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## „Effects of high and low cycling cadences on oxygen parameters in the m. vastus lateralis"


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# Kurzzusammenfassung

Um die physiologischen Reaktionen von verschiedenen Trittfrequenzen während einer Radintervallbelastung zu untersuchen, suchten 16 moderat-trainierte TriathletInnen das Labor für 3 verschiedene Testungen and 3 Tagen auf. Während des ersten Testtags wurde eine diagnostische Spiroergometrie zur Feststellung des $P_{max}$, VT und RCP durchgeführt. An den anderen beiden Testtagen absolvierten die Probanden ein Radintervallprogram. Das Intervallprogram wurde in zufälliger Reihenfolge einmal mit hoher (90 rpm) und einmal mit niedriger (60 rpm) Trittfrequenz durchgeführt. Das Intervallprogram inkludierte 4x5 min Intervalle mit ∆25% (niedrige Intensität) und 4x3 min Intervalle mit ∆70% (hohe Intensität). Die Belastungsintervalle wurden durch eine 3-minütige aktive Erholungsphase von 80 W getrennt. Lokale Muskelsauerstoff Parameter – $m\dot{V}O_2$ - (HHb Konzentrationsveränderungen und TSI) wurden während des gesamten Intervallprogramms mittels Nahinfrarotspektroskopie (NIRS) aufgezeichnet. NIRS wurde am rechten Vastus lateralis Muskel platziert. Außerdem wurden kardiorespiratorische Parameter ($\dot{V}O_2$, VE, RER, HR und [La]) aufgezeichnet. Die kardiorespiratirischen Parameter zeigten signifikante Unterschiede ($p < 0.05$) zwischen den zwei Trittfrequenzen und Intensitäten. $m\dot{V}O_2$ - Parameter unterschieden sich zwischen den beiden Trittfrequenzen nicht voneinander ($p > 0.05$). Außerdem wurde eine zeitliche Veränderung (Zeitkinetik) bei allen kardiorespiratorischen Parametern gefunden, wobei keine Zeitkinetik für $m\dot{V}O_2$ Parameter gefunden wurde. Weiters wurden Geschlechterunterschiede in allen Parametern festgestellt. Die Ergebnisse dieser Studie zeigen, dass verschieden Trittfrequenzen während dem Radfahren unterschiedliche kardiorespiratorische und metabolische Reaktionen in moderat-trainierten TriathletInnen auslösen und dass jene kardiorespiratorische Parameter eine Ermüdung der Probanden während des Intervalltests darstellen. Da für lokale $m\dot{V}O_2$ -Parameter weder ein Unterschied zwischen den beiden Trittfrequenzen, noch ein Ermüdungseffekt gefunden wurde, besteht die Annahme dass lokale Muskelsauerstoffparameter keine leistungsdeterminierenden Faktoren im Radfahren sind.

# Abstract

To evaluate physiological responses upon different cadences during a cycling interval test, 16 moderately trained triathletes visited the laboratory on 3 different occasions. During the first visit, the subjects completed a GXT to determine $P_{max}$, VT and RCP. During visit two and three the subjects completed an interval exercise. All three tests were conducted on subject´s individual bicycles. The exercise was conducted at high (90 rpm) and low (60 rpm) cadences in a randomised order. The cycling interval test included 4x5 min intervals at ∆25% (low intensity) and 4x3 min intervals at ∆70% (high intensity), with recovery periods of 3 min at 80 W. m$\dot{V}O_2$ parameters (HHb concentration changes and TSI) were recorded throughout the tests using Near-infrared spectroscopy (NIRS) on the right m. vastus lateralis. Additional, cardiorespiratory and metabolic parameters ($\dot{V}O_2$, VE, RER, HR and [La]) were recorded. Significant differences ($p < 0.05$) between the two cadences and intensities were found for cardiorespiratory and metabolic variables. No significant differences ($p > 0.05$) were found vor m$\dot{V}O_2$ parameters. Time effects during the interval exercise were found in all cardiorespiratory parameters, indicating an effect of fatigue. No time effect occurred in m$\dot{V}O_2$ parameters. Gender differences were found for all parameters. The results of this study show that different cycling cadences generate different cardiorespiratory and metabolic responses in moderate trained athletes. The lack of differences and time effects found for local m$\dot{V}O_2$ parameters indicate that these values may be no important performance determining parameters in cycling. These findings could be important for (cadence specific) training applications, whereas further research is required to compare and interpret these results.

# Content

# 1 Cycling as an endurance sport

Cycling is a high velocity cyclic endurance sport. Important key factors that are performance related in endurance sports (e.g. cycling) are maximal aerobic power at maximal oxygen consumption ($\dot{V}O_{2max}$), body composition, muscle fibre composition, lactate threshold (LT), gross efficiency (GE), training and nutrition strategy. The major difference between elite and sub-elite is the power produced at $\dot{V}O_{2max}$. Such maximal power outputs typically range from at least 5.5 up to 7.6 $W \cdot kg^{-1}$ for top cyclists. Furthermore, high level cycling performance requires a high respiratory compensation point (RCP). Power output at RCP has shown to be a better predictor of cycling performance than $\dot{V}O_{2max}$ (Atkinson et al. 2003).In contrast to walking or running on a level surface, which requires negligible net-work per cycle, cycling at a constant power output requires net positive work to be performed against the resistance provided at the cranks; the majority of which is performed by the knee and hip extensors (i.e. m. vastus lateralis) (Brennan et al. 2019).

Besides these physiological factors, cycling performance is influenced by pedal power, pedal cadence, air resistance, rolling resistance, pacing strategy, rider position and bike design. However, there are several other factors that influence the relationship between power and cycling velocity. Cycling is based on cyclic joint movements which are characterised by various frequencies and amplitudes. These parameters, together with the anthropometric characteristics of the subject, determine the distance travelled per cycle. In cycling, the distance travelled for each thrust on a pedal with a given crank length is independent of the amplitude of the joint motions. In fact, for any given speed, the gear chosen and, therefore, the force applied determines the distance travelled. In other words: The cyclist is rather free to choose the coupling between force and frequency that allows him to develop a given power and to reach a given velocity. In cycling, the force opposing the movement at a given speed is constant, but the applied force to the cranks is cyclic in nature: In the middle of the cycling thrust the applied force is higher than the force opposing but in the initial and final part it is lower. (Mognoni and Di Prampero 2003). The transfer of power from the human body to the drive train of the bicycle depends upon many factors regarding rider´s position: Crank length, longitudinal foot position on the pedal, pedal cadence, seat height and seat-tube angle. Furthermore, the cyclist´s performance is influenced by the bicycle profile and cyclist´s attire (Faria et al. 2005).

In cycling, performance is determined by the average speed ($m \cdot s^{-1}$), and speed is determined by the energy turnover rate ($J \cdot s^{-1}$) and the work economy ($J \cdot m^{-1}$) (speed=energy turnover rate/work economy) (Di Prampero 1986).

Key muscle groups of cycling are m. vastus lateralis, gluteus maximus, rectus femoris, gastrocnemius, tibialis anterior and soleus. The m. vastus lateralis is a powerful knee extensor. During the downstroke of seated cycling, extensor moments are needed at all lower extremity joints. The m. v. lateralis is observed to be activated earlier in the upward recovery phase and is active longer into the subsequent downward power phase when moving from a sitting on the saddle to a standing position (i. e. during uphill cycling). When uphill cycling, the magnitude and activity of the gluteus maximus, whose function is hip extension, is higher in the standing position than when seated on the saddle. However, the gluteus maximus does not appear to increase extensor moment but rather serves to enhance pelvis stabilisation (Faria 1978).

Cycling can be performed in all terrains and environments. Physical resistive forces present in the environment (e.g. hills or winds) influence greatly the relationship between cycling power and overall cycling velocity. Kyle & Caiozzo (1986) stated, that cycling speeds above 15 $km \cdot h^{-1}$ on level ground, air resistance becomes the major resistive force. The wind influences the cycling speed almost linear (Broker et al. 1999). Air resistance increases as an approximate squared function of cycling velocity therefore, the aerodynamic drag becomes important. Air resistance is the primary energy cost factor at high speeds. The aerodynamic resistance represents > 90% of the total resistance the cyclist encounters at speeds > 30 km/h. At speeds > 50 km/h, aerodynamic resistance is the most performance-determining variable (Faria et al. 2005). Therefore, riders position on the bicycle becomes more important: The aerodynamic advantage from a reduced cyclist's body frontal area when cyclists assume a forward crouched upper body position is well established (Faria et al. 2005).

However, when the cyclist´s upper body configuration is changed from the upright to the aero position there is a significant increase in $\dot{V}O_2$, heart rate (HR) and respiratory exchange ratio (RER). In a study by Gnehm et al. (1997) subjects cycled at 70% of their individual $\dot{V}O_{2max}$ in either an upright-, hands on drops- or hands on clip-on aero-handlebars- bike position at 90 rounds per minute (rpm). Metabolic values ($\dot{V}O_2$, HR and RER) were significant higher in the aero position than in the upright position ($p = 0002$). In fact, the study demonstrated that the aero position requires a higher metabolic cost of

approximately 37 W and decreases net mechanical efficiency of approximately 3%. However, a 30-35% reduction of drag can be expected when changing from upright into the aero position. At the subjects' level of power output (303 W), this reduction of drag would be equivalent to a gain of mechanical power of almost 100 W. It can be surmised that the benefits of reducing air drag in an aero position far outweigh the disadvantage of a slightly reduced mechanical efficiency incurred in this position. (Gnehm et al., 1997).

The drafting cyclists benefit from riding into a lower pressure vortex created by the leading cyclist. Following cyclists may therefore experience a 30% reduction in the required power output compared with the lead rider (Broker et al., 1999). In general, reduces drafting energy cost by as much as 40%. The reduced drag force has the effect of lowering $\dot{V}O_2$, HR, ventilation (VE) and blood lactate concentration ([La]) while cycling when compared with remaining on the front (Faria et al., 2005).

Like the wind resistance, uphill cycling influences the cycling speed linear. During uphill cycling, speeds frequently slow to such an extent that aerodynamic drag is no longer important (Martin et al., 1998). Rolling resistance becomes the major resistance force during uphill cycling, because it can influence power output more than drag at low riding speeds (I. E. Faria, 1978). Rolling resistance is the result of the compression of either the wheel or the ground or both. As the bicycle wheel rolls, the same total area of the tyre and road remain in contact. The greater the area of this “patch” of the tyre and road that are in contact, the greater the resistance will be. Wheel radius, tyre pressure and road surface influence rolling resistance.

Furthermore, the riders body mass and that of the bicycle becomes the most important determinant during uphill cycling, as it has to be lift up against the gravity (Faria et al., 2005).

During uphill cycling, there is a change in muscle recruitment: While uphill cycling the cyclist must overcome the force of gravity and gravity-induced resistance of the body mass. In response to increased resistance, cyclists frequently switch from the conventional sitting position to a less economical standing posture. Consequently, the cyclist can then exert more force on the pedals. Millet et al. (2002) investigated the effects of standing versus seated position. The different positions affect metabolic responses: standing cycling position increases HR and VE. However, the power output during a maximal 30-s sprint was 25-30% greater in the standing position. However, the preferred position during uphill cycling is individual: While some cyclists elect to rise off the saddle and push harder gears (i.e. 39 x 17-21) at relatively low cadence, remain others on the saddle and push hard gears. Standing on the pedals changes the range of motion of the hip joint

substantially during the crank revolution and furthermore, the centre of body mass is modified by standing on the paddles.

For competitive cyclists, finishing in the absolute quickest time possible is the goal. In other words, the goal is to maintain the highest sustainable speed for a given period. In order to do so, there exist different pacing strategies. Common among racing cyclists is a variable pace strategy throughout the race. However, steady pace riding compared with variable pacing has shown to be better for a 20 km time-trial (TT). Furthermore, findings suggest, that to produce the best possible race time it may be best not to vary > 5% for either the slow or fast portion of the race (Faria et al., 2005).

# 2 Physiological demands in cycling

## 2.1 Pulmonary oxygen uptake ($\dot{V}O_2$)

Respiratory muscles work rhythmically and continuously providing an essential function to oxygenate tissues throughout the life. A primary determinate of sports performance is the ability of the cardiovascular system to deliver $O_2$ to the working muscle. For the major forms of whole-body exercise such as walking, running, and cycling the control of upstream is into the $O_2$-transport pathway. The $\dot{V}O_2$ kinetics response is measured most conveniently breath-by-breath using rapidly responding gas analysers and applying algorithms that estimate changes of alveolar $O_2$ uptake (Bassett & Howley, 2000).

The overall $\dot{V}O_2$ demand is higher in exercising muscles than in resting muscles. When exercise intensity increases, so is the $O_2$ demand (Moore et al., 2008). Within seconds-to-minutes following the onset of severe intensity exercise, pulmonary $\dot{V}O_2$ may increase from a resting value of ~0.25 $L.min^{-1}$ up to its maximum value for the individual which, in the extreme, may exceed 5 to 6 liter per min. For constant-load exercise of moderate intensity $\dot{V}O_2$ increases, reaching a constant value (steady state) within about 3 min. The time constant does not vary appreciably with work rate at this intensity.

Fast $\dot{V}O_2$ kinetics mandates a smaller $O_2$ deficit, less substrate-level phosphorylation and high exercise tolerance. Slow $\dot{V}O_2$ kinetics incurs a high $O_2$ deficit, presents a greater challenge to homeostasis and presages poor exercise tolerance. Because of the very limited non-oxidative muscle energy stores, at the transition from rest to exercise there must be a coordinated pulmonary, cardiovascular, and muscular system response to increase rapidly the flux of $O_2$ from atmosphere to muscle mitochondria allowing aerobic ATP production. That $\dot{V}O_2$ does not rise immediately to its steady state suggests that a finite metabolic capacitance may have evolved as a crucial feature of the energy transfer pathways (Poole & Jones, 2012).

Exercise training causes a speeding of the $\dot{V}O_2$ kinetics within very few training bouts and the $\dot{V}O_2$ slow component is reduced by exercise training and often by improved muscle $O_2$ delivery. Trained endurance athletes exhibit extremely fast $\dot{V}O_2$ kinetics whereas detraining, aging and the predations of many chronic disease conditions slow $\dot{V}O_2$ kinetics. Thus, at the transition to exercise, elite cyclists and marathon runners may achieve near constant exercising $\dot{V}O_2$ within 30 to 40 s. In contrast, aged individuals or those suffering

from chronic heart failure or pulmonary disease may require several minutes or more to reach steady state and will consequently, for a given increase in $\dot{V}O_2$, incur a much larger $O_2$ deficit which is associated with premature fatigue (Poole & Jones, 2012).

When cadence increases, so is $O_2$ demand. $\dot{V}O_2$ during pedalling exercise at a given power output increases linearly or exponentially according to the increase in power output and pedal cadence: $\dot{V}O_2$ reaches a steady state only during a low power output exercise below VT and increases linearly with increasing power output. However, during a high-power output exercise - above RCP - the $\dot{V}O_2$ shows a continuous exponential increase until the end of the exercise. This effect is called the slow component of $\dot{V}O_2$ kinetics (Whipp & Wasserman, 1972).

The presence of a slow component in $\dot{V}O_2$ kinetics implies that during an incremental exercise test, after the RCP has been exceeded the $\dot{V}O_2$ to power output relationship has to rise exponentially. The power output at which $\dot{V}O_2$ starts to rise non-proportionally to the power output has been called “the change point in $\dot{V}O_2$”. The $\dot{V}O_2$ slow component can also be described as an additional “slow phase” of $\dot{V}O_2$ (Zoladz et al., 1998).

At work rates that engender a lactic acidosis, however, an additional slow phase of $\dot{V}O_2$ is superimposed upon the underlying kinetics: This is of delayed onset and prolongs the time to steady state over the range within which the increases in [La]. At higher work rates i.e. above what has been termed “critical power” a steady state is unattainable. $\dot{V}O_2$ continues to rise until the end of the test or until exhaustion and will eventually drive $\dot{V}O_2$ to $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ is reached progressively earlier the higher the work rate: it is therefore a fundamental determinant of exercise tolerance (Whipp, 1994).

It was found, that the slow phase of $\dot{V}O_2$, was linearly correlated with the [La] increase. Work rates less than approximately 50% of the difference between $\dot{V}O_{2max}$ and LT result in [La] and $\dot{V}O_2$ curves that reach recognizable asymptotes. At higher work rates, both the [La] and $\dot{V}O_2$ curves continue to rise to the point of fatigue (Roston et al., 1987).

For moderate intensity exercise (<RCP) $\dot{V}O_2$ increases within the first breath (Phase I), there is then a brief surcease prior to the start of the exponential increase of $\dot{V}O_2$ (Phase II) to the steady state (Phase III). In particular, the approach of $\dot{V}O_2$ to its steady state

value within is reached in about 2 to 3 min in a healthy young individual during moderate exercise intensity.

At higher work rates in the serve intensity domain, $\dot{V}O_2$ may either rise rapidly and exponentially to $\dot{V}O_{2max}$ or evince a $\dot{V}O_2$ slow component that increases systematically, driving $\dot{V}O_2$ to $\dot{V}O_{2max}$. Furthermore, there exists a still higher exercise intensity domain, termed “extreme”, where the work rate is so great that fatigue intervenes in < 140 s before $\dot{V}O_{2max}$ can even be achieved (Poole & Jones, 2012).

Pulmonary $\dot{V}O_2$ responds generally as a linear first-order system; at least in the moderate domain. Specifically, following a high work rate “impulse” [e.g., 0 → 500 W (10 s) → 0 W] $\dot{V}O_2$ rises almost instantaneously to a value that is proportional to the impulse area and then declines exponentially to baseline. For the “step” (e.g., 0 → 50 W) in the moderate domain $\dot{V}O_2$ increases exponentially, after a brief delay, to the steady state. There is often a tendency for the primary component $\dot{V}O_2$ kinetics to become slower at higher work rates, especially above the RCP. An explanation is that the slower overall $\dot{V}O_2$ kinetics reflects the increasing contribution to force production of muscle fibers that are higher in the recruitment hierarchy (i.e., type II fibres). There is evidence to suggest that these “higher-order” fibers might have slower $\dot{V}O_2$ kinetics (and also lower efficiency) relative to early-recruited fibers (i.e. type I fibers) (Poole & Jones, 2012).

In general, exercise training improves cardiovascular function and increases vascular transport capacity of skeletal muscle. Blood flow capacity increases within a training period of four weeks. Muscles composed predominantly of slow-twitch fibers (type I fibers) have increased capillarization, arteriolar density and oxidative capacity when compared with white muscle composed mainly of fast twitch fibers (type II fibers). Muscle fiber recruitment depends on duration and intensity of the exercise. At low intensities, deep, high oxidative fibers are recruited and produce the majority of force, while at increasing intensities, fast-twitch fibers are recruited progressively, so that during high-intensity exercise (e.g. sprints) all fibers are active (Laughlin & Roseguini, 2008). The fibres of each muscle have an optimum length for force production and will experience a hyperbolic decrease in force capacity as shortening velocity increases (Gordon et al., 1966).

Ventilation (VE) has the function to facilitate $CO_2$ removal at rates which increase from ~ 0.2 liter per min at rest to, in the extreme, over 6 liter per min during maximal exercise. Thus, VE is coupled closely to $CO_2$ exchange (Poole & Jones, 2012).

The respiratory exchange ratio (RER) is the ratio between the amount of $CO_2$ and oxygen used during any kind of exercise or movement. A high RER indicates that carbohydrates are being predominantly used, whereas a low RER suggests lipid oxidation (Mezzani, 2017).

Measuring $\dot{V}O_2$ is the golden standard of laboratory tests for determining actual fitness status.
During progressive exercise testing, the first ($VT_1$) and second ventilatory threshold ($VT_2$ or respiratory compensation point - RCP), have been shown to be important determinants of performance and fitness level in endurance exercise (Lucía et al., 1999). Furthermore, two of the most important physiological determinants of endurance performance are an athlete's maximum oxygen uptake ($\dot{V}O_{2max}$) and the fractional use of $\dot{V}O_{2max}$ (% of $\dot{V}O_{2max}$) during competition. $\dot{V}O_{2max}$ is defined as the highest rate at which oxygen can be taken up and utilized by the body during severe exercise. Maximal cardiac output is the principal limiting factor for $\dot{V}O_{2max}$ during cycling or running tests (Bassett & Howley, 2000). $\dot{V}O_{2max}$ is not only limited by the cardiac output but also by the oxygen carrying capacity, and in some cases the pulmonary system (Bassett & Howley, 2000).

$VT_1$ represents the first increase in minute ventilation (VE) that is proportional to the increase in $CO_2$ output ($VCO_2$) generated by the $HCO_3^-$ buffering of lactic acid. As a result, the ventilatory equivalent for oxygen increases with no change in the ventilatory equivalent for $CO_2$. RCP, in turn, represents a high work intensity at which blood lactic accumulation increases considerably and is accompanied by an additional hyperventilation in an attempt to buffer acidosis. At this exercise intensity, both the equivalent for oxygen and $CO_2$ show a marked increase whereas end-tidal partial pressure of $CO_2$ starts to decrease (Davis, 1985). VT appears to be an indicator of endurance performance as $\dot{V}O_2$, efficiency and HR at VT remain stable. (Lucía et al., 2000).

Billat et al. (1999) investigated the effects of different pedal cadences and stride frequencies on the $\dot{V}O_2$ slow component. The subjects in this study were well-trained triathletes, who cycled and ran to exhaustion at a stride frequency and pedal cadence lower than their freely chosen cadence (FCC) (for cycling: 82.5 ± 10.0 rpm). They found no difference between the time to exhaustion, the low cadence (for cycling: 74.3 ± 8 rpm) and the FCC, respectively ($p = 0.80$) and no difference for the amplitude of the $\dot{V}O_2$ slow

component between running stride, cycling cadence (low cadence and FCC) was found ($p > 0.05$). The authors therefore concluded, that the $\dot{V}O_2$ slow component is not influenced by the cadence for each cyclic exercise (running and cycling).

## 2.2 Metabolic demands

Measuring physiological responses to exercise is a valid and reliable method to predict performance. The most commonly measured responses include not only $\dot{V}O_2$ but also HR and [La] (Lucía et al., 2000). For energy supply pyruvate is metabolised by $O_2$. When $O_2$ is not available (i.e during high intensities), [La] arises in the muscle. At RCP, [La] begins to increase exponentially (Adeva-Andany et al., 2014).

In endurance sports, the prescription of training loads is often based on HR data. HR is an indicator of exercise intensity up to levels close to $\dot{V}O_{2max}$, and it is possible to use reliable HR monitors during training sessions for immediate feedback to the athlete and for later analysis (Lucía et al., 2000). Lucía et al. (2000) found that despite a significant improvement ($p < 0.05$) in performance throughout a training season in competitive professional road cyclists ($\dot{V}O_{2max} \sim 75.0\ mL \cdot kg^{-1} \cdot min^{-1}$) with increased power output that are associated with LT, VT, or RCP, HR values were stable ($p > 0.05$). These results lead to the suggestions, that HR values are sufficient to adequately prescribe training loads. These results are in agreement with those found by Nimmerichter et al. (2011). In that study no difference between power output and HR were found when the total season (training and competition data) of professional cyclists were analysed ($p = 0.15$).

It is well-known, that during steady-state prolonged exercise, a progressive rise in HR and decline in stroke volume (SV) synthesize a phenomenon known as cardiovascular drift ($CV_{drift}$), which may result in a subsequent drop in mean arterial pressure and cardiac output, which decreases performance (Kounalakis & Geladas, 2012). Kounalakis & Geladas (2012) found a faster $CV_{drift}$ during a 90 minute cycling bout at 60% $\dot{V}O_{2max}$ with 80 rpm than with 40 rpm ($p < 0.05$). Cardiac output was determined during minutes 18–25, 42– 47, 59–65, and 82–88, with duplicate measurements separated by 2 min to ensure physiological steady state. Although $CV_{drift}$ was found in both cadences, at 80 rpm the observed drop in cardiac output, the higher decrease in SV, and the greater increase in HR indicated that $CV_{drift}$ was aggravated at the faster cadence. $\dot{V}O_2$ continued to be higher at 80 rpm than at 40 rpm, and increased progressively at 80 rpm throughout exercise. The

authors assumed that $CV_{drift}$ was most probably because of a reduced cardiac filling time. The significant correlation found in the study between SV decline and HR increase indicates a connection between these two variables ($r = -0.66$ at 40 rpm and $r = -0.71$ at 80 rpm; $p < 0.05$)..

In addition, Kawaguchi et al. (2006) investigated the effects of 60 min submaximal (50% $\dot{V}O_{2max}$) constant load cycling on cardiorespiratory parameters. The authors found a significant decrease in SV and an increasing HR indicating the occurrence of $CV_{drift}$ in all participants. After 40 min of exercise, there was a significant increase in $\dot{V}O_2$. After 30 min of exercise cardiac output remained unchanged ($p > 0.05$), but there was a significant decrease in SV and a proportionate increase in HR, thus indicating the occurrence of $CV_{drift}$. Moreover, the respiratory $\dot{V}O_2$ measured by indirect spirometry demonstrated a significant increase ($p < 0.01$). Further, HR has been shown to drift upwards by as much as 20 bpm during exercise lasting 20-60 min, despite unchanged work rates and steady or decreasing plasma lactate concentrations (Kindermann et al., 1979).

## 2.3 Cycling cadences and physiological responses

Increasing cadence while maintaining power output is associated with a greater metabolic cost due to the increased mechanical internal work required to spin the legs, especially at low power output (Coast & Welch, 1985; Gotshall et al., 1996; Marsh & Martin, 1997; Kounalakis & Geladas, 2012 and Formenti et al., 2015). Furthermore, it has been found, that theoretical models of predictions of $\dot{V}O_2$ can be improved by considering athlete´s cycling cadences (Formenti et al., 2015).

In general, higher cadences create higher internal work (increase in EMG-signal from the muscles), thus more type l fibers are activated (Ahlquist et al., 1992). The higher internal work results in a reduced external work (force on the pedal) (Takaishi et al. 2002). High pedal cadences with reduction of force to the pedal, reduce the force used per pedal stroke. Consequently, muscle fatigue is reduced in type ll fibres. However, when pedal cadence is increased without a reduction in force to the pedal or a harder gear is employed, type ll muscle fibres become progressively recruited. Type ll muscle fibers have a lower mitochondrial density than type l fibres and therefore are more dependent on glycolysis ATP turnover (Faria et al., 2005). So it is possible to manipulate both resistance and cadence to maintain the same overall power output. For example, cycling with a low cadence and high pedal forces may produce the same power output as cycling with high

cadence and low pedal forces. In both conditions, the joint ranges of motion remain relatively similar and only the velocity of movement varies (Brennan et al., 2019).

The most efficient cadence exerts in the lowest $O_2$ uptake, VE, [La], HR and RPE (rates of perceived exhaustion) at a defined fixed work rate. When an most efficient cadence is found, it increases with work rate. (Gaesser & Brooks, 1975). The most efficient cadence has shown to be between 50 and 65 rpm and increases with power output. Furthermore, the increase in energy expenditure observed when pedalling slower than 'most efficient' during a bicycle ergometry is more pronounced at high power outputs than at low outputs. The increase in response to pedalling faster than most 'efficient' is less pronounced at high power outputs than at low outputs. Thus, there is appreciable interaction between cadences and power output in achieving the 'most efficient' rate in bicycle ergometry (Seabury et al., 1977; Coast & Welch, 1985). Most studies that assessed a rather wide range of cadences reported that the lowest pedalling rate is most effective (Gaesser & Brooks, 1975; Lucía et al., 2001).

However, it has been found that the effect of cadence on $\dot{V}O_2$ is gradually reduced when increasing intensity (i.e. during an incremental exercise test (Sidossis et al., 1992 and Boone et al., 2015). Unlike $\dot{V}O_2$, cadences per se do not have any influence on HR, which increases just linearly with $\dot{V}O_2$.

Hagberg et al. (1981) conducted a study where subjects cycled 5 min exercise bouts at 80% $\dot{V}O_{2max}$ on their road-racing bicycles on a treadmill to simulate competitive cycling. The exercise bouts were completed in a random order at 60, 75, 90, 105 and 120 rpm in order to examine the most efficient cadence. $\dot{V}O_2$, RER, HR, [La] and RPE were assessed during all bouts. Cyclists were also studied during a series of unloaded trials to assess the effects of varying cadences independent of external workload. During the unloaded cycling bouts, RPE, [La], and RER were not different from resting values and did not differ significantly at the various pedal cadences. $\dot{V}O_2$ and [La] were quadratically (u-form) related to pedal cadence ($p < 0.05$). The authors stated, that the quadratically relationship between the parameters and the cadences may be the additional work done to move the legs more frequently at the higher pedal cadences. Furthermore, the unloaded cycling bouts show that simply "moving the legs" more frequently does have a significant effect on many of the observed parameters: $\dot{V}O_2$, VE and HR. Those parameters were also significantly affected by the different cadences during unloaded cycling. However, RER, [La] and RPE were not elevated above resting level during the unloaded cycling rides.

During loaded cycling all parameters were best fit by quadratic models, whereas RPE and HR increased linearly with an increase in cadences. One explanation for rising [La] levels could be attributed to a modification of muscle blood flow during the different cadences. As cadence increases, less absolute work (and therefore force) is required per pedal stroke. Since blood flow during the time of contraction is known to be related to the relative fore of contraction, an increased pedal frequency will cause less occlusion of blood flow during the contraction period. This more uniform blood flow would affect $\dot{V}O_2$ very little but could affect [La] levels. $\dot{V}O_2$ could be affected to the various muscles that may be used at higher cadences to stabilize the trunk and to eliminate irrelevant motion. However, the results demonstrated that competitive cyclists are most efficient at an average pedalling cadence of 91 rpm. All variables measured in that study except HR and RPE tended to increase when cadence was either above or below the preferred cadence (Hagberg et al., 1981).

Coast & Welch (1985) designed an experiment to examine the optimal pedal cadence. Five trained cyclists performed progressive maximal tests. Each at cadences of 40, 60, 80, 100 and 120 rpm. $\dot{V}O_2$ and HR, derived during the tests, were plotted against cadence for power outputs of 100, 150, 200, 250, and 300 W. They found significant differences in $\dot{V}O_2$ and HR between the cadences ($p < 0.05$). The lowest point of the $\dot{V}O_2$ and HR curve was taken as the optimal pedal rate; i.e., the pedal rate which elicited the lowest HR or $\dot{V}O_2$ for a given power output (the most “efficient” cadence as already mentioned). When the optimum was plotted against power output the variation was linear. Therefore, the authors could show, that an optimal cadence in that group exists and it increases linear with power output.

The subjects in the study of Ahlquist et al. (1992) exercised 30 min at 85% of their aerobic capacity at cadence of either 50 or 100 rpm. Muscle biopsy samples of the m. vastus lateralis were taken immediately prior to and after exercise to identify the used muscle fiber type. They concluded, when cycling at the same intensity, 50 rpm leads to greater type II fiber glycogen depletion. This is attributed to the increased muscle force required to meet the higher resistance per cycle at the lower pedal frequency.

In a study by Gotshall et al. (1996) seven cyclists were asked to cycle 15 min at 200 W at cadences of 70, 90 and 110 rpm. $\dot{V}O_2$, HR, SV, cardiac output, blood pressure and vascular resistance were determined. The authors found that HR, SV, cardiac output and blood pressure were increasing, and vascular resistance decreased, with increased

cadence. Even though the workload was constant, the increase in cadence resulted in a more effective skeletal-muscle pump which increased muscle blood flow and venous return. These results could be partly explaining the natural selection of higher cadences by cycling athletes.

Brisswalter et al. (2000) have shown that the theoretical energetically optimal pedalling rate, corresponding to the lowest point of the $\dot{V}O_2$ - cadence relationship shifted progressively over the duration of exercise towards a higher pedalling rate (from 70 to 86 rpm) which was closer to the freely chosen one. Therefore, a minimum of energy cost seems not to be a relevant parameter for the choice of cadence, at least in a non-fatigued state. A change of muscle fibre recruitment pattern with exercise duration would explain the shift in energetically optimal rate towards a higher pedal rate. If there is a particular tendency for professional cyclists to increase their FCC as power output increases, it may hypothesise that they have physiologically adapted and have become more economical, or efficient at higher workload.

However, Deschenes et al. (2000) found that there are no significant differences in $\dot{V}O_2$ between 30 min cycling at 50-55% $\dot{V}O_{2max}$ with 40 and 80 rpm, respectively ($p > 0.05$). Furthermore, the different cadences had no effect on VE or RER. Mean arterial pressure was similar for both cadences, whereas there was a trend ($p = 0.08$) for mean arterial pressure to be higher during the final minute of cycling at 40 rpm compared with the same time point during the 80-rpm session. Recovery of mean arterial pressure was determined to be significantly slower after cycling at 40 than at 80 rpm ($p < 0.05$). Like the arterial pressure, HR at 40 rpm was significantly higher than at 80 rpm during the final minute of exercise. Unlike at the faster cadence, pedalling at 40 rpm appeared to have a cumulative effect; there was a significant increase in HR from the 15th to the 30th min of exercise. Furthermore, plasma cortisol was elevated by the 30th min of cycling at 40 rpm and remained so throughout a 15-min recovery period. The authors revealed that pedalling rate significantly influenced RPE ($p < 0.05$). At 15 min of exercise, similar RPE scores were reported for each cadence. However, by the 30th min of exercise, subjects sensed their efforts to be more strenuous when pedalling at 40 compared with 80 rpm. EMG recordings showed, that during the force-production phase of pedalling, muscle recruitment was significantly greater while cycling at 40 than at 80 rpm, indicating more intense muscle contraction at the slower cadence. This could be due to a greater recruitment of higher threshold motor units at the slower cadence and/or a faster firing rate of the same motor units activated at 80 rpm. The higher RPE scores during 40 rpm may

be explained by the cadence-specific differences in cardiovascular responses. Therefore, HR and local factors, including muscle force, regulate the RPE during exercise (Deschenes et al., 2000).

Lepers et al. (2001) examined the effect of different pedalling cadences upon various physiological responses (HR, $\dot{V}O_2$, and VE) during endurance cycling exercise. The subjects, representing well-trained triathletes, cycled three times for 30 min each at an intensity corresponding to 80% of their maximal aerobic power output. The first test was performed at FCC, the two others at FCC -20% and FCC +20%. They found a significant time effect in $\dot{V}O_2$ and VE ($p < 0.01$) The values increased mainly at the 5th and 30th min. It was suggested this could occur due to $CV_{drift}$, previously described. No significant effect was found between the different cadences ($p > 0.05$). This could be explained by the relatively small range of cadences used in the present study. The only difference observed between cadences in this study occurred in VE in the first part of the exercise ($p < 0.01$). It could be shown, that VE was significantly higher at FCC +20% compared to FCC -20% at the 5th and 15th min but not at the 30th min. For $\dot{V}O_2$ and HR no significant effect of cadence was observed. The investigators suggested, that well-trained triathletes can easily adapt to the changes in cadence used habitually during racing.

In a study by Takaishi et al. (2002) the authors investigated the effects of cycling experience and pedal cadences. The subjects conducted 4-min cycle bouts with cadences of 50, 75, 85, and 95 rpm (in random order) with 2 min of exercise for active rest at 100–140 W (depending on subject´s fitness level) between the exercises. Significant main effects and an interaction for the cycling experience and cadence in comparisons of the $\%\dot{V}O_{2max}$ and $\%HR_{max}$ were found. The $\%\dot{V}O_{2max}$ value for untrained was significantly larger than that for cyclists at 85 rpm. The effect of pedal cadence at 85 rpm was higher than those at 50 and 75 rpm in untrained subjects. For $\%HR_{max}$ there were no differences among the values at 50 and 75 rpm, but at 85 rpm, the value for untrained was significantly larger than that for cyclists. For triathletes, the value of $\%HR_{max}$ at 85 rpm was higher than that at 50 rpm. Cyclists had no differences in either $\%\dot{V}O_{2\ max}$ or $\%HR_{max}$ among the pedal cadences. Suggesting that trained cyclists are able to cope with different cadences.

Mora-Rodriguez & Aguado-Jimenez (2006) investigated if pedalling cadences in a range from 80 to 120 rpm affects final power output during a continuous incremental test. Nine competitive, well-trained road cyclists participated in this study. The FCC (89 ± 1.4 rpm) during the preliminary $\dot{V}O_{2max}$ test was not statistically different from 80 and 100 rpm but

was 34% below 120 rpm ($p < 0.05$). $W_{max}$ achieved before exhaustion during the incremental cycling test was similar during the 80- and 100-rpm trials. During 120-rpm trial, however, $W_{max}$ was reduced approximately 9% because of exhaustion ($p < 0.05$). Furthermore, VE during 120 rpm was higher than during the 80- and 100-rpm trials at all stages ($p < 0.05$). During the 120-rpm trial RCP (power output at ventilatory anaerobic threshold) was reduced 11% below the 80-rpm trial ($p < 0.05$). The authors suggested that in trained cyclists RCP is a suitable predictor of the effects of cadence on performance.

Moore et al. (2008) tested the effects of moderate cycling intensities (50 and 65% of $\dot{V}O_{2max}$) at different cadences (80 and 100 rpm) on SV, HR and $\dot{V}O_2$. The authors found that $\dot{V}O_2$ was greater at 100 rpm than at 80 rpm for both workloads ($p = 0.05$). Furthermore, HR was higher at 100 rpm than at 80 rpm in both workloads ($p < 0.05$). Interestingly [La] values remained unchanged at both cadences at 50% $\dot{V}O_{2max}$, whereas it was significant higher at 100 rpm than at 80 rpm at the higher workload corresponding 65% $\dot{V}O_{2max}$ ($p < 0.05$).

Jacobs et al. (2013) investigated the effect of different cadences (60, 80 and 100 rpm) on HR, [La] and RPE during 8 min trials at an exercise intensity of 75% $P_{max}$. HR was lower at 60 rpm than at 80 rpm ($p = 0.026$) and 100 rpm ($p < 0.001$), and HR at 80 rpm was less than at 100 rpm ($p < 0.001$). Additionally, HR was higher at minute 8 than at minute 4 ($p < 0.001$) regardless of cadence. Furthermore, [La] at 100 rpm was greater than at 60 ($p = 0.039$) and 80 rpm ($p = 0.048$) regardless of time. Furthermore, [La] at minute 8 was higher than at minute 4 ($p = 0.001$) regardless of cadence. RPE was higher at 100 rpm than at 60 rpm ($p = 0.010$) and 80 rpm ($p = 0.003$) at minute 8.

Zorgati et al. (2015) found no significant difference in $\dot{V}O_2$, HR and [La] during an exercise until exhaustion – test at 90% $\dot{V}O_{2max}$ between 40 and 90 rpm ($p > 0.05$). Furthermore, the authors reported a similar time to exhaustion between those two cadences. The lack of difference in the cardiorespiratory and metabolic parameters may be due to the high exercise intensity. Considering, that the effect of cadence on $\dot{V}O_2$ is gradually reduced according to the increase of power outputs (Boone et al., 2015).

Hirano et al. (2015) examined the effects of a single bout of exercise at different cadences on physiological responses. The defined cadences were 35, 50 and 75 rpm. The physiological responses of interest were $\dot{V}O_2$, VE, HR and [La]. It was found that exercise

at 35 and 50 rpm resulted in significantly lower $\dot{V}O_2$, VE, HR, and [La] than exercise at 75 rpm ($p < 0.01$).

Nimmerichter et al. (2015) conducted a study where trained subjects had to cycle 2 trials on a flat course and 2 uphill trials with 60 and 90 rpm, respectively. The authors also found higher end exercise $\dot{V}O_2$ values and a higher HR during cycling at 90 rpm ($p < 0.001$).

When Graham et al. (2018) investigated the effects of different cadences (60 and 90 rpm) on TT performance in professional cyclists, they found a significant higher HR ($p = 0.03$), $\dot{V}O_2$, [La] and RPE at 90 rpm than at 60 rpm ($p < 0.001$).

In general, cardiorespiratory and metabolic demands such as $\dot{V}O_2$, [La] and HR increase with increasing intensity and increasing cadence ( Hagberg et al., 1981 and Hirano et al., 2015) while cycling at moderate power outputs (Zoladz et al., 1998). However, it has been found that the effect of cadence on $\dot{V}O_2$ is reduced with increasing intensity ( Boone et al., 2015 and Zorgati et al., 2015). Moreover, RPE has shown to be higher at those cadences (Nimmerichter et al., 2012, Graham et al., 2018, Kounalakis & Geladas, 2012, Hirano et al., 2015, Mitchell et al., 2019, Moore et al., 2008). Regardless of power output, increasing cadence during unloaded cycling leads to an u-form response of $\dot{V}O_2$ (Hagberg et al., 1981).

## 2.4 Influences of different cadences on efficiency

During locomotion an imaging flow of energy takes place from the metabolism to the environment, with some efficiency. Mechanical efficiency of cycling i.e. 20–25% is much less than that of running at 45–70%. Muscle tension during running is applied for a shorter duration and at higher levels than in cycling which has an isometric contraction phase (Ettema & Lorås, 2009).

Cycling performance is strong related to pedal power. Since power is defined as mass x acceleration, the pedal power and pedal cadence are essential factors in cycling performance (Atkinson et al., 2003). Furthermore, pedal cadence and gear ratio influence the gross efficiency (GE) and therefore overall cycling performance. Therefore, efficiency is largely influence by cycling cadence and power output (Mora-Rodriguez & Aguado-Jimenez, 2006).

Pedalling rate is widely accepted as an important factor affecting cycling performance. Several factors influence the efficiency of cadences. These factors include crank length, body position, linear and angular displacements, velocities and accelerations of body segments and forces in joints and muscles (Faria et al., 2005). Cadences have an influence on GE in low intensity exercicses. A range of cadences can be observed in equally performing riders during any particular stage of a cycling competition (i.e., 80–99 rpm for flat stages; 62–80 rpm for high mountain ascents (Lucía et al., 2001).

GE describes the ratio between mechanical work (power) accomplished and energy expended. Both variables indicate a linear or slightly exponential relationship. Whereat the linear relationship occurs in low work rates and the slightly exponential relationship in higher work rates (Gaesser & Brooks, 1975). GE of an energy converting system is the ratio of free energy outflow over the total free energy inflow. In muscle contraction, the free energy outflow is work. The energy cost is usually expressed as metabolic cost, which is often calculated from $\dot{V}O_2$ and [La] production. Efficiency is a parameter describing a quality of the energy flow that runs through that particular system. Aside from that, delta efficiency (∆efficiency) involves ∆power and ∆metabolic rate, where ∆power stands for increment of power and ∆metabolic rate for the increment of metabolic rate with increasing work rate.

As mentioned above, the two most obvious variables that affect efficiency are cadence and work rate. Gaesser & Brooks (1975) found, that during steady state exercises efficiency decreases with increasing cadence speed. However, mainly work rate influences GE. Indeed, factors other than work rate, including cadence, explain less than 10% of the variation in GE. Indicating, that the influence effect of cadences on GE decreases when power output increases (Ettema & Lorås, 2009).

GE during cycling has been reported to be between 18 and 28% (amateur competitive cyclists up to professional cyclists). There is no significant difference in GE between amateur and professional cyclists at low workloads. However, professional cyclists have a tendential greater GE, due to higher workloads accomplished. For professionals, the ability to sustain high power outputs for long periods as well as a high GE are required for success. Complementing these characteristics would be a high level of muscle capillarisation to supply enough oxygen and substrate to sustain the large energy outputs (Lucía et al., 2001).

Maximal strength training has shown to increase efficiency in the working muscle. Barrett-O'Keefe et al. (2012) have identified the trained skeletal muscle as the site of this improved work efficiency. This study was able to document that the metabolic demand has been reduced in the muscle because of the maximal strength training. Another hypothesis is, that muscular strength improvement creates a decrease in the fraction of maximal pedal force necessary for each pedal thrust, thereby shifting the pattern of muscle fiber recruitment toward type I fibers, resulting in reduced energy expenditure (Hickson et al., 1988).

Chavarren & Calbet (1999) showed in seven competitive road cyclists, that the relationship between power output and GE increased parabolic ($r = 0.94$, $p < 0.05$). Regardless of cadence, GE improved with increasing exercise intensity ($p < 0.001$). Conversely, GE decreases with increasing cadence ($p < 0.001$). Moreover, interactions were found between these two factors: The effect of pedalling cadence on GE decreased as a linear function with increasing power output ($r = 0.98$, $p < 0.001$). Therefore, the impact of cadence on efficiency seems most remarked at lower work rates (Chavarren & Calbet, 1999). Similiar results were found by Samozino et al. (2006), who investigated the GE-cadence relationship during 4 constant power output-tests (40, 80, 120 and 160 W) in which the cadence varied in five bouts from 40 to 120 rpm.

Foss & Hallén (2005) investigated the effect of different cadences on TT performance. 14 male elite cyclists performed two or five TT at different cadences (60, 80, 100, 120 rpm or freely chosen cadence). They were instructed to complete a TT (about 30 min) as fast as possible. The total workload was the same between the TTs. GE at 80 rpm was larger than at the other cadences ($p < 0.05$). This study shows that elite cyclists perform better at 80 rpm compared to 60, 100, 120 rpm and freely chosen cadence (90 -105 rpm) in a TT lasting about 30 min. The highest GE was achieved at 80 rpm, while the average energy turnover rate was highest at 100 rpm, indicating less development of fatigue during 100 rpm. The optimal cadence found in this study (80 rpm) is lower than the cadence typically preferred by elite cyclists (90–105 rpm). Based on the finding that the most efficient cadence increases with increasing workload, one cannot exclude the idea that professional cyclists are more efficient at cadences even higher than 80 rpm. The study showed that the difference in performance between 80 and 100 rpm was about 30 s for a 30-min TT. This is significant, but probably within the range of error for the self-experienced feeling of maximal performance.

In a study by Jacobs et al. (2013) moderate trained subjects performed three 8-minute trials at cadences of 60, 80, and 100 rpm at 75% of $P_{max}$. $\dot{V}O_2$ and RER were used to calculate efficiency and economy. It was also found that as cadence increased, efficiency decreased. In fact, cycling at 60 rpm was more efficient than at 80 rpm ($p = 0.031$) and 100 rpm ($p < 0.001$), and cycling at 80 rpm was more efficient than 100 rpm ($p < 0.001$). Efficiency was also influenced by duration of cycling time because it decreased from minute 4 to minute 8 ($p < 0.001$). As cadence increased, economy also tended to decrease ($p < 0.001$). These results are in opposition with those of Lucía et al. (2001) and Mora-Rodriguez & Aguado-Jimenez (2006). The contradictory results may be because of the training status of the subjects. This study condicted the experiments with trained local cyclists, whereas Lucía et al., 2001 studied 8 world-class professional cyclists.

Similar results were observed by Nimmerichter et al. (2015) who investigated effects of different cadences on GE under field conditions. They conducted four trials of 6 min duration at 90% of ventilatory threshold (VT) on flat and uphill terrain assessing cadences of 60 rpm and 90 rpm, respectively. They found, that GE was significantly affected by the different cadences ($p = 0.029$). In fact, GE was higher at 60 rpm than at 90 rpm ($p < 0.01$). These results are consistent with those previous found by Davison et al. (2000), who found an decrease in GE when cadence increased ($p < 0.05$).

Recently, Graham et al. (2018) examined the effects of different cadences on TT performance in female professional cyclists. The subjects conducted two laboratory TTs at 60 and 90 rpm, respectively. The subjects cycled the 8 km TTs with individual maximal effort. The randomized TT testing sessions were completed 1 week apart. The “distance” of 8 km was based on power output provided directly by the cycle ergometer. The authors found a significant ($p < 0.05$) faster TT finishing time, higher power output ($p = 0.003$) and higher GE ($p < 0.001$) during 60 rpm. During 100 rpm there was a higher HR, $\dot{V}O_2$, [La] and RPE ($p < 0.05$).

Most studies found, that GE increases when power output increases and cadence decreases (Gaesser & Brooks, 1975; Davison et al., 2000; Nimmerichter et al., 2015; Graham et al., 2018). However other studies, like Sidossis et al. (1992) found, that in well-trained cyclists at a high intensity (80% of $\dot{V}O_{2max}$), there were no differences in GE between cadences of 60, 80, or 100 rpm ($p > 0.01$). Therefore, it is possible that long-term training results in the ability to use higher cadences without changing the metabolic cost of the work performed. Furthermore, the effect of cadence on GE decreases when power

output increases, indicating that during high power outputs there are no significant differences between high and low cadences, respectively.

For high-intensity cyclic exercises (cycling, running, rowing etc.) the time to exhaustion is inversely proportional to the rate the work is performed. This hyperbolic relationship between power and endurance was originally described by Hill (1925). The author investigated physiological fatigue in runners, swimmers and rowers. The basis for the model is the fact, that a relationship between time and distance is evident. While high intensities are maintainable only for shorter times, decreasing intensity results in increasing distance.

McNaughton & Thomas (1996) found, that a significant greater time to exhaustion is present when pedalling at 50 rpm than at 90 rpm ($p < 0.01$) or 110 rpm ($p < 0.001$). Maximum sustainable power output during cycle ergometry was higher at 50 rpm than at either 90 or 110 rpm. At the intermediate cadence endurance performance was higher than at the high but lower than at the lowest cadence. However, that study was conducted with subjects, who were non-competitive, recreational cyclists.

However, Kohler & Boutellier (2005) proposed that the preferred (higher) cadence of competitive cyclists can be explained by the inherent properties of muscle fibers that are described by Hill (1938) as functions relating muscle force and heat liberation to shortening velocity. These functions contain constants that depend on muscle size and fiber type. Thus, each muscle has its own force–velocity relationship and its own heat liberation. According to the author´s calculation, the “most powerful” cadence at a workload of 50 – 300 W lies between 80 and 115 rpm if the cross-sectional areas of both fiber types are equal. (It should be noted that using the “most powerful” cadence is not a synonym for maximal power output. Using the most powerful cadence means that the greatest power is produced for a given cross-sectional area. In other words: Using the most powerful cadence, a given power can be produced by activating the least number of muscle fibers) (Holmes, 2006).

Using the most powerful (higher) cadence results in a higher energy turnover at a given workload than using the most efficient (lower) cadence. In competitions of short duration, the energy aspect may not be important. In a long-distance race, however, the importance of energy supply increases: using the most powerful (higher) cadence may result in early exhaustion of the cyclist. In track cycling and sprints, cadences of 120 rpm and more are observed because the duration of the race is rather short, and the most powerful cadence

is more advantageous. In very long-distance races, low cadences between 50 and 60 rpm are preferred because energy uptake and turnover rate is limited and using the most efficient cadence is more advantageous than using the most powerful one. However, the authors concluded, that it would be too simple to state that the optimal cadence decreases linear from the most powerful to the most efficient cadence as the race duration increases. Although this is theoretically true, the most advantageous cadence is a function of many variables i.e.: energy supply, fiber type, fatigue, workload, duration etc (Kohler & Boutellier, 2005).

However, Emanuele & Denoth (2012) stated that the power – cadence relationship for endurance cycling can be well fitted with a quadratic relationship, whereas the optimal cadence at first increases with increased power output and then the power output decreases with further increase in cadence (up to 120 rpm).

It has been shown that at the same power output and cadence, crank inertial load is higher during level than during uphill cycling because crank inertia increases as a quadratic function of the gear ratio (Fregly et al., 2000). In addition, an increase in crank inertia is accompanied by an increase in peak crank torque and therefore it is suggested, that cyclists prefer higher cadences during level ground cycling to reduce peak crank torque (Ernst A. Hansen et al., 2002 and Jacobs et al., 2013).

The study of Neptune & Hull (1998) investigated the effect of different cadences (75, 90 and 105 rpm) on neuromuscular parameters (e.g. muscle force, activation and stress). The authors found that an optimal cadence, which was similar to the preferred cadence for the power output assessed, neuromuscular parameters were minimised compared to lower or higher cadences. Furthermore, the results showed that neuromuscular parameters were minimized at the 90-rpm pedalling rate.

Coyle et al. (1992) investigated the efficiency of muscle fiber types during an exercise intensity of 50 and 70% of $\dot{V}O_{2max}$ in trained cyclists. The percentage of type I and II muscle fibers was determined from biopsies of the m. vastus lateralis muscle. The subjects were cycling on a ergometer at 80 rpm at work rates of 50 and 70% of $\dot{V}O_{2max}$. GE was calculated as the ratio of work accomplished $\cdot$ min$^{-1}$ to caloric expenditure-min$^{-1}$, whereas $\Delta$-efficiency was calculated as the slope of this relationship between approximately 50 and 70% $\dot{V}O_{2max}$. The type I fibers were positively correlated with $\Delta$-efficiency ($r = 0.85$; $p < 0.001$) and GE ($r = 0.75$; $p < 0.001$) during cycling. The authors stated, when trained cyclists

exercise under these conditions (50 – 70% $\dot{V}O_{2max}$), Type I muscle fibers appear to be substantial more efficient than type II muscle fibers. That is a result of a lower rate of ATP turnover as reflected by a lower $\dot{V}O_2$ while performing exercise at that given output.

Professional cyclists have a high amount of type I muscle fibres (fatigue resistant and long duration movements) rather than type II fibres (faster and stronger short duration movements). Further evidence of the importance of type I fibres are the cadences used typically by professional cyclists. These relatively high (> 90 rpm) FCC do not tend to minimize $\dot{V}O_2$ but rather minimize force per pedal stroke - a method to reduce the recruitment of type II fibres (Lucía et al., 2001).

### 2.4.1 Freely chosen cadence (FCC)

Preferred cadences (or freely chosen cadences - FCC) vary with power and terrain. At the same power output, low cadences cause pedal force to be high in each pedal thrust. Exactly how high the pedal force in each pedal thrust becomes, is a result of the combination of power output and cadence. When cycling at a constant power output, pedal force increases with a decrease in cadence. It has been reported that the cadence choice affects the cycling adaptation at different biological levels regardless of the sport background. Thus, subjects adopt cadences that minimise either the oxygen demand, muscular activity, joint moments or pedal forces applied to the cranks for a given power output. Cadence selection has been shown to play a role across metabolic responses by affecting efficiency, onset of fatigue, and maximal sustainable power output (Brisswalter et al., 2000). During prolonged strenuous cycling, or during intensive cycling at high effort, cyclists typically find it difficult to apply anything other than their freely chosen cadence. Still, it is of course possible, with volitional control and exertion, to apply particular cadences that are considerably higher or lower than the freely chosen cadence (Hansen & Rønnestad, 2017)

Depending on the activation conditions, peak muscle power may be observed at faster cadences than peak efficiency. As such, it may not be possible to maximise power and efficiency at the same cadence, which may impact on a cyclist's preferred cadence during cycling (Brennan et al. 2019).

As in the previous chapter described, experienced cyclists or triathletes usually select a relative high pedalling cadence, close to 80-90 rpm. The reasons behind the choice of such a cadence are still controversial and are certainly multi-factorial. Several assumptions

relating to neuromuscular, biomechanical or physiological parameters have previously been proposed (Billat et al., 1999; Lepers et al., 2001). Furthermore, the reasons that experienced cyclists adopt a higher cadence around 90 rpm during endurance cycling is not well explained by energy expenditure which is lower at low cadences (Takaishi et al., 2002).

Takaishi et al. (1994) investigated the difference in the preferred cadence between cyclists and non-cyclists. Cadences in this study were 45, 60, 75, 90 and 105 rpm at 150 and 200 W. They found that the peak pedal force significantly decreased with increasing cadence in both groups. Further, the decrease in non-cyclists was higher than that for cyclists at each cadence despite the same power output. Also, the iEMG amplitude for m. vastus lateralis and medialis increased in non-cyclists with rising cadence, however, cyclists did not show such a significant increase. On the other hand, iEMG data for m. biceps femoris showed a significant increase in cyclists while there was no increase in non-cyclists. The authors suggested, that cyclists have a certain pedalling skill, allowing them a positive utilization for knee flexors up to the higher cadences, which would contribute to a decrease in peak pedal force and which would alleviate muscle activity for the knee extensors. Furthermore, the pedalling skills that decrease muscle stress influence the preferred cadence selection.

In addition, Takaishi et al. (1996) found, that the pedal cadence that one prefers for a constant power output depends on neuromuscular fatigue in the working muscle rather than energy expenditure and that the pedal cadence for lower neuromuscular fatigue shifts to a higher cadence with cycling experience ($p < 0.05$). In other words, it was found that the preferred cadence (80 - 90 rpm) of the cyclists, who participated in that study, was coincident to the rate at which minimal neuromuscular fatigue took place, although $\dot{V}O_2$ at these cadences was significantly higher than at 70 rpm and/or 60 rpm.

These results are consistent with those from Marsh & Martin (1997) who observed, that preferred cadences in trained cyclists are close to 90 – 100 rpm. Those high cadences were much higher than those spontaneously chosen by untrained or less-trained subjects in that study. These data also suggest that a specific cycling training engenders an improvement in pedal skill leading to higher pedal rate. Furthermore, the authors concluded that cycling experience and minimization of aerobic demand are not critical determinants of preferred cadence in well-trained individuals. It was speculated that less-trained non-cyclists, who cycled at a higher intensity, may have selected lower preferred cadence to reduce aerobic demand.

A study by Davison et al. (2000) found a significant higher FCC (~ 80 rpm) in a 6 km uphill climb with 6% gradient than in 1 km uphill climb with 12% gradient (~ 60 rpm) ($p < 0.05$). The higher cadence found in the flatter climb is similar to the most economical cadence reported for level riding ( Hagberg et al., 1981; Coast & Welch, 1985). However, on the steeper gradient climb, where riders produce high power outputs, a lower cadence was found. In addition several of the participants chose to ride out of the saddle in a standing position on the steeper climb, where it is more difficult to maintain higher cadences. Standing on the pedals allows the involvement of other muscle groups to assist force production. The authors concluded that it is possible that the most effective cadence decreases as the gradient of a climb increases.

On flat courses or TT competitions, the preferred pedal cadence in professional cyclists is around 90 rpm. Whereas cyclist prefer a significant lower cadence (70 rpm, $p < 0.01$) during uphill cycling (e.g. high mountain passes ~ 15 km distance) (Lucía et al., 2001). The high cadences appearing on flat TT reduce the force used per pedal stroke, and lower muscle fatigue. Furthermore, cyclists increase the pedalling rates because their aerodynamic posture does not allow high force production. In addition, a decline in RPE is more pronounced at the highest power outputs. Although professional cyclists select high cadences, they have to push hard gears in order to meet the high requirements of competition. Thus, the selection of slower pedalling rates would imply the use of extremely high gears during long periods.

Gear measurement uses two numbers (e.g. 53/19), where the first is the number of teeth in the front chainring and the second is the number of teeth in the rear sprocket. High speeds (~ 50 km/h) can only be reached at pedal rates greater than 90 rpm, because lower cadences would require the use of hard gears (i.e.: 60 x 11 at 80 rpm). Another example: To reach ~ 50 km/h at 60 rpm, one would have to use an extremely high and non-existent gear ratio of 80 x 12. This is supported by the fact, that pedalling cadence was significantly correlated to cycling speed during TT ($r = 0.66$, $p < 0.05$) suggesting that the ability to adopt high cadences (90 rpm) at high power outputs is an important determinant of TT performance (Lucía et al., 2001).

The reasons for choosing lower cadences during uphill cycling are still not clear. Lucía et al. (2001) suggested, that the specific conditions of climbing performance (negative effect of the force of gravity and the high rolling resistance because of lower velocities) are too demanding for most cyclists to overcome the higher cardiorespiratory work, e.g. $\dot{V}O_2$, VE

and HR, related to high pedalling rates. Therefore, professional cyclists spontaneously adopt the more economical lower cadences on uphill cycling, because as mentioned above, cadences between 50 – 60 rpm producing the highest efficiencies.

Nesi et al. (2005) investigated, if the FCC could be used as an index of cycling performance. In this study, 13 competitive cyclists cycled at their individual FCC during a graded exercise test (GXT) test and a supra-threshold constant power test (∆50-intensity = VT+[Pmax-VT]*0.5). The authors found a strong relationship ($r = 0.86$; $p < 0.001$) between exhaustion time, determined during the constant power test, and the preferred cadence. Indicating an increase in time to exhaustion when cadence increased. Furthermore, a relationship ($r = 0.66$; $p < 0.001$) between $P_{max}$, determined during the GXT, and preferred cadence during the constant power test was found. Indicating a higher $P_{max}$ with higher cadences.
However, these findings are in disagreement with other studies, which have demonstrated that high pedal rates (90 and 100 rpm) lead to a decrease in exercise tolerance and exhaustion time compared to low cadences (50 and 60 rpm) (e.g.. Kohler & Boutellier, 2005; Emanuele & Denoth, 2012).

Hansen & Ohnstad (2008) investigated how “robust” the FCC are during submaximal cycling. The authors examined the effect of increases in loading on the cardiopulmonary system (changed by exposure to acute simulated altitude of 3.000 m above sea level) and mechanical load (increasing power output). The results show, that the increase in loading on the cardiopulmonary system as well as in the mechanical load did not cause participants ($N = 8$) to change their pedalling rhythm ($p > 0.05$). With small changes in their pedalling rates, the participants could have reduced the loading on $\dot{V}O_2$ demands (by choosing slower cadences) and the mechanical loading (by choosing higher cadences). The results indicate, that the freely chosen cadence is primarily a robust voluntary motor rhythm. Similar results were previously found by Takaishi et al. (1994).

Ludyga et al. (2016) investigated the effect of the type of regulation of work rate on the relationship between this work rate and FCC. In that study subjects cycled either “free” on a competition bike mounted on an electromagnetic roller or on an ergometer, which enables a constant work rate, independent of cadence. The authors found a clear difference ($p < 0.05$) in FCC between both groups. While the ergometer group maintained a constant FCC during all work rates (100, 150 and 350 W), the group on the “free” roller increased their FCC from low to high work rates (from 72 rpm at 100 W to 106 rpm at 350 W). It was hypothesised, that this difference is due to “action and reaction” manners: The

subjects on a fixed ergometer are reacting on a change made by the experimenter, the work rate increases because of a higher resistance of the ergometer. The subjects may initially reduce cadence because of the mismatch between the propulsive and resistive forces. The most intuitive way to obtain the new higher work rate is to increase the propulsive force rather than cadence. The situation is different when cycling freely on a roller system: The subjects were asked to increase the work rate to a new level. As a result, the cyclist must act, not react, to obtain that new level. The first natural way is to simply increase cadence in the given gear or/and choose a heavier gear. The authors pose that the easiest way to react to the changed work rate condition is the first to increase cadence and then possibly change gear.

## 2.5 Differences between triathletes and cyclists

Triathlon is a multidiscipline endurance sport that involves swimming, cycling and running. In order to balance the training demands of the three disciplines, triathletes most often practice two or three disciplines in one training session or complete separate training sessions for different disciplines with only short recovery periods, i.e. 2 – 4 h (Chapman et al., 2007).

Considering the differences between cyclists and triathletes, there is an obvious difference in the position on the bike. Millet et al. (2002) examined the effects of cycling position (seated or standing) during level-ground and uphill cycling on GE and economy. Eight trained cyclists performed five trials of 6-min duration at 75% of $P_{max}$ either on a velodrome or during the ascent of a hill in seated or standing position. GE and economy were calculated by using the mechanical power output that was measured by a crank set and energy consumption by a portable gas analyser. In addition, each subject performed three 30-s maximal sprints on a cycle ergometer or in the field either in seated or standing position. The authors found, that GE and economy were not different between level seated, uphill seated, or uphill standing conditions ($p < 0.05$). HR was significantly ($p < 0.05$) higher in standing position. Further, in the uphill cycling trials, VE was higher ($p < 0.05$) in standing than in seated position. The average 30-s power output was higher ($p < 0.01$) in standing than in seated position or on the stationary ergometer. The different positions on the bicycle not only have an effect on cardiorespiratory parameters but also on the selected cadence. The main difference between the two seated conditions is the higher cadence in the ground-level compared to the uphill trials (90 vs 59 rpm) ($p < 0.05$). The cadence was ~60 rpm in the two uphill conditions and ~90 rpm in level cycling. These

results are in agreement with the results from Lucía et al. (2001). Further, as previous described, trained cyclists cadence higher than the unskilled cyclists (Lucía et al., 2001).

Similar results were found by Gnehm et al. (1997), who stated, that although the frontal area will be lower and the drag coefficient is reduced when using aerobars, this position may be less efficient and oxygen uptake and heart rate may be increased.

Vercruyssen et al. (2001) indicated a reduction in FCC from 90 to 82 rpm towards the energetically optimal cadence following 1 h 15 min of cycling. The decrease in FCC with increase in exercise duration has been exclusively found in trained triathletes, who compete swimming, cycling and running. Choosing lower cadences in triathletes may be interpreted as an adaption of the movement pattern to reduce the oxygen demand rather than the peripheral parameters (i.e. fore applied to the cranks). An additional hypothesis for that may be that there is a specific adaption of the neuromuscular system induced by the multidiscipline training.

Chapman et al. (2007) investigated the muscle recruitment among different athlete groups: triathletes (multidiscipline trained athletes), highly trained cyclists (single-discipline athletes) and novice cyclists (single-discipline non trained cyclists). EMG activity of five leg muscles (i.e. m. tibialis anterior, tibialis posterior, peroneus longus, gastrocnemius lateralis and soleus) was recorded during cycling using intramuscular fine-wire electrodes. The subjects cycled during all cycling bouts at their FCC (60 – 95 rpm). Athlete´s feedback was provided to modify work rate until a RPE of 15 was obtained. The authors reported, that muscle recruitment was less developed in triathletes than in high trained cyclists ($p = 0.02$). For example, the muscle activity decreased with increasing cadence (the amplitude and duration of muscle activity were greater at higher cadences) in triathletes when compared to cyclists. A reason for that may be training volume: A low training volume (< 100 km per week) would result in more extensive and more variable muscle coactivation and decreased modulation of muscle activity at increased cadence when compared to professional cyclists (around 400 km per week). Differences between these groups may reflect interference with motor learning in triathletes due to multidiscipline training, adaptations to muscle recruitment in triathletes which occur to maximise multidiscipline performance, differences in tissue morphology, lower limb power or kinematic variations. For example, greater individual variance, or greater variation in leg muscle recruitment between pedal strokes, may minimise fatigue and therefore lead to improved performance during the subsequent run.

The findings suggest that the concept of the optimal cadence is not unique and must take into account changes in metabolic, neuromuscular or biomechanical factors during cycling. In the most studies, the optimal cadence lies between 60 and 80 rpm. However, studies show a conflict between the energetically optimal cadence (i.e. the cadence at which oxygen demand is minimal) and the FCC, that is spontaneous selected by endurance athletes. Based on the studies the energetically optimal cadence is representing a relatively low cadence (50 – 60 rpm) and the FCC a relatively high cadence (80 - 100 rpm) (Skovereng et al., 2016).

Some studies indicated that the choice of high cadences could be attributed to the magnitude of forces applied to the crank during each pedalling cycle as well as the crank inertial load. High trained athletes generally choose higher cadences (around 90 rpm) than untrained athletes, whereas FCC decreases when cycling uphill. Indicating that critical determinants of cadence selection relate generally to the neuromuscular and biomechanical responses rather than the metabolic responses. The term ‘‘optimal cadence’’ may differ depending on whether the term refers to most economical, maximum power producing, less fatiguing or most comfortable cadence. However, for a competitive cyclist, the optimal cadence is the cadence that produces the best performance (= speed or power). During short-term exercise (3–8 min), performance is very much determined by $\dot{V}O_{2max}$, which is probably insignificantly affected by muscular fatigue. During prolonged exercise (> 30 min), muscle fatigue plays a greater role for performance and muscle fatigue may be dependent on cadence. Thus, it has been hypothesised that the reason why competitive cyclists do not choose the most efficient cadence is that fatigue develops faster at low cadences than at higher cadences (Foss & Hallén, 2005).

# 3 Principles of endurance training

Key physiological variables that are positively related to successful endurance performance are a high $\dot{V}O_{2max}$, $P_{max}$, LT, RCP and fractional utilization of $\dot{V}O_{2max}$ ($\%\dot{V}O_{2max}$). The aim of the various training methods to date is to improve one or more of those key variables (Faria et al., 2005).

Cyclists are often exposed to high training loads and, together with their coaches, are constantly challenged to find the optimal balance between training load and recovery. This challenge is enhanced by the varied response to identical training loads between athletes. Individualized training prescription, based on a cyclist's training status and levels of fatigue, is likely to improve adaptation to training (Capostagno et al., 2016).

Success in competitive cycling is dependent on high muscular and aerobic power as well as an effective application of the power to the crank system. According to the training principle of specificity, most cyclists perform the majority of their training on the cycle, including different types of training that promotes specific muscular strength and endurance, as well as technique and efficiency (Kristoffersen et al., 2014).

## 3.1 Continuous moderate intensity training

Endurance training adaptations result in a changed relationship between $O_2$ supply and $O_2$ utilization due to central adaptations and an expected rise in capillary density as well as in the number and size of mitochondria in skeletal muscle. Exercise training increases the number of capillaries per square millimetre of muscle in humans. The regions of the muscle that exhibit increased oxidative capacity also exhibit increased capillarity. Therefore, endurance training increases capillarity in slow-twitch fibers but not in fast-twitch fibers. In contrast, capillarity is increased in muscles with fast-twitch fibers during high intensity training, but not in muscles with slow-twitch fibers (Laughlin & Roseguini, 2008).

A moderate continuous training describes an exercise that is performed in a continuous manner and at lower intensities. Continuous moderate endurance training causes an increase in mitochondrial enzyme activities, which improves performance by enhancing fat oxidation and decreasing lactic acid accumulation at a given $\dot{V}O_2$ (Bassett & Howley, 2000).

Endurance exercise training leads to an increased vasodilation in skeletal muscle (McAllister et al., 2005). It has been shown that skeletal muscle capillarization requires weeks to months to manifest in response to exercise training and changes in capillary density appear to be develop less at higher exercise intensities (MacInnis & Gibala, 2017).

Skeletal muscle mitochondrial density regulates substrate metabolism during submaximal exercise, with increased mitochondrial content promoting a greater reliance on fat oxidation and a proportional decrease in carbohydrate oxidation. When the intensity and duration of exercise are held constant, mitochondrial content has been shown to plateau after about five days of training. However, when the intensity increased progressively, mitochondrial content continues to rise for at least several weeks. Furthermore, training volume has been suggested to be a primary determinant of the exercise induced increase in mitochondrial content in humans (MacInnis & Gibala, 2017).

Furthermore, skeletal muscle blood flow capacity is increased by exercise training due to this structural vascular re-modeling and/or altered control of vascular resistance. The importance of each of these mechanisms varies throughout muscle tissue due to interactions of muscle fiber-type composition and muscle fiber recruitment patterns during exercise. Furthermore, the importance of each of these mechanisms varies throughout muscle tissue due to interactions of muscle fiber-type composition and muscle fiber recruitment patterns during exercise. The distribution of vascular adaptive changes varies with mode of training. Exercise training for as little as 4 weeks has been reported to increase blood flow capacity – measured by reactive hyperemic responses of occlusion of blood flow. Blood flow increases by at least two primary mechanism: Structural remodelling of the vascular tree (capillary bed and arterial tree) and by altered vasomotor reactivity of arteries and arterioles. Furthermore, muscle fiber type composition influences blood flow powerful, whereas during low intensities mainly the high-oxidative, slow-twitch fibers are active. During high intensity exercise fast-twitch fibers are recruited progressively in order to active all fibers during high-intensity exercises (e.g. sprints) (Laughlin & Roseguini, 2008).

Coyle et al. (1991) compared a group of elite national cyclists with good regional cyclists and showed that the elite cyclists had a 23% higher muscle capillary density. Capillary density was significantly correlated with the average absolute work rate for a 1 h performance. These results indicate, that high-trained cyclists have a training-induced better relationship between $O_2$ supply and $O_2$ utilization.

Knowledge of the exercise intensity required during cycling competition provides valuable information of the prescription of training regimens. In general, intensity during endurance exercise can be split in four intensity zones: (1) recovery = 50% of $\dot{V}O_{2max}$; (2) moderate aerobic (50-70% of $\dot{V}O_{2max}$; (3) intense aerobic = 70-90% of $\dot{V}O_{2max}$; (4) anaerobic (over the individual anaerobic threshold) = around 90% of $\dot{V}O_{2max}$ (Faria et al., 2005). It is noteworthy that there are several other approaches to describe training intensity zones.

Nimmerichter et al. (2011) monitored the power output of professional cyclists the season. The authors observed power output distributions of 73% for the low-intensity Zones 1 (30%) and 2 (43%); 22% for the moderate-intensity Zones 3 (15%) and 4 (7%), and 5% for the high intensity zones.

## 3.2 Interval training

Endurance athletes include various forms of (high intensity) sessions as part of their training to enhance competitive performance. The term “interval training” can be characterised as performing repeated bouts of exercise interspersed with recovery periods within a training session. This definition implies that several variables can be modified to describe such training sessions. The modification of number, duration and intensity of the exercise bout, as well as for the recovery phase, affect the impact of the training (Stepto et al., 1999). Depending on the specific protocol employed, this type of training can elicit adaptations resembling endurance or strength training, or a mix of the two (MacInnis & Gibala, 2017).

Training volume is the product of exercise intensity, exercise duration (i.e. time per session), and training frequency (i.e. sessions per week). When a rise in training volume no longer augments fitness, cyclists employ intervals to intensify their training load. Short and intense sprint training can enhance maximum short-term power output and $\dot{V}O_{2max}$ (Faria et al., 2005).

In general, interval training achieves its performance enhancing effects through improvements of $\dot{V}O_{2max}$, anaerobic threshold and economy (Paton & Hopkins, 2004). During high-intensity interval exercise lasting more than a few seconds, adenosine triphosphate (ATP) is resynthesized by both aerobic and anaerobic processes. The ability to resynthesize ATP may limit performance in many sports. Thus, if possible, the training of athletes for sports involving high-intensity exercise should improve the athletes' ability to release energy both aerobically and anaerobically (Tabata et al., 1996).

High intensity interval training is conducted at intensity that elicits about 85–95% of $HR_{max}$. In contrast, sprint interval training is characterized by efforts performed at intensities equal to or greater than the pace that would elicit $\dot{V}O_{2max}$, including 'all-out' or 'supra maximal' efforts. Higher intensities of exercise elicit a greater metabolic signal than moderate intensities. ATP turnover is greater for higher intensities of exercise, which also rely more on carbohydrate oxidation and utilize more glycogen than do lower intensities of exercise (MacInnis & Gibala, 2017).

Sprint interval training has shown to produce the greatest relative increase in contractile activity in fast-twitch fibers and this type of training produces the largest relative increase in oxidative capacity, capillary density and blood flow. In contrast, endurance exercise training produces greatest relative increase in contractile activity in the slow-twitch fibers (Laughlin & Roseguini, 2008). Interval training has also shown to improve $\dot{V}O_{2max}$ by increasing cardiac output (Bassett & Howley, 2000). While the effect of interval training duration on $\dot{V}O_{2max}$ is not completely clear, it is suggested that longer high intensity interval bouts (3-4 min) increased $\dot{V}O_{2max}$ to a greater extent than shorter interval bouts (MacInnis & Gibala, 2017).

A maximum performance enhancement for TTs of medium duration (e.g. 40 km) has been shown for interval trainings consisting work bouts with a duration of 3-6 min and an intensity of ~85% of $P_{max}$. The intensity represents the average intensity during a 40 km - TT. Therefore, the most effective interval trainings for improvement of TT performance are intervals near race pace. The findings are therefore in accord with the principle of specificity. Furthermore, very short high-intensity intervals (~30 s at ~175%$P_{max}$) can also enhance endurance performance. This rather unexpected training effect of high-intensity work bouts could be due to an enhanced fatigue resistance, perhaps by altering skeletal muscle buffering capacity or an increased recruitment of motor units after training (Stepto et al., 1999).

Lindsay et al. (1996) could show that high intensity training increases $P_{max}$ and fatigue resistance of competitive cyclists and improves their 40 km TT performances. The cyclists in that study replaced 15 ± 2% of their ~300 km/week low intensity training with high intensity training, which took place on six days and consisted of 6-8 5-min repetitions at 80% of $P_{max}$, with 60-s recovery between work bouts. After 4 weeks of training, high intensity training significantly improved 40 km TT performance ($p < 0.001$) and time to

fatigue at 150% $P_{max}$ ($p < 0.01$). Furthermore, $P_{max}$ increased significant after the high intensity training ($p < 0.05$).

Tabata et al. (1996) found that a high-intensity intermittent training programme achieved bigger gains in $\dot{V}O_{2max}$ than a programme of 60 minutes of moderate-intensity cycling (70% $\dot{V}O_{2max}$) for a total of 5 hours per week for 6 weeks. The short-term, high- intensity training sessions consisted of eight all-out work bouts, each lasting 20 seconds, with 10 seconds of rest. This group cycled for a total of only 20 minutes per week, yet their $\dot{V}O_{2max}$ improved by 15% ($p < 0.01$). It has been shown that moderate-intensity aerobic training improves the maximal aerobic power but does not change anaerobic capacity, whereas high-intensity intermittent training improves both anaerobic and aerobic energy supplying systems significantly ($p < 0.01$), probably through imposing intensive stimuli on both systems.

Stepto et al. (1999) investigated the effect of varying intensity during an interval training on 40km time-trial performance in 20 male endurance cyclists. The subjects performed a 25 km TT test, an incremental test to determine maximal power output ($P_{max}$) and a simulated 40 km time-trial on an ergometer. The subjects were randomly assigned to one of five types of interval-training session: 12 x 30 s at 175% $P_{max}$, 12 x 60 s at 100% $P_{max}$, 12 x 2 min at 90% $P_{max}$, 8 x 4 min at 85% $P_{max}$, or 4 x 8 min at 80% $P_{max}$. These interval sessions were conducted additionally (six sessions over three weeks) to their usual aerobic training. The results observed after three weeks show, that the greatest performance enhancement showed the (8 x 4 min at 85% $P_{max}$) – group and the (12 x 30 s at 175% $P_{max}$) – group ($p < 0.005$). The other interval trainings showed no significant effect for 40 km time-trial performance. These results indicate that for improving specific TT performance a minimum of duration as well as intensity is required.

Similar results were found by Laursen et al. (2002). This study showed that four high-intensity interval-training sessions performed over 2 weeks improve VT, RCP, and $P_{max}$ ($p < 0.05$). For each of the four high intensity sessions, subjects were required to complete 20 x 1 min bouts of cycling at 100% of $\dot{V}O_{2max}$; each bout was separated by 2-min recovery at 50 W. However, despite improvements of VT, RCP and $P_{max}$, there was no change in $\dot{V}O_{2max}$.

## 3.3 Strength training and endurance performance

For short duration endurance performance supramaximal and explosive sport-specific resistance training has shown to have the most beneficial effects. For long duration endurance performance is enhanced most by intervals of maximal and supramaximal intensities, whereas resistance training has tendential smaller effects. In more detail, the largest improvements in $\dot{V}O_{2max}$ occur with maximal-intensity interval training, with intervals lasting 2-10 min. The largest improvement for economy occurs with explosive resistance training (Paton & Hopkins, 2004).

Heavy strength training can be defined as “all training aiming to increase or maintain a muscle or a muscle group’s ability to generate maximum force” (Knuttgen, 2019). The training load is between 1 to 15 repetition of the one-repetition maximum (1-RM). Explosive strength training is defined as exercise with external loading of 0-60% of 1-RM and maximal mobilization (0% of 1-RM equal body weight). Strength training contributes to enhance endurance performance by improving economy, delaying fatigue, improving anaerobic capacity and enhancing maximal speed (Rønnestad & Mujika, 2014).

Hickson et al. (1988) conducted a study where endurance athletes performed maximal strength training including each 3x5 sets of parallel squats, knee flexions and toe raises additional to their endurance training. All exercises were performed with as much weight as possible. With the exception of the toe raises, this resistance initially was about 80% of 1-RM. Strength training was performed three days/week for ten weeks, whereas endurance training remained constant during this phase. After ten weeks leg strength was increased by an average of 30%, but muscle fibres in the vastus lateralis (fast and slow twitch) were unchanged ($p > 0.05$). Maximal $\dot{V}O_2$ was also unchanged by heavy-resistance training during cycling and treadmill running, however, short-term endurance (4-8 min) was increased by 11 and 13% ($p < 0.05$) during cycling and running, respectively. Long-term cycling to exhaustion at 80% $\dot{V}O_{2max}$ increased from 71 to 85 min ($p < 0.05$) after the addition of strength training. These results indicate that certain types of endurance performance, particularly those requiring fast-twitch fiber recruitment, can be improved by strength-training supplementation.

Bastiaans et al. (2001) investigated the effect of explosive strength training on cycling performance in 14 competitive cyclists. The 14 athletes were divided into two groups (explosive strength training group and control group), whereas the strength training group received a training program where 37% of the whole training consisted of explosive-type

strength training, whilst the control group received endurance training only. The strength training consisted of four series of squats, leg press, leg pull and step-ups (i.e. 30 repetitions). Short-term performance was measured during a 30-s ergometer test at a fixed cadence of 60 rpm. TT performance, short-term performance, maximal workload ($W_{max}$), GE as well as $\Delta$-efficiency were measured before, after four weeks of training and at the end of the training program (after nine weeks). Results showed that simulated TT improved in both groups, whereas the increase in the strength training group was significant ($p < 0.05$). The increase in $W_{max}$ occurred in both groups, whereas the increase was somewhat higher for the strength training group than for the control group. A short-term performance decrease occurred in the control group ($p < 0.05$), whereas no changes were observed in the explosive strength training group. Correlation analysis showed that the improvement in TT correlated significantly with the increase in $W_{max}$ ($r = 0.61$, $p < 0.05$). Therefore, the authors concluded that replacing a portion of the endurance training by strength training with similar total training volume has an significant effect on TT performance compared to endurance training only. Furthermore, endurance performance determinants (i.e. TT and short-term performance) increased only in the strength training group ($p < 0.05$). These results suggest that adaptions in endurance performance occur faster after combined strength and endurance training.

GE is relative hard to improve with training. However, resistance training has mainly benefits on GE (Paton & Hopkins, 2004). Coyle (2005) reported an increase in efficiency over a period of 7 years of training and competing in one of the most outstanding cyclists of modern times from about 21–23%. The authors proposed that biochemical adaptations may have caused this improvement (i.e., a greater contribution from aerobically-efficient type I fibres).

In addition, Barrett-O'Keefe et al. (2012) showed that maximal strength training increases work efficiency. In that study five trained subjects took part in an 8-week maximal strength training intervention consisting of half-squats with an emphasis on the rate of force development during the concentric phase of the movement. Pre- and post- training measurements of pulmonary $\dot{V}O_2$, single-leg blood flow, and single-leg arterial-venous oxygen difference were performed, to allow the assessment of skeletal muscle $\dot{V}O_2$ ($m\dot{V}O_2$) during submaximal cycling exercise (60% of $\dot{V}O_{2max}$). Following the strength training, there was a significant reduction in pulmonary $\dot{V}O_2$ in submaximal cycling. This change in efficiency has shown to be due to a reduction in blood flow, while arterial-venous

oxygen difference remained constant. Thus, an increase in muscle efficiency has been confirmed.

Rønnestad et al. (2015) investigated the effect of 25 weeks heavy strength training in elite cyclists. Although the authors found no significant change in $\dot{V}O_{2max}$ from pre- to post-intervention, an increased $P_{max}$ was found in the strength training group ($p < 0.05$), while there was no significant change in the control group (endurance training only) ($p < 0.05$). Furthermore, power output at 4mmol/L [La] and mean as well as maximal power output during the 40-min maximum effort trial increased in the strength training group ($p < 0.05$) while no change was shown in the control group.

A likely mechanism for improved performance after combined strength and endurance training is (altered) muscle fiber type recruitment pattern. After prolonged cycling some type I fibers may be exhausted and the less economical type II fibers gradually increase their contribution to exercise. It is suggested that strength training increases the maximum strength of type I fibers and postpones their time to exhaustion (Rønnestad & Mujika, 2014).

Falz et al. (2019) investigated the acute effect of continuous moderate intensity exercise training with high intensity exercises and strength exercises. The continuous moderate intensity training was performed for 25 min at 70% $HR_{max}$, whereas the high intensity training was performed for 4x4 bouts at 85-95% $HR_{max}$. Strength training consisted of five different exercises that were performed using subject´s own body weight. Strength exercises were squats, push-ups, isometric back extension, isometric leg raise and inverted rows. The authors found different mean intensity factors ($p < 0.05$), such as the mean power output between the interventions, despite the same training time. This leads to different physiological response. For continuous moderate intensity training, subjects spent approximately 90% of the exercise duration in the training impact zone; whereas for high intensity training, they spent 68% of the exercise duration in the training impact zone. High intensity interval training resulted in significantly higher blood pressure in, which resulted in a higher cardiac workload. During strength training, lower values of SV and blood pressure were shown compared to that of the endurance methods, whereas it should be noted that the blood pressure measurements were taken during the 20-s resting phase. Furthermore, large differences in the SV and HR values were observed for the different strength exercises. In particular, the SV for squats reached comparable values to that for high intensity training. Despite an equal training duration and a similar acute metabolic

response, the authors found large differences with regard to the training impact time and the cardiopulmonary response.

## 3.4 Cadence-specific training

Training with imposed low or high cadences are commonly applied by competitive athletes and are widely denoted “power training”, “cadence training”, or “functional strength training”. Terms like these signals that this kind of training has a substantial effect on muscle strength. Further that, any potential performance enhancing effect is related to mechanisms involved in adaptations to heavy strength training (Hansen & Rønnestad, 2017). As previously described, low cadences cause pedal force to be high in each pedal thrust. Low cadences have shown to result in a lower demand on cardiorespiratory factors (i.e.: $\dot{V}O_2$ and HR) as well as lower RPEs.

Koninckx et al. (2010) compared the effects of strength training (for leg extensors) with isokinetic cycling training at 80 rpm on $P_{max}$ (assessed from five maximal 5s sprints at 40 and 120 rpm) and endurance performance (determined by measuring power, HR and [La] during GXT and a 30-min performance test). $P_{max}$ increased at all cadences ($p < 0.05$), however $P_{max}$ was not enhanced at 120 rpm in the isokinetic training group but was enhanced in the weight training group. All subjects, regardless of the training regime, increased their mean power output during the 30-min test ($p < 0.05$). However, there was no difference in power output for the two different training regimes ($p > 0.05$). The authors suggested, that the lack in $P_{max}$ improvement at very high cadences (120 rpm) in the isokinetic cycling group is due to an impairment in pedalling technique (impaired intra-muscular coordination above habitual cadences). Moreover, it could be due to the inability to generate a rapid knee extension at the onset of the downstroke, which results in the lower power output. The possible implication of the enhanced power output at 120 rpm in the weight training group but not in the isokinetic cycling group is that resistance training at low cadences could impair maximal power production at high cadence in cyclists.

In a study of Paton et al. (2009) authors stated that high intensity training bouts (80% peak power output) at lower cadences (60 – 70 rpm) enable the athletes to higher power outputs during trainings sessions at five sets of 30s-maximal cycling intervals. After four weeks of training, the low cadence group increased their $\dot{V}O_{2max}$ ($p < 0.01$), while no change occurred in the high cadence group. A reason for that could be the greater achieved mean power with the lower cadence. However, the resulting difference in training load appears unlikely to explain the difference in performance. The authors therefore suspect other

adaptions resulting from the higher forces in the muscle was responsible. The mean changes in the testosterone concentration in the low cadence training group was higher than in the high cadence training group. Since testosterone is a key anabolic hormone, which is strongly associated with strength gains, the higher performance in the low cadence group could be due to the higher testosterone concentration.

Nimmerichter et al. (2011) also showed, that workouts of 2-20 min at low cadences (40 – 60 rpm) improve performance in professional cyclists over the season. The authors concluded, that not only successful riders train more (higher training volume) but also at higher intensities to improve their strength. Further, it was shown, that the time spent for non-cycling activities, which included a main part of weight training, was related to performance ($r = –0.8$, $p = 0.02$).

Further, Nimmerichter et al. (2012) conducted a 4-week interval training including high and low cadences during flat and uphill cycling. The results show that higher pedalling forces caused by using lower cadences ($p = 0.029$) provide a potentially higher training stimulus with a crossover positive effect to uphill time-trial performances. Furthermore, there was a significant higher end-exercise $\dot{V}O_2$ value during 90 rpm cycling ($p < 0.001$). High-cadence intervals on level ground are more likely to enhance flat time-trial power output with no crossover to uphill time-trial. The study showed that training stimulus with the same power output, but different cadences might result in specific and consequently different adaptations.

Hirano et al. (2015) observed that $\dot{V}O_2$ and HR were lower during cycling at low cadences compared to higher cadences ($p < 0.01$). Furthermore, power output was higher at low cadences compared to power output at high cadences. After two weeks of five weekly sessions with low cadence cycling, the increases in power output at LT was significantly larger than after high cadence sessions in previously untrained males ($p = 0.048$). The authors suggested that high pedal forces with a concomitant low muscle oxygenation, caused by pedalling at low cadence, constituted the peripheral stimuli for aerobic improvements.

Ludyga et al. (2016) investigated the effects of cadence-specific training on brain cortical activity as well as endurance performance. 36 subjects ($\dot{V}O_{2max}$: 52.6 ± 6 ml $min^{-1}kg^{-1}$) conducted a four-week training period, which includes 4 h of basic endurance training and four cadence-specific 60 min sessions weekly. The subjects were randomly assigned to

either a high cadence (120–140 rpm), low cadence (60 rpm) or control group (only basic endurance training with a freely chosen cadence). The training intensities were matched between the groups and controlled by HR targets as percentages of the individual LT. In contrast to the control group, the high cadence and low cadence groups achieved similar improvements of $\dot{V}O_{2max}$ over the four-week training intervention ($p < 0.001$), whereas no significant differences were observed in the control group. The authors reported, that the similar improvements of $\dot{V}O_{2max}$ between high cadence and low cadence groups are due to the matched intensity between the two training groups.

Whitty et al. (2016) conducted a study where trained cyclists performed a series of submaximal exercises at 60%$W_{max}$ at cadences of 50, 70, 90, 110 rpm and their FCC. After the testing, the subjects were randomly assigned to a high (20% above FCC) or low cadence (20% below FCC) group for 18 interval-based training sessions over six weeks. Both groups improved their performance ($p = 0.05$), increasing their total distance ($p < 0.01$) and reaching higher average power outputs ($p = 0.02$ for high cadence group and $p = 0.03$ for low cadence group) during cycling bouts. However, they found that the low cadence interval training group significantly improved TT results of short duration ($p = 0.04$) due to an increase in strength development or potential neuromuscular adaptations.

However, Kristoffersen et al. (2014) found no significant effects of low cadence training (40 rpm) at moderate intensity (73–82% of $HR_{max}$) on $\dot{V}O_{2max}$ ($p = 0.28$), cycling performance (maximal power output) ($p = 0.10$) or GE ($p = 0.21$) in trained veteran cyclists (age 47 ± 6 years, $\dot{V}O_{2max}$ 57.9 ± 3.7ml·kg$^{-1}$·min$^{-1}$). The low cadence group performed interval training as group sessions on spinning bikes two times a week, in addition to their usual training. All participants were instructed on how to perform the low cadence interval training (5 x 6 min at a HR of 73–82% of $HR_{max}$ measured at the $\dot{V}O_{2max}$ test prior the intervention period), with 3 min active rest at freely chosen cadence and low intensity (60–72% of $HR_{max}$) in-between. Not only there were no difference in cardiorespiratory measures further, the freely chosen cadence did not change after the twelve-week training program. These findings are in contrast to the previous described studies (Paton et al., 2009 and Nimmerichter et al., 2012) that have demonstrated positive effects of low cadence training. This disagreement might be due to the extremely low cadence (40 rpm) used in the study by Kristoffersen et al. (2014), whereas in the study by Paton et al. (2009) and Nimmerichter et al. (2012) the training was performed with higher cadences (70 rpm). As Lucía et al. (2001) described, the optimal cadence of professional road cycling is reported around (71 ± 1 rpm) during uphill cycling. Therefore, a cadence of 40 rpm might

have been too low to fulfill the training principle of specificity, meaning that the transfer value to ordinary cycling is too low. Moreover, interval training at higher intensity as used in the study by Nimmerichter and colleagues may have been more optimal to improve aerobic capacity than at moderate intensity. Furthermore, it has been demonstrated that the effects of resistance training on muscle force are specific for the contraction velocities used in training, indicating, that the contraction velocity of 40 rpm could be too low for improving performance (Behm & Sale, 1993).

## 3.5 Gender differences

Although competitive female cycling is growing, the scientific literature on female cycling is still limited. Most of the research to date has focused on the male population, particularly professional or elite male cyclists (Graham et al., 2018). Fewer studies have examined other groups such as trained non-cyclists and untrained males (i.g. McNaughton & Thomas, 1996). Especially the studies examining female cyclists has been restricted to identifying characteristics of the professional or elite female cyclist (Impellizzeri et al., 2008) with only one study detailing the power output demands of female road cycling (Ebert et al., 2005). There is some suggestion that the hormonal changes may alter exercise metabolism during prolonged exercise (Isacco et al., 2012) or influence performance in hot environments (Janse de Jonge, 2003).

Impellizzeri et al. (2008) investigated the differences in morphological and physiological characteristics of elite female mountain bikers with road cyclists of different specialties and competitive level. In brief, the authors found that TT and flat specialists showed higher body mass, body surface and frontal area compared with MTB athletes and climbers. Absolute physiological parameters were generally higher in TT athletes than the other groups. The same parameters normalized by body mass were similar between TT athletes, climbers and MTB athletes but higher compared to flat specialists ($p < 0.01$).

Ebert et al. (2005) document the power output generated by elite female road cyclists who achieved success in flat as well as in hilly World Cup races to characterise the demands of women's cycling racing. Hilly and flat stages were raced at a similar cadence (75 ± 8 rpm vs. 75 ± 4 rpm, $p = 0.93$) but higher speed ($p = 0.008$) and power output ($p = 0.04$). This relative low cadence for professional cyclists (see i.e. Lucía et al., 2001) occurs due to the non-stop cadence measurement during the races: This low cadence value includes the time when the rider was going downhill at very fast speeds and not pedalling. Further analysis of the study data revealed that female cyclists spent the majority of race time

between 80 and 100 rpm, which is in agreement with previous findings in male professional cyclists ( Lucía et al., 2001 and Foss & Hallén, 2005).

Furthermore, it was found that during flat World Cup races, professional female cyclists produce a higher absolute (192 ± 21 W) and relative (3.3 W $kg^{-1}$) power output compared with hilly World Cup competitions (169 ± 17 W and 3.0 W $kg^{-1}$, respectively). This power output profile is different from the one reported for male stage races: Possible reasons for this discrepancy may be that male races involve hill-climbs of up to 30 km, while the longest climb for females in the World Cup races was about 6 km. The greater climbing distance and number of ascents during male races may have contributed to the higher average power outputs in the hilly races compared to females, because of the sustained medium to high power outputs that are required during hill-climbing. Furthermore, team tactics can also influence the intensity of a race because the pace in men's races is often controlled by the teams who are in contention to win. This is less obvious in women's racing due to the reduced number of teams (Ebert et al., 2005).

Graham et al. (2018) conducted a study with recreational female cyclists. They investigated the effect of cadences on TT performance, whereas finishing time was faster during the lower cadence of 60 rpm compared to a higher cadence of 100 rpm ($p < 0.05$). These results are in agreement with previous findings of studies conducted with male subjects.

Because research is limited, it is difficult to know whether findings from previous studies on male cyclists can be generalized to female cyclists, especially in light of some differences reported in the power output profile of female compared to male competitions (Ebert et al., 2005, Impellizzeri et al., 2008).
However, Sanders et al. (2019) recently collected HR, RPE and power output data of men´s (n = 3024) and women´s (n = 667) professional races during four years. Higher values were observed for distance, duration and power output in men´s races, whereas intensity and time spend in high-intensity HR zones is higher in women´s races.

# 4 Near-Infrared Spectroscopy (NIRS)

The main limitation of the whole body $\dot{V}O_2$ measurements resides in the impossibility to discriminate between the exercising muscles and the rest of the body, as well as between different muscles engaged in the exercise. Over the past two decades several techniques have provided the temporal fidelity necessary for resolution of arterial or muscle blood flow kinetics and $m\dot{V}O_2$ kinetics. These include: thermodilution, ultrasound and near-infrared spectroscopy (NIRS) which, in combination with pulmonary $\dot{V}O_2$ measurements, have been used to resolve microvascular flow dynamics in human muscle. However, the $\dot{V}O_2$ measured on the whole body does not reflect the contribution of single muscles or tissues. During a low-to-moderate intensity of exercise (i.e. daily activity), the skeletal muscle deeply relies on oxidative metabolism. During exercise, skeletal muscle $O_2$ consumption ($m\dot{V}O_2$) can rise 50-fold, with abrupt increases in $O_2$ delivery of up to 10-fold. Oxidative metabolism is the primary method of energy production in skeletal muscle, and changes in oxidative muscle metabolism are useful in understanding muscle function in both healthy and diseased conditions (Hamaoka et al., 2011).

The NIRS technology allows real time, non-invasive investigation of skeletal muscle oxygenation and consumption ($m\dot{V}O_2$) during exercise from the oxygen-dependent absorption characteristics of infrared light. It provides an index for oxygenated haemoglobin ($HbO_2$), deoxygenated haemoglobin (HHb), tissue oxygenation ($StO_2$) (which allows the measurement of the tissue oxygenation index in percentages: TSI%) and total haemoglobin (tHb), which gives indices of the overall local $m\dot{V}O_2$. Since the end of the 1980s, NIRS technology has been utilized to investigate local muscle oxidative metabolism at rest and during different exercise modalities (Shastri et al., 2019).

NIRS is an affordable and portable technology, which enables the assessment of skeletal muscle haemodynamic through relative concentrations and concentration changes of $HbO_2$ and HHb (Lucero et al., 2018). The strength of NIRS for measuring skeletal muscle oxidative metabolism is its possible application on even frail or vulnerable populations, and that it can be employed in both laboratory- and field-based studies. NIRS devices use wavelengths in the range of 700–900 nm, as this range has a much better penetration into biological tissue than visible light (Hamaoka et al., 2011).

Three different NIRS techniques are used: (i) the continuous-wave modality, based on constant illumination of the tissue, simply measures the attenuation of light through the

tissues; (ii) frequency-domain instruments, illuminating the tissues with intensity modulated light, measure both the attenuation and the phase shift of the emerging light; and (iii) the time-domain technique, illuminating the tissues with short pulses of light, detects the shape of the pulse after propagation through the tissues. The continuous-wave modality is the most widely used oximetry approach. Continuous-wave based systems offer the advantages of low cost and easy transportability. Typical depth sensitivity of the continuous wave based imagers is approximately 1.5 cm and the spatial resolution is limited to approximately 1 cm (Ferrari et al., 2011).

NIRS instrumentation has been used mainly by investigating thigh or forearm muscle groups at single measurement sites. First-generation of NIRS instruments provided only oxygenation changes expressed in arbitrary units or concentration changes in $O_2Hb$ and HHb (with respect to an initial value arbitrarily set equal to zero) and were expressed in $\mu mol.cm^{-1}$. The NIRS oximeters introduced in the 1990s offer the advantage of a continuous measure of regional $O_2Hb$ saturation on a scale from 0 to 100%. From the intensity values measured at the different detectors (optical light), a light attenuation slope is calculated and inserted in an equation to estimate the absorption coefficient of the tissue, and then $O_2Hb$ and HHb. This allows determination of the ratio $O_2Hb$ to total Hb ($O_2Hb$ + HHb), and thus tissue $O_2Hb$ saturation (in percentages) (Perrey & Ferrari, 2018). The increase in concentration changes of HHb should represent an estimate of $m\dot{V}O_2$ (Grassi & Quaresima, 2016). $m\dot{V}O_2$ is therefore measured by evaluating the rate of decline in Hbdiff (Hbdiff = $HbO_2$ – HHb) (De Blasi, 1997).

The component of the NIRS signals would predominantly come from the capillaries. Since in normal conditions, all regions of the muscle receive nearly-fully oxygenated arterial blood, oxygenation changes detected by NIRS would mainly reflect changes in capillary (Hb-related) and intracellular (Myoglobin-related) $O_2$ levels. In any case, the whole diffusion pathway, including vascular Hb and intracellular Mb, would desaturate during exercise with a similar time course. Therefore, a greater contribution of Mb than Hb to the NIRS HHb signal would not invalidate the interpretation that changes in this signal reflect fractional $O_2$ extraction (Grassi & Quaresima, 2016).

The NIRS signals are the result of the weighted average of the oxygen saturations of the heme group of hemoglobins (Hb) in the vascular bed (small arteries, arterioles, capillaries, venules, small veins) and the heme group of Mb in the muscle fibers. Considering that capillaries contribute to >90% of the total blood volume in muscle, and, under normal

conditions, all regions of the muscle receive nearly fully oxygenated arterial blood, oxygenation changes detected by NIRS would mostly reflect changes in capillary (Hb related) and intracellular (Mb-related) oxygen levels (Perrey & Ferrari, 2018).

Changes of $O_2Hb$ and HHb can indicate the interplay of anaerobic/aerobic metabolism and tHb can be reflective of recruitment strategies (Bastiaans et al., 2001). However, it is important to note, that the decrease in the oxygenation level demonstrated by NIRS does not primary reflect the occurrence of anaerobic energy production but a change in $HbO_2$ and HHb concentration regarding aerobic metabolism (precisely dissociation of $O_2$ from HHb) in mitochondria in the muscle (Takaishi et al., 2002). Tissue muscle oxygenation does not directly reflect $m\dot{V}O_2$ but reflects the balance between oxygen supply and consumption

The relationship between $\dot{V}O_2$, blood flow and $O_2$ extraction can be expressed by the Fick equation, based on the principle of mass conservation. As described by the Fick equation, skeletal $m\dot{V}O_2$ is the product of blood flow and the difference between arterial and venous $O_2$.The Fick principle has been applied to the measurement of cardiac output (= volume of blood pumped by the heart; product of HR and stroke. Therefore, a given reduction in skeletal $m\dot{V}O_2$ may be achieved by a reduction in either the difference between arterial and venous $O_2$, blood flow, or reductions in both variables (Barrett-O'Keefe et al., 2012).

The calculations for $m\dot{V}O_2$ are made with the assumption that tHb remains constant. It has already been stated that $HbO_2$ can only be measured reliable, if tHb remains constant. Because a change in haematocrit and blood volume affect the pathlength of near-infrared light (Ijichi et al., 2005). Therefore, it is required to use arterial occlusion for the reliable measurement of $HbO_2$ and subsequently to calculate total $m\dot{V}O_2$ during exercise. Arterial occlusions are used to control inflow and outflow of blood to the limb (De Blasi, 1997). In other words, during arterial occlusion an absolute value for $m\dot{V}O_2$ can be calculated, under the assumption that tHb remains constant (due to the occlusion), from the decreasing slope of $HbO_2$ (Hopker et al., 2017). However, there is ample evidence suggesting that changes in HHb are less influenced by changes in tHb compared to changes in $HbO_2$ (Grassi & Quaresima, 2016). It has been found that when blood flow is interrupted, as it is the case in arterial occlusion, then the HHb rate represents the driving force proportional to $\dot{V}O_2$ (Honig et al., 1992).

Muscle blood flow is heterogeneous across the muscle and can vary widely depending on the following factors: (i) the region measured; (ii) the exercise modality used; and (iii) the units used to report muscle blood flow. It is concluded, that oxygen extraction in muscle was depend upon blood flow, if blood flow increased, oxygen extraction decreases and vice versa. (Lucero et al., 2018). The concentration changes in tHb have been said to reflect microvascular blood volume and blood volume reflects local $O_2$ diffusing capacity. (Ijichi et al., 2005).

Temperature, humidity, exercise duration and intensity can influence blood flow and therefore tHb and as a result $HbO_2$ (Shastri et al., 2019). Further, $HbO_2$ measurements by NIRS in exercising skeletal muscle can be confounded by changes in skin blood flow, which can increase in order to facilitate heat loss during exercise. It was also found that most of the warming-induced increase of tHb resulted from an increase in $HbO_2$ with a relatively small contribution by HHb. (Grassi & Quaresima, 2016).

Muscle blood flow is highly stratified within and among contracting skeletal muscles. Therefore, highly recruited and oxidative muscles and muscle fibers (fiber type I) can receive a prodigious blood flow whereas that to their low oxidative and glycolytic counterparts (fiber type II) is constrained to a small fraction of this. Moreover, NIRS has been used to measure oxygenation changes in skeletal muscle haemoglobin under different conditions, such as ischemia and extreme exercise.

NIRS investigations in human muscle have confirmed non-invasively that muscle fractional $O_2$ extraction does not increase immediately following the onset of contractions. There is an initial decrease of the HHb signal over the first few seconds of exercise prior to its rapid subsequent increase. Furthermore, unlike pulmonary $\dot{V}O_2$ kinetics, $m\dot{V}O_2$ evidences no delay during the onset of an exercise. In conclusion, beyond the first few seconds of muscle contractions the $m\dot{V}O_2$ kinetics provide a close analogy of the pulmonary $\dot{V}O_2$ kinetics. Muscle blood flow kinetics ($O_2$ delivery to the muscles) is faster than $m\dot{V}O_2$ kinetics ($O_2$ extraction) during both low- and high-intensity exercise. (Poole & Jones, 2012).

TSI% can signal the balance between muscle oxygen supply and demand. (Tanaka et al., 2018). By utilizing the values derived from NIRS, TSI% can be calculated, as the ratio $HbO_2$ / tHb. Since it includes $HbO_2$, however, also TSI% may be more significantly

influenced by skin blood flow compared to HHb. The oxygenation variable is expressed as a percentage. (Grassi & Quaresima, 2016).

One would expect higher TSI oscillation at lower cadences due to greater intramuscular pressure during contraction as well as longer time of contraction. However, authors found that skeletal muscle TSI% is not substantially affected during cycling for short periods of time at constant, moderate exercise intensity at cadences between 30 and 110 rpm ($p > 0.05$) (Shastri et al., 2019) It has also been found that NIRS can quantify the rate of oxidative metabolism at rest and after exercise (Hamaoka et al., 2007).

The majority of the retrieved studies examined oxygenation of the m. vastus lateralis. Because force application of a knee extensor muscle such as the m. vastus lateralis is important in a number of sports (e.g. cycling, running, alpine skiing). In cycling, several endurance exercise protocols (GXT, submaximal work rate, TT, and repeated sprints) were used for evaluating the effects of interventional training and training status level, as well as the time course of the NIRS responses during and after exercise (Perrey & Ferrari, 2018).

Recently, Lucero et al. (2018) verified the reliability of NIRS-derived muscle blood flow and $m\dot{V}O_2$ responses from low- to moderate intensity dynamic exercise. In that study, measurements of muscle blood flow and $m\dot{V}O_2$ were collected in the m. vastus lateralis of twelve healthy, physically active adults. The participant completed six stages of progressive intensity (5, 10, 15, 20, 25 and 30% of maximal voluntary contraction) 90° rhythmic isotonic knee extension exercise on a dynamometer. Estimates of muscle blood flow were assessed as the relative change in the tHb signal derived through occlusion. $m\dot{V}O_2$ was calculated as the rate of change in the Hb difference signal ($[\Delta HbDif] = [\Delta HbO_2] - [\Delta HHb]$). Mean values for all intensities were greater than the previous (lower) intensity. Mean muscle blood flow was correlated linearly with exercise intensity and was directly proportional to $m\dot{V}O_2$ ($r = 0.92$, $p < 0.05$). Further, mean values for TSI% were substantially less during exercise than at rest. All parameters were correlated with % of maximal voluntary contraction.

Chance et al. (1992) was the first author who investigated the recovery time for HHb after exercise. The study was conducted with professional rowers. The main findings showed that reoxygenation times following exercise increased with work intensity from 70% to 100% maximal voluntary contraction and that recovery from maximal deoxygenation in the

quadriceps muscle group was prolonged and extended with a higher intensity of exercise. Furthermore, the recovery time for $HbO_2$ was related to [La] ($r^2 = 1.00$), indicating a Bohr effect (describing the inversely relation to acidity and to the concentration of carbon dioxide) with the unloading of $O_2$. In summary, Chance et al. (1992) suggested that the reoxygenation times determined using NIRS provided a non-invasive indication of the degree of localized $O_2$ delivery stress. Furthermore, it was suggested that individual differences in deoxygenation and reoxygenation changes during both exercise and recovery could serve as an indication of training state of the athlete, i.e., accelerated recovery of deoxygenation in trained vs. les-trained. Furthermore, this finding could have application in areas such as athletic training, rehabilitation, and sports medicine.

This suggestion was verified by Brizendine et al. (2013) who investigated the recovery rate of $m\dot{V}O_2$ between endurance trained athletes and an untrained control group. By measuring the recovery rate, the authors wanted to evaluate the ability of NIRS measurements to detect differences in mitochondrial function between these two groups. The trained group with high muscle oxidative capacity based on self-reported history of strenuous endurance training and the control group with lower muscle oxidative capacity based on a lack of strenuous endurance training. $m\dot{V}O_2$ was calculated as the slope of change in $HbO_2$ and HHb during arterial occlusion. Twitch electrical stimulation was performed using 15 s of continuous electrical stimulation. The intensity was adjusted for each individual to produce twitch contractions at the maximal tolerable level. Immediately after each bout of electrical stimulation, a series of 10–18 brief arterial occlusions using a blood pressure cuff were applied to measure the rate of recovery of $m\dot{V}O_2$ back to resting level. The authors found no difference in $m\dot{V}O_2$ resting levels between trained and untrained subjects ($p = 0.42$). However, the difference in end-exercise recovery of $m\dot{V}O_2$ were statistically significant ($p < 0.001$). These results indicate that endurance trained athletes have a greater mitochondrial capacity than inactive controls.

At ramp incremental exercise metabolic demand and $\dot{V}O_2$ rises linearly. However, recent studies have shown that NIRS measurements of local HHb within the active tissues (i.e. m. vastus lateralis) do not display the same profile that is associated to systemic measurements ($\dot{V}O_2$), indicating that central and peripheral profiles of $O_2$ extraction and blood flow are different. The authors found that increases in HHb reflect a nonlinear relationship between microvascular muscle oxygen supply and muscle oxygen consumption during incremental ramp exercise. The mechanistic implications of study findings are that in most healthy subjects, muscle blood flow increased at a faster rate than

m$\dot{V}O_2$ early in the exercise test (low to moderate intensities) and slowed progressively as maximal work rate was approached. At heavy work rates the kinetics of m$\dot{V}O_2$ and muscle blood flow were similar, yielding a linear relationship and plateau in HHb (Ferreira et al., 2007).

Similar results were found for both, ramp and step incremental exercises. The HHb response hereby follows a similar sigmoid pattern to incremental steady-state (step) and non-steady-state (ramp) exercise in trained cyclists. The sigmoid HHb pattern is the reflection of an initial rapid increase in blood flow in the low- to moderate-intensity domain, followed by a less pronounced increase of blood flow as work rate increases during the ramp exercise. In other words: At the beginning of the test (low to moderate intensity) blood flow increases rapidly linear until a plateau occurs. At the last phase of the test blood flow increases further, but not that steep. For HHb there is a slow increase at the beginning of the test which becomes rapidly greater when intensity increases. This progress indicates a sigmoid similar curve. This pattern is an expression of a nonlinear blood flow / HHb relationship, related to changes in muscle fiber-type recruitment (Boone et al., 2010).

The concentration changes of HHb signal during ramp incremental exercise has been described to display a linear increase in relation to the increase in metabolic demand (i.e., $\dot{V}O_2$) until a breakpoint leading to a plateau-like response occurs despite the continuous increase in $\dot{V}O_2$. This breakpoint in the concentration changes of HHb signal, has been indicated as a reliable measure that can be used as the demarcation point for the exercise intensity closely associated with the $\dot{V}O_2$ at the RCP, and it has been proposed as a tool that can differentiate between heavy and very heavy exercise intensity domains (Murias et al., 2013).

Inglis et al. (2017) investigated whether the plateau of HHb observed during the end of incremental ramp exercise, reflects the upper limit of $O_2$ extraction or whether further extraction is possible beyond the plateau. Eleven young healthy men performed an incremental ramp test exercise. HHb, as an index of $O_2$ extraction, was measured using NIRS on the m. vastus lateralis. A leg blood flow occlusion was performed at rest and immediately after the ramp test. The authors found no significant difference between the leg blood flow measurement at rest and HHb plateau ($p > 0.05$) or between the baseline measurements ($p > 0.05$). HHb values at blood flow measurement directly after the ramp test were significantly greater than all other time points ($p < 0.05$). If the plateau in the HHb signal reflects the upper limit of $O_2$ extraction, then elimination of tissue blood flow

(occlusion) should result in no further increase in the HHb signal. Since this is not the case, this result suggests that the observed plateau in the HHb signal toward the end of a ramp incremental test is not representative of an upper limit in $O_2$ extraction in the m. vastus lateralis. The study demonstrated that there is a $O_2$ extraction reserve in the m. vastus lateralis at the end of an incremental ramp cycling test and that further extraction beyond this plateau is possible.

## 4.1 Influences of training on m$\dot{V}O_2$

Increased oxygen supply and oxygenation responses were observed to support the beneficial effect of training on muscle function. NIRS technology is therefore appropriate for determining exercise training intensity. Although the use of NIRS is relatively new in the context of sports science, it has proven to be a reliable non-invasive tool to determine peripheral muscle oxygenation during and after many types of sports. Understanding how groups of muscles respond in 'real-time' within set motor tasks and across endurance training regimens could help coaches to continuously assess whether exercises are targeting the muscle groups they were expecting to be targeted, and inform modification, if required (Perrey & Ferrari, 2018).

Costes et al. (2001) investigated, if training interventions have an effect on m$\dot{V}O_2$ parameters. The authors hypothesized that local adaptations (increased capillary density, decreased lactic acid) would increase oxygenation in the muscle (i.e. reduce deoxygenation effect). Before and after 4 weeks of endurance training (120 min × 6 days/week at 70–80% $HR_{max}$), all subjects performed a 15-min bout of steady-state cycling exercise at both 50% and 80% of $\dot{V}O_{2max}$. It was found that during exercise at the 80% work rate $StO_2$ was significantly greater after training ($p < 0.05$), i.e.less deoxygenation occurred as a result of training at the same relative work rate. However, tHb was significantly increased after training at both the 50% and 80% work rates. Furthermore, they found a significant relationship ($r = 0.42$, $p = 0.03$) between [La] and $StO_2$ at the end of both bouts of exercise. [La] was significantly ($p < 0.05$) lower after training whatever the intensity of exercise. Furthermore, it was found that the accumulation of [La] and the decrease in $StO_2$ were related during the course of each bout of exercise. After training, this relationship was weaker, reflecting the stability of both measurements during high-intensity exercise. The influence of [La] on $StO_2$ was then reduced or suppressed after training, which could reflect an improved oxidative metabolism. However, the changed tHb could have caused the greater $StO_2$ values.

However, Neary (2004) claimed that there is no physiological reason why there would be a difference in the oxygen cost at a given power output regardless of whether it was before or after a period of training. One reason for the higher $StO_2$ values found by Costes et al. (2001) could be the fact that the subjects exercised at a lower mean percentage (i.e., 80% vs. 73%) of $\dot{V}O_{2max}$ during the post-test. Alternatively, it could indicate a unique finding by Costes et al. (2001) that the reduced deoxygenation after training was related to an increased arterial-venous oxygen difference.

### 4.1.1 Limitations of NIRS

However, when the probe is applied on the skin overlying a muscle of interest, NIRS instruments can assess only a relatively small (2 to 6 cm) and superficial volume of skeletal muscle tissue. It is generally accepted that the depth of penetration of the NIR light in tissues roughly corresponds to half of the distance between the light source and the detector. The fact that the NIR light has to cross the skin and subcutaneous fat in order to reach the underlying skeletal muscle tissue inevitably causes a sensitivity problem and represents a "contamination" of the signal of interest, which is the one coming from skeletal muscle. Furthermore, the area of muscle investigated by the NIRS probe may not represent, in terms of fiber types, fiber activation and the matching of $m\dot{V}O_2$ and blood flow, a reliable picture of the situation in the whole muscle. Subcutaneous adipose tissue greatly influences the NIRS signal intensity from the muscle tissue below. In conclusion, skeletal muscle fractional $O_2$ extraction is the main variable that can be noninvasively evaluated by NIRS (Grassi & Quaresima, 2016).

Although $m\dot{V}O_2$ values during exercises are generally less examined than other physiological responses (HR, [La] and $\dot{V}O_2$), the knowledge of the local muscle oxygen values are additional important factors for determining performance and influence training interventions in cycling (Jacobs et al., 2013). The selection of cadences during cycling are not only determined by power output and the overall $\dot{V}O_2$ but also by the degree of (de)oxygenation in exercising skeletal muscles.

## 4.2 Effects of cycling exercise on muscle oxygen parameters

Endurance training adaptations in general result in a changed relationship between $O_2$ supply and $O_2$ utilization due to central adaptations and an expected rise in capillary density as well as in the number and size of mitochondria in skeletal muscle (Holloszy,

2008; Laughlin & Roseguini, 2008). Considering these adaptations of oxygenation in muscle in relation to the subjects' aerobic fitness, there is a better regulation between $O_2$ uptake and consumption in the response to a change in the cadence, suggesting smaller or no differences between different cadences and m$\dot{V}O_2$ during high intensity exercise (Zorgati et al., 2015).

Takaishi et al. (2002) compared parameters derived from NIRS on m. vastus lateralis between untrained subjects, triathletes and cyclists. The authors wanted to investigate the effects of cycling experience and pedal cadences on m$\dot{V}O_2$ parameters. In particular, the peak and nadir of blood volume were defined as muscle oxygenation and these values were observed. Regarding the peaks of oxygenation, the mean value for cyclists was larger than those for untrained and triathletes. In general, the mean value at 85 rpm was larger than that at 50 rpm ($p < 0.05$). For the nadir of oxygenation, values significantly increased with the increase in cadence for untrained and triathletes but not for cyclists. At 85 rpm, the value for untrained was significantly larger than that for cyclists ($p < 0.05$). In general, mean value for 85 rpm was significantly larger than those for 50 and 75 rpm ($p < 0.05$). These results are suggesting that endurance training increases muscle oxidative capacity by means of increases in capillary density and enzyme activity. Untrained subjects needed to exert more anaerobic energy production for muscle contraction as a result of a lack of pedalling skill (peak pedal force). Furthermore, ballistic muscle contractions for pedal thrusts in untrained subjects may recruit a higher percentage of fast twitch fibers, which are inferior in oxidative capacity. It is important to keep in mind, that the decrease in the oxygenation level demonstrated by NIRS does not reflect the occurrence of anaerobic energy production but a change in $HbO_2$ and HHb concentration regarding aerobic metabolism. The authors concluded, that cyclists and triathletes have acquired developments, contributing to an increase in muscle oxidative capacity through their daily training, and that this capacity of the cyclists is higher than that of the triathletes. Therefore, the resultant order (cyclists<triathletes<untrained) of the nadir level of oxygenation at 75 and 85 rpm may indicate that the measure nadir of muscle oxygenation is available to estimate the extent of $O_2$ extraction (aerobic energy production) in the working muscle. These results demonstrated that NIRS measures could be used to differentiate between fitness levels (i.e., experience) based on the oxygenation and blood volume vs. crank angle pattern.

Ferreira et al. (2006) found no significant difference in $\Delta$HHb ($p = 0.94$) during incremental cycling exercise at 60 and 100 rpm, respectively. Even through $\dot{V}O_2$ was higher at 100 rpm

compared to 60 rpm ($p < 0.05$). This result indicates no impairment of muscle blood flow with the increase in contraction frequency. The similar muscle oxygenation and greater muscle metabolism at 100 rpm than at 60 rpm indicates a higher blood flow at 100 rpm, so that the ratio between $\dot{V}O_2$ and blood flow was similar during exercise at 60 and 100 rpm.

Kounalakis & Geladas (2012) monitored local muscle blood values with NIRS derived changes in $StO_2$, tHb, $HbO_2$ and HHb haemoglobin during cycling bouts at two different cadences (40 and 80 rpm). The cycling bouts lasted each 2h at an exercise intensity corresponding 60% of $\dot{V}O_{2max}$. The authors found lower tHb and $HbO_2$ values in the m. vastus lateralis at 80 rpm ($p < 0.05$), whereas HHb and $StO_2$ values remained the same between the cadences. The reduction in cardiac output (reduced SV and elevated HR) at the higher cadence could have affected the regional muscle blood values, tHb and $HbO_2$, directly. Lower tHb and $HbO_2$ could also be attributed to the reduced blood filling time in the muscle as contractions become faster in the higher cadence. Furthermore, the higher respiratory work, which can be assumed from the higher VE and $\dot{V}O_2$ at 80 than at 40 rpm, could potentially impair leg blood flow, causing a redistribution of blood from the locomotor to the respiratory muscles or, alternatively, reduce blood availability in both locomotor and respiratory muscles. However, HHb and $StO_2$ were not affected of the different cadences. The authors concluded that cadence, does not seem to threaten muscle oxygenation during prolonged cycling at least under a specific externally controlled (moderate) exercise intensity.

Zorgati et al. (2013) also examined the effect of pedal cadence on the $m\dot{V}O_2$, defined as the change in HHb, during moderate exercise intensity (80% of VT). Moderate trained subjects performed 6-min bouts of cycling at 40 and 100 rpm, respectively. The HHb value, provided from the NIRS on the m. vastus lateralis, showed no shift when changing the pedal cadence, whereas pulmonary $\dot{V}O_2$ was higher at 100 rpm than at 40 rpm ($p < 0.001$). Therefore, $O_2$ extraction was independent of the cadences. However, the elevated cadence increased $m\dot{V}O_2$ which suggests a greater heterogeneity in the muscle $O_2$ delivery and consumption and further, an increase in muscle blood flow during higher cadences.

Jacobs et al. (2013) investigated the $m\dot{V}O_2$ parameter TSI% during different cadences in moderately trained subjects. While cycling 3x8 min at 75% $P_{max}$ at cadences of 60, 80 and 100 rpm, NIRS-derived measures of TSI% were recorded. TSI was higher at 80 rpm than

at 60 ($p = 0.018$) and 100 rpm ($p = 0.008$) at minute 4, but at minute 8, TSI at 80 rpm was still higher than at 100 rpm ($p = 0.017$) but not different than at 60 rpm ($p = 0.209$). These findings are interesting because as subjects increased cadence from 60 to 80 rpm, their $\dot{V}O_2$ also increased. These findings seem contradictory since $O_2$ dissociation typically increases in parallel with $\dot{V}O_2$ to facilitate peripheral oxygen uptake.

Hirano et al. (2015) found lower $HbO_2$ concentrations during cycling at 35 rpm compared to cycling at 75 rpm ($p < 0.01$). Furthermore, pedal forces per single cycle were significantly higher at the lower pedalling rate ($p < 0.01$). The authors concluded that a lower pedalling rate requires greater pedal force per cycle to achieve the same work rate as that performed at a higher pedalling rate. Thus, higher and/or longer muscle force generation and contraction during cycling restricts peripheral blood flow and delivery, and thus decreases muscle oxygenation (Hirano et al., 2015; Takaishi et al., 2002)).

In another study by Zorgati et al. (2015) the degree of $m\dot{V}O_2$ of trained and untrained subjects were compared. The subjects cycled until exhaustion at 90% $\dot{V}O_{2\ max}$ at 40 and 100 rpm, respectively. Since $m\dot{V}O_2$ was continuously monitored using NIRS on the m. vastus lateralis, a significant higher ($p < 0.05$) deoxygenation at 40 rpm than in 100 rpm was found in untrained subjects but not in triathletes. Furthermore, the time to exhaustion was significant higher at 40 rpm than at 100 rpm in untrained ($p < 0.001$) but not in triathletes ($p = 0.65$). That there were no differences in $m\dot{V}O_2$ between the two cadences in trained subjects, but differences in untrained, could be due to the high exercise intensity. A change in the pedalling cadence affected the $m\dot{V}O_2$ only at the beginning of the exercise and concomitantly with the increase in pulmonary $\dot{V}O_2$. Regarding the second half of the exercise, the results (no difference in HHb and $\dot{V}O_2$ at 40 and 100 rpm, respectively) suggest that the muscles in trained subjects are able to coordinate muscle oxygen delivery and muscle oxygen consumption, after some delay, independently of the cadence. While untrained are not able to coordinate this rate. These observations can also explain the lack of difference in performance between the two cadences in triathletes. Another explanation in the different results between trained and untrained could be the fact, that the preferred cadences in trained subjects are generally higher (Lucía et al., 2001). A cadence of 100 rpm was likely closer to the preferred cadence of triathletes than of untrained subjects. The consequence was that triathletes were able to maintain at 100 rpm the performance they obtained at 40 rpm.

Skovereng et al. (2016) investigated the effect of cadence on $m\dot{V}O_2$ and joint specific power in the m. vastus lateralis and m. vastus medialis muscle. At a relative low work rate of 75% LT participants cycled at six 4-min stages at different cadences (i.e. 60, 70, 80, 90, 100 and 110 rpm). $m\dot{V}O_2$ was calculated as the slope of linear change in HHb during arterial occlusion. Again, a subsequent increase in cadence resulted in increase of $\dot{V}O_2$, [La], HR and RPE ($p < 0.05$). Furthermore, an increase in cadence led to increased HHb. This effect of cadence on HHb was similar for both muscles. $StO_2$ decreased with increasing cadence ($p < 0.05$). This effect of cadence on desaturation was also similar for both muscles. For $HbO_2$ there was a main effect of cadence leading to a decrease in $HbO_2$ with increasing cadence ($p < 0.05$). What is more, the authors found an increased knee joint specific power when cycling cadence increased and a decrease in relative hip joint contribution ($p < 0.05$). However, considering $m\dot{V}O_2$, there is a difference in the effect of cadence in the $m\dot{V}O_2$ of the m. vastus lateralis and m. vastus medialis at a cadence of 110 rpm compared to 60 rpm. Increasing cadence did not affect m. vastus medialis ($p = 0.28$). Fast twitch fibers have a higher potential for force generation at high contraction speeds. The authors suggested that the larger proportion of fast twitch fibers in the m. vastus lateralis can contribute to the finding of increasing $m\dot{V}O_2$ in the m. vastus lateralis and not in the m. vastus medialis at a high cadence. Higher tHb values were found in the m. vastus lateralis compared to the m. vastus medialis ($p < 0.05$). However, different cadences did not affect tHb ($p = 0.24$), indicating that the change in cadence did not affect blood volume changes in either of the muscles. The authors concluded, that higher pedal cadences lead to higher HHb and lower $StO_2$ values, and that the difference in $m\dot{V}O_2$ between the two muscles shows that differences between two mono-articular knee extensors occur when cyclists change cadence at a constant external work rate.

Also Skovereng et al. (2017) compared different cadences on joint specific power and $m\dot{V}O_2$ (slope of linear change in HHb) in m. gastrocnemius and m. tibialis anterior. The subjects cycled at a cadence of 60, 70, 80 90, 100 and 110 rpm, like in the previous study. Subjects cycled 6x4 min stages with increasing cadence at an external work rate corresponding to 75% of the work rate where subjects had a blood lactate concentration of 4 $mmol \cdot L^{-1}$ (previous determined). Like in their previous study, the authors found that increasing cadences led to increased $\dot{V}O_2$, HR, [La] and RPE; as well as HHb and decreased $StO_2$ and $HbO_2$ in the m. gastrocnemius and m. tibialis anterior (all $p < 0.05$). The overall $m\dot{V}O_2$ in the m. tibialis anterior was lower than that in the m. gastrocnemius. The effect of cadence was different for both muscles: The m. tibialis anterior $m\dot{V}O_2$ increased above 80 rpm, whereas increasing cadence did not lead to a significant increase

in m. gastrocnemius $m\dot{V}O_2$ ($p = 0.12$). For tHb, there was no effect of cadence found in both muscles ($p = 0.13$). Increasing cadence led to a decrease in ankle power and an increase in ankle joint angular velocity above 80 rpm. The increase in $m\dot{V}O_2$ of the m. tibialis anterior coincides with the increase in the ankle joint velocity at cadences above 80 rpm. These findings indicate that high cadences lead to increased $m\dot{V}O_2$ in m. tibialis anterior which cannot be explained by an increase in power and thus is suggest being due to other mechanisms, such as greater energy requirements for controlling foot motion at higher angular velocity.

Recently, Mitchell et al. (2019) conducted tests where subjects cycled 6-min constant-load trials at 10% below their previously determined RCP while pedalling at 60, 90, and 120 rpm, and a freely chosen cadence (94.3 ± 6.9 rpm). Muscle blood flow was determined on four leg muscles (m. vastus medialis, m. semitendinosus; m. gastrocnemius and m. vastus lateralis) using NIRS. The authors found that $\dot{V}O_2$ increased with increasing cadence. Furthermore, they found a higher muscle blood flow in the m. vastus medialis and semitendinosus ($p < 0.01$), but muscle blood flow remained unchanged in the m. vastus lateralis ($p = 0.06$). Furthermore, blood flow was significantly (or approached significance) correlated with the increase in $\dot{V}O_2$ with increasing cadence ($p < 0.001$ for m. gastrocnemius; $p = 0.09$ for m. vastus lateralis; $p = 0.06$ for m. vastus medialis; $p = 0.09$ for m. semitendinosus).

Recently, Shastri et al. (2019) investigated $m\dot{V}O_2$ parameters in the m. vastus lateralis during low exercise intensities (70% and 90% VT) and different cadences (30, 50, 70, 90, and 110 rpm). The authors investigated the $m\dot{V}O_2$ parameters $HbO_2$, HHb and TSI. They found an overall higher cardiorespiratory and metabolic response during the higher intensity of 90% VT. At each exercise intensity, increasing cadence was associated with a greater metabolic response. Furthermore, $HbO_2$ was significant greater at the higher intensity ($p < 0.05$). However, no difference has been found between HHb and TSI during the different intensities and cadences ($p > 0.05$). The authors concluded, that an increase in power output (external mechanical work rate) is a greater determinant of the physiological response to cycling exercise than an increase in cadence (internal mechanical work rate), unless individuals cycle at very low power output levels like in that study.

Kawaguchi et al. (2006) investigated the effects of 60 min submaximal (50% $\dot{V}O_{2max}$) constant load cycling on $m\dot{V}O_2$ in the m. vastus lateralis. Generally, the tHb, $HbO_2$ and

HHb demonstrated an initial decrease at the onset of exercise, followed by a gradual increase until approximately 10 min of exercise. Thereafter, all parameters attained a temporary steady state. During the last 20 min of exercise, tHb and $HbO_2$ showed a systematic increase, whereas HHb showed a decrease over the same time period. This finding implies that the balance between oxygen supply and delivery at muscular level changed at that intensity. Furthermore, the increase in tHb during the last 20 min indicates an increased localized blood volume, which is known to be accompanied by a systematic increase in $HbO_2$. What is more, the authors suggested, that the increase in respiratory $\dot{V}O_2$ during prolonged exercise is attained by an increase in tHb.

Hopker et al. (2017), investigated the effects of 2h moderate (60% of maximal aerobic power) constant load cycling on GE and $m\dot{V}O_2$ derived using NIRS of the m. vastus lateralis. The authors found no significant change in $HbO_2$ ($p = 0.27$), however, HHb increased significantly at 90 and 120 min of constant load cycling ($p = 0.03$). Furthermore, GE declined significantly during the constant load cycling exercise ($p < 0.01$). Hopker et al. (2017) suggested, that the main reason for reduced GE and increased $m\dot{V}O_2$ is the progressive peripheral fatigue encountered during the 2 h exercise. During the 2 h cycling there could have been an increase in the ATP cost of muscle contraction and an increased ATP cost of power production, which in turn might have contributed to the increased $m\dot{V}O_2$.

As described in the previous chapter, it is well stablished that low cadences are more efficient (lower HR, lower VE, lower RER, lower [La]) (Lucía et al., 2001). Higher cadences require a higher demand of the general cardiorespiratory and metabolic work (Shastri et al., 2019) as well as higher $m\dot{V}O_2$ at moderate and high intensities. There are several recent studies that were investigating the effect of pedalling cadence on $m\dot{V}O_2$, reflecting HHb and TSI or other values such like $HbO_2$ and $StO_2$, by using NIRS. It is summarized that there are different $m\dot{V}O_2$ responses not only in different cadences, but also among trained and untrained subjects, respectively ( (Takaishi et al., 2002 and Zorgati et al., 2015). The balance between local muscle HHb and $HbO_2$ at high intensities has shown to be affected by cadence in untrained subjects, but not in trained subjects, suggesting due to training adaptations. Ferreira et al. (2006) have reported no significant difference ($p = 0.94$) in $m\dot{V}O_2$ and cadences during incremental exercise. However, significant differences in $m\dot{V}O_2$ have been found in moderate exercise (Mitchell et al., 2019, Kounalakis & Geladas, 2012; Zorgati et al., 2013; Skovereng et al., 2016 and Hopker et al., 2017) and heavy exercises (Zorgati et al., 2015). Moreover, there are different responses in trained and untrained subjects.

It is generally accepted that HHb increases and $HbO_2$ decreases while cycling at increasing power output associated with increased metabolic rate ((Takaishi et al., 2002), Hirano et al., 2015 and Skovereng et al., 2016).The findings show that the magnitude of significant changes in vastus lateralis oxygenation levels quantified using NIRS and was related to various stressors (exercise intensity, duration of exercise, training status) (Perrey & Ferrari, 2018).

However, to date researchers also found rather contradictory results regarding $m\dot{V}O_2$ values. An increase in cadence was associated with greater HHb ( Skovereng et al., 2016; Skovereng et al., 2017 and Hopker et al., 2017), no difference in HHb concentration changes ( Ferreira et al., 2006; Kounalakis & Geladas, 2012; Zorgati et al., 2013 and Shastri et al., 2019), or reduced HHb ( Zorgati et al., 2015).

An increase in cadence was also associated with either greater $HbO_2$ ( Ferreira et al., 2006; Hirano et al., 2015), no difference in $HbO_2$ (Zorgati et al., 2015), or reduced $O_2Hb$ ( Kounalakis & Geladas, 2012, Skovereng et al., 2016). These apparently contradictory findