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Cardiorespiratory and metabolic response to different sinusoidal work rates

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ABSTRACT

Cardiorespiratory and Metabolic Response to Different Sinusoidal Work Rates

Cardiorespiratory fitness is one of the most important health criteria in seemingly healthy individuals and nearly all patient populations. Alongside the traditional tests for cardiorespiratory functional evaluation, a test protocol was proposed based on the intensity of the exercise that varies according to a sine wave model. This approach engenders an incessantly fluctuating response in which the response to one cycle is repeated. Indeed, by the periodic occurrence of increasing and decreasing phases, an evaluation of the physiological response kinetic to similar work rate perturbations can be obtained in a single testing session. Sinusoidal protocol pattern depends on several factors: period (T), amplitude (AMP) and midpoint (MP), therefore, modulating one or all of these factors yield various responses of the heart-lung-muscle integrated system. So far, previous studies administered sine wave protocols to investigate changes in the physiological responses induced by changes in T, pedal frequency, or exercise intensity (below and above the lactate threshold). In particular, those studies focused on changes in the temporal responsiveness of the cardiorespiratory and metabolic parameters, measured as the time delay (T_D, the latency between mechanical work rate and physiological responses). To date, no study has investigated the possible differences in the cardiorespiratory and metabolic kinetic induced by different sine waves AMP in two protocols sustained by the aerobic metabolism (exercise intensity below the critical power (CP)). Additionally, given that none of the previous studies has ever explored the influence of fatigue on sinusoidal

responses, and considering that all the previously reported results were obtained by overlapping, rather than averaging, the analysis of each cycle, it is worth investigating if a cycle-by-cycle analysis may reveal any sign of fatigue with the cycle's number progresses. Therefore, the aim of this study was two-folds: i) to assess the cardiorespiratory and metabolic response kinetic between two exhausting sinusoidal protocols differing in MP and AMP: $30CP_{-30}$ and $50CP_{-50}$, and ii) to compare the traditional data analysis approach with a cycle-by-cycle evaluation. In this latter, the possible impact of fatigue on the kinetics characteristics of cardiorespiratory and metabolic parameters will be explored. Ten active male (age: 25.7 ± 1.5 yrs; stature: 1.80 ± 0.06 m; body mass: 75.0 ± 2.7 kg; maximum oxygen uptake ($\dot{V}_{O_2\,max}$): 3905±182 ml·min^{-1}, CP: 216 ± 10 W) contributed to the study that was conducted in accordance with the Basic Principles of the Declaration of Helsinki. After determining individual $\dot{V}_{O_2\,max}$ and CP on a cycle ergometer, they randomly underwent two sinusoidal work rates ($50CP_{-50}$: AMP= 50W, MP =CP-50W; $30CP_{-30}$: AMP= 30W, MP =CP-30W) with a period of 4 minutes, until exhaustion. Expiratory ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}_{O_2}$), carbon dioxide output ($\dot{V}_{CO_2}$), and heart rate (f_H) responses were fitted by the sinewave function that minimized the residuals. AMP, MP and T_D were assessed for all the physiological variables. The T_D was determined for: i) peak (T_{D_MAX}); ii) down-ward MP crossing (T_{D_DOWN}); iii) nadir (T_{D_MIN}); and iv) up-ward MP crossing (T_{D_UP}) of all parameters for each cycle in $50CP_{-50}$ and $30CP_{-30}$. After examining the normality with Shapiro-Walk test, a two-way ANOVA for repeated measures was applied to test the presence of differences between the two protocols and among cycles,

taking the first cycle as the reference condition. To determine the presence of fatigue a regression analysis was also applied for exploring possible relationship between each variables and time. Lastly, a paired samples t-test also evaluated the differences between the traditional approach and the cycle-by-cycle analysis. Despite the lower AMP and higher MP values obtained on all the physiological parameters during $30CP_{-30}$ compared to $50CP_{-50}$, no difference was found in the T_Ds values in both protocols, regardless of which T_D was considered. In some investigated variables, the impact of fatigue on AMP was dissimilar between the two protocols. Indeed, none of the physiological parameters showed any sign of fatigue in $30CP_{-30}$, while a significant increase in $\dot{V}_E$ and $\dot{V}_{CO_2}$was observed in $50CP_{-50}$. In regards to a possible impact of fatigue on MP, the $\dot{V}_E$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$responses remained steady, whereas a significant rise was observed in f_H in both protocols. Refer to a possible effect of fatigue on T_Ds, no difference was found among cycles in both protocols.Taken together, these findings indicated that the respiratory and metabolic parameters responded similarly among all the cycles and were not influenced by the onset of fatigue. On the contrary, the f_H showed a different response in the MP since the beginning of the protocol therefore, the traditional analysis ventures the lack of possible alterations in cardiac response.

In conclusion, T_Ds of the cardiorespiratory and metabolic variables are not affected by the work rate intensity and oscillation AMP when the exercise is mostly aerobic (below CP). Additionally, a cycle-by-cycle analysis is recommended especially when the steadiness of all the cardiorespiratory and metabolic parameters cannot be ensured.

1. INTRODUCTION

1.1. Physical Exercise Testing

1.1.1. Why Exercise testing?

Exercise testing is a valuable tool in the assessment of the prescription of exercise and training, patient functional capacity and diagnosis of cardiopulmonary disease. There are several different modes and modalities available for exercise testing that can provide different types of information to the athletes, to improve their fitness or cardiopulmonary status and the clinician, to efficiently manage patients. Through cardiopulmonary exercise testing, ventilatory function and gas exchange, as well as heart rate (f_H), and blood pressures, are measured to provide detailed information on the cardiovascular, pulmonary, metabolic and muscular systems (Nelson et al., 2016).

1.1.2. Exercise modalities

The exercise modality (or mode) can be characterized by the type of energy demand (aerobic or anaerobic), muscle action (continuous and rhythmical, dynamic resistance, or static), or a combination of the energy systems and muscle action. Walking, cycling, and swimming are examples of continuous, rhythmical aerobic/ anaerobic activities; jumping, sprinting, and weight lifting are anaerobic and/ or dynamic resistance activities (Plowman et al., 2013).

1.1.3. What is cardiopulmonary exercise testing (CPET)?

Simply, exercise testing (ET) is used to evaluate the body's reaction to a measured exercise stress. Exercises able to elicit significant cardiovascular responses that may not be present at rest are proposed to assess the function of the cardiovascular system. One of The most important measures provided by ET is functional capacity. Determined by calculating the amount of metabolic equivalents (METs) an individual can perform, with 1 MET defined as an oxygen use of 3.5 $mlO_2 \cdot min^{-1} \cdot kg^{-1}$, that is the energy an average person expends seated at rest. Noticeably, this measure of exercise quantification is considered as relatively simplistic and does not provide much clinical utility. CPET allows a more dependable assessment of function, as well as an ability to provide prognostic or clinical information by the use of ET with addition of respiratory gas exchange.

CPET directly measures inhaled and exhaled gases to determine the maximal oxygen uptake ($\dot{V}_{O_2\,max}$), which is a more accurate quantification of functional capacity. This information can then be used for a better functional evaluation in the clinical setting. CPET capitalizes on this principle and measures the fraction of oxygen (O_2) and carbon dioxide (CO_2) in expired gas and expired air volume, which can then be used to determine $\dot{V}_{O_2}$ per unit time, $\dot{V}_{CO_2}$ and $\dot{V}_E$. These measures assume a paramount role to physician during testing evaluation. The degree of supervision needed will be determined by the testing facility and the risk level of the patients. The American College of Sports Medicine (ACSM) recommends that a physician does not need to be present for a person of low risk (1 or

fewer risk factors: Fasting serum total cholesterol) during ET (ACSM, 2018). In the case of moderate risk, a physician should be present during maximal testing but does not need to be present for submaximal testing. A high-risk individual should have a physician present during any ET (Nelson et al., 2016).

1.1.4. Equipment and Protocols of CPET

Performance of CPET requires the ability to measure 3 responses during inspiration and expiration: (1) the concentration of O_2; (2) the concentration of CO_2; and (3) a quantification of ventilation ($\dot{V}_E$, which depends on both tidal volume and respiratory rate).This measurement requires the participant to wear a facemask that covers the mouth and nose or a mouthpiece and a nose clip. CPET software systems can rapidly analyze the inhaled and exhaled gases. Although a wide variety of tests are available, the mostly used test is a symptom-limited, incremental test, which typically occurs by a progressive increase in work rate at a small fixed interval; however, newer, high-intensity constant load tests also are being used increasingly (Nelson et al., 2016).

The treadmill and the cycle ergometer are the most commonly used devices for this ET, but other devices also could be used, depending on which grade of the task specificity is required (Nelson et al., 2016). It is important to realize that ergometer-based tests generally affect the maximum $\dot{V}_{O_2}$, because of the involvement of different proportion of muscle mass. Traditionally, all the protocols generally involve a “warm-up” period with an initial

low load followed by a progressive increase in effort; typically with a stepwise progression until maximal effort level is reached, followed by a “cooldown” or recovery period. The most widely used treadmill protocol is the Bruce protocol, first described by Robert Bruce in 1973. The Bruce protocol was developed as a clinical test to evaluate patients with suspected coronary heart disease, although it can also be used to estimate cardiovascular fitness. The Bruce protocol is a standard test in cardiology and is composed of multiple exercise stages of 3 minutes each. At each stage, the gradient and speed of the treadmill are elevated to increase work output (Nelson et al., 2016).

Although the Bruce protocol is one of the protocols most commonly used for ET, other exercise protocols have been proposed for cardiopulmonary evaluations, one of which is the Balke protocol in which the speed remains constant at 3.3 mph whereas the slope is increased by 1% at each minute. This gradual approach is sometimes preferred because of the more steady increase in work load; however, the test requires twice as much time to reach maximal state. Despite the popularity and widespread use of the stepwise Bruce and Balke protocols, a ramp protocol will more consistently compared with other protocols (Myers et al., 1991). The key is that the exercise intensity should be individualized for the fitness level and be task-specific.

1.1.5. Incremental exercise *vs* constant load

The incremental test has proved to be valuable for determining maximum aerobic capacity, lactate and ventilatory thresholds, pulmonary $\dot{V}_{O_2\,max}$ and maximum exercise ventilation. It could be meaningless for determining work efficiency (which is relationship in $\dot{V}_{O_2}$ and work rate) influenced by the characteristic of the test. The incremental test requires that the workload is continuously increased without recovery pauses until exhaustion. The duration of the loads and the extent of their increase vary according to the types of protocols.

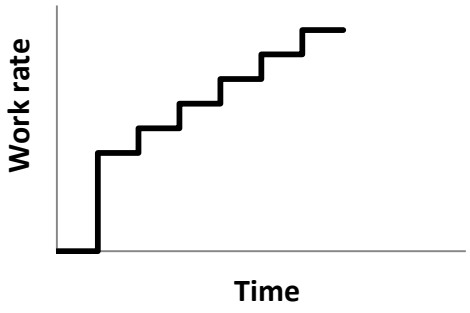

Figure1. Incremental step protocol

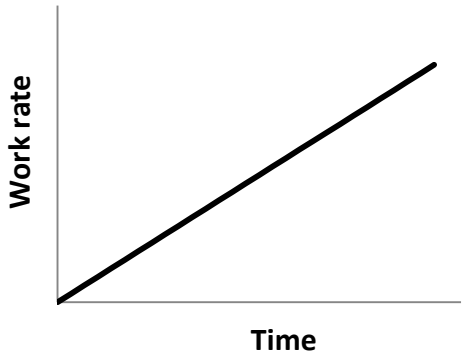

Figure2. Incremental ramp protocol

The first protocol (fig.1) is called "incremental step test" with each load remaining constant for a certain period of time (from 30s to 3 min, depending on the aim of the study). Another incremental protocol is represented in figure 2 and is obtained by a continuous increase of the load over time. In continuous ramp incremental protocols, work rate increments are administered without any resting period in between (Riboli et al., 2017; Adami et al.,

2013). The exercise protocol should be terminated when maximal heart rate is achieved; however, there are other indications for terminating the protocol early for the safety of the participant such as moderate-to-severe angina symptoms, a decrease in systolic blood pressure of >10 mm Hg from baseline despite an increase in workload, ataxia, dizziness, near-syncope, signs of poor perfusion, sustained arrhythmia such as ventricular tachycardia, and/or ST-segment elevation on electrocardiogram. Another indication to stop the test early is the patient's desire to stop determined by administering the Borg Rating of Perceived Exertion Scale (RPE) which is a rating scale of perceived exertion and may be useful to assess fatigue and assist in assessing the functional capacity (Borg, 1970).

The constant load test consists of an exercise in which the level of work does not vary from the beginning to the end of each fraction, thus it is called square wave test (fig. 4). In this case the load is maintained constant for a fixed time or until exhaustion. This kind of protocol can be performed as single test if the duration of the test is short. In contrast, if it lasts long several sessions might be considered (fig. 3) or, after an adequate recovery period able to bring the subject back to almost basal conditions, as a sequence of subsequent constant tests, each at a higher load than the previous one.

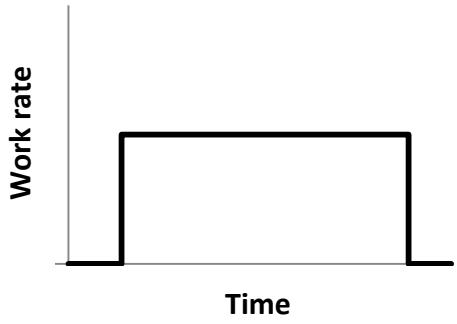

Figure3. Constant load protocol

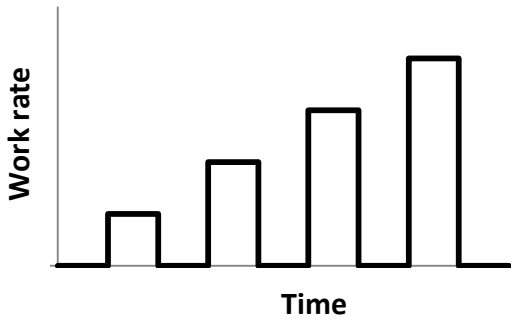

Figure4. Square wave protocol

1.1.6. Continuous *vs* discontinuous incremental exercise

In the incremental step test it is possible to observe an increase in the linear load up to exhaustion; on the contrary, in the square-wave test the load increases discontinuously. In discontinuous square-wave incremental protocol, also known as incremental intermittent Astrand-type test, each work load lasts 3 to 6 minutes, with resting periods in between. This kind of protocol, during low (37- 45% $\dot{V}_{O_2\,max}$and moderate (46-63% $\dot{V}_{O_2\,max}$) work rates (ACSM, 2018), allows the cardiorespiratory and metabolic responses to reach the steady state; consequently, we have a precise correspondence between mechanical and metabolic aspects. However, in the square wave test it is not always possible to obtain the steady state due to the intervention of the slow component, which occurs when the load intensity is higher than the gas exchange or lactate accumulation thresholds: in this case a progressive increase in $\dot{V}_{O_2}$appears at the end of the rising phase eventually result in steady-state

responses and continues to increase until the end of the test. Therefore, the incremental step test has the limitation of not allowing a perfect coupling between mechanical and metabolic power; instead the square wave protocol has a good correspondence between the mechanical power and the metabolic responses for the first loads below the lactate accumulation threshold; however in the last loads the responses are overestimated (Poole et al., 1997). In addition, there are advantages and disadvantages for these kinds of tests. The benefit of ramp incremental test is its short duration; whereas the incremental square wave test demonstrates a better $\dot{V}_{O_2}$ /PO correspondence despite its longer duration.

1.2. Physiological Thresholds

1.2.1. Lactate threshold

There are two physiological boundaries identified over the years. Among them, one of the most used involves the measure of the lactate concentration $[La^-]_b$ in the blood. By convention, the lactate concentration is expressed in mM or mmol / l.

The lactate threshold (LT) measurement is widely accepted because;

- It is a predictor of athletic performance in endurance exercises, often with greater accuracy than $\dot{V}_{O_2\,max}$.
- It can be used to determine the intensity of training suited to the actual metabolic and aerobic dynamics of the muscles.

During an exercise it is customary to measure the appearance of lactate in the muscle or blood to be able to indirectly analyze the anaerobic cellular metabolic processes (McArdle et al., 2009). In fact the concentration of lactate in the blood varies according to the metabolic intensity of the exercise: in exercises of low metabolic intensity (37- 45% $\dot{V}_{O_2\,max}$) (ACSM 2018) the concentration of lactate in the blood does not vary with respect to the values of rest, because the aerobic metabolism is able to cover the energy needs of muscles and therefore under these conditions there is not substantially accumulation of lactate. For more intense exercises, but still lower than $\dot{V}_{O_2\,max}$ blood lactate increases in the first minutes, to reach a stable value within 10 minutes of exercise. This condition is satisfied for all the exercise intensities at which a balance between lactate production and disposal is fulfilled. The highest work rate presenting this balance is called maximal lactate steady state (MLSS) (Plowman et al., 2013).

Finally, in very intense exercises (64-90% $\dot{V}_{O_2\,max}$), where the energy demand is higher than that corresponding to $\dot{V}_{O_2\,max}$, the concentration of blood lactate continues to increase exponentially throughout the test (Alloatti et al., 2002). Different approaches have been proposed to determine the exercise intensity at which lactate begins to accumulate. Some authors defined the lactate threshold as the highest value of oxygen consumption at which lactate accumulation above the baseline value of 1 mM does not occur. Instead, the value of $\dot{V}_{O_2}$ to which the concentration of lactate in the blood increases above 4 mM is defined as the onset of blood lactate accumulation (OBLA) (McArdle et al., 2009). The terms of

lactate threshold and OBLA therefore represent two different points of blood lactate concentration and power. The 4 mM value of the OBLA corresponds to the maximum power that a subject can maintain for a prolonged time. In reality, there is a great inter-individual variability in relation to the maximum lactate concentration compatible with resistance to prolonged work. The physiological parameter by definition correlated with the crossing of the lactic acid threshold should be represented by the lactate produced by the muscles involved during the activity; in fact blood lactate values do not always reflect the lactate concentration of the muscles that are working (McArdle et al., 2009).

However, since the lactate molecule is small and spreads quite well and rapidly in the body's aqueous compartments, the quantity contained in the blood can be considered a sufficiently reliable and reproducible index of that produced at the muscular level (Dal Monte et al., 1999). Furthermore, the method of taking blood from the ear lobe or from the fingertip compared to the method using a muscle biopsy is a faster and less invasive method (Plowman et al., 2013).

In addition, the introduction of the micro method for determining the blood lactate concentration, which requires a few microliters (1-2 drops) of blood taken from the ear lobe or from the fingertip, has overcome all the problems related to the trauma of the technique of sampling from the brachial vein (Dal Monte et al.,1999). Mader et al. (1976) proposed a method based on the determination of the lactate curve during some tests at increasing loads. The duration of each test was 5-6 minutes, with a pause between each load and the

next one sufficient to take blood (generally 30 seconds) (Dal Monte et al., 1999). The aim is to highlight the passage of lactic curve through the standard value of 4Mm. On the basis of empirical observations, it can be seen that this value can be proposed as a point of passage through the LT since, normally, the corresponding workloads can be tolerated very long, independently from the individual endurance performance skills; while labor intensities such as to exceed this value determine a progressive accumulation of lactate (Heck et al., 1985). According to the method presented by Mader (fig.5 [A]), from the graph of blood lactate concentration has a function of the intensity of the exercise (expressed as load, as $\dot{V}_{O_2}$) it is possible to determine, on the abscissa axis, the intensity corresponding to the concentration of 4mM of blood lactate (Dal Monte et al.,1999).

Several graphical and mathematical techniques have been used for the exact identification of the point corresponding to 4 Mm. One of the most widely used procedures is to construct a polynomial interpolating. However, some authors did not consider a standard lactate value valid for all to be sufficiently acceptable, which can be objectively recognized as the moment from aerobic to anaerobic metabolism, since through direct procedures extremely variable individual values of lactate concentration are observed (from 1.8 to 10 mM), therefore, various methods have been proposed that are based on the identification of a point or a curve area, constructed with blood lactate values taken during tests at increasing loads (Dal Monte et al., 1999). One of these methods (Yoshida et al., 1984) considers the lactic acid threshold as the point at which the lactate concentration increases by 1 mM

above the basal value (fig. 5 [B]). Other authors (Simon et al., 1981) identified the lactate accumulation threshold at the point where the tangent to the curve exceeds 45 ° (fig. 5 [C]). Similar method was proposed by Keul et al. (1979) which suggested to take into consideration the point at which the tangent to the curve of lactate is identified with an angle of 51 ° (corresponding to the slope of 1 mM in 3 minutes) (fig. 5 [D]).

Cheng et al. (1992) calculated LT using the D_{max} method. This method identifies the threshold as the point of the polynomial regression curve that presents the maximum distance, measured perpendicularly, from the straight line (chord) that combines the minimum and maximum lactate concentration (fig. 5 [E]). Bishop et al. (1998) determined the threshold through a modification (D_{max} MOD) of the D_{max} method. In this case the lactic threshold is identified with the point on the third-order polynomial curve which has the maximum distance, measured perpendicularly, from the straight line joining the first point preceding the increase in lactate concentration to more than 0.4 mM on end point of lactate (Dal Monte., 1999) (fig. 5 [F]).

Finally, Heuberger in one of his studies (Heuberger et al., 2018) notes that the 4 mM method, the D_{max} method and the modified D_{max} method show the best repeatability and have the highest correlation with endurance performances. Furthermore, since the modified D_{max} was previously referred to as a valid MLSS estimate, he recommends using this method when analyzing the blood lactate curve.

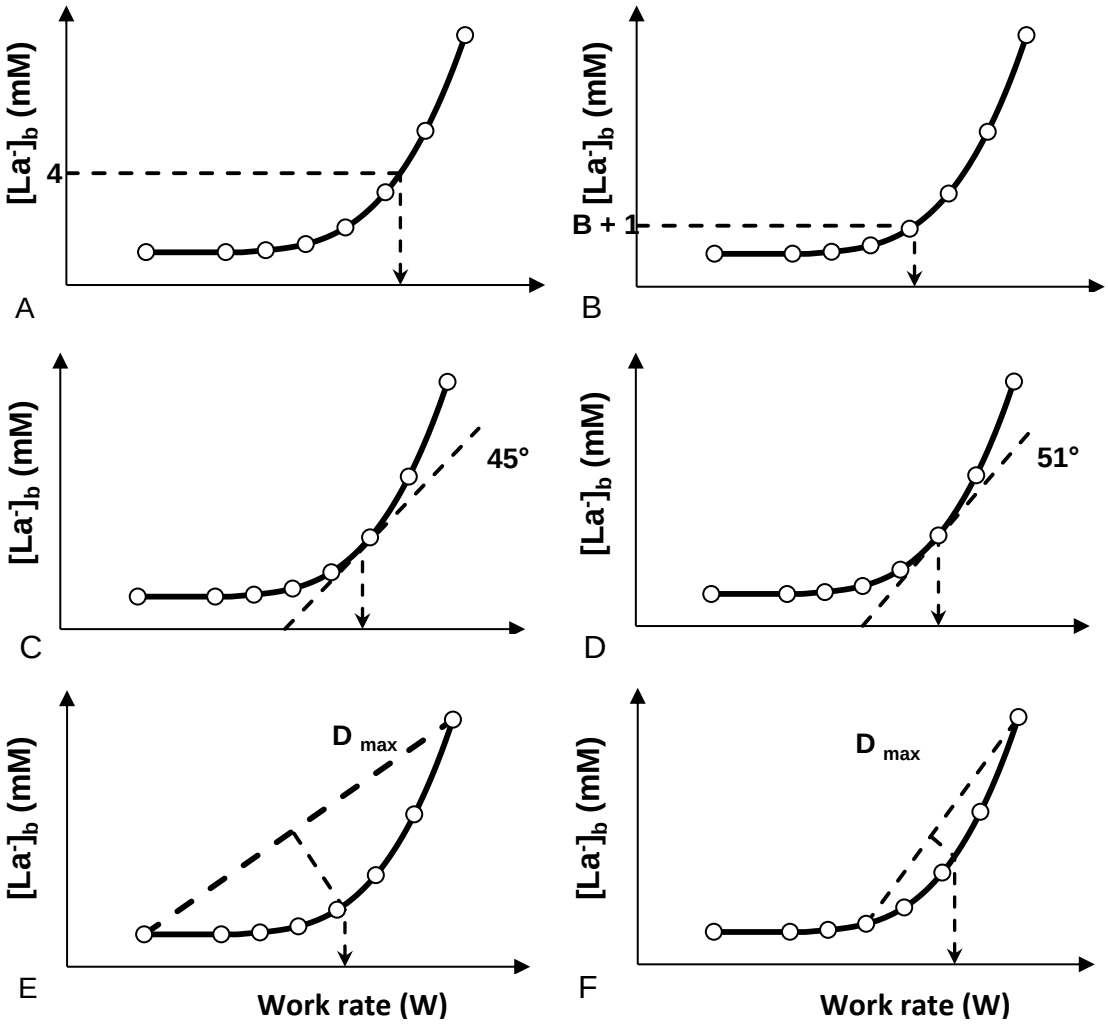

Figure5. Methods for determining the lactate accumulation threshold: A) 4 mM (Mader); B) B+ 1mM (Yoshida); C) tangent of 45 ° (Simon); D) tangent of 51 ° (Keul); E) D_{max} (Cheng); F) D_{max} MOD (Bishop).

