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Effect of cigarette smoking on cardiorespiratory and metabolic response to different protocols in young active males

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Abstract

Cigarette smoking is one of the most impacting risk factors for cardiopulmonary morbidity and a major cause of mortality. It leads to systemic modifications that alter the cardiorespiratory and metabolic response at rest and during exercise. Indeed, stimulates the sympathetic nervous system, elevating the heart rate (HR) and cardiac work. Likewise, tar impairs O_2 diffusion across the alveolar-capillary barrier, and carbon monoxide reduces O_2 transport and extraction. The analysis of the cardiorespiratory and metabolic transitions at different work rates can provide insights in the O_2 transport and delivery mechanisms. Hence, this dissertation aimed to provide a comprehensive examination of the effect of cigarette smoking on the cardiorespiratory and metabolic responses to exercise in young, physically active males. The smokers, compared to a no-smoker group, were evaluated in different exercise protocols: i) moderate intensity square-wave work rates to assess the cardiorespiratory and metabolic kinetics; ii) incremental stepwise protocols to determine the maximum oxygen uptake ($\dot{V}_{O_2max}$), a benchmark for cardiorespiratory fitness, recovery kinetics, and exercise capacity; iii) moderate and iv) heavy intensity sinusoidal work rates. Cigarette smoking has detrimental effects on cardiorespiratory and metabolic response to different exercise protocols in young, physically active males. Despite young age, high fitness level and similar pulmonary function, the smokers were characterized by slower cardiorespiratory and metabolic kinetics during moderate exercise and during the recovery of an incremental test. Interestingly, there were no differences between the smokers and controls during both sinusoidal exercises, except for a shorter time to exhaustion that may suggest peripheral dysfunction.

1 Introduction

1.1 Classification of aerobic exercise intensities

Aerobic exercise can be classified into various intensity domains, corresponding to different physiological responses. Four exercise intensity domains have been most commonly identified: i) moderate (below the lactate threshold, LT); ii) heavy (between LT and a critical intensity of exercise, that can be approximated using different approaches, with critical power, CP being one of them; iii) severe (above CP and sustained until maximum pulmonary oxygen uptake, $\dot{V}_{O_2max}$, is achieved); and iv) extreme (intensities resulting in task failure before $\dot{V}_{O_2max}$ is attained).

1.2 Physiological response to exercise

1.2.1 Cardiovascular response to exercise

The responses of the cardiovascular system to physical exercise are complex. The system must increase blood flow to the muscles to satisfy their augmented demands for O_2 and nutrients, and to augment the exchange of respiratory gases in the pulmonary circulation (Plowman et al., 2013).

Cardiac response to a constant work rate

Cardiac output ($\dot{Q}$), that is the volume of blood the heart pumps per minute, is calculated as:

$$\dot{Q} = SV \cdot HR$$

where, SV is stroke volume that is the volume of blood ejected from the heart ventricle per beat, HR is the number of beats per minute.

At the onset moderate-intensity exercise (<LT), $\dot{Q}$ increases to a steady-state plateau within the first ~2 minutes. Attainment of a steady-state reflects the fact that $\dot{Q}$ is sufficient to transport the O_2 needed to support the metabolic demands of the active muscles. $\dot{Q}$ increases owing to an increase in both SV and HR, which both level off within ~2 minutes (Figure 1).

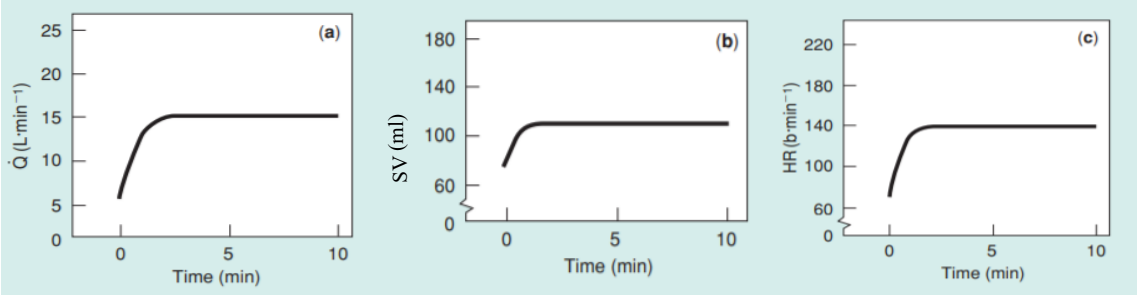

Figure 1: Schematic representation of cardiac output ($\dot{Q}$, panel a), stroke volume (SV, panel b) and heart rate (HR, panel c) response to a moderate constant work rate (modified from Plowman et al., 2013)

As shown in Figure 2, when a heavy exercise is performed (>LT & <CP, approximately 60–85% $\dot{V}_{O_2max}$) the initial increase in $\dot{Q}$ is brought about by a rise in both SV and HR. After the initial increase, SV plateaus and then exhibits a negative drift that is offset by a positive drift for HR. For exercise intensities up to approximately 50% $\dot{V}_{O_2max}$, SV increases rapidly during the first minutes of exercise and soon attains a plateau. During work that requires more than 50% $\dot{V}_{O_2max}$, the response of SV is not intensity dependent, and SV remains relatively constant during the first 30 min of heavy exercise. As for moderate exercise, the increase in SV is due to an increased end-diastolic volume by venous return, leading to the Frank-Starling mechanism, and decreased end-systolic volume by increased contractility owing to sympathetic nerve stimulation. The negative drift in SV, due to a venous return reduction, after approximately 30 min of heavy exercise

is most likely due to thermoregulatory stress, plasma loss and a redirection of blood to the cutaneous vessels to dissipate heat (Rowell & O'Leary, 1990). HR increases rapidly during the first 1–2 min of exercise, with the magnitude of the increase being dependent on the intensity of exercise. The rapid increase in HR is provoked by parasympathetic withdrawal and sympathetic activation. After approximately 30 minutes of heavy exercise HR begins to drift upward in proportion to the decrease in SV, so that $\dot{Q}$ is constant. The changes observed in HR and SV during prolonged constant work rate heavy intensity exercise are defined as cardiovascular drift. Cardiovascular drift may be associated with rising body temperature. The combination of exercise and heat stress produces competing regulatory demands, specifically competition between skin and muscle for large fractions of $\dot{Q}$. SV decreases as a result of vasodilation, a progressive increase in the fraction of blood being directed to the skin in an attempt to dissipate heat from the body, and a loss of plasma volume. The magnitude of cardiovascular drift is also strongly affected by fluid ingestion.

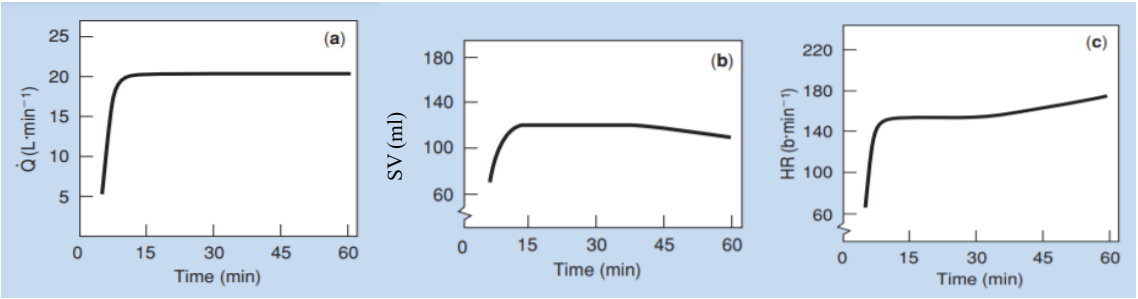

Figure 2: Schematic representation of cardiac output ($\dot{Q}$, panel a), stroke volume (SV, panel b) and heart rate (HR, panel c) response to a heavy constant work rate (modified from Plowman et al., 2013)

Cardiac response to incremental work rates

During incremental exercise tests, the initial increase in $\dot{Q}$ is due to increase in SV and HR. At about 40–50% $\dot{V}_{O_2max}$, SV plateaus and further increases in $\dot{Q}$ are attained exclusively by an increase in HR until a plateau at maximal exercise (Figure 3).

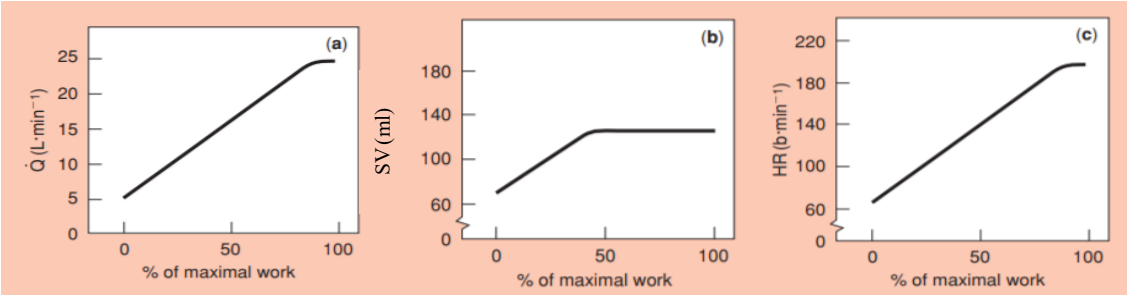

Figure 3: Schematic representation of cardiac output ($\dot{Q}$, panel a), stroke volume (SV, panel b) and heart rate (HR, panel c) response to an incremental work rate (modified from Plowman et al., 2013)

1.2.2 Pulmonary response to exercise

Alterations in ventilation ($\dot{V}_E$) maintain the proper gas concentrations to support rapid gas exchange. $\dot{V}_E$, that is the volume of air inspired or expired per minute, is computed as follow:

$$\dot{V}_E = V_T \cdot f_R$$

where, V_T is tidal volume that is the volume of air that moves in or out of the lungs with each respiratory cycle and f_R is the respiratory rate that is the number of breaths per minute.

$\dot{V}_E$ response to a constant work rate

During moderate exercise, $\dot{V}_E$ increases linearly with pulmonary oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$), averaging between 20 and 25 l of air for each liter of

O_2 consumed. $\dot{V}_E$, in this case, increases mainly through increases in V_T, while at higher exercise intensities, f_R plays a more important role.

$\dot{V}_E$ response to an incremental test

At higher levels of incrementally more intense submaximal exercise, $\dot{V}_E$ increases disproportionately to $\dot{V}_{O_2}$ (i.e., there is a marked and precipitous increase in $\dot{V}_E/\dot{V}_{O_2}$ during this portion of graded exercise). At this point, $\dot{V}_E$ no longer links tightly to O_2 demand at the cellular level. In fact, the "excess" ventilation comes directly from CO_2 release from the buffering of hydrogen ions that accumulate from increased glycolysis (see chapter below).

1.2.3 Metabolic response to exercise

$\dot{V}_{O_2}$ response to an incremental test

When an incremental test is performed, $\dot{V}_{O_2}$ increases as a linear function of work rate. In some individuals, there may be a defined plateau in $\dot{V}_{O_2}$, defined as $\dot{V}_{O_2max}$, where further increases in work rate produce little or no change in $\dot{V}_{O_2}$ (Figure 4) (Poole & Richardson, 1997). $\dot{V}_{O_2max}$, a benchmark for fitness evaluation, determines the integrated functioning of the pulmonary, cardiovascular and muscle systems to transport (diffusive and conductive) and utilize O_2 (mitochondria oxidative phosphorylation) (Poole & Jones, 2017).

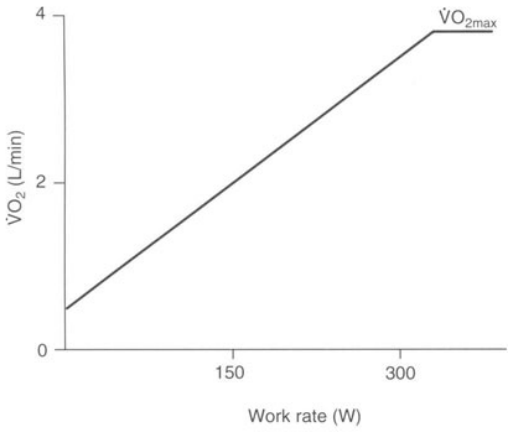

Figure 4: Schematic representation of the relationship between pulmonary oxygen uptake ($\dot{V}_{O_2}$) and work rate during an incremental test on a cycle ergometer to task failure (Poole & Richardson, 1997)

1.2.3.1 $\dot{V}_{O_2}$ kinetics

During a constant work rate of moderate intensity (<LT), the $\dot{V}_{O_2}$ kinetics follow an exponential function as follow:

$$\Delta\dot{V}_{O_2}(t) = \Delta\dot{V}_{O_2}ss\left(1 - e^{-t-tD/\tau}\right)$$

where $\Delta\dot{V}_{O_2}(t)$ is the increase in $\dot{V}_{O_2}$ above baseline at any point in time (*t*), $\Delta\dot{V}_{O_2}ss$ is the increase in $\dot{V}_{O_2}$ from baseline to steady-state, t_D is the time delay between the increase in work rate and the increase in $\dot{V}_{O_2}$, and τ is the time constant of the increase in $\dot{V}_{O_2}$.

This exponential kinetics can be divided into three phases.

1.2.3.1.1 Phase I

Phase I represents the early (15-25 seconds), rapid increase in $\dot{V}_{O_2}$(Murias et al., 2011; Wasserman et al., 1974; Xu & Rhodes, 1999). This phase is mainly due to the rise in $\dot{Q}$ and, thus pulmonary blood flow (Xu & Rhodes, 1999).

1.2.3.1.2 Phase II

Phase II reflects the muscle $\dot{V}_{O_2}$ (Barstow & Mole, 1987; Ferretti, 2015; Poole & Jones, 2012). Following a phase I, $\dot{V}_{O_2}$ rises exponentially toward a steady-state, that is defined as phase III (Ferretti et al., 2017). $\dot{V}_{O_2}$ attains steady-state after about 3 minutes in healthy, young subjects and increases linearly with work rate at a gain of 9 - 11 ml $O_2 \cdot W^{-1} \cdot min^{-1}$. With a square-wave transition from rest to a moderate exercise work rate, the time constant of the primary component of the $\dot{V}_{O_2}$ kinetics corresponds to that of phosphocreatine breakdown and no lactate accumulation occurs during the exercise transient (Binzoni et al., 1992; di Prampero et al., 2003; Rossiter et al., 2002).

1.2.3.1.3 Slow component

When a constant work rate is performed above LT, $\dot{V}_{O_2}$ slowly increases leading to the so-called slow component phenomenon (Ferretti, 2015; Gaesser & Poole, 1996; Jones et al., 2011; Poole et al., 1994; Zołądz & Korzeniewski, 2001). For constant work rates performed above LT but below CP, the $\dot{V}_{O_2}$ slow component delays the attainment of steady-state. For constant work rates performed above CP, the $\dot{V}_{O_2}$slow component drives $\dot{V}_{O_2}$to maximum. The $\dot{V}_{O_2}$ slow component is not negligible; indeed, in the severe domain, its magnitude can be above 1 l min^{-1}, which represents more than 25% of the total increase in $\dot{V}_{O_2}$ above the pre-exercise baseline (Figure 5) (Poole et al., 1994). The $\dot{V}_{O_2}$ slow component by definition increases the oxygen cost of work that shortens the time to exhaustion and impairs power generating capabilities at $\dot{V}_{O_2max}$ (Korzeniewski & Zoladz, 2015; Poole et al., 1994). For these reasons, the $\dot{V}_{O_2}$ slow component phenomenon has

received great interest because its study is likely to enhance our basic understanding of muscle energetics, metabolic control, and the determinants of the efficiency of skeletal muscle contraction (Jones et al., 2011). Indeed, the $\dot{V}_{O_2}$ slow component represents a progressive loss of skeletal muscle contractile efficiency and is associated with the fatigue development (de Almeida Azevedo et al., 2022), marking nonlinearities between the work rate and physiological responses during both steady-state and transient exercise modalities (Casaburi et al., 1977; Wasserman et al., 1973). Although multiparameter model fitting can usefully isolate the $\dot{V}_{O_2}$ primary component and assess the amplitude of the $\dot{V}_{O_2}$ slow component, physiologically justifiable parameterization of the $\dot{V}_{O_2}$ slow component remains elusive (Poole & Jones, 2012).

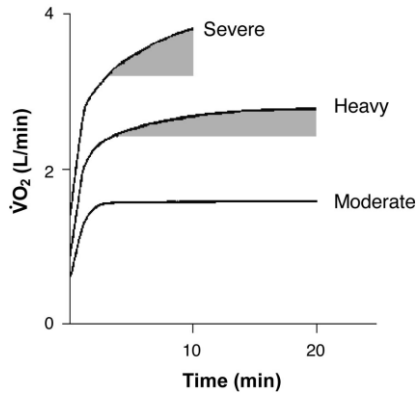

Figure 5: Schematic representation of $\dot{V}_{O_2}$ response to constant work rate exercise of moderate, heavy, and severe exercise domains. As shown, the steady-state is either delayed (heavy) or not achieved (severe) because of the $\dot{V}_{O_2}$ slow component (shaded area) (Jones & Poole, 2005).

A multitude of factors have been identified as possible causes of the $\dot{V}_{O_2}$ slow component, such as recruitment of type II muscle fibers, lactate and hydrogen ion accumulation, elevated arterial catecholamine concentration, augmented muscle temperature, enhanced proton leak through the inner mitochondrial membrane, increased activation of additional muscle groups, and increased respiratory muscle activity (Jones et al., 2011; Harry B.

Rossiter, 2011; Zołądz & Korzeniewski, 2001), but their role in the mechanism responsible for the origin of $\dot{V}_{O_2}$ slow component remains unclear (Korzeniewski & Zoladz, 2015) (Figure 6).

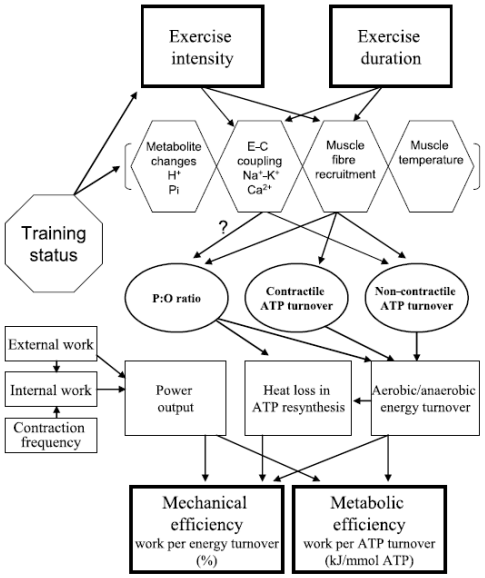

Figure 6: Schematic representation of the most likely determinants of muscular efficiency during dynamic exercise (Jones et al., 2011)

An increase in muscle temperature does not seem to cause the muscle $\dot{V}_{O_2}$ slow component to a significant extent (Jones et al., 2011; Krustrup et al., 2004). Indeed, to increase $\dot{V}_{O_2}$ it may be necessary to elevate muscle temperature above 43°C (Gaesser et al., 1984), which is unlikely during exercise. Muscle pH was only lowered during the intense cycling in which the $\dot{V}_{O_2}$ slow component was observed, indicating that muscular acidosis may be causing the decrease in efficiency (Jones et al., 2011). However, no effect on the $\dot{V}_{O_2}$ slow component was observed after infusion of adrenaline in humans, which increased blood lactate concentration and reduced pH (Gaesser et al., 1994). Variation in the muscle fiber recruitment pattern has been investigated by measurements of single

muscle fiber content of PCr and glycogen. In the study conducted by Krustrup et al. (2004), it was found that type I fiber recruitment is predominant during moderate-intensity exercise, whereas both type I and type II fibers were recruited during heavy intensity exercise. Furthermore, these data indicated that more type II fibers were recruited from 3 to 6 min where the $\dot{V}_{O_2}$ slow component was apparent. Generally, it seems that recruitment of additional muscle fibers and a shift in fiber-type recruitment toward type II fiber play an important role in the development of the muscle $\dot{V}_{O_2}$ slow component during constant work rate exercise, whereas changes in muscle temperature and acidity seem to be of little importance (Jones et al., 2011).

1.2.3.1.4 Kinetics evaluation

During the onset of a constant work rate moderate exercise, $\dot{V}_{O_2}$as a function of time can be fit by a monoexponential model in the form of:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2}\text{bsl} + \text{AMP}\left(1 - e^{-\,t-tD/\,\tau}\right)$$

where $\dot{V}_{O_2}(t)$ is $\dot{V}_{O_2}$ at any time (*t*), $\dot{V}_{O_2}$bsl represents the $\dot{V}_{O_2}$ at baseline, AMP is the amplitude of the increase in $\dot{V}_{O_2}$ above baseline, τ is the time constant defined as the time necessary for $\dot{V}_{O_2}$ to increase to 63% of the remaining AMP, and t_D is the time delay between the increase in work rate and the increase in $\dot{V}_{O_2}$. The evaluation of cardiorespiratory and metabolic response to a constant work rate exercise provides a fundamental parameter of aerobic energy production that reflects the ability to adapt to exercise indicative of daily life activity (Guazzi et al., 2017; Poole & Jones, 2012). In particular, $\dot{V}_{O_2}\tau$ is an important index that can classify the population according to the

exercise tolerance and also to cardiorespiratory disease (Poole & Jones, 2012). Accordingly, trained endurance athletes show extremely fast $\dot{V}_{O_2}$ kinetics, whereas detraining, aging and the presence of many chronic disease conditions are characterized by slow $\dot{V}_{O_2}$ kinetics. The onset of exercise in elite cyclists and marathon runners is characterized by a $\dot{V}_{O_2}\tau$ up to 25 s. In contrast, aged individuals or those suffering from chronic heart failure or pulmonary disease may require six or more minutes to reach steady-state (Poole & Jones, 2012).

1.2.4 Physiological thresholds

The concept of threshold-based exercise intensity has been applied to assess and classify cardiorespiratory fitness and health, for exercise prescription, and to determine the outcomes of specific interventions. In literature there are different approaches to detect the ‘‘threshold’’ separating heavy from very heavy exercise (i.e., sustainable from unsustainable constant-power exercise) (Keir et al., 2015).

1.2.4.1 Lactate threshold

Proportional to the work rate increase at heavy intensity, the metabolic demand rises and consequently glycolytic metabolism. Glycolysis, which is a sequence of different enzymatic reactions, converts glucose to pyruvate. This conversion resynthesizes adenosine triphosphate (ATP) and reduces nicotinamide adenine dinucleotide (NADH) from oxidized NAD (NAD^+). In the final step of anaerobic glycolysis, pyruvate is converted to lactate by the enzyme lactate dehydrogenase (LDH), providing NAD^+ that allows the anaerobic glycolysis to proceed (Phypers & Pierce, 2006).

During exercise it is useful to assess the blood lactate concentration ([La^-]) because it is sensitive to changes in exercise intensity (Beneke et al., 2011). During moderate intensity steady-rate exercise, [La^-] does not rise beyond values observed at rest (about 1-1.5 $mmol \cdot l^{-1}$). Exercise intensity related to the onset of blood lactate accumulation (OBLA) denotes the lactate threshold (LT). Graded incremental exercise tests are commonly utilized to evaluate aerobic endurance performance capacity. Typically, this protocol elicits an exponential rise in [La^-] (Figure 7).

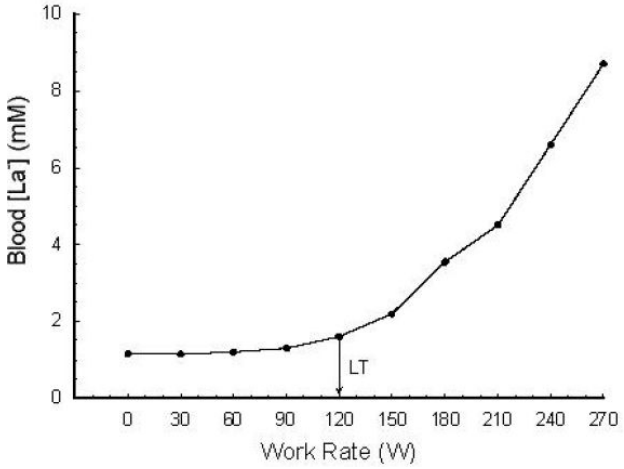

Figure 7: A typical lactate response [La^-] to an incremental exercise (Goodwin et al., 2007)

Over the years, several LT concepts with different methodologies have been proposed (Faude et al., 2009). Mader et al. (1976) proposed 4 $mmol \cdot l^{-1}$ as a fixed value that represents the OBLA, being the most frequently used method (Figure 8A) (Faude et al., 2009). However, some authors do not consider a fixed lactate value valid for all type of subjects to be appropriate. Yoshida et al., (1984) considered LT as the point at which [La^-] increased by 1 $mmol \cdot l^{-1}$ above the basal value (Figure 8B). Other authors identified the LT as the point where the tangent to the curve between [La^-] and work rate exceeds

45° and 51° (Figure 8C, D, respectively) (Simon et al., 1983; Keul et al, 1979). Cheng et al. (1992) determined LT using the D_{max} method, that identifies LT as the maximum distance from [La^-] polynomial regression curve to the line formed by its endpoints (Figure 8E). Bishop et al. (1998) modified this last method determining the D_{mod} method. In this case, LT is equated as the maximum distance from [La^-] polynomial regression curve to the line formed by the first point preceding the increase in lactate concentration to more than 0.4 mmol·l^{-1} and by the end point of lactate (Figure 8F). The 4 mmol·l^{-1}, the D_{max} and the modified D_{mod} methods have been demonstrated to have the best repeatability; moreover the D_{mod} method has been proved to have the highest predictability in long lasting cycling performances (Heuberger et al., 2018).

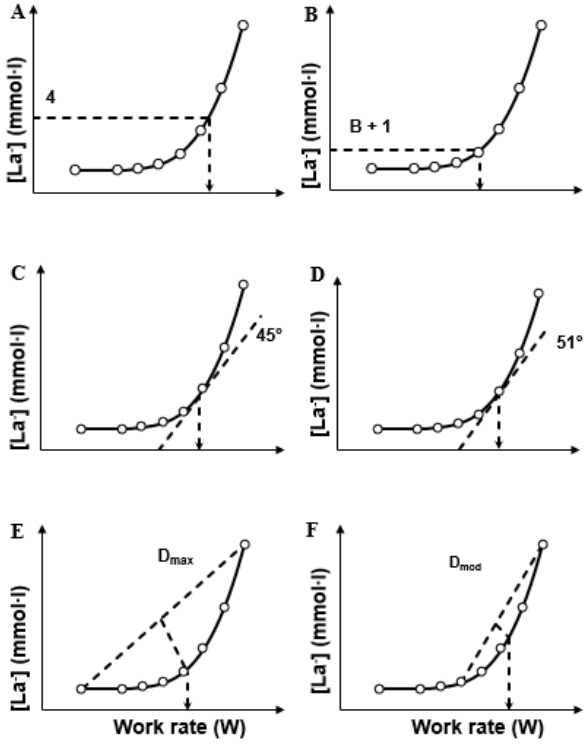

Figure 8: Methods for determining the lactate threshold: A) 4 mmol·l^{-1} (Mader et al., 1976); B) Basal (B) +1mmol·l^{-1} (Yoshida et al, 1984); C) tangent of 45° (Simon et al., 1983); D) tangent of 51° (Keul et al., 1979); E) D_{max} (Cheng et al., 1992); F) D_{mod} (Bishop et al., 1998).

1.2.4.2 Ventilatory thresholds

The ventilatory thresholds are assessed by non-invasive methods that indirectly measure the evaluation of muscle metabolic responses through the use of ventilatory and gas exchange approaches (Wasserman et al., 1973). The first ventilatory threshold is the point at which $\dot{V}_E$ increases disproportionally with the $\dot{V}_{O_2}$ while maintaining proportionality with $\dot{V}_{CO_2}$ (Wasserman et al., 1973). The disproportionate increase in $\dot{V}_E$ relative to $\dot{V}_{O_2}$ is due to the excess of CO_2 that comes directly from hydrogen ion (H^+) buffering in the blood and increased cytosolic ATP turnover (Jamnick et al., 2020). Excess H^+ combine with bicarbonate ions ($HCO3^-$) to form carbonic acid (H_2CO_3) which rapidly breaks down into CO_2 and water (H_2O), as schematized in the following reaction catalyzed by the carbonic anhydrase enzyme:

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow H_20 + CO_2$$

The increase in CO_2 stimulates the respiratory center (medulla and pons) to raise $\dot{V}_E$. The first ventilatory threshold (VT_1) is determined as the first inflection point in $\dot{V}_E$, as a systematic increase in $\dot{V}_E/\dot{V}_{O_2}$ and in end-tidal oxygen pressure (Pet_{O_2}), and the point where end-tidal carbon dioxide pressure (Pet_{CO_2}) begins to plateau (Jamnick et al., 2020; Wasserman et al., 1973). With the increasing of exercise intensity, a further increase in H+ concentration causes a growth in $\dot{V}_E$ that is not able to remove the excess of H^+. This point, defined as second ventilatory threshold (VT_2), is characterized by a break point in $\dot{V}_E$, in $\dot{V}_E/\dot{V}_{CO_2}$, and a falling down of Pet_{CO_2} after an apparent steady-state (Jamnick et al., 2020; Wasserman, Whipp, Koyl, et al., 1973) (Figure 9).

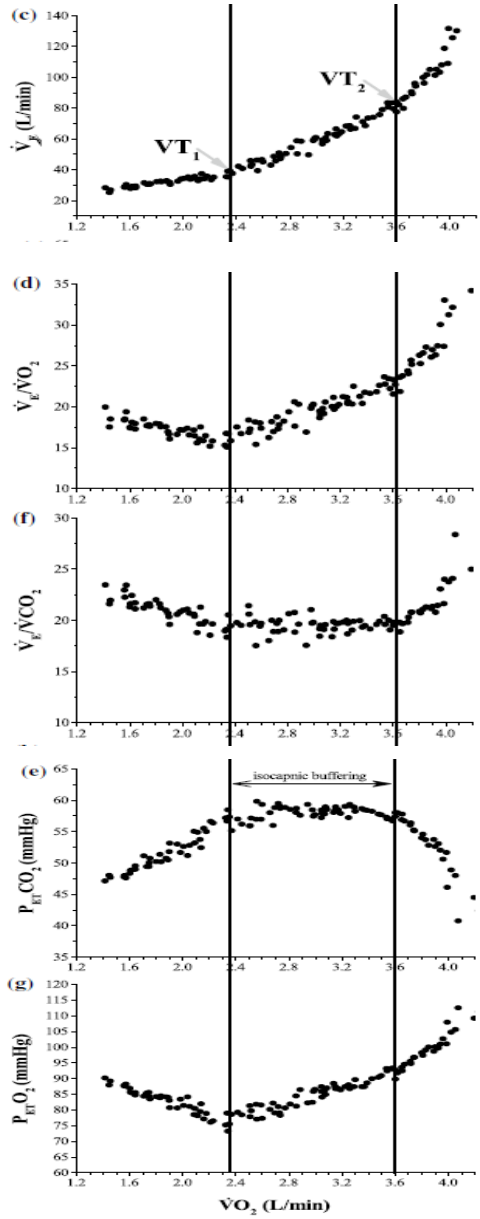

Figure 9: Breath-by-breath gas-exchange and ventilatory variables of a representative participant. The solid vertical line intersecting the abscissa of each figure indicates the oxygen uptake ($\dot{V}_{O_2}$). Note from ***c*** *the change-point in minute ventilation ($\dot{V}_E$) versus $\dot{V}_{O_2}$ (VT2). This rise in $\dot{V}_E$ is confirmed to be hyperventilation (and not hyperpnea) because it is coincident with a fall in pressure of end tidal carbon dioxide expiration (Pet_{CO_2}) from a period of stability (i.e., "isocapnic buffering" period* ***e*** *and by a rise in $\dot{V}_E/\dot{V}_{CO_2}$ (****f****). (Keir et al., 2022)*

1.2.4.3 Critical power

To investigate the effects of fatigue during constant-load protocols, several studies adopted the CP as the exercise intensity threshold that differentiates the heavy from the severe exercise domain (Black et al., 2017; Burnley & Jones, 2018; Poole et al., 2016; Walsh, 2000). CP is a threshold of oxidative metabolism separating exercise intensity domains within which the physiological responses to exercise can (<CP) or cannot (>CP) become stable (Poole et al., 2016). CP is considered to represent the greatest metabolic rate that results in 'wholly-oxidative' energy provision. This means that energy supply through substrate-level phosphorylation reaches a steady-state and that there is no further accumulation of [La^-] or breakdown of intramuscular phosphocreatine. Among all the physiological thresholds, CP is built objectively on the assessment of mechanical work rate and exercise exhaustion time (Jones et al., 2019), yet remaining a critical metabolic rate marker (Barker et al., 2006; Vanhatalo et al., 2016).

Originally CP was identified as the mechanical power output that could be sustained 'indefinitely' or for 'a very long time without the occurrence of fatigue' (Monod & Scherrer, 1965). However, this definition should be considered theoretical, because no exercise can ever be undertaken indefinitely (Poole et al., 2016). Typically, CP is assessed by a series of 3 to 5 time-to-exhaustion trials at severe exercise intensities (i.e., 90-115% maximal mechanical aerobic power, $\dot{W}_{max}$) (Mattioni Maturana et al., 2016; Morton, 2006; Vanhatalo et al., 2007). CP can be mathematical calculated as the hyperbolic relationship between mechanical power and the duration for which that speed or power output can be sustained (ordinate) (Jones et al., 2010; Smith et al., 1999) (Figure 10).

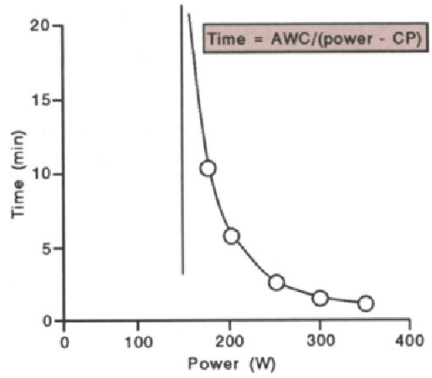

Figure 10: Nonlinear power-time relationship shown graphically and mathematically (Hill, 1993)

Another mathematical approach is to calculate CP with a linear regression for the points, yielding an intercept that corresponded to CP (Figure 11). Both models using two time-to-exhaustion trials lasting between 7 and 20 min could give accurate estimations of CP (Mattioni Maturana et al., 2018).

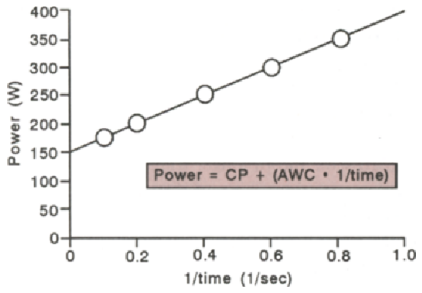

Figure 11: Linear power-1/time relationship shown graphically and mathematically (Hill, 1993)

1.2.5 Recovery after exercise

1.2.5.1 Heart rate recovery indexes

At the end of exercise, autonomic regulation gradually returns to the baseline condition and HR comes back to baseline values as a consequence of this autonomic readjustment.

HR recovery responses can be fitted by a first-order exponential decay function. It can be split in two phases: fast and slow recovery (Peçanha et al., 2014). The fast phase includes the first minute of recovery and characterizes a period in which there is an abrupt and rapid decrease in HR (Imai et al., 1994; Peçanha et al., 2014). This phase is mainly due to vagal reactivation. The slow phase, defined as a more gradual decay, comprises the period after the fast phase until HR returns to baseline values (Peçanha et al., 2014). This phase is determined by vagal reactivation and sympathetic withdrawal (Figure 12).

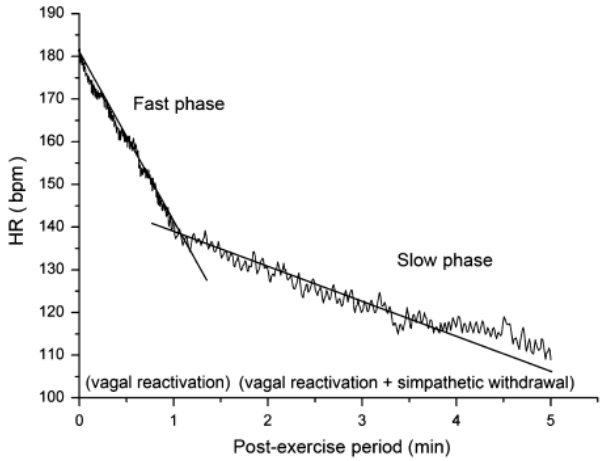

Figure 12: Heart rate (HR) recovery phases. The fast phase, a rapid decline in HR, is promoted predominantly by cardiac vagal reactivation. The slow phase, in turn, is characterized by a more gradual decline in HR and is promoted by vagal reactivation and sympathetic withdrawal (Peçanha et al., 2014).

As heart rate recovery (HRR) indexes of the fast phase, HRR30s and HRR60s are calculated as differences between peak HR at the end of exercise and the HR value reached after 30 s and 1 min of recovery, respectively (Peçanha et al., 2017). These differences can be expressed in absolute terms (i.e., number of beats per minute) or percentage units (i.e., the percentage of HR decay in relation to HR at peak). There are some advantages of using HRR30s and HRR60s to assess cardiac autonomic recovery after exercise. First, they are easy to calculate. Second, it is reported that HRR30s is less

dependent on exercise work rate (Arai et al., 1989; Buchheit et al., 2007), which allows for appropriate comparisons between exercise studies with different intensities (Peçanha et al., 2017).

The T30 index quantifies the HR decay in the first 30s of recovery applying a semilogarithmic analysis. Indeed, T30 is calculated by fitting the natural logarithm of HR in the first 30 s of recovery into a first-degree polynomial. The index is expressed as the negative reciprocal of the slope of the resulting line (−1/slope) (Imai et al., 1994; Peçanha et al., 2017). While the T30 index is almost independent of sympathetic activation and exercise work rate (Imai et al., 1994), its calculation requires mathematical processing that is not trivial. Equipment to register HR on a beat-by-beat basis is required. Moreover, the T30 index is also more susceptible to spurious values due to artefact or arrhythmias (Arduini et al., 2011). Last, the HR in the first 5 or 10 s of recovery can show a plateau or even an additional increase in relation to exercise HR, especially after high-intensity exercise (Arduini et al., 2011). In these circumstances, the linear fitting of HR in the first 30 s is seriously impaired and the slope values may not represent the expected HRR behavior (Peçanha et al., 2017).

Lastly, τ, that is the time necessary to reach the 63% of the remaining response amplitude, is a marker of the slow phase (Imai et al., 1994) (Figure 13).

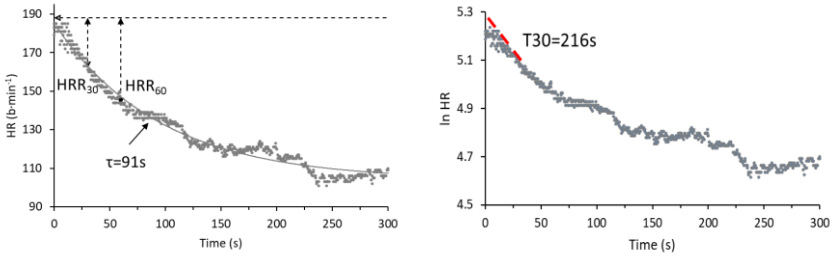

Figure 13: Heart rate recovery (HRR) indexes: HRR_{30}, HR_{peak}– HR at 30s of recovery; HRR_{60}, HR_{peak}– HR at 60s of recovery; T30, negative reciprocal slope of the linear regression of HR natural logarithm vs time in the first 30 s of recovery

A slower HR recovery is an indicator of autonomic dysfunction and has been associated with an increased risk of all-cause mortality (Peçanha et al., 2017). As HR recovery reflects the cardiac autonomic recovery, it is a valuable prognostic tool (Peçanha et al., 2017).

1.2.5.2 Ventilatory response

During the recovery phase, $\dot{V}_E$ can be fit with a monoexponential function. The recovery can be divided into a fast and a slow component. The fast component is controlled by two mechanisms: (1) cerebral input from afferent impulses from the brain's psychomotor area to the respiratory center in the medulla and (2) extra-thoracic mechanoreceptor stimulation from proprioceptors in active muscle. Two factors regulate the slower component: i) a reflex, originating from muscle chemoreceptors sensitive to progressive physiochemical changes within active muscle as exercise progresses and ii) a humoral mechanism. At the end of exercise, $\dot{V}_E$ decreases precipitously when neurogenic input stops. $\dot{V}_E$ is then regulated exclusively by humoral factors from the recovering musculature (Dejours, 1963; Forster et al., 2012).

1.2.5.3 Metabolic response

In the recovery phase, $\dot{V}_{O_2}$ does not return to resting values immediately but remains elevated above resting levels for some period of time. The increase in $\dot{V}_{O_2}$ is defined as "excess post-exercise oxygen consumption" (EPOC) and consists of a rapid and a prolonged component (Figure 14) (Borsheim & Bahr, 2003). During this phase, $\dot{V}_{O_2}$ returns to baseline values with a temporal profile closely resembling the primary on kinetics. There is a certain domain dependency that dictates the off kinetics (i.e., presence of slow component or not) and symmetry/asymmetry with the on kinetics. $\dot{V}_{O_2}$ kinetics in the off transient reflects the combined temporal characteristics of many physiological processes as the homeostasis of the resting condition is restored. These include dynamics of $\dot{Q}$, muscle blood flow, muscle $\dot{V}_{O_2}$ (locomotory, respiratory, cardiac, and accessory), partial refilling of O_2 stores in venous blood and muscle, and energetic costs associated with hormonal, thermal, and metabolic derangements (Poole & Jones, 2012).

