020a; McArdle et al., 2010). However, the validity of HR as an index of metabolic intensity over time has been recently questioned; an increase of HR over time, totally or partially dissociated from the slow component of $\dot{V}O_2$, has been observed during constant work exercise in healthy adults (Zuccarelli et al., 2018) and individuals with obesity (Zuccarelli et al., 2021).

In particular, HR seems to display a positive slope as a function of time (*i.e.* a HR slow component) at all exercise intensities (*i.e.* below the gas exchange threshold (GET), moderate exercise domain), while $\dot{V}O_2$ shows this behavior only at intensities exceeding GET (*i.e.* heavy and severe exercise intensity domains) (Zuccarelli et al., 2018). A slow increase of HR over time during exercises lasting over 10 min has been classically described under the name of "cardiovascular drift" (Coyle & González-Alonso, 2001;

Mostardi et al., 1974; Trinity et al., 2010; Watso & Farquhar, 2019), in association with a parallel increase in body temperature (T°) due to dehydration and hyperthermia (Coyle & González-Alonso, 2001; Mostardi et al., 1974; Trinity et al., 2010; Watso & Farquhar, 2019) and with a decrease (Coyle & González-Alonso, 2001; Mostardi et al., 1974; Trinity et al., 2010; Watso & Farquhar, 2019) in stroke volume (SV). More recently, it was suggested that an increase in HR over time (*i.e.,* a "true slow component") occurs even before 10 min of exercise, unrelated to the dehydration, hyperthermia, or a decrease in SV that characterize the traditionally described "cardiovascular drift" (Zuccarelli et al., 2018). However, the physiological underpinnings of this phenomenon remain to be fully elucidated (Zuccarelli et al., 2018). Moreover, the relationship between the slow component of HR and intensity domain has received very little attention. Whatever the physiological cause of the slow component of HR may be, the practical implication of this phenomenon is that prescribing exercise intensity based on HR targets leads to an unanticipated, undesired, and domain-specific reduction in work rate/metabolic intensity during a prolonged exercise session that is intended to provide a constant training load (Zuccarelli et al., 2018, 2021). The results of the above studies performed on adult males, healthy and with obesity, have raised the awareness of the scientific community on this perhaps overlooked problem in exercise implementation. In this context, menopausal women represent a large and increasing portion of the population in which accurate exercise prescription is crucial to promoting and maintaining health (Dąbrowska-Galas et al., 2019; Daly et al., 2019; Dugan et al., 2018). As women entering this status may experience increments in resting heart rate and systolic and diastolic blood pressure (Nio et al., 2015; Rael et al., 2021), the development of a research-informed framework for optimal exercise prescription requires the collection of population-specific data (Bull et al., 2020; Riebe et al., 2018). Such information is essential to grant the desired stimulus is maintained throughout the exercise sessions (Iannetta, Inglis, Mattu, et al., 2020). Therefore, in the present study, we analyzed the HR, SV, $\dot{V}O_2$, and T° kinetics during constant work exercise performed at different relative intensities and domains in a group of postmenopausal women with the aim to *i)* verify the existence of an increase over time of HR across domains; *ii)* quantify the amplitude of this phenomenon; *iii)* establish the relationship between the slow component of HR and that of $\dot{V}O_2$ across domains; *iv)* quantify the relationship between the slow component of HR and other physiological variables (*i.e.* kinetics of $\dot{V}O_2$, SV, T° and relative exercise intensity) towards a possible prediction model.

Methods

Participants:

Eighteen recreationally active postmenopausal women (Table 1) were recruited by advertisement within the local community and agreed to participate in this study. Inclusion criteria were female sex and age between 45 and 65 years, menopause (i.e., absence of menstrual cycles for a minimum of 12 months), exclusion criteria were smoking, and any medical condition or therapy that could influence the physiological responses during testing. The subjects were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate in the study. All procedures were approved by the Committee for Approval of Human Research - CARU of the University of Verona (n.16-2019).

Protocol:

After medical clearance and anthropometric measurements (Body mass (digital scale, Seca877, Seca, Leicester, UK), height (vertical stadiometer, Seca, Leicester, UK) and skinfolds thickness (Holtain T/W skinfold caliper, Holtain limited, UK) (Ferrari et al., 2022), subjects visited the laboratory on six occasions within a maximum of one month. On the first visit, they performed a ramp incremental test to exhaustion on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL). On the successive appointments, each separated by a minimum of 2 days, subjects performed, in a randomized order, five constant work exercises on the same cycle ergometer, respectively at 40, 50, 60, 70, and 80% of their $\dot{V}O_{2max}$, as determined from the ramp incremental test, each lasting 30 min or until exhaustion.

Participants were instructed to avoid caffeine consumption and physical activity respectively for at least 8 h and 24 h before each testing session. Tests were conducted at the same time of the day in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity). Ergometer position was chosen during the first ramp incremental test and recorded for the successive appointments. Moreover, to minimize the variability of glycogen oxidation, participants consumed the following standardized meal 2 hours before all the testing sessions: 500cc of water and 2 $g \cdot kg^{-1}$ of low glycaemic index carbohydrates (Colosio, Lievens, et al., 2020a).

Ramp incremental protocol:

The ramp incremental protocol consisted of a 3-min baseline cycling at 30 watts, followed by a 10-15 watt·min-1 increase in power output (PO) until volitional exhaustion.

Participants were asked to pick a self-selected cadence in the range of 70-90 rpm and to maintain it throughout all tests. Breath-by-breath pulmonary gas exchange, ventilation, and heart rate were continuously measured using a metabolic cart (Quark B2, Cosmed, Italy) (de Roia et al., 2012). Capillary blood samples (20 µl) were drawn from the ear lobe before and at the 1st, 3rd, 5th, and 7th min after exhaustion. Samples were immediately analysed using an electro-enzymatic technique (Biosen C-Line, EKF Diagnostics, Barleben, Germany) and the highest value was considered as the peak of blood lactate accumulation ($[La^-]_{max}$) for the incremental test.

Constant work protocol:

The constant work exercise consisted of a 3-min baseline cycling at 30 watts, followed by an instantaneous increase in PO that was maintained for 30 min or until exhaustion. The absolute PO for each of the five trials was chosen so that it would elicit a $\dot{V}O_2$ equal to 40, 50, 60, 70, and 80% of the previously identified $\dot{V}O_{2max}$. To this aim, the individual $\dot{V}O_2$/PO relationship derived from the incremental exercise was corrected for the $\dot{V}O_2$ mean response time and slow component, by applying the mathematical model recently proposed by Caen et al (Caen et al., 2020).

Breath-by-breath pulmonary gas exchange, ventilation, and HR were continuously measured with the same method described for the ramp incremental.

SV was determined continuously by electrical bioimpedance meter (PhysioFlow®, Manatec type PF05L1, Paris, France) that uses resting blood pressure (ERKA, Perfect Aneroid Clinica 48, Hamburg, Germany) and changes in transthoracic impedance during cardiac ejection to calculate SV (Charloux et al., 2000). After skin preparation, six electrodes were used as *per* the manufacturer's instructions: 2 at the base of the neck, 2 on the back at the same level as the xiphoid process, and 2 on the chest.

Tympanic temperature was taken as a proxy of core body temperature and was measured by an infrared thermometer (Braun® ThermoScan, Lausanne, Switzerland) (Morán-Navarro et al., 2019) from the inner ear at rest, during the last minute of baseline cycling, and at the 3rd, 5th, 10th, 15th, 20th, 25th, and 30th min of exercise.

<u>*Data-analysis*</u>

For both the incremental and constant work protocols, gas exchange variables were sampled breath-by-breath while HR, SV, and CO were sampled beat by beat. Aberrant data-points (that lay 3 standard deviations from the local mean) were removed, and

thereafter data were linearly interpolated at 1-s and then mediated at 5-s intervals. For the incremental test, the gas exchange threshold (GET) and respiratory compensation point (RCP) were independently determined with the standard technique by three experts (Beaver et al., 1986). Respiratory exchange ratio (R) was calculated as carbon dioxide production/ $\dot{V}O_2$. $\dot{V}O_{2max}$ and peak PO were determined, respectively, as the average $\dot{V}O_2$ of the last 30-s of exercise and the highest mechanical PO achieved upon exhaustion during the ramp incremental exercise (Colosio et al., 2019).

For each constant work exercise, we calculated: 1) a one-minute mean of $\dot{V}O_2$, HR, and SV for the last minute of baseline and the for the 1st, 3rd, 5^{th,} and then for every 5th additional min until the end of the exercise (i.e. 30th min or exhaustion); 2) the individual slow components of the $\dot{V}O_{2,}$ HR, SV, quantified as the slope of the linear fitting of the 1-s interpolated data, from the 5th min to the end of the exercise and named sc$\dot{V}O_{2,}$ scHR, scSV, respectively; 3) the individual slow components of T° quantified as the slope of the linear fitting of the 5-min data, from the 5th min to the end of the exercise and named scT°. For each intensity, all the slow components were expressed in absolute units; scHR and sc$\dot{V}O_2$ were also expressed relative to the individual reserve calculated as the difference between maximal and resting values observed during the ramp incremental protocol and were named %scHR and %sc$\dot{V}O_2$, respectively.

Finally, each trial was further classified as belonging to the moderate, heavy, and severe domain based on the mean $\dot{V}O_2$ at the 5th-min of the exercise with the following rule:

if $\dot{V}O_2$ at the 5th-min < $\dot{V}O_2$ at GET → MODERATE domain → score 1

if $\dot{V}O_2$ at the 5th-min > $\dot{V}O_2$ at GET and < $\dot{V}O_2$ at RCP → HEAVY domain → score 2

if $\dot{V}O_2$ at the 5th-min > $\dot{V}O_2$ at RCP → SEVERE domain → score 3

<u>*Statistical analysis*</u>*:*

Data description: All data are presented as mean ± standard deviation (SD). After assumption verification (i.e., normality, homogeneity of variance), a one-way repeated measure ANOVA was performed to compare the value at the 5th min and the slow component of $\dot{V}O_2$, PO, HR, SV, T° across the different intensities relative to %$\dot{V}O_{2max}$.

To verify the presence and amplitude of scHR in exercise domains and its relationship with sc$\dot{V}O_2$: %sc$\dot{V}O_2$ and %scHR were compared with a control value equal to 0 and to

each other across the three exercise intensity domains by two-way RM-ANOVA (variable and domain). Post-hoc analyses were performed using the Holm-Sidak method.

To test the possible interaction among the above variables and scHR we propose a regression including sc$\dot{V}O_2$, scHR, scSV, scT, intensity domains, %$\dot{V}O_{2max}$ and %RCP follow by multiple linear regression proceed as follows: A Pearson Correlation Coefficient was calculated between the slow component of $\dot{V}O_2$, HR, SV, T°, exercise domain, %$\dot{V}O_{2max}$, and %RCP.

To develop a multiple linear model for the prediction of the individual increments over time of scHR we proceed as follows: i) the parameters were ordered by correlation coefficient with scHR; ii) a forward multiple regression was initially run including these variables: sc$\dot{V}O_2$, scSV, scT°, intensity domains, %$\dot{V}O_{2max}$ and %RCP; this analysis identified nonsignificant ($p \geq 0.05$) and cross-correlated predictors (*i.e.*, correlation coefficient > ± 0.70) that were discarded from the model. Then, a forward multiple regression was run again until significant and non-oss-correlated predictors and the best model were identified (Field, 2005). Power analysis was conducted a *priori*, based on the expected standard deviation of heart rate seen during constant load exercise in previous article (Zuccarelli et al., 2018, 2021) as the main variable. In order to identify significant differences, with an α error of 0.05 and a statistical power ($1 - \beta$) of 0.95, a n value of 8 subjects was necessary (G*Power 3.1). All statistical analyses were performed using SigmaPlot version 14.0, and α was set in advance at the 0.05 level. Statistical significance was accepted when $p < \alpha$.

Results

Subjects' characteristics are reported in Table 1. The average time from menopause was 4 ± 3 yrs (from 1 to 8); while the average BMI was indicative of a normal weight population, the rather high average $\dot{V}O_{2max} \cdot kg^{-1}$ (36.4 ± 5.3 $ml \cdot min^{-1} \cdot kg^{-1}$) and the results of the IPAQ questionnaire (2250 ± 1340 MET/wk) were indicative of a moderately-active to active lifestyle.

Subjects' mean ± SD of $\dot{V}O_{2max}$, peak PO, and HR_{max}, measured at the end of the ramp incremental, were 2.12 ± 0.26 $l \cdot min^{-1}$, 172 ± 22 watts, and 171 ± 9 bpm respectively. A plateau (*i.e.,* an increase in $\dot{V}O_2$ <50% of the expected based on the increase in PO) was present in 16 of the 18 subjects. Furthermore, the values of $\%HR_{max}$, $R_{max,}$ and $[La^-]_{max}$ upon exhaustion (101 ± 5 %, 1.14 ± 0.24, and 8.6 ± 1.1 $mmol \cdot l^{-1}$, respectively) indicate

that a maximal effort was reached. Subjects' GET and RCP were detected at $\dot{V}O_2$ of 1.20 ± 0.18 l·min^{-1} (57 ± 9 %VO_{2max}) and 1.72 ± 0.18 l·min^{-1} (81 ± 5 %VO_{2max}) respectively.

For constant work exercises, an overview of the subjects' mean ± SD response at the 5th min of exercise intensity is reported in table 2; the profiles of the variables are displayed as a function of time in figure 1. A one-way repeat measure ANOVA on values at 5th min showed, as expected, a significant effect of relative exercise intensity (i.e., %$\dot{V}O_{2max}$) on PO, $\dot{V}O_2$, and HR (for all variables p<0.001) while no main effect of intensity was found on either SV (p=0.28) or T° (p=0.55). Post-hoc analysis revealed significant differences between all the intensities for PO, $\dot{V}O_2$, and HR (for all intensities p<0.001).

Subjects' mean ± SD of sc$\dot{V}O_2$, scHR, scSV, and scT° are reported in table 3 as increments in absolute units per min across exercise intensity (*i.e.* %$\dot{V}O_{2max}$). A one-way repeated measure ANOVA revealed a main effect of relative exercise intensity on sc$\dot{V}O_2$, scHR, and scT° (p<0.001) and no effect on scSV (p=0.12). On sc$\dot{V}O_2$, the post-hoc analysis revealed a significantly lower value for the 40% when compared to all the intensities above 60%$\dot{V}O_{2max}$ (p<0.001, for all the conditions), while 50% was significantly lower compared to all the values above 70%$\dot{V}O_{2max}$ (p<0.001, for all the conditions). When comparing the scHR, all the values increased from 40 to 80%$\dot{V}O_{2max}$ and were significantly different from each other (p<0.05 for all the conditions). Lastly, on the scT°, the post-hoc analysis revealed a significantly greater value at 80% compared to all the intensities (p<0.001, for all the conditions); likewise, the value at 70% was greater compared to the intensities at 40 and 50%$\dot{V}O_{2max}$ (p<0.001, for both conditions).

Subjects' mean ± SD of %sc$\dot{V}O_2$ and %scHR were respectively: 0.05 ± 0.07 %min^{-1} and 0.22 ± 0.15 %min^{-1} for the moderate domain; 0.15 ± 0.12 %min^{-1} and 0.49 ± 0.22 %min^{-1} for the heavy domain and 0.21 ± 0.11 %min^{-1} and 0.80 ± 0.17 %min^{-1} for the severe domain. %sc$\dot{V}O_2$ and %scHR are plotted as a function of %$\dot{V}O_{2max}$ in figure 2. A two-way repeat measure ANOVA (domain and variable) on the %sc$\dot{V}O_2$ and %scHR in the moderate, heavy, and severe domains showed a main effect of the domain and the variable (for both factors, p<0.001). A post hoc analysis showed that both variables, in all domains, were significantly different from a control column equal to zero. Moreover, %scHR was significantly greater compared to %sc$\dot{V}O_2$ in all domains (p<0.001). Regarding the %sc$\dot{V}O_2$, heavy and severe domains were not different from each other, but both were significantly greater compared to the moderate domain. Finally, the %scHR significantly increased from moderate to heavy to severe intensity domain (p<0.001 for all post hoc comparisons across domains). The results of the Pearson correlation analysis

between scHR and sc$\dot{V}O_2$, scSV, scT°, exercise intensity (expressed both as %$\dot{V}O_{2max}$ and %RCP) and domain (i.e., 1, 2, 3) are presented in Table 4 as coefficients of determination (r-squared). All the above-listed variables, except for scSV, had a significant moderate to high correlation with scHR.

Moreover, the iterative application of forward multiple linear regression identified the following significant, not cross-correlated predictors of scHR: sc$\dot{V}O_2$, scT° and %RCP and the following predicting equations for the individual scHR:

(1) scHR ($b \bullet min^{-2}$) = -0.315 + (0.00777 x intensity expressed in %RCP) + (0.0337 x sc$\dot{V}O_2$) + (2.416 x scT°)

$r^2 = 0.76$; SEE = 0.13.

Relative exercise intensity, as indicated by %RCP was the single most relevant predictor of scHR. The use of this single predictor as opposed to the above more complex equation, yield the following equation and performance:

(2) scHR ($b \bullet min^{-2}$) = -0.498 + (0.0120 x intensity expressed in %RCP)

$r^2 = 0.66$; SEE = 0.15.

Table 1 Overview of anagraphic, anthropometrics, and functional characteristics

	#	Age	T-MP	Height	Weight	BMI	$\dot{V}O_{2max}$	GET	RCP
		(yrs)	*(yrs)*	*(cm)*	*(kg)*	$(kg \cdot m^{-2})$	$(ml \cdot min^{-1} \cdot kg^{-1})$	$\%\dot{V}O_{2max}$	$\%\dot{V}O_{2max}$
Mean	18	54.0	4.2	163.9	59.0	22.0	36.4	57.0%	81.2%
SD		± 3.6	± 2.7	± 5.5	± 8.1	± 3.0	± 5.3	± 8.9%	± 4.7%

Values are expressed as mean ± SD. Age, time from menopause (T-MP), height, weight, body mass index (BMI), maximal oxygen consumption ($\dot{V}O_{2max}$), gas exchange threshold (GET), respiratory compensation point (RCP).

Table 2 Variables at 5th min of constant work exercises

$\dot{V}O_{2max}$	PO	$\dot{V}O_2$	HR	SV	T°
%	*(Watt)*	*($l \cdot min^{-1}$)*	*(bpm)*	*(ml)*	*(°C)*
40	35 ± 9	0.92 ± 0.11	100 ± 13	91.1 ± 12.5	37 ± 0.2
50	51 ± 14[a]	1.11 ± 0.17[a]	109 ± 12[a]	98.6 ± 15.2	36.9 ± 03
60	74 ± 14[ab]	1.31 ± 0.20[ab]	121 ± 12[ab]	101.0 ± 12.7	36.9 ± 0.3
70	96 ± 16[abc]	1.53 ± 0.22[abc]	136 ± 12[abc]	98.4 ± 10.3	37.0 ± 0.3
80	120 ± 17[abcd]	1.75 ± 0.26[abcd]	153 ± 10[abcd]	100.4 ± 9.8	37.0 ± 0.2
Main effect	**P-value <0.001**	**P-value <0.001**	**P-value <0.001**	P-value 0.28	P-value 0.55

Mean ± SD of Power Output (PO), Oxygen uptake ($\dot{V}O_2$), Heart Rate (HR), Stroke Volume (SV), and Body Temperature (T°) at the 5th min of different exercise intensities relative to the $\dot{V}O_{2max}$. Main effects for the relative exercise intensity are shown in the bottom line of the tables. [a] indicates a significant difference from 40%; [b] indicates a significant difference from 50%; [c] indicates a significant difference from 60%; [d] indicates a significant difference from 70%;

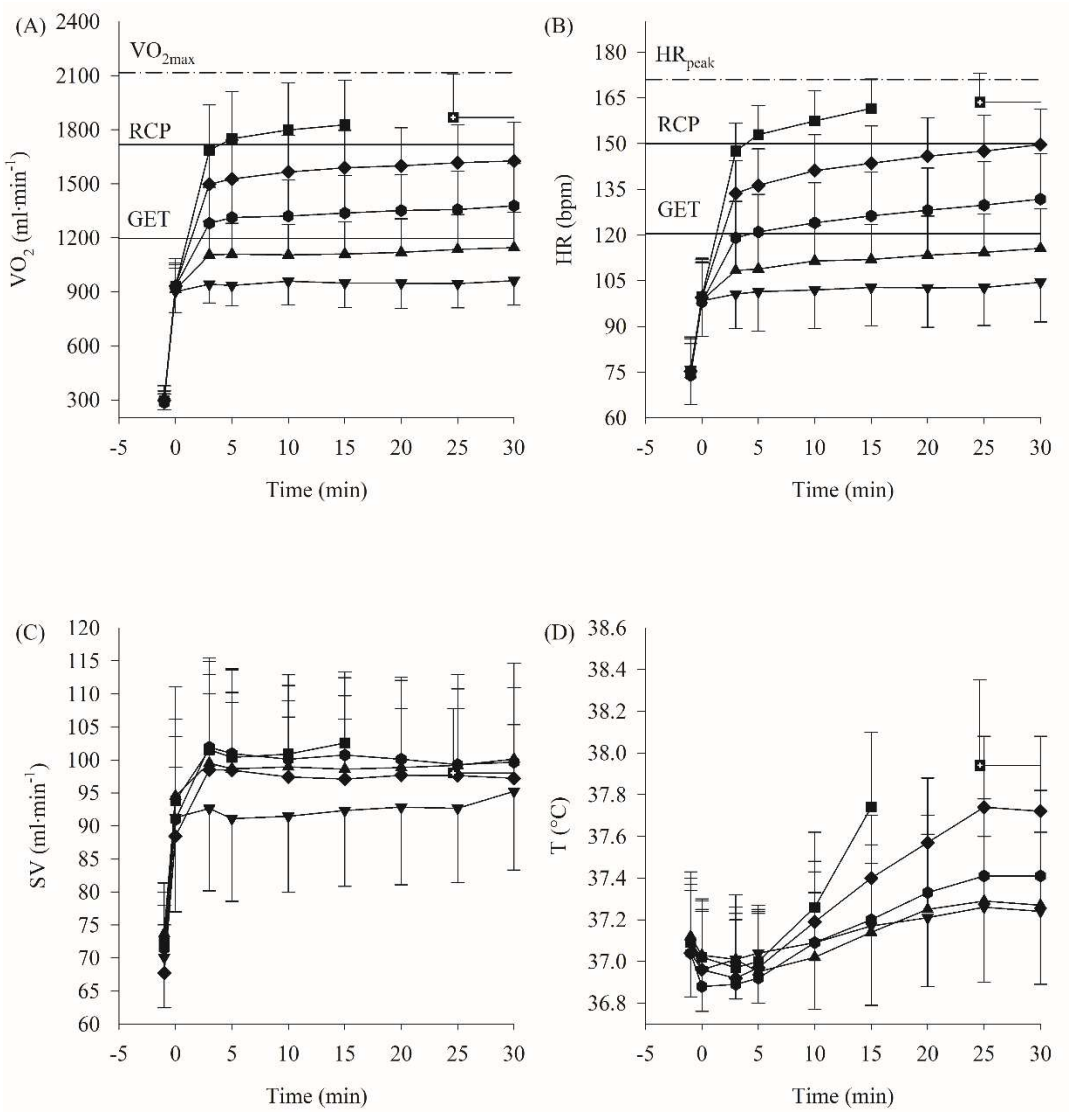

Figure 1. Data collected during CWR at 40 (▼), 50 (▲), 60 (●), 70 (♦), and 80 (■) % of V̇O2max are plotted as a function of time. Mean ± SD of heart rate (A); Oxygen Uptake (B); Stroke Volume (C) and Body Temperature (D). The three horizontal line plots in panels (A) and (B) indicate from the lowest to the highest, gas exchange threshold (GET), respiratory compensation point (RCP), and peak value as identify from the ramp test.

Table 3 Variable' slow component at different exercise intensities.

$\dot{V}O_{2max}$	sc$\dot{V}O_2$	scHR	scSV	scT
%	*(ml·min^{-2})*	*(bpm·min^{-2})*	*(ml·min^{-1})*	*(°C·min^{-1})*
40	0.31 ± 0.83	0.11 ± 0.10	0.11 ± 0.19	0.01 ± 0.01
50	1.13 ± 1.32	0.25 ± 0.13[a]	-0.05 ± 0.16	0.01 ± 0.01
60	2.38 ± 1.57[a]	0.42 ± 0.15[ab]	-0.08 ± 0.32	0.02 ± 0.01
70	3.18 ± 2.02[ab]	0.57 ± 0.18[abc]	-0.13 ± 0.25	0.03 ± 0.01[ab]
80	4.01 ± 2.21[ab]	0.70 ± 0.16[abcd]	-0.03 ± 0.33	0.06 ± 0.02[abcd]
Main effect ***(p value)***	**<0.001**	**<0.001**	0.08	**<0.001**

Means ± SD absolute increments per minute of Oxygen uptake (sc$\dot{V}O_2$), Heart Rate (scHR), Stroke Volume (scSV), and Body Temperature (scT°) at different intensities relative to $\dot{V}O_{2max}$. Main effects for the relative exercise intensity are shown in the bottom line of the tables; [a] indicates a significant difference from 40%; [b] indicates a significant difference from 50%; [c] indicates a significant difference from 60%; [d] indicates a significant difference from 70%;

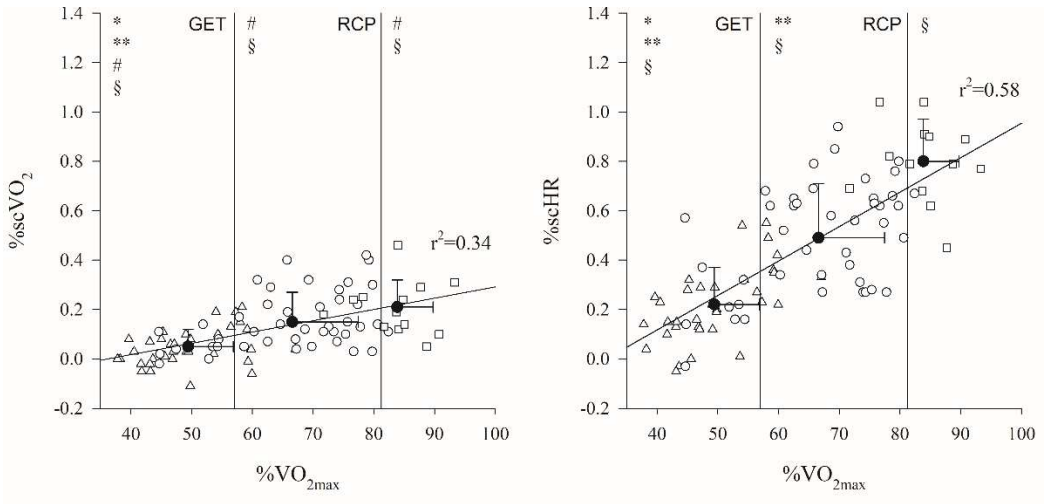

*Figure 2. Individual data (white symbols) of %slow component per minute of oxygen uptake ($\dot{V}O_2$) (left panel) and Heart Rate (HR) (right panel) are plotted as a function of $\%\dot{V}O_{2max}$. Data points falling in the moderate domain are indicated by Δ, in the heavy domain by o, and in the severe domain by □. Regression lines with the coefficient of determination are shown. The two vertical lines in each panel indicate the group-average value of gas exchange threshold (GET) and respiratory compensation point (RCP), expressed as $\%\dot{V}O_{2max}$. The three black dots (●) in each graph indicate mean ± SD values of $\%sc\dot{V}O_2$ and %HR grouped by intensity domain. Relative to each domain group, * indicates a significant difference from heavy and ** from the severe domains; # indicates a significant difference from %scHR within the same domain; § indicates a significant difference from "0".*

Table 4 Coefficient of determinations between the slope of variables.

	scSV *(ml·min^{-1})*	**scT** *(°C·min^{-1})*	**scV̇O$_2$** *(ml·min^{-2})*	**Intensity** *(domain)*	**Intensity** *(%V̇O$_{2max}$)*	**Intensity** *(% of RCP)*
scHR *(bpm·min^{-2})*	-0.07*	0.52***	0.46***	0.51***	0.63***	0.66***
scSV *(ml·min^{-1})*		0.01	0.00	0.02	0.07*	0.06*
scT *(°C·min^{-1})*			0.33***	0.34***	0.61***	0.47***
scV̇O$_2$ *(ml·min^{-2})*				0.21***	0.33***	0.29***
Intensity *(domain)*					0.51***	0.62***
Intensity *(%V̇O$_{2max}$)*						0.91***

Coefficient of determinations between Slope of Heart rate (HR), stroke volume (SV), body temperature (T), oxygen uptake ($\dot{V}O_2$), domains, relative exercise intensity as $\%\dot{V}O_{2max}$ and %RCP. Significant effect: **p value*<0.05; ***p value*<0.01; *p value*<0.001***;

Discussion

The purpose of this study was: i) to confirm the presence, ii) describe the amplitude, iii) and the relationship with $\dot{V}O_2$ of the scHR in all exercise domains in postmenopausal women; iv) to verify the possible relationship between the scHR and the slow component of $\dot{V}O_2$, stroke volume, and body temperature across domains/exercise relative intensities towards the development of a possible prediction model. The study confirmed, in the specific post-menopausal population, the existence of a scHR that increases in proportion to the intensity and across domains. In all domains, the scHR over time is significantly larger than the sc$\dot{V}O_2$. The scHR over time can be accurately predicted from a simple equation based on %RCP only.

The individual and anthropometric characteristics (BMI and %BF) of the subjects enrolled in the study were in line with what we expected from the existing literature for a healthy post-menopausal population (Moreira et al., 2014). However, the average relatively high value of $\dot{V}O_{2max}$ of 36.4 ± 5.3 $ml \cdot min^{-1} \cdot kg^{-1}$ places these mean between the 75^{th} and 80^{th} percentile of the age-specific ACSM (American College of Sports Medicine) fitness distribution (Riebe et al., 2018). The phenomenon of a delayed increase in HR over time during constant work exercises of prolonged duration has been previously described in association with changes in SV and T° in male individuals under the name of "cardiovascular drift" (Coyle & González-Alonso, 2001; Trinity et al., 2010). Two recent studies, with an overall sample size of 33 male-only, individuals (17 healthy adults and 16 individuals with obesity), specifically investigated the existence of the scHR in relation to intensity domains and $\dot{V}O_2$ kinetics; however, the above studies did not simultaneously record changes in SV and T° possibly associated with the cardiovascular drift phenomenon (Zuccarelli et al., 2018, 2021). By contextually analyzing the time-course of body temperature, cardiovascular and metabolic variables during constant work exercise, the current study contributes to fill a lack of knowledge on the existence and the relationship with other cardiometabolic variables of the scHR, across relative intensities and domains in post-menopause women. In addition, this is the first study to examine more than one intensity in a single domain. In agreement with previous findings in adult males, our data confirm the presence of a scHR in all domains of exercise, including moderate. While it had been previously reported that the amplitude of the scHR increases from the moderate to the heavy to the severe domain (Zuccarelli et al., 2018, 2021), our study is the first to demonstrate that the dynamics of the scHR is a linear function of exercise intensity.

Interestingly, the absolute and relative amplitude of the scHR in our study were markedly smaller (*i.e.,* ~0.21 bpm and 0.22 % per minute) than the values reported in young males at a comparable intensity of exercise (*i.e.,* ~1 bpm and 1.5 % per minute for moderate-intensity exercise) (Zuccarelli et al., 2018). The difference may be partially explained by the fact that our determination of the scHR was calculated from the 5th min of exercise (*i.e.,* when a temporary steady-state in HR is typically reached) (de Roia et al., 2012) as opposed to the 3rd min of exercise, when HR may still be raised in the least fit individuals (Adami et al., 2011). In fact, a smaller dynamic of HR (*i.e.,* ~0.18 bpm and 0.12 % per minute, more similar to the values in our current study) had been previously documented in endurance-trained athletes between the 10th and the 60th min of constant, moderate-intensity sessions (Coyle & González-Alonso, 2001; González-Alonso & Calbet, 2003). Moreover, a smaller potential for HR excursion typically characterizes older individuals with a smaller HR reserve compared to younger ones (Riebe et al., 2018); therefore, the ~25 years age difference in the populations of the studies could at least partially explain the observed smaller HR dynamic over time in our older sample compared to younger adults (Zuccarelli et al., 2018). The above findings and considerations suggest that future studies aimed at quantifying the dynamics of HR over time during constant work exercise should be mindful of the time window in which the phenomenon is measured and ensure that different ages and sexes are evaluated.

Regarding the relationship with other physiological variables, in agreement with previous work (Zuccarelli et al., 2018, 2021) our data confirm a greater amplitude of the scHR compared to the sc$\dot{V}O_2$ in all domains of exercise, with a ratio of 2/1 roughly for all domains. Moreover, our study demonstrates a significant yet mild correlation between scHR and sc$\dot{V}O_2$, which suggests that scHR is only partially related to metabolism (*i.e.,* <50% of the variability in scHR is explained by changes in sc$\dot{V}O_2$). The slow component of the $\dot{V}O_2$ kinetics is a very well-described phenomenon that has been discussed in detail in several reviews (Grassi et al., 2015; Poole et al., 2016; Poole & Jones, 2012; Rossiter, 2011). While the physiological underpinnings of this phenomenon remain to be fully elucidated, it is generally agreed that i) the sc$\dot{V}O_2$ is absent in the moderate-intensity domain of exercise, ii) it is present in the form of a delayed exponential (and increased gain) in the heavy domain and iii) in the form of a linear projection to $\dot{V}O_{2max}$ in the severe domain of exercise (González-Alonso & Calbet, 2003; Poole et al., 2016). We would like to specify that our study was focused on the quantification of the amplitude of the sc$\dot{V}O_2$ rather than on the accurate determination of its kinetics, which would have been impossible with only one exercise repetition. The choice of using a linear function

aimed at reducing the impact of the variability of the $\dot{V}O_2$ signal on the identification of the amplitude of the change over time rather than endorsing a linear over an exponential fitting of the response in the heavy exercise domain.

The data from the current study agree with the literature in that we find an increase in the sc$\dot{V}O_2$ with increasing intensity. However, the sc$\dot{V}O_2$ over time different from 0 in the moderate domain seems in contrast with previous findings in healthy young males (Zuccarelli et al., 2018) and adults affected by obesity (Zuccarelli et al., 2021). In the majority of studies, the sc$\dot{V}O_2$ in the moderate intensity is quantified as the difference between the $\dot{V}O_2$ at the 3rd-6th min to the 10th of exercise. As such, small differences in breath by breath $\dot{V}O_2$, in a small group of individuals may be difficult to confirm. In our study, the large sample size, the long exercise duration (*i.e.,* 30 min), and the comparison with 0 may have emphasized a difference that is statistically significant, yet practically very small (total amplitude of ~20 ml·min^{-1} that is well below the minimum detectable difference for this variable (*i.e.,* ~100 ml·min^{-1}) (Keir et al., 2015). In agreement with this view, a drift in $\dot{V}O_2$ comparable to that observed in our study (*i.e.,* ~1 ml·min^{-2}) had been described previously in a group of young individuals exercising for 60 min in the moderate domain (Trinity et al., 2010). Alternatively, it is also plausible that in our sample of post-menopausal women, a delayed "metabolic shift" between aerobic and anaerobic metabolisms (Colosio, Caen, et al., 2020) may characterize the moderate domain of exercise.

The notion that $\dot{V}O_2$ slowly projects to its max when a constant-load exercise above the heavy to severe boundary is performed is supported by several studies on $\dot{V}O_2$ kinetics (39-41). Therefore, the fact that in our study $\dot{V}O_2$ did not reach its peak at the higher intensities (*i.e.,* 80% $\dot{V}O_{2max}$) may be somewhat surprising. Yet, a $\dot{V}O_2$ upon exhaustion about 6-7% lower compared to $\dot{V}O_{2max}$ has been previously described in individuals exercising up to 10% above the heavy to severe boundary for 30 min (Hill et al., 2021; Iannetta, Inglis, et al., 2018). We hypothesize that, while a slow component of $\dot{V}O_2$ is always present above critical intensity, which would theoretically project to $\dot{V}O_{2max}$ (Murias et al., 2018), this projection may be very slow in the lower portion of the severe domain (Hill et al., 2021; Lucia et al., 2000), to the extent that the subject may not reach $\dot{V}O_{2max}$ within the window of observation or before exhaustion.

The physiological basis of the slow increase in HR over time has received less attention than the sc$\dot{V}O_2$. Its origin has been attributed to either an indirect consequence of the exercise/temperature-related reduction of SV or to the direct chronotropic effect of

hyperthermia/catecholamines/signaling from exercising muscles (Escourrou et al., 1982; Jose et al., 1970; Trinity et al., 2010). A progressive decrease of SV and mean arterial pressures have been described as part of the cardiovascular drift phenomenon that occurs when an exercise between 50 and 75%$\dot{V}O_{2max}$ is prolonged above 10th min (Trinity et al., 2010); in this context, larger tendencies to a decrease have been described for the more prolonged exercises (Coyle & González-Alonso, 2001; Fritzsche et al., 1999; Trinity et al., 2010). The peripheral displacement of blood volume in conjunction with thermoregulatory increases in skin blood flow has been reported to reduce venous return, in turn lowering SV and causing a secondary increase in HR in order to maintain a constant cardiac output (Trinity et al., 2010). Alternatively, recent literature suggests that the observed reduction of SV over time is in fact secondary to the reduction of ventricular filling time that is caused by the increase in HR (Trinity et al., 2010). The continuous increase in HR observed overtime after the 10th min of a constant work exercise >45%$\dot{V}O_{2max}$ has been attributed to the direct effect of either temperature or circulating catecholamine on the sinoatrial node or via muscle thermoreflexes (Trinity, Pahnke, Lee, & Coyle, 2010). Our study did not observe significant SV decreases at all exercise intensities; this confirms previous work on exercises of a comparable or shorter duration (Trinity et al., 2010; Zuccarelli et al., 2018). Moreover, in line with previous studies, we found no correlation between the scHR and changes of SV over time (Zuccarelli et al., 2018, 2021). On the contrary, T° was significantly increased after 30 min of exercise (~0.2 °C in the moderate exercise, ~0.5 °C in the heavy domain, and ~0.8 °C during the severe domain), with values similar to those reported in the literature for exercises of a comparable duration (Trinity et al., 2010; Zuccarelli et al., 2021). Moreover, a significant mild-to-good correlation was found between increments of T° and HR (r^2=0.52). The above finding confirms in post-menopausal women and in a larger range of intensities the findings of previous studies in adult males (Davies et al., 1976; Fritzsche et al., 1999; Saltin & Hermansen, 1966) and supports the role of heat accumulation in determining the HR increases over time. Larger changes in T° (*i.e.,* ~1 °C vs. 0.2-0.5 °C of our study) and stronger correlations with the HR drift (*i.e.,* r^2=0.95) had been reported in young males for exercises of double the duration conducted in the upper-end of the moderate-intensity domain (Fritzsche et al., 1999). The higher relative intensity compared to our study (57% *vs* 49%$\dot{V}O_{2max}$ in our study) is the likely cause for the larger heat accumulation in the study mentioned above compared to the current study. In fact, a linear relationship was previously described between heat accumulation and relative exercise intensities between 25% and 75%$\dot{V}O_{2max}$ (Saltin & Hermansen, 1966). Our data, enclosed between 40 to

80%$\dot{V}O_{2max}$, are in line with these observations and show significant scT° increments associated with exercise intensity expressed both as %RCP as well as %$\dot{V}O_{2max}$ (r^2=0.66 and r^2=0.63 respectively). In summary, our data support the hypothesis that the scHR is at least in part attributable to the increases in T° as previously described in adult men (Trinity et al., 2010; Zuccarelli et al., 2021).

Whatever the causes of scHR may be, this phenomenon has practical implications in the field of exercise prescription, yet this phenomenon is often ignored in both healthy adults (Zuccarelli et al., 2018) and clinical populations (Zuccarelli et al., 2021). When exercise is anchored to HR targets and HR demonstrates an increase over time that is dissociated from $\dot{V}O_2$, a time-dependent reduction workload (approximately 14% in 17 min) will be observed along with a decrease in the metabolic load of the training session (Adami et al., 2011; Zuccarelli et al., 2018). Our findings align with the above reports. To obtain a gross estimate of the effect of ignoring the scHR on the overall training load of a constant HR-target session, we proceeded as follows: we first calculated the PO that corresponds to a given target HR based on the HR/PO relationship either of the 5th (PO@5) or the 30th min of exercise (PO@30). Then we calculated the $\dot{V}O_2$ at the 5th (CL5) and 30th min (CL30) of a constant load trial performed at PO@5, by using the $\dot{V}O_2$/PO relationship of the 5th and 30th min; we also calculated the $\dot{V}O_2$ at the 30th min (CT30) for PO@30 by using the $\dot{V}O_2$/PO relationship of the 30th min. Finally, we calculated the total session VO_2 as the area of the triangles defined by the above points on a $\dot{V}O_2$/time relationship for either a constant load or a constant HR target training session. We translated these values into kcal using the energy equivalent of O_2. Based on the above procedure and on the embedded assumptions in our data, the practical impact of ignoring the sc HR while prescribing exercise anchored to HR targets is a reduction in PO from ≃6 to ≃15% and in $\dot{V}O_2$ from ≃1% to ≃12% over a 30-min session. Importantly, the metabolic load reduction would have a marginal impact on the energy expenditure (≃5% reduction in Kcal per 30min session); however, an undesired switch in the domain from heavy to moderate and severe to heavy could occur at the intensities closer to the respective boundaries. In this context, to be able to predict the scHR would be very useful to avoid an undesired reduction in both absolute and relative training load. The prediction of scHR derived from exercise intensity relative to RCP (equation 2), can be used to dynamically correct HR targets based on the anticipated HR drift. This strategy allows maintaining the target metabolic intensity/training load throughout prolonged exercise in postmenopausal women.

Interestingly, relative exercise intensity, as indicated by %RCP was the single most relevant predictor of scHR. Recent data have raised the attention on the limitations of using $\%\dot{V}O_{2max}$ for the definition and the implementation of exercise intensity domains (Iannetta et al., 2021; Iannetta, Inglis, Mattu, et al., 2020). In fact, the $\%\dot{V}O_{2max}$ that corresponds to the metabolic intensities that separate moderate from heavy and heavy from severe exercise have been found to be more variable than expected between-subjects. Our finding that % of the intensity at the heavy to severe boundary (as measured by RCP) is the single most relevant predictor of the scHR, stronger than $\%\dot{V}O_{2max}$, corroborates the importance of the direct measurement of these boundaries at the individual level rather than the use of an average value in order to grant that a desired and homogeneous stimulus is administered through exercise. In our study we used RCP as a marker of the heavy to severe boundary (Keir et al., 2015), yet the correspondence/equivalence among different indexes of this critical intensity has been the object of an unsettled discussion (Broxterman et al., 2018; Keir, Pogliaghi, et al., 2018). While entering in this debate is beyond this paper, it is our contempt that different indexes of the heavy to severe boundary (*e.g.* RCP, critical power, maximum lactate steady state, deoxy hemoglobin deflection point) all occur at an identical metabolic intensity (Keir et al., 2015; Keir, Pogliaghi, et al., 2018) and, provided that they are determined with the appropriate protocols and that they are correctly "transaleted" in homogeneous units (Caen et al., 2020; Iannetta, Inglis, Pogliaghi, et al., 2020), they should be considered equivalent. Importantly, the submaximal nature of the heavy to severe boundary and the possibility to measure or estimate it with different methodological approaches (Fontana et al., 2015, 2016; Iannetta, Fontana, et al., 2018; Keir et al., 2015; Keir, Pogliaghi, et al., 2018) make the implementation of this measure feasible on a large scale.

Limitations:

The developed predictive equation does not take into account factors that may potentially affect heart rate kinetics, such as age, sex, altitude, fatigue, overtraining, nutrition, and hydration. Further studies are needed to verify the accuracy of the proposed predictive equation outside of the specific population, to consider sex, age, and other factors that could play a role as predictors of scHR, and to understand the mechanistic bases of this phenomenon better.

Conclusions:

This investigation demonstrated that a scHR is present in all domains of exercise, its amplitude being larger with increasing intensity and about twice as large as the sc$\dot{V}O_2$. Whenever the implementation of the workload is impossible or impractical, and exercise is prescribed on HR targets, we need to be mindful of the mismatch between the slow components of HR and metabolic load/$\dot{V}O_2$. An adjusted HR target over time would grant that the desired stimulus is maintained throughout the exercise session in a given individual.