1.2.2. Ventilatory Thresholds

Another procedure commonly adopted to determine the transition from a prevalently aerobic to a predominantly anaerobic metabolism involves the expiratory ventilation signal. Physical activity induces increases in $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. Up to a mild to moderate load intensity, $\dot{V}_E$ grows linearly with $\dot{V}_{O_2}$ and with $\dot{V}_{CO_2}$ and in this case, increases mainly due to the increase in tidal volume. At higher intensities, however, the respiratory rate plays a more important role. These adjustments allow a complete blood arterialization: in fact P_{O2} and P_{CO2} alveolar remain similar to the rest values. During a submaximal exercise, in a young and healthy subject the respiratory equivalent, i.e. the ratio between pulmonary ventilation and oxygen consumption ($\dot{V}_E/ \dot{V}_{O_2}$), is maintained at about 25 (25 liters of ventilated air for every liter of O_2 consumed). On the contrary, at more intense levels of activity, $\dot{V}_E$ increases more, and in a no longer proportional manner, with respect to $\dot{V}_{O_2}$. The respiratory equivalent can therefore reach values of 30-40 liters of air per liter of oxygen consumed (McArdle et al., 2009).

The term ventilatory threshold indicates the point where the pulmonary ventilation increases disproportionately relative to increase in oxygen consumption during graded exercise. At this point, pulmonary ventilation no longer links tightly to oxygen demand at cellular level. In fact, the “excess” ventilation comes directly from carbon dioxide’s release from the buffering of excess hydrogen ions (H^+) that begins to accumulate from increased

glycolysis. Sodium bicarbonate in the blood buffers almost all of the lactic acid generated in anaerobic metabolism to sodium lactate in the following reaction:

$$Lactic\ acid + NaHCO_3 \rightarrow Na\ Lactate + H_2CO_3$$

$$\uparrow\downarrow$$

$$H_2O + CO_2$$

The excess H^+ are combined with bicarbonate ions (HCO_3^-) to form carbonic acid (H_2CO_3) which breaks down into CO_2 and water (H_2O). The increase of the $\dot{V}_E$ thus determined would therefore serve to remove the CO_2 in excess. The $\dot{V}_E$ therefore, increases with respect to oxygen consumption and determines an increase in the respiratory quotient $R= \dot{V}_{CO_2}/ \dot{V}_{O_2}$ above 1. It is believed that the increase in $\dot{V}_E$ and the increase of R above 1 indicate that amount of O_2 required by the muscles is greater than that actually reaching the mitochondria; this causes an increase in the energy produced through anaerobic metabolism. Therefore, the ventilatory threshold corresponds to the point beyond which anaerobic metabolism increases (McArdle et al., 2009; Wasserman et al., 1973).

In fact, during an incremental test two points of inflection can be distinguished: the first one corresponds to the initial increase of $\dot{V}_E$ and $\dot{V}_{CO_2}$ with respect to $\dot{V}_{O_2}$ (compensated ventilatory threshold) which allows keep blood pH constant; the second in which the $\dot{V}_E$

rises more, also with respect to $\dot{V}_{CO_2}$, and in which there is a lowering of pH (uncompensated ventilation threshold). The observation of the kinetics of respiratory parameters in relation to the intensity of physical exercise offers the possibility of identifying the transition point between the different types of metabolism even without having to resort to direct measurement of blood lactate concentration. Several methods have been proposed to identify the ventilatory thresholds of a subject.

Among the methods proposed, the one that undoubtedly had the greatest diffusion is the one suggested by Wasserman in 1973, based on the determination of the breaking point of the linear relationship between $\dot{V}_E$ and $\dot{V}_{O_2}$ for the reasons explained above. This method presupposes the measurement of gas exchanges breath-by-breath and the adoption of a protocol with short duration loads and with modest intensity increments. Wasserman proposed the use of a ramp protocol in which the load varies almost continuously for the duration of the test (Wasserman et al., 1973). For this method we could also directly relate the $\dot{V}_E$ with the workload, assuming a linear relationship between this and $\dot{V}_{O_2}$; in this way it is possible to express the ventilatory threshold in terms of the corresponding unit of work load (fig. 6).

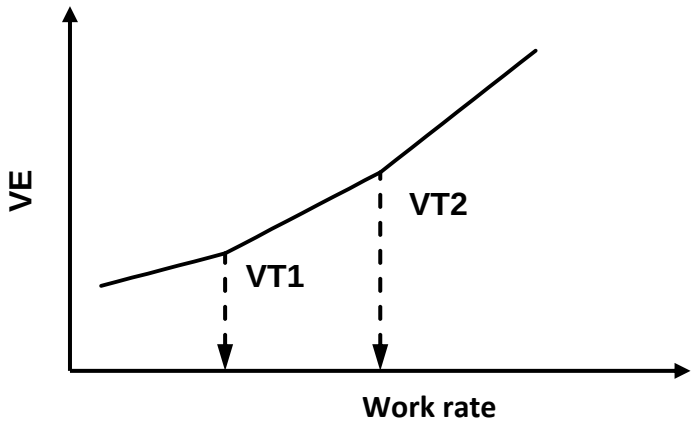

Figure6. Method for identifying the ventilatory threshold: ratio $\dot{V}_E$ and workload

Other authors suggest using other ventilatory and metabolic parameters for a better identification of the points of inflection, such as the ventilator equivalents $\dot{V}_E / \dot{V}_{O_2}$ and $\dot{V}_E / \dot{V}_{CO_2}$ the relationship between $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$(V-Slope method) and the end-expiratory pressure of O_2, CO_2, Pet_{O2} and Pet_{CO2} (Wasserman et al., 1999). In regards to the method of respiratory equivalents, the ventilatory threshold can be observed by increasing the respiratory equivalent for the use of O_2, ($\dot{V}_E / \dot{V}_{O_2}$), without a parallel increase in the respiratory equivalent for the production of CO_2 ($\dot{V}_E / \dot{V}_{CO_2}$), which on the contrary reaches its minimum value (fig 7) (Mezzani et al., 2009).

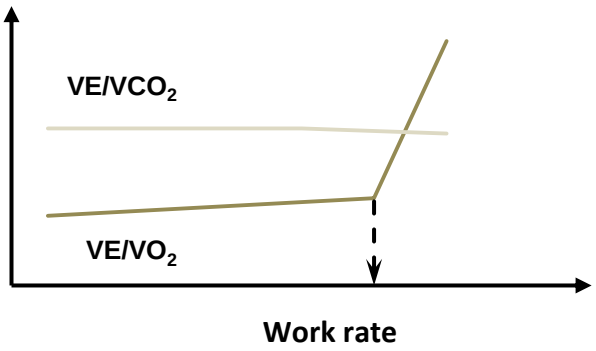

Figure7. Method for identifying the ventilatory threshold: ratio between respiratory equivalents

The V-slope method of determining the anaerobic threshold makes use of the fact that $\dot{V}_{CO_2}$ plotted against $\dot{V}_{O_2}$ shows a slope of slightly less than 1 for work below the anaerobic threshold. A line of best fit for points obtained from the start of exercise is drawn through this plot to obtain the initial slope (fig. 8) (Schneider et al., 1993).

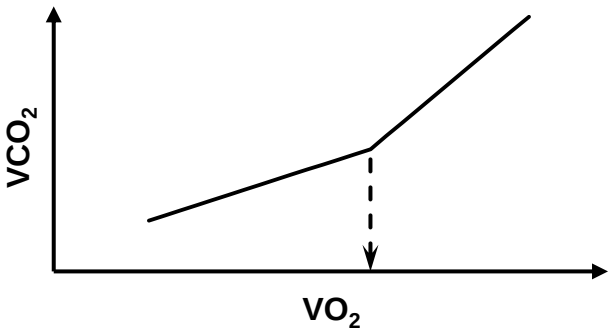

Figure8. Method for identifying the ventilatory threshold: ratio $\dot{V}_{CO_2}$ / $\dot{V}_{O_2}$

Finally, with the end-expiratory pressure method, the compensated ventilation threshold is identified with the beginning of the increase of the partial expiratory pressure of O_2; the uncompensated ventilation threshold corresponds to the point at which the partial expiratory pressure of the CO_2 begins to decrease (fig. 9).

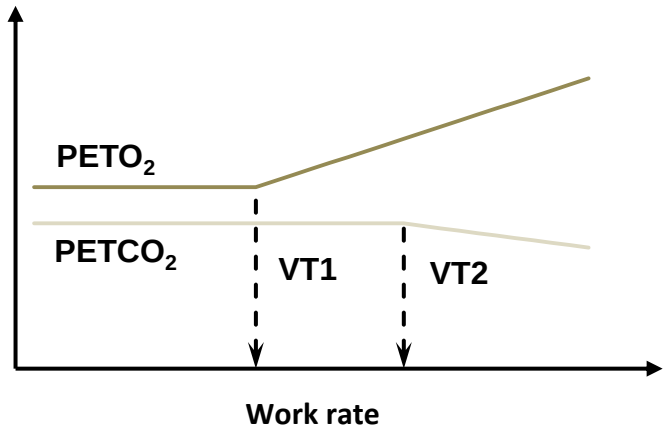

Figure9. Method for identifying the ventilatory threshold: ratio Pet_{O_2} and Pet_{CO2}

1.2.3. Critical Power

The relationship between fatigue and performance has always interested many scholars, both in the field of Physiology and in that of physical activity in the sports and medical fields. In 1965, Monod and Sherrer were the first to introduce the concept of critical power (CP); they noticed a hyperbolic relationship between the power supplied and the time in

which the power can be maintained. They therefore defined the CP as the maximum load that can be sustained "for a long time without fatigue", stating that, at a theoretical level, if the imposed load is less than or equal to the CP, exhaustion cannot occur and therefore activity can be protracted to infinity (Monod et al.,1965). Physiologically, CP represents the highest sustainable load from oxidative metabolism, above which an alteration of homeostasis occurs, leading to an increase in blood lactate, decrease in blood pH, and ventilation increase (Jones et al., 2010). It has also been reported that CP had a higher value than the blood lactate accumulation threshold and the ventilatory thresholds (Hill, 1993; Pringle et al., 2002).

As previously mentioned, the relationship between load and the corresponding time–of-exhaustion can be described through a non-linear function: a hyperbolic curve (Monod et al., 1965). As shown in the graph (fig. 10), on the x-axis (x) the load values are set, while on the y-axis the exhaustion time for each load is shown. Since CP is defined as the maximum load that can be supported at infinity (t_lim), it corresponds mathematically to the vertical asymptote, parallel to the y-axis.

By rearranging the factors, the hyperbolic relationship can be transformed into a linear one. Indeed, by plotting the sustained load (y) and the reciprocal of its exhaustion time (x) a linear function is obtained (fig. 11) (jones et al., 2010). In this case, CP is represented by the intercept of the regression line. With this model, in theory, only two points are needed to determine the CP; however, to reduce the errors in the estimate, it is advisable to carry out three to five tests (Hill, 1993), with different intensities (Mattioni Maturana et al.,

2018). Alternatively, a single-session test can be proposed to determine the CP (Vahnatalo et al., 2007 and Mattioni Maturana et al., 2016).

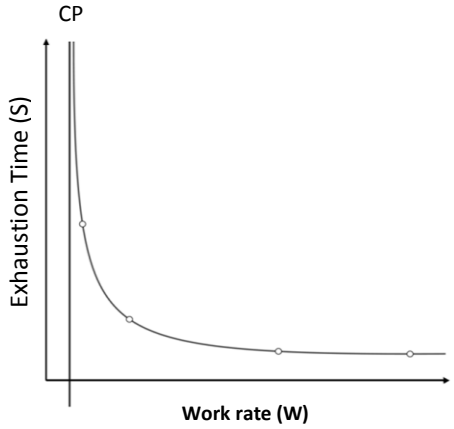

Figure10. Hyperbolic model for the determination of CP

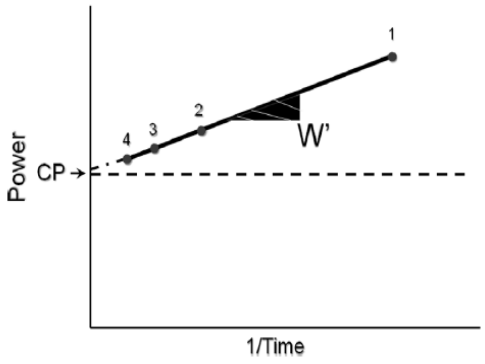

Figure11. Linear model for the determination of CP

1.3. Cardiovascular system

1.3.1. Cardiovascular system components

The cardiovascular system consists of the continuous linkage of a pump, a high-pressure distribution circuit, exchange vessels, and a low-pressure collection and return circuit. If stretched in a line, the 100,000 miles of blood vessels of an average-sized adult would encircle the earth about four times. Figure 12 presents a schematic view of the cardiovascular system including the major arteries (McArdle et al., 2009).

1.3.2. The Heart

The heart provides the impetus for blood flow. Situated in the mid-center of the chest cavity, about two thirds of its mass lies to the left of the body's midline. The four-chambered muscular organ weights 0.32 liters for an average-sized adult male and 0.27 liters for an average-sized female and pumps about 0.070 liters, or 70 mL, on each beat. At rest, the heart's output of blood averages 7193 liters daily, or 94635295 liters over a 75-year lifetime. For a person average fitness status, the maximum output from a household faucet turned wide open (McArdle et al., 2009). Functionally, the heart has two separate pumps. The hollow chambers on the right side of the heart (right heart) perform two crucial functions:

- Receiving blood returning from throughout the body;
- Pump blood to the lungs through the pulmonary circulation.

Instead, the left side of the heart (left heart):

- Receives oxygenated blood from the lungs
- Pumps blood into the thick-walled, muscular aorta for distribution throughout the body in the systemic circulation.

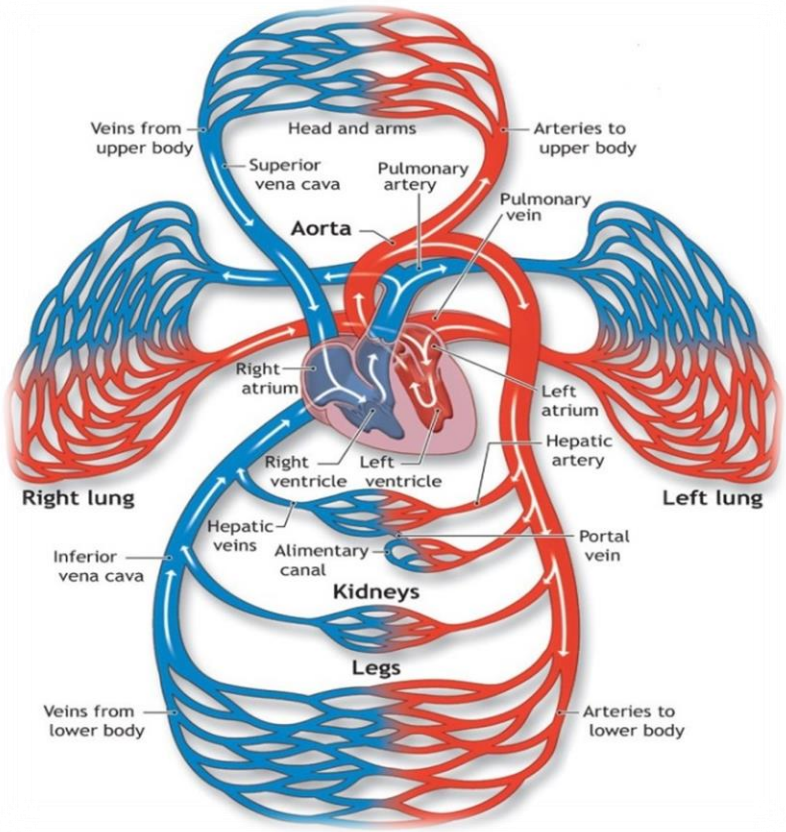

Figure.12 Schematic view of the cardiovascular system indicating the heart and pulmonary and systemic vascular circuits.

A thick, solid muscular wall or interventricular septum separates the heart's left and right sides. The atrioventricular valves within the heart provide one-way blood flow from the right atrium to the right ventricle via the tricuspid valve, and from the left atrium to the left ventricle through the mitral or bicuspid valve. The semilunar valves, located in the arterial wall just outside the heart, prevent blood from flowing back into the heart between contractions.

The heart wall is made up of muscle tissue, called myocardium. This muscle tissue is striated, with a high capillary density and numerous mitochondria. Unlike the other tissues, the heart muscle has its own excitation system. In the posterior wall of the right atrium there is a small mass of specific myocardial tissue, called the sinoatrial node. This node spontaneously depolarizes and repolarizes maintaining the electrical stimulus necessary for cardiac contraction. The stimulation and depolarization of a cell spread to all the surrounding cells, thus activating the myocardium as if it were a single unit.

The electrochemical stimulus, which arises from the atrial sinus node, propagates through the atria to another agglomerate of specific tissue located near the tricuspid valve, called the atrioventricular node. In the passage through the atrioventricular node, there is a physiological delay of about 0.10 s: in this way the atria have sufficient time to contract and expel blood towards the ventricle. From the atrioventricular node originates the atrioventricular bundle or bundle of His, which rapidly transmits the impulse to the ventricles through highly specialized conduction fibers, called Purkinje fibers. These fibers form branches that penetrate distinctly into the right ventricle and left ventricle, and

transmit the pulse at a rate that is about six times higher than that of normal ventricular fibers. The passage of the impulse in the ventricles in turn stimulates the common myocardial cells, allowing the almost simultaneous contraction of both ventricles (Alloatti et al., 2002).

1.3.3. Cardiac output

Cardiac output expresses the amount of blood pumped by the heart during a 1-min period. The maximal value reflects the functional capacity of the cardiovascular system. Output from the heart, as with any pump, depends on its rate of pumping f_H and quantity of blood ejected with each stroke volume (SV). Cardiac output ($\dot{Q}$) computes as follows:

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

The average values for $\dot{Q}$, f_H and SV for endurance-trained and untrained men at rest:

Untrained: $\dot{Q} = 70\ bpm \cdot 71\ mL =$ "~$4900\ mL/min$

Trained: $\dot{Q} = 50\ bpm \cdot 100\ mL = 5000 mL\ /min$

Systemic blood flow increases directly with intensity of physical activity. $\dot{Q}$ increases rapidly during transition from rest to steady-rate exercise. Thereafter, $\dot{Q}$ rises gradually until

it plateaus when blood flow meets the exercise metabolic requirements. This leads to an increase in both f_H and SV. We therefore arrive at $\dot{Q}$ values which, in the case of maximal exercises, in elite athletes dedicated to aerobic sports, can exceed 35 to 40L.min^{-1}. These increases are achieved by increasing, with respect to the rest values, at least 3 times of f_H and at least 2 times of range SV.

The average values for $\dot{Q}$, f_H and SV for endurance-trained and untrained men during maximal physical activity:

Untrained: $\dot{Q} = 195\ bpm\ \cdot 113\ mL =$ "~$22000\ mL/min$

Trained: $\dot{Q} = 195\ bpm\ \cdot 179\ mL =$ "~$35000\ mL/min$

1.3.4. Nervous and humoral control of the cardiovascular system

The cardiovascular system must be adjusted to adapt blood pressure and flow to the needs of the various tissues at any time. In resting conditions, the organs with the highest oxygen consumption and blood flows are the encephalon, the heart and the kidneys. When the muscle tissue is in a state of activity, the blood flow that reaches it can rise up to values 30-40 times the resting value. Changes in blood flow also occur at the level of the skin to ensure thermoregulation processes. In this situation, the control system must ensure that, in order not to compromise vital functions, sending more blood to other districts does not come at the expense of fundamental organs such as the heart and brain. The control of the

circulation is carried out through different mechanisms, tightly integrated: at the local level the regulation processes act on the microcirculation; while to maintain the pressure values inside the circle the activity of the heart, of the arterial and venous vessels, and the volume of blood are regulated (Alloatti et al., 2002).

1.3.5. Adjustment of cardiac output

When intense muscular work is done, $\dot{Q}$ can increase about four times the resting value. The increase may be induced either by changes in SV and/or by an increase in f_H. The heart is able to modify the SV either autonomously (intrinsic regulation) or following the action of the autonomic nervous system (extrinsic regulation). The intrinsic regulation concerns in particular the contraction force; while the parasympathetic systems (belonging to the autonomic nervous system), in addition to regulating contractility, also act on the heartbeat frequency.

1.3.6. Intrinsic regulation

In cardiac muscle the developed contraction force depends on the length of the muscle fibers before the contraction begins. The relationship between the initial length of the muscle fibers (that is proportional to the end-diastolic volume and in turn to the end-diastolic pressure) and the force of contraction of the heart is known as the Frank-Starling law (fig. 13).

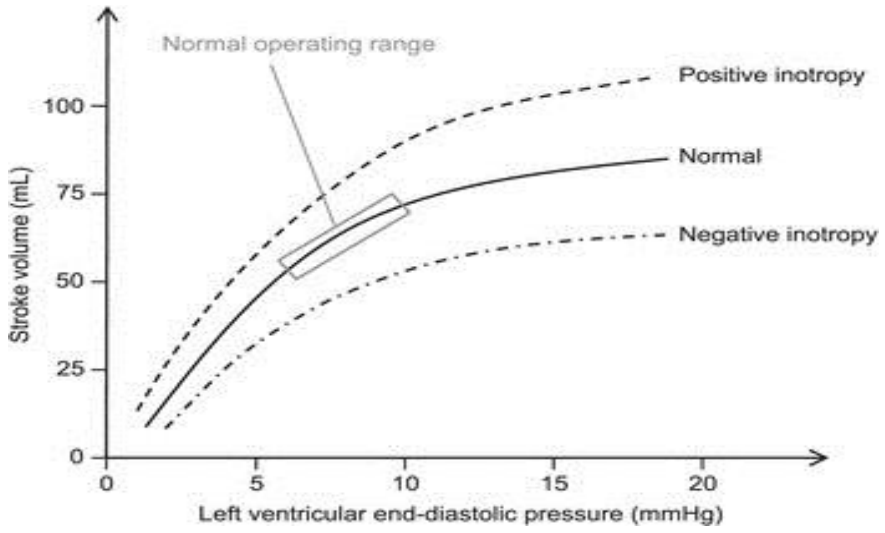

Figure13. Frank-Starling curve and influence of noradrenaline. The Frank-Starling law expresses the relationship between the length of the muscle fibers and the force of contraction of the heart. This relationship can be influenced by numerous factors, such as norepinephrine, which moves the curve to the left and upwards, so that a higher force is developed for the same ventricular volume.

The law explains that the length of the cardiac muscle fibers is a function of the volume, and consequently of the pressure, of blood present inside the heart chambers at the end of the diastole so that the higher is the pressure, the longer are the fibers and the higher is the stroke volume. In this way the heart is able to adapt to even considerable variations in the volume of blood that fills it independently of the autonomic nervous system. Several factors can change the volume of blood contained in the heart. Among these, the most important is the extent of venous return, which, during physical activity, tends to increase both due to the pump action exerted by the muscles in activity and due to the increase in muscle tone of the vessels venous both for the greater attraction towards the thoracic cavity exercised by the increase in the depth of the breath (McArdle et al., 2009).

1.3.7. Extrinsic regulation

As for extrinsic regulation, the autonomic nervous system modulates not only the contraction force (inotropic effect), but also the frequency (chronotropic effect), the excitability (batmotropic effect) and the conduction speed in heart (dromotropic effect). The excitatory action on the heart muscle is exerted by the norepinephrine, freed from the terminations of the sympathetic system, and by the adrenaline, released into the circulation by the adrenal medulla. The sympathetic system determines the increase in f_H and conduction speed by acting on the pacemaker cells of the sinoatrial node and on the cardiac conduction system (fig. 14).

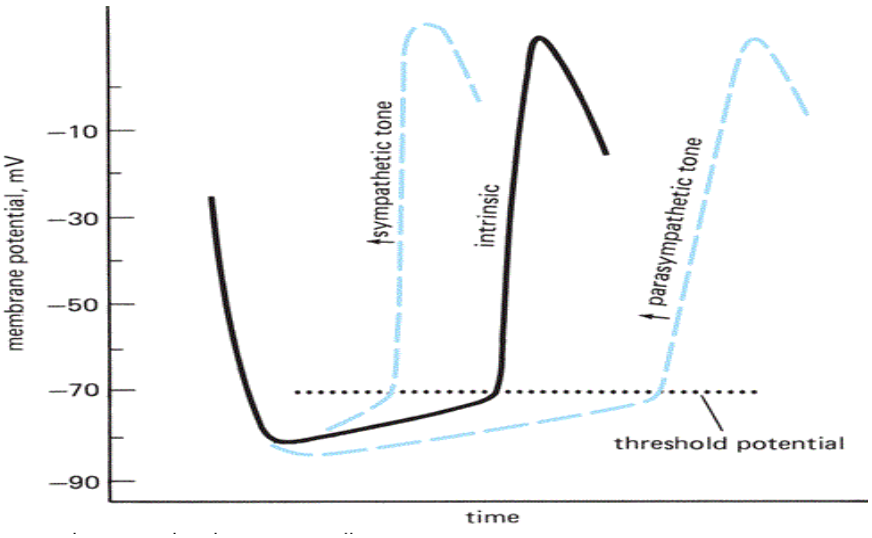

Figure 14 - Effects of the variation of sympathetic and parasympathetic tones on the action potential of a pacemaker cell. The sympathetic noradrenaline mediator allows an increase in the slope of the spontaneous depolarization phase; an effect of this is an increase in f_H (positive chronotropic action). In contrast, the parasympathetic system, through the mediator acetylcholine, causes a decrease in the slope of spontaneous depolarization, causing a slowing of the f_H (negative chronotropic action).

The increase in contractility induced by stimulation of the sympathetic system leads to an increase in SV with the same ventricular volume, which translates into a greater ejection fraction. Finally, the sympathetic system increases the speed with which the muscle contracts and releases, in order to adapt the cardiac function to the appropriate frequency (fig. 15).

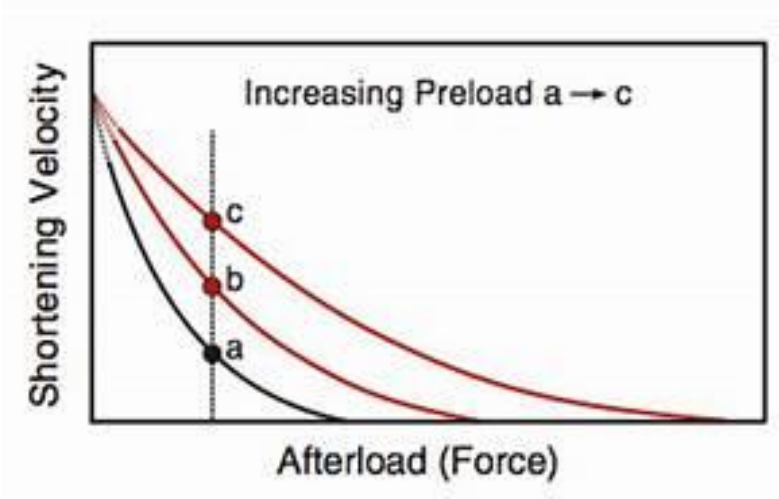

Figure15. Effects of noradrenaline on the force-speed relationship. The upward shift of the curve indicates an increase in the developed voltage and contraction speed. The effects are the basis of the positive inotropic action of noradrenaline.

On the contrary, the parasympathetic system reduces f_H, conduction velocity and the contraction force of the heart. Acetylcholine, a mediator freed from the vagus nerve endings, acts mainly on the cells of the conduction system and the atrium, while its action on the ventricle is minor (Alloatti et al., 2002). The main nerve centers responsible for

regulating the activity of the cardiovascular system are located in the trunk of the brain, in the hypothalamus and in the cerebellum. The signals coming from the aortic and carotid baroceptors, from the other pressure receptors and from the chemoreceptors arrive at the nucleus of the solitary tract, located at the level of the bulb. This center performs an inhibitory tonic action on the heart and on the vessels; moreover, signals are sent from it to other regions of the bulb, such as the ambiguous nucleus. This nucleus contains the motor neurons of the vagus nerve, which exerts inhibitory effects on the cardiac pacemaker and, in part, on the cardiac contractility. Still inside the bulb, there is a ventro-lateral rostral group of neurons, which exerts a tonic action on the sympathetic neurons, which acts at the level of the heart, blood vessels and medullary of the adrenal gland (Alloatti et al., 2002; McArdle et al., 2009).

1.3.8. Influence of breathing

During the respiratory cycle there is a natural variation in f_H called respiratory sinus arrhythmia; this phenomenon is particularly evident in children. It is characterized by an increase in f_H during inspiration and a decrease in f_H during expiration. The increase in f_H during inspiration comes from the temporary inhibition of the ambiguous nucleus (or cardio- inhibitory center), which causes a reduction in vagal output at the sinoatrial node and shortening of inter- beat interval (Mortola et al., 2016).

Furthermore, an expansion of the thoracic activity during the inspiration phase cause a greater venous return, which cause an increase in f_H through stimulation of stretch receptors, located in the atrium. The afferent branch of the reflex is represented by vagal afferents, which at the central level inhibit the vagal tone and increase the sympathetic tone (Bainbridge reflex). The venous return also causes a direct mechanical effect, with a consequent increase in f_H due to the stretching of the cells of the atrial sinus node.

1.3.9. Cardiovascular response to exercise

The adaptations of the cardiovascular system to physical exercise are very complex: in fact, it is necessary to provide a greater flow of blood to the muscles to satisfy the increased demands for O_2 and nutrients, and to favor an increase in the exchange of respiratory gases in the pulmonary circulation. This latter aspect is crucial as serious changes in blood pressure, could cause hypo-perfusion of the central nervous system (Plowman et al., 2013).

Cardiovascular response during an incremental exercise

During an incremental exercise, $\dot{Q}$ has a straight increase as a function of the workload reaching a plateau at the maximum intensity of the activity. The initial increase is due to both an increase in SV and an increase in $f_{H.}$ Instead, for loads exceeding 40–50% of the maximum load, the continuous increase in $\dot{Q}$ in untrained individuals is almost completely implemented by the increase in f_H: in fact, in these subjects SV initially grows linearly and then reaches a plateau at about 40–50% of the maximum load (Åstrand et al., 1964;

Higginbotham et al., 1986). The exact SV response to incremental exercise continues to be debated (González- Alonso, 2008; Warburton et al., 2008).

As indicated above, it has traditionally been believed that SV plateaus at approximately 50% of. $\dot{V}_{O_2\,max}$ in untrained individuals. However, there appears to be considerable inter individual variability in this response, and many laboratories have reported an increase in the SV at maximal exercise in most endurance athletes and some untrained individuals (Ferguson et al., 2001; Gledhill et al., 1994; Warburton et al., 1999). In contrast, other researchers have documented a decrease in SV at the maximal exercise (Mortensen et al., 2005 and some researchers contend that after an initial increase (due to the skeletal muscle pump returning the pooled venous blood to the heart), the SV remains essentially unchanged during the incremental maximal exercise (Rowland, 2005). Much of the controversy is undoubtedly associated with difficulties in measuring SV during maximal exercise, with the use of different exercise protocols, and with individual variability. The end-diastolic volume increases largely because of the return of blood to the heart by the active muscle pump and the increased sympathetic outflow to the veins causing venoconstriction and augmenting venous return. The end-diastolic volume decreases because of augmented contractility of the heart, which ejects more blood and leaves less in the ventricle (Poliner et al., 1980). The f_H increases in a rectilinear fashion throughout much of the submaximal (~120–170 b·min−1) portion of incremental exercise and plateaus at maximal exercise (Astrand and Rhyming, 1954; Hale, 2008). Myocardial cells can contract at over 300 b·min−1 but rarely exceed 210 b·min−1 because a faster f_H would not allow for

adequate ventricular filling. Thus, SV and ultimately cardiac output would decrease. Consider the simple analogy of a bucket brigade. Up to a point it is useful to increase the speed of passing buckets under the water source, but the maximum rate is limited because of the time required for the buckets to be filled with water. The maximal amount of oxygen an individual can take in, transport, and utilize ($\dot{V}_{O_2\,max}$) is usually measured during an incremental maximal exercise test. Although $\dot{V}_{O_2\,max}$ is considered primarily a cardiovascular variable, it also depends on the respiratory and metabolic systems. , $\dot{V}_{O_2\,max}$ can be defined by rearranging the Fick equation to the following equation:

$$\dot{V}O_{2\,max} = \dot{Q}_{max} \cdot (C_aO_2 - C_vO_2)_{max}$$

Since the $\dot{Q}$ grows linearly during the incremental exercise and the diffusion C_aO_2-C_vO_2 increases until it flattens to about 60% of the maximum load, even $\dot{V}O_2$ has a trend straight and a plateau with maximum intensity. However, not all subjects are able to reach this plateau during an incremental test. As for the systolic blood pressure (SBP), it increases linearly until it reaches the plateau with maximal exercise, even reaching values above 200 mmHg in highly trained individuals. This increase is caused by the increase in $\dot{Q}$, which exceeds the simultaneous decrease in total peripheral resistance (TPR), directly related to vasodilation. Diastolic arterial pressure (DBP) on the other hand, remains constant (or has little significant variation), because vasodilation in the vascular system of active muscles is

balanced by vasoconstriction. The TPR decreases with a negative curvilinear trend and reaches the minimum value at maximum exercise. This decrease in TPR reflects the maximum vasodilation in the active tissue in response to the need for an increase in blood flow; moreover the decrease of the TRP allows the mean arterial pressure (MAP) increase slightly. Finally, $\dot{Q}$ increases from about 5 (l / min) (rest condition) up to 25 (l / min) (maximal operating condition). As for its distribution, the most considerable change affects the direct blood flow to the active muscles, which covers 88% of the entire blood flow. On the contrary, at the level of the skin, kidneys and splanchnic organs, the blood flow decreases considerably; while the blood supply to the brain and heart is maintained (Plowman et al., 2013).

Cardiovascular response during a light (37-45% $\dot{V}_{O_2\,max}$) to moderate (46-63% $\dot{V}_{O_2\,max}$) intensity exercise at constant load (ACSM, 2018)

At the beginning of the exercise of mild to moderate intensity, the $\dot{Q}$ increases in the first 2 minutes until it reaches a plateau; it grows due to an initial increase in SV and f_H, which level off within 2 minutes. The plateau denotes that a steady state has been reached, ie a condition in which the energy required to perform the exercise is balanced by the energy supplied aerobically. The SV increases rapidly at the beginning of the exercise due to an increase in venous return, which in turn determines an increase in end-diastolic volume. This increase generates an elongation of the myocardial fibers, which can be contracted more strongly, as described by the Law of Frank Starling. Myocardial contractility is also

enhanced by the sympathetic nervous system, which is activated during physical activity. Simultaneously with the increase in SV, the f_H immediately rises to the beginning of the activity as an effect of the inhibition of the parasympathetic system and, as the exercise continues, it increases further thanks to the activation of the sympathetic nervous system (Plowman et al., 2013).

Since the increase in SBP due to the increase in $\dot{Q}$, it has a trend very similar to the latter: it is in fact characterized by an initial increase followed by a plateau, once the steady state is reached. The SBP would be even higher if it were not for the fact that the TPR decreases, thus partially compensating for the increase in $\dot{Q}$: DBP, on the other hand, remains relatively constant due to peripheral vasodilation, which facilitates blood flow to the active muscles. The modest increase in SBP and the lack of a significant change in DBP mean that MAP increases only slightly.

The TPR decreases due to vasodilation in active muscles; this vasodilation derives mainly from the influence of local chemical factors, which reflect an increase in metabolism. The decrease in TPR has two important implications: first, the dilation of the vessels supplying the active muscle causes a decrease in resistance, which leads to an increase in blood flow, thus increasing the availability of O_2 and nutrients; secondly, reduced resistance prevents MAP from dramatically increasing. Indeed, the increase in MAP is determined by the relative changes in $\dot{Q}$ and TPR. Even in the constant load exercise, the double product (DP) grows in relation to increases in f_H and SBP, reflecting the greater demand for oxygen at the level of the myocardium during physical activity (Plowman et al., 2013).

Finally, the Q̇ increases from about 5-6 l / min, in the rest condition, to 10 l / min. With light exercise, the most noticeable change in its distribution is the increased percentage of blood supply to active muscles, from 21% to 47%. Blood flow to the skin also increases to meet the need for thermoregulation of the exercise. On the contrary, the blood supply to the heart and the encephalon remains constant, while both renal and splanchnic blood flow decrease to a limited extent (Plowman et al., 2013).

Cardiovascular response to a constant load of moderate (46-63% $\dot{V}_{O_2\,max}$) - high intensity (64-90% $\dot{V}_{O_2\,max}$) (ACSM, 2018)

Similarly to exercises with mild to moderate workloads, Q̇ increases rapidly during the first two minutes, to then reach a plateau and remain constant; however, the absolute value of the plateau is greater during high intensity than in mild to moderate exercise. The increase in Q̇ results from an increase in SV and f_H. As in mild to moderate aerobic exercise, the increase in SV is thought to be due to greater venous return, which leads to the Frank-Starling mechanism, and to greater contractility, due to stimulation of the sympathetic nerve; therefore, the variations in SV occur because the diastolic volume increases and the end-systolic volume decreases. The diastolic volume increases mainly due to the increase of the venous return of the blood to the heart, due to the muscular pump and the greater vasoconstriction; end-systolic volume, on the other hand, decreases due to increased contractility of the heart, which effectively expels more blood. If the exercise continues for about 30 minutes, the SV decreases gradually, remaining above the rest values; this

downward shift is often attributed to thermoregulatory stress, which reduces venous return causing vasodilation, plasma loss and a greater supply of blood to the skin vessels to dissipate heat. This hypothesis suggests that f_H increases to compensate for a decrease in SV, in order to keep $\dot{Q}$ constant. On the contrary, other scholars suggest that the downward drift of SV is due to a reduced filling time of the chambers of the heart, caused by an increase in f_H, which in turn caused the increased sympathetic nerve activity. f_H increases rapidly in the first 1-2 minutes of exercise, through inhibition of the parasympathetic nervous system and activation of the sympathetic nervous system; after which, in the steady state, the curve levels out. After about 30 minutes of intense physical exercise, the f_H begins to rise again; this increase is proportional to the decrease in SV. As a result, $\dot{Q}$ remains constant during the exercise (Plowman et al., 2013).

The SBP response is characterized by a rapid initial increase during the first 1-2 minutes, a steady state plateau and a negative drift, following vasodilation and the consequent decrease of TPR. The DBP on the other hand, remains constant (or has a non-significant variation); the result is a modest increase in MAP. As in the mild-moderate exercise, the magnitude of the increase in MAP depends on a noticeable decrease in the TPR, which decreases rapidly in the first few minutes, reaches a plateau, and then has a slight negative drift during the physical exercise to continue of time. The cause of this last phenomenon is the vasodilation at the level of the active muscles and the skin. Finally, since both f_H and SBP have a considerable increase during intense work, the DP increases considerably with

the beginning of the exercise, and then stabilizes in the stationary state; however, a modest shift of the curve towards the top could occur after about 30 minutes of activity, for an increase in f_H higher than the SBP reduction. During a constant moderate-high intensity exercise, the $\dot{Q}$ also reaches values close to 20 (l / min). Once again, a noticeable increase in blood flow to active muscle (71%) and to the skin is evident, to meet the demands of thermoregulation. The percentage of $\dot{Q}$ in the heart remains relatively constant, as well as the amount of cerebral blood flow; while both renal and splanchnic blood flow are further reduced as exercise intensity increases (Plowman et al., 2013).

1.4. Pulmonary response

1.4.1. Respiratory system: anatomy and function

The respiratory system consists of the upper airways, which include the nose, the nasal cavities, the paranasal sinuses and the pharynx, and from the lower airways, formed by the larynx, trachea, bronchi and lungs (fig.16).

1.4.2. Respiratory system's functions:

- Provide a large area for gas exchange between air and circulating blood;
- Conducting air from and to the pulmonary exchange surfaces;
- Protect respiratory surfaces from dehydration, temperature changes and other environmental changes;

- Defend the respiratory system itself and other tissues from invading pathogenic microorganisms;
- Allow phonation;
- Adjust blood volume and pressure;
- Controlling the pH of body fluids

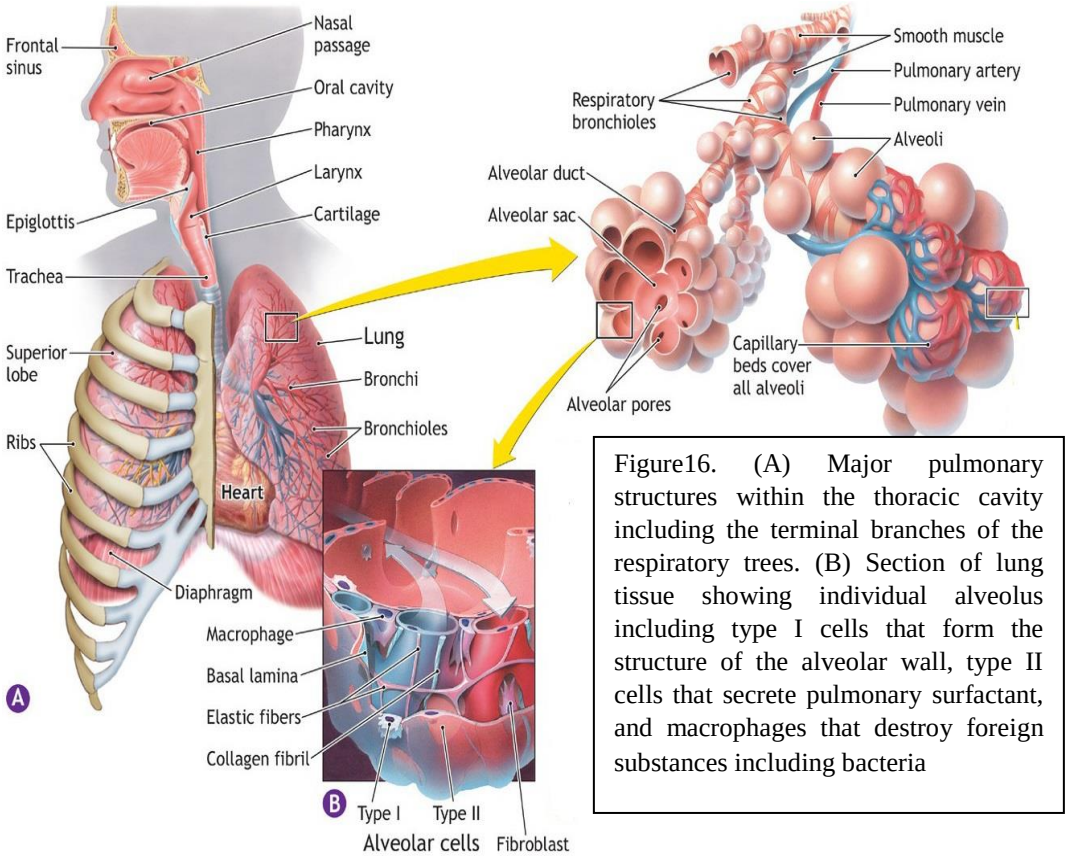

Figure16. (A) Major pulmonary structures within the thoracic cavity including the terminal branches of the respiratory trees. (B) Section of lung tissue showing individual alveolus including type I cells that form the structure of the alveolar wall, type II cells that secrete pulmonary surfactant, and macrophages that destroy foreign substances including bacteria

1.4.3. Breathing

Breathing consists of:

- Pulmonary ventilation, ie the process by which air is moved in and out of the lungs;
- External respiration, which involves the exchange of O_2 and CO_2 between the lungs and the blood through the diffusion mechanism;
- Perfusion and internal respiration ie transport of respiratory gases in the blood and exchange of O_2 and CO_2 at the cellular or tissue level; Cellular respiration or the use of O_2 by cells to produce energy (Plowman et al., 2013).

1.4.4. Ventilation

The air, after passing through the nasal cavities and / or the mouth, the pharynx and the larynx, enters the trachea, which is subdivided into two main bronchi, which in turn are distributed in various generations to the alveoli. In the first 16 generations the airways have only the function of transporting gases; while, starting from the 17th generation, the first alveoli begin to appear, which allow gas exchange between air and blood. The movement of the air from the external environment to the lungs depends on two factors: the pressure gradient (ΔP) and the resistance (R); the relationship between these factors is expressed by the equation:

$$\dot{V} = \frac{\Delta P}{R}$$

The R is the sum of the forces opposing the gas flow; it is due to the friction of the tissues during inhalation and exhalation the friction of the gas molecules with the airway walls and the viscosity of the fluid. To generate an air flow, it is necessary that the ΔP be greater than the R. Furthermore, the gases move from a higher pressure to lower pressure environment. Therefore, for inspiration to take place, the pressure must be higher in the external environment than in the lungs; on the contrary, for expiration, the pressure in the alveoli must be greater than the external one. Since at a constant temperature the pressure of a gas is inversely related to its volume (Boyle's law), an increase in lung volume during inhalation allows a lowering of pressure and a displacement of air inside the alveoli; vice versa, a decrease in lung volume causes an increase in pressure and consequently the leakage of air (Plowman, Smith, 2013). In resting conditions, the normal respiratory rate (f_R) is about 12 acts per minute and the tidal volume (V_T), ie the volume of air that enters with a quiet inspiration, is about 500 ml. Consequently, total lung ventilation ($\dot{V}_E$), ie the ventilated air volume per minute, corresponds to about 6 l.

$$\dot{V}_E = V_T \cdot f_R = 500 \text{ ml} \cdot 12 \text{ b/min} = 6 \text{ l}$$

A part of the inhaled air does not reach the alveoli and therefore does not participate in the gas exchange with the blood. The term anatomical dead space identifies this portion of air. In healthy adult subjects this volume is approximately 150 ml. Therefore, following an inspiration of 500 ml, only 350 ml arrive in the alveoli and become available for gas

exchange. Alveolar ventilation is the amount of air that actually reaches the alveolar compartment in one minute (McArdle et al., 2009).

1.4.5. Diffusion

The gas exchange of O_2 and CO_2 occur at the level of the alveolus-capillary membrane, consisting of the alveolus and the alveolar capillary. It has an average thickness of 0.5 μm. The movement of gases through the membrane is regulated by Fick's law, expressed by the formula:

$$\dot{V}_{gas} = D_{gas} \cdot \frac{SA}{d} \cdot (P_1 - P_2)$$

of which V gas is the volume of gas that diffuses in the unit of time; D_{gas} is the gas diffusion coefficient; d is the distance to be traveled, ie the average thickness of the membrane; SA is the area of the exchange surface, P_1 - P_2 is the difference in gas pressure on the sides of the membrane. Therefore the amounts of O_2 and CO_2 transferred in the unit of time, respectively from the alveolus to the blood and from the blood to the alveolus, are influenced by all these variables. However, additional factors not present in the equation affect the V gas, such as the contact time of the blood on the exchange surface and the volume of blood exposed on the same surface. The contact time is about 750 ms in rest conditions, while it drops to 350 ms in conditions of intense physical activity. The maximum time for reaching the equilibrium between the partial pressures on the two sides of the alveolar-capillary membrane is about 300 ms. There is therefore a safety margin that

allows the complete balance between the partial pressures on the two sides of the membrane, before the blood leaves the exchange area. The amount of blood present in the pulmonary capillaries at each instant amounts to about 60-140 ml in resting conditions; in conditions of intense physical exercise it can even reach 1000 ml (Alloatti et al.,2002).

1.4.6. Perfusion

In the pulmonary circulation, at arterial level, the pressure fluctuates between 25 mmHg and 12 mmHg; in the capillary it goes from 12 mmHg to 8 mmHg; while at venous level it reaches 5 mmHg. The pulmonary circulation, compared to the systemic one, is therefore a low pressure and low resistance circulation. Capillary pressure tends to increase with increased $\dot{Q}$, as occurs during physical exercise. To prevent this increase from causing a capillary break, two phenomena occur: the phenomenon of recruitment and the phenomenon of distension. In the first case also the closed capillaries in resting conditions tend to open for a purely mechanical mechanism. While the second phenomenon acts at the level of the cross section of the individual capillaries, which, as the pressure increases, tend to expand. In both mechanisms, the overall cross section increases. If the resistance of the vessels (R) is equal to**:**

$$R = \frac{8 \cdot \eta \cdot L}{\pi \cdot r^4}$$

Increasing the radius (r), the resistances are reduced; consequently the capillary bed offers a much lower resistance compared to a rest situation, avoiding capillary breakage. Moreover,

this system allows the capillary bed to receive not only 5 (l / min) of blood, as it happens in rest conditions, but also 25-30 l / min, as happens during a maximum exercise.

1.4.7. Nervous and humoral control of the respiratory system

Changes in pulmonary and alveolar ventilation determine a change in the composition of respiratory gases in arterial blood. Ventilation control is performed by means of chemical and nervous mechanisms and is aimed at the constant maintenance of the composition of respiratory gases in arterial blood in all physiological situations (Alloatti et al., 2002).

Chemical regulation

The chemicals that regulate alveolar ventilation are the CO_2, O_2 and the hydrogen ions (H^+). A hypoxic situation (decrease in P_{O_2}hypercapnia (increase in P_{CO_2} or a decrease in the pH of arterial blood situation (decrease in P_{O_2}), hypercapnia (increase in P_{CO_2}) or a decrease in the pH of arterial blood, causes a stimulation of $\dot{V}_E$, Conversely, an increase in P_{O_2}, a decrease in P_{CO_2} and an increase in pH are factors that cause a decrease in $\dot{V}_E$. Furthermore, the respiratory system is much more sensitive to an increase in arterial P_{CO_2} compared to a decrease in P_{O_2}: a minimal increase in arterial P_{CO_2} induces an increase in $\dot{V}_E$, which allows its elimination and return to its normal basic values in a short time. The levels of P_{CO_2}, $P_{O_2}$and pH in arterial blood are recognized by specific sensors, the chemoreceptors. The

chemoreceptors are present both at the level of the carotid bifurcation and of the aortic arch (peripheral chemoreceptors) and of the bulb of the brainstem (central chemoreceptors). The former are also sensitive to minimal variations in P_{O_2}, P_{CO_2}, and arterial pH; while the latter are sensitive only to alterations of P_{CO_2} and pH. Peripheral chemoreceptors are innervated by glossopharyngeal and vagus nerves, through which they send input at the centers of the breath. P_{O_2} and arterial P_{CO_2}synergistically determine the ventilatory response, as can be seen from the graphs below (fig.17).

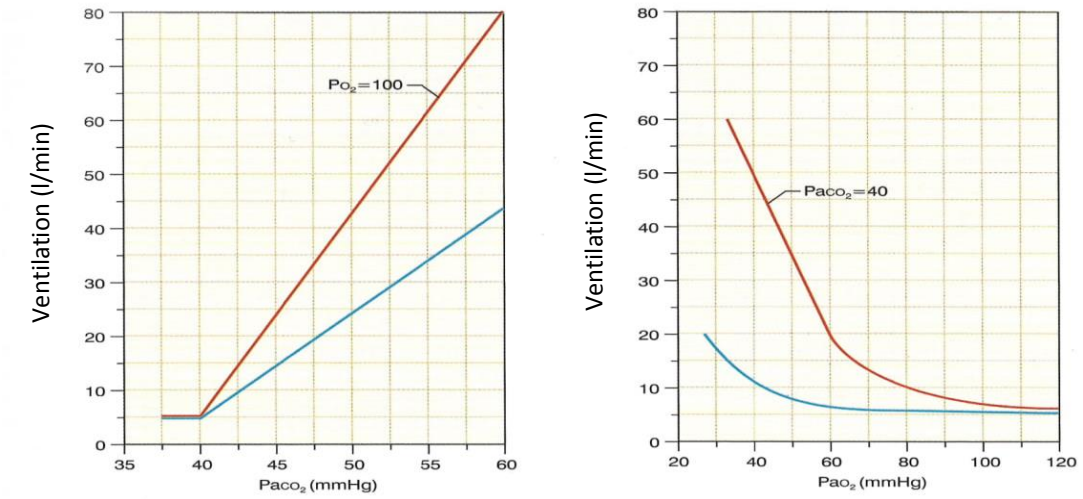

Figure.17 - Ventilatory response (l / min) to changes in PaCO2 (mmHg) and PaO2 (mmHg). The relation between $\dot{V}_E$ and $PaCO_2$ (blue curve in the first graph) has a linear trend and, as the $PaCO_2$ increases, the $\dot{V}_E$ increases. If the PaO_2 is kept constant PaO_2 = 100 mmHg), the relation remains linear, but the line has a greater slope (red curve). In the relation between $\dot{V}_E$ and PaO_2 (second graph), the $\dot{V}_E$ varies imperceptibly as diminuire PaO_2 decreases until it reaches values of 60 mmHg (blue curve); for lower values of PaO_2 we see an increase in $\dot{V}_E$, which corresponds to a proportional decrease in $PaCO_2$. If this is kept constant on the base values (PaCO2) = 40 mmHg), adding to the inspired air discrete quantities of CO_2, a much clearer response (red curve) will already be at those levels of PaO_2 in which there is a minimum ventilatory response. Both graphs demonstrate the presence of synergism at the chemoreceptor level.

The central chemoreceptors are located at the level of the ventral portion of the bulb. The increase in P_{CO_2}, in interstitial and cephalo-rachidian fluid of the brainstem stimulates bulbar chemoreceptors, both directly and indirectly, by increasing the hydrogen ion concentration due to the hydration reaction of CO_2 with formation of acid carbon that dissociates in hydrogenions and bicarbonates (Alloatti et al., 2002).

Nerve regulation

Nerve regulation is allowed due to the presence of inspiratory and expiratory neurons located in the bulb. The activity of these neurons is synchronous with the respiratory rhythm. At the bridge level, structures have been identified that influence ventilation: the pneumotaxic center and the apneustic center (fig.18).

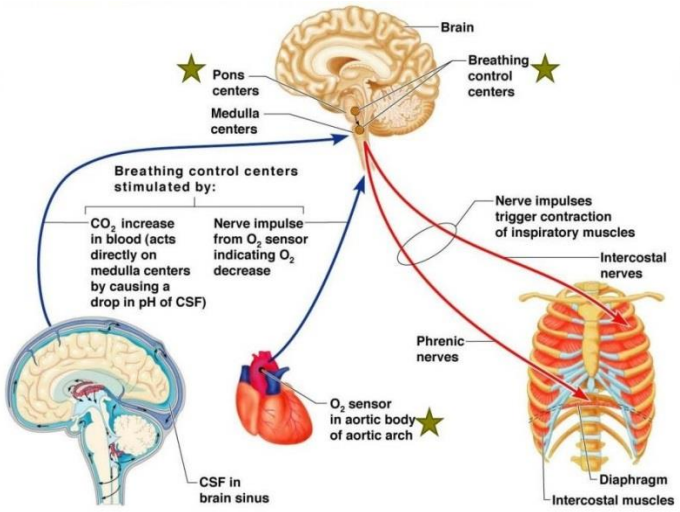

Figure18. Scheme of the neural regulation of respiration

In a normal subject, the section of vague nerves cause a decrease in the ventilatory frequency although the depth of the single beats increases: in normal conditions the vague carries stimuli generated by pulmonary distension (pulmonary tensoreceptors) which reach the apneustic center with effect inhibitory, interrupting the tonic discharge on the inspiratory neurons. The vagal afferents therefore have the purpose of interrupting the inspiratory act facilitating the increase of the ventilatory frequency. In the absence of the function of the pneumotaxic center, the effect of the vague nerves becomes evident, since, as its inhibition on the center of the apnea is lacking, the inspiratory act increases at the expense of the expiratory one (phenomenon of apneusi). The pneumotaxic center, in addition to inhibiting the apneustic center, also has the function of simultaneously stimulating the expiratory neurons, so as to path from inspiratory to the expiratory phase. Inspiratory cells are able to self-excite and send impulses to the diaphragm via the phrenic nerve. When inspiratory neurons are excited, they inhibit expiratory neurons, and vice versa. Therefore, the rhythmic alternation of inhalation and expiration is an intrinsic property of the inspiratory and bulbar expiratory neurons which mutually inhibit each other and whose activity is controlled by other structures. The rhythmic impulses that depart from these neurons discharge on the diaphragm through the phrenic nerve and on the other spinal motor neurons that innervate all the muscles of respiration (Alloati et al., 2002).

1.4.8. Respiratory response to exercise

During the exercise the energy demand increases, which depends mostly on the availability of O_2. To supply the necessary oxygen, the respiratory system must respond

by intervening pulmonary ventilation, external and internal respiration. Lung ventilation increases to improve alveolar ventilation; external respiration is regulated to maintain the relationship between ventilation and perfusion, and internal respiration responds with greater extraction of oxygen by muscles. These changes in breathing not only provide adequate oxygenation for the muscles, but also play an important role in maintaining the acid- base balance, which in turn is closely related to CO_2 levels (Plowman et al., 2013).

Respiratory response during an incremental exercise

The response to an increase in metabolic demand due to physical activity is $\dot{V}_E$, called hyperpnea. For low-moderate intensity, up to about 50-75% of the maximum workload, the $\dot{V}_E$ increases linearly; after which a linearity interruption occurs and a second, faster, linear increment follows. This continuous increase up to around 85-95% of the maximum workload, when a second interruption of the linearity is realized again; the slope of the third rise is even more pronounced. The increase in $\dot{V}_E$ is caused by different factors, such as the excess of CO_2 deriving from the buffering of lactic acid, the presence of catecholamines, an increase in body temperature, some feedback from the proprioceptors of skeletal muscle. At low to moderate workloads, the variation of $\dot{V}_E$ is obtained mainly through an increase in V_T on the contrary, during very high workloads, the depth of breathing can decrease, defining an inverted and truncated U-shape. Therefore, when the V_T reaches the highest value, any further increase in $\dot{V}_E$ can occur only as a result of an

increased respiratory rate, which during an incremental exercise assumes an exponential trend (Plowman et al., 2013).

Respiratory response during a light to moderate intensity exercise at constant load

The increase in $\dot{V}_E$ in the first 2 minutes of the exercise is characterized by a triphasic response. At the beginning of the exercise, there is a first rapid increase in $\dot{V}_E$, defined as phase 1, which is maintained for about 10-20 seconds. Phase 2 is defined by a slower exponential increase, generally completed in 2-3 minutes, which leads to a plateau condition. Phase 3 is represented by the steady state. The stationary level achieved depends on a number of factors, including the workload, the fitness status of the individual and the environmental conditions.

The initial increase in $\dot{V}_E$ occurs mainly due to an increase in V_T. In theory, the V_T could vary from the rest level to the one corresponding to the limits of the vital capacity, in reality; it rarely reaches more than 50-65% of the vital capacity, before a plateau occurs. The contribution of f_R to the increase in $\dot{V}_E$ is minimal and gradual (Plowman et al., 2013).

Respiratory response during a constant load exercise of moderate-high intensity

The $\dot{V}_E$ increases with higher values than those achieved during a sub-maximal exercise at low-moderate intensity, stabilizing following a steady state. The achievement of the steady

state may take longer than the lower work intensities and is often not maintained for the entire duration of the exercise. After about 30 minutes, a gradual increase in $\dot{V}_E$ may occur, although the workload remains constant. As for the constant low-moderate intensity exercise, the initial change of $\dot{V}_E$ is mainly due to an increase in V_T; subsequently, with the continuation of physical activity, the V_T could undergo a modest decrease. Therefore the increase of $\dot{V}_E$ in the final phase of the administration of the load derives mainly from the increase over time of the ventilatory frequency (Plowman et al., 2013).

1.5. Metabolic Response

The metabolic response to exercise is indispensable for muscle contraction. The human body is provided with a sophisticated and complex neurohormonal system designed to guarantee proper fuel supply to working out muscles. Our ancestors' survival was strictly dependent on an efficient energy system capable of producing energy quickly for high intensity physical activity (flight/fight situations) and of maintaining prolonged physical activity required for migrations. These different needs (speed and endurance) account for the diversity of our muscle fibers. Based upon the myoglobin content and time-to-peak tension, muscle fibers may be either twitch type I (red or slow fibers) or twitch type II (white or fast fibers). Type I fibers have high oxidative capacity and are utilized for endurance work, type II fibers have high glycolytic capacity and are recruited for short bursts of rapid, heavy work (De Feo et al., 2003).

1.5.1. ATP :The ultimate source of energy

Independently of the type of fibers, the ultimate source of energy for their contraction is ATP. Actomyosin-ATPase hydrolysis of ATP allows action-myosin cross-bridge formation and release, with consequential muscle contraction. ATP turnover is very high: in a typical cell an ATP molecule is consumed within 1 min. Since intracellular stores are small, ATP must be continually and rapidly regenerated; the maximal peaks of ATP turnover rate are reached with muscle work. For example, a resting human consumes about 1.6 kg of ATP/h; during strenuous exercise the rate of ATP utilization may be as high as 30 kg/h. In order to avoid a fall of ATP availability, muscle fibers utilize 3 energy sources: a) the posphocreatine (PCr) shuttle; b) anaerobic sources; and c) oxidative phosphorylation. The first energetic source is PCr which can readily transfer its phosphoryl group to ATP. This reaction is catalyzed by creatine kinase: PCr+ADP+H+D ATP +Cr. The abundance of PCr and its high phosphoryl transfer potential relative to that of ATP make it a highly effective P anaerobic buffer. PCr shuttle resynthesizes ADP to ATP during the first sec of intense physical exercise; for example, this system can sustain a 100-m top sprinter during the first 30-40 m. The next source of ATP production is anaerobic glycolysis: glucose+2 Pi+2 ADP α2 lactate+2 H_2O+2 ATP. Anaerobic glycolysis is an important ATP source at the onset of intense exercise, especially when elevated plasma catecholamines trigger glycogenolysis. Anaerobic glycolysis is a characteristic metabolic pathway of fast-twitch muscle fibers where it can preserve ATP availability for about the first minute of activity. In the presence of oxygen, obligatory for more protracted muscle contraction, glycolytic reactions occur at a slower rate and play a minor contribution to ATP formation. The third and most efficient

ATP source for skeletal muscle is the oxidative phosphorylation of carbohydrates, fats and proteins. Initially, intracellular glycogen and free fatty acids (FFA) are the primary substrates to go into the oxidative pathway. However, if exercise is prolonged, also the oxidation of circulating glucose turns out to be a significant ATP source. The contribution of branched chain amino acids and of proteins in general to ATP formation during exercise is very limited (less than 2%); only, if glycogen stores are severely depleted it can rise to ~10%. Oxidative reactions take place in the mitochondria, where fuels are completely oxidized to CO_2 via the Krebs cycle and oxidative phosphorylation, in which ATP is generated as electrons flow to oxygen. Adequate fuel supply to working muscle is guaranteed by an integrated hormonal response.

During exercise, the concentration of plasma insulin declines whereas that of the counter-regulatory hormones glucagon, catecholamines, GH and F increases. As a result of the modified hormonal milieu, lypolysis and glycogenolysis rapidly increase, new glucose is synthesized owing to a sustained increase of gluconeogenesis, promoted by counter-regulatory hormones and enhanced supply of precursors (FFA, lactate, glycerol and branched chain amino acids) (De Feo et al., 2003).

1.5.2. Relative contribution of glucose and FFA to ATP production during exercise

The recent availability of stable isotope techniques and of nuclear magnetic resonance spectroscopy, along with the classic studies made with indirect calorimetry and tissue biopsy, has permitted to establish the contribution of glucose and FFA to ATP production in exercising humans. The first conclusion that can be drawn from these studies is that energy flux is determined by relative exercise intensity. At rest, most of energy (about 60%) for non-contracting skeletal muscle derives from lipid oxidation. During moderate intensity exercise lipid and glucose are equally oxidized by working muscles. At greater exercise intensities carbohydrates become the primary energy source whereas FFA flux and oxidation are inversely related to exercise intensity. The rate of FFA utilization declines below the basal values at sub-maximal (>80%) exercise intensities; under these conditions the energy supply of working skeletal muscle becomes strictly dependent on muscle glycogen and blood glucose. The above conclusions, derived from tracer studies, were anticipated by the classic studies performed with indirect calorimetry. Since 1939, Christensen et al. demonstrated that respiratory exchange ratio ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) approximated the unit, i.e. shifted to carbohydrate oxidation, as relative effort increased. More recently, O'Brien et al. estimated carbohydrate oxidation from respiratory gas exchange ratios in men during slow and fast treadmill marathon runs. Before starting, carbohydrates contributed to whole oxidation by about 50%; after 30 min the contribution of carbohydrate oxidation peaked to about 80% in the slower run and to almost 95% in the faster run.

During the last part of both marathon runs, carbohydrate oxidation progressively declined to be replaced by a rising contribution of fat oxidation to total fuel oxidation. The shift from carbohydrate to lipid oxidation usually occurs after 100 min of a marathon race and it is caused by the depletion of muscle glycogen. It has been described as "hitting the wall" because it is associated with a reduction of the running speed and the feeling of fatigue (De Feo et al., 2003).

Endurance training improves racing performance through several additive mechanisms. $\dot{V}_{O_2\,max}$ during exercise is augmented by enhanced cardiopulmonary capability, increased muscle capillary density and by reduced blood viscosity. For instance, $\dot{V}_{O_2\,max}$ of a 40-yr-old untrained healthy man is normally ~40 ml/kg/min, whereas age-matched endurance athletes can reach values of 80 ml/kg/min. Furthermore, endurance training has important positive effects on the oxidative capacity of skeletal muscle through the increase of the cellular content of mitochondria, pyruvate dehydrogenase and hexokinase.

The increase of mitochondrial mass alters the pattern of energy substrate utilization permitting a given respiratory rate to be accomplished at higher ATP/ADP ratio. At a given power output (PO), the higher ATP/ADP ratio decreases the rate of glycogenolysis, glycolysis and lactate accumulation favoring lipid respect to glucose oxidation. An elegant study by Friedlander et al. has shown that a 10-week training program is sufficient to reduce by ~25% oxidative glucose disposal rate at 65% of $\dot{V}_{O_2\,max}$. Thus, genetically

advantaged subjects, i.e. those with red fibers rich of mitochondria, and endurance trained athletes are capable to better oxidize fat and, consequently, to spare glycogen for the final fraction of the race. Other main effects of endurance training on carbohydrate metabolism regard increased insulin-sensitive and exercise-sensitive blood glucose transport to skeletal muscle. Insulin-mediated glucose transport is enhanced by exercise mainly through better translocation on muscle cells of glucose transporters GLUT 4, promoted by adenosine monophosphate protein kinase activation. Insulin-independent glucose transport is enhanced by endurance training probably by augmented endothelial and muscle production of nitric oxide in working muscles. The effects of training on insulin sensitivity are beneficial also during recovery from exercise because of better rates of muscle and liver glycogen resynthesis (De Feo et al., 2003).

1.5.3. Metabolic response to exercise

$\dot{V}_{O_2}$ is the amount of oxygen absorbed, transported and used at the cellular level; it is equal to the difference between the amount of inspired oxygen and the amount of exhaled oxygen. Similarly, the $\dot{V}_{CO_2}$ is the amount of carbon dioxide generated during the metabolism, mainly by cellular respiration; it coincides with the difference between the amount of exhaled carbon dioxide and the amount of inspired carbon dioxide (Plowman, Smith, 2013).

The quantity of a gas is given by the product of the volume of air and the percentage of gas; therefore it can be said that:

$$\dot{V}_{O_2} = \left(\dot{V}_I \cdot \%FIO_2\right) - \left(\dot{V}_E \cdot \%FEO_2\right)$$

$$\dot{V}_{CO_2} = \left(\dot{V}_E \cdot \%FECO_2\right) - \left(\dot{V}_I \cdot \%FICO_2\right)$$

Metabolic response during an incremental exercise

As the workload in this type of exercise continues to increase, the values of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ also undergo a continuous increase, which reflects the increase in energy required. Therefore, $\dot{V}_{O_2}$ has a rectilinear course up to the maximum intensity of the load, which is followed by a flattening of the curve (Plowman et al., 2013). In particular, during the first phase of operation, the $\dot{V}_{O_2}$ increases rapidly, proportionally to the intensity of the exercise; instead, depends on the characteristics of the incremental test, in the second part of the activity, when the intensity is higher, the $\dot{V}_{O_2}$ decreases its rate of increase, until it reaches a condition in which there is no longer any increase. The point at which the $\dot{V}_{O_2}$ reaches the plateau is defined as $\dot{V}_{O_2\,max}$(McArdle et al., 2018). The difference between the latter and the basal $\dot{V}_{O_2}$ is defined as reserve oxygen consumption. However, not all subjects are able to reach this plateau during an incremental test.

Metabolic response during a light to moderate intensity exercise at constant load

At the beginning of a constant load exercise, the speed of use of the ATP instantly increases to a level as high as the work is more intense; subsequently it remains unchanged, only to fall abruptly to the levels of rest in the instant in which the work ceases. $\dot{V}_{O_2}$, on the other hand, follows the ATP utilization rate with a certain delay: it increases until it reaches the steady state in 2-4 minutes. Consequently, in the first minutes $\dot{V}_{O_2}$ is insufficient to the resynthesis of all the ATP needed for muscle activity. However, the speed of ATP resynthesize must equal that of its utilization, since the muscle does not tolerate a decrease in its concentration. Therefore, at the beginning of the work, a part of the necessary ATP is resynthesized from different sources of $\dot{V}_{O_2}$ (Alloatti et al., 2002). "Oxygen debt" is defined as the difference between total oxygen consumption during activity and that would have been used if the steady state of oxygen had been reached from the beginning (McArdle et al., 2018). The oxygen debt is therefore a measure of the amount of energy that, during an aerobic exercise, before reaching the steady state, is "borrowed" from other energy sources than $\dot{V}_{O_2}$. At the end of the work, while the use of ATP by the muscles is reduced to rest values almost instantaneously, the $\dot{V}_{O_2}$ returns to the rest values with a trend similar to that observed at the beginning. In this phase, the non-oxidative energy sources used in the first minutes of work are reconstituted at the expense of O_2, consumed in excess of the rest value (Alloatti et al., 2002).

Metabolic response during a moderate-high intensity exercise at constant load

In constant load exercise, the curve of $\dot{V}_{O_2}$ has an exponential trend: it tends to rise rapidly at the beginning to then progressively slow down as the $\dot{V}_{O_2}$ approaches the equilibrium value with the load; from that moment the $\dot{V}_{O_2}$ should have a steady state. However, this happens only for workloads below the LT; above this intensity and below the $\dot{V}_{O_2\,max}$ (about 90-95% of $\dot{V}_{O_2\,max}$), the $\dot{V}_{O_2}$ after the rapid ascent phase, tends to gradually increase, which is called "slow component" of $\dot{V}_{O_2}$. It is usually expressed as the difference in $\dot{V}_{O_2}$ in between the end and the third minute of exercise, the point at which the plateau phase should begin (Dal Monte et al., 1999). There are various hypotheses regarding the origin of the slow component; among them the one concerning the accumulation of lactic acid takes in particular importance (Gaesser et al., 1996).

1.6. Rate of Perceived Exertion (RPE) Scale

The study of human performance and perceived exertion during physical activity has been an area of considerable scientific interest and research over the last 50 years. The symptom of exertion is unique to an individual and can be used as a subjective estimate of the work intensity undertaken across a variety of populations. The intensity of work is important because of the risks of musculoskeletal injuries and disorders arising from a mismatch between the worker's capability and the physical demands of their job (Williams, 2017).

The RPE scale, developed by Swedish researcher (Gunnar Borg, 1970), is a tool for measuring an individual's effort and exertion, breathlessness and fatigue during physical work and so is highly relevant for occupational health and safety practice. In its simplest terms, it provides a measure of how hard it feels that the body is working based on the physical sensations that the subject experiences, including increased heart rate, increased respiration or breathing rate, increased sweating and muscle fatigue. The scale is a very simple numerical list. Participants are asked to rate their exertion on the scale during the activity, combining all sensations and feelings of physical stress and fatigue. They are told to disregard any one factor such as leg pain or shortness of breath but to try to focus on the whole feeling of exertion. This number gives an indication of the intensity of activity allowing the participant to speed up or slow down movements. The scale takes seconds to complete and can be researcher or self-administered and used on a single occasion or multiple times. '9' corresponds to 'very light' exercise which, for a healthy person, is equivalent to walking slowly at his or her own pace for several minutes. '13' feels 'somewhat hard' but the individual still feels able to continue. '17' is 'very hard' (Williams, 2017).

A healthy person can continue but must push themselves beyond their feeling of being very fatigued. '19' is extremely strenuous exercise for most people, the hardest they have ever experienced. The unusual scaling, ranging not from '0' to '20' but from '6' to '20' is related to the high correlation between the scale and f_H. Thus, a Borg RPE scale of 6 corresponds to a heart rate of 60 beats/min in a healthy adult, 8–80 beats/min and so on

although for individuals on beta blocker therapy (e.g. for hypertension) studies have suggested that the therapy increases the RPE due to altered metabolism in the muscles. This increase in intensity occurs at all work rates. Borg also developed the Borg CR10, a Category-Ratio (CR) scale anchored at number 10, representing an extreme intensity of activity. It is a general intensity scale with special anchors to measure exertion and pain. The individual is asked to circle or tick the number that best describes breathlessness, on average, over the last 24 h (Williams, 2017).

1.6.1. Prediction of Maximal Exercise Capacity and CP using the RPE

The ability to estimate maximal functional capacity with acceptable accuracy from submaximal exercise testing is advantageous for monitoring training status. It has been known for some time that the RPE elicited from submaximal increments in a graded exercise test (GXT) can be used to provide estimations of $\dot{V}_{O_2\,max}$ that are as good as, or better than, heart rate. Morgan and Borg observed that the linear relationship of RPE and work rate during a GXT in physically active and sedentary men permits extrapolation to a theoretical end point, enabling the prediction of maximal work capacity with better accuracy than heart rate. Studies have confirmed the efficacy of submaximal RPE to estimate $\dot{V}_{O_2 max}$ or maximal work rate from standard GXT in healthy active and sedentary groups, club runners, and able-bodied and paraplegic athletes; from ramped protocols in sedentary and athletic subjects; and from randomized workloads in competitive cyclists. The RPE from the 20-m shuttle-run test may also be used to predict $\dot{V}_{O_2\,max}$. Other evidence

also suggested that the RPE can be used to estimate a 1-repetition maximum in adults and children, predict maximal performance of intermittent vertical-jump exercise, and describe the physiological demands of such exercise. A further innovative application of the RPE is in the calculation of CP, which has been used extensively to assess athletes' endurance capacity. However, the necessity for subjects to perform a number of exhaustive efforts on separate days has precluded its routine use. Nakamura et al. (2008) validated a "perceived exertion threshold" procedure by which the rate of increase in the RPE (as assessed by RPEs of 14–17) across exhaustive efforts was regressed against PO. This method of estimating critical power was shown to be remarkably accurate and reliable and to avoid causing exhaustion (Eston, 2012).

1.7. Fatigue

Fatigue represents the decline in muscle tension or force capacity with repeated stimulation or during a given time period. This definition also encompasses perceptual alterations of increased difficulty to achieve a desired submaximal or maximal exercise outcome. Fatigue occurs from interrupting the chain of events between the central nervous system and muscle fiber, regardless of the reason. Four examples include:

- Exercise-induced alterations in levels of central nervous system neurotransmitters serotonin, 5 hydroxytryptamine, dopamine, and Acetylcholine in various brain

regions, along with the neuromodulators ammonia and cytokines secreted by immune cells alter one's psychic or perceptual state to disrupt ability to exercise.

- Reduced glycogen content of the active muscle fibers relates to fatigue during prolonged intense exercise. This "nutrient fatigue" occurs even with sufficient oxygen available to generate energy through aerobic pathways. Depletion of phosphocreatine (PCr) and a decline in total adenine nucleotide pool (ATP +ADP + AMP) also accompanies the fatigue state in prolonged submaximal exercise (McArdel et al., 2009).
- Oxygen lack and increased level of blood and muscle lactate relate to muscle fatigue in short-term, maximal exercise. The dramatic increase in H^+ in the active muscle dramatically disrupts the intracellular environment. Alterations in contractile function in anaerobic exercise also relate to five factors: (1) PCr depletion, (2) changes in myosin ATPase, (3) impaired glycolytic energy transfer capacity from reduced activity of the key enzymes phosphorylase and phosphofructokinase, (4) disturbance in the T-tubule system for transmitting the impulse throughout the cell, (5) and ionic imbalances. Downregulation in muscle Na^+, K^+, and Ca^{2+} release, distribution, and uptake alters the myofilament activity and impairs muscular performance, even though nerve impulses continue to bombard the muscle fiber.

- Fatigue occurs at the neuromuscular junction when an action potential fails to cross from the motor neuron to the muscle fiber (McArdel et al., 2009).

1.7.1. Cardiovascular response to fatigue

Regarding to the fatigue, incremental aerobic exercise is characterized by a large increase in the f_H, which contributes to an increased cardiac output. The treadmill exercise response also shows a modest increase in the systolic blood pressure (SBP) and a relatively stable or decreasing diastolic blood pressure (DBP). Aerobic exercise is said to impose a "volume load" on the heart. Increased venous return leads to increased SV, which contributes to an increased cardiac output. In contrast, fatiguing static exercise is characterized by a modest increase in the f_H, but a dramatic increase in the BP (pressor response). Mean BP increases as a result of increased SBP and DBP. Static exercise is said to impose a "pressure load" on the heart. Increased MAP means that the heart must pump harder to overcome the pressure in the aorta (Plowman et al., 2013).

1.7.2. Respiratory response to fatigue

One exception involves exercise-induced diaphragm fatigue. During both short and long-duration incremental or constant load exercise ≤ 80% $\dot{V}_{O_2\,max}$, the diaphragm does not fatigue. However, at more than 80% $\dot{V}_{O_2\,max}$ intensity continued to exhaustion, the diaphragm does fatigue. The consequence of this fatigue is a decrease in exercise tolerance. Because the diaphragm is a muscle, it is logical to attempt to use specific training to increase its resistance to fatigue. One study (Enright et al., 2006) documented increased diaphragm thickness with increased performance and PO. A second study (Verges et al., 2007) achieved increased fatigue resistance of respiratory muscles after respiratory muscle

training; however, cycling endurance did not change. During strenuous exercise, healthy individuals over breathe at higher levels of oxygen consumption. The hyperventilation response generally decreases alveolar P_{CO2} and slightly increases alveolar P_{O2}. Exercise conditions that trigger hyper ventilation induced reductions in arterial carbon dioxide restrict cerebral blood flow, which may compromise oxygen delivery to active brain areas and contribute to central fatigue (Plowman et al., 2013).

1.7.3. Metabolic response to fatigue

Metabolic fatigue results from a reduced production of ATP linked to enzyme changes, changes in membrane transport mechanisms, and changes in substrate availability. Enzymes in particular, the rate-limiting enzymes in the metabolic pathways can be inactivated by high hydrogen ion concentrations (low pH). The hydrogen ion attaches to these enzyme molecules and in so doing changes their size and shape and thus their ability to function. Phosphofructokinase is thought to be particularly sensitive, although oxidative enzymes can also be affected. At the same time, changes occur in membrane transport mechanisms (either to the carriers in the membrane or to the permeability channels). These changes affect the movement of molecules across the cell membrane and between the cytoplasm and organelles such as the mitochondria. Energy substrate availability can be inhibited by a high concentration of hydrogen ions. Glycogen breakdown is slowed by the inactivation of the enzyme glycogen phosphorylase. Fatty acid utilization is decreased because lactic acid inhibits mobilization. Thus, a double-jeopardy situation occurs. With

fatty acid availability being low, a greater reliance is placed on carbohydrate sources at the time when glycogen breakdown is inhibited. At the same time, phosphocreatine breakdown is accelerated, leading to a faster depletion of substrate for ATP regeneration. Thus, both the inactivation of enzymes and the decrement in substrate availability will lead to a reduction in the production of ATP and, ultimately, a decrement in performance (Plowman et al., 2013).

1.8. Slow component of $\dot{V}_{O_2}$ kinetics

What has so far eluded definitive mechanistic explanation is the slow component of the $\dot{V}_{O_2}$ response which obtains for all supra-lactate threshold work rates in the heavy and severe exercise domains. Despite the magnitude of the slow component (up to 1.5L O_2/min), and its association with the ventilatory and acid-base responses as well as the fatigue process, established texts have largely chosen to ignore this feature of the exercise response. Indeed, acknowledgement of the presence of the slow component challenges our understanding of muscle energetics and exposes serious flaws in the O_2 deficit concept (Poole et al., 1997).

1.8.1. Mechanistic bases of $\dot{V}_{O_2}$ slow component

There is an extensive list of factors which have been considered as putative mediators of the $\dot{V}_{O_2}$ slow component. These include:

- body and/or muscle temperature
- cardiac and ventilatory muscle work
- auxiliary muscle work
- metabolic stimulation associated with exercise-induced alterations in potassium, hormonal (e.g. catecholamines) or metabolic (lactate) profiles
- muscle pH
- shifts in the blood O_2 dissociation curve resulting from changes in muscle temperature
- blood gas and acid-base changes
- dietary intake
- muscle fiber energetics or recruitment profile.

Since the demonstration that the predominant portion of the slow component arises from within the exercising muscles (Poole et al. 1991), this reduces substantially the list of candidate mediators given above. Of those remaining, most can be rejected on the basis of experimental findings. Specifically:

- Elevated lactate stimulates metabolism in tissue with gluconeogenic capabilities (liver and kidney), and skeletal muscle does have the requisite gluconeogenic enzymes. However, elevating blood lactate under iso-pH conditions does not increase muscle $\dot{V}_{O_2}$ in the electrically-stimulated dog gastrocnemius.

- Catecholamine infusion stimulates $\dot{V}_{O_2}$ in humans at rest, but evokes no increase of $\dot{V}_{O_2}$ during heavy exercise.

Whereas elevated muscle temperature uncouples mitochondrial function and lowers the yield of ATP per unit of O_2 utilised, in the intact organism, altered body and presumably muscle temperature does not always change pulmonary $\dot{V}_{O_2}$ during exercise. Furthermore, it is possible to stabilise leg $\dot{V}_{O_2}$ in the face of rising muscle temperature.

- A shift to the right in the O_2 dissociation curve due to increased temperature and P_{CO_2} and reduced pH will certainly aid O_2 offloading. However, we contend that this cannot represent a primary mechanism for the elevation of $\dot{V}_{O_2}$ in and of itself, because it can only explain the increase of $\dot{V}_{O_2}$ towards the predicted level. It is crucial to appreciate that the $\dot{V}_{O_2}$ slow component represents an increase of $\dot{V}_{O_2}$ above that predicted on the basis of the sublactate threshold $\dot{V}_{O_2}$-work rate relation or work efficiency.

- Elevated extracellular potassium substantially elevates the metabolic rate of isolated xenopus (frog) muscle but venous plasma potassium levels demonstrate a profile markedly different to that of muscle $\dot{V}_{O_2}$ during severe intensity exercise.

- Decreased muscle pH may compromise muscle contractile function. The impact of altered exercise muscle $[H^+]$ in the absence of other potentially confounding metabolic events remains to be established.

In contrast to those factors considered above, there is compelling evidence that some features of the motor unit recruitment pattern may be important in the aetiology of the $\dot{V}_{O_2}$ slow component. For instance: (i) fast twitch motor units are energetically less efficient than slow twitch motor units, and progressive increases in exercise intensity above the lactate threshold would be expected to recruit a larger population of these fibers; (ii) integrated quadriceps electromyogram recordings indicate a correlation between increased fiber activation and the slow component; (iii) Coyle et al. (1992) reported that cyclists with a greater proportion of fast twitch fibers had a higher $\dot{V}_{O_2}$ and reduced efficiency during heavy intensity constant load exercise; and (iv) Gaesser et al. (1996) demonstrated that the $\dot{V}_{O_2}$ slow component was significantly greater in individuals cycling at a cadence of 100 vs 50 rpm, in keeping with the concept that higher cadences mandate recruitment of more fast twitch fibers. The tight association between the temporal profile of blood lactate and $\dot{V}_{O_2}$ slow

component may be the consequence of an increasing recruitment of fast twitch highly glycolytic fibers. In addition to the motor unit recruitment pattern, there is some evidence that substrate availability and utilization also may affect pulmonary $\dot{V}_{O_2}$ to a greater extent than that calculated simply from the calorigenic yield of fat vs carbohydrate. Specifically, ingestion of glucose polymer increased pulmonary $\dot{V}_{O_2}$ by 300ml during 10 minutes of severe intensity exercise. Also, there was preliminary evidence which indicated that manipulation of testosterone affected exercise $\dot{V}_{O_2}$ profoundly (Poole et al., 1997).

1.9. Sinusoidal loads

Alongside the traditional tests for cardiorespiratory functional evaluation, a new test protocol was proposed in the 1970s based on the intensity of the workload that varies according to a sine wave model (Wigertz, 1970). This protocol has the advantage of producing a continuously fluctuating workload, in which there is an alternation between periodic phases of increasing and decreasing work, which reflects the variable effort (and the consequent cardiovascular and respiratory responses) to which it can be subjected an athlete during a long-term physical activity or a sedentary subject during a daily life activity.

The sine wave function that represents the workload can be described using the formula:

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

Where AMP is the amplitude, T the period, t the time variable, φ the phase and MP the midpoint around which the function oscillates. The physiological cardiorespiratory responses can therefore be estimated from the phase shift, understood as a time delay between the physiological response and the mechanical stimulus (which depends on the speed of the metabolic processes to adapt to the load), and on the amplitude, understood as the reactivity of the cardiorespiratory variables in response to sinusoidal exercise (Casaburi et al., 1977; Bakker et al., 1980; Haouzi et al., 1992, Fukuoka et al., 2002).

Although this protocol has not been the subject of numerous studies, several authors investigated how the physiological responses to a sinusoidal load changed with the variation of its period. Wigertz observed that, varying the periods of the sinusoidal load from 0.75 to 15 minutes, amplitude of the parameters and f_H decreased with decreasing period (Wigertz, 1970). The same results were reported in the Miyamoto study (Miyamoto et al., 1983), which also found an increase in the phase shift between workload and cardiorespiratory responses as the period decreased. It was noted that the dynamic response of cardiac output was strongly influenced by the pattern of f_H, while it is not significantly conditioned by SV, which did not change appreciably with the applied sinusoidal load.

Finally Miyamoto also reported, as emerges also from other studies (Casaburi et al., 1977; Bakker et al., 1980; Fukuoka et al., 1991), that the time constants of $\dot{V}_E$ and $\dot{V}_{CO_2}$ were strongly correlated, reaching, in agreement with the previous authors, that hyperpnea during the non-stationary state could be explained by the cardio-dynamic hypothesis (Potts et al., 1992; Wasserman et al., 1977). In a recent study (Nicolò et al., 2018) the close association between $\dot{V}_E$ and $\dot{V}_{CO_2}$ results to be mediated by the V_T, which was continuously adjusted according to the levels of the respiratory frequency.

Fukuoka (Fukuoka et al., 1990; Fukuoka et al. 1997) found that, for periods of medium to long sinusoids (4-16 minutes), T_D between the load and the response of the physiological parameters was longer at the level of crest and shorter at the slope-down MP curves, this distortion was the result of sluggish increase and steeper descent of the response. It therefore, described the dynamics of respiratory parameters with a trend that was not sinusoidal. However, it was reported that this feature was not evident with short periods (1-2 minutes). The same author, in another study described how the curve response of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ had a period equal to that of the trend. However, it was reported that the response of the respiratory quotient $\dot{V}_{O_2} / \dot{V}_{CO_2}$ was not represented sinusoidally. This distortion seemed to be determined by the phase delay between the response of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, and from the ratio between the AMP of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. In further studies, it was investigated how the participant's cardiorespiratory response changed, modified other protocol parameters, such as pedal frequency and work rate. (Casaburi 1978 and Haouzi 1993). Casaburi (1978),

keeping the load constant and sinusoidally varying the pedaling frequency from 40 rpm to 80 rpm, reported that the fluctuations of $\dot{V}_E$ were strictly in phase with those of $\dot{V}_{CO_2}$ and their AMPs were highly correlated. The variation of the pedaling frequency, therefore, produced a respiratory response dependent on the effect of $\dot{V}_{CO_2}$; therefore the influence of the limbs used in the effort did not have an appreciable role in the response of the ventilation. This provided further evidence that exercise hyperpnea was related to the flow of CO_2 in the circulation and not from afferent nerve impulses from active muscles (Wigertz, 1970; Casaburi et al., 1977; Bakker et al., 1980; Fukuoka et al., 1991).

On the contrary Wells (2007) observed in one of his studies that $\dot{V}_E$ responded faster and to a greater extend when the speed of treadmill varied rather than its inclination; therefore concluded that the frequency of limb movements was a significant factor in hyperpnea. Haouzi (1993) in his study gave the subjects two protocols, respectively with workload above and below the lactic acid threshold. The results showed a significant difference in responses depending on the intensity of the load. Both in the kinetics of $\dot{V}_{O_2}$and in that of f_H, the AMP of the sinusoid decreased and the phase delay increased significantly during the above threshold test compared to the one below the threshold. Moreover, in both protocols, as the number of sinusoids continued, no significant changes in AMP, phase and MP found in the response of $\dot{V}_{O_2}$; on the contrary, with regard to the kinetics of f_H the decrease in AMP and the increase in phase and MP, delay were significant in the above

threshold test. As a consequence, the fluctuation of parameter $\dot{V}_{O_2}/ f_H$ was reduced. In another study, the difference in the response between walking and pedaling was evaluated, with equivalent metabolic demand in the two types of exercise (Fukuoka et al., 2017). In addition to clearly showing different dynamics depending on the periods of oscillation of the load (with physiological parameters during movement that follow the trend more rapidly), the response of $\dot{V}_E$ was correlated to $\dot{V}_{CO_2}$.

However, in movement, when the periods of sinusoid of the load were shortened, the AMP of the $\dot{V}_E$ decreased sharply, while the phase shift increased, with respect to the parameter $\dot{V}_{CO_2}$. Other authors, however, had investigated how the dynamics of cardiorespiratory responses to a sinusoidal load was influenced by the age of the participants. Haouzi (Haouzi et al., 1992) studied the ventilatory response during sinusoidal exercise in children of prepural age (10-13yrs) and in adults (22-37 yrs) with not significantly different state level of form, showing that the kinetics ventilatory was faster in children than in adults. Fukuba et al. (1999), by contrast, investigating the response of f_H in children (10-13yrs) and adults (22-37 yrs.), concluded that the control of f_H during sinusoidal exercise was not significantly different between children and adults, with the exception of small difference in the response delay in the case of sinusoid with short period.

Also in a recent study (Ebine et al., 2018) the kinetics of cardiorespiratory variables was investigated in young subjects (21 ± 2 years) and elderly (67 ± 5 years). The results showed

that the AMP and phase shift in the responses of $\dot{V}_E$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were similar in both subject types; they were also independent of sinusoidal oscillation periods. In contrast, the f_H response slowed significantly in the elderly compared to young people.

In the same way Cunningham et al. (1993), comparing in his study young (22-28 years) and elderly women (62-73 years), It was noted that the kinetics of cardiorespiratory responses slowed down after reducing the width of sinewave in elderly women. This was probably due to multiple factors, such as the age and fitness level of the participants. In fact, older women who had a higher level of physical activity had less slow response.

The influence of physical activity and age on the kinetics of the responses was studied by Fukuoka et al. (2002), who reported that the average T_D of the f_H response did not present a significant difference between young (20-39 years) and elderly subjects (50-69 years), but was more closely related to the subjects' level of physical activity. The same author, in another study (Fukuoka et al., 1995), in which it was tested a sinusoidal load on long-distance runners, football players and untrained men, reported greater AMP and a significant minor T_D in the response of f_H to runners. It also reported that overall there was a significant correlation between the AMP of the f_H kinetics and $\dot{V}_{O_2max}$ of the subjects, as well as between the T_D and the same $\dot{V}_{O_2max}$.

In the study by Tiedt (1975) it emerged that the response of f_H was faster in trained subjects than in untrained subjects. The author was one of the first to point out that the individual time constant decreased as the subject's training level increased, thus arguing that its

analysis turns out to be a suitable criterion for estimating the physical performance of individuals during the exercise. From the application point of view, the sinusoidal load test was used by Fukuoka et al. (1997) to evaluate the effects of a period of football training. The parameters investigated were $\dot{V}_{O_2 max}$, ventilatory threshold and the kinetics of cardiorespiratory parameters (Fukuoka et al., 1997). During the training period, there was a significant increase in $\dot{V}_{O_2\,max}$ and the ventilatory threshold; while in the sinusoidal exercise the AMP of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, $\dot{V}_E$, and f_H remained unchanged. Moreover the phase variations of the trend of $\dot{V}_{O_2}$ and of f_H did not change significantly, contrary to those of $\dot{V}_{CO_2}$ and $\dot{V}_E$, which continued to increase significantly with the progress of training. These results suggested that football training did not significantly effect on the development of $\dot{V}_{O_2}$ and f_H kinetics during submaximal exercise, but appreciably increased $\dot{V}_{O_2\,max}$ and ventilatory threshold values.

Finally, in the clinical setting, a study on high-intensity sinusoidal exercise was conducted for patients with chronic obstructive pulmonary disease (COPD) (Porszasz et al., 2013). In this study it was shown that a fast fluctuation of the sinusoidal work rate (60 s), with excursions up to 120% of maximum aerobic power (MAP) did not induce limitations in patients with severe COPD. In fact, during the high-frequency sinusoidal exercise there was a slight and prolonged fluctuation of the ventilatory response. This allowed sustaining a high intensity exercise for a prolonged time, an element that seems to be important for the effectiveness of achieving the physiological effects in these patients.

From the studies carried out it emerges that different parameters (period, pedaling frequency, type of activity) and factors (age, physical level) have been taken into consideration to evaluate cardio-respiratory responses to a sinusoidal load. Nevertheless only two studies have investigated the differences in the responses going to modify the intensity of the exercise (Haouzi, 1993; Nicolò et al., 2018). Moreover in the literature no data are still evident in which sinusoidal loads with different amplitudes have been compared. Finally, many studies have characterized physiological responses through a single sinusoidal pattern; however this method does not allow verifying if there are variations between the various cycles of the sinusoid with the progress of the exercise.

From the studies carried out, it emerged that the different parameters characterizing sinusoidal protocols (T, MP, and AMP) might influence the response of the heart-lung-muscle integrated system. Noticeably, to reduce the measure variance of the physiological variables, the approach traditionally applied to analyze the signal uses to average or overlap the cycles. However this method might present a limitation as it is not able to detect any drift in the signal. Indeed, in case of long-lasting protocols, rather than exhausting exercises, the physiological variables may show signs of fatigue that, with the traditional approach would be masked by the overlapping procedure.

2. AIMS

Considering that from the studies carried out, it emerged that the different parameters characterizing sinusoidal protocols (T, MP, and AMP) might influence the response of the heart-lung-muscle integrated system. No study has ever explored the cardiorespiratory and metabolic response to two sine wave protocols of severe intensity, differing in midpoint and amplitude and that the results reported by previous studies were obtained by overlapping/averaging the cycles, with the risk of masking possible signs of fatigue.

Therefore, the aims of this study were to compare the cardiorespiratory and metabolic response between two different exhausting sinusoidal protocols differing in MPs and AMPs, both of severe intensity. Additionally, a comparison between the traditional approach analysis and a newly proposed cycle-by-cycle evaluation to detect the possible impact of fatigue on the kinetics of cardiorespiratory and metabolic parameters during work rates performed.

3. MATERIAL AND METHODS

Table 1 shows the characteristics of the participants. Maximum values obtained during ramp test are reported along with LT. Twenty male participants adhered to the study but only ten participants could complete the protocol. With the study progression, six participants spontaneously dropped out to the study before completion and two subjects were excluded because of the presence of extra systoles during the sinusoidal effort. Moreover, after reanalyzing the data the other two participants were excluded because they did not reach the exhaustion. Therefore, only ten participants completed the entire protocols.

All participants were clinically healthy and free of any medical conditions and treatments that could interfere with metabolic and cardiorespiratory responses to exercise.

Being familiar with cycling ii) having a good level of fitness status; and iii) practicing endurance activities at least three times a week, were inclusions criteria. After having been informed about the procedure and the purpose of the study, participants gave their written informed consent. The ethics committee of the local university approved the study, which was performed in accordance with the principles of the 1964 Declaration of Helsinki.

	Mean ± standard error (SE)
n	10
Age (yr)	25.7±1.5
Body mass (kg)	75±2.7
Stature (m)	1.80±0.02
BMI(kg/m^2)	22.1±0.6
$\dot{V}_{O_2 max}$ ($ml \cdot min^{-1}$)	3905±182
$\dot{V}_{E\ Peak}$($l \cdot min^{-1}$)	146±6.8
$f_{H Peak}$ (bpm)	180±2.6
MAP (W)	270 ± 1
CP (W)	216 ± 10
LT (mM)	3.7 ± 03

Table1. Mean values of the demographic characteristics of the participants. Mean ± standard error.

3.1. Experimental design

All participants visited the laboratory on eight separate days. During the first visit, each subject was evaluated from an anthropometric point of view; they were also allowed to familiarize themselves with the test protocol and the cycle ergometer. In the second session, they were subjected to an incremental test on a cycle ergometer to assess their $\dot{V}_{O_{2max}}$ and the MAP. Subsequently, four exhaustion tests at different percentages of MAP (from 90 to 110%) were randomly administered to determine CP. Finally, once the CP had been identified, two sinusoidal load tests were randomly performed differing in midpoints and amplitude. At least 48 hours of recovery were required between each test. At the end of the tests, data analysis was carried out to assess the responses of the cardio-respiratory parameters to the imposed sinusoidal mechanical load.

3.2. Experimental procedures

Each test, was performed on an electromechanically braked cycle ergometer (839E, Monark, Vansbro, Sweden), and was conducted in a room with constant temperature (20 ± 1 ° C) and relative humidity (50 ± 5%). Regarding the use of the cycle ergometer, the height of the saddle, the saddle-handlebar distance and the connection of the pedals have been adapted to each participant and have been kept constant within each test to guarantee a greater repeatability condition. Participants were allowed to self-select their cadence within the range of 85-100 and were instructed to maintain this cadence throughout the protocol and for all subsequent visits. Exercise intolerance was defined as the moment at which

participants could not maintain their self-selected cadence for greater than 10 s despite strong verbal encouragement.

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3.2.1. Incremental step test

$\dot{V}_{O_2\,max}$ and MAP were assessed by a continuous step test. After 3 minutes of rest, for baseline measurements, the test began with a 4-minute warm-up phase at 100W. Afterwards, the load increased by 25W every 2 minutes until exhaustion (Riboli et al. 2017) (Figure 19). After eliminating spurious data, the average of the values recorded in the last 30 s of each load was calculated for each variable. $\dot{V}_{O_2\,max}$ was determined by the plateau attained by the relationship between $\dot{V}_{O_2}$ and the PO. In case a plateau was not evident, the highest $\dot{V}_{O_2}$ value was retained as $\dot{V}_{O_2\,max}$ whenever the participant was unable to complete the two minutes at a given work or when one of the following conditions was met: (i) a lack of increase in heart rate (f_H) between successive workloads and ii) a respiratory exchange ratio value higher than 1.1 (Adami et al., 2013). MAP was defined as the lowest PO able to elicit $\dot{V}_{O_{2max}}$ and was determined by the work rate at which the $\dot{V}_{O_2}$/PO regression line intercepted the $\dot{V}_{O_2\,max}$value (Ferretti, 2014). A 20 µl blood sample was collected from the ear lobe in basal condition, at the end of the warm-up and every workload step and analyzed to determine blood lactate concentration $[La^-]_b$ by an enzymatic-amperometric device (BST Labtrend, Berlin, Germany), which was previously calibrated according to manufacturer's instructions (BST systemic solution, Berlin,

Germany). The relationship between $[La^-]_b$ and work rate has been obtained and used to determine LT through the D_{max} method (Heuberger et al., 2018**).** Additionally, in basal conditions, during the warm-up and at the end of the loads the subjects were asked for the perception of effort (Borg, 1982), declined in general (with a 6-20 scale), muscular (with a CR10 scale) and respiratory (with a CR10 scale) aspect.

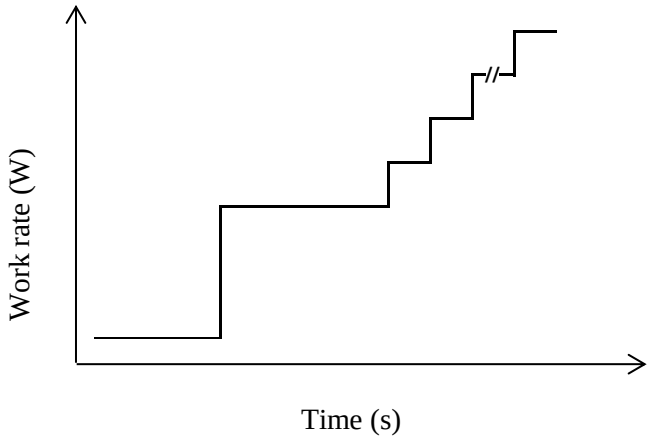


Figure19. Step incremental test protocol

3.2.2. Critical Power assessment

To determine CP, on different days, four tests at constant load were randomly administered till exhaustion. Mechanical loads were selected in the range between 90 and 110% MAP in order to obtain a range of times to exhaustion between 2 and 15 min (Whipp et al. 1982). Each test included three minutes of rest, for baseline measurements, five minutes of warm-up at 100W, a five minutes recovery phase and a three minutes unloaded pedaling. Subsequently, the work rate was set at one of the target load percentages (figure.20).

The time-to-exhaustion (TTE) was calculated for each test subtracting the time to start the application of the target load from the final time of the test. The determination of CP was carried out by calculating the intercept of the linear relationship between PO and the reciprocal of the TTE (Jones et al. 2010).

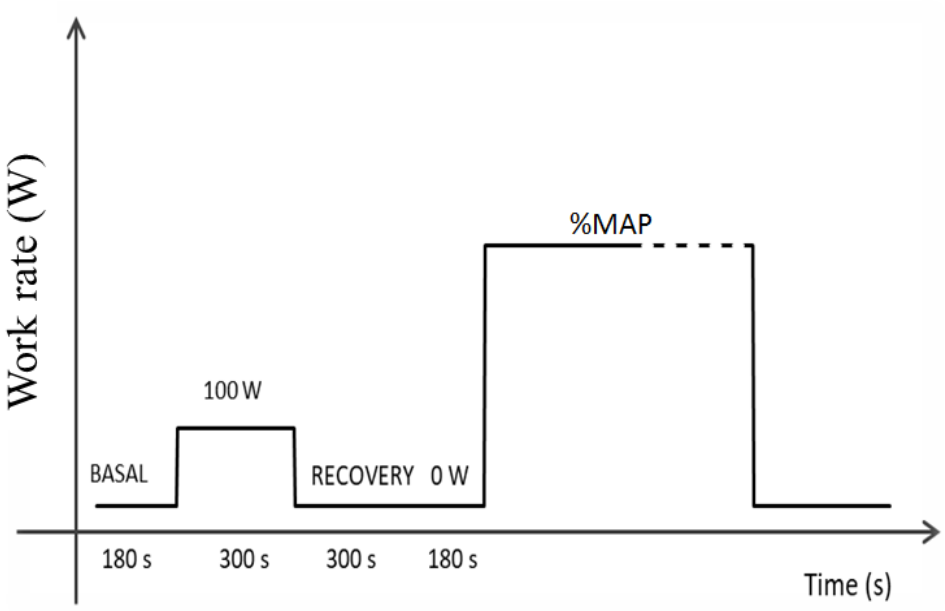


Figure20. Example of a constant load test used to determine the critical power.

3.2.3. Sinusoidal load test

In the two sinusoidal sessions the protocol consisted of 3 minutes of rest, for basal measurements, 3 minutes of warm up at 50 W, 3 minutes of warm up at the individual CP, followed by a sinusoidal load test, until exhaustion. The PO pursued according to:

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

Where MP, AMP, T corresponded to the midpoint, the amplitude and the period of the sine wave cycle, respectively.

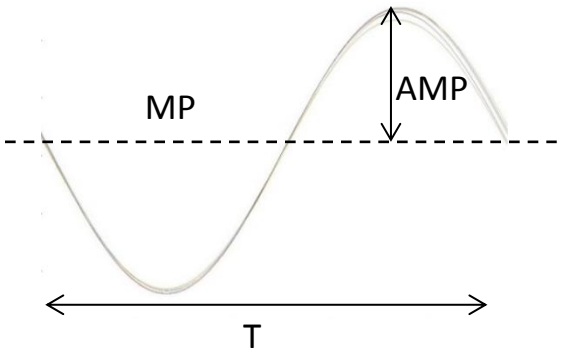


Figure21. Sinewave components

Two different sinusoidal protocols were randomly administered on different days with 48-h recovery in between. MP and AMP were chosen to provide a workload entirely below the CP able to elicit a significant cardiorespiratory response. Therefore, based on a previous pilot study, one sinusoidal protocol presented a MP of 30W below CP and AMP of 30W

(30CP$_{-30}$) while the second one combined a MP of 50W below CP and AMP equal to 50W (50CP$_{-50}$).

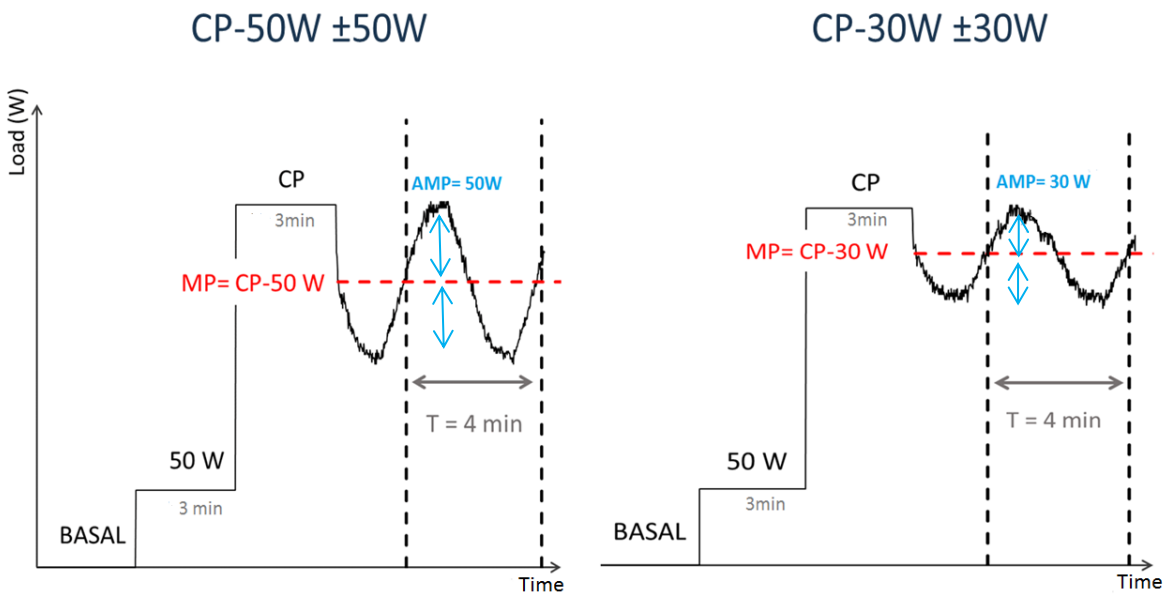


Figure22. Sinusoidal experimental design

The choice of 4-minute period (T) took into account that the time constant of $\dot{V}_{O_2}$ is around 40 s to reach 63% of its expected transition amplitude (Keir et al, 2018) therefore, the ascent phase of the work rate (from the MP to the positive peak) should have lasted at least 1 minute. The next three minutes were necessary to entirely complete the other three phases (decrease to the MP, reach the minimum and rise to the successive MP value).

During every test $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ were determined on a breath-by-breath basis with a metabolimeter (Quark b^2, Cosmed, Italy). f_H, was continuously monitored by a heart rate

transmitter belt (T34, Polar, Finland). The flow meter and gas analyzers were carefully calibrated, following the manufacturer's instructions, with a 3-liter syringe (model 5530, Hans-Rudolph, Shawnee, KS, USA) and with a gas mixture composed for the 16 % from O_2, for 5% from CO_2 and for the remaining part from N_2. Furthermore, PO and the pedaling frequency were simultaneously integrated to metabolic data. $[La^-]_b$ was determined every cycle by analyzing 20 µl blood sample collected from the ear lobe. To verify that the Lactate concentration remained almost steady and below the lactate threshold. For this reason we wanted to record the values of blood lactate concentrations for each sinusoid cycle.

3.3. Data analysis

Analysis of all the cardiorespiratory and metabolic parameters response to the sinusoidal protocol was conducted by custom built software (Matlab, Mathworks). During the analysis, each cycle was analyzed separately and the parameters (MP, AMP, T and T_D) of the sine wave function that fitted the data with the lowest residuals were obtained for all the parameters. To avoid distortions introduced by the transition from warm-up at CP to MP, the first two minutes were excluded by the analysis. During the analysis, different T_Ds have been determined, each referred to four hallmarks. Indeed, the latency of cardiorespiratory and metabolic responses with respect to mechanical loading was determined for: i) positive peak (T_{D_MAX}); ii) downward MP crossing (T_{D_DOWN}); iii) nadir (T_{D_MIN}); and iv) upward MP crossing (T_{D_UP}).

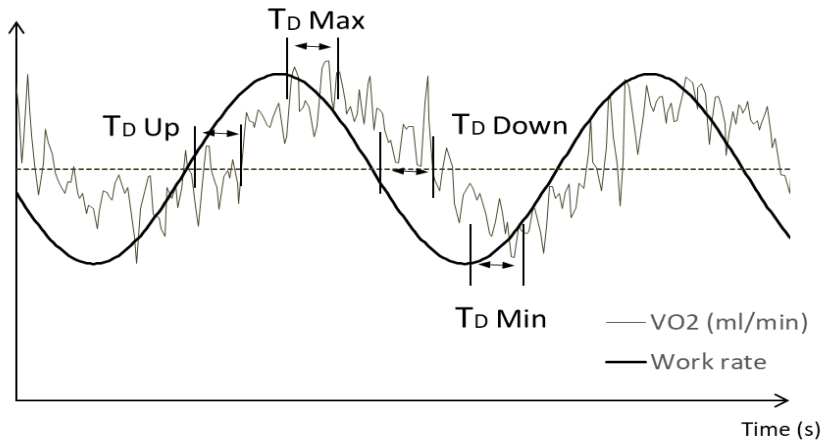


Figure23. Sinusoidal T_D hallmarks

These T_Ds depend on both AMP and the speed of the metabolic processes to adapt to the load, and reflect the reactivity of the cardiorespiratory variables in response to sinusoidal exercise (Casaburi et al., 1977; Bakker et al., 1980; Haouzi et al., 1992, Fukuoka et al., 2002). Given that the kinetic during increasing phase might differ from the decreasing phase due to O_2 deficit (Fukuoka, 1997), we have considered four different hallmarks to better follow the time difference between sinusoidal work loading and each response curve for cardiorespiratory and metabolic parameters in on and off transient.

3.4. Statistical analysis

Commercial software (SPSS 22.0, IBM, Armonk, USA) was utilized to perform the statistical analysis. Normal distribution of the data and homogeneity of variances were

examined by Shapiro-Walk and Levene's tests. To determine the mean difference in $\dot{V}_E$, $\dot{V}_{O_2}, \dot{V}_{CO_2}$ and f_H among cycles and between protocols, a two-way ANOVA for repeated measurements was applied, with time as within group factor and protocol as between-groups factor. For within group analysis, a Bonferroni post-hoc statistic was used to test for differences between each cycle and the first cycle, which was considered as the reference condition. Lastly, a paired samples t-test also evaluated the differences between the traditional approach and the cycle-by-cycle analysis. Sample size calculation was based on a previous investigation (Haouzi 1993), considering the changes in phase shift of metabolic parameters at two sinusoidal protocols with different midpoints. Computation was done through statistical software G-Power 3.1 (Düsseldorf, Germany). From this study, an average Cohen's ds effect size (ES) of ~0.86 was obtained. By considering this ES, two-tail effect, $\alpha = 0.05$, and required power $(1 - \beta) = 0.80$, the desired sample size resulted in 13 participants. The mean difference between each cycle and the first one and between the two protocols ($50CP_{-50}$ and $30CP_{-30}$) was reported with the corresponding 95% confidence intervals (CI). The magnitude of changes was assessed using partial η^2 (η_p^2), which was classified as small if <0.06, medium if between 0.06 and 0.14, and large if >0.14 (Cohen 2013). The significance level was set at $\alpha<0.05$. Data are reported as mean ± standard error (SE).

4. RESULTS

4.1. Cycle to exhaustion in two protocols

In regards to the number of the cycles-to-exhaustion, no significant difference was observed between the two protocols (figure 24).

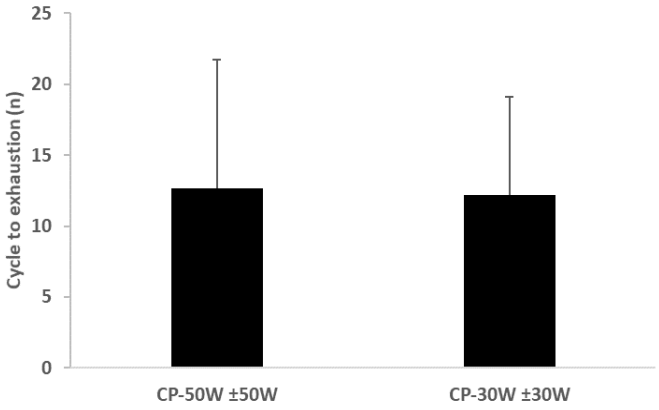


Figure24. Mean values of the cycles-to exhaustion determined in the two protocols.

4.2. Midpoint (MP)

As expected, differences in MP were found in all the investigated variables. Indeed, higher values were found in $30CP_{-30}$ compared to $50CP_{-50}$ in $\dot{V}_E$ (η_p^2: 0.543; F= 9.505; P=0.003; figure 25 panel a), $\dot{V}_{O_2}$ (η_p^2: 0.515; F= 9.549; P=0.006; figure 25, panel b), $\dot{V}_{CO_2}$ (η_p^2: 0.539; F= 10.527; P=0.003; figure 25, panel c) and f_H (η_p^2: 0.614; F= 14.308; P=0.001; figure 25, panel d). In addition, significant differences were observed in all the variables except of

$\dot{V}_{O_2}$ between the mean value and the cycles within each protocol separately. In regards to a possible effect of the fatigue, beyond the $\dot{V}_{CO_2}$ (figure 25, panel c), $\dot{V}_E$ (figure 25, panel a) and $\dot{V}_{O_2}$ (figure 25, panel b) responses which remained almost steady, whereas a significant rise was observed in f_H in both protocols (figure 25, panel d).

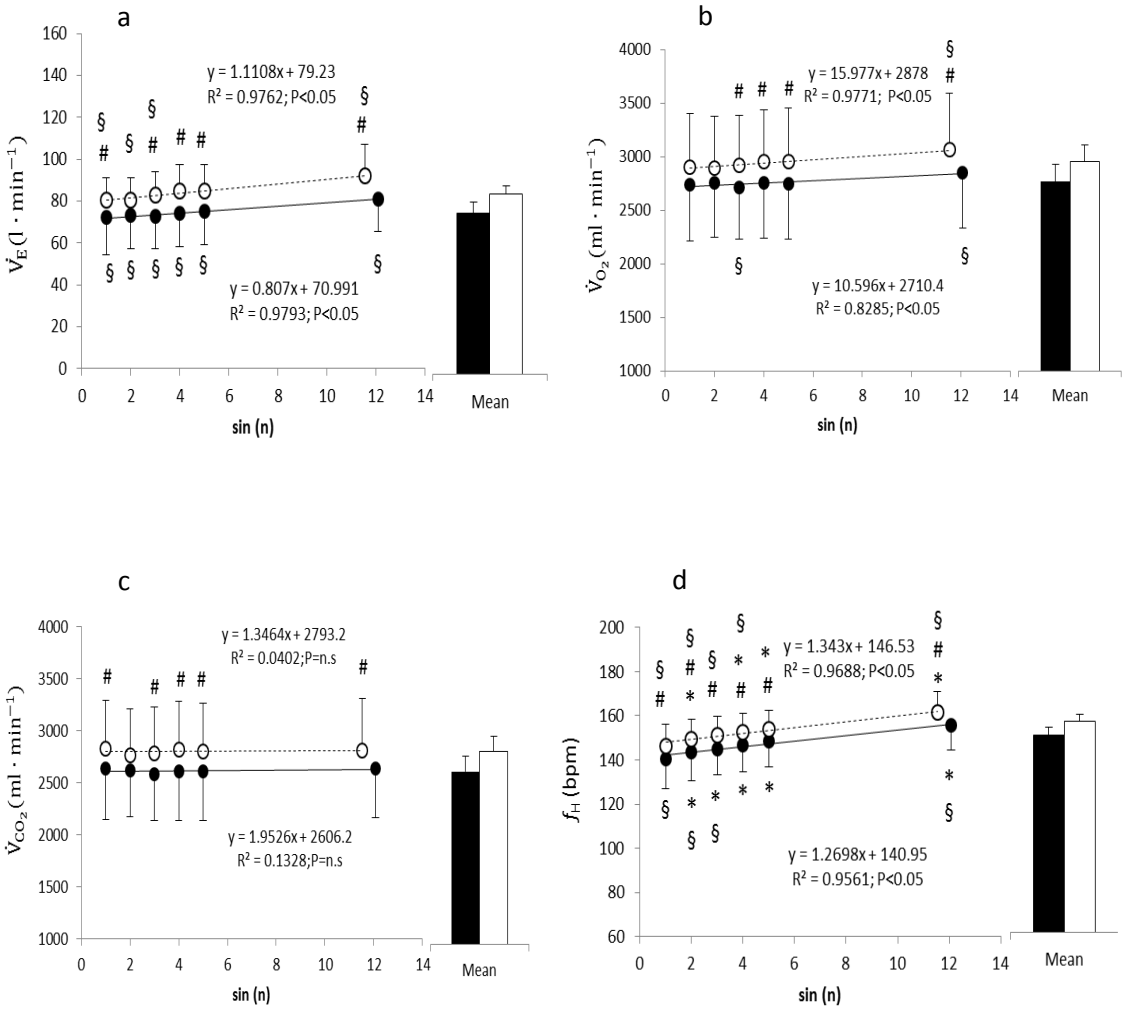
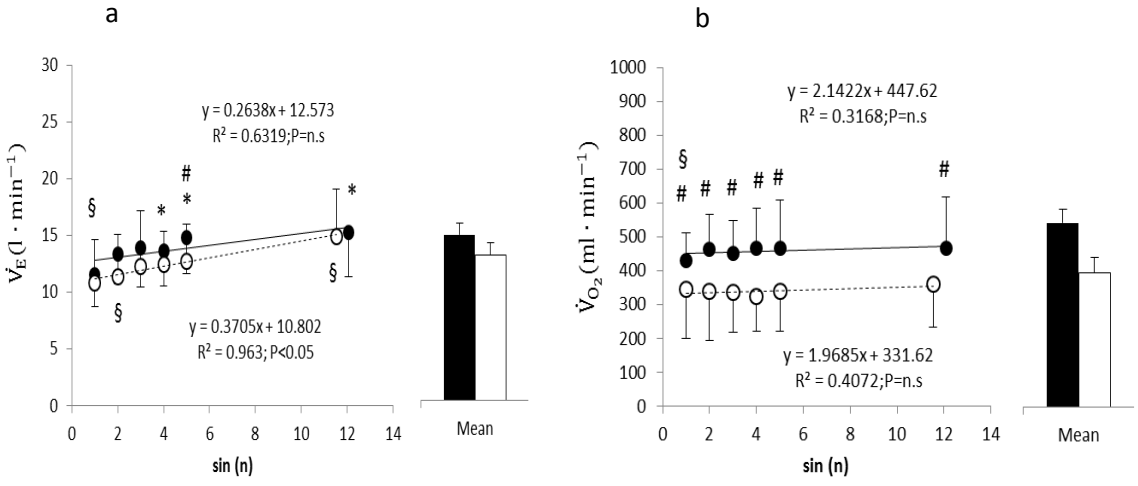


Figure25. Mean MP values obtained in both protocols. Panel a, b, c, and d refer to $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and f_H, respectively as the number of sinusoid cycles varies (1-5 + last). The vertical columns indicate mean ± SD. ○: 30 CP-$_{30}$W; ●: 50 CP-$_{50}$W. #P<0.05 vs 50CP-$_{50}$; * P<0.05 vs first cycle; § P<0.05 vs Mean value.

4.3. Amplitude (AMP)

As predicted, AMP of 30CP$_{-30}$ was significantly lower in $\dot{V}_E$ (η_p^2: 0.222, F= 2.281; P=0.024; figure 26, panel a), $\dot{V}_{O_2}$ (η_p^2: 0.690, F= 17.796; P=0.001; figure 26, panel b), $\dot{V}_{CO_2}$(η_p^2: 0.832; F= 44.623; P=0.001; figure 26, panel c) and f_H (η_p^2: 0.876; F= 63.291; P=0.001; figure 26, panel d) compare to 50CP$_{-50}$. Moreover, significant differences were found beyond all the variables in some cycles compare to the mean value within each protocol separately. Differently to MP, in some variables of interest the impact of fatigue on AMP was dissimilar between the two protocols (figure 26). Indeed, none of the physiological parameters showed any sign of fatigue in 30CP$_{-30}$, while a significant increase in $\dot{V}_E$ (figure 26, panel a) and $\dot{V}_{CO_2}$(figure 26, panel c) was observed in 50CP$_{-50}$.



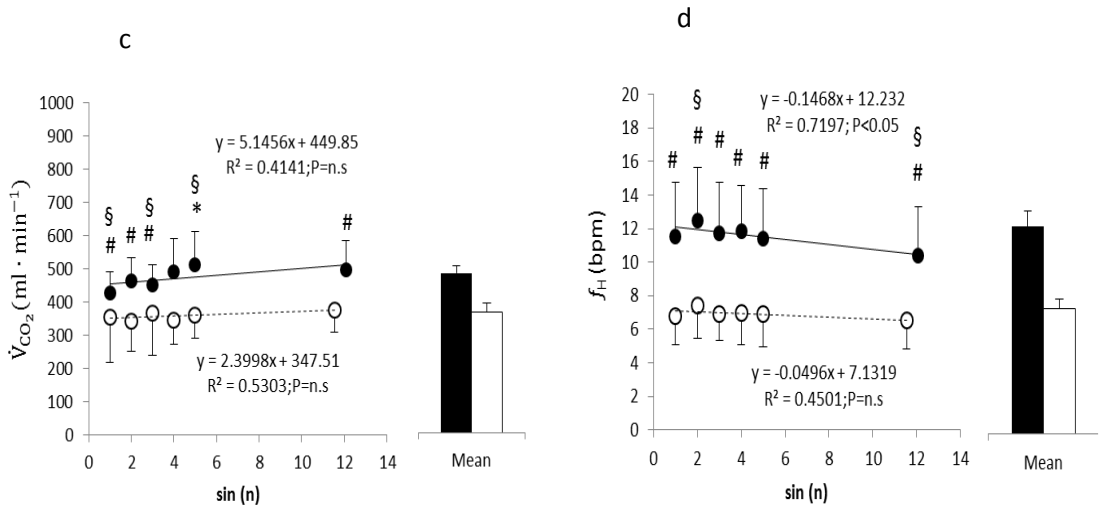


Figure26. Mean AMP values obtained in both protocols. Panel a, b, c, and d refer to $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and f_H, respectively as the number of sinusoid cycles varies (1-5 + last). The vertical columns indicate mean ± SD. ○: 30 CP-$_{30}$W; ●: 50 CP-$_{50}$W. #P<0.05 vs 50CP-$_{50}$; * P<0.05 vs first cycle; § P<0.05 vs Mean value.

4.4. Time delay (T_D)

None of the respiratory parameters showed any significant variation in the all hallmarks ($T_{D\text{-}UP}$, $T_{D\text{-}MAX}$, $T_{D\text{-}DOWN}$ and $T_{D\text{-}MIN}$) between the two protocols (figure 27,28,29 and 30 panel a, b, c and d). Indeed, the T_D values of all the variables of interest were similar in the two work rates regardless of which T_D was considered. In addition, significant differences were found in f_H in some cycles compare to the mean value in both protocols. Refer to the possible influence of fatigue, no difference was found among the all cycles in both protocols, whereas significant rising regressions were observed in f_H in all hallmarks.

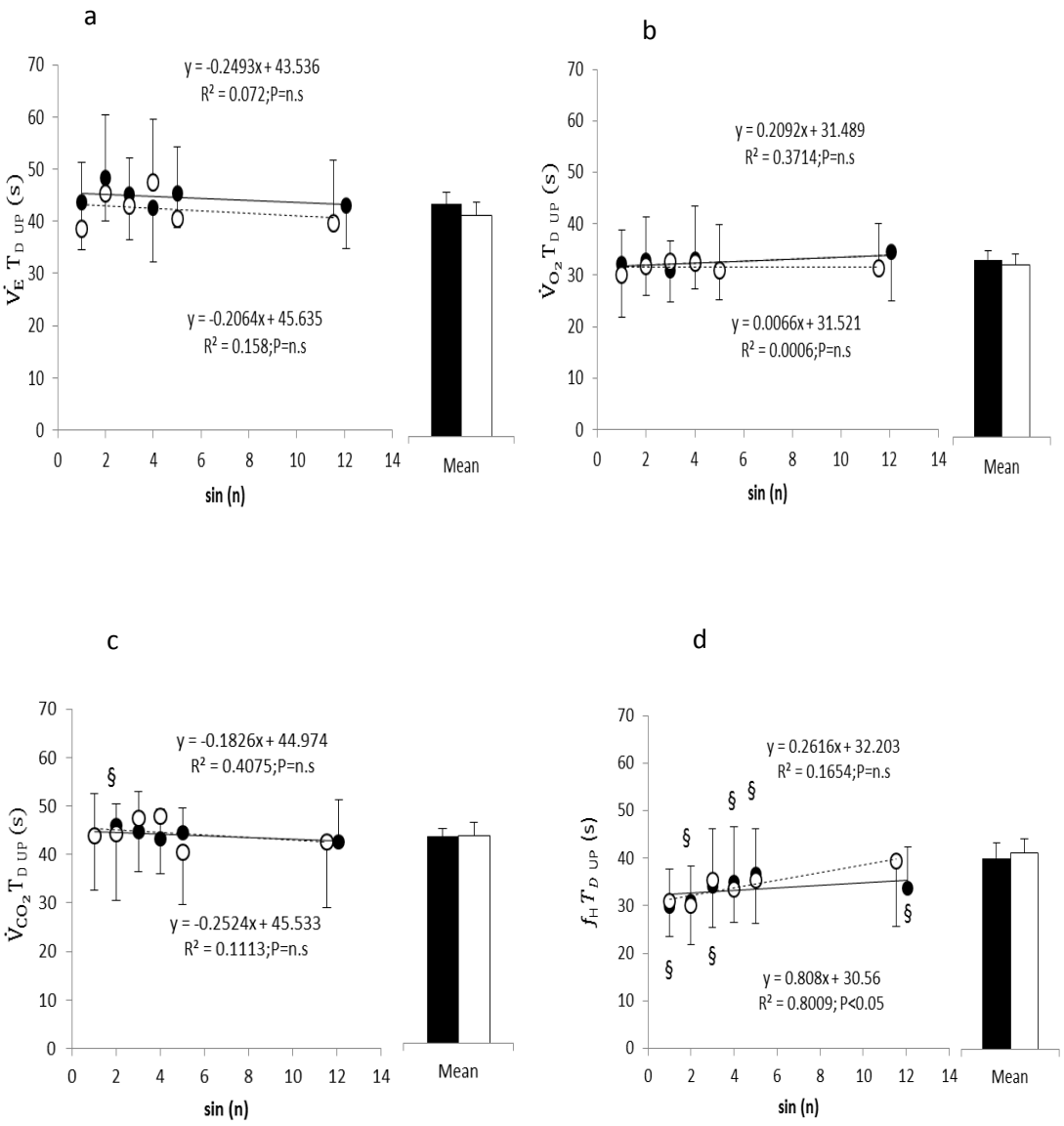


Figure27. Mean T_{D_UP} values obtained in both protocols. Panel a, b, c, and d refer to $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and f_H, respectively as the number of sinusoid cycles varies (1-3 + last). The vertical columns indicate mean ± SD. ○: 30 CP-$_{30}$W; ●: 50 CP-$_{50}$W. #P<0.05 vs 50CP-$_{50}$; * P<0.05 vs first cycle; § P<0.05 vs Mean value.

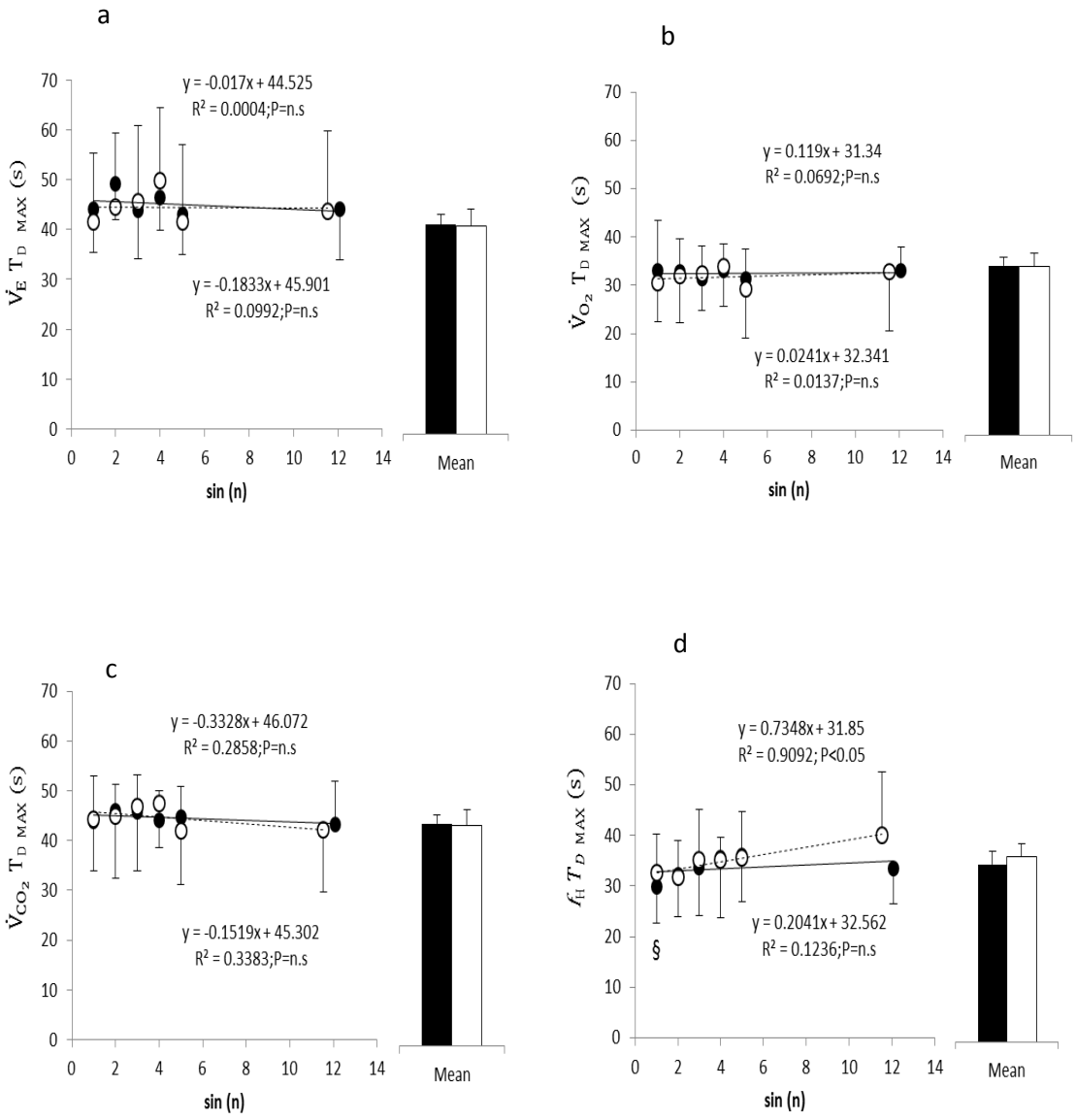


Figure28. Mean T_{D_MAX} values obtained in both protocols. Panel a, b, c, and d refer to $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and f_H, respectively as the number of sinusoid cycles varies (1-5 + last). The vertical columns indicate mean ± SD. ○: 30 CP-$_{30}$W; ●: 50 CP-$_{50}$W. #P<0.05 vs 50CP-$_{50}$; * P<0.05 vs first cycle; § P<0.05 vs Mean value.

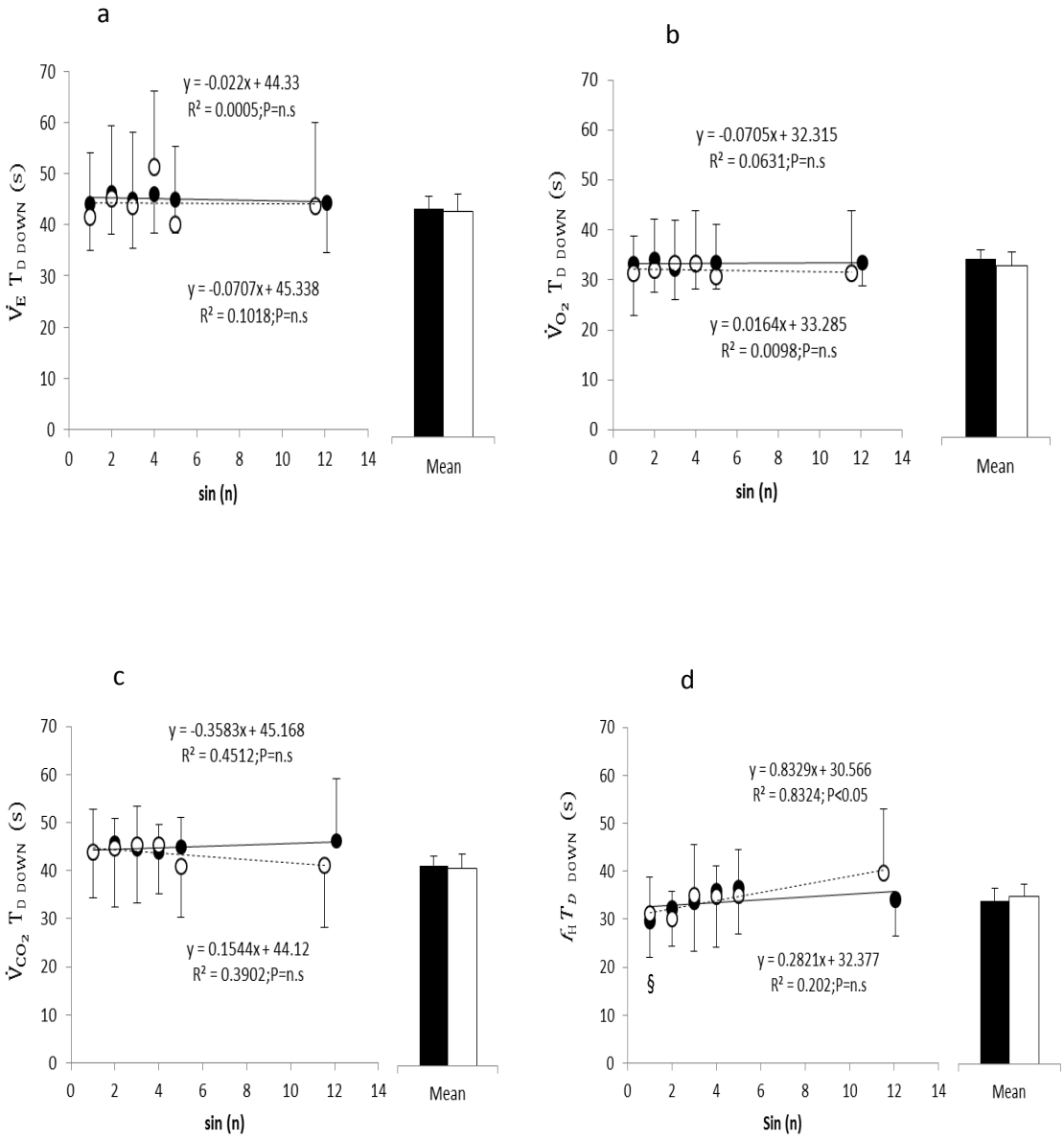


Figure29. Mean T_{D_DOWN} values obtained in both protocols. Panel a, b, c, and d refer to $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and f_H, respectively as the number of sinusoid cycles varies (1-5 + last). The vertical columns indicate mean ± SD. ○: 30 CP-$_{30}$W; ●: 50 CP-$_{50}$W. #P<0.05 vs 50CP-$_{50}$; * P<0.05 vs first cycle; § P<0.05 vs Mean value.

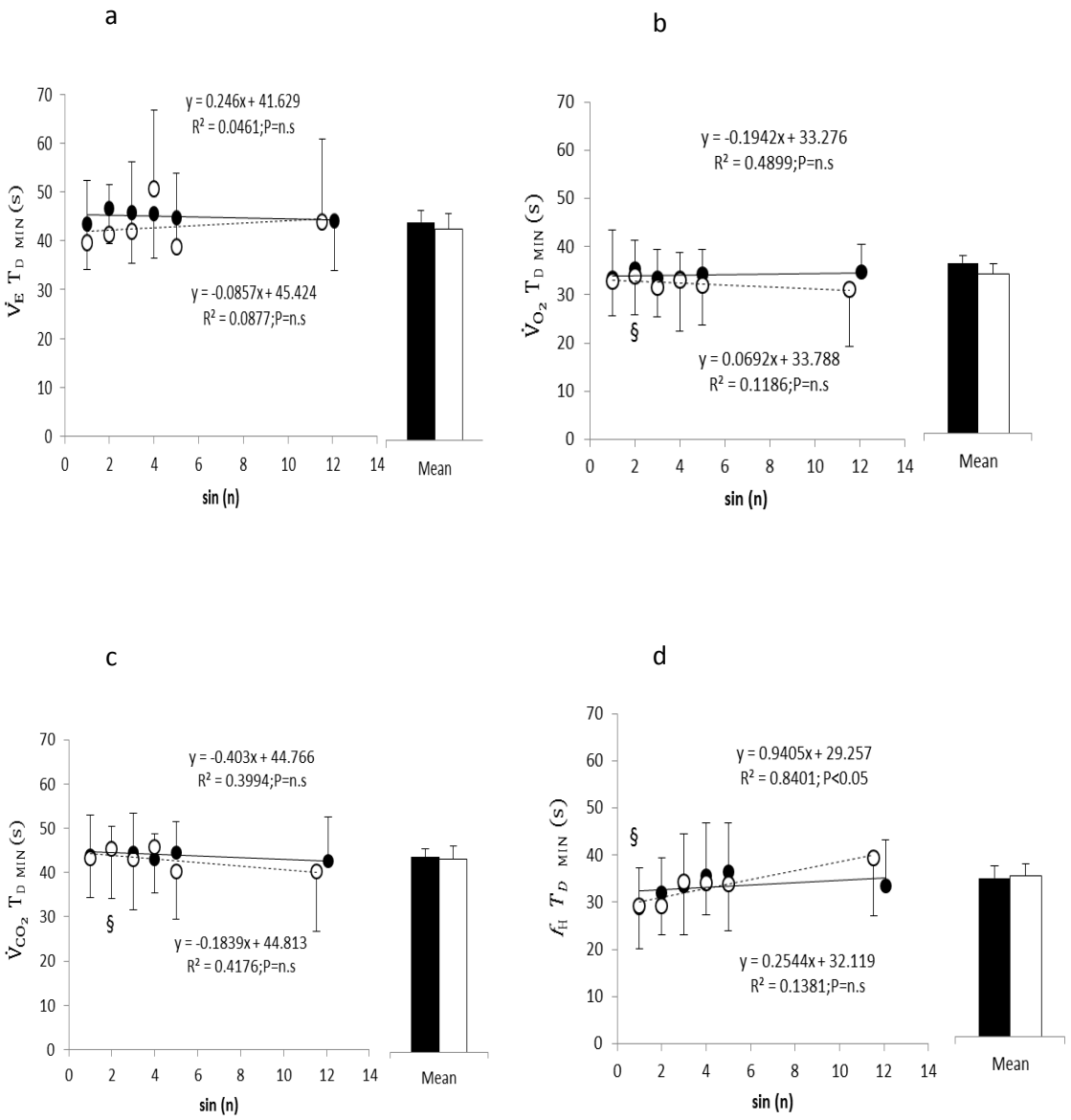


Figure30. Mean T_{D_MIN} values obtained in both protocols. Panel a, b, c, and d refer to $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and f_H, respectively as the number of sinusoid cycles varies (1-5 + last). The vertical columns indicate mean ± SD. ○: 30 CP-$_{30}$W; ●: 50 CP-$_{50}$W. #P<0.05 vs 50CP-$_{50}$; * P<0.05 vs first cycle; § P<0.05 vs Mean value.

4.5. Blood lactate $[La^-]_b$

In regards to the concentrations of ($[La^-]_b$) obtained during the different sinusoid cycles (figure 31), there was not any significant difference between two protocols. In regards to the effect of fatigue no significant rise was observed in $50CP_{-50}$ and $30CP_{-30}$ (fig. 31).

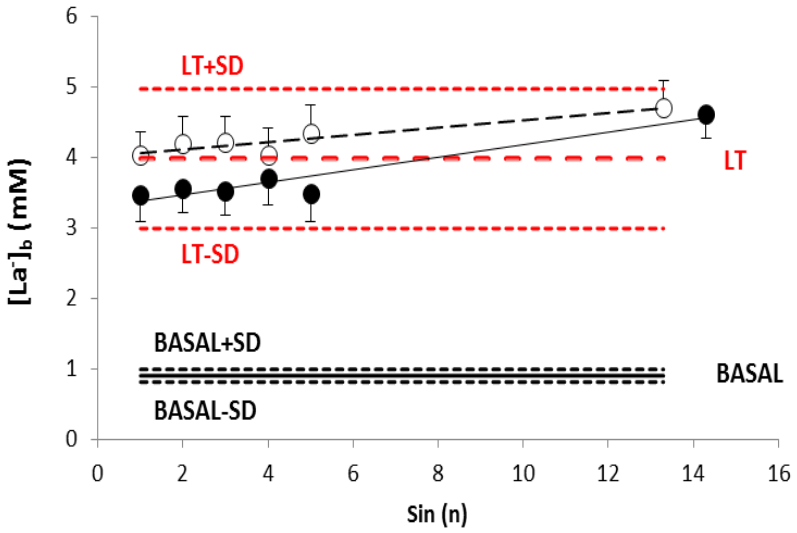


Figure31. Mean $[La^-]_b$ values detected during the different sinusoid cycles during both protocols. The average of the subjects' lactate threshold along with basal values is also shown. ○: $30CP_{-30}$; ●$50CP_{-50}$.

4.6. Rate of perceived exertion (RPE)

In regards in, RPE, no difference was found between the two protocols in the general (G) and muscular (M) RPE (figure 32, panel a, and b respectively). On the contrary, respiratory (R) RPE was significantly higher during $30CP_{-30}$ in the fourth cycle compared to $50CP_{-50}$ (figure 32, panel c). In regards to a possible impact of fatigue there were significant

differences among the cycles compare to the first cycle (figure 32, panel a, b and c respectively).

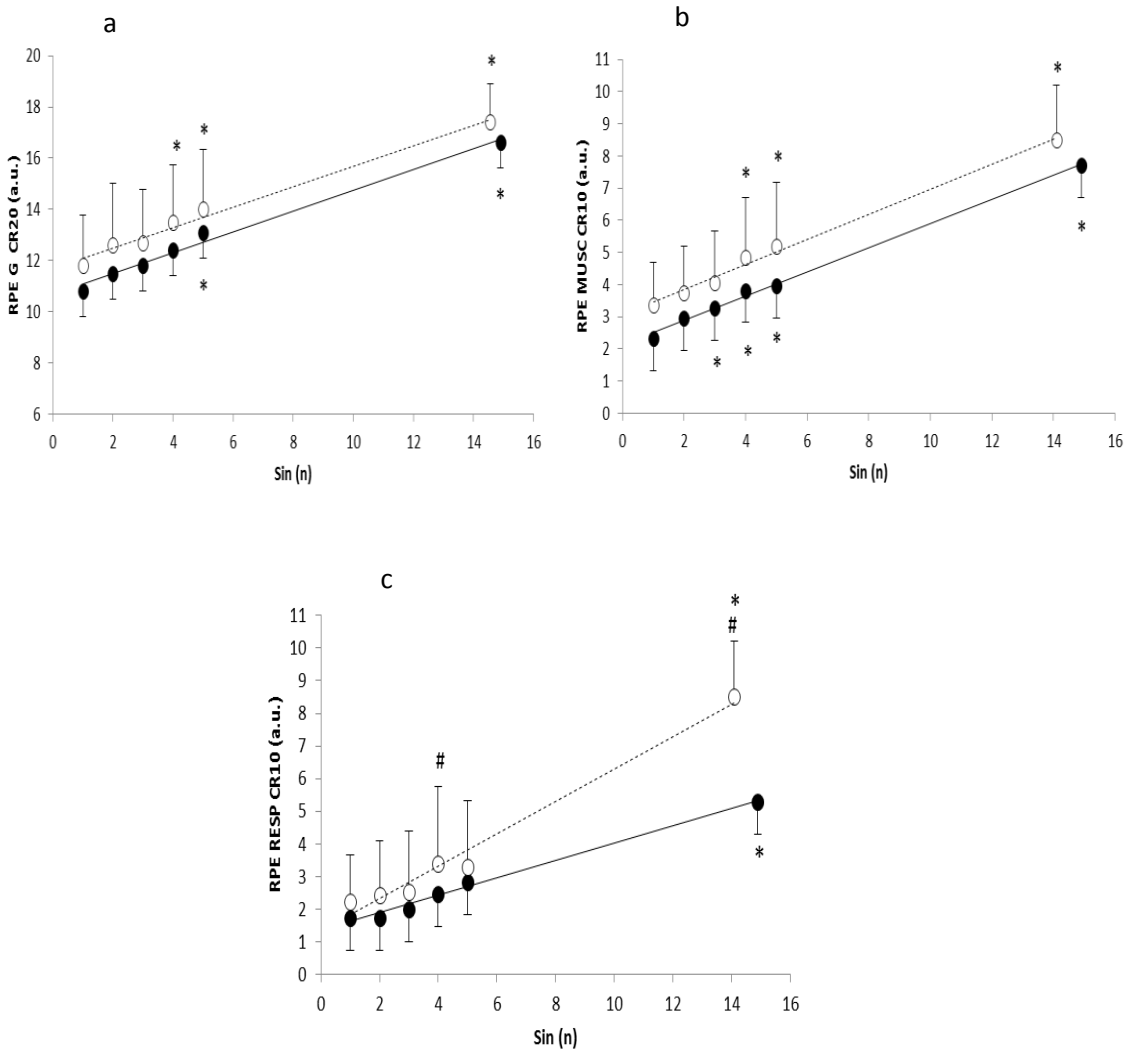


Figure32. Mean RPE values obtained in both protocols. Panel a, b, c, and d refer to G,M and R respectively as the number of sinusoid cycles varies (1-5 + last). ○: 30 CP-$_{30}$W; ●: 50 CP-$_{50}$W. *P<0.05 vs 50CP-$_{50}$; # P<0.05 vs last cycle.

5. DISCUSSION

To determine the cardiorespiratory and metabolic responses to sinusoidal work rates under aerobic conditions, two sinusoidal exercises of severe intensity were administered. Both protocols were set to be completely below the CP to ensure a condition of the oxidative metabolism (Keir et al. 2018). The two protocols differed also for sinewave amplitude (30W and a 50W, with peak-to-nadir amplitude of 60W and 100W, respectively) to evaluate the cardiorespiratory and metabolic responses to exercise intensities with different oscillation amplitudes. The present findings revealed differences in amplitude and midpoint of all the investigated variables between the two protocols, whereas time delays were similar, regardless of the exercise intensity/oscillation. Additionally, the novel approach adopted to analyze the variables of interest (cycle-by-cycle) showed to be suitable in detecting signs of the onset of fatigue during time to exhaustion protocols, thus making the use of the traditional approach questionable, because in those tests all values averaged as one value while in sinewave protocol the average of each cycle can be obtained.

5.1. 30CP-30 *vs* 50CP-50

The results related to the comparison of the two protocols witnessed some preliminary expectations and rejected others. Indeed, significant differences were found in the AMPs of the cardio-metabolic responses, with values determined during $30CP_{-30}$ significantly lower in $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and f_H compared to $50CP_{-50}$. The lower peak-to-nadir delta work rate found during $30CP_{-30}$ was the main reason for the lower values found on the metabolic and

cardiac variables. It is intuitive that a total work rate oscillation of 60W is less demanding than a change of 100W. Indeed, the amount of oxygen required to sustain a limited work rate rise is inevitably below the amount required to sustain a wider increase. As a consequence, being the $\dot{V}_{CO_2}$ produced during moderate activities predominantly the result of the oxidative metabolism, its production is destined to be reduced proportionally to the reduction in $\dot{V}_{O_2}$. The lower metabolic demand associated to a lower work rate which produces the different metabolic alterations. Indeed, the linear relationship between $\dot{V}_{O_2}$ and f_H makes these two variables concordant so that a reduction in one parameter reflects a proportional reduction in the other one.

The other prediction confirmed by the results relates to the MPs of the physiological variables. Indeed, higher values were found in $30CP_{-30}$ in $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$and f_H compared to $50CP_{-50,}$ thus reflecting the higher mean work rate of the former. The well-known gain in the $\dot{V}_{O_2}$ *vs* PO relationship (Ozyener et al, 2001) may explain the differences in cardiorespiratory and metabolic variables amplitude between the two protocols given that a higher work rate requires a higher energy demand (and then higher $\dot{V}_{O_2}$) and, in turn, a higher f_H (to promptly deliver O_2 to peripheral districts and remove $\dot{V}_{CO_2}$ from periphery). A higher $\dot{V}_{O_2}$ results also in a higher $\dot{V}_{CO_2}$ which, in turn, is responsible for a higher $\dot{V}_E$, that is necessary to deliver O_2 to the alveoli and dispose $\dot{V}_{CO_2}$ from the lungs. In regard to T_D, none of the cardiorespiratory and metabolic parameters showed any significant variation in $T_{D\ UP,}$ $T_{D\ MAX,}$ $T_{D\ DOWN}$ and $T_{D\ MIN}$ between $50CP_{-50}$ and $30CP_{-30.}$ These T_DS

finding indicated that the cardiorespiratory kinetics adaptation are independent by the work rate intensity and oscillation AMP when the exercise is mostly aerobic (below CP).

5.2. Fatigue effect on amplitude (AMP)

In some variables of interest the effect of fatigue on AMP was dissimilar between the two protocols. Indeed, none of the physiological parameters showed any sign of fatigue in $30CP_{-30}$, while a significant increase in $\dot{V}_E$ and $\dot{V}_{CO_2}$ was observed in $50CP_{-50}$. However, since only just Haouzi et al., 1993 analyzed how kinetics vary as the number of sinusoid cycles progresses and since this author only studied the behaviors of $\dot{V}_{O_2}$ and f_H, it is not possible to find a comparison in the literature on the reason behind $\dot{V}_E$ and $\dot{V}_{CO_2}$ AMP trends. In many studies it was investigated how the entire sinusoidal response varies in relation to the length of the period and it appeared that with short periods the AMP tends to decrease, due to the decrease in time that does not allow the variable to fully develop its response (Wigertz, 1970; Miyamoto et al., 1983).

5.3. Fatigue effect on mid-point (MP)

Refer to a possible impact of fatigue on MP, $\dot{V}_{E,}$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values remained almost steady, whereas a significant rise was observed in f_H in both protocols. The presence of a positive trend for f_H was confirmed by the regression analysis. Taking all these considerations together, the MPs can be considered constant along the cycle, confirming

the steady condition of the oxidative metabolism. This result, then, is in agreement with the finding of Haouzi which did not find any fatigue effect on $\dot{V}_{O_2}$ MP (Haouzi et al., 1993).

In the present investigation the progressive rise of f_H MP suggest an influence of fatigue. A possible explanation of this behavior may include the theory of the "cardiovascular drift" which states that during long-term, moderate to heavy aerobic exercise, $\dot{Q}$, SV, f_H, SBP, and rate-pressure product increase rapidly (Plowman et al, 2013). Once steady-state is achieved, cardiac output remains relatively constant owing to the downward drift of SV and the upward drift of f_H. This cardiovascular drift is associated with rising body temperature. Similarly to the study of Hayashi (2006) in which an increase in $\dot{V}_E$ during prolonged submaximal exercise was observed and described as "ventilatory drift", in the present investigation a rise in MP of $\dot{V}_E$ occurred. The previously published study did not give any explanation. However, a possibility is that the increase in $\dot{V}_E$ might reflect augmented input from chemoreceptors. In that case, hyperthermia-induced reductions in blood pH, increases in chemo sensitivity and/or increases in plasma norepinephrine derived from increased sympathetic nerve activity lead to increases in peripheral chemoreceptor activity (Crandall, 1999; Seals et al., 1988). Another reason might be the activity of group III and IV muscle afferents, induced by an increase in muscle temperature, and increased activity of the ventral respiratory group, induced by an increase in brain temperature, lead to an increase in $\dot{V}_E$ (Hayashi et al., 2006).

5.4. Fatigue effect on time- delay (T_D)

Regarding the T_D of the cardiorespiratory variables, the averages of the values obtained are in line with the values previously published (Miyamoto et al., 1983; Fukuoka et al., 1990; Fukuoka et al., 1997). Similarly to these studies, findings of the present investigations have shown T_D in $\dot{V}_{O_2}$ shorter than $\dot{V}_{CO_2}$ response. This phenomenon could be explained by the larger solubility of CO_2 than O_2 in the body fluid and tissue (Fukuoka et al., 1990). In a study by Fukuoka and coll (1997) 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 $\dot{V}_E$ which was inconsistent with our finding. The author employed O_2 deficit to examine this asymmetry. At the crest of work loading, O_2 deficit increased progressively with increasing work load because a gradual delay in aerobic metabolic activity occurred. In contrast, trough of work loading, the time difference was less influenced by O_2 deficit when most of the O_2 debt was repaid. Therefore, the change in O_2 deficit may have caused a difference of between curves responses. However, O_2 deficit cannot completely explain the asymmetry of the response curve. It was demonstrated that at heavy exercise, $\dot{V}_{O_2}$ kinetics were faster in the on-transient than off-transient whereas at a subthreshold work load, $\dot{V}_{O_2}$ kinetics for on- and off-transients were symmetrical, which was in line with our finding (Ozyener 2001, Cleuziour 2003). They speculated that the dynamic asymmetries of $\dot{V}_{O_2}$ might be attributed to reduced O_2 utilization, which may be ascribable to faster recruitment of anaerobic metabolism and to some extent, to the contribution of a slow component. However, in the

present study none of these mechanisms seem to have taken place as in both protocols we found symmetrical cardiorespiratory responses.

Regarding f_H $T_{D,}$ the higher values found in the last cycle in $30CP_{-30}$ may be interpreted as a sign of fatigue. Neuronal factors originating from the central cortex and/or muscle, and the dynamics of muscle blood flow transporting humoral stimuli to chemoreceptor sites might be responsible for this behavior (Fukuoka et al., 1997). Another hypothesis could be the intervention of the sympathetic and parasympathetic nervous system. In fact, during physical activity the first increase in f_H is allowed by the regulation of the parasympathetic nervous system that is inhibited; while with the progress of the exercise there is a greater intervention of the sympathetic nervous system. The action of the parasympathetic system is beat-by-beat, therefore very rapid because: the SA node and the AV node are very rich in acetylcholinesterase which rapidly degrades the Acetylcholine present in the synaptic space and the latter acts directly on the potassium channels without the intervention of second messengers. On the contrary, the sympathetic system is slower because noradrenaline is re-caught by the axonic terminals and not enzymatically degraded; moreover, the connection between β receptors and Ca^{2+} channels occurs through a second messenger. Therefore, it could be assumed that the first sinusoid cycles were more influenced by the parasympathetic nervous system (which allowed a fast f_H adjustment to work rate) while, after a few minutes, there was a greater intervention of the sympathetic nervous system which had a slower regulation causing a greater delay in the kinetics. These results are in line with literature (Casaburi et al., 1977; Bakker et al., 1980; Miyamoto et al., 1983;

Fukuoka et al., 1991) and could support the hypothesis of cardio-dynamics theory, according to which hyperpnea during the non-stationary state depends on the increase in $\dot{Q}$ which allows CO_2 to quickly reach the alveolar capillaries (Potts et al., 1992; Wasserman et al., 1977).

5.5. Blood lactate concentration $[La^-]_b$

Observing the $[La^-]_b$ detected during sinusoidal cycles, no difference was found between the two protocols values. This result seems to indicate a similar involvement of the lactic anaerobic metabolism. Comparing these values with the values corresponding to the LT, it appears that both work rates engaged the subjects' lactic system at a level similar to the LT. The absence of any statistical difference between the first cycle and the latter cycles indicates that there was not any net lactate accumulation.

5.6. Rate of Perceived exertion (RPE)

The study of the differences in the kinetics of cardiorespiratory and metabolic parameters that occurred between the various cycles of the protracted sinusoids until exhaustion was based on the assumption that fatigue occurs as the exercise progresses. As the number of sinusoid cycles varied, it observed that indeed a condition of fatigue in participants and perceived exertion occurred, declined in its three forms, increased significantly in both protocols. This was also supported by the statistical analysis, in which a significant

difference emerged between the first and the last cycle. In particular, by examining the RPE values, we noted that the participants reached exhaustion due to muscular fatigue. In fact the average of the values recorded in the last cycle of sinusoid with regard to general RPE (6-20) was about 17; the muscular RPE (CR10) was about 8, while the values of the respiratory RPE (CR10) did not exceed 6 in both protocols.

6. STUDY LIMITATIONS AND FUTURE PERSPECTIVES

There were some limitations in our study. First of all, we investigated only a T of 4 minutes, exploring other Ts and AMPs could give other new information. Another limitation to this investigation is the lack of a statistical analysis on the reliability of the cycle-by-cycle analysis. It would be effective to do the reliability test. Lastly, there were lack of cardiac output and peripheral tissue oxygenation measurements during the data collection. It would be useful to extend the study to the participants with different characteristic, gender and age. In the future, this protocol could be adapted to subjects suffering from cardiorespiratory diseases to assess the progression of the disease and the effectiveness of a therapeutic intervention and / or a training program.

7. CONCLUSION

From the results of this study it can be concluded that applying sinusoidal work rates in under the CP, T_Ds response between two protocols was similar and also no differences are present among the four hallmarks (T_{D_UP}, T_{D_MAX}, T_{D_DOWN} and T_{D_MIN}), thus suggesting a symmetrical behaviour during the rising and decreasing phase of the work rate. This could be explained by the intensity of the exercise that was not so high as to produce different cardio responses as well as different breathing patterns. Moreover, the cycle-by-cycle analysis, in addition to the traditional average of the different sinusoids, made it possible to explore the kinetics of the responses throughout the duration of the test and evaluate the fatigue effect. In fact, it was concluded that in this type of loads the effect of fatigue appears, also evidenced by the increasing values of RPE general and muscular.

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9. APPENDIX (ABBREVIATIONS)

MAP = Maximum aerobic power

$\dot{V}_E$ = Respiratory ventilation

$\dot{V}_{E\,max}$ = Maximum respiratory oxygen

$\dot{V}_{O_2}$ = Oxygen uptake

$\dot{V}_{O_2\,max}$ =Maximum oxygen uptake

$\dot{V}_{O_2 r}$ = Oyxygen uptake reserve

f_H = Heart rate

f_{Hmax} = Maximum heart rate

$f_{H\,r}$ = Heart rate reserve

$\dot{V}_{CO_2}$ = Carbon dioxide production

$\dot{V}_{CO_2\,max}$ = Maximum carbon dixide production

Pet_{O_2} = End-tidal pressure of oxygen

Pet_{CO_2}= End-tidal pressure of carbon dioxide

$\dot{Q}$ = Cardiac output

SV = Stroke volume

AMP = Amplitude

MP = Midpoint

T_D = Time delay

T = Period

t = Time

CP = Critical power

O_2 = Oxygen

CO_2 = Carbon dioxide

f_R = Respiratory frequency

V_T = Tidal volume

P_{O_2} = Partial pressure of oxygen

P_{CO_2} = Partial pressure of carbone dioxide

$[La^-]_b$ = Acid lactate concentration

LT = Lactate threshold

RPE = Rate of perceived exertion scale

sin = Sinusoide

VT_1 = Firstventilatory threshold or compensated

VT_2 = Second ventilatory threshold or not compensated

R = Respiratory quotient

W= Watts

PO= Power output

t_{lim} = Exhaustion time

$\dot{V}_E/\dot{V}_{O_2}$ = Oxygen ventilatorty quotient

$\dot{V}_E/\dot{V}_{CO_2}$ = Carbon dioxide ventilatory quotient