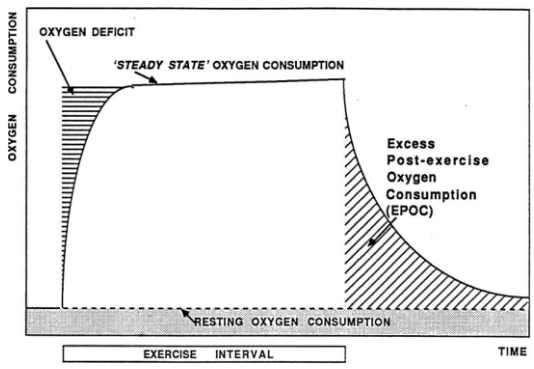

Figure 14: Oxygen uptake response to a constant work rate of moderate domain (<LT) (Laforgia et al., 2006)

$\dot{V}_{O_2}$ kinetics during recovery after exercise is a marker of physical fitness and cardiovascular health (Bellefleur et al., 2016).

1.3 Testing protocols

The analysis of the cardiorespiratory responses to physical exercise provides valuable insight into the efficiency of the heart-lung-muscle integrated system (Poole & Jones, 2017). The protocols commonly proposed for functional evaluation are the incremental and square-wave work rate tests (Giada et al., 2008; Müller et al., 2015).

1.3.1 Square-wave exercise

The square-wave test, a procedure during which the work rate increases rapidly and then remains constant. If it last greater than 3-6 minutes and remains below LT, it allows the cardiorespiratory and metabolic system to reach a steady-state, thus enabling a correspondence between the mechanical power and the metabolic demand (Poole & Jones, 2012). This test permits investigation of the dynamic profile of the cardiorespiratory responses through the analysis of their transition kinetics between different work rates.

1.3.2 Incremental exercise

The incremental protocols can be classified in continuous and discontinuous. The incremental tests allows the determination of $\dot{V}_{O_2max}$, $\dot{W}_{peak}$ as well as the ventilatory and lactate thresholds (Giada et al., 2008; Poole & Jones, 2017; Wasserman, Whipp, Koyal, et al., 1973).

1.3.2.1 Continuous protocol

Ramp and stepwise tests are continuous protocols. In continuous incremental protocols, work rate increments are performed without any resting periods. The ramp protocol is characterized by almost-imperceptible increases in work rate every few seconds (Figure 15).

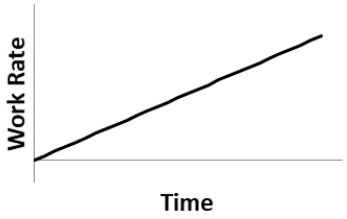

Figure 15: Representation of an example of a ramp test

The stepwise test consists of continuous, incremental steps where the work rate is increased after a more prolonged period of time (generally 1 or 2 minutes) (Figure 16).

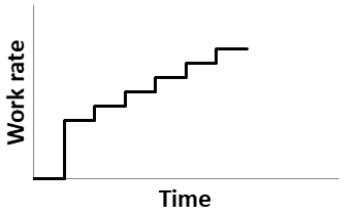

Figure 16: Representation of an example of a stepwise test

1.3.2.2 Discontinuous protocol

The Incremental Intermittent Astrand Test (IIAT) is an intermittent protocol that is characterized by a series of incremental square-wave increases in work rate (usually

lasting 4 - 5 minutes) interspaced with recovery periods for the cardiorespiratory response to return to baseline values (Figure 17).

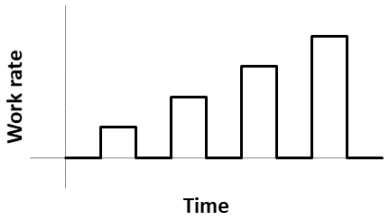

Figure 17: Representation of an example of an incremental intermittent Astrand test (IIAT)

Continuous and discontinuous protocols provide similar $\dot{V}_{O_2max}$ values, but $\dot{W}_{max}$ is higher in the continuous incremental ramp test compared to IIATt (Riboli et al., 2017). This difference can be explained by the faster increase in work rate in the continuous incremental exercise, while the IIAT allows for a better matching of work rate and metabolic rate for the duration of the single step (Riboli et al., 2017).

1.3.3 Sinusoidal exercise

Sinusoidal work rate protocols recently have been re-proposed because of their advantage of simulating long-lasting life activities, in which the intensity oscillates continuously (Yamazaki et al., 1996). Moreover, contrary to the constant work rate protocol, their sinusoidal nature permits examination of the cardiorespiratory response several times (Casaburi et al., 1977).

The sinusoidal protocols are characterized by a work rate that varies continuously, alternating increasing and decreasing phases. The mechanical power, as a function of time (t), fluctuates in a sinusoidal manner according to the following equation:

$$f(t) = MP + AMP \cdot \sin\left(\frac{2\pi}{T}t\right)$$

where, MP is the midpoint value around which the work rate oscillates; AMP is the amplitude the peak deviation of the function from the MP; T is the cycle's duration.

Previous studies have reported that the relationship between cardiorespiratory kinetics and various factors, such as T, changes (Casaburi et al., 1977; Miyamoto et al., 1983; Wigertz, 1970). It has been observed that, when T grows from 0.75 to 15 minutes, HR and $\dot{V}_E$ are characterized by an increase in AMP (Wigertz, 1970). These findings are in agreement with the study of Casaburi et al. (1977) that also found an increase in the phase shift (t_D), defined as the time interval between the mechanical work rate and the physiological response, when T decreases.

To better understand the cardiorespiratory and metabolic response to a sub-threshold sinusoidal cycle, Fukuoka et al. (1997) assessed four t_Ds: at maximum and minimum of the sinusoidal pattern, and at upward and downward MP (Figure 18)

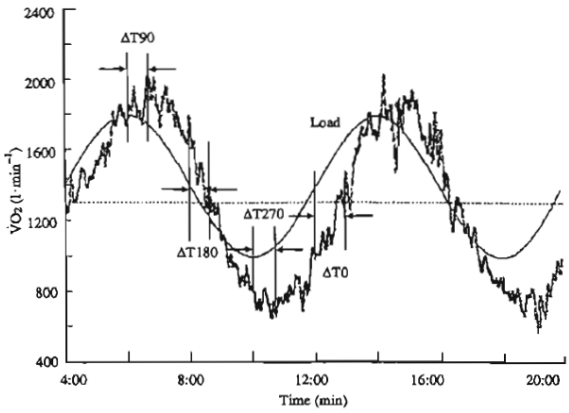

Figure 18: Example of $\dot{V}_{O_2}$ response to sinusoidal work rate with a period of 8 min. Solid line, work rate; ΔT0-ΔT270. time differences between work rate at O°, 90° and 270° and $\dot{V}_{O_2}$ response at the midpoint of upslope or downslope, at crest, and at the trough. Dotted line designates mean value in response of $\dot{V}_{O_2}$ during sinusoidal exercise (Fukuoka et al., 1997)

In that study, the authors provided evidence that the respiratory and metabolic response to sinusoidal work rate had a steeper down-slope and sluggish up-slope at higher T of work rate (i.e., T of 4-16 min), but at lower T of work rate was almost sinusoidal in form. The time difference between sinusoidal work loading and each response curve was greater at the crest than at the trough for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and $\dot{V}_E$. This asymmetry may be due to the fact that at the crest of work loading, O_2 deficit increased progressively with rising work rate because a gradual delay in aerobic metabolic activity occurred. In contrast, at the trough of work loading, the time difference was less affected by O_2 deficit (Fukuoka et al., 1997).

Successive studies (Bakker et al., 1980; Miyamoto et al., 1983) demonstrated a strong correlation between the time constants of $\dot{V}_E$ and $\dot{V}_{CO_2}$ (Figure 19), justifying that hyperpnea during the non-stationary state may be explained by the cardiodynamic hypothesis (Haouzi, 2006; Wasserman et al., 1974).

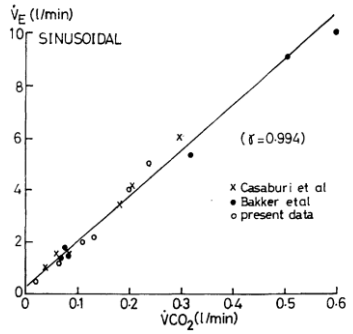

Figure 19: Relationship between the mean-to-peak amplitude of $\dot{V}_E$ and $\dot{V}_{CO_2}$ responses to sinusoidal work rate. The data are based on the studies of Bakker et al. (1980), Casaburi et al. (1977) and Miyamoto et al. (1983) presented there as present data

According to these findings, Casaburi et al. (1978) provided further evidence that hyperpnea during the non-stationary state is linked to $\dot{V}_{CO_2}$ to the central circulation,

changing the sinusoidal work rate between different pedaling frequencies (40-80 rpm). Indeed, changes in pedaling frequency did not generate a ventilatory response independent of $\dot{V}_{CO_2}$ effect. This provides further evidence that exercise hyperpnea was related to the $\dot{V}_{CO_2}$ in the circulation and not from afferent nerve impulses from the exercising limbs. On the contrary, Wells (2007) noted that $\dot{V}_E$ responded faster and to a greater amplitude when the speed of treadmill varied rather than its inclination; therefore concluded that the frequency of limb movements may be a significant factor in hyperpnea. Miyamoto and colleagues (1983), investigated the effect of T changes also on HR, $\dot{Q}$ and SV. They found that $\dot{Q}$ is strongly influenced by HR changes, rather than SV which did not follow a sinusoidal pattern and remained almost constant. Investigating deeply the HR response to sinusoidal work rate, Sone (1996) suggested that the contribution of the withdrawal of cardiac parasympathetic activity to rises in HR with increases in exercise intensity during sinusoidal exercise were greater at lower HR, and that the cardiac parasympathetic system was more activated during HR decreases than during HR increases at the same HR In other words, the complex parasympathetic HR regulation during sinusoidal exercise, depending on the level and the direction of the change (increase or decrease) in HR, may be influenced by parasympathetic and sympathetic contribution.

Haouzi et al. (1993) compared the $\dot{V}_{O_2}$ and HR kinetics during short-lasting sinusoidal exercise between moderate and heavy exercise intensities (below and above LT but below CP), thus investigating the effects of different MPs. During heavy exercise, they found a decrease in AMP and an increase in t_D in both the investigated variables compared to moderate exercise (Haouzi et al., 1993). The longer HR t_D was interpreted to be due to

the prevalent contribution of sympathetic activity during the heavy exercise (Haouzi et al., 1993; Maciel et al., 1986; Rowell & O'Leary, 1990). The blunted $\dot{V}_{O_2}$ response found during heavy intensity sinusoidal exercise was interpreted as an expression of the slower dynamics (Haouzi et al., 1993; Whipp et al., 1986).

Previous studies have investigated the effects of aging (Cunningham et al., 1993; Ebine et al., 2018; Haouzi et al., 1992) and physical fitness (Tiedt et al., 1975; Fukuoka et al., 1995; Fukuoka et al., 2002) on the relationships between the cardiorespiratory and metabolic responses to sinusoidal exercise. Indeed, Cunningham and colleagues (1993) and Ebine and colleagues (2018), comparing the cardiorespiratory and metabolic responses to sinusoidal work rate between women of different ages, found that time constants increase with aging. The slowing of $\dot{V}_E$ and $\dot{V}_{CO_2}$ has been explained as an attenuation of the responsiveness of the carotid bodies, while the slowing of $\dot{V}_{O_2}$ may be due to changes in phosphocreatine system at the muscular level, while autonomic control changes may be responsible for HR slowing down (Cunningham et al., 1993). Similar results have been obtained by Haouzi et al. (1992), who found that children have faster ventilatory kinetics than adults. In contrast, the HR response to sinusoidal exercise does not significantly change between adults and children with the exception of small difference in the t_D during short T sinusoidal protocols (Fukuba et al., 1999; Fukuoka et al., 2002).

The influence of physical activity on the cardiorespiratory and metabolic responses to sinusoidal exercise was investigated by Fukuoka and colleagues (1995; 2002) and Tiedt and colleagues (1975). These authors, comparing American football players, distance runners and untrained subjects, found an increase in AMP and decrease in t_D of the

cardiorespiratory and metabolic variables in the athletes with higher fitness levels. These results may be explained by the higher $\dot{V}_{O_2max}$ participants having better O_2 delivery and extraction. These findings are highlighted by Fukuoka (1997) who found a decreased t_D of $\dot{V}_E$ and $\dot{V}_{CO_2}$ after a football training.

Lastly, sinusoidal exercise also has been applied in the clinical setting, where patients with chronic obstructive pulmonary disease (COPD) performed high-intensity sinusoidal exercise (Porszasz et al., 2013). In that study, it was observed that sinusoidal exercise, with certain parameters (i.e., smaller T), allows COPD patients to sustain a high intensity exercise for a prolonged time.

1.4 Effect of cigarette smoking

In 2019, more than 1 billion people were estimated to be current tobacco smokers, hence, the massive health and economic costs of the tobacco epidemic are likely to rise in the coming decades (Global Burden of Disease, 2017). Large cohort studies have observed that, at minimum, 50% of chronic tobacco smokers will die from causes directly linked to smoking, and that tobacco smokers have an average life expectancy that is 10 years shorter than that of people who have never smoked (Doll et al., 2005; Reitsma et al., 2021). Indeed, cigarette smoking is considered to be one of the most important risk factors for future cardiovascular morbidity and a major cause of mortality (Organization WHO, 2011). Cigarette smoking, both acute and chronic, can induce alterations of the local functionality of different apparatuses and systemic remodeling. Indeed, according to King (1987), cigarettes include some 260 different compounds, of which the most well-known

and investigated are carbon monoxide (CO), nicotine and tar, which are responsible for many health abnormalities.

1.4.1 On cardiovascular system

Nicotine directly stimulates sympathetic neurotransmission on the central nervous system by exciting ganglionic sympathetic neurotransmission and by accelerating catecholamine release by postganglionic nerve endings (Benowitz & Gourlay, 1997). This sympathetic activation increases myocardial contractility and HR (Chaabane et al., 2016; Kobayashi et al., 2004; Papathanasiou et al., 2007) through β_1-adrenergic receptor stimulation (Narkiewicz et al., 1998) and increases vasomotor tone by stimulating α_2-adrenoreceptors (Adamopoulos et al., 2008). In addition, as a result of sympathetic predominance, a reduction in vagal cardiac control and a depression of baroreflexes has been observed (Lucini et al., 1996).

Tobacco is a trigger for generalized vascular inflammation that affects the tunica media of blood vessels, causing a global vasoconstriction (Amato et al., 2013; De Tarso Muller et al., 2019; Yilmaz et al., 2007). Indeed, smoking has been indicated as a cause of inflammation by simultaneously increased proinflammatory and decreasing anti-inflammatory cytokines (Arnson et al., 2010). Cigarette smoking has been demonstrated to stimulate oxidative stress, altering vascular tone (Rahman & Laher, 2007). Indeed, smoking increases the production of reactive oxygen species, which decrease the activity of nitric oxide synthase (NOS) and inhibit nitric oxide (NO) production by the endothelium (Peluffo et al., 2009; Valavanidis et al., 2009). Moreover, active smoking reduces total body antioxidant levels, which increases endothelial dysfunction and arterial

stiffness (Doonan et al., 2010). It has been demonstrated that the chronic smoking negatively impacts endothelial function independent of the type of cigarette with even the lowest amount of cigarettes inducing maximal damage on flow mediated dilation (Amato et al., 2013).

It has also been observed that chronic cigarette smoking causes an impairment on diastolic function of the heart (Gulel et al., 2007; Yilmaz et al., 2007). This adverse effect may be due to the increased arterial stiffness that is associated with structural alterations in the vascular media, including calcification (Mahmud & Feely, 2003), increased collagen and reduced elastin content (Lakatta & Levy, 2003). The aforementioned impairment in regulation of NO synthesis may be another possible mechanism explaining the impairment of ventricular relaxation in smokers (Yilmaz et al., 2007).

Regarding the relationship between cigarette smoking and blood pressure, cigarette smoking acutely exerts a persisting pressor and tachycardia effect through stimulation of the sympathetic nervous system. However, this evidence is not unequivocal. Available data on cigarette smoking and blood pressure do not point to a clear, direct causal relationship between these two cardiovascular risk factors, a concept supported by the evidence that lower blood pressure values are not observed after chronic smoking cessation. Nevertheless, smoking, affecting arterial stiffness and wave reflection, might have greater detrimental effects on central blood pressure, which is more closely related to target organ damage than brachial blood pressure (Figure 20) (Virdis et al., 2010).

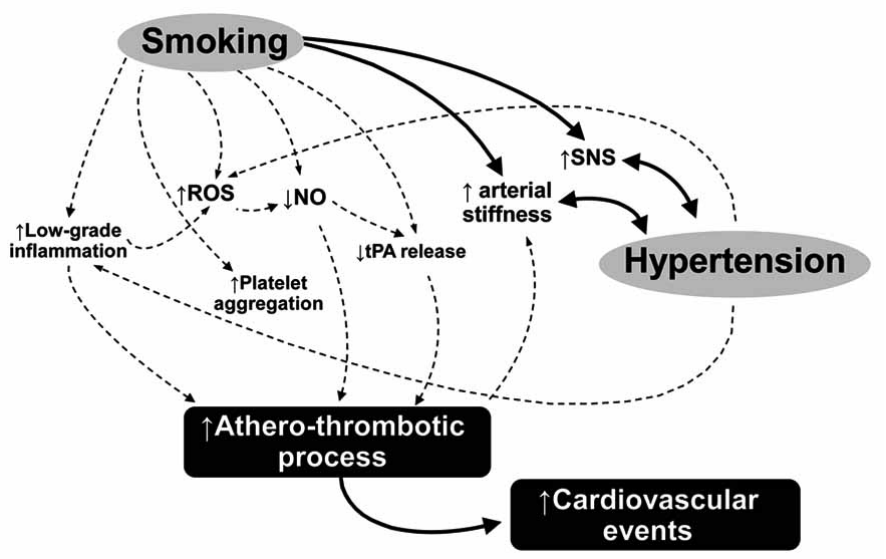

Figure 20: Schematic representation of the most important mechanisms whereby smoking, hypertension (broken lines), and their association (continuous lines), can accelerate the atherothrombotic process (Virdis et al., 2010)

1.4.2 On pulmonary system

Current smokers show increased airway wall thickness than never-smokers, independent of age (Telenga et al., 2017), and proportional to cigarette exposure (i.e. more packyears smoked) and that current-smoking was associated with a narrower airway lumen (Donohue et al., 2012). Thicker airway walls, in current-smokers, may be due to the effects of continuous smoke exposure on the epithelium that produces pro-inflammatory cytokines causing remodeling (Takizawa et al., 2001).

The work of the inspiratory muscles at rest increases (Clini et al., 2016; Elbehairy et al., 2016) as well as $\dot{V}_E$ (McDonough & Moffatt, 1999; Rotstein et al., 1991) to counterbalance the aforementioned remodeling and reduced $\dot{V}_{O_2}$ (Kimura et al., 2007; McDonough & Moffatt, 1999). The reduced $\dot{V}_{O_2}$ is due to CO, produced by the incomplete combustion of carbon contained in tobacco, that combines more readily with

haemoglobin (Hb) than O_2 according to this reaction (De Tarso Muller et al., 2019; Klausen et al., 1983; McDonough & Moffatt, 1999):

$$HbO_2 + CO \Rightarrow COHb + O_2$$

where, HbO_2 is oxyhaemoglobin, COHb is carboxyhaemoglobin. It is very difficult to reverse this equation in favour of O_2 binding because the Hb affinity for CO is approximately 225 times that for O_2 (Rietbrock et al., 1992).

In an animal model study (Wawryk-Gawda et al., 2020), an increased thickness of the blood-air membrane that impaired diffusion has been demonstrated. A reduction in O_2 diffusion across the pulmonary membrane-capillary interface is also provoked by tar, a product of tobacco combustion that increases pulmonary airway resistance (Nadel & Comroe, 1961). It has also been noted that cigarette smoking activates neutrophil and macrophage elastases, which damage the elastic fibers and lead to emphysema (Wawryk-Gawda et al., 2020). Moreover, nicotine may provoke the induction of the fibroblast differentiation into myofibroblasts. The latter cause pro-fibrotic extracellular matrix protein secretion. Consequently, the accumulation of collagen within lung tissue leads to fibrosis and decreases the the surface area for gas exchange (Blaauboer et al., 2014).

1.4.3 On musculo-skeletal system

In the literature, there is a lack of in-depth analysis regarding the effects of smoking on the “muscle-mitochondria compartment” as a possible limiting factor in non-COPD smokers. Despite reduced muscle strength and/or mass in smokers (Degens et al., 2015; Kok et al., 2012; Wüst et al., 2008), chronic nicotine-induced sympathetic nerve over-

stimulation may offset the negative consequences of this (Mündel & Jones, 2006). Muscle wasting after chronic cigarette smoking might be associated to increased ubiquitin-mediated proteolysis (Rom et al., 2012; Talukder et al., 2011) and to inhibition of anabolic pathways and protein synthesis in the quadriceps (Degens et al., 2015; Madani et al., 2018). Previous studies have demonstrated changes in muscle fiber endotype toward a less oxidative profile (Krüger et al., 2015; 2018). While, decreased muscle capillarization is still controversial (Nogueira et al., 2018; Wüst et al., 2008).

The mitochondrion is a key target for smoking toxicity, leading to reduced respiration, decreased ATP content, and increased production of free radicals in a dose- and time-dependent manner (Madani et al., 2018; Neves et al., 2016). Moreover, other metabolic derangements that may impair the physical performance include: a considerable (10%) rise in energy expenditure at rest compared to nonsmokers (Hofstetter et al., 1986), impaired sarcoplasmic reticulum calcium uptake in myofibers (Nogueira et al., 2018), and reduced insulin-dependent glycogen recovery from exercise (Jensen et al., 1995).

1.4.4 On exercise performance

The aforementioned alterations may affect the cardiorespiratory and metabolic responses to exercise. Smokers exhibit an intolerance to sub-maximal and maximal exercise (Bernaards et al., 2003; Gläser et al., 2011; Kobayashi et al., 2004; Mendonca et al., 2011; Papathanasiou et al., 2007). Exercise intolerance has been noted as an increase in submaximal HR (Kobayashi et al., 2004; Mendonca et al., 2011) and as the slower HR recovery kinetics because of the aforementioned nicotine over stimulation of sympathetic nervous system (Kobayashi et al., 2004). No studies about respiratory and metabolic

responses to a submaximal exercise have been conducted. The impact of acute cigarette smoking on the cardiorespiratory and metabolic kinetics has been assessed by only one study (Rotstein et al., 1991). A considerable lengthening of the physiological kinetics was observed after an acute consumption of three cigarettes immediately before the test, compared to 24-h smoking wash-out in the same group. To our knowledge, no study has yet investigated the effect of chronic smoking on aerobic performance.

In response to maximal exercise, a reduction in $\dot{V}_{O_2max}$ (Bernaards et al., 2003; Kobayashi et al., 2004; Lee & Chang, 2013; Sven et al., 2010) and HR because of chronotropic incompetence (Bernaards et al., 2003; Sven et al., 2010), as well as reduced LT and ventilatory thresholds as result of bioenergetic derangements have been observed (Lauria et al., 2017; Miyatake et al., 2011).

These abnormalities occur not only in a sedentary middle age population, but also in young athletes (Chaabane et al., 2016; Saranovic et al., 2019).

2 Aim

It is well known that tobacco cigarettes have detrimental health effects that may lead to different types of diseases such as COPD, heart disease, type 2 diabetes and lower bone density (Sengbusch et al., 2021). The negative impact of smoking on the lungs and the heart begs the question how cigarette smoking affects physical activity and exercise. Hence, this dissertation aimed to provide a comprehensive examination of the effect of cigarette smoking on the cardiorespiratory and metabolic responses to exercise in young, physically active males. Young smokers were chosen as a physiological model to evaluate the impact of the cigarette-induced abnormalities on the heart-lung-muscle system during exercise, avoiding any other comorbidities and any other affecting factors (i.e., gender, aging, or sedentary lifestyle).

The smokers were evaluated in four different studies using different exercise protocols: i) moderate intensity square-wave work rates to assess some markers of cardiopulmonary fitness and exercise tolerance; ii) incremental stepwise protocols to determine $\dot{V}_{O_2max}$, a benchmark for cardiorespiratory fitness, recovery kinetics, and exercise capacity; iii) moderate intensity sinusoidal work rates to investigate the cardiorespiratory responses; and iv) heavy intensity sinusoidal work rate protocols to investigate the cardiorespiratory responses. It was hypothesized that, despite high physical activity, cigarette smokers would have exercise intolerance during all exercise protocols, slower cardiorespiratory and metabolic kinetics during both on and off phases, reduced recovery capacity after maximal exercise, and shorter time to exhaustion during sinusoidal work rate exercise coupled with longer t_Ds of both exercise intensities.

3 Study 1: The impact of chronic cigarette smoking on cardiorespiratory and metabolic kinetics in young physically active males

3.1 Aim

The aim of this project was to evaluate the impact of cigarette smoking on both the on- and off-phases of cardiorespiratory and metabolic kinetics during moderate exercise in young, physically active males compared to a group of well-match non-smokers. We hypothesized that resting HR would be greater and the on- and off-phase HR kinetics would be slower in the smokers compared to the non-smokers. Moreover, the two groups should not differ in $\dot{V}_{O_2max}$ because they are sportsmen of the same fitness level (A. R. Morton & Holmik, 1985).

3.2 Materials and Methods

3.2.1 Participants

Based on pilot testing, the optimal sample size was computed using a statistical software (G-Power 3.1, Dusseldorf, Germany), expecting a large Cohen's d effect size (1.4) in τ differences between groups. Considering a required power $(1 - \beta) > 0.80$ and an $\alpha < 0.05$, the optimal sample size was 20 participants (n = 10 for smokers and n = 10 for non-smokers). 28 volunteers were recruited, but 2 were excluded from the study after having showed ectopic beats and extrasystoles, 3 were excluded because of fitness level (too unfit), and 3 dropped out for personal reasons. Hence, nine smokers and eleven age-matched non-smokers completed the protocol. Their demographic characteristics are shown in Table 1. Inclusion criteria for smokers were at least 6 cigarettes per day and the

continued use of cigarette smoking for at least two years (Okuyemi et al., 2002). For both groups exclusion criteria were: i) presence of cardiovascular and respiratory diseases; ii) presence of musculoskeletal impairments; and iii) taking medications that alter cardiovascular and respiratory responses. After being fully informed about the purpose and the experimental procedures, participants provided written informed consent, which was approved by the local University Ethical Committee (#77/20) and was performed in accordance with the latest principles of the Declaration of Helsinki.

	CTRL	**Smokers**	**p value**
Number	11	9	
Age (year)	23.8 ± 3.3	21.3 ± 2.0	n.s.
Body mass (kg)	79.0 ± 10.0	78.5 ± 6.2	n.s.
Stature (m)	1.80 ± 0.08	1.80 ± 0.07	n.s.
Cigarettes per day	-	12 ± 5	
Smoking history (year)	-	6 ± 2	

Table 1: Demographic characteristics of smoker and control group (CTRL) expressed as mean ± standard deviation (SD).

3.2.2 Study design

Participants reported to the laboratory four times with at least 48 hours between each test. On the first day, after familiarization and anthropometric assessments, pulmonary function was evaluated. On the second session, participants underwent an incremental cycle-ergometer test to determine $\dot{V}_{O_2max}$, $\dot{W}_{max}$ as well as the lactate and ventilatory thresholds. During the third and the fourth visits, the participants performed two square-wave work rate protocols to determine cardiorespiratory and metabolic kinetics to moderate exercise.

3.2.3 Experimental procedures

All experimental sessions were conducted in a climate-controlled laboratory (with constant temperature of 20 ± 1°C and relative humidity of 50 ± 5%) at approximately the same time of the day to minimize possible bias induced by circadian rhythms. Each day of testing, participants were asked to abstain from caffeine and any other stimulant substances for at least 12 hours, and to refrain from heavy exercise for at least 24 hours prior to testing. Smokers were asked to smoke the last cigarette 1.5 hours before the test to allow 5-16% elimination of blood COHb levels in order to avoid the acute effects of cigarette smoking (McDonough & Moffatt, 1999).

Familiarization, anthropometric and pulmonary function assessments

During the first session, the participants were equipped with a facemask and wearable devices to familiarize them with the equipment for cardiopulmonary exercise testing. The optimal saddle height, handlebars angle of inclination, and foot position over the pedal were defined for each participant (Peveler, 2008; Wang et al., 2020). Body mass and stature of each volunteer were measured using a mechanical scale equipped with a stadiometer (Asimed, Samadell; Barcelona). Participants then underwent a spirometric plethysmographic evaluation to determine VC and dynamic lung volumes (forced vital capacity, FVC; forced expiratory volume, FEV_1; peak expiratory flow, PEF; and maximum voluntary ventilation, MVV). Maximal inspiratory and expiratory pressure (MIP and MEP, respectively) were measured at the mouth using a portable manometer equipped with a mouthpiece (S&M Instrument Company Inc., mod. PortaResp,

Doylestown, PA). After familiarization with the manometer, participants repeated the maneuvers three times and the highest value was recorded.

Incremental exercise test

On the second visit, $\dot{V}_{O_2max}$ and $\dot{W}_{max}$ were determined through a stepwise incremental test. After three minutes of basal recordings and four minutes of warm-up cycling at 100 W, work rate was increased by 25 W every two minutes until task failure (Figure 21). Participants were asked to maintain the pedaling rate between 60-70 rpm. Exercise was terminated when the participant failed to maintain the cadence within 5 rpm of the imposed range for more than 5 s (Brickley et al., 2002).

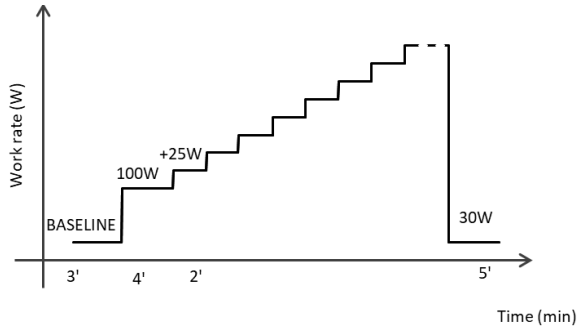

Figure 21: Stepwise test protocol

[La^-] was assessed at baseline, at the end of each work rate and at minutes 1, 3 and 5 of recovery. At the same time of blood sample collections, RPE was asked on a general (RPE_{GEN}; Borg 6-20), respiratory and muscular (RPE_{MUSC} and RPE_{RESP}, respectively; CR-10) standpoints.

Kinetics assessments

During the third and the fourth visits, participants performed two square-wave transitions to a moderate intensity work rate that elicited a $\dot{V}_{O_2}$ corresponding to 90% of VT_1.

Participants completed four trials each including cycling 6 minutes at 20 W, 6 minutes at 90% VT_1, and 8 minutes at 20 W. Each trial was separated by a 30-min resting recovery while the participant was seated on a chair (Murias et al., 2011) (Figure 22).

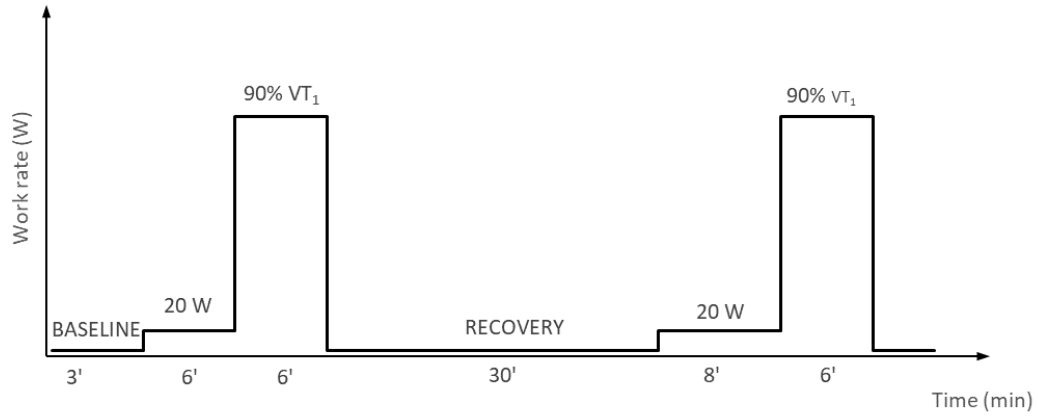

Figure 22: Square wave protocol for the kinetics assessment. VT_1, first ventilatory threshold

3.2.4 Measurements

Tests were performed on an electro-mechanically braked cycle ergometer (mod. 839E, Monark, Sweden). During the experiments, work rate and cadence were continuously recorded. $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, f_R, and respiratory exchange ratio (RER) were measured on a breath-by-breath basis by a metabolic unit consisting of a turbine flowmeter, a zirconium oxygen sensor and an infrared CO_2 meter (Quark b^2, Cosmed, Rome, Italy). According to the manufacturer's instructions, the turbine and gas analyzers were calibrated before each test by means of a 3-l syringe (mod. 5530, Hans-Rudolph, Shawnee, KS, USA) and a certified gas mixture of known concentrations (16% O_2, 5% CO_2, balance N_2), respectively. Simultaneously, HR was continuously acquired with a heart rate monitor (S810, Polar Electro Oy, Kempele, Finland).

3.2.5 Data analysis

All data were analyzed off-line. Cardiorespiratory and metabolic data were filtered by deleting points outside of three standard deviation of the local mean (Lamarra et al., 1987).

From the incremental test, $\dot{V}_{O_2max}$ was identified as the value obtained from the plateau in the relationship between $\dot{V}_{O_2}$ and $\dot{W}$. In the event that the plateau did not occur, the highest value of $\dot{V}_{O_2}$ was used if: i) HR did not increase between consecutive loads; ii) RER > 1.10 (Adami et al., 2013). $\dot{W}_{max}$ was determined as the mechanical work rate corresponding to the intersection of the line representing the linear relationship between $\dot{V}_{O_2}$ and $\dot{W}$ and the line corresponding to the plateau of $\dot{V}_{O_2}$.

VT_1 was identified as the $\dot{V}_{O_2}$ at which $\dot{V}_{CO_2}$ began to increase out of proportion to $\dot{V}_{O_2}$ with a systematic increase in $\dot{V}_E/\dot{V}_{O_2}$ and Pet_{O_2}, whereas $\dot{V}_E/\dot{V}_{CO_2}$ and Pet_{CO_2} were stable (Wasserman et al., 1973). $\dot{V}_{O_2}$ values corresponding to the VT_1 are reported as the average of three experienced independent observers analysis.

For the kinetic analyses, the data of each transition were linearly interpolated to 1-s intervals and time aligned such that time 0 represented the onset of exercise. After the exclusion of the initial 20 seconds, that represent the phase I (Murias et al., 2011), all the transients were averaged together (Keir et al., 2014; Murias et al., 2014). Then, both the on- and off- phase were fit by a mono-exponential of this form:

$$(t) = Y_0 + AMP\left[1 - e^{-(t-TD)/\tau}\right]$$

Where, $Y(t)$ is the cardiorespiratory variable at any point in time, Y_0 constitutes the value before the transient, AMP is the amplitude of the increase/decrease from the Y_0 to the asymptote, τ is the time necessary to reach the 63% of the remaining response, and TD is time delay between the change in work rate and the initial change in the cardiorespiratory variable.

3.2.6 Statistical analysis

Descriptive statistics were used assess participant characteristics. The Shapiro-Wilk test was applied to check for normal distribution. Unpaired Student's t-tests were used to determine differences in cardiorespiratory and metabolic variables between the two groups. The Hedge's g effect size with 95% CI was calculated and interpreted as: 0.00–0.19: trivial; 0.20–0.59: small; 0.60–1.19: moderate; 1.20–1.99: large; ≥ 2.00: very large (Hopkins et al., 2009). All statistical analyses were performed using a statistical software (SigmaPlot ver. 12.5, Systat Software, Inc., San Jose, CA USA). The significance level was set at $p < 0.05$. Results are presented as mean ± standard deviation (SD).

3.3 Results

The main pulmonary function outcomes are shown in Table 2.

	CTRL	Smokers	p value
VC (l)	5.5 ± 0.6	5.5 ± 0.6	n.s.
FVC (l)	5.8 ± 0.7	5.7 ± 0.7	n.s.
FEV_1 (l)	5.0 ± 0.6	4.7 ± 0.6	n.s.
PEF ($l \cdot s^{-1}$)	10.5 ± 1.7	7.8 ± 3.4*	n.s.
MVV ($l \cdot min^{-1}$)	191 ± 23	175 ± 20	n.s.
MIP (cmH_2O)	113 ± 20	120 ± 18	n.s.
MEP (cmH_2O)	127 ± 25	128 ± 15	n.s.

*Table 2: Respiratory function test parameters in smoker and control groups (mean ± standard deviation, SD). VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume during the first second of the test; PEF, peak expiratory flow; MVV, maximal voluntary ventilation; MIP, maximal inspiratory mouth pressure; MEP, maximal expiratory mouth pressure. * p <0.05 vs CTRL*

The resting values of the main cardiorespiratory and metabolic variables are indicated in Table 3.

	CTRL	Smokers	p value
HR ($b \cdot min^{-1}$)	73 ± 8	77 ± 12	n.s.
$\dot{V}_E$ ($l \cdot min^{-1}$)	12 ± 2	11 ± 2	n.s.
f_R ($b \cdot min^{-1}$)	18 ± 4	16 ± 4	n.s.
V_T (l)	0.7 ± 0.1	0.7 ± 0.2	n.s.
$\dot{V}_{O_2}$ ($ml \cdot min^{-1}$)	364 ± 55	330 ± 72	n.s.
$\dot{V}_{CO_2}$ ($ml \cdot min^{-1}$)	311 ± 56	278 ± 62	n.s.

*Table 3: Resting values of cardiorespiratory and metabolic variables in smoker and control groups (mean ± standard deviation, SD). HR, heart rate; $\dot{V}_E$, expiratory ventilation; f_R, respiratory frequency; V_T, tidal volume; $\dot{V}_{O_2}$, pulmonary oxygen uptake; $\dot{V}_{CO_2}$, carbon dioxide production. * p <0.05 vs CTRL*

Although similar values between smokers and CTRL were found for $\dot{V}_{O_2 max}$ (3410 ± 328 vs 3586 ± 310 $ml \cdot min^{-1}$, respectively) and HR_{max} (185 ± 9 vs 184 ± 9 $b \cdot min^{-1}$, respectively), the smokers exhibited a lower $\dot{W}_{max}$ compared to CTRL (247 ± 22 vs 275

± 25 W, respectively, p = 0.019; g = 1.11, moderate), $\dot{V}_{E\,max}$ (126 ± 11 vs 140 ± 17 l·min^{-1}, respectively, p = 0.04; g = 0.99, moderate). $f_{R\,max}$ (49 ± 7 vs 58 ± 10 b·min^{-1}, respectively, p = 0.031; g = 1.01, moderate). Similar results have been found for $V_{T\,max}$ in smokers and CTRL group (2.6 ± 0.4 vs 2.5 ± 0.4 l, respectively) and $\dot{V}_{CO_2 max}$ (3856 ± 391 vs 3853 ± 363 ml·min^{-1}, respectively). Smokers exhibited greater $Pet_{CO_2\,max}$ (40 ± 5 vs 32 ± 2 mmHg, respectively, p <0.001; g = 1.90, large) and tended to have lower $Pet_{O_2\,max}$ (115 ± 4 vs 118 ± 3 mmHg, respectively, p = 0.056; g = 0.88, moderate).

The cardiorespiratory and metabolic kinetics results are shown in Table 4 while a representative subjects fitting is shown in Figures 23.

		ON			OFF		
		CTRL	**Smokers**	**p value**	**CTRL**	**Smokers**	**p value**
Yo	HR (b·min^{-1})	81±9	85±10	n.s.	139±15	139±16	n.s.
	$\dot{V}_{O_2}$ (ml·min^{-1})	751±92	688±222	n.s.	2492±285	2373±206	n.s.
	$\dot{V}_E$ (l·min^{-1})	19±2	18±5	n.s.	60±7	58±6	n.s.
	$\dot{V}_{CO_2}$ (ml·min^{-1})	653±89	582±184	n.s.	2414±281	2287±217	n.s.
AMP	HR (b·min^{-1})	55±9	50±12	n.s.	54±9	52±14	n.s.
	$\dot{V}_{O_2}$ (ml·min^{-1})	1697±243	1648±256	n.s.	2084±258	1962±211	n.s.
	$\dot{V}_E$ (l·min^{-1})	41±8	39±5	n.s.	45±6	43±7	n.s.
	$\dot{V}_{CO_2}$ (ml·min^{-1})	1791±253	1788±198	n.s.	2016±263	1920±251	n.s.
τ	HR (b·min^{-1})	44±7	52±11*	p=0.04 g=1.13 $CI_{95\%}$=0.20-2.06	35±9	45±15	n.s.
	$\dot{V}_{O_2}$ (ml·min^{-1})	36±12	27±4*	p=0.03 g=1.05 $CI_{95\%}$=0.13-1.98	36±5	43±7*	p= 0.02 g=1.06 $CI_{95\%}$=0.13-1.98
	$\dot{V}_E$ (l·min^{-1})	48±8	70±30*	p=0.02 g=1.36 $CI_{95\%}$=0.40-2.32	57±11	67±26	n.s.
	$\dot{V}_{CO_2}$ (ml·min^{-1})	62±28	46±6	n.s.	52±10	62±20	n.s.

*Table 4: Initial values (Y_0), amplitude (AMP), tau (τ) of the on- or off- transients in controls (CTRL) and smokers for heart rate (HR) pulmonary oxygen uptake ($\dot{V}_{O_2}$), expiratory ventilation ($\dot{V}_E$) and carbon dioxide production ($\dot{V}_{CO_2}$). *p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD).*

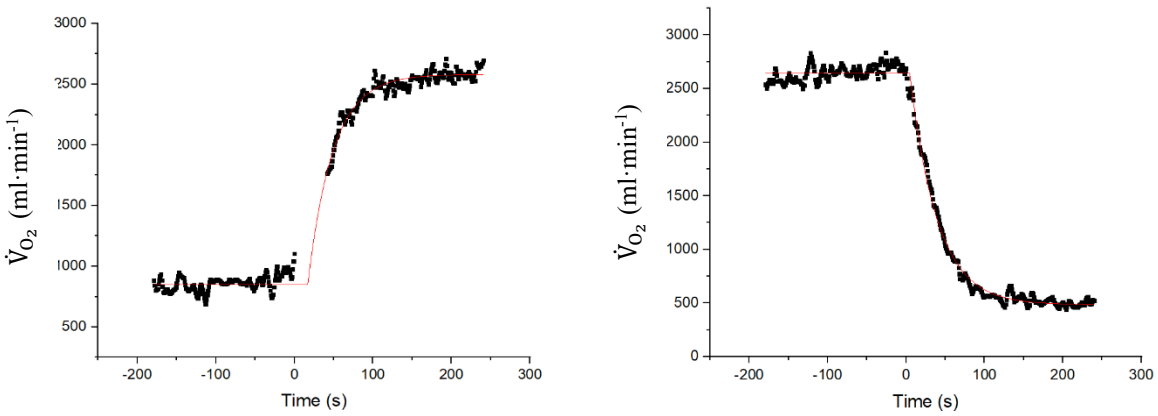

Figure 23: Representative subject of pulmonary oxygen uptake ($\dot{V}_{O_2}$) fitting during phase on (left panel) and phase off (right panel)

4 Study 2: Cardiorespiratory and metabolic kinetics during recovery from an incremental test in young physically active male chronic cigarette smokers

4.1 Aim

The aim of this project was to determine the impact of chronic cigarette smoking on the kinetics of cardiorespiratory and metabolic recovery after a maximal incremental test in young, physically active smokers. We hypothesized that, compared to controls, chronic cigarette smokers would have: i) greater resting HR, ii) slower cardiorespiratory and metabolic recovery kinetics, and iii) greater HRR indexes.

4.2 Materials and Methods

4.2.1 Participants

Ten smokers and twelve age-matched non-smokers completed the protocol. Their characteristics are shown in Table 5.

	CTRL	Smokers	p value
Number	13	10	
Age (year)	23.5 ± 2.9	21.5 ± 2.4	n.s.
Body mass (kg)	77.6 ± 9.1	77.0 ± 7.4	n.s.
Stature (m)	1.80 ± 0.08	1.79 ± 0.06	n.s.
Cigarettes per day	-	12 ± 5	
Smoking history (year)	-	6 ± 2	

Table 5: Characteristics of smoker and control groups (CTRL) expressed as mean ± standard deviation (SD).

4.2.2 Study design

Participants reported to the laboratory two times with at least 48 hours between each visit. On the first day, after familiarization and anthropometric assessments, pulmonary function was evaluated. On the second session, participants underwent an incremental cycle-ergometer test to determine $\dot{V}_{O_2max}$, $\dot{W}_{max}$ as well as cardiorespiratory and metabolic variables recovery kinetics.

4.2.3 Experimental procedures

All experimental sessions were conducted in a climate-controlled laboratory (with constant temperature of 20 ± 1°C and relative humidity of 50 ± 5%) at approximately the same time of the day to minimize possible bias induced by circadian rhythms. Each day of testing, participants were asked to abstain from caffeine and any other stimulant substances for at least 12 hours, and to refrain from heavy exercise for at least 24 hours prior to testing. Smokers were asked to smoke the last cigarette 1.5 hours before the test to allow 5-16% elimination of blood COHb levels in order to avoid the acute effects of cigarette smoking (McDonough & Moffatt, 1999).

Familiarization, anthropometric and pulmonary function assessments

During the first session, the participants were equipped with a face mask and wearable devices to familiarize them with the equipment for cardiopulmonary exercise testing. The optimal saddle height, handlebars angle of inclination, and foot position over the pedal were defined for each participant (Peveler, 2008; Wang et al., 2020). Body mass and stature of each volunteer were measured using a mechanical scale equipped with a stadiometer (Asimed, Samadell; Barcelona). Participants then underwent a spirometric plethysmographic evaluation to determine VC and dynamic lung volumes (FVC, FEV_1,

PEF and MVV). Maximal inspiratory and expiratory pressure (MIP and MEP, respectively) were measured at the mouth using a portable manometer equipped with a mouthpiece (S&M Instrument Company Inc., mod. PortaResp, Doylestown, PA). After familiarization with the manometer, participants repeated the maneuvers three times, and the highest value was recorded.

Incremental exercise test

On the second visit, $\dot{V}_{O_2max}$ and $\dot{W}_{max}$ were determined through a stepwise incremental test. After three minutes of basal recordings and four minutes of warm-up cycling at 100 W, work rate was increased by 25 W every two minutes until task failure Participants were asked to maintain the pedaling rate between 60-70 rpm. Exercise was terminated when the participant failed to maintain the cadence within 5 rpm of the imposed range for more than 5 s (Brickley et al., 2002). After exercise cessation, an active 5-min recovery at 30 W at 30 rpm was included.

4.2.4 Measurements

Tests were performed on an electro-mechanically braked cycle ergometer (mod. 839E, Monark, Sweden). During the experiments, work rate and cadence were continuously recorded. $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, f_R, and RER were measured on a breath-by-breath basis by a metabolic unit consisting of a turbine flowmeter, a zirconium oxygen sensor and an infrared CO_2 meter (Quark b^2, Cosmed, Rome, Italy). According to the manufacturer's instructions, the turbine and gas analyzers were calibrated before each test by means of a 3-l syringe (mod. 5530, Hans-Rudolph, Shawnee, KS, USA) and a certified gas mixture of known concentrations (16% O_2, 5% CO_2, balance N_2), respectively. Simultaneously,

HR was continuously acquired with a heart rate monitor (S810, Polar Electro Oy, Kempele, Finland).

20 µl arterialized blood samples were taken at baseline, at the end of each work rate and at minutes 1, 3 and 5 of recovery of the stepwise incremental test from the ear lobe and analyzed by an enzymatic-amperometric system (Labtrend, Bio Sensor Technology GmbH, Berlin, Germany) to determine [La^-].

4.2.5 Data analysis

All data were analyzed off-line. Cardiorespiratory and metabolic data were filtered by deleting points outside of three standard deviation of the local mean (Lamarra et al., 1987).

From the incremental test, $\dot{V}_{O_2max}$ was identified as the value obtained from the plateau in the relationship between $\dot{V}_{O_2}$ and $\dot{W}$. In the event that the plateau did not occur, the highest value of $\dot{V}_{O_2}$ was used if: i) HR did not increase between consecutive loads; ii) RER > 1.1 (Adami et al., 2013). $\dot{W}_{max}$ was determined as the mechanical work rate corresponding to the intersection of the line representing the linear relationship between $\dot{V}_{O_2}$ and $\dot{W}$ and the line corresponding to the plateau of $\dot{V}_{O_2}$.

For the kinetic analyses, the data of the off- transition were interpolated to 1-s intervals and fit by a mono-exponential of this form:

$$Y(t) = Y_0 + AMP\,[1 - e^{-(t-TD)/\tau}]$$

where, $Y(t)$ is the cardiorespiratory variable at any point in time, Y_0 is the value at the beginning of recovery (i.e., exercise peak), AMP is the decrease from Y_0 to the asymptote,

τ is the time necessary to reach the 63% of the remaining response, and TD is time delay between the change in work rate and the initial change in the cardiorespiratory variable. HRR30s and HRR60s were calculated as differences between peak HR at the end of exercise and the HR value at 30 s and 60 s into recovery, respectively (Peçanha et al., 2017). T30 was measured by fitting the natural logarithm of HR in the first 30 s of recovery into a first-degree polynomial. The index was expressed as the negative reciprocal of the slope of the resulting line (−1/slope) (Imai et al., 1994; Peçanha et al., 2017).

4.2.6 Statistical analysis

Descriptive statistics were used to assess participant characteristics. The Shapiro-Wilk test was applied to check for normal distribution. Unpaired Student's t-test were used to determine the differences in cardiorespiratory and metabolic variables between the two groups. The Hedge's g effect size with 95% CI was calculated and interpreted as follows: 0.00–0.19: trivial; 0.20–0.59: small; 0.60–1.19: moderate; 1.20–1.99: large; ≥ 2.00: very large (Hopkins et al., 2009). All statistical analyses were performed using a statistical software (SigmaPlot ver. 12.5, Systat Software, Inc., San Jose, CA USA). The significance level was set at $p < 0.05$. Results are presented as mean ± standard deviation (SD).

4.3 Results

The main pulmonary function outcomes are shown in Table 6.

	CTRL	Smokers	p value
VC (l)	5.5 ± 0.6	5.4 ± 0.6	n.s.
FVC (l)	5.8 ± 0.7	5.7 ± 0.7	n.s.
FEV_1 (l)	4.9 ± 0.6	4.7 ± 0.6	n.s.
PEF ($l \cdot s^{-1}$)	10.5 ± 1.8	7.7 ± 3.2*	p = 0.01 g=1.00 $CI_{95\%}$=0.12-1.87
MVV ($l \cdot min^{-1}$)	193 ± 21	175 ± 18*	p = 0.04 g=0.88 $CI_{95\%}$=0.02-1.75
MIP (cmH_2O)	116 ± 19	120 ± 17	n.s.
MEP (cmH_2O)	125 ± 28	126 ± 15	n.s.

*Table 6: Respiratory function test parameters in smoker and control groups (mean ± standard deviation, SD). VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume during the first second of the test; PEF, peak expiratory flow; MVV, maximal voluntary ventilation; MIP, maximal inspiratory mouth pressure; MEP, maximal expiratory mouth pressure. * p <0.05 vs CTRL*

The resting values of the main cardiorespiratory and metabolic variables are indicated in Table 7.

	CTRL	Smokers	p value
HR ($b \cdot min^{-1}$)	74 ± 8	77 ± 12	n.s.
$\dot{V}_E$ ($l \cdot min^{-1}$)	12 ± 2	11 ± 2	n.s.
f_R ($b \cdot min^{-1}$)	19 ± 5	16 ± 4	n.s.
V_T (l)	0.7 ± 0.1	0.7 ± 0.2	n.s.
$\dot{V}_{O_2}$ ($ml \cdot min^{-1}$)	359 ± 51	331 ± 68	n.s.
$\dot{V}_{CO_2}$ ($ml \cdot min^{-1}$)	310 ± 51	276 ± 58	n.s.

*Table 7: Resting values of cardiorespiratory and metabolic variables in smoker and control groups (mean ± standard deviation, SD). HR, heart rate; $\dot{V}_E$, expiratory ventilation; f_R, respiratory frequency; V_T, tidal volume; $\dot{V}_{O_2}$, pulmonary oxygen uptake; $\dot{V}_{CO_2}$, carbon dioxide production. * p <0.05 vs CTRL*

Although similar values between smokers and CTRL were found for $\dot{V}_{O_2 max}$ (3341 ± 378 vs 3637 ± 315 $ml \cdot min^{-1}$, respectively) and HR_{max} (184 ± 9 vs 184 ± 9 $b \cdot min^{-1}$,

respectively), the smokers exhibited a lower $\dot{W}_{max}$ compared to CTRL (242 ± 25 vs 275 ± 24 W, respectively, p = 0.004; g = 1.30, large), $\dot{V}_{E\,max}$ (125 ± 11 vs 140 ± 16 l·min^{-1}, respectively, p = 0.014; g = 1.08, moderate). $f_{R\,max}$ (48 ± 7 vs 58 ± 9 b·min^{-1}, respectively, p = 0.01; g = 1.13, moderate). Similar results have been found for $V_{T\,max}$ in smokers and CTRL group (2.6 ± 0.4 vs 2.5 ± 0.4 l, respectively) and $\dot{V}_{CO_2 max}$ (3778 ± 442 vs 3917 ± 385 ml·min^{-1}, respectively). Smokers exhibited greater $Pet_{CO_2\,max}$ (40 ± 4 vs 35 ± 5 mmHg, respectively, p = 0.02; g = 1.01, moderate) and similar $Pet_{O_2\,max}$ (116 ± 4 vs 118 ± 3 mmHg, respectively).

The results of the cardiorespiratory and metabolic kinetics are shown in Table 8. The HRR indexes are presented in Figure 24.

		CTRL	Smokers	p value
Yo	HR (b·min^{-1})	184±10	184±9	n.s.
	$\dot{V}_{O_2}$ (ml·min^{-1})	3607±271	3813±453	n.s.
	$\dot{V}_E$ (l·min^{-1})	141±13	125±10	p=0.006 g=1.23 $CI_{95\%}$=0.33-2.13
	$\dot{V}_{CO_2}$ (ml·min^{-1})	3917±349	3341±377	n.s.
AMP	HR (b·min^{-1})	76±10	78±7	n.s.
	$\dot{V}_{O_2}$ (ml·min^{-1})	2836±269	2754±321	n.s.
	$\dot{V}_E$ (l·min^{-1})	114±14	125±10	p=0.04 g=0.90 $CI_{95\%}$=0.03-1.76
	$\dot{V}_{CO_2}$ (ml·min^{-1})	3279±355	3324±416	n.s.
τ	HR (b·min^{-1})	110±32	108±26	n.s.
	$\dot{V}_{O_2}$ (ml·min^{-1})	66±18	96±15*	P<0.001 g=1.73 $CI_{95\%}$=0.77-2.70
	$\dot{V}_E$ (l·min^{-1})	101±21	120±12*	p=0.01 g=1.08 $CI_{95\%}$=0.20-1.96
	$\dot{V}_{CO_2}$ (ml·min^{-1})	103±24	126±17*	p=0.02 g=1.02 $CI_{95\%}$=0.14-1.89

*Table 8: Initial values (Y_0), amplitude (AMP), tau (τ) of the off- transients in controls (CTRL) and smokers for heart rate (HR) pulmonary oxygen uptake ($\dot{V}_{O_2}$), expiratory ventilation ($\dot{V}_E$) and carbon dioxide production ($\dot{V}_{CO_2}$). *p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD).*

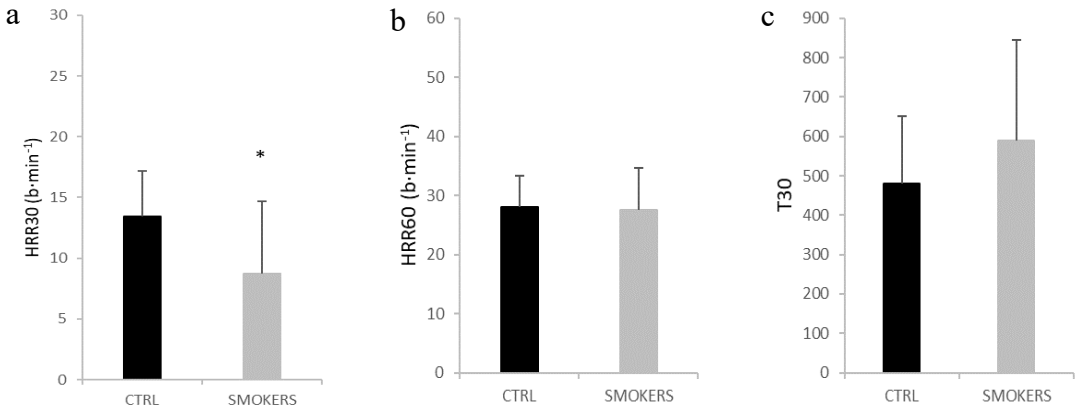

*Figure 24: Heart rate recovery (HRR) indexes in smokers (grey bars) and controls (CTRL, black bars). * p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD).*

5 Study 3: Effect of chronic cigarette smoking on cardiorespiratory and metabolic responses to moderate intensity sinusoidal work rate

5.1 Aim

The aim of this study was to: i) comprehensively compare the cardiorespiratory and metabolic responses to a sinusoidal exercise below the LT between smokers and nonsmokers; ii) evaluate the effect of chronic smoking on the cardiorespiratory and metabolic responses to the sinusoidal protocol continued task failure. We hypothesized that the smokers would have a shorter time to exhaustion and a longer time delay between the mechanical and the respiratory sines (t_{DS}) compared to the control group.

5.2 Materials and Methods

5.2.1 Participants

Based on pilot testing, a statistical software (G-Power 3.1, Dusseldorf, Germany) was used to compute the optimal sample size, expecting a large Cohen's d effect size (1.2) in t_D differences. Considering a required power $(1 - \beta) = 0.80$ and an $\alpha = 0.05$, the desired sample size was 8 participants for each group. The participant characteristics are shown in Table 9.

	CTRL	Smokers	p value
Number	8	8	
Age (year)	22.6 ± 1.4	22.1 ± 2.2	n.s.
Body mass (kg)	74.3 ± 7.9	79.9 ± 4.4	n.s.
Stature (m)	1.79 ± 0.10	1.79 ± 0.06	n.s.
Cigarettes per day	-	11 ± 5	
Smoking history (year)	-	6 ± 2	

Table 9: Participant characteristics of smoker and control groups (CTRL) expressed as mean ± standard deviation (SD).

5.2.2 Study design

Participants reported to the laboratory three times with at least 48 hours between each visit. On the first day, participants were familiarized with the exercise protocols and anthropometric characteristics were assessed. On the second day, participants underwent an incremental cycle-ergometer test to determine $\dot{V}_{O_2max}$, $\dot{W}_{max}$, and LT. During the third visit, participants performed a sinusoidal protocol of moderate intensity.

5.2.3 Experimental procedures

All experimental sessions were conducted in a climate-controlled laboratory (with constant temperature of 20 ± 1°C and relative humidity of 50 ± 5%) at approximately the same time of the day to minimize possible bias induced by circadian rhythms. Each day of testing, participants were asked to abstain from caffeine and any other stimulant substances for at least 12 hours, and to refrain from heavy exercise for at least 24 hours prior to testing. Smokers were asked to smoke the last cigarette 1.5 hours before the test

to allow 5-16% elimination of blood COHb levels in order to avoid the acute effects of cigarette smoking (McDonough & Moffatt, 1999).

Familiarization, anthropometric and pulmonary function assessments

During the first session, the participants were equipped with a facemask and wearable devices to familiarize them with the equipment for cardiopulmonary exercise testing. The optimal saddle height, handlebars angle of inclination, and foot position over the pedal were defined for each participant (Peveler, 2008; Wang et al., 2020). Body mass and stature of each volunteer were measured using a mechanical scale equipped with a stadiometer (Asimed, Samadell; Barcelona). Participants then underwent a spirometric plethysmographic evaluation to determine VC and dynamic lung volumes (FVC, FEV_1, PEF and MVV). Maximal inspiratory and expiratory pressure (MIP and MEP, respectively) were measured at the mouth using a portable manometer equipped with a mouthpiece (S&M Instrument Company Inc., mod. PortaResp, Doylestown, PA). After familiarization with the manometer, participants repeated the maneuvers three times, and the highest value was recorded.

Incremental exercise test

On the second visit, $\dot{V}_{O_2max}$ and $\dot{W}_{max}$ were determined through a stepwise incremental test. After three minutes of basal recordings and four minutes of warm-up cycling at 100 W, work rate was increased by 25 W every two minutes until task failure. Participants were asked to maintain the pedaling rate between 60-70 rpm. Exercise was terminated when the participant failed to maintain the cadence within 5 rpm of the imposed range for more than 5 s (Brickley et al., 2002).

[La^-] was assessed at baseline, the end of each work rate and minutes 1, 3 and 5 of recovery. At the same time of blood sample collections, RPE was asked on a general (RPE_{GEN}; Borg 6-20), respiratory and muscular (RPE_{MUSC} and RPE_{RESP}, respectively; CR-10) standpoints.

Sinusoidal test

During the third visit, the participants performed a sinusoidal work rate task at moderate intensity until exhaustion. The sinusoidal protocol (LT-50) had the MP set 50 W below LT, the AMP of 50 W and T of four minutes (Figure 44). The 4-min T was decided according to the time constant of the primary component of $\dot{V}_{O_2}$ kinetics (at most 40 s below LT) to reach 63% of its expected transition AMP (Keir et al, 2018); hence, the rising phase of the work rate (from MP to the positive peak) should last at least one minute. The next three minutes were therefore required to complete the other three phases (decrease to MP, reaching the minimum nadir, and rising to the successive MP). Additionally, a 4-min T should be reasonably short enough to minimize the possible slow component occurrence within the same sine (Haouzi et al., 1993).

The protocols involved 3 min of baseline recordings, followed by a warm-up of 3 min at 50 W and 3 min at their individualized LT value, after which the sinusoidal exercise began in its downward midpoint crossing toward the nadir (Figure 25).

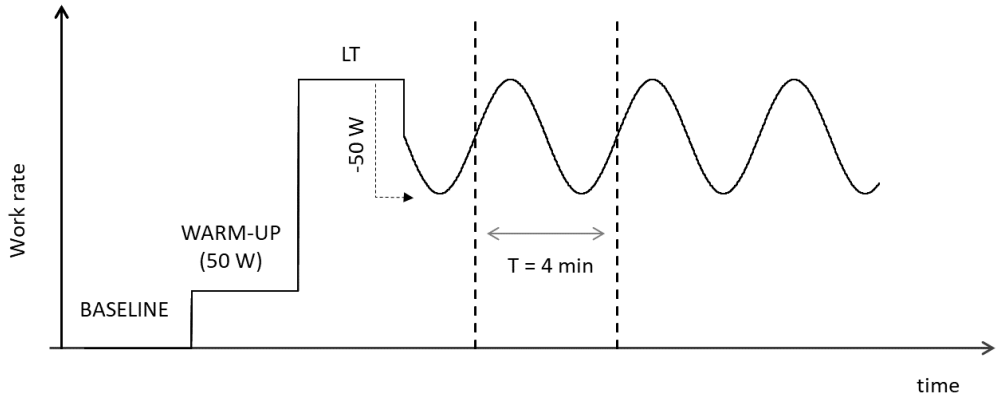

Figure 25: Illustration of the sinusoidal work rate. LT, lactate threshold; T, period.

[La^-] and RPE values (RPE_{GEN}, RPE_{MUSC} and RPE_{RESP}, respectively) were determined at baseline, the end of LT warm-up and the end of each cycle (corresponding to downward midpoint crossing).

5.2.4 Measurements

Tests were performed on an electro-mechanically braked cycle ergometer (mod. 839E, Monark, Sweden). During the experiments, work rate and cadence were continuously recorded. $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, f_R, and RER were measured on a breath-by-breath basis by a metabolic unit consisting of a turbine flowmeter, a zirconium oxygen sensor and an infrared CO_2 meter (Quark b^2, Cosmed, Rome, Italy). According to the manufacturer's instructions, the turbine and gas analyzers were calibrated before each test by means of a 3-l syringe (mod. 5530, Hans-Rudolph, Shawnee, KS, USA) and a certified gas mixture of known concentrations (16% O_2, 5% CO_2, balance N_2), respectively. Simultaneously,

HR was continuously acquired with a heart rate monitor (S810, Polar Electro Oy, Kempele, Finland).

20 μl arterialized blood samples were taken from the ear lobe and analyzed by an enzymatic-amperometric system (Labtrend, Bio Sensor Technology GmbH, Berlin, Germany) to determine [La^-].

5.2.5 Data analysis

All experimental data were analyzed off-line. Any ectopic beats or extrasystoles were eliminated from the HR trace. The respiratory and gas exchange responses were edited of spurious breaths that resulted from swallowing, coughing, sighing or premature ending of breath, by deleting values outside three standard deviation (SD) from the local mean (Lamarra et al., 1987).

LT was determined by the D_{max} modified method (Bishop et al. 1998), which is considered the best approach in endurance activities (Heuberger et al., 2018).

The first two minutes of the sinusoidal exercise were excluded from the analysis to avoid any distortion in the cardiorespiratory responses induced by the transition of the work rate intensity from LT (during the 3 min warm-up phase) to the MP (sinusoidal phase). The fundamental sinusoid harmonic that best represents the progress of each cardiorespiratory variable was identified through a custom-built software (Matlab 2019b, MathWorks Inc., Natick, USA). For each cycle, AMP, MP, and t_Ds were calculated for all cardiorespiratory and metabolic variables. As shown in Figure 26, t_D was calculated as the time shift between the mechanical load and the cardiorespiratory response. Two different t_Ds were

determined according to the following hallmarks: i) $t_{D\ max}$: maximum of the sinusoidal pattern and ii) $t_{D\ min}$: minimum of the sinewave (Fukuoka et al., 1997).

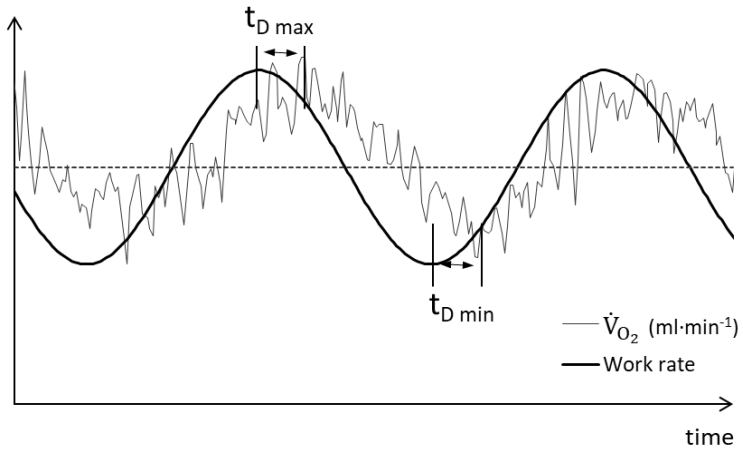

Figure 26: Raw signals of a representative participant during two cycles of a sinusoidal protocol. Black line: work rate; grey line: pulmonary oxygen uptake, $\dot{V}_{O_2}$. $t_{D\ max}$, maximum of the sinusoidal pattern; $t_{D\ min}$, delay at the minimum of the sinewave.

All parameters were determined on a cycle-by-cycle basis for all the cardiorespiratory and metabolic variables.

5.2.6 Statistical analysis

Descriptive statistics were used to describe the study sample characteristics. The Shapiro-Wilk test was applied to check for normal distribution. A two-way analysis of variance (2-way ANOVA) was used to assess the presence of differences in AMP, MP, and t_{DS} between the two groups and sinusoidal cycles. For all pairwise multiple comparisons, the Bonferroni's correction was applied. An unpaired Student's t-test was used to test for differences between the number of cycles completed during the two sinusoidal exercises by the two groups. The Hedge's g effect size with $CI_{95\%}$ was calculated and interpreted as follows: 0.00–0.19: trivial; 0.20–0.59: small; 0.60–1.19: moderate; 1.20–1.99: large; ≥

2.00: very large (Hopkins et al., 2009). All statistical analyses were performed using a statistical software (IBM SPSS Statistics v. 26, Armonk, NY, USA). The significance level was set at $\alpha < 0.05$. Unless, otherwise stated, results are presented as mean ± standard deviation (SD).

5.3 Results

The main pulmonary function outcomes are shown in Table 10.

	CTRL	Smokers	p value
VC (l)	5.5 ± 0.6	5.5 ± 0.6	n.s.
FVC (l)	5.8 ± 0.7	5.8 ± 0.7	n.s
FEV_1 (l)	4.9 ± 0.7	4.7 ± 0.6	n.s
PEF ($l \cdot s^{-1}$)	10.1 ± 1.7	8.8 ± 1.9	n.s
MVV ($l \cdot min^{-1}$)	184 ± 22	175 ± 21	n.s
MIP (cmH_2O)	108 ± 21	121 ± 18	n.s
MEP (cmH_2O)	120 ± 24	128 ± 16	n.s

*Table 10: Respiratory function test parameters in smoker and control groups (mean ± standard deviation, SD). VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume during the first second of the test; PEF, peak expiratory flow; MVV, maximal voluntary ventilation; MIP, maximal inspiratory mouth pressure; MEP, maximal expiratory mouth pressure. * p <0.05 vs CTRL*

The resting values of the main cardiorespiratory and metabolic variables are indicated in Table 11.

	CTRL	Smokers	p value
HR (b·min^{-1})	72 ± 9	78 ± 13	n.s
$\dot{V}_E$ (l·min^{-1})	12 ± 2	10 ± 2	n.s
f_R (b·min^{-1})	18 ± 4	15 ± 4	n.s
V_T (l)	0.8 ± 0.1	0.7 ± 0.2	n.s
$\dot{V}_{O_2}$ (ml·min^{-1})	374 ± 36	320 ± 70	n.s
$\dot{V}_{CO_2}$ (ml·min^{-1})	323 ± 35	274 ± 65	n.s

*Table 11: Resting values of cardiorespiratory and metabolic variables in smoker and control groups (mean ± standard deviation, SD). HR, heart rate; $\dot{V}_E$, expiratory ventilation; f_R, respiratory frequency; V_T, tidal volume; $\dot{V}_{O_2}$, pulmonary oxygen uptake; $\dot{V}_{CO_2}$, carbon dioxide production. * p <0.05 vs CTRL*

Although similar values between smokers and CTRL were found for $\dot{V}_{O_2 max}$ (3385 ± 341 vs 3623 ± 302 ml·min^{-1}, respectively) and HR_{max} (185 ± 10 vs 184 ± 10 b·min^{-1}, respectively), the smokers exhibited a lower $\dot{W}_{max}$ compared to CTRL (246 ± 24 vs 278 ± 29 W, respectively, p = 0.03; g = 1.15, moderate), $\dot{V}_{E\,max}$ (125 ± 12 vs 142 ± 16 l·min^{-1}, respectively, p = 0.03; g = 1.11, moderate), and $f_{R\,max}$ (47 ± 7 vs 58 ± 10 b·min^{-1}, respectively, p = 0.02; g = 2.00, very large). Similar results have been found for $V_{T\,max}$ in smokers and CTRL groups (2.7 ± 0.4 vs 2.5 ± 0.5 l, respectively) and $\dot{V}_{CO_2 max}$ (3815 ± 396 vs 3870 ± 334 ml·min^{-1}, respectively). Smokers exhibited greater $Pet_{CO_2\,max}$ (40 ± 5 vs 32 ± 3 mmHg, respectively, p <0.01; g = 1.72, large) and tended to have lower $Pet_{O_2\,max}$ (115 ± 4 vs 119 ± 3 mmHg, respectively, p = 0.08; g = 0.89, moderate).

There were no differences for LT between smokers and CTRL (200 ± 26 vs 220 ± 29 W, respectively).

The cardiorespiratory and metabolic responses to the LT-50 protocol are shown in Figures 27-31. The smokers completed fewer cycles compared to CTRL (10 ± 2 vs 18 ± 6, respectively, $p = 0.001$; $g = 1.91$, large).

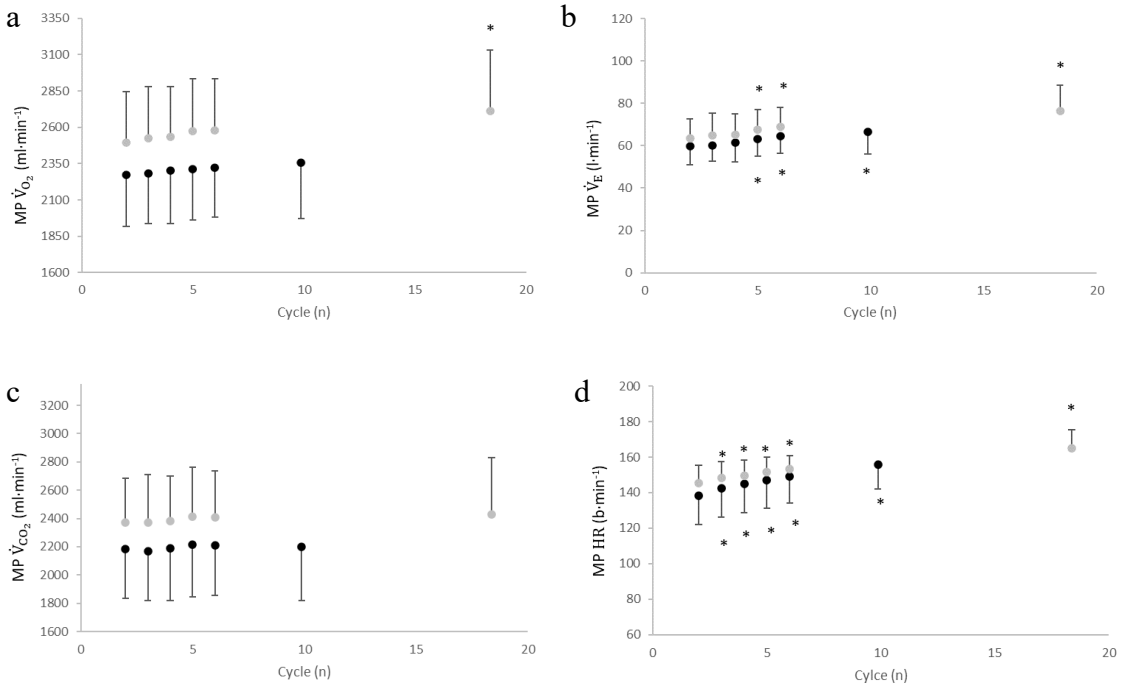

*Figure 287: Midpoint (MP) response of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

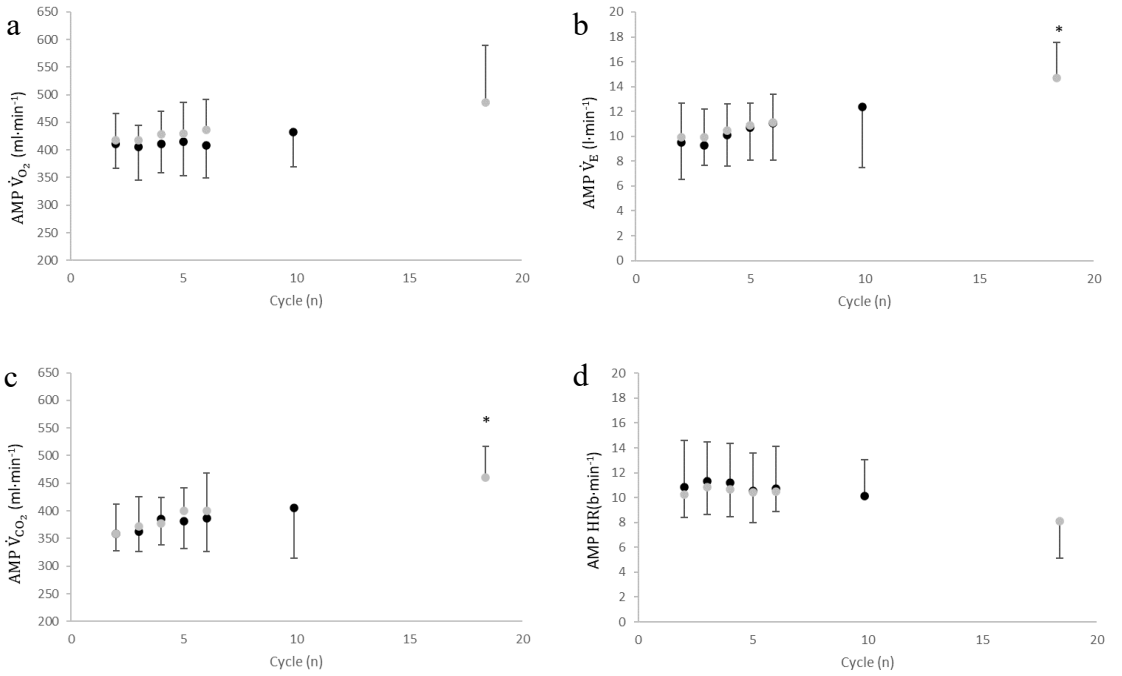

*Figure 278: Amplitude (AMP) response of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), heart rate, carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

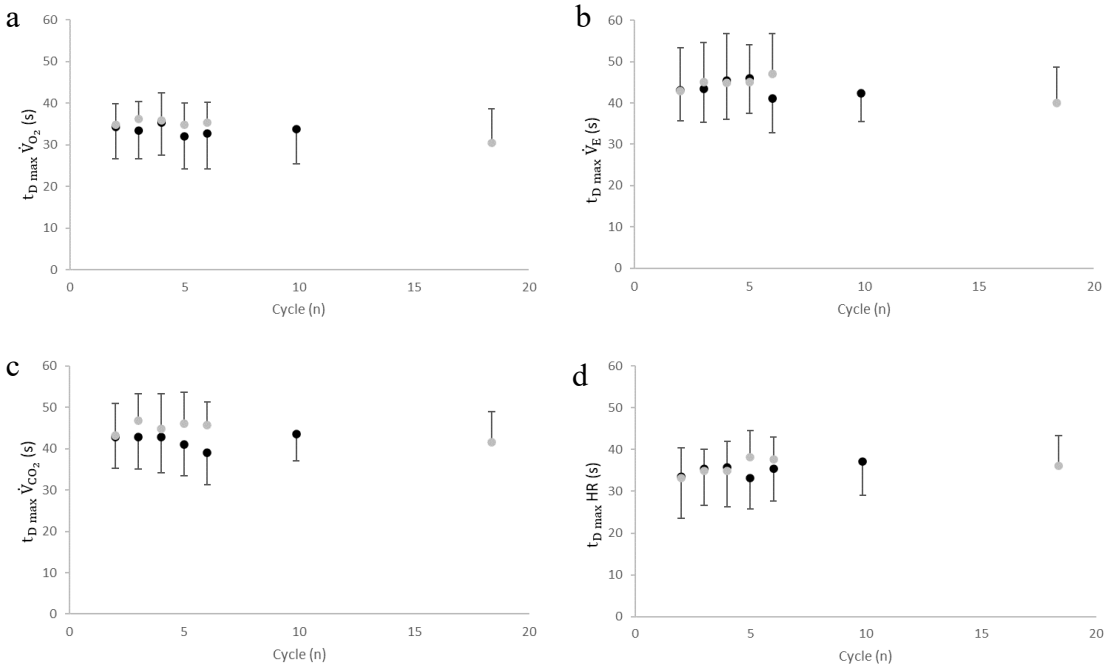

*Figure 2929: Time delay at the maximum of the sinewave ($t_{D\,max}$) of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

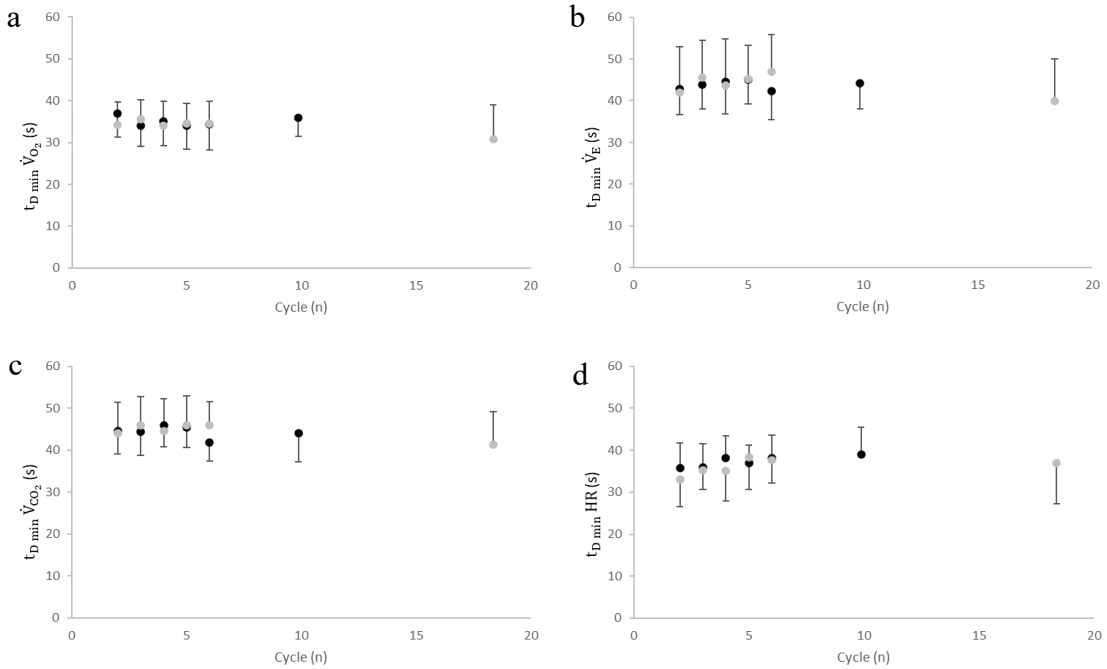

*Figure 3030: Time delay at the minimum of the sinewave ($t_{D\,min}$) of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

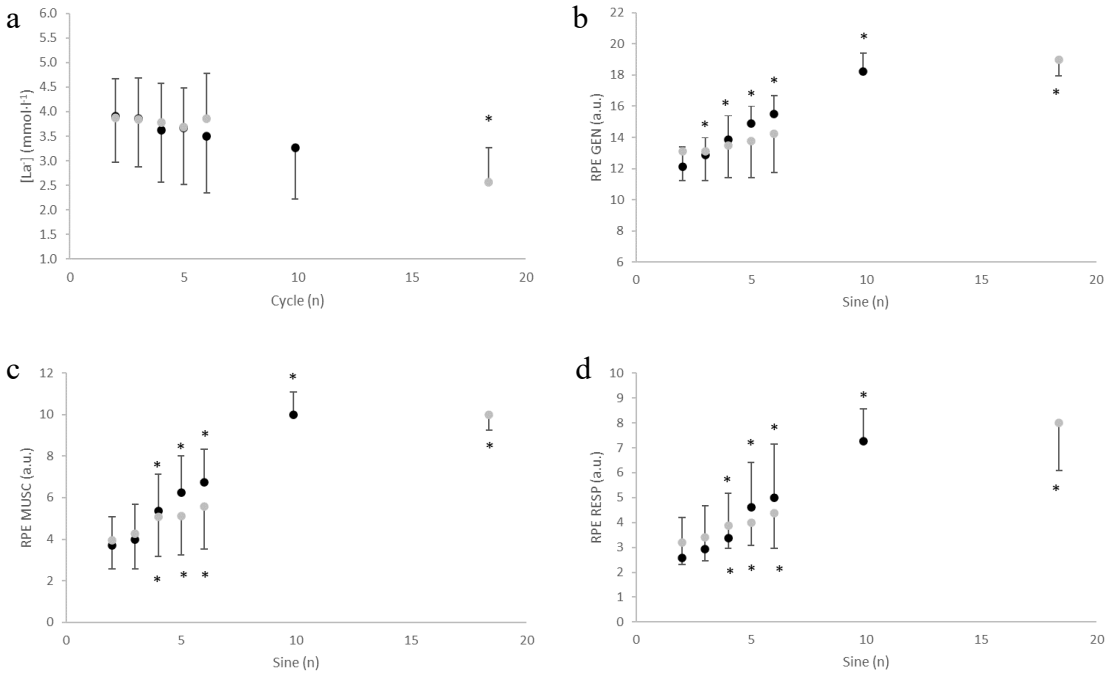

*Figure 31: Blood lactate concentration [La⁻] (panel a), ratings of perceived exertion on a general (RPE_{GEN}; Borg 6-20, panel b), respiratory (RPE_{MUSC}; CR-10, panel c) and muscular (RPE_{RESP}, panel d)) for each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

6 Study 4: Effect of chronic cigarette smoking on cardiorespiratory and metabolic responses to heavy intensity sinusoidal work rate

6.1 Aim

The aim of this study was to: i) comprehensively compare the cardiorespiratory and metabolic responses to a sinusoidal exercise greater than LT between smokers and nonsmokers; ii) evaluate the effect of chronic smoking on the cardiorespiratory and metabolic responses to the sinusoidal protocol continued until exhaustion. We hypothesized that the smokers would have a shorter time to exhaustion and a longer time delay between the mechanical and the respiratory sines (t_{DS}) compared to the control group.

6.2 Materials and Methods

6.2.1 Participants

Eight smokers and eight age-matched non-smokers completed the protocol. Their characteristics are shown in Table 12.

	CTRL	Smokers	p value
Number	8	8	
Age (year)	23.2 ± 1.5	25.0 ± 1.4	n.s.
Body mass (kg)	74.3 ± 7.9	79.9 ± 4.4	n.s
Stature (m)	1.79 ± 0.10	1.79 ± 0.06	n.s
Cigarettes per day	-	12 ± 5	
Smoking history (year)	-	6 ± 2	

Table 12: Participant characteristics of smoker and control group (CTRL) expressed as mean ± standard deviation (SD).

6.2.2 Study design

Participants reported to the laboratory three times with at least 48 hours between each visit. On the first day, participants were familiarized with the exercise protocols and anthropometric characteristics were assessed. On the second day, participants underwent an incremental cycle-ergometer test to determine $\dot{V}_{O_2max}$, $\dot{W}_{max}$, and LT. During the third visit, the participants performed a sinusoidal protocol of heavy intensity.

6.2.3 Experimental procedures

All experimental sessions were conducted in a climate-controlled laboratory (with constant temperature of 20 ± 1°C and relative humidity of 50 ± 5%) at approximately the same time of the day to minimize possible bias induced by circadian rhythms. Each day of testing, participants were asked to abstain from caffeine and any other stimulant substances for at least 12 hours, and to refrain from heavy exercise for at least 24 hours prior to testing. Smokers were asked to smoke the last cigarette 1.5 hours before the test to allow 5-16% elimination of blood COHb levels in order to avoid the acute effects of cigarette smoking (McDonough & Moffatt, 1999).

Familiarization, anthropometric and pulmonary function assessments

During the first session, the participants were equipped with a facemask and wearable devices to familiarize them with the equipment for cardiopulmonary exercise testing. The optimal saddle height, handlebars angle of inclination, and foot position over the pedal were defined for each participant (Peveler, 2008; Wang et al., 2020). Body mass and stature of each volunteer were measured using a mechanical scale equipped with a

stadiometer (Asimed, Samadell; Barcelona). Participants then underwent a spirometric plethysmographic evaluation to determine VC and dynamic lung volumes (FVC, FEV_1, PEF and MVV). Maximal inspiratory and expiratory pressure (MIP and MEP, respectively) were measured at the mouth using a portable manometer equipped with a mouthpiece (S&M Instrument Company Inc., mod. PortaResp, Doylestown, PA). After familiarization with the manometer, participants repeated the maneuvers three times, and the highest value was recorded.

Incremental exercise test

On the second visit, $\dot{V}_{O_2max}$ and $\dot{W}_{max}$ were determined through a stepwise incremental test. After three minutes of basal recordings and four minutes of warm-up cycling at 100 W, work rate was increased by 25 W every two minutes until task failure. Participants were asked to maintain the pedaling rate between 60-70 rpm. Exercise was terminated when the volunteer failed to maintain the cadence within 5 rpm of the imposed range for more than 5 s (Brickley et al., 2002).

$[La^-]$ was assessed at baseline, the end of each work rate and minutes 1, 3 and 5 of recovery. At the same time of blood sample collections, RPE was asked on a general (RPE_{GEN}; Borg 6-20), respiratory and muscular (RPE_{MUSC} and RPE_{RESP}, respectively; CR-10) standpoints.

Sinusoidal test

During the third visit, the participants performed a sinusoidal work rate task at heavy intensity until exhaustion. The sinusoidal protocol (LT50) had the MP set at LT, the AMP of 50 W and T of four minutes. The 4-min T was decided according to the time constant

of the primary component of $\dot{V}_{O_2}$ kinetics (at most 40 s) to reach 63% of its expected transition AMP (Keir et al, 2018); hence, the rising phase of the work rate (from MP to the positive peak) should last at least one minute. The next three minutes were therefore required to complete the other three phases (decrease to MP, reaching the minimum nadir, and rising to the successive MP). Additionally, a 4-min T should be reasonably short enough to minimize the possible slow component occurrence within the same sine (Haouzi et al., 1993).

The protocol involved 3 min of baseline recordings, followed by a warm-up of 3 min at 50 W and 3 min at their individualized LT value, after which the sinusoidal exercise began in its downward midpoint crossing toward the nadir (Figure 32).

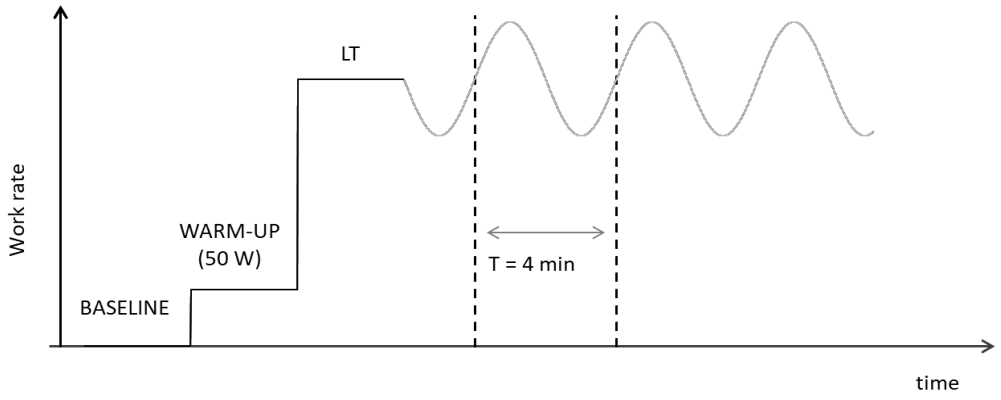

Figure 32: Illustration of the sinusoidal work rate. LT, lactate threshold; T, period.

[La^-] and RPE values (RPE_{GEN}, RPE_{MUSC} and RPE_{RESP}, respectively) were determined at baseline, the end of LT warm-up and the end of each cycle (corresponding to downward midpoint crossing).

6.2.4 Measurements

Tests were performed on an electro-mechanically braked cycle ergometer (mod. 839E, Monark, Sweden). During the experiments, work rate and cadence were continuously recorded. $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, f_R, and RER were measured on a breath-by-breath basis by a metabolic unit consisting of a turbine flowmeter, a zirconium oxygen sensor and an infrared CO_2 meter (Quark b^2, Cosmed, Rome, Italy). According to the manufacturer's instructions, the turbine and gas analyzers were calibrated before each test by means of a 3-l syringe (mod. 5530, Hans-Rudolph, Shawnee, KS, USA) and a certified gas mixture of known concentrations (16% O_2, 5% CO_2, balance N_2), respectively. Simultaneously, HR was continuously acquired with a heart rate monitor (S810, Polar Electro Oy, Kempele, Finland).

20 µl arterialized blood samples were taken from the ear lobe and analyzed by an enzymatic-amperometric system (Labtrend, Bio Sensor Technology GmbH, Berlin, Germany) to determine [La^-].

6.2.5 Data analysis

All experimental data were analyzed off-line. Any ectopic beats or extrasystoles were eliminated from the HR trace. The respiratory and gas exchange responses were edited of spurious breaths that resulted from swallowing, coughing, sighing or premature ending of breath, by deleting values outside three standard deviation (SD) from the local mean (Lamarra et al., 1987).

LT was determined by the D_{max} modified method (Bishop et al. 1998), which is considered the best approach in endurance activities (Heuberger et al., 2018).

The first two minutes of the sinusoidal exercise were excluded from the analysis to avoid any distortion in the cardiorespiratory responses induced by the transition of the work rate intensity from LT (during the 3 min warm-up phase) to the MP (sinusoidal phase). The fundamental sinusoid harmonic that best represents the progress of each cardiorespiratory variable was identified through a custom-built software (Matlab 2019b, MathWorks Inc., Natick, USA). For each cycle, AMP, MP, and t_{D}s were calculated for all cardiorespiratory and metabolic variables. t_{D} was calculated as the time shift between the mechanical load and the cardiorespiratory response. Two different t_{D}s were determined according to the following hallmarks: i) $t_{D\,max}$: maximum of the sinusoidal pattern and ii) $t_{D\,min}$: minimum of the sinewave (Fukuoka et al., 1997). All parameters were determined on a cycle-by-cycle basis for all the cardiorespiratory and metabolic variables.

6.2.6 Statistical analysis

Descriptive statistics were used to describe the study sample characteristics. The Shapiro-Wilk test was applied to check for normal distribution. A two-way analysis of variance (2-way ANOVA) was used to assess the presence of differences in AMP, MP, and t_{D}s between the two groups and sinusoidal cycles. For all pairwise multiple comparisons, the Bonferroni's correction test was applied. An unpaired Student's t-test was used to test for differences between the number of cycles completed during the two sinusoidal exercises by the two groups. The Hedge's g effect size with $CI_{95\%}$ was calculated and interpreted as follows: 0.00–0.19: trivial; 0.20–0.59: small; 0.60–1.19: moderate; 1.20–1.99: large; ≥ 2.00: very large (Hopkins et al., 2009). All statistical analyses were performed using a statistical software (IBM SPSS Statistics v. 26, Armonk, NY, USA). The significance

level was set at $\alpha < 0.05$. Unless, otherwise stated, results are presented as mean ± standard error (SE).

6.1 Results

The main pulmonary function outcomes are shown in Table 13.

	CTRL	Smokers	p value
VC (l)	5.5 ± 0.7	5.5 ± 0.6	n.s.
FVC (l)	5.8 ± 0.7	5.8 ± 0.7	n.s.
FEV_1 (l)	4.9 ± 0.7	4.7 ± 0.6	n.s.
PEF ($l \cdot s^{-1}$)	10.1 ± 1.7	8.8 ± 1.9	n.s.
MVV ($l \cdot min^{-1}$)	184 ± 22	175 ± 21	n.s.
MIP (cmH_2O)	108 ± 21	121 ± 18	n.s.
MEP (cmH_2O)	120 ± 21	128 ± 16	n.s.

*Table 13: Respiratory function test parameters in smoker and control groups (mean ± standard deviation, SD). VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume during the first second of the test; PEF, peak expiratory flow; MVV, maximal voluntary ventilation; MIP, maximal inspiratory mouth pressure; MEP, maximal expiratory mouth pressure. * p <0.05 vs CTRL*

The resting values of the main cardiorespiratory and metabolic variables are indicated in Table 14.

	CTRL	Smokers	p value
HR (b·min^{-1})	72 ± 9	78 ± 13	n.s.
$\dot{V}_E$ (l·min^{-1})	12 ± 2	10 ± 2	n.s.
f_R (b·min^{-1})	18 ± 4	15 ± 4	n.s.
V_T (l)	0.8 ± 0.1	0.7 ± 0.2	n.s.
$\dot{V}_{O_2}$ (ml·min^{-1})	364 ± 55	320 ± 70	n.s.
$\dot{V}_{CO_2}$ (ml·min^{-1})	311 ± 56	274 ± 65	n.s.

*Table 14: Resting values of cardiorespiratory and metabolic variables in smoker and control groups (mean ± standard deviation, SD). HR, heart rate; $\dot{V}_E$, expiratory ventilation; f_R, respiratory frequency; V_T, tidal volume; $\dot{V}_{O_2}$, pulmonary oxygen uptake; $\dot{V}_{CO_2}$, carbon dioxide production. * p <0.05 vs CTRL*

Although similar values between smokers and CTRL were found for $\dot{V}_{O_2 max}$ (3385 ± 341 vs 3623 ± 302 ml·min^{-1}, respectively) and HR_{max} (185 ± 10 vs 184 ± 10 b·min^{-1}, respectively), the smokers, compared to CTRL, exhibited a lower $\dot{W}_{max}$ (244 ± 24 vs 278 ± 29 W, respectively, p = 0.03; g = 1.15, moderate), $\dot{V}_{E\,max}$ (125 ± 12 vs 142 ± 16 l·min^{-1}, respectively, p = 0.013; g = 1.11, moderate), and $f_{R\,max}$ (47 ± 7 vs 58 ± 10 b·min^{-1}, respectively, p = 0.026; g = 2.00, very large). Similar results have been found for $V_{T\,max}$ in smokers and CTRL groups (2.7 ± 0.4 vs 2.5 ± 0.5 l, respectively) and $\dot{V}_{CO_2 max}$ (3814 ± 396 vs 3870 ± 334 ml·min^{-1}, respectively). Smokers exhibited greater $Pet_{CO_2\,max}$ (40 ± 5 vs 32 ± 3 mmHg, respectively, p <0.01; g = 1.72, large) and tended to have lower $Pet_{O_2\,max}$ (115 ± 4 vs 119 ± 3 mmHg, respectively, p = 0.08; g = 0.89, moderate).

There was no difference in LT between smokers and CTRL 200 ± 26 vs 220 ± 29 W, respectively).

The results of the cardiorespiratory and metabolic response to the LT50 protocol are shown in Figures 33 - 37. There were differences in the completed cycles between smokers and CTRL (4 ± 2 vs 5 ± 2, respectively, $p = 0.16$).

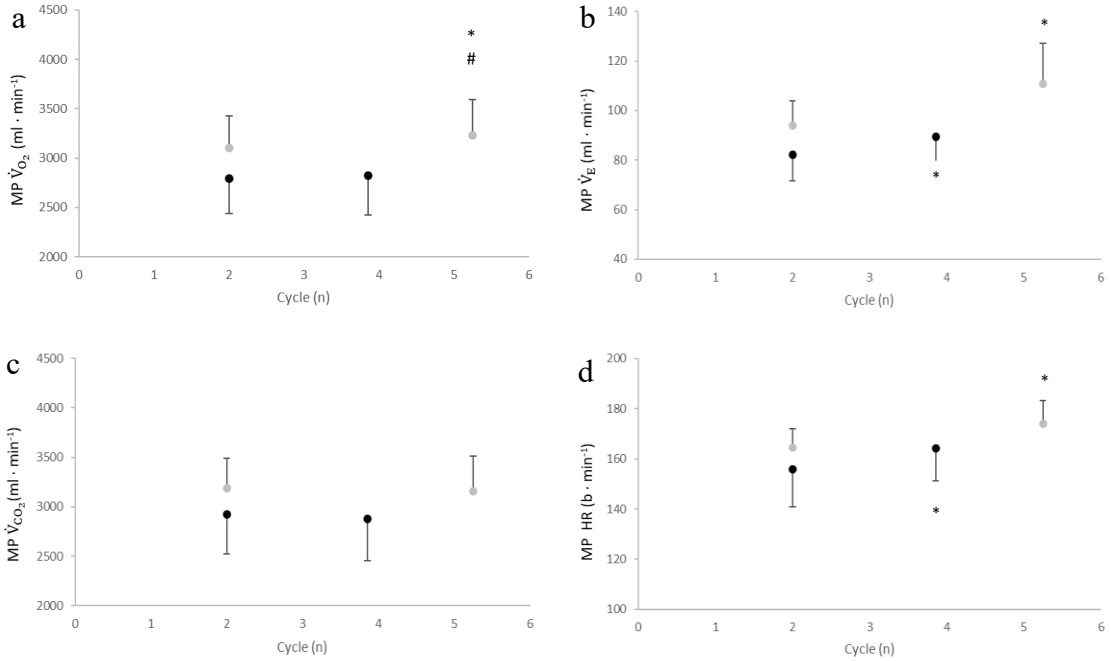

*Figure 34: Midpoint (MP) response of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

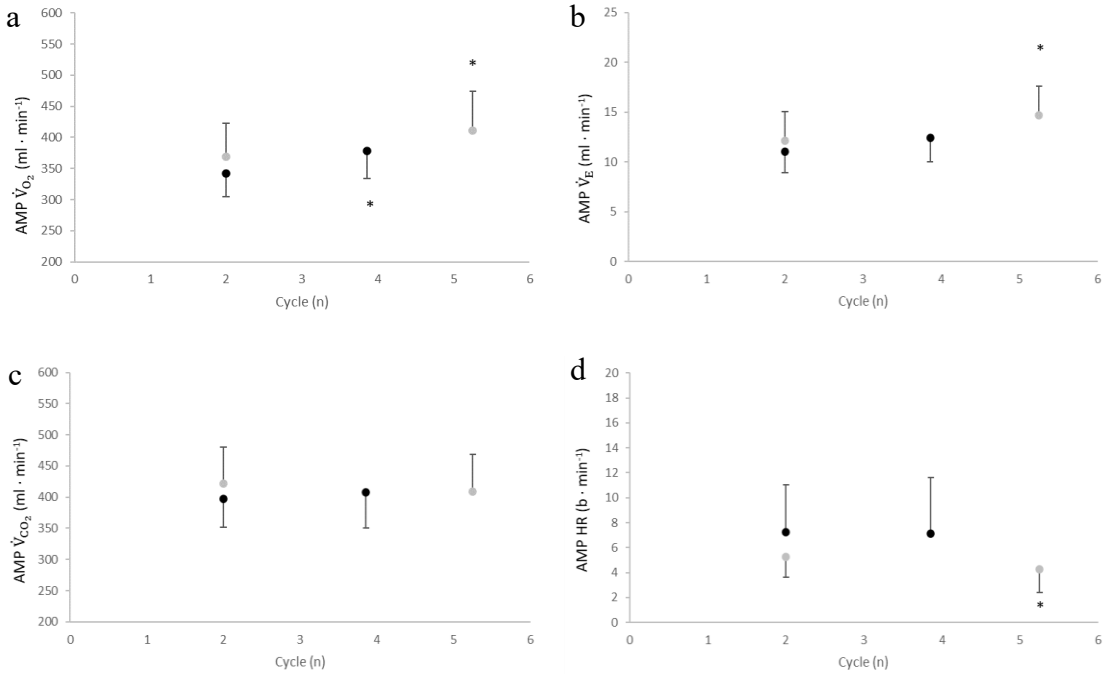

*Figure 33: Amplitude (AMP) response of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

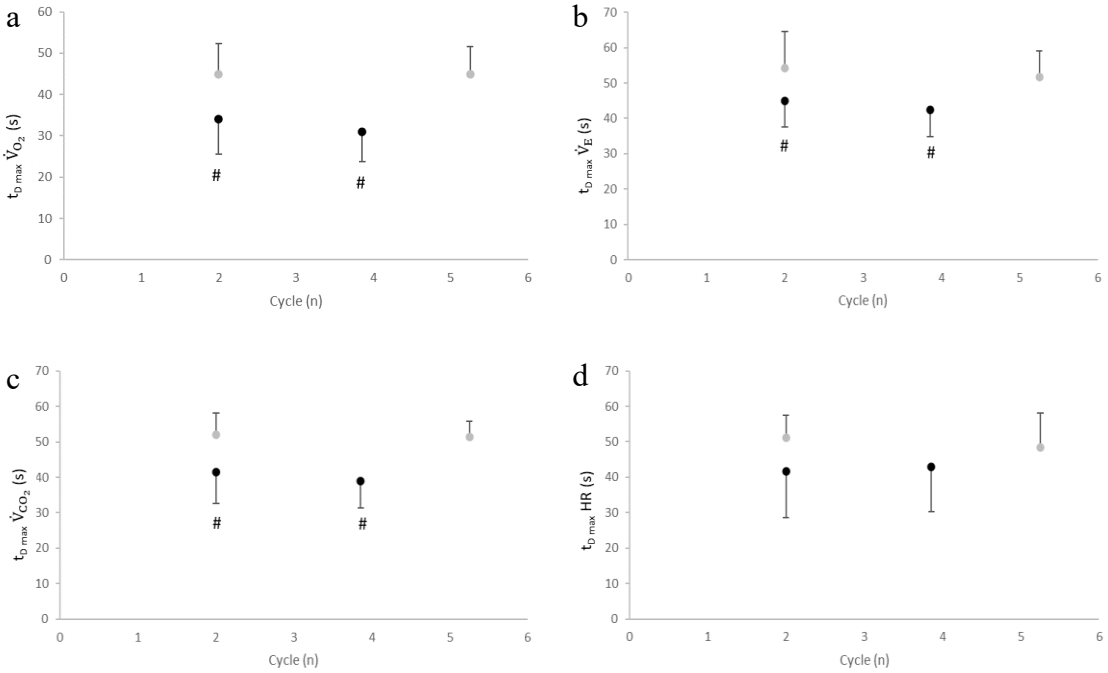

*Figure 355: Time delay at the maximum of the sinewave ($t_{D\,max}$) of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

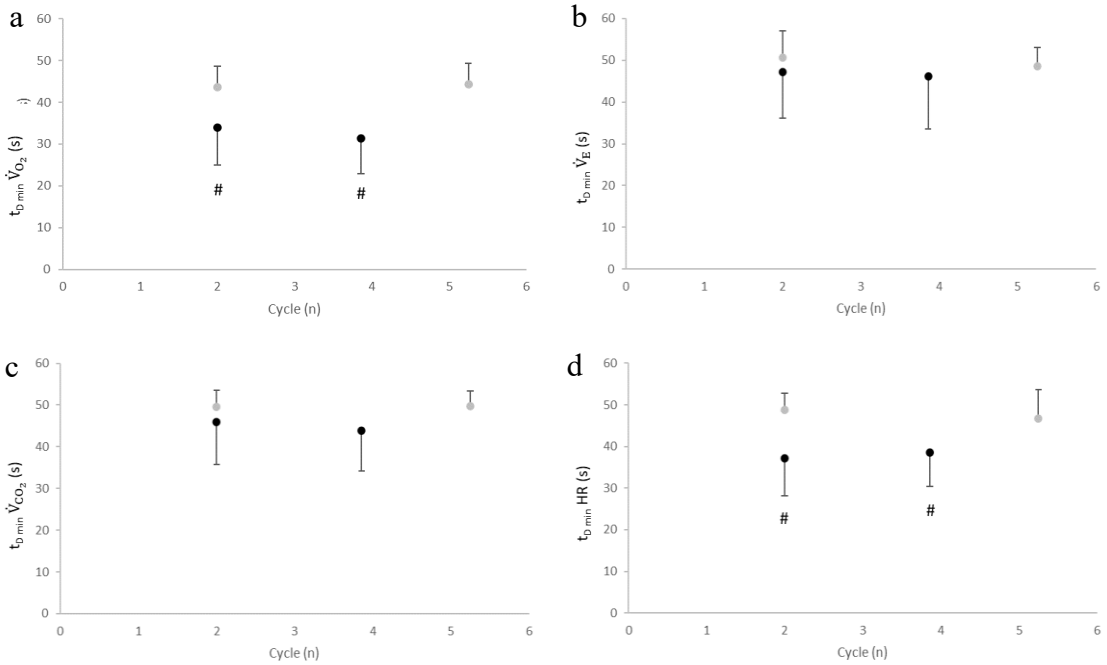

*Figure 36: Time delay at the minimum of the sinewave ($t_{D\,min}$) of pulmonary oxygen uptake, $\dot{V}_{O_2}$ (panel a), ventilation, $\dot{V}_E$ (panel b), carbon dioxide production, $\dot{V}_{CO_2}$ (panel c) and heart rate, HR (panel d) to each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; # p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

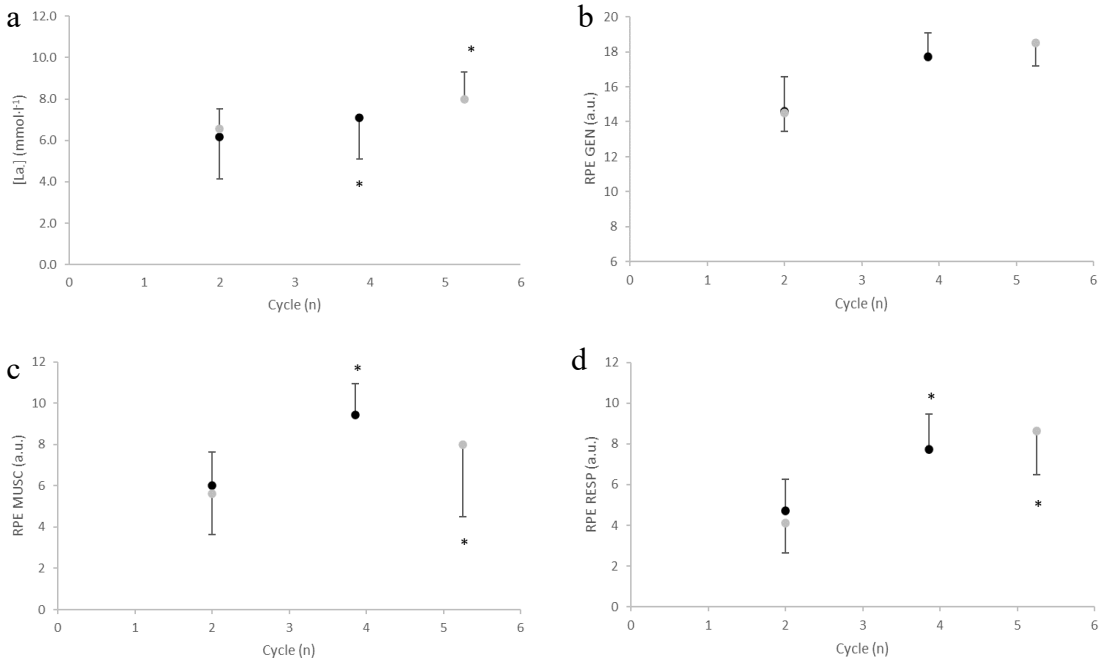

*Figure 36: Blood lactate concentration [La$^-$] (panel a), rates of perceived exertion on a general (RPE_{GEN}; Borg 6-20, panel b), respiratory (RPE_{MUSC}; CR-10, panel c) and muscular (RPE_{RESP}, panel d)) of each cycle in smokers (black circles) and CTRL (grey circles). *p<0.05 vs cycle 1; $^{\#}$p<0.05 vs CTRL. Data are shown as mean ± standard deviation (SD)*

7 DISCUSSION

This dissertation aimed to comprehensively examine the effect of chronic cigarette smoking on the cardiorespiratory and metabolic responses to different exercise protocols in young, physically active males.

The main finding of the present dissertation is that, despite the young age, high fitness level and relatively short smoking history, cigarette smoking negatively affected the cardiorespiratory and metabolic responses to exercise. In particular, smokers demonstrated blunted cardiorespiratory and metabolic kinetics during both on- and off-transients for moderate exercise and during the incremental test recovery phase. During the sinusoidal exercises, the only difference between smokers and CTRL was the lower number of cycles completed. However, similar fatigue development between groups appears to have occurred in both sinusoidal protocols as MP was greater and AMP lower in almost all the cardiorespiratory and metabolic variables.

$\dot{V}_{O_2max}$ is a benchmark for fitness evaluation, that determines the integrated functioning of the pulmonary, cardiovascular and muscle systems to transport (conductive & diffusive) and utilize (muscle mitochondria) O_2 (Poole & Jones, 2017). Some studies have found that young smokers had a lower $\dot{V}_{O_2max}$ (Gläser et al., 2013; Klausen et al., 1983; Kobayashi et al., 2004; Mendonca et al., 2011). To avoid different fitness levels between the two groups and to better understand the smoking-induced alterations, we matched CTRL and smokers for $\dot{V}_{O_2max}$.

Although the smokers and the CTRL had similar $\dot{V}_{O_2max}$ values, the smokers stopped exercise at a lower $\dot{W}_{max}$. This may be due to a decrease in skeletal muscle fatigue

resistance that has previously been observed in young healthy smokers (aged 22.2 ± 2.5 years) with a relative short smoking history (2.5 ± 3.1 pack years) (Morse et al., 2007). The main factors related to skeletal muscle fatigue resistance are: oxygen delivery to the muscle, oxygen transport from the capillary to the muscle fibers, fiber-type composition, and oxidative capacity expressed as mitochondrial volume and activity (Wüst et al., 2008). There is a smoking-induced impairment of muscle tissue oxygenation expressed in reduced capillary density (Tang et al., 2010) and fiber cross-sectional area (Nogueira et al., 2018; Tang et al., 2010).

Although the increase in $\dot{V}_E$ concurrently with exercise intensity was similar between the two groups, the smokers exhibited lower $\dot{V}_{E\,max}$, linked to a lower $f_{R\,max}$. This outcome may be related to the fatigue of the respiratory muscles (Melliti et al., 2021). Indeed, it has been reported that rapid, shallow breathing can reduce fatigue of the respiratory muscles and maximum inspiratory muscle effort and, therefore, optimize the O_2 cost of breathing. In fact, for a given $\dot{V}_E$ the combination of smaller V_T and higher f_R is most efficient by decreasing loading because of the increase in elastic forces against which smokers needed to breath and raise the endurance of the inspiratory muscles. This strategy is advantageous initially, but in the long term becomes ineffective as dead space increases and the O_2 cost of breathing increases with f_R growing (Melliti et al., 2021).

Similar findings has been obtained by Elbehairy et al. (2016), who hypothesized that smokers adopt a specific strategy to limit the maximal voluntary activation of the mechanically stressed diaphragm and minimize the respiratory discomfort. Accordingly, in an autopsy study a greater stiffness in the lung parenchyma, due to alterations in the structure of the collagen fibers, has been reported in smokers' lung tissues (Karimi &

Razaghi, 2018). In the same study, it was reported that chronic smoking (~7 years) causes lung tissue stiffness through pulmonary fibrosis, which can affect the structure and mechanical performance of the lungs (Karimi & Razaghi, 2018). It also has been found that maximal exercise can exacerbate pulmonary oxidative stress by increasing the accumulation and/or activation of neutrophils and alveolar macrophages in the lungs in smokers with characteristics similar to our study's population (Taito et al., 2012). Despite these differences in $\dot{V}_E$ between smokers and age-matched control, no differences in pulmonary function parameters were found, except for a reduction in PEF. As PEF is dependent on expiratory muscle strength, airway resistance, and alveolar pressure (Antunes et al., 2016), its reduction can be interpreted as an increase in airway resistance and a decrease in expiratory muscle strength (Behera et al., 2013).

According to previous studies (Nicola et al., 2014; Rodrigues et al., 2013), no differences in the other pulmonary function parameters were noted in the two groups. This is probably due to young age, in which their exposure to cigarette smoke had not yet impaired their lung function, or to high fitness level, that increases respiratory efficiency, compensating for the smoking-induced detrimental effects (Lorensia et al., 2021). Further tests are necessary to reveal possible smoking-induced impairments in the cardiorespiratory and metabolic responses to exercise in smokers.

From the results of the first study, cigarette smoking seems to affect both the on- and off-phases of the main cardiorespiratory and metabolic kinetics during a moderate exercise, slowing down the response. Despite a similar $\dot{V}_{O_2max}$, the smokers exhibited a lower work rate at VT_1 likely because of the aforementioned skeletal muscle impairments. However,

no differences in cardiorespiratory and metabolic variables at VT_1 were detected between the two groups, which is likely due to age and exercise habits (Miyatake et al., 2011).

The first project revealed slower $\dot{V}_E$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ on-kinetics during a moderate exercise in smokers. Although the exact mechanisms of slowed on-kinetics are not still fully understood, Chiappa et al. (2008) demonstrated this may be linked to a slowed O_2 delivery and utilization at the onset of exercise in COPD patients. In fact, it is commonly recognized that alterations in the diffusive and/or convective transport of O_2 to skeletal muscle mitochondria (Hughson et al., 2001), autonomic imbalance (Heindl et al., 2001), blood flow redistribution from peripheral to respiratory muscles (Borghi-Silva et al., 2008; Richardson et al., 1999), derangements in muscle vasodilatation capacity (Gaenzer et al., 2001) and increased intrathoracic and/or pleural pressures may contributed to the attenuated $\dot{V}_{O_2}$ on-kinetics in the smokers.

Despite HR being similar at the peak of the incremental test between the groups, the τ of the on-transient was longer in the smokers. Considering that the increase in HR from rest to 50–60% $\dot{V}_{O_2max}$ is mainly dependent on vagal withdrawal (Tulppo et al., 1997), the slower HR on-kinetics may be explained by both an autonomic imbalance and the effects of breathing mechanisms on venous return (Mendonca et al., 2011).

According to Chevalier's study (1963), the slower $\dot{V}_{O_2}$ recovery response to a moderate exercise in smokers can be explained by a larger O_2 debt. Evidence of such hypothesis comes in COPD patients that showed increased recovery time for PCr (Kutsuzawa et al., 1995) as well as decreased activity of several oxidative enzymes (Sauleda et al., 1995).

The off-$\dot{V}_{O_2}$ kinetics are affected by the dynamics of $\dot{Q}$, muscle blood flow, partial refilling of O_2 stores in venous blood and muscle, and metabolic derangements (Poole & Jones, 2012). In fact, a slower $\dot{V}_{O_2}$ can be ascribed to a reduced venous return, with a consequent decrease in ventricular filling and SV (Lonsdorfer-Wolf et al., 2003; Okuno et al., 2011). However, SV remained constant and was not different between the two groups during the moderate exercise in our study. These findings do not support the hypothesis of a reduction in cardiac pre-load in smokers. Peripheral muscle dysfunction could be one of the factors that contributed to the longer $\dot{V}_{O_2}$ kinetics. Indeed, it has been shown that $\dot{V}_{O_2}$ kinetics during recovery are related to re-oxygenation of peripheral skeletal muscle evaluated by near-infrared spectroscopy (NIRS) in COPD patients (Okamoto et al., 2003).

The respiratory and metabolic responses are exacerbated during the recovery phase of the incremental test. The longer HRR30 in HR is in agreement with the nicotine-induced reduction in vagal cardiac modulation and a shift of the sympatho-vagal balance towards greater sympathetic dominance during exercise conditions.

To simulate longer-lasting and oscillating activities (Yamazaki et al., 1996) and to examine the cardiorespiratory response several times (Casaburi et al., 1977), sinusoidal protocols of different intensity domains have been conducted. In the moderate sinusoidal exercise, the smokers performed a fewer number of cycles. This outcome may be explained by the before-mentioned peripheral muscle limitations, considering that there were no differences between the two groups in cardiorespiratory and metabolic parameters. Similar results were obtained for LT50, where no differences were found in the number of completed cycles. These findings seem to suggest that sinusoidal protocols,

when correctly set, could be applied as a form of exercise training more tolerable for populations with cardiorespiratory limitations. This is in accordance with the study of Porszasz et al. (2013), in which severe COPD patients were able to sustain high intensity sinusoidal exercise for a prolonged time, despite ventilatory and peripheral limitations.

To indirectly examine the development of fatigue, both sinusoidal protocols were executed until exhaustion. In both cases, it seems that fatigue developed to similar levels between the two groups but was delayed for CTRL. The presence of fatigue development was indicated in both protocols for some of the cardiorespiratory and metabolic variables. The progressive increase in HR MP LT50 and LT-50 may be the result of a gradual hyper-activation of the sympathetic branch (Rowell & O'Leary, 1990), an increase in body core temperature (González-Alonso et al., 1997; Rowell & O'Leary, 1990; Zuccarelli et al., 2018), and/or a gradual decrease in hydration status (Coyle & González-Alonso, 2001; González-Alonso et al., 1997).

$\dot{V}_{O_2}$ MP remained steady in smokers under both sinusoidal protocols. Considering that there were no differences in $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and [La^-], the rise observed in $\dot{V}_E$ MP may be associated with thermoregulatory effects (Powers et al, 1982). Indeed, the increase in body temperature increases anaerobic metabolites that stimulate signaling by central or peripheral chemoreceptors or muscle metaboreceptors to increase $\dot{V}_E$ (Gaudio & Abramson, 1968; Hayashi et al., 2006). Moreover, the sensory feedback from group III and IV muscle afferents have been shown to play a crucial role in exercise hyperpnea (Amann et al., 2010; Hayashi et al., 2006). In contrast, the CTRL group $\dot{V}_{O_2}$ MP increased in both sinusoidal protocols. LT50, other factors may have contributed to the increase in $\dot{V}_{O_2}$, $\dot{V}_E$ and HR MP in addition to the mechanisms previously discussed

(thermoregulation, muscle mechano-sensitive afferent feedback). Indeed, the growth of the cardiorespiratory and metabolic responses could be an effect of the slow component, which is mainly related to the recruitment of fast-twitch fibres and to a shift towards fatty acid utilization during long-lasting exercise (Burnley & Jones, 2018; Ferretti, 2015; Jones et al., 2011). The greater [La^-], greater $\dot{V}_{O_2}$ AMP as well as the greater $\dot{V}_E$ AMP observed in our study for LT50 seems to support this hypothesis.

The lack of difference between groups for t_{DS} for all the investigated variables of both sinusoidal exercises may be accounted for by the oscillating nature of the work rate in the present protocol. Indeed, the off phase of the sine wave (from the zenith to the nadir) may be considered a sort of priming exercise. Prior exercise, depending on intensity and duration, could accelerate $\dot{V}_{O_2}$ kinetics (Carter et al., 2004; Koppo & Bouckaert, 2001; Poole & Jones, 2012). Indeed, by favouring muscle vasodilatation (Jones et al., 2006; Poole & Jones, 2012) and improving local microvascular O_2 availability (Gurd et al., 2009), prior exercise may increase the $\dot{V}_{O_2}$ primary component amplitude and reduce the amplitude of slow component through priming effects on type II fibres (Burnley et al., 2000; Carter et al., 2005; Dimenna et al., 2008; Gerbino et al., 1996).

8 STUDY LIMITATIONS

As with all studies, this dissertation comes with some known limitations. This dissertation lacks data about lung diffusion, the amount of carboxyhemoglobin, and the determination of oxygen extraction at the muscle level to provide a more comprehensive picture of the integrated heart-lung-muscle system.

As future perspective, it would be interesting to extend the study in a sample with different characteristics, such as sex, age, fitness level and cigarette consumption/history. Lastly, it would be helpful to assess the role of different exercise modalities and types of exercise training to counterbalance the cigarette smoking negative effects.

9 CONCLUSIONS

In conclusion, chronic cigarette smoking has detrimental effects on cardiorespiratory and metabolic response to different exercise protocols in young, physically active males. Despite young age and high fitness level, the smokers were characterized by slower cardiorespiratory and metabolic kinetics during moderate exercise and during the recovery of an incremental test. Interestingly, there were no differences between the smokers and CTRL during both sinusoidal exercises, except for a shorter time to exhaustion that may suggest peripheral dysfunction.

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