CHAPTER

4

"Mind the drift": a Comprehensive Equation Predicts the Heart Rate Slow Component Across Exercise Intensities, Age, and Sex

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Abstract

Heart rate slow component ($_{sc}$HR) is an intensity-dependent HR increment that emerges during constant-load exercises, partially dissociated from metabolism. If ignored, when prescribing exercise with HR targets, the $_{sc}$HR will reduce training intensity over time. **Purpose:** To characterize $_{sc}$HR across intensities, sex, and age to develop and validate a predictive equation. **Method:** 66 healthy individuals (35 females; 35 ± 13 yrs) performed: i) a ramp-test until volitional exhaustion for gas exchange threshold (GET), respiratory compensation point (RCP) and $\dot{V}O_{2max}$ and ii) 6x9-min constant-load trials (2<GET, 2 >GET/<RCP, 2>RCP/<$\dot{V}O_{2max}$). $_{sc}$HR was calculated by linear fitting from the 5th min exercise (b•min^{-2}). A step-wise multiple-linear equation was developed to predict $_{sc}$HR based on individual and exercise variables. The validity of the equation was tested on an independent sample by a Pearson correlation and Bland Altman analysis between the measured and estimated HR during constant exercises. **Results:** $_{sc}$HR increases with intensity and is larger in males (p<0.05). A multiple-linear equation predicts $_{sc}$HR based on relative exercise intensity to RCP, sex, and age (r^2=0.54, SEE=0.61). In the independent sample, we found an excellent correlation between the measured and estimated HR (r^2=0.98, p<0.001) with no significant bias (-0.27 b•min^{-1}, z-score=-1.2) and a fair precision (±4.13 b•min^{-1}). **Conclusion:** The dynamic of $_{sc}$HR can be accurately predicted in a heterogeneous sample accounting for the combined effects of relative intensity, sex, and age. The above equation informs the adjustment of HR targets over time to maintain the desired metabolic stimulus throughout aerobic exercise sessions.

Introduction

Aerobic physical activity induces specific metabolic stimuli and adaptations able to improve the cardiometabolic fitness in a given individual in a dose-response manner (Gronwald et al., 2020; McLaughlin & Jacobs, 2017). As such, aerobic exercise is a fundamental ingredient of any training intervention for health promotion (Riebe et al., 2018). The exercise prescription dose is typically quantified by these four elements: frequency, intensity, time, and type of exercise (according to the FITT scheme) (Garber et al., 2011; Herold et al., 2019). Frequency, time, and type are relatively easy to determine, manipulate, and monitor, while intensity remains the most complex and elusive term of an exercise prescription dose (Iannetta, Inglis, Mattu, et al., 2020; Jamnick et al., 2020).

Exercise intensity can be expressed in "absolute" or "relative" terms (Herold et al., 2019). Absolute intensity refers to the energy required to perform a specific activity and can be measured through metabolic equivalent as oxygen uptake (Jamnick et al., 2020). Instead, relative intensity refers to the stress imposed on the body's homeostasis during exercise and is typically expressed as a percentage of anchor measurements, such as maximal or reserve oxygen uptake ($\%\dot{V}O_{2max}$ and $\%\dot{V}O_{2R}$) (Flück, 2006; Jamnick et al., 2020; Jones et al., 2008; Perry et al., 2010; Vanhatalo et al., 2016). The implementation of either absolute or relative exercise intensity outside of a laboratory environment typically entails the transition of the metabolic equivalent in the form of an external load such as speed, watt, or pace that elicits the desired metabolic intensity (e.g., $\dot{V}O_2$ or $\%\dot{V}O_{2max}$) (Bourgois, Mucci, et al., 2023; Caen et al., 2020). Whenever this approach is impossible or impractical, heart rate (HR) is commonly used as an easy-to-measure and inexpensive intensity index to prescribe and monitor exercise intensity in both clinical and sports settings (Achten & Jeukendrup, 2003; Hofmann & Tschakert, 2011). The prescription of exercise intensity using HR targets relies on the existence and constancy over time of a linear relationship between HR and oxygen uptake (as either $\dot{V}O_2$ or $\%\dot{V}O_{2max}$) (Colosio et al., 2018; Colosio, Lievens, et al., 2020a). As a result, guidelines for exercise intensity prescription typically include HR targets ($\%HR_{max}$ or %HR reserve) to obtain specific metabolic stimuli and, in turn, to generate the desired training adaptations (Riebe et al., 2018).

However, recent studies have raised our awareness of a problem that has been underappreciated: during prolonged constant-work exercise, a time-dependent mismatch emerges between HR and oxygen consumption as a result of a slow rise in HR, partially independent of metabolism (e.g., Heart rate slow component) (Baldassarre et al., 2022;

Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021). Then, the failure of the heart rate to attain a steady state response hinders the accurate association of a univocal $\%HR_{max}$ or HR target to any exercise intensity (Iannetta, Inglis, Mattu, et al., 2020; Teso, Colosio, et al., 2022). As a result of maintaining a constant HR over time, the workload is progressively reduced throughout the exercise session, which in turn leads to an undesired reduction in the metabolic stimulus that was intended to be constant (Iannetta, Inglis, Mattu, et al., 2020; Zuccarelli et al., 2021).

Heart rate slow component ($_{sc}$HR) has been repeatedly described in different male-only populations (i.e., healthy adults (Zuccarelli et al., 2018) and people suffering from obesity (Zuccarelli et al., 2021)) and in a group of postmenopausal women (Teso, Colosio, et al., 2022). This phenomenon is present in all exercise domains, with an amplitude that appears larger with increasing intensity (Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021). Interestingly, the only study so far conducted in women found the $_{sc}$HR to be 1/3 smaller compared to what was reported for young males (Teso, Colosio, et al., 2022). In our former study (Teso, Colosio, et al., 2022), we hypothesized that the observed smaller dynamic of HR could be related to i) a higher potential for HR excursion (i.e., a larger HR reserve) in younger individuals compared to older and ii) a higher absolute heat production in younger and fitter males compared to older females that may have affected the core temperature over time. In the above-cited study, we also proposed that HR targets could be adjusted over time to ensure a constant metabolic intensity and provided a population-specific equation to predict $_{sc}$HR in a given individual. Yet, a model with external validity needs to be developed to grant an accurate prediction of $_{sc}$HR at a given intensity, accounting for the possible role of sex, age, and fitness in this dynamic.

Therefore, the present study aimed to develop and validate a mathematical model that, by accurately predicting the dynamics of HR during exercises of different intensities in individuals of both sexes, different ages, and fitness levels, would allow adjusting HR targets to maintain a desired metabolic stimulus during prolonged sessions.

To this aim, we performed a two-step study: i) development of a comprehensive model for $_{sc}$HR prediction across different intensities, sexes, ages, and cardiorespiratory fitness; ii) evaluation of the validity of the developed model using an independent sample of individuals. We hypothesized that age and sex would predictably affect the HR slow component in a given intensity domain.

Methods

Participants

A total of one hundred and one healthy subjects (53 females) were recruited by advertisement within the local community and agreed to participate in this two step-study. The whole group was randomly subdivided into two subgroups of unequal size balanced for sex and age. The size required for each step was determined based on the power analysis reporting in the statistics analysis section. As a result, sixty-five individuals (35 females, 21–62 yrs) participated in *step 1*, *development of the prediction equation,* and thirty-six (18 females, 22-65 yrs) in step 2, *validation of the prediction equation* (see respectively, Table 1 and Table 3 for participants' characteristics). Inclusion criteria were individuals of both sexes and age between 18 and 70 years. Exclusion criteria were smoking and any medical condition or therapy that could influence the physiological responses during testing. Moreover, they were fully informed about the study procedures and the potential risks and discomfort associated with the exercise testing before agreeing to sign a written informed consent. The study was approved by the Ethics Committee of the University of Verona (CARP) and conducted in conformity with the Declaration of Helsinki (no. 16-2019).

Protocol

After medical clearance, subjects' main anthropometric measurements were collected (body mass (digital scale, Seca877, Seca, Leicester, UK) and height (vertical stadiometer, Seca, Leicester, UK)) (Ferrari et al., 2022).

During Visit 1, all participants performed a ramp incremental test until volitional exhaustion on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) for the determination of gas exchange threshold (GET), respiratory compensation point (RCP), and maximal parameters ($\dot{V}O_{2max}$ and HR_{max}).

On the successive appointments, subjects performed the following constant work rate exercises: *step 1*, participants performed six, 9-min constant-work rate exercises: two below GET (i.e., moderate domain), two between GET and RCP (i.e., heavy domain) and two above RCP (i.e., severe domain); *step 2,* participants performed three, constant-work rate exercises lasting 15-min or until exhaustion: one in each domain. The constant work rate order was randomized and counterbalanced. Participants were instructed to avoid caffeine consumption and physical activity at least 8 h and 24 h before each visit, respectively (de Roia et al., 2012). All visits were separated by at least 48 hours and

completed within 30 days. Tests were conducted at the same time of the day (±2 hrs) in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity). The cycloergometer position was set at the first visit to the lab and recorded for successive tests. To minimize the variability of glycogen oxidation, participants consumed a standardized meal (i.e., 500cc of water and 2 $g \cdot kg^{-1}$ of low glycaemic index carbohydrates) two hours before attending the laboratory (Colosio, Lievens, et al., 2020a).

Ramp Incremental Protocol

The ramp incremental test consisted of a 2-min rest, followed by a 4-min warm-up at 30-100 watts and thereafter power output increments of 10-30 W every minute until volitional exhaustion (Boone & Bourgois, 2012; Keir, Paterson, et al., 2018; Poole et al., 2008). The warm-up load and ramp increment were customized to reach the individual's exhaustion between 8 and 12 min, as described in detail elsewhere (Poole et al., 2008). Participants were asked to pick a self-selected cadence between 70-90 rpm and to maintain it for all tests. Breath-by-breath pulmonary gas exchange and heart rate were continuously measured using a metabolic cart (Quark B2, Cosmed, Italy). Moreover, the perceived effort (RPE) ratingas collected using a Borg 6-20 scale 20min from the end of the ramp incremental test (Borg, 1982).

Constant Work Protocol

All the constant work rate exercises were preceded by a 3-min freewheeling cycling warm-up followed by an instantaneous increase in power output.

For *step 1*, the exercise intensity of the six trials was chosen as follows:

i) Moderate trials: 33% (M1) and 66% (M2) of the difference between rest $\dot{V}O_2$ and $\dot{V}O_2$ at GET

ii) Heavy trials: 33% (H1) and 66% (H2) of the difference between $\dot{V}O_2$ at GET and RCP

iii) Severe trials: 33% (S1) and 66% (S2) of the difference between $\dot{V}O_2$ at RCP and $\dot{V}O_{2max}$

For *step 2*, the exercise intensity was chosen as follows:

i) Moderate trials: 50% of the difference between rest $\dot{V}O_2$ and $\dot{V}O_2$ at GET

ii) Heavy trials: 50% of the difference between $\dot{V}O_2$ at GET and RCP

iii) Severe trials: 50% of the difference between $\dot{V}O_2$ at RCP and $\dot{V}O_{2max}$

To identify the power output that elicits the above $\dot{V}O_2$ targets, the individual $\dot{V}O_2$/Power output relationship derived from the incremental exercise was corrected for the $\dot{V}O_2$ mean response time and slow component by applying the mathematical model recently proposed by Caen et al. (Caen et al., 2020).

Breath-by-breath pulmonary gas exchange, ventilation, and HR were continuously measured using the same method of the ramp incremental test. RPE was collected using a Borg 6-20 scale 20min from the end of each constant work rate (Borg, 1982).

Data-analysis

Breath by breath gas exchange variables and HR were treated as follows: Aberrant data points (that lay 3 standard deviations away from the local mean) were removed; thereafter, data were linearly interpolated at 1-s and then mediated at 5-s intervals (Colosio, Teso, et al., 2020). GET and RCP were determined from the ramp incremental test by three experts independently using the standard technique (Beaver et al., 1986). $\dot{V}O_{2max}$ (in absolute and relative to the body weight) and HR_{max} were determined as, respectively, the average $\dot{V}O_2$ of the last 30-s and the highest HR achieved before exhaustion during the ramp incremental exercise (Colosio et al., 2019). In each constant work rate, we calculated:

For *step 1*: i) oxygen pulse ($\dot{V}O_2$/HR) at the 5th min and 9th min of exercise; ii) $_{sc}$HR, as the slope of the HR/time linear fitting from the 5th min to the end of the exercise and expressed in both absolute units (b•min^{-2}) and relative to the $\dot{V}O_2$ at the 5th min ($_{sc}$HR/$_{5min}\dot{V}O_2$);

For *step 2*: the mean HR value at the 5th min and last minute of exercise;

Statistical analysis

Data description. All data are presented as mean ± SD. After assumption verification (i.e., normality, homogeneity of variance), a mixed RM-ANOVA was performed to compare the values at the 5th min of power output, $\dot{V}O_2$, HR, and %RCP, as well as the value of RPE, across exercise intensities with sex as between-subjects factor.

To verify the amplitude of $_{sc}$HR and its relationship with $\dot{V}O_2$. Oxygen pulse ($\dot{V}O_2$/HR) between the 5th and the 9th min was compared across intensities and sex by a mixed two-way RM-ANOVA (time x intensity x sex). Moreover, $_{sc}$HR and $_{sc}$HR/$_{5min}\dot{V}O_2$,

were compared by a mixed RM-ANOVA across intensities with sex as a between-subjects factor. A Post-hoc analysis was performed using the Holm Sidak method (Field, 2005).

To develop a multi-linear model for the prediction of the individual $_{sc}$HR, we proceeded as follows: i) the sex parameter was classified as male = *score 0* and female = *score 1;* ii) relative exercise intensity to RCP (%RCP) was calculated based on the mean individual $\dot{V}O_2$ measured at the 5th minute for each trial; iii) then a forward multiple linear regression was initially run including these variables: *Age, Sex, %RCP,* HR_{max}, $\dot{V}O_2$ *at the* 5^{th} and $\dot{V}O_{2max} \cdot kg^{-1}$. This analysis identified non-significant ($p \geq 0.05$) and cross-correlated predictors (*i.e.*, correlation coefficient > ± 0.70) that were discarded from the model. Subsequently, a forward multiple regression was rerun until significant, non-cross-correlated predictors were identified, and the best prediction model was found (Field, 2005).

To test the validity of the developed equation. In *step 2*, we proceed as follows: i) quantified the $_{sc}$HR amplitude by comparing the HR measure at the 5th min with the last minute of exercise by a paired t-test; ii) estimated the HR at the end of the exercise with the following formula:

$$HR@end = HR@5min + (_{sc}HR \times (time-5))$$

where $_{sc}$HR was estimated using the previously developed equation derived from *step 1,* and time is in minutes from the start of the exercise; iii) lastly we compared the measured and the estimated HR at the end of all exercises by a mixed RM-ANOVA (method x sex), Pearson correlation, and Bland Altman analysis.

A power analysis was conducted a *priori* (G*Power 3.1). To develop a valid prediction model, the sample size necessary required for *step 1* was 60 individuals. Moreover, based on the standard deviations of the primary outcomes ($_{sc}$HR for *step 1* and within-subject variability of HR for *step 2*) detected in previous studies (Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021), a minimum of 18 subjects were required to identify significant differences with an α error of 0.05 and a statistical power (1 − β) of 0.80. All statistical analyses were performed using SigmaPlot (version 14.0). Statistical significance was accepted when $p < 0.05$.

Results:

Step 1:

Participants' characteristics and maximal parameters derived from the ramp incremental test are reported in Table 1. The average body mass index and cardiorespiratory variables were indicative of normal weight and active population (Garber et al., 2011).

Participants' HR and $\dot{V}O_2$ responses during the constant-workload exercise are shown in Figure 1, while physiological responses at the 5th min are reported in Table 2. A mixed RM-ANOVA on power output, $\dot{V}O_2$, HR, %RCP, and RPE found, as expected, a significant effect of relative exercise intensity (for all variables $p<0.001$), while a significant main effect regarding sex was found for power output and $\dot{V}O_2$ only (Power output and $\dot{V}O_2$ $p<0.01$; HR $p=0.11$; %RCP $p=0.39$; RPE $p=0.28$). The post-hoc analysis results are shown in Table 2.

A mixed two-way RM-ANOVA on $\dot{V}O_2$/HR found a significant main effect of time and intensity (respectively, $p<0.05$ and $p<0.01$), while no sex effect was found. Regarding the time effect, $\dot{V}O_2$/HR at the 9th min was lower compared to the 5th min for both intensities in moderate and heavy (mean difference ± SD: M1 -0.17 ± 0.73, M2 -0.32 ± 0.88, H1 -0.45 ± 0.80 and H2 -0.60 ± 0.77 ml· b· min^{-1}) while no difference was found in the intensities in the severe domain (mean difference: S1 -0.13 ± 0.74, S2 0.14 ± 0.72 ml· b· min^{-1}). The post-hoc analysis for intensity showed no difference between intensities within each domain (i.e., S2 vs. S1, H2 vs. H1, and M2 vs. M1, $p>0.05$).

The $_{sc}$HR in absolute values and relative to the 5th min of $\dot{V}O_2$ are shown in Figure 2. A mixed RM-ANOVA on $_{sc}$HR found a significant main effect of relative exercise intensity and sex ($p<0.001$ and $p<0.05$) and no interaction ($p=0.48$). Moreover, when the $_{sc}$HR was expressed relative to the $_{5min}\dot{V}O_2$, a significant main effect of intensity was confirmed ($p<0.001$), while the effect of sex disappeared ($p=0.24$) with no interaction ($p=0.18$). The post-hoc analysis for sex within each intensity is shown in Figure 2. Notably, the post-hoc analysis for intensity within each domain (i.e., S2 vs. S1, H2 vs. H1, and M2 vs. M1) showed no difference for either $_{sc}$HR or $_{sc}$HR/$_{5min}\dot{V}O_2$ ($p>0.05$ for all comparisons).

The iterative application of forward multiple linear regression to estimate $_{sc}$HR indicated that the most relevant and not cross-correlated predictor was %RCP along with sex and age. $\dot{V}O_2$ at the 5th minute, HR_{max}, and $\dot{V}O_{2max}\cdot kg^{-1}$ were all significant predictors of the $_{sc}$HR but cross-correlated with %RCP and/or age and discard from the analysis. Then, the following predicting equation for the individual $_{sc}$HR was found:

$_{sc}HR\ (b \cdot min^{-2}) = -0.0514 + (0.0240 \text{ x intensity expressed in \%RCP}) - (0.0172 \text{ x age}) - (0.347 \text{ x Sex})$

$r^2 = 0.53$; SEE = 0.61

Table 1. Anagraphic, anthropometric, and cardiorespiratory variables of the Step 1 subjects.

Group	**#**	**Age**	**BMI**	**HR_{max}**	**$\%HR_{max}$**	**$\dot{V}O_{2max}$**	**R_{max}**	**GET**	**RCP**
		(yrs)	*($kg \cdot m^{-2}$)*	*($b \cdot min^{-1}$)*	*%*	*($ml \cdot min^{-1} \cdot Kg^{-1}$)*		*$\%\dot{V}O_{2max}$*	*$\%\dot{V}O_{2max}$*
Males	31	33 ± 9	24 ± 2	182 ± 11	97±1	51.0 ± 11.0	1.20±0.1	62.0 ± 6.8	86.2 ± 5.3
Females	34	37 ± 15	22 ± 2	180 ± 11	96±2	41.4 ± 7.1	1.13±0.04	62.2 ± 7.1	83.2 ± 6.0
Total	65	35 ± 13	23 ± 2	181 ± 12	96±4	45.5 ± 10.0	1.17±0.08	62.1 ± 7.1	84.5 ± 6.0

Data are expressed as mean ± SD: Body mass index (BMI), maximal heart rate (HR_{max}), percentage of theoretical HR_{max} ($\%HR_{max}$), maximal oxygen uptake ($\dot{V}O_{2max}$), maximal respiratory exchange ratio (R_{max}), gas exchange threshold (GET), respiratory compensation point (RCP).

Table 2. Variables at the 5th min during the constant-workload exercises.

Sex	**CWR**	**PO**	**$\dot{V}O_2$**	**HR**	**%RCP**	**RPE**
		(Watt)	*($ml \cdot min^{-1}$)*	*($b \cdot min^{-1}$)*	*(%)*	
Male	M1	26.0 ± 16.1	1.06 ± 0.23	#89.2 ± 9.0	33.3 ± 8.2	7.1 ± 1.0
	M2	96.2 ± 16.9[a]	1.77 ± 0.36[a]	111.5 ± 13.5[a]	57.5 ± 12.3[a]	8.0 ± 1.8
	H1	#186.85 ± 35.9[ab]	#2.58 ± 0.51[ab]	137.6 ± 10.4[ab]	80.4 ± 6.0[ab]	10.8 ± 2.5[ab]
	H2	#212.4 ± 37.7[ab]	#2.79 ± 0.56[abc]	143.5 ± 9.5[ab]	90.2 ± 6.2[abc]	13.1 ± 1.9[ab]
	S1	#260.2 ± 44.4[abc]	#3.27 ± 0.63[abcd]	156.9 ± 12.3[abc]	105.4 ± 8.5[abcd]	15.5 ± 1.8[abcd]
	S2	#268.4 ± 45.3[abcd]	#3.33 ± 0.64[abcd]	161.8 ± 9.3[abcd]	109.3 ± 10.5[abcd]	17.5 ± 2.1[abcd]
Female	M1	45.1 ± 29.9	0.99 ± 0.18	106.0 ± 15.2	49.5 ± 9.9	6.7 ± 0.8
	M2	73.0 ± 30.0[a]	1.30 ± 0.17[a]	118.8 ± 15.2[a]	62.7 ± 10.0[a]	7.9 ± 1.3
	H1	107.4 ± 42.5[ab]	1.67 ± 0.45[ab]	137.4 ± 21.22[ab]	80.4 ± 8.3[ab]	10.1 ± 1.6[ab]
	H2	127.8 ± 40.0[ab]	1.91 ± 0.47[ab]	150.0 ± 18.7[ab]	92.6 ± 7.0[abc]	11.4 ± 2.9[ab]
	S1	154.8 ± 40.5[ab]	2.12 ± 0.46[ab]	161.7 ± 13.1[abc]	105.3 ± 8.5[abcd]	14.5 ± 1.6[abcd]
	S2	165.5 ± 38.47[ab]	2.21 ± 0.47[abc]	167.1 ± 13.0[abc]	112.7 ± 10.6[abcd]	14.9 ± 3.7[abcd]
Intensity effect		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Sex effect		**<0.01**	**<0.001**	=0.11	=0.39	=0.28

Data are expressed as Mean ± SD: Power Output (PO), Oxygen uptake ($\dot{V}O_2$), Heart Rate (HR), relative intensity to RCP (%RCP) at the 5th minute, and ratings of perceived exertion (RPE) during the different constant workload exercise (M1, M2, H1, H2, S1, and S2) in males and females. The main effects for the relative exercise intensity and sex are shown in the bottom line of the tables. [a] indicates a significant difference from M1, [b] from M2, [c] from H1, [d] from H2, and [e] from S1; Lastly, [#] indicates a significant difference from females at the same relative exercise intensity.

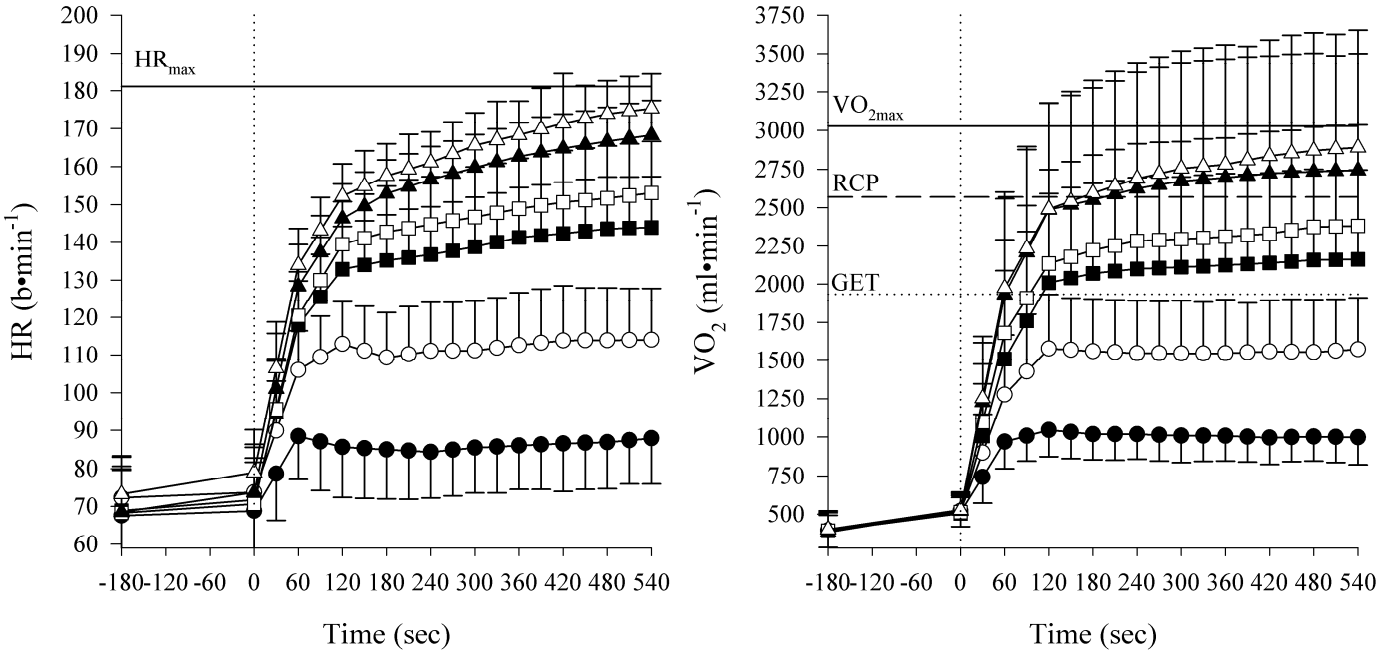


Figure 1. Data are expressed as Mean± SD of the heart rate (HR) and oxygen uptake ($\dot{V}O_2$) during the different constant work rate exercise (M1 ●; M2 ○; H1 ■; H2 □; S1 ▲; S2 Δ). As identified from the ramp test, peak values are displayed as horizontal lines in both panels. GET and RCP were plotted as, respectively, dotted-line and dash-line.

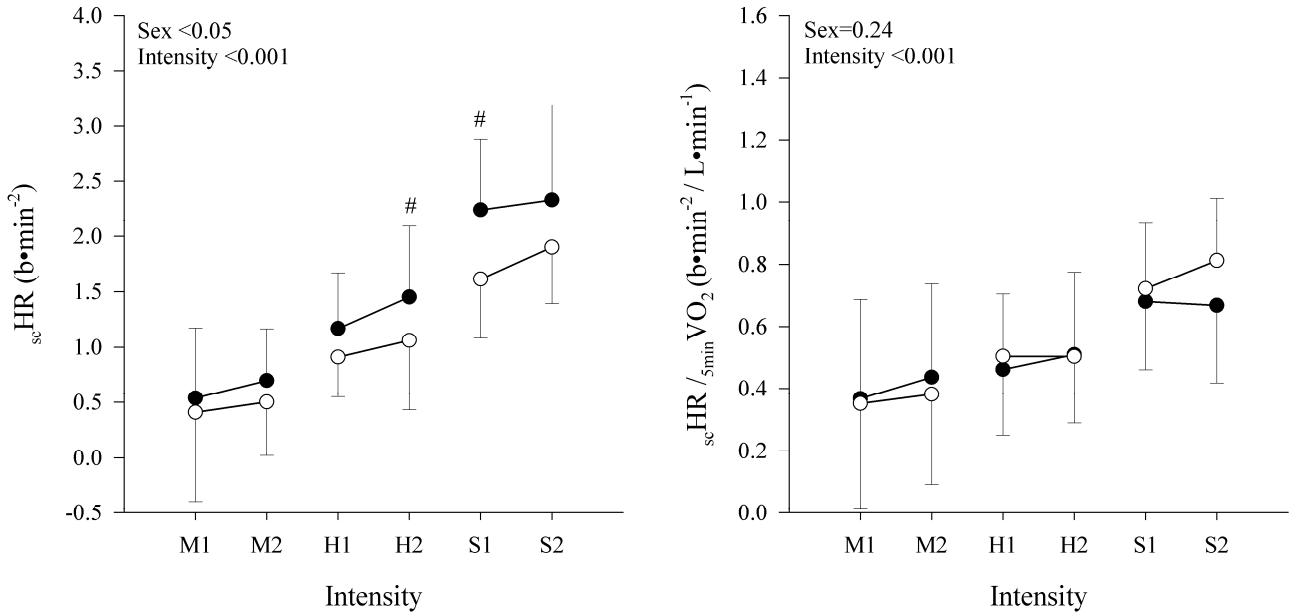


Figure 2. Absolute HR slow component ($_{sc}$HR) (left panel) and relative to the $\dot{V}O_2$ at the 5th min (right panel) are displayed as mean ± SD during different constant workload exercises (M1, M2, H1, H2, S1, and S2). Males (●) and females (○). $^{\#}$indicates a significant difference from females at the same exercise intensity.

Step 2:

To investigate the external validity of the present equations, we tested the model on an independent sample (Table 3 for individual features). A paired t-test showed significant increments in the HR from the 5th min to the end (12 ± 3 min) of the exercise (129 ± 21 vs 136 ± 24 b·min^{-1}, $p<0.05$).

A mixed RM-ANOVA showed higher end-exercises HR in females compared to males ($p<0.001$), however no difference between the measured and estimated HR ($p=0.86$) and no interaction (sex x method, $p=0.82$) (respectively estimated and measured HR for: females, 146 ± 27 and 145 ± 26 b·min^{-1}; males, 126 ± 19 and 127 ± 18 b·min^{-1}).

Figure 3 displays correlation and Bland Altman analysis performed between the measured and estimated end-exercises HR, indicating an excellent correlation and correspondence with small, non-significant bias of -0.01 ± 4.09 b·min-1 (LoA: lower = -7.84 b·min^{-1}, upper = 7.81 b·min^{-1}; z score = -0.04).

Table 3. Anagraphic, anthropometric, and cardiorespiratory variables of the Step 2 subjects.

Group	**#**	**Age**	**Height**	**Weight**	**BMI**	**HR_{max}**	**$\dot{V}O_{2max}$**	**GET**	**RCP**
		(yrs)	*(cm)*	*(kg)*	*($kg \cdot m^{-2}$)*	*(bpm)*	*($ml \cdot min^{-1} \cdot Kg^{-1}$)*	*$\%\dot{V}O_{2max}$*	*$\%\dot{V}O_{2max}$*
Males	18	47 ±18	175.7 ±10.1	75.1 ±8.9	25 ±2	170 ±17	40.6 ±11.1	60.1 ±7.2	84.2 ±5.1
Females	18	47 ±16	164.3 ±6.2	56.6 ±6.9	21 ±2	175 ±10	40.8 ±7.0	62.4 ±9.9	82.2 ±5.4
Total	36	47 ±16	170.6 ±10.0	66.0 ±12.0	23 ±3	173 ±14	40.7 ±9.2	61.1 ±8.5	83.1 ±6.0

Data are expressed as means ± SD: age, height, weight, body mass index (BMI), maximal Heart Rate (HR_{max}) and oxygen consumption ($\dot{V}O_{2max}$), gas exchange threshold (GET), and respiratory compensation point (RCP).

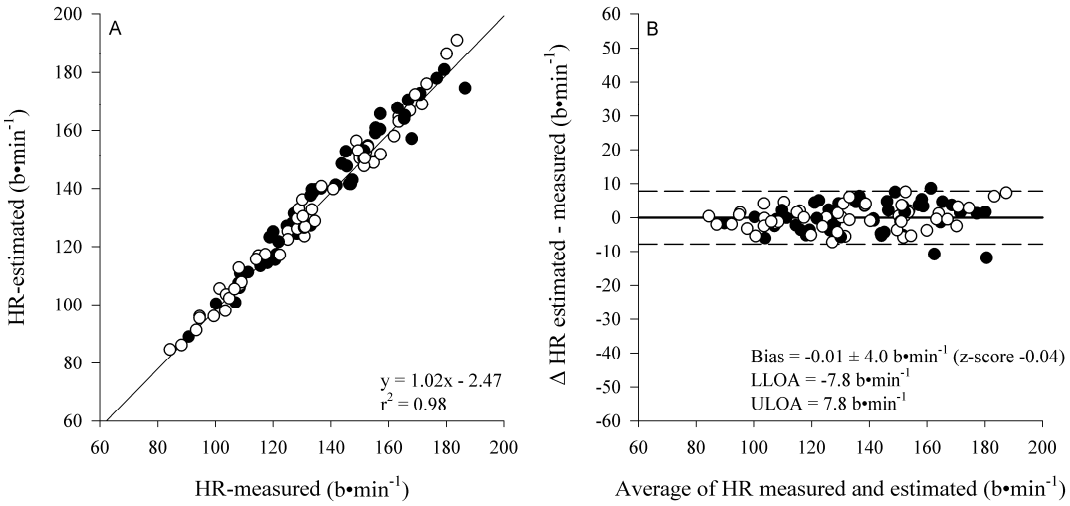


Figure 3. Pearson correlation and Bland Altman analysis between the measured and estimated HR at the end of exercise. Males (●) and females (○). Since no interactions effects sex x method were detected, cumulative coefficient of determination (r^2), bias, z-score, and precision are shown.

Discussion

The purpose of this study was to develop a comprehensive prediction model for $_{sc}$HR across exercise intensities in both sexes and different ages and to test the validity of the developed model using an independent sample of individuals.

The study confirmed the presence of a $_{sc}$HR in all exercise domains, which proportionally increases with relative intensity. Moreover, the study demonstrated for the first time a significant effect of sex and age on the amplitude of the $_{sc}$HR at a given intensity. Finally, the study developed a comprehensive equation that, by including %RCP, sex, and age, accurately predicted $_{sc}$HR in an independent sample of males and females of different ages across the three domains.

The individual and anthropometric characteristics of the subjects enrolled in the study were in line with what we expected from the existing literature for healthy, active individuals (Riebe et al., 2018; Tur & Bibiloni, 2019).

In agreement with previous findings on either male only (Zuccarelli et al., 2018, 2021) or female only (Teso, Colosio, et al., 2022) population our data confirmed the presence of $_{sc}$HR in all domains (including the moderate) and confirmed its relationship with relative exercise intensity (Baldassarre et al., 2022; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021). In male individuals, previous study, described higher $_{sc}$HR slope in all domains compared to our male sample (i.e., mean respectively of ~0.9, ~2.9, and 6.7 b·min^{-2} versus ~0.55, ~1.35, ~2.2 b·min^{-2}, for moderate, heavy and severe domain) (Zuccarelli et al., 2018). This discrepancy may be partially explained by a 10-years age difference in our population. Age is well known to be linearly related to the individual potential of the HR excursion. Higher HR excursions typically characterize young individuals with higher HR reserves compared to older ones (Riebe et al., 2018). Corroborating the above hypothesis our study found age to be a significant negative predictor of the $_{sc}$HR (see equation 1). Moreover, this discrepancy may result from the different methodologies adopted for calculating $_{sc}$HR, i.e., linear fitting from the 3rd minute of exercise in the previous study versus the 5th in our work. It is plausible that, in the least fit individuals, HR may not reach a steady state at the third minute of exercise, possibly leading to an apparently larger $_{sc}$HR (Adami et al., 2011).

Regarding the female population, the $_{sc}$HR in our current study was approximately double the size of data reported in postmenopausal women (i.e., mean respectively of ~0.22, ~0.99, and ~1.8 b·min^{-2} versus ~0.21, ~0.31, and ~0.99 b·min^{-2} for moderate, heavy, and severe domains) (Teso, Colosio, et al., 2022). Again an age difference of ~25 years could

at least partially explain the observed higher $_{sc}$HR dynamic over time in younger compared to postmenopausal women (Teso, Colosio, et al., 2022). It remains to be determined if the phase of the menstrual cycle may affect the dynamic of the $_{sc}$HR component, possibly explaining the differences between normally-menstruating and postmenopausal women. A previous study, that explored the effect of the menstrual cycle on the cardiovascular drift, appears to exclude such a possibility. In fact, the cycle phase was found to affect the absolute HR at given workload, yet not its time-dependent dynamic.

To our knowledge, this is the first study directly comparing the $_{sc}$HR between age-matched adults of both sexes, across several exercise intensities and domains. Our data show a $_{sc}$HR in females approximately 1/3 lower than males at the same relative exercise intensity.

A possible explanation for sex difference in $_{sc}$HR may derive from metabolic heat production on one hand and heat dissipation capacity on the other hand, both of which will affect core temperature and, possibly, the HR drift over time (Gagnon & Kenny, 2012; Rowell, 1974; Yanovich et al., 2020). The metabolic heat production for a given relative exercise intensity will be higher in individuals with higher absolute $\dot{V}O_{2max}$ (e.g., for trained vs. untrained, young vs. old, and male vs. female, heavier vs. lighter individuals) (Gagnon & Kenny, 2012; Yanovich et al., 2020). Indeed: i) males and females differ anthropometrically, with the first being generally heavier and taller, as well as displaying higher $\dot{V}O_{2max}$ values (Santisteban et al., 2022) and ii) $\dot{V}O_{2max}$ decreases with aging of about ~10% every 10yrs. In the present study, males were, on average, ~17% heavier and ~7% taller compared to female participants and consequently presented a ~30% higher $\dot{V}O_{2max}$ and ~25% higher absolute $\dot{V}O_2$ value at the 5th minute in each matched relative exercise intensity. Although neither $\dot{V}O_{2max}\cdot kg^{-1}$ nor $\dot{V}O_2$ at the 5th minute were found to be a significant predictor of $_{sc}$HR (due to their cross-correlation with relative exercise intensity to RCP), when the individual $_{sc}$HR were normalized for the $\dot{V}O_2$ at the 5th minute, sex difference disappeared, suggesting that absolute oxygen uptake does play a role in the observed sex differences in $_{sc}$HR.

Moreover, the ability to eliminate heat (dissipation) through vasodilation and sweating is affected by body dimensions (mainly related to the ratio between body surface area and mass), sex, age, and aerobic fitness (that affect threshold and sensitivity of the sweating mechanism) (Gagnon & Kenny, 2012; Kenny et al., 2008; Yanovich et al., 2020). Unfortunately, no measures of body temperature, sweating rate, or peripheral blood flow

were taken in our study to allow comparison between sexes and confirm our hypothesis of heat accumulation's contribution to HR dynamics during exercise.

In summary, we think it is fair to hypothesize that metabolic heat production (i.e., absolute $\dot{V}O_2$) and temperature regulation capacity may play a role in the discrepancy observed between sexes and ages. The aforementioned findings and considerations suggest that further investigation is needed to understand the physiological underpinnings of the $_{sc}$HR dynamics.

Whatever the causes of $_{sc}$HR may be, this phenomenon has practical implications in exercise prescription. Whenever exercise is anchored to HR targets, the presence of a $_{sc}$HR that is dissociated from $\dot{V}O_2$ will cause a time-dependent reduction workload (approximately 14% in 17 min) along with a decrease in the metabolic load of the training session (Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021). Notably, at the intensities closest to the exercise intensity boundaries, a time-dependent workload decline could also cause an undesired switch to the lower-intensity exercise domain, with important implications for training outcomes (Jamnick et al., 2020; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021).

In this context, the prediction equation for $_{sc}$HR developed and validated in the current study (equation 1) provides the means to dynamically adapt HR targets over time, avoiding an undesired reduction in absolute and relative training load. This strategy would allow the maintenance of the desired metabolic stimulus throughout an exercise session in a heterogeneous population.

LIMITATIONS

The developed predictive equation does not take into account factors that may potentially affect HR kinetics, such as fatigue, overtraining, nutrition, hydration, and environmental conditions such as temperature and humidity that were controlled for in our study. Moreover, the validity of the predictive equation of $_{sc}$HR needs to be confirmed in longer exercise sessions.

PERSPECTIVE

This investigation demonstrated that $_{sc}$HR is present in all exercise intensity domains, with its amplitude being larger with increasing intensity in males compared to females and in younger compared to older individuals. Whenever the implementation of workload is impossible or impractical, and exercise is prescribed based on HR targets, we need to be mindful of the mismatch between HR and metabolic load that emerges over time. The

adjustment of the HR target over time, which is made possible by our generalizable predictive equation, would grant that the desired stimulus is maintained throughout the exercise session in a given individual.

CHAPTER

5

Testing the Performance of a Novel Strategy that "Minds the Gap" in HR Prescription During a Prolonged Exercise Session

Massimo Teso, Alessandro L Colosio, Jan Boone, Silvia Pogliaghi

Abstract:

During prolonged exercise, heart rate partially dissociated from metabolism displaying the so called heart rate slow component ($_{sc}$HR). If ignored when prescribing exercise with HR targets, the $_{sc}$HR will reduce training intensity. A recent study suggested that $_{sc}$HR can be predicted with a function (model) based on relative exercise intensity, sex, and HR_{max} and therefore account for this issue. **Purpose:** To validate the model in maintaining the desired metabolic stimulus during prolonged exercise and across domains. **Method:** 10 subjects (4 females, 26 ± 4 yrs) performed on a cycle ergometer 30-min trials in both the moderate and heavy domain using two protocols: a constant work rate (CWR) and a HR-target based (model) exercise. We calculated: i) the difference (Δ) between the 5th and 30th min in PO, VO_2, HR, and [LA^-] for each protocol and domain; ii) bias in HR between protocols at 1-min intervals. ΔPO, ΔVO_2, ΔHR, Δ[LA^-] were compared with a control equal to "0" by a one-way RM ANOVA. HR bias were compared between protocols, domains and with a control equal to "0" by a two-way RM ANOVA. **Results:** during CWR, increments in HR were detected in both domains (ΔHR 7.6 ± 1.8 and 18.7 ± 1.9 b·min^{-1} in moderate and heavy, ≠0, $p<0.001$). Increments in VO_2 was detected in the heavy domain only (ΔVO_2 heavy 168 ± 150 ml·min^{-1}, ≠0, $p<0.01$) while no difference in [LA^-] and PO occurred ($p>0.05$ for both comparisons). On the contrary, during the model, all variables increased significantly over time ($p<0.05$, for all comparisons). Lastly, model overestimate HR above 12th min of exercise with a positive HR bias between protocols that emerged in both domains (≠0, $p<0.01$). **Conclusions:** we confirm a $_{sc}$HR in both moderate and heavy domains. When used to guide exercise, the previously proposed predictive model can accurately estimate the HR increments only up to 12 min, while for more prolonged exercises it does not grant the stability of training intensity. Further studies are needed to characterize the HR increments in prolonged exercise.

Introduction

Regular physical activity is able to maintain and enhance cardiometabolic health and function throughout life in any individual (Borde et al., 2015; Gronwald et al., 2020; McLaughlin & Jacobs, 2017). To this aim, exercise training needs to be administered in an appropriate and individualised dose, for which intensity is a key ingredient (Herold et al., 2019; Riebe et al., 2018). In clinical and sports settings, one of the most used methods to prescribe exercise intensity remains the Heart Rate (HR, in absolute or relative to the individual maximal or reserve value) (Achten & Jeukendrup, 2003; Weltman et al., 1989). This is based on the existence of a linear relationship between HR and pulmonary oxygen uptake (either $\dot{V}O_2$ or $\%\dot{V}O_{2max}$) (Achten & Jeukendrup, 2003; Riebe et al., 2018).

However, an increase in HR occurs over time during constant work rate (CWR) exercise in all exercise intensity (also moderate intensity, *i.e.,* also below the gas exchange threshold (GET)) (Baldassarre et al., 2022; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021).

Because this rise in HR is slowly developing during CWR exercise, it has been termed "*slow component*" of the HR kinetics, although it is only partially related to $\dot{V}O_2$ kinetics slow component (Teso, Colosio, et al., 2022). The HR slow component typically refers to the increase in HR during the first 15-20 min of exercise after the transient phase (i.e., first 2-3 min of exercise); and might be the result of the skin blood vasodilation by nitric oxide or the results of blood catecholamine or hyperthermia (Baldassarre et al., 2023; Souissi et al., 2021). For exercises lasting over 20 min, increments in HR have been termed *cardiovascular drift* and attributed to hyperthermia, with decreased stroke volume as either driving cause or secondary effect (Coyle & González-Alonso, 2001; Laginestra et al., 2023). Having said this, weather the *HR slow component* and *cardiovascular drift* are two distinct phenomena or not and if/how they interact are still to be elucidated along with their physiological underpinnings.

In terms of exercise prescription, the practical consequence of neglecting these phenomena is an undesired decrease of metabolic intensity when prolonged aerobic exercise is prescribed using HR targets (as either HR or %HRmax) (Baldassarre et al., 2022; Iannetta, Inglis, Mattu, et al., 2020; Zuccarelli et al., 2021). However, this aspect is not often considered (Lafrenz et al., 2008; Murphy et al., 2009; Teso, Colosio, et al., 2022; Wingo, Ganio, et al., 2012; Zuccarelli et al., 2018).

To date, the HR slow component received considerable attention and has been described at and in function of different exercise intensities (Baldassarre et al., 2022; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018, 2021). Moreover, factors like the level HR_{max} reached

and biological sex has been associated with the magnitude of the HR slow component, and used to develop a predictive model (Teso, Colosio, et al., 2022; Zuccarelli et al., 2018) of the HR slow response ($b \bullet min^{-2}$), up to 15 min (Teso, Rizzo, et al., 2022). What remains to be determined is if this predictive equation retains its validity in more prolonged exercise sessions (i.e., 30-45 min, typical exercise prescription for cardiometabolic health and cardiorespiratory fitness) when the cardiovascular drift phenomenon may start to dictate the slow HR response. In fact, the transition between the HR slow component and the cardiovascular drift has received less attention compared to the extensive characterisation of the slow component alone, and the different physiological responses underpinning these two could translate in different HR increases. In this "land in between", the different physiological responses aimed at maintaining the perfusion of organs as well as body temperature may affect the amplitude of the slow HR response, also in relation to the absolute and relative intensity of exercise, environmental conditions, and dehydration (Coyle & González-Alonso, 2001; Souissi et al., 2021; Teso, Colosio, et al., 2022; Teso, Rizzo, et al., 2022).

Given the importance of the HR slow responses for exercise prescription both for short and long duration exercise sessions, this study was aimed at testing the performance of the existing predictive model in i) predicting HR throughout the earlier (5-15 min) and later (15-30 min) phases of a prolonged exercise session; ii) maintaining a desired, constant metabolic intensity over time, in two different domains. We hypothesized that the model-predicted HR would not differ from that measured during a constant work rate exercise at the same intensity and that the metabolic stimulus ($\dot{V}O_2$ and $[La^-]$) at the 5^{th} min of exercise would not significantly change over time across exercise intensities when model-predicted HR targets are used.

Methods:

Participants:

Ten healthy individuals were recruited by advertisement within the local community and agreed to participate in this study (Tab 1). Inclusion criteria were individuals aged between 20 and 35 of both sexes. Exclusion criteria were smoking and any medical condition or therapy that could influence the physiological responses during testing. The subjects were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate. All procedures were approved by the Committee for Approval of Human Research - CARU of the University of Verona.

Protocol

After medical clearance and anthropometric measurements (Body mass (digital scale, Seca877, Seca, Leicester, UK), height (vertical stadiometer, Seca, Leicester, UK), and skinfolds thickness (Holtain T/W skinfold calliper, Holtain limited, UK) (Ferrari et al., 2022), subjects visited the laboratory on five occasions within a maximum of two weeks. On the first visit, they performed a ramp incremental test (RI) to exhaustion on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) to determine thegas exchange threshold (GET), respiratory compensation point (RCP) and maximal parameters (VO_{2max} and HR_{max}). On the following appointments, each separated by a minimum of 2 days, subjects performed on the same cycle ergometer, in a randomized order, four different cycling exercises trials of 30 min , two in the moderate (below GET) and two in the heavy domain (between GET and RCP) of exercise. Participants were instructed to avoid caffeine consumption and physical activity, respectively, for at least 8 h and 24 h before each testing session. Tests were conducted at the same time of the day in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity). The ergometer position was chosen during the first visit and recorded for successive appointments. Moreover, to minimize the variability of glycogen oxidation, participants consumed the following standardized meal two hours before all the testing sessions: 500cc of water and 2 $g \cdot kg^{-1}$ of low glycaemic index carbohydrates [37].

Ramp incremental protocol

The ramp incremental protocol consisted of a 4-min baseline cycling at 50 for women and 100 watts for men, followed by respecively 15 or 30 $watt \cdot min^{-1}$ increase in power output until voluntary exhaustion. Participants were asked to pick a self-selected cadence in the 70-90 rpm range and maintain it throughout all protocols. Breath-by-breath pulmonary gas exchange, ventilation, and heart rate were continuously measured using a metabolic cart (Quark B2, Cosmed, Italy) (de Roia et al., 2012).

Trials

Subject performed in a randomized order, two protocol per domain as described below. Each trial in both protocols lasted 33 minutes and consisted of 3-minute freewheeling cycling followed by an instantaneous increase in power output. The initial absolute power output for the two intensities was chosen as follows:

i) Moderate trials (M): 66% of the difference between $\dot{V}O_2$ at rest and GET

ii) Heavy trials (H): 66% of the difference between $\dot{V}O_2$ at GET and RCP

To identify the power output that elicits the $\dot{V}O_2$ target of the two intensities, the individual $\dot{V}O_2$/power output relationship derived from the incremental exercise was corrected for the $\dot{V}O_2$ mean response time and slow component by applying the mathematical model recently proposed by Caen et al. (Caen et al., 2020).

The two protocol performed were: i) a constant work rate protocol where power output was maintained for the entire session (*CWR,* $M_{cwr,}$ and H_{cwr}, respectively, for moderate and heavy domains) and an ii) HR *target* model where, after the initial 5 min, power output was adjusted by an operator every 30 sec whenever the measured HR exceeded by ±2 beats the HR target derived from the equation model described below. (*Model*, $M_{model,}$ and H_{model}, respectively, for moderate and heavy domains), To this aim, the HR/PO relationship from the ramp test was determined. The HR *target* was determined as follows:

$HR(t) = HR_{@5min} + ({}_{sc}HR \times (t - 5))$

Where HR(t) is the increase of HR at time t above the rest (i.e., HR *target*), $HR_{@5min}$ is the mean HR at the 5th min, and ${}_{sc}HR$ estimated with the predictive equation model:

(1) ${}_{sc}HR\ (b \cdot min^{-2}) = -0.0514 + (0.0240 \times \text{intensity expressed in \%RCP}) - (0.0172 \times \text{age}) - (0.347 \times \text{Sex})$

Where: a) intensity expressed in %RCP, calculated based on the mean $\dot{V}O_2$ at the 5th min of each trial relative to the individual value at RCP; b) sex as score 0 and 1 for male and female, respectively.

In both protocols, we measured breath-by-breath pulmonary gas exchange, ventilation, and HR with the same method described for the ramp incremental protocol. Capillary blood samples (20 µl) were drawn from the ear lobe during the freewheeling and at the 3rd, 10th, and 30th min of exercise and immediately analyzed using an electro-enzymatic technique (Biosen C-Line, EKF Diagnostics, Barleben, Germany).

Data -analysis:

In both protocols, gas exchange variables and HR were treated as follows: aberrant data points (3 standard deviations away from the local mean) were removed; thereafter, data were linearly interpolated at 1-s and then averaged at 5-s intervals (Colosio, Teso, et al.,

2020). GET and RCP were identified by three experts independently using the standard technique (Beaver et al., 1986). $\dot{V}O_{2max}$ and HR_{max} were determined, respectively, as the average $\dot{V}O_2$ of the last 30-s of exercise and the highest HR achieved upon exhaustion (Colosio et al., 2019).

Lastly, we calculated: i) a 1-min mean every minute of $\dot{V}O_2$, HR, and PO from the rest to the end of exercise; ii) the Δ difference between the 30th and the 5th min for PO (ΔPO), $\dot{V}O_2$ ($\Delta\dot{V}O_2$), HR (ΔHR) and [LA^-] (Δ[LA^-]); and iii) the bias HR every minute between *CWR* and *model* protocols .

Statistical analysis

Data description. All data are presented as mean ± SD, after assumption verification (i.e., normality, homogeneity of variance). A two way RM anova was performed to compare PO, $\dot{V}O_2$, %RCP, HR, and [LA^-] across exercises domains and protocols.

To test the performance of the equation model in i) maintaining a desired, constant metabolic intensity over time, a two-way RM ANOVA was performed to compare ΔPO, $\Delta\dot{V}O_2$, ΔHR, Δ[LA^-] across exercise domains and protocols and versus a control value equal to "0" and ii) predicting HR during prolonged exercise session a one-way ANOVA was performed to compare the HR bias over time versus a control value equal to "0".

Post hoc analyses were performed using the Holm-Sidak method. Power analysis was conducted a priori, based on the expected SD of HR seen during constant load exercise in previous articles (Baldassarre et al., 2022; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021) as the main variable. To identify significant differences, with an α error of 0.05 and a statistical power (1 − β) of 0.95, a n value of 10 subjects was necessary (G*Power 3.1). All statistical analyses were performed using SigmaPlot version 14.0, and α was set in advance at the 0.05 level. Statistical significance was accepted when $P < \alpha$.

Results

Participants' characteristics and maximal parameters derived from the ramp incremental test are reported in Table 1. The average BMI and $\dot{V}O_{2max}$ were indicative of a normal weight and active population (Garber et al., 2011). Subjects' GET and RCP were detected at $\dot{V}O_2$ values of 1.96 ± 0.42 l·min^{-1} (64 ± 4% $\dot{V}O_{2max}$) and 2.63 ± 0.54 l·min^{-1} (86 ± 4% $\dot{V}O_{2max}$), respectively.

For both protocols, an overview of the participants' mean ±SD responses at the 5th min of exercise are reported in Table 2; the profiles of the variables are displayed as a function of time in Figure 1. A two-way RM ANOVA (domain x protocol) on PO, $\dot{V}O_2$, %RCP, HR, and [LA^-] values at 5th min showed, as expected, a significant effect of domain only (for all variables, $p<0.001$), whereas no main effect of protocols was found in any variable.

To test if the model became different from CWR protocol:

A two-way RM ANOVA (domain x protocol) on ΔPO, $\Delta\dot{V}O_2$, ΔHR, Δ[LA-] showed a significant effect of domain and protocol for all variables ($p<0.05$ for all comparisons). The post-hoc analysis results are shown in Table 3.

To test when the equation model became different from the CWR protocol:

Bias between HR measured during both *model* and CWR protocol is shown in Figure 2. A two-way RM ANOVA shows a main effect for time and domain ($p<0.001$ for both). HR bias became significantly different from "0" from 11th and 13th min moderate and heavy domain exercise, respectively ($p<0.01$ for both comparisons).

Table 1. *Anagraphic, anthropometric, and functional characteristics of the subjects*

Group	**#**	**Age**	**BMI**	**HR_{max}**	**$\%HR_{max}$**	**$\dot{V}O_{2max}$**	**R_{max}**	**GET**	**RCP**
		(yrs)	*($kg \cdot m^{-2}$)*	*($b \cdot min^{-1}$)*	*%*	*($ml \cdot min^{-1} \cdot Kg^{-1}$)*		*$\%\dot{V}O_{2max}$*	*$\%\dot{V}O_{2max}$*
Males	6	26 ± 2	24 ± 2	189 ± 1	98 ± 4	51.4 ± 4.6	1.30 ± 0.09	60 ± 3	83 ± 3
Females	4	24 ± 2	20 ± 1	192 ± 1	99 ± 4	47.9 ± 5.8	1.17 ± 0.04	64 ± 2	85 ± 3
Total	10	25 ± 2	22 ± 2	191 ± 2	98 ± 5	49.4 ± 6.5	1.22 ± 0.09	62 ± 4	84 ± 4

Values are expressed as mean ± SD: Body mass index (BMI), maximal heart rate (HR_{max}), percentage of theoretical HR_{max} ($\%HR_{max}$), maximal oxygen uptake ($\dot{V}O_{2max}$), maximal respiratory exchange ratio (RER_{max}), gas exchange threshold (GET), and respiratory compensation point (RCP).

Table 2. *Variables at the fifth minute for both protocols and domains.*

Domain	Protocol	PO	$\dot{V}O_2$	%RCP	HR	[LA^-]
		(Watt)	*($ml \cdot min^{-1}$)*	*(%)*	*($b \cdot min^{-1}$)*	*[$mmol^{-1}$]*
Mod	***model***	69.3 ± 20.1	1492.1 ± 277.8	55.3 ± 7.1	110.8 ± 18.2	1.28 ± 0.48
	CWR	69.3 ± 20.1	1491.6 ± 322.7	55.2 ± 8.3	109.9 ± 19.6	1.29 ± 0.76
Hvy	***model***	164.7 ± 49.9*	2617.1 ± 549.3*	96.9 ± 4.72*	142.3 ± 4.5*	4.69 ± 1.93*
	CWR	164.7 ± 49.9*	2473.4 ± 632.4*	91.6 ± 5.02*	146.9 ± 9.6*	4.87 ± 1.67*
Domain (*p*)		***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
Protocol (*p*)		*=1.00*	*=0.62*	*=0.87*	*=0.54*	*=0.68*

*Mean ± SD of Power Output (PO), Oxygen uptake ($\dot{V}O_2$), relative intensity to RCP (%RCP), heart rate (HR), and Lactate ([LA^-]), at the 5^{th} minute in both protocols (model and CWR) and exercise domains (Mod and Hvy). The main effects of the domain and protocol are shown in the bottom line of the table. *indicates a significant difference from M for the same protocol.*

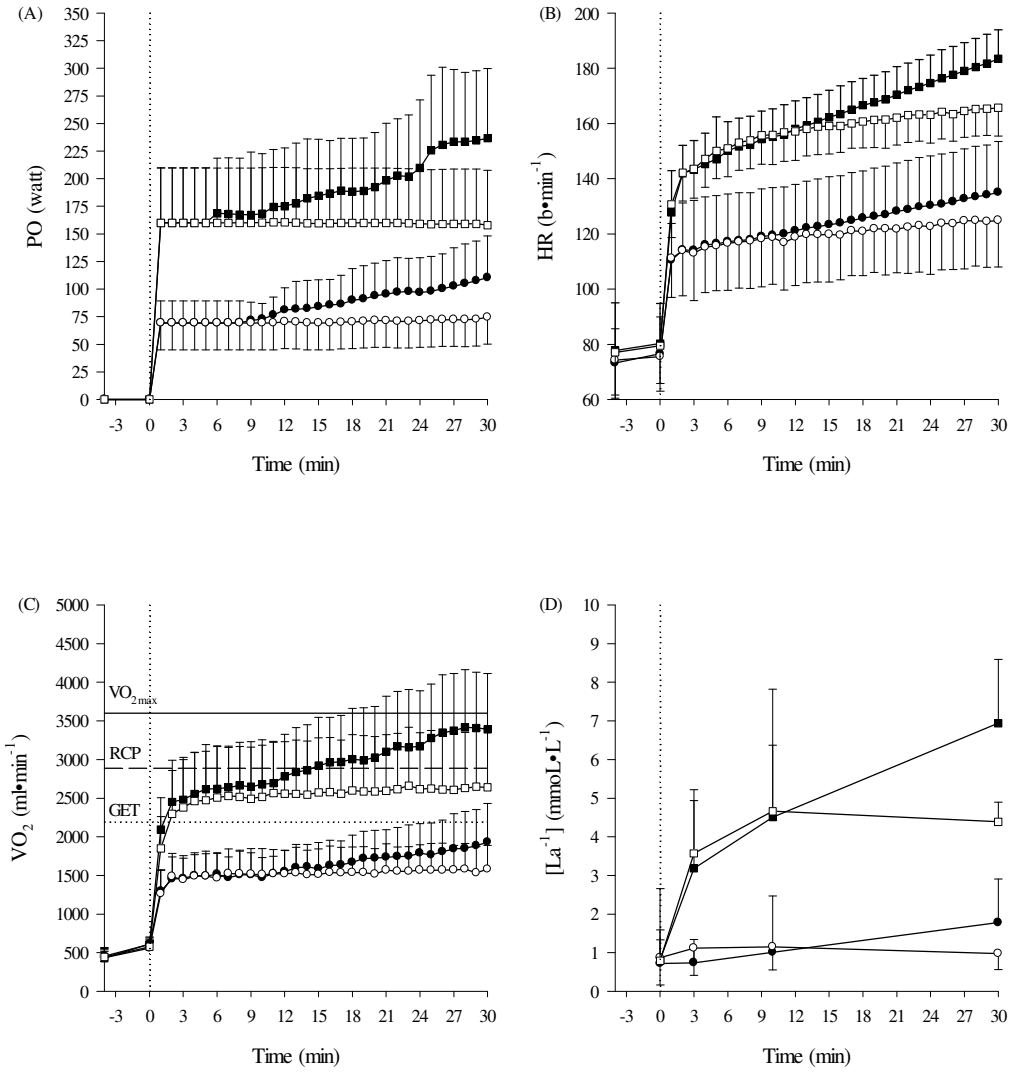


Figure 1. Data collected during the two domains (Moderate & Heavy) on both protocols (model & CWR): M_{model} (●) M_{cwr} (○), H_{model} (■),and H_{CWR} (□) plotted as a function of time. Mean ± SD of power output (A), Heart Rate (B), oxygen uptake (C), and Lactate accumulation (D). The three horizontal line plots in panel C indicate from the lowest to the highest: GET, RCP, and peak value.

Table 3. *Difference between the 30th and the 5th min of exercise.*

Domains	Protocol	ΔPO	$\Delta\dot{V}O_2$	ΔHR	Δ[LA⁻]
		(Watt)	*(ml · min⁻¹)*	*(b · min⁻¹)*	*[mmol⁻¹]*
Mod	***model***	41.2 ±14.2#	438.1 ±135.7#	19.4 ±5.0#	0.76 ±1.13#
	CWR	0.0 ±0.0§	88.9 ±99.8§	7.6 ±1.8§#	-0.17 ±0.42
Hvy	***model***	68.0 ±9.0*#	775.3 ±113.7*#	37.0 ±7.3*#	2.43 ±1.65*#
	CWR	0.0 ±0.0§	168.0 ±150.0*§#	18.7 ±1.9*§#	-0.27 ±0.51§
Domain (p)		***<0.5***	***<0.001***	***<0.001***	***<0.001***
Protocol (p)		***<0.001***	***<0.001***	***<0.001***	***<0.001***

Mean ± SD of the difference between the 30th and the 5th min of Power Output (ΔPO), Oxygen uptake ($\Delta\dot{V}O_2$), Heart Rate (ΔHR), and [Lactate] (Δ*[LA⁻]) during both protocols (model vs CWR) and domains (M vs H). The main effects of domain and protocol are shown in the bottom line of the tables.* **indicates a significant difference from the moderate domain in the same protocol.* *§ indicates a significant difference from model in the same domain;* *# indicates a significant difference from "0".*

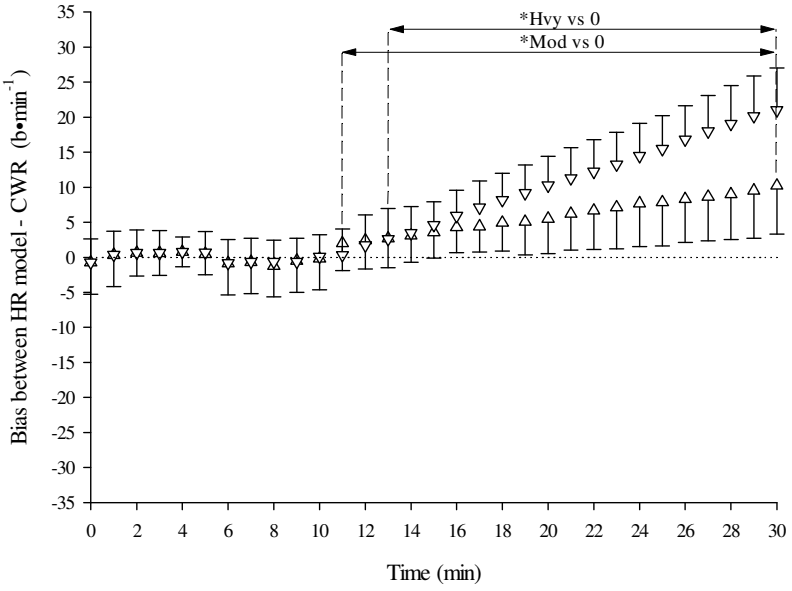


*Figure 2. Mean ± SD of the HR bias over time between HR measured during the model and CWR protocol on moderate and heavy domains. Moderate (△) and heavy domain (▽). *indicate the significant difference from "0".*

Discussion

This study aimed to test the performance of the previously developed multiple linear equation in predicting the delayed increase in HR and maintaining a constant metabolic stimulus over time across exercise domains. This study confirmed the presence of a slow component of HR during a constant workload exercise in both the moderate and heavy domains and confirmed its relationship with relative exercise intensity. However, the predicted slow component of HR was significantly larger than the actual response in both domains. As a result, when HR targets predicted from the multiple linear equation were used to guide exercise intensity, the absolute intensity, rather than remaining constant, rose over time; consequently, the metabolic load ($\dot{V}O_2$ and [La^-]) departed from the response of the constant workload exercise measured at the 5^{th} minute for both the moderate and heavy domain. In more detail, the HR prediction model accurately estimated the delayed increase in HR up to the 11^{th} min of exercise in the moderate and 13^{th} min in the heavy domain (i.e., the early phase of the response called the HR slow component) yet not thereafter. Therefore, the HR prediction derived from the originally formulated model is confirmed as an effective strategy to maintain a constant metabolic intensity in short-duration exercises only; above 10-15 min, however, this approach loses its accuracy.

The individual and anthropometric characteristics of the subjects enrolled in the study were in line with what we expected from the existing literature for healthy and active individuals (Riebe et al., 2018). However, the average relatively high value of VO_{2max}, in both males and females (respectively: 51.4 ±4.6 and 47.9 ±4.3 $ml \cdot min^{-1} \cdot Kg^{-1}$), places them between the 75^{th} and 80^{th} percentiles of the age- and sex-specific American College of Sport Medicine fitness distribution (Riebe et al., 2018).

The measured metabolic intensity aligned with the desired metabolic target during the first 5 min of each exercise trial. The average $\dot{V}O_2$ of ~1.5 ± 0.3 and 2.5 ± 0.6 $l \cdot min^{-1}$ placed the two tested intensities respectively at 2/3 of the moderate and the heavy domain. This confirms the validity of the approach used for the translation of a desired metabolic target derived from a ramp incremental protocol into an equivalent load for a constant exercise (Caen et al., 2020). Moreover, there was no difference in the metabolic load (both $\dot{V}O_2$ and [LA^-]) between trials at the same intensity up to the 5^{th}-minute exercise, confirming no significant between-days variability.

In agreement with the literature, our findings confirmed the absence of increments in $\dot{V}O_2$ over time in the moderate domain beyond the 5^{th} min of the constant workload trials as

well as the existence of a $\dot{V}O_2$ slow in the heavy domain, with an amplitude similar to that observed in comparable conditions (i.e. ~168 ml·min^{-1}) (Colosio et al., 2021; Gaesser & Poole, 1996; Poole & Jones, 2012).

Regarding the heart rate slow component, our data confirmed its presence during constant workload exercises with a lower slope in the moderate compared to the heavy (respectively increments of ~0.30 vs ~0.75 b·min^{-2}) (Teso, Colosio, et al., 2022; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018). The absolute slopes of HR in our study appear to be roughly in between the values previously reported for longer (i.e., cardiovascular drift, ~0.18 b·min^{-2} in the moderate domain) (Coyle & González-Alonso, 2001; González-Alonso & Calbet, 2003) and shorter exercises duration (i.e., HR slow component, ~0.56 and ~1.15 b·min^{-2} in the moderate and heavy domain, respectively) (Baldassarre et al., 2022; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018, 2021) in young individuals at comparable exercise intensities. We can hypothesize that in our study, in which the slope of the scHR was calculated from the 5th to the 30th min, the earlier and later components of the slow adjustment of HR could coexist, leading to an overall slope of the adjustment that is close to the average of the two components.

The delayed increase in HR during constant-load exercises has been originally described as secondary to or causing a reduction of stroke volume (Bourgois et al., 2023; Coyle & González-Alonso, 2001; Souissi et al., 2021). This phenomenon has been typically observed starting from ~20 min into a constant load exercise in the moderate domain and up to 1-2h and has been named “Cardiovascular drift” (Coyle & González-Alonso, 2001; Laginestra et al., 2023; Souissi et al., 2021). More recently, an early increment in HR that emerges after the initial steady state (i.e., ~3-5 min) has been described (Baldassarre et al., 2022; Teso, Colosio, et al., 2022; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018, 2021). This phenomenon has been typically studied during exercises of shorter duration (up to 15-20min), not associated with a drop in stroke volume and only partially linked to a slow increase in VO_2, is known under the name of HR slow component (Baldassarre et al., 2022; Teso, Colosio, et al., 2022; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018, 2021). Its physiological underpinnings remain elusive and its origin has been attributed to the direct chronotropic effect of hyperthermia, nitric oxide, catecholamines and/or signalling from exercising muscles (Souissi et al., 2021; Zuccarelli et al., 2018). While the distinction between HR slow component and cardiovascular drift is not universally accepted (Zuccarelli et al., 2018), previous studies reported higher increases in HR in the initial phase of exercise (i.e., $_{sc}$HR) compared to the cardiovascular drift, suggesting an

attenuation of the dynamics over time (Ganio et al., 2006; Souissi et al., 2021; Wingo, Ganio, et al., 2012; Wingo, Salaga, et al., 2012; Zuccarelli et al., 2018).

Compatible with the idea of a distinction between an earlier and a later slow HR response, our study observed a faster increment of HR up to ~12 min of exercise. This earlier increase overlapped the prediction of our model protocol in which an estimation of the slow component of HR was used to guide the exercise. The model prediction estimated the slow component of HR based on relative exercise intensity, sex, and HR_{max} and had been previously validated for exercise up to 15 min in a wide age range and both sexes (study 2 of this thesis) (Teso, Rizzo, et al., 2022). Our current data further confirm the accuracy of the prediction model, yet limiting its validity over time. In fact, above ~12 min, the slope of the HR increment was overestimated by the equation model, driving the Heart Rate excessively high and causing a rise over time in the metabolic load ($\dot{V}O_2$ and $[LA^-]$) in both domains.

To the best of our knowledge, no studies have developed an equation to estimate the slow HR kinetics during the cardiovascular drift (i.e., up to 1-2h;) or to describe it relative to the subjects' anthropometric and/or fitness characteristics. Our current findings appear to indicate that above ~12-15 min the HR slow response is attenuated by ~50% compared to that observed between 5 and 15 min. This indicates that a more complex mathematical model is probably required to dynamically correct the HR target and maintain the desired metabolic intensity throughout exercises prolonged above 15 min.

In conclusion, this investigation demonstrated that the slow increment in HR, from the initial steady state and up to 30-min constant exercise, is not accurately tracked by a single linear model but is characterized by a more complex time-dependent change. To deal with this unanticipated complexity, further studies will need to clarify if the HR slow component and the cardiovascular drift represent distinct phases of the adjustment and if they can be linked to specific physiological underpinnings.

Whatever the definitions and the physiological bases of the observed phenomena, we need to be mindful of the existence of a slow HR response over time whenever the exercise is prescribed using HR targets to avoid undesired reduction in power output and metabolic load over time.

Limitations. The developed predictive equation does not consider factors that may potentially affect HR kinetics, such as fatigue, overtraining, nutrition, hydration, and

environmental conditions such as temperature and humidity that were controlled for in our study.

CHAPTER

6

Characterization of the Heart Rate Slow Component and Development of a Dynamic HR-target Model

Massimo Teso, Alessandro L Colosio, Jan Boone, Silvia Pogliaghi

Abstract:

A predictive linear equation was validated to estimate the heart rate slow component ($_{sc}$HR) across exercise intensities. However, a time-dependent change in the $_{sc}$HR kinetics emerges during prolonged exercise. **Purpose:** to characterize $_{sc}$HR kinetics in prolonged exercise to develop and validate a predictive equation. **Method:** 19 individuals (10 females; 25 ± 3 yrs) performed: ii) 2x30-min constant-load trials (1<GET, 1 >GET/<RCP). From the 5th min exercise, different fitting models were performed on the $_{sc}$HR. The best-fitting model was determined by the least sum of the squared residual (SQR) across domains (two-way RM ANOVA, model X domain). An equation model was developed to predict HR in prolonged exercise (*dynamic* HR-*target)* based on individual characteristics and fitting parameters. In an independent sample, we tested the ability of the developed equation to maintain a constant metabolic stimulus in both moderate and heavy domains. Power output (PO), oxygen uptake ($\dot{V}O_2$) and lactate concentration [LA^-] from the 5th to the 30th min of exercise were compared during exercise prescribed using i) *dynamic* HR-*target* and ii) *clamped* HR (two-way RM ANOVA, protocol x domain). **Results:** A double linear fitting showed less SQR ($p<0.001$) by detecting a reduction in the $_{sc}$HR slope from the ~13th min in both domains (time delay ~808"; reduction ~48%). During *clamped* HR exercise, PO, $\dot{V}O_2$, and [LA^-] decreased ($\neq 0$, $p<0.05$ in all comparisons, respectively of 14, 10, and 15%) while using the *dynamic* HR-*target* all variables remained stable in both domains ($\neq 0$, $p>0.5$ in all comparisons). **Conclusion:** The dynamic of $_{sc}$HR can be accurately predicted, accounting for the combined effects of relative intensity, sex, age, and time. The above equation informs the adjustment of HR targets over time to maintain the desired metabolic stimulus throughout aerobic exercise sessions.

Introduction

Regular aerobic exercise is a powerful "medicine" producing several long-term adaptations that improve health, mobility, and well-being in a dose-response manner (McLaughlin & Jacobs, 2017; Riebe et al., 2018). Aerobic exercise is typically prescribed in terms of absolute or relative metabolic stimulus to achieve a specific intensity component of the dose and, in turn, of desired long-term adaptations(Garber et al., 2011; Riebe et al., 2018). Exercise intensity is often prescribed using a fixed or *clamped* heart rate (HR) target, corresponding to a given percentage of maximal or reserve HR (Gormley et al., 2008; Ivey et al., 2007; Macko et al., 2005; Nybo et al., 2010; Piepoli et al., 2016). Rooted in the linear relationship between HR and oxygen uptake ($\dot{V}O_2$) (Legge & Banister, 1986) and the continuous tracking of HR (Achten & Jeukendrup, 2003; Brooks A. et al., 2005; Colosio et al., 2018; Colosio, Lievens, et al., 2020a), the *clamped* HR approach has allowed easy and low-cost monitoring of the intensity element of the exercise dose during practice in both clinical and sports settings (Achten & Jeukendrup, 2003; Gormley et al., 2008; Ivey et al., 2007; Macko et al., 2005; Nybo et al., 2010; Piepoli et al., 2016).

However, the validity of HR as an index of metabolic intensity has been recently questioned (Iannetta, Inglis, Mattu, et al., 2020; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018). During prolonged constant work exercise, an increase in HR over time called the HR slow component ($_{sc}$HR), has been described in healthy adults (Zuccarelli et al., 2018), people with obesity (Zuccarelli et al., 2021), and postmenopausal women (Teso, Colosio, et al., 2022). Only marginally related to the $\dot{V}O_2$ slow component, the $_{sc}$HR is observed in all domains, its amplitude increasing as a function of relative intensity (Teso, Colosio, et al., 2022; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018). Ignoring the presence of the $_{sc}$HR when prescribing exercise based on *clamped* HR targets will result in an undesired reduction of the $\dot{V}O_2$ and workload over time (Baldassarre et al., 2022, 2023; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021). To grant that the desired stimulus is maintained throughout the exercise session, a predictive equation able to estimate the $_{sc}$HR across different relative exercise intensities has been developed and validated for exercise sessions up to 15 min in a heterogeneous population (both sex and wide-age range) (Teso, Rizzo, et al., 2022).

(2) $_{sc}$HR ($b \cdot min^{-2}$) = -0.0514 + (0.0240 x intensity expressed in %RCP) - (0.0172 x age) - (0.347 x Sex)

Previous studies that analyzed the HR increments during constant-load exercises in different time windows (i.e., < or > 15 min) reported higher increases in HR in the initial phase of the exercise (i.e., <15min) compared to the later phase (i.e.,> 15 min), suggesting an attenuation of the dynamics over time (Coyle & González-Alonso, 2001; Ganio et al., 2006; Logan-Sprenger et al., 2012; Souissi et al., 2021; Stone et al., 2021; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018). Indeed, in study number 3, in which we tested the validity of our predictive equation in prolonged exercise (1), we found that above ~13 min, the slope of the HR increment was overestimated by our equation, driving the heart rate excessively high and causing an undesired rise over time in the metabolic load ($\dot{V}O_2$ and [LA^-]). This would indicate that after the initial 15-min phase, the slow increment in HR may not be accurately tracked by a single linear equation but may require more complex time-dependent modeling.

The mechanism underlying the increments in heart rate observed above 3-5 and up to 15-20 min of exercise is still unclear (Souissi et al., 2021; Zuccarelli et al., 2018). Some authors suggest that the increase in heart rate, usually associated with increased skin blood flow and cardiac output, could be partially related to the β-adrenergic stimulation (Zuccarelli et al., 2018). On the contrary, above ~15-20min into exercise, it has been suggested that the increase in the heart rate is secondary to i) a later-onset reduction in stroke volume due to diastolic fatigue (altered calcium reuptake would induce decreased left ventricular compliance and, therefore, reduced ventricular filling) (Souissi et al., 2021) ii) hyperthermia-induced vasodilation (Laginestra et al., 2023; Wingo & Cureton, 2006) and/or ii) sweating-related hypovolemia (reduced venous return and ventricular filling) (Ganio et al., 2006; Wingo, Ganio, et al., 2012). Based on the above, it is plausible that the complex nature of the earlier and later components of the HR slow increase may be better represented by a more complex equation.

Therefore, in order to determine if more complex modelling is necessary for the accurate anticipation of the $_{sc}$HR in exercise sessions lasting >15 min, we performed a two-step study to:

In step 1, develop a comprehensive modelling strategy for the characterization of the time-dependent change in the HR in prolonged exercise by testing the performance of different fitting models (linear, double linear) and the predictive equation model (1);

In step 2, test the performance of the comprehensive model in maintaining a constant metabolic stimulus ($\dot{V}O_2$ and [LA^-]) in an independent sample by comparing exercise

prescribed using a *dynamic* HR-*target* derived from the comprehensive model versus a standard *clamped* HR approach.

Methods

Participants

A total of thirty-five healthy individuals were recruited by advertisement within the local community and agreed to participate in this two-step study. The whole group was randomly subdivided into two subgroups balanced for sex and age. As a result, nineteen subjects (10 female) participated in *step 1, characterization of the HR slow component kinetics,* and sixteen (8 female) in *step 2, validation of the equation model for prolonged exercise* (see respectively, Table 1 and Table 5 for participants' characteristics). Inclusion criteria were individuals of both sexes aged between 20 and 36. Exclusion criteria were smoking and any medical condition or therapy that could influence the physiological responses during testing. The subjects were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate. The study was approved by the Ethics Committee of the University of Verona (CARP) and conducted in conformity with the Declaration of Helsinki (no. 16-2019).

Protocol

After medical clearance, the subjects' main anthropometric measurements of body mass (digital scale, Seca877, Seca, Leicester, UK) and height (vertical stadiometer, Seca, Leicester, UK) were collected (Ferrari et al., 2022) and a total of 3 to 5 visit were performed on the following days.

During visit 1, all participants performed a ramp incremental test to exhaustion on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) for gas exchange threshold (GET), respiratory compensation point (RCP) and maximal parameters detection (VO_{2max} and HR_{max}). On the following appointments: *step 1* participants performed two constant work rate exercises, one in the moderate (<GET) and one in the heavy domain (>GET, <RCP); *Step 2* participants' performed in both moderate and heavy domain two different protocols i) an HR *clamp* protocol and ii) a *dynamic HR-target model* based on the developed equation derived from *step 1*. Within each step, trials were randomized and counterbalanced. Participants were instructed to avoid caffeine consumption and physical activity, respectively, for at least 8 h and 24 h before each

testing session (de Roia et al., 2012). All visits were separated by at least 48 hours and completed within 30 days. Tests were conducted at the same time of the day in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity). Ergometer position was chosen during the first ramp incremental test and recorded for the successive appointments. Moreover, to minimise the variability of glycogen oxidation, participants consumed the following standardised meal two hours before all the testing sessions: 500cc of water and 2 $g \cdot kg^{-1}$ of low glycaemic index carbohydrates, as detailed elsewhere (Colosio, Lievens, et al., 2020a).

Ramp incremental protocol

The ramp incremental protocol consisted of a 4-min baseline cycling at 50-100 watts, followed by a 15-30 $watt \cdot min^{-1}$ increase in power output until voluntary exhaustion (Boone & Bourgois, 2012; Keir, Paterson, et al., 2018). Participants were asked to pick a self-selected cadence in the 70-90 rpm range and maintain it throughout all protocols. Breath-by-breath pulmonary gas exchange, ventilation, and heart rate were continuously measured using a metabolic cart (Quark B2, Cosmed, Italy) (de Roia et al., 2012). Capillary blood samples (20 µl) were drawn from the ear lobe before and at the 1^{st}, 3^{rd}, $5^{th,}$ and 7^{th} min after exhaustion. Samples were immediately analysed using an electro-enzymatic technique (Biosen C-Line, EKF Diagnostics, Barleben, Germany), and the highest value was considered the peak of blood lactate concentration for the incremental test.

Trials

All trials for both steps lasted 33 minutes and consisted of 3-minute freewheeling cycling followed by an instantaneous increase in power output. The initial absolute power output for the two intensities was chosen as follows:

i) Moderate trials: 66% of the difference between $\dot{V}O_2$ at rest and GET

ii) Heavy trials: 66% of the difference between $\dot{V}O_2$ at GET and RCP

To identify the power output that elicits the $\dot{V}O_2$ target of the two intensities, the individual $\dot{V}O_2$/power output relationship derived from the incremental exercise was corrected for the $\dot{V}O_2$ mean response time and slow component by applying the mathematical model recently proposed by Caen et al. (Caen et al., 2020).

In *step 1*, power output was maintained for the constant work rate protocol.

In *step 2*, after the initial 5 min, two different protocols were proposed: i) HR *clamp*, the HR was maintained constant over time; ii) *dynamic HR-target model (HR_{DTM}),* the HR was kept on the HR *target* derived from the equation developed in *step 1*.

To this aim, power output was adjusted by an operator every 30 sec whenever the live-measured HR exceeded the clamped or target value by ±2 beats. The power output adjustment was derived from the individual ramp test's HR/Power output relationship.

Breath-by-breath pulmonary gas exchange, ventilation, and beat-by-beat HR were measured with the same method described for the ramp incremental. Capillary blood samples (20 µl) were drawn from the ear lobe during the freewheeling and at the 3rd, 10th, and 30th min during exercise and immediately analyzed with the same equipment described for the ramp incremental.

Data -analysis

For all protocols, gas exchange variables and HR were treated as follows: aberrant data points (3 standard deviations away from the local mean) were removed. Thereafter, data were linearly interpolated at 1-s. GET and RCP were independently identified by three experts using the standard technique (Beaver et al., 1986). $\dot{V}O_{2max}$ and HR_{max} were determined, respectively, as the average $\dot{V}O_2$ of the last 30-s of exercise and the highest HR achieved upon exhaustion (Colosio et al., 2019).

Moreover, in all protocols, we calculated: i) 1-min means of $\dot{V}O_2$, HR, and power output from the rest to the end of exercise; ii) the mean $\dot{V}O_2$ at the 5th min of each trial was expressed relative to the respiratory compensation point (%RCP).

For *Step 2* only, we calculated the difference between the 30th and the 5th min for power output (ΔPO), $\dot{V}O_2$ ($\Delta\dot{V}O_2$), HR (ΔHR), and [LA^-] (Δ[LA^-]).

Time course description models:

For each constant work rate exercise, the time course of the $_{sc}$HR was estimated using our original predictive equation (Teso, Rizzo, et al., 2022). I.e.,:

$$HR(t) = HR_{@5min} + (_{sc}HR \times (t - 5)$$

Where HR(t) is the increase of HR at time t above the rest, time is expressed in minutes, and $_{sc}$HR is estimated as follows:

$_{sc}$HR (b·min^{-2}) = -0.0514 + (0.0240 x intensity expressed in %RCP) - (0.0172 x age) - (0.347 x Sex)

Where sex as score 0 and 1 for male and female, respectively;

Kinetics analysis:

$_{sc}$HR kinetics were mathematically evaluated during transitions from the 5th minute to the end of each constant workload exercise using the 1s interpolated data. The early adjustment of HR was excluded from the analysis (300s). Thus, HR kinetics analysis dealt mainly with the slow component of the response. The functions of the fitting equations used were the following:

1) *Linear :*

$HR(t) = HR_{@5min} + S_{lin}(t)$;

2) *Double linear :*

If (t < time delay):

$HR(t) = HR_{@5min} + S_{dbl1}(t)$;

Else:

$HR(t) = (HR_{5min} + S_{dbl1}(\text{time delay})) + S_{dbl2}(t - \text{time delay})$

Where S_{lin}, $S_{dbl1,}$ and S_{dbl2} are the angular coefficients of the linear regression of HR versus time *t.* Time delay were determined to yield the lowest sum of squared residuals and were constrained to be >300s. S_{dbl2} relative to the S_{dbl1} was also calculated (S_{dbl2}/S_{dbl1}).

Lastly, we calculated the sum of squared residuals (RSS) as the sum of the squared values of the residuals (deviations of predicted from actual measured values of data) for both the fitting model and our predictive equation.

Statistical analysis

All data are presented as mean ± SD after assumption verification (i.e., normality, homogeneity of variance).

Step 1.

Data description. A one-way RM ANOVA was performed to compare the value at the 5th min of power output, $\dot{V}O_2$, %RCP, HR, and [LA^-] across exercise domains.

Time course analysis. Data fitting by linear and double linear functions was performed by the least squared residuals (RSS) method. A two-way RM ANOVA was performed to compare the slopes (S_{lin}, S_{dbl1}, $S_{dbl2,}$ and $_{sc}$HR) and the sum of the squared residuals across models and domains. That is to say, when equation 2, *double linear,* provided a better fit of the data, a time-dependent change in the HR slow component was present. A one-way RM ANOVA was performed to compare the time delay and S_{dbl2}/S_{dbl1} between moderate and heavy domains.

Lastly, a correction strategy to the originally predictive equation was proposed based on the outcome of the kinetic analysis.

Step 2.

Data description. A two-way RM ANOVA was performed to compare the value at the 5th min of power output, $\dot{V}O_2$, %RCP, HR, and [LA^-] across protocols and exercise domains.

To test the performance of the predictive equation model (i.e., dynamic HR-target model) in maintaining a constant metabolic stimulus compared to an HR clamp protocols. Within both protocols, a two-way RM ANOVA (time x domain) was performed to compare the 30th to the 5th min value of power output, $\dot{V}O_2$, HR, and [LA^-] across domains. Lastly, ΔPO, $\Delta\dot{V}O_2$, ΔHR, and Δ[LA^-] were compared across domains and protocols by a two-way RM ANOVA.

The Post-hoc analysis was performed using the Holm Sidak method. Power analysis was conducted a priori, based on the observed standard deviation of HR and $\dot{V}O_2$ detected during constant workload exercise in previous articles (Baldassarre et al., 2022; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021) as the main variable. To identify significant differences, with an α error of 0.05 and a statistical power ($1 - \beta$) of 0.95, a n. value of 16 subjects was necessary (G*Power 3.1). All statistical analyses were performed using SigmaPlot version 14.0, and α was set in advance at the 0.05 level. Statistical significance was accepted when $P < \alpha$.

Results

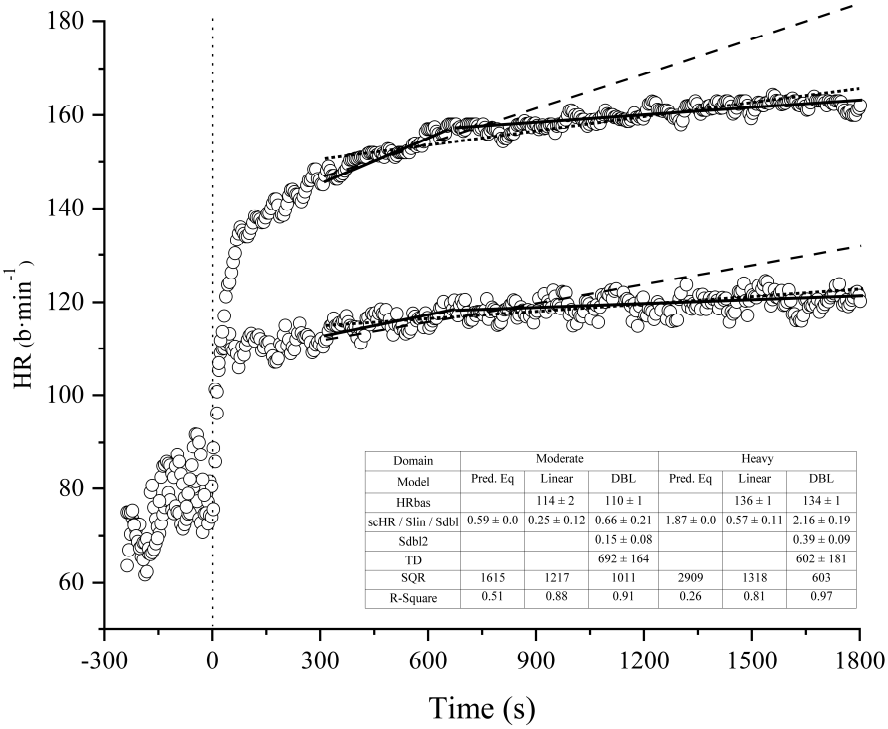
Step 1

Step 1 Participants' characteristics and maximal parameters derived from the ramp incremental test are reported in Table 1. The average BMI and $\dot{V}O_{2max}$ were indicative of a normal weight and active population (Garber et al., 2011). Subjects' GET and RCP were detected at $\dot{V}O_2$ values of 1.99 ± 0.52 L·min^{-1} (65 ± 4% $\dot{V}O_{2max}$) and 2.71 ± 0.61 L·min^{-1} (86 ± 4% $\dot{V}O_{2max}$), respectively.

An overview of the participants' mean ± SD responses at the 5th min of exercise is reported in Table 2. A one-way RM ANOVA on power output, $\dot{V}O_2$, %RCP, HR, and [LA^-] values at 5th min showed, as expected, a significant effect of the domain (for all variables, $p<0.001$).

Time course description models:

The main outcomes of the predictive equation and fitting models are shown in Table 3. A two-way RM ANOVA (model x domain) found a main effect for the model on the RSS ($p<0.01$), while a domain effect was detected on both RSS and slopes within all models ($p<0.05$ for all comparisons). In brief, double linear fitting shows the best fitting RSS, while our predictive equation was inaccurate in estimating the dynamic of HR. However, in both domains, the estimated $_{sc}$HR did not differ from the first portion of the fitted slope of the double linear fitting (S_{dbl1}) (moderate $p=0.11$ and heavy $p=0.18$). All the Post-hoc analyses are shown in Table 3. Lastly, on the double linear fitting, the S_{dbl2} relative to the S_{dbl1} significantly differed between domains (respectively for moderate and heavy domain: 58 ± 19 vs. 46 ± 12%, $p<0.05$).



Domain	Moderate			Heavy		
Model	Pred. Eq	Linear	DBL	Pred. Eq	Linear	DBL
HRbas		114 ± 2	110 ± 1		136 ± 1	134 ± 1
scHR / Slin / Sdbl	0.59 ± 0.0	0.25 ± 0.12	0.66 ± 0.21	1.87 ± 0.0	0.57 ± 0.11	2.16 ± 0.19
Sdbl2			0.15 ± 0.08			0.39 ± 0.09
TD			692 ± 164			602 ± 181
SQR	1615	1217	1011	2909	1318	603
R-Square	0.51	0.88	0.91	0.26	0.81	0.97

Figure 1. Heart rate (HR) in a representative subject during moderate and heavy constant work exercise. Linear (dot line) and double linear fitting (line) and the slow component estimate from our original predictive equation (dash line) are shown.

Table 1. *Anagraphic, anthropometric, and functional characteristics of the step 1 participants'.*

Group	**#**	**Age**	**BMI**	**HR_{max}**	**$\%HR_{max}$**	**$\dot{V}O_{2max}$**	**R_{max}**	**GET**	**RCP**
		(yrs)	*($kg \cdot m^{-2}$)*	*($b \cdot min^{-1}$)*	*%*	*($ml \cdot min^{-1} \cdot Kg^{-1}$)*		*$\%\dot{V}O_{2max}$*	*$\%\dot{V}O_{2max}$*
Males	9	27 ± 6	24 ± 3	189 ± 1	100 ± 4	48.1 ± 8.6	1.30 ± 0.09	64 ± 5	84 ± 4
Females	10	23 ± 8	21 ± 1	192 ± 1	99 ± 4	44.9 ± 6.3	1.17 ± 0.04	65 ± 4	87 ± 4
Total	19	25 ± 7	22 ± 3	191 ± 2	100 ± 4	46.4 ± 7.5	1.22 ± 0.09	65 ± 4	86 ± 4

Values are expressed as mean ± SD. Body mass index (BMI), maximal heart rate (HR_{max}), percentage of theoretical HR_{max} ($\%HR_{max}$), maximal oxygen uptake ($\dot{V}O_{2max}$), maximal respiratory exchange ratio (R_{max}), gas exchange threshold (GET), and respiratory compensation point (RCP).

Table 2. *Variables at the fifth minute in both domains.*

Domain	**Power output**	**$\dot{V}O_2$**	**%RCP**	**HR**	**[LA^-]**
	(Watt)	*($L \cdot min^{-1}$)*	*(%)*	*($b \cdot min^{-1}$)*	*[$mmol \cdot L^{-1}$]*
Moderate	70.3 ± 29.1	1.59 ± 0.34	56.1 ± 6.1	110 ± 15	1.29 ± 0.69
Heavy	168.7 ± 39.9	2.57 ± 0.64	92.2 ± 4.0	149 ± 9	4.89 ± 1.78
Domain (*p*)	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

Mean ± SD of Power Output, Oxygen uptake ($\dot{V}O_2$), relative intensity to the respiratory compensation point (%RCP), heart rate (HR), and lactate accumulation ([LA^-]) at the 5^{th} minute during constant work rate exercise in both domains (Moderate and Heavy). The main effects of the domain are shown in the bottom line of the tables.

Table 3. *Model parameters determined during constant work rate exercise.*

	Moderate			**Heavy**		
	Predictive Equation	***Linear***	***Double Linear***	***Predictive Equation***	***Linear***	***Double Linear***
$HR_{@5min}$ *($b \cdot min^{-1}$)*	108 ± 14	111 ± 15	107 ± 13	144 ± 8	148 ± 9	145 ± 9
$_{sc}HR$ / S_{lin} / S_{dbl}	0.67 ± 0.16	0.38 ± 0.20*	0.56 ± 0.27$^{\#}$	1.34 ± 0.20^{ç}	0.63 ± 0.16^{ç*}	1.22 ± 0.48$^{ç\#}$
S_{dbl2} *($b \cdot min^{-2}$)*			0.30 ± 0.19*			0.47 ± 0.18$^{ç*\#}$
TD *(s)*			823 ±217			793 ±193
RSS	2695 ± 554	1812 ± 731*	1575 ± 622$^{*\#}$	2997 ± 804^{ç}	1450 ± 1106^{ç*}	824 ± 445$^{ç*\#}$

Mean ± SD of the main outcomes of the predictive equation, linear and double linear fitting models. HR at the 5th min ($HR_{@5min}$), estimated HR slow component from the predictive equation ($_{sc}HR$), linear slope (S_{lin}), slope of the double linear fitting before (S_{dbl1}) and above (S_{dbl2}) the time delay (TD) and the sum of the squared residuals (RSS). ^{ç} *indicates a significant difference from the moderate domain within the same model .* indicates a significant difference from the predictive equation within the domain.* $^{\#}$ *indicates a significant difference from the linear fitting within the domain.*

Correction strategy for prolonged exercise:

To develop a predictive equation able to estimate the HR slow component before and after the detected time delay (i.e., mean of the two domains of 808 sec), a correction strategy based on the relationship between S_{dbl2} and S_{dbl1} (i.e., 58 and 46% respectively for the moderate and heavy domain) was implemented on our original formula as follows:

If time < 808 sec:

$_{sc}$HR (b·min^{-2}) = -0.0514 + (0.0240 x intensity expressed in %RCP) - (0.0172 x age) - (0.347 x Sex)

Else and moderate domain:

$_{sc}$HR (b·min^{-2}) = (-0.0514 + (0.0240 x intensity expressed in %RCP) - (0.0172 x age) - (0.347 x Sex)) **x 0.58**

Else and heavy domain:

$_{sc}$HR (b·min^{-2}) = (-0.0514 + (0.0240 x intensity expressed in %RCP) - (0.0172 x age) - (0.347 x Sex)) **x 0.46**

Step 2.

Step 2 Participants' characteristics and maximal parameters derived from the ramp incremental test are reported in Table 4. The average BMI and $\dot{V}O_{2max}$ were indicative of a normal weight and active population (Garber et al., 2011). Subjects' GET and RCP were detected at $\dot{V}O_2$ values of 1.89 ± 0.32 L·min^{-1} (64 ± 5% $\dot{V}O_{2max}$) and 2.55 ± 0.40 L·min^{-1} (86 ± 6% $\dot{V}O_{2max}$), respectively.

Participants' responses at the 5th minute in both protocols and domains are reported in Table 5, while the profiles of the variables are displayed as a function of time in Figure 3. A two-way RM-ANOVA (domain X protocol) on power output, $\dot{V}O_2$, %RCP, HR, [LA$^-$] values at 5th min shows a significant effect of relative exercise intensity only (p<0.001, for all variables). The post-hoc analysis results are shown in Table 5.

ΔPO, ΔHR, Δ$\dot{V}O_2$, and Δ[LA$^-$] between protocols in each domain are reported in Table 6. A two-way RM ANOVA (domain X protocol) on ΔPO, ΔHR, Δ$\dot{V}O_2$ and Δ[LA$^-$] showed a significant effect of domains and protocol for all variables (p<0.001). In brief, in both domains, on the HR *clamp* protocol, HR remained constant over time while $\dot{V}O_2$ and

[LA^-] were significantly lower at the 30^{th} compared to the 5^{th} min ($p<0.001$ for all comparisons). On the contrary, on the *dynamic HR-target model*, HR increases in both domains (i.e., accounting for the HR slow component and its time-dependent change) while power output, $\dot{V}O_2$, and [LA^-] remain constant. The post-hoc analysis results are shown in Table 6.

Table 4. *Anagraphic, anthropometric, and cardiorespiratory variables of the step 2 participants'.*

Group	*N*	**Age**	**BMI**	**HR_{max}**	**$\%HR_{max}$**	**$\dot{V}O_{2max}$**	**RER_{max}**	**GET**	**RCP**
		(yrs)	*($kg \cdot m^{-2}$)*	*(bpm)*	*%*	*($ml \cdot min^{-1} \cdot Kg^{-1}$)*		*$\%\dot{V}O_{2max}$*	*$\%\dot{V}O_{2max}$*
Males	8	28 ± 2	22 ± 4	190 ± 3	102 ± 6	46.2 ± 8.1	1.27 ± 0.05	62 ± 6	83 ± 5
Females	8	24 ± 3	22 ± 2	191 ± 2	98 ± 4	41.8 ± 6.4	1.14 ± 0.05	65 ± 3	88 ± 5
Total	16	26 ± 3	22 ± 3	190 ± 3	101 ± 6	44.0 ± 7.4	1.20 ± 0.08	64 ± 5	86 ± 6

Values are expressed as mean ± SD. Body mass index (BMI), maximal heart rate (HR_{max}), percentage of theoretical HR_{max} ($\%HR_{max}$), maximal oxygen uptake ($\dot{V}O_{2max}$), maximal respiratory exchange ratio (RER_{max}), gas exchange threshold (GET), respiratory compensation point (RCP).

Table 5. *Variables at the fifth minute during exercise in both protocols and domains.*

Domain	Protocol	Power output	$\dot{V}O_2$	HR	%RCP	[LA-]
		(Watt)	*($L \cdot min^{-1}$)*	*($b \cdot min^{-1}$)*	*(%)*	*[$mmol \cdot L^{-1}$]*
Moderate	**HR_{DTM}**	73.4 ± 19.8	1.45 ± 0.27	121 ± 15	57.3 ± 8.7	1.05 ± 0.77
	HR_{clamp}	73.4 ± 19.8	1.51 ± 0.27	120 ± 16	59.8 ± 8.8	1.22 ± 0.65
Heavy	**HR_{DTM}**	140.8 ± 30.4*	2.11 ± 0.37*	158 ± 11*	84.7 ± 8.6*	4.55 ± 1.67*
	HR_{clamp}	140.8 ± 30.4*	2.20 ± 0.47*	155 ± 10*	87.8 ± 11.9*	4.06 ± 1.29*
Domain (*p*)		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Protocol (*p*)		1.00	0.47	0.78	0.11	0.10

*Variables at the 5^{th} minute in both dynamic HR-target (HR_{DTM}) and HR clamp across moderate and heavy domains. Mean ± SD of power output, oxygen uptake ($\dot{V}O_2$), relative intensity to the respiratory compensation point (%RCP), heart rate (HR), and lactate accumulation [LA^-]. The main effects of protocols and domains are shown in the bottom line of the tables. *indicates a significant difference from the moderate domain for the same protocol.*

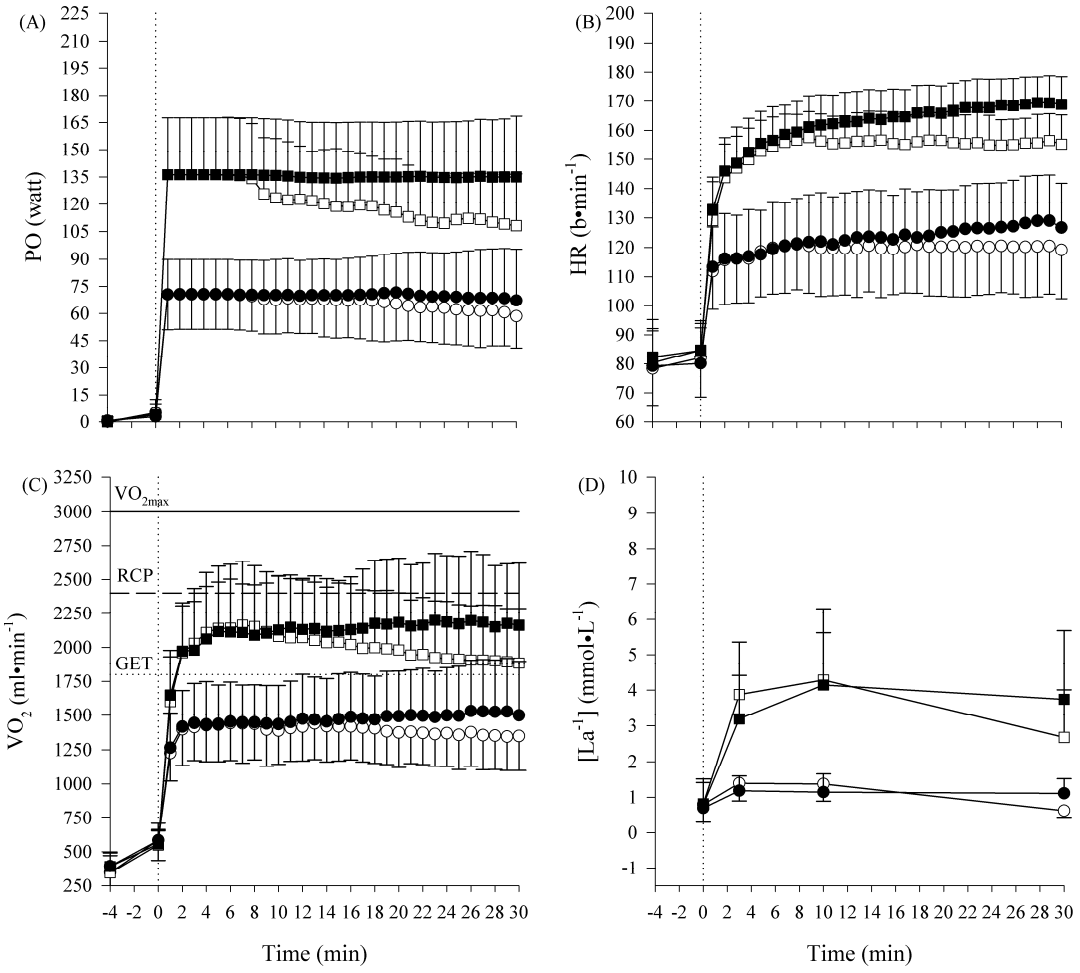


Figure 2. Mean ± SD of power output (A), Heart Rate (B), oxygen uptake (C), and Lactate concentration (D) during exercise in both protocols and domains are plotted as a function of time. Moderate domain: HR_{clamp} (○) and HR_{DTR} (●); Heavy d□main: HR_{clamp} (□) and HR_{DTM} (■). The three horizontal line plots in panel C indicate the lowest to the highest: gas exchange threshold (GET), respiratory compensation point (RCP), and maximal value of oxygen uptake ($\dot{V}O_{2max}$).

Table 6. *Differences between the 5th and 30th min of exercise in both protocol and domains.*

Domain	Protocol	**ΔPO** *(Watt)*	**$\Delta\dot{V}O_2$** *($ml \cdot min^{-1}$)*	**ΔHR** *($b \cdot min^{-1}$)*	**$\Delta[LA^-]$** *($[mmol \cdot L^{-1}]$)*
Moderate	***HR_{DTM}***	2.2 ± 14.2	77.9 ± 141.7	9.7 ± 5.0$^{\#}$	-0.09 ± 0.24
	HR_{clamp}	-10.2 ± 9.4$^{\S\#}$	-100.0 ± 86.3$^{\S\#}$	0.1 ± 1.8§	-0.42 ± 0.42$^{\#}$
Heavy	***HR_{DTM}***	-3.1 ± 9.0	42.9 ± 103.7	13.8 ± 7.3$^{\#}$	-0.48 ± 0.87
	HR_{clamp}	-21.1 ± 14.4$^{*\S\#}$	-296.61 ± 107.7$^{*\S\#}$	0.8 ± 1.9§	-1.39 ± 0.69$^{*\S\#}$
Domain (*p*)		*=0.57*	***<0.05***	*=0.09*	***<0.01***
Protocol (*p*)		***<0.001***	***<0.001***	***<0.001***	***<0.001***

Mean ± SD of the difference between the 5th and 30th min of Power Output (ΔPO), Oxygen uptake ($\Delta\dot{V}O_2$), Heart Rate (ΔHR), and Lactate accumulation ($\Delta[LA^-]$) *during both protocols (model vs clamp) in each domain. The main effects of domain and protocol are shown in the bottom line of the table.* **indicates a significant difference from Moderate within the same protocol. § indicates a significant difference from the model within the same domain;* $^{\#}$ *indicates a significant difference between the 30th and the 5th min within protocol and intensity.*

Discussion

The purpose of this study was to i) characterize the slow component of heart rate kinetics during prolonged exercise and across domains with the aim of developing a comprehensive prediction equation for $_{sc}$HR (step 1) and test its performance in maintaining a constant metabolic stimulus ($\dot{V}O_2$ and [LA^-]) over time and across domains (step 2).

The study confirmed the existence of a slow component of heart rate that increases in proportion to relative exercise intensity. Step 1 of this study enlightened a time-dependent reduction in the slope of the HR increments during constant work rate exercises that appears with a time delay of ~13 minutes. While our data confirm the validity of the original predictive equation (1) in estimating the HR slow component of the earlier phase of the adjustment (i.e., 5- ~13 min), above 13 min, a correction strategy based on the kinetics analysis was developed to account for the complexity of the phenomenon. In step 2, a dynamic HR-target model based on a comprehensive equation allowed the attainment of a constant metabolic stimulus ($\dot{V}O_2$ and [LA^-]) over time, while a reduction in power output, oxygen uptake, and lactate accumulation was confirmed during a clamped HR exercise.

In agreement with previous findings in individuals of comparable average fitness and weight, our data confirmed the presence of a slow component of heart rate in all domains (including the moderate) and its direct relationship with relative exercise intensity (Baldassarre et al., 2022, 2023; Teso, Colosio, et al., 2022; Zuccarelli et al., 2018, 2021).

To our knowledge, this is the first study that analysed the time course of the HR adjustment during 30-min constant load trials using both linear and double-linear fitting models. Our data indicate that the dynamics of the $_{sc}$HR in exercises lasting over 15 min are better represented by a double linear fitting, incorporating a faster early adjustment followed by a slower one after ~13-min. Therefore, the duration of the exercise session must be considered when comparing studies that use single linear fitting for detecting HR increments (Baldassarre et al., 2022; Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018, 2021). In fact, in our study, the slope of the $_{sc}$HR during the early phase of the exercise (i.e., S_{dbl1}, 5 to ~13min) was similar to the values reported in previous experiments of <15min duration for both moderate (respectively, 0.56 vs. 0.55 b·min^{-2}) and heavy domain (respectively, 1.22 vs. 1.35 b·min^{-2}) for comparable population (Teso, Rizzo, et al., 2022; Zuccarelli et al., 2018). However, since a time-dependent reduction in the HR slope over time was observed after ~13 min during both moderate (~-42%) and heavy

exercise (~-64%), the slope of this later phase (i.e., S_{dbl2}, 13 to 30min) was more similar to what had been previously reported in studies analysing the HR drift in longer exercise durations (i.e.,10 to 45-90min) (i.e., moderate domain, 0.18 vs. 0.25 b·min^{-2} in the current study) (Coyle & González-Alonso, 2001; Ganio et al., 2006; Lafrenz et al., 2008; Logan-Sprenger et al., 2012; Souissi et al., 2021; Stone et al., 2021; Wingo, Ganio, et al., 2012; Wingo, Salaga, et al., 2012)

The finding of an earlier and later phase of the HR adjustment in exercise of intermediate duration (i.e., up to 30 min) is not completely surprising since, during exercise, continuous time-dependent changes in the cardiovascular response have been described in relation to different physiological mechanisms (Souissi et al., 2021). In the early phase (5-15min), dilation of resistance vessels and increase in sympathetic outflow results in increased venous return, contractility, and heart rate (van de Vegte et al., 2019). Both central and peripheral mechanisms might be partially modulated by the direct influence of blood catecholamines and nitric oxide on the active vasodilation and to the intrinsic cardiac nervous system (Souissi et al., 2021; van de Vegte et al., 2019). Indeed, previous findings showed that nitric oxide-induced 40-50% of the total cutaneous vasodilation during exercise in humans and led to positive inotropic and chronotropic responses in dogs in vivo (Souissi et al., 2021; van de Vegte et al., 2019; Zanzinger, 1999). However, there is no study yet that has assessed the direct influence of nitric oxide on the slow component of the heart rate. Moreover, in proportion to the intensity of the exercise, the adrenal medulla is also activated, releasing epinephrine into the circulation with a further positive chronotropic response (Hackney, 2006; Orizio et al., 1988a; van de Vegte et al., 2019).

After 15 minutes, heart rate increments during exercise can be amplified/affected by hyperthermia and dehydration (Coyle & González-Alonso, 2001; Ganio et al., 2006; Lafrenz et al., 2008; Laginestra et al., 2023; Souissi et al., 2021; Stone et al., 2021; Trinity et al., 2010; Wingo, Ganio, et al., 2012; Wingo, Salaga, et al., 2012). The increased thermoregulatory need and cutaneous circulation vasodilation during exercise are suggested to be one of the leading causes for the HR increments affecting the filling pressures and preload (Laginestra et al., 2023). Moreover, Trinity J. et al. propose a direct effect of hyperthermia on intrinsic HR or the indirect effect of increasing sympathetic outflow (Trinity et al., 2010). Alternatively, post-exercise assessments have indicated impaired left ventricular contractility and relaxation as a possible cause of the HR increments (Souissi et al., 2021). However, this is detectable following >45 min of

prolonged exercise compared with the early onset (>15min) typically observed for cardiovascular drift (Laginestra et al., 2023). Thus, the phenomena seem to be temporally unrelated. Lastly, a decreased blood volume due to progressive dehydration during exercise may decrease stroke volume and affect the heart rate by altering the preload condition (Ganio et al., 2006; Logan-Sprenger et al., 2012; Wingo, Ganio, et al., 2012).

In agreement with the existence of a time-dependent cardiovascular response during exercise that might be enlightened by our approach (double linear), our original predictive equation, developed using shorter experiments (<15min), appeared to retain its validity up to ~13 min of exercise. Moreover, the $_{sc}$HR slope in the later phase of the adjustment appeared to represent a predictable fraction of the $_{sc}$HR of the earlier adjustment. Therefore, we decided to maintain the original estimating equation (Teso, Rizzo, et al., 2022)(validated on a large and heterogeneous population) to calculate dynamic HR targets and simply apply a time-dependent correction factor to it. In *step 2,* we tested the performance of the *dynamic HR-target model* in maintaining a constant metabolic stimulus in comparison to the commonly used *clamped* HR prescription strategy. In the *clamped* HR, we confirmed progressive decrements of power output and $\dot{V}O_2$ similar to that reported in previous studies of comparable duration and population (Baldassarre et al., 2022, 2023; Zuccarelli et al., 2018, 2021) (respectively, mean power output -14% vs. -14% and $\dot{V}O_2$ -9% vs. -10% in the previous study). To obtain a gross estimation of the impact of the *clamped* HR strategy on the overall energy expenditure (Kcal) of a session, we calculated the integral of the difference between $\dot{V}O_2$ of the *clamped* HR and that of a hypothetical steady state $\dot{V}O_2$ exercise from the 5th min. Using an energy equivalent of O_2 of 5 kcal·lO_2^{-1}, a reduction in the total energy expenditure of ~ 7 and 13% would occur for a 30- and 60-min exercise session, respectively. Importantly, in addition to the reduction in overall energy expenditure, the use of the *clamped* HR caused a shift in domain from heavy to moderate. Notably, during the heavy exercise domain, in 20% of the subjects (3/16) $\dot{V}O_2$ progressively decreased from slightly below RCP at the beginning of the exercise to below GET at the end of the 30-min task. Moreover, in the remaining subjects, $\dot{V}O_2$ at 30 min was only ~ 100 ml·min^{-1}, above GET (Fig 5); we can hypothesize that a shift in the moderate domain would occur before the ~43th min of exercise in these healthy young subjects. These observations appear in agreement with previous data reported by our group in postmenopausal women (Teso, Colosio, et al., 2022). While quantitative impact of keeping HR clamped during training sessions in different populations remains to be evaluated in future studies, our data confirm the

importance of accounting for the $_{sc}$HR for an accurate quantification of the intensity element of the exercise-therapy dose (Teso, Colosio, et al., 2022; Zuccarelli et al., 2018).

Regarding the *dynamic HR-target model*, the heart rate target, based on the estimated heart rate slow component, led to the maintenance of a constant power output, $\dot{V}O_2$, and [LA^-] over time in both moderate and heavy domains (as can be seen from Tab.7). In this context, the predictive equation for $_{sc}$HR (along with the correction strategy developed *step 1*) provides the means to dynamically adapt HR targets over time, avoiding an undesired reduction in absolute and relative training load. This strategy would allow the maintenance of the desired metabolic stimulus throughout an exercise session in a heterogeneous population.

Conclusions

This investigation demonstrates that a time-dependent change in the slow component of the heart rate appears early during constant work exercise and is better fitted by a double linear rather than a simple linear equation. The developed predicted equation with a correction strategy that accounts for the reduction in the slope of the increments in heart rate allows the maintenance of a constant metabolic stimulus over time—on the contrary, using a *clamped* heart rate if we prescribe exercise using a clamped heart rate, a progressive reduction in oxygen uptake in both moderate and heavy domains is likely to occur. Whenever workload implementation is impossible or impractical, and training is defined at a clamped heart rate, we need to be mindful of the mismatch between the slow component of heart rate and metabolic load. The proposed intensity and time-dependent heart rate target would grant that the desired stimulus is maintained throughout the exercise session in a given individual.

Limitations

The developed predictive equation does not consider factors potentially affecting HR kinetics, such as fatigue, overtraining, nutrition, hydration, and environmental conditions, such as temperature and humidity. Other studies are needed to verify the accuracy of the proposed predicted equation by taking into account these factors that could play a role as predictors of the $_{sc}$HR to understand the mechanistic bases of this phenomenon better.

CHAPTER

7

General discussion

Regular physical activity promotes and preserves health throughout a person's life in a dose-response manner. The optimal dose of exercise can be quantified through the elements of frequency, intensity, time, and type according to the "FITT" scheme. Frequency, time, and type are relatively easy to determine, manipulate, and monitor, while exercise intensity is arguably the most critical and elusive component of an exercise prescription dose (Iannetta, Inglis, Mattu, et al., 2020; Jamnick et al., 2020). Nowadays, heart rate (HR) remains one the most common, easy, and low-cost methods to prescribe exercise intensity in sports and clinical settings (Achten & Jeukendrup, 2003; Ivey et al., 2007; Macko et al., 2005; Nybo et al., 2010; Piepoli et al., 2016). The prescription of exercise intensity using HR targets requires the continuous monitoring of HR and relies on the existence and constancy over time of a linear relationship with the oxygen uptake ($\dot{V}O_2$) (in absolute, relative to max/reserve, or metabolic equivalents) (Achten & Jeukendrup, 2003; Legge & Banister, 1986). However, the validity of HR as an index of metabolic intensity over time has been recently questioned; an increase of HR over time totally or partially dissociated from the slow component of $\dot{V}O_2$ has been observed at all exercise intensities, from the moderate to severe domain (Zuccarelli et al., 2018).

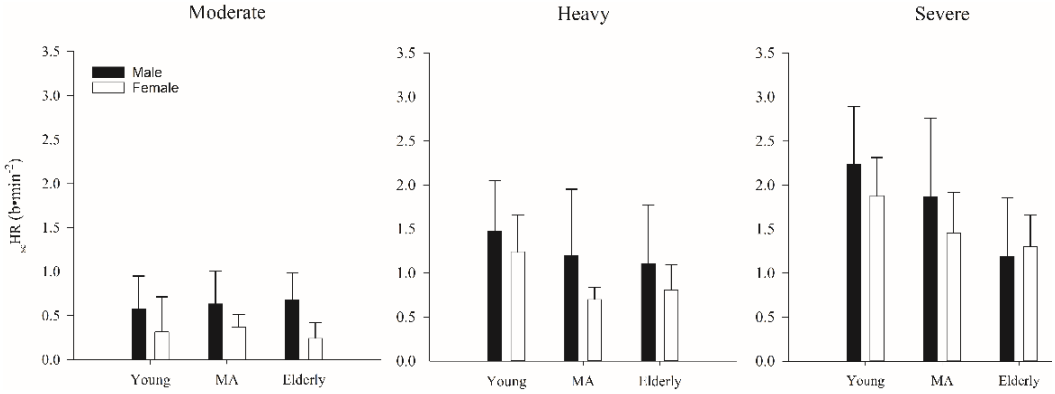
The first part of the thesis, composed of two studies, tested the existence and quantified the amplitude of the dynamic of the so-called HR "slow component" ($_{sc}$HR) across exercise intensities and domains in a heterogeneous population. The physiological underpinnings of this phenomenon have been investigated. In particular, the first study focused on the two main putative mechanisms, i.e., a decreasing stroke volume and increasing body temperature over time, behind the time- and intensity-dependent heart rate increase. Moreover, we established the possible relationship of the HR slow component dynamic with physiological and anthropometric variables (e.g., $\dot{V}O_2$, $\dot{V}O_{2max}\cdot kg^{-1}$) as well as individual sex and age. Thus, an equation model that is able to estimate the slope of the $_{sc}$HR was proposed and validated. In the second part of the thesis, we tested the hypothesis of a time-dependent dynamic of the $_{sc}$HR. Lastly, the performance of our approach in maintaining a constant metabolic stimulus during prolonged aerobic exercise sessions was validated in the fourth study.

Heart rate slow component across intensity domains, age, and sex:

The delayed increase in HR during prolonged, constant exercise has been widely investigated in young males with several different exercise durations (<15min, HR slow component; >15-20min cardiovascular drift) (Baldassarre et al., 2023; Coyle & González-Alonso, 2001; Ganio et al., 2006; Murphy et al., 2009; Souissi et al., 2021; Wingo, Ganio, et al., 2012; Wingo, Salaga, et al., 2012; Zuccarelli et al., 2018, 2021). Only two studies specifically investigated this phenomenon in females (Logan-Sprenger et al., 2012; Stone et al., 2021) however, only examining the cardiovascular drift (i.e., >15-20min) when dehydration and hyperthermia were shown to amplify the dynamic of the HR increments (Ganio et al., 2006; Laginestra et al., 2023; Wingo & Cureton, 2006). In addition, the amplitude of the HR slow component and its possible relationship with exercise intensity have not been analyzed, as almost all studies evaluated the moderate domain (Coyle & González-Alonso, 2001; Souissi et al., 2021; Wingo, Ganio, et al., 2012; Zuccarelli et al., 2018).

In the first two studies, the $_{sc}$HR during prolonged exercise across intensities has been analyzed in a wide age spectrum (22-63 years) and in both sexes. We demonstrated that the dynamics of the HR increments is a linear function of the exercise intensity with greater accuracy when expressed relative to the heavy-to-severe intensity boundaries than to the maximal aerobic power. Previous studies report that $\%\dot{V}O_{2max}$ elicits a large inter-individual variation in the metabolic responses to exercise; in this context, using a threshold-based method for establishing the relative exercise intensity might better normalize the metabolic stimulus for individuals with varying fitness levels.

Lastly, we demonstrated an overall sex- and age-dependent $_{sc}$HR with an amplitude higher in males (10 to 30% across domains) but progressively reduced by age (up to 50% in the severe domain).



Adapted from study 2. Slow component of heart rate ($_{sc}$HR) observed during the first 15 min of exercise across domains in 101 participants. Females (white) and males (black) subdivided into three age groups (young <36; Middle age, MA >36 and <55 and elderly >55yrs).

Physiological underpinning of the HR slow component:

Cardiovascular adjustment occurs during exercise to provide the necessary supply of oxygen and nutrients to meet the demands of the working muscle dictated by the exercise task (Rowell 1993).

During prolonged exercise, a delayed increase in heart rate persists even if a steady state in the oxygen uptake has been reached (Baldassarre et al., 2023; Souissi et al., 2021; Wingo, Ganio, et al., 2012; Zuccarelli et al., 2018). It has been hypothesized that this phenomenon is due to hyperthermia-induced vasodilation and dehydration-induced hypovolemia caused by prolonged exercise. However, this phenomenon has been linked to increased HR after 10-20 min of exercise, when a progressive decline in stroke volume has also been observed. Thus, the physiological underpinning of the observed HR slow component during the early phase of exercise 5 to 15-30 min needs to be better elucidated.

In addition, for exercise exceeding the moderate to heavy boundaries, the interplay between the $\dot{V}O_2$ and HR slow component is an issue that needs to be clarified.

In our first study, we investigated the relationship between the HR and the $\dot{V}O_2$ slow component in postmenopausal women and concurrent changes in stroke volume and body temperature from the 5th to the 30th min of exercise and across a wide intensity range. In agreement with previous work (Zuccarelli et al., 2018, 2021) the $_{sc}$HR was present in the moderate domain and, with double the size compared to the $_{sc}\dot{V}O_2$, in

the heavy and severe. Moreover, we demonstrated that hyperthermia and metabolism significantly affect the $_{sc}$HR dynamic, with a rise in body temperature being a better predictor than $_{sc}\dot{V}O_2$. On the contrary, $_{sc}$HR was only weakly correlated with a non-significantly reduction (i.e., trend) in the observed stroke volume over time, confirming the hypothesis of the previous study (Laginestra et al., 2023; Zuccarelli et al., 2018). In conclusion, the above findings confirm, in postmenopausal women, in a wide range of intensities, the role of heat accumulation in determining the HR increases (Davies et al., 1976; Fritzsche et al., 1999; Saltin & Hermansen, 1966).

We hypothesized that the reported sex and age differences in the amplitude of the $_{sc}$HR might be partially attributable to the metabolic heat production and heat dissipation capacity, which will affect core temperature and, possibly, the HR drift over time (Gagnon & Kenny, 2012; Rowell, 1974; Yanovich et al., 2020).

Indeed, males and females differ anthropometrically, with the first being generally heavier and taller, as well as displaying higher $\dot{V}O_{2max}$ values (Santisteban et al., 2022). The metabolic heat production for a given relative exercise intensity is higher in individuals with higher absolute $\dot{V}O_{2max}$ (e.g., for trained vs. untrained, young vs. old, and male vs. female, heavier vs. lighter individuals) (Gagnon & Kenny, 2012; Yanovich et al., 2020). Therefore, the reported higher $_{sc}$HR in males in the second study at matched relative exercise intensity can be partially due to the ~30% higher absolute $\dot{V}O_2$ observed in males during exercise. In agreement with this hypothesis, when $_{sc}$HR is normalized for the $\dot{V}O_2$ at the 5th minute, the sex difference in the $_{sc}$HR disappears.

Moreover, the ability to eliminate heat (dissipation) through vasodilation and sweating is affected by body dimensions (mainly related to the ratio between body surface area and mass), sex, age, and aerobic fitness (that affect threshold and sensitivity of the sweating mechanism) (Gagnon & Kenny, 2012; Jay & Kenny, 2007; Kenny et al., 2008; Yanovich et al., 2020). In particular, while the larger surface/mass ratio in women may represent an advantage for heat dissipation, the lower swelling rate vs men may represent a disadvantage. In our study, we did not measure heat dissipation therefore we have no means to quantify the overall effect of sex and body geometry on this variable. However, the fact that when exercise is matched for

metabolic heat production, the differences in the HR between sexes disappear would suggest that a major role is played by heat production (Gagnon & Kenny, 2012).

Similarly, we describe a decrease in the $_{sc}$HR amplitude with aging (up to 7 $b \cdot min^{-1}$ in 10-minute exercise) that can be partially due to the lower absolute $\dot{V}O_2$ observed in older people compared to young individuals. A decrease in $\dot{V}O_{2max}$ with aging is a well-documented phenomenon quantified by about ~10% every 10 years (Kim et al., 2016). The aging $\dot{V}O_{2max}$ decline may, therefore, be partially related to the lower HR excursion that characterizes older individuals compared to young ones (Christou & Seals, 2008). Moreover, reductions in intrinsic maximal heart rate and the chronotropic responsiveness to β-adrenergic stimulation might also contribute to the lower HR slow component dynamic with aging (Christou & Seals, 2008)

In summary, we think it is fair to hypothesize that metabolic heat production (i.e., absolute $\dot{V}O_2$), temperature regulation capacity, and the potential for maximal HR excursion may all play a role in the discrepancy observed between sexes and ages. However, further investigation is needed to fully understand the physiological underpinnings of the $_{sc}$HR dynamics.

Time-dependent dynamics of the heart rate slow component:

Since the time series of the heart rate are very similar to those of the $\dot{V}O_2$ (i.e., approximately exponential), HR kinetics analysis at the exercise onset (i.e., <3min) has been described using the same 3-phase model proposed for the $\dot{V}O_2$ (Stirling et al., 2008; Zuccarelli et al., 2018).

However, unlike the $\dot{V}O_2$, HR never reaches a true steady state due to the emergence of the slow component of heart rate and, later during exercise, of the cardiovascular drift phenomenon. In the fourth study, we analyzed the time series of HR kinetics by using a double linear function over a 30-min constant load exercise. A ~50% drop in the slope of the HR increments has been detected in almost all subjects with a time delay of ~13 minutes. Which mechanism(s) could be responsible for the more pronounced HR increase during the early phase of the exercise (i.e., 5 to 13min HR slow component) compared to the later phase (i.e., cardiovascular drift) can not be derived from the present studies. However, from study 1, we can hypothesize that, for

exercises within a 30-minute duration, the mechanism may not include the progressive decline in stroke volume previously described in longer-duration exercises (i.e., cardiovascular drift).

From the onset of exercise, HR adjustment (inotropic and chronotropic) mainly occurs due to changes in myocardial cells and indirectly from the change in smooth muscle cells surrounding blood vessels (Brooks A. et al., 2005). Myocardial cells and muscle cells respond to different stimuli: i) neural (sympathetic nervous system) (Coote & Bothams, 2001; Fu & Levine, 2013; ii) hormonal (noradrenaline and epinephrine) (Orizio et al., 1988a); iii) local chemical (such as nitric oxide) (Zanzinger, 1999), and mechanical (such as the degree of stretch) (Rowell, 1974; Souissi et al., 2021).

In the early phase of exercise (i.e., 5-15min), the slow component of HR has been hypothesized due to the effects of blood catecholamines (Baldassarre et al., 2023; Hackney, 2006; Orizio et al., 1988a) increase in body temperature (Teso, Colosio, et al., 2022; Zuccarelli et al., 2021) and/or release of nitric oxide (Souissi et al., 2021; van de Vegte et al., 2019). In proportion to the intensity of the exercise, the adrenal medulla is activated, releasing epinephrine into the circulation with a possibly positive chronotropic response (Hackney, 2006; Orizio et al., 1988a; van de Vegte et al., 2019). Hyperthermia- and nitric-oxide-induced vasodilation during exercise can be responsible for the further increment in HR (Souissi et al., 2021; van de Vegte et al., 2019; Zanzinger, 1999).

Further study is needed to assess the direct influence of blood catecholamines, nitric oxide, and hyperthermia on the slow component of HR.

Model-predicted heart rate slow component:

Based on the assumption that HR and $\dot{V}O_2$ are linearly related (Åstrand & Ryhming, 1954) intensity prescription is often defined in terms of a fixed percentage of heart rate reserve or peak (Gormley et al., 2008; Ivey et al., 2007; Legge & Banister, 1986; Macko et al., 2005; Nybo et al., 2010; Piepoli et al., 2016). Alternatively, the HR corresponding to the individual ventilatory thresholds can be easily determined during routinely performed incremental tests (also referred to as gas exchange threshold, GET, and respiratory compensation point (RCP) (Beaver et al., 1986; Shimizu et al., 1991).

Whatever approach is used, the HR target associated with a desired, initial metabolic target (desired $\dot{V}O_2$) would entail a progressive decrease in the metabolic stimulus over time due to the emergence of the HR slow component and cardiovascular drift (Iannetta et al., 2023; Zuccarelli et al., 2018).

In this context, the possibility of estimating a priori the increments in heart rate over time and the use of a time-adjusted HR target would help avoid the reduction in $\dot{V}O_2$ and work rate reported during fixed HR (clamp) exercise (Baldassarre et al., 2022, 2023; Zuccarelli et al., 2021).

To deal with this problem, in the second study, we developed a model to estimate the $_{sc}$HR based on the relative exercise intensity, sex, and age. Across different intensities and heterogenous populations, the predictive model appeared to retain its validity up to ~13 min of exercise. Moreover, the $_{sc}$HR slope in the later phase of the adjustment appeared to represent a predictable fraction of the $_{sc}$HR of the earlier adjustment. Therefore, we decided to maintain the predictive model (validated on a large and heterogeneous population) to calculate dynamic HR targets and simply apply a time-dependent correction factor to it. The performance of the predictive model in maintaining a constant metabolic stimulus in comparison to the commonly used *clamped* HR prescription strategy was tested in our fourth study. If in the *clamped* HR, we confirmed a progressive decrement of power output and $\dot{V}O_2$ (Baldassarre et al., 2022, 2023; Zuccarelli et al., 2018, 2021), during exercise prescription with our model a constant power output, $\dot{V}O_2$, and lactate concentration over time was reach. In this context, the predictive equation for $_{sc}$HR (along with the correction strategy developed in *step 1* of study four provides the means to dynamically adapt HR targets over time, avoiding an undesired reduction in absolute and relative training load. This strategy would allow the maintenance of the desired metabolic stimulus throughout an exercise session in a heterogeneous population.

Conclusions

This investigation demonstrates that a time-dependent change in the slow component of the heart rate appears early during constant work exercise and is better fitted by a double linear rather than a simple linear equation. Further study will need to clarify if the HR slow component and the cardiovascular drift represent distinct adjustment phases and if they can be linked to specific physiological underpinnings.

The developed predicted equation with a correction strategy that accounts for the reduction in the slope of the increments in heart rate allows the maintenance of a constant metabolic stimulus—on the contrary, using a *clamped* heart rate if we prescribe exercise using a clamped heart rate, a progressive reduction in oxygen uptake in both moderate and heavy domains is likely to occur. Whenever workload implementation is impossible or impractical, and training is defined at a clamped heart rate, we need to be mindful of the mismatch between the slow component of heart rate and metabolic load. The proposed intensity and time-dependent heart rate target would grant that the desired stimulus is maintained throughout the exercise session in a given individual.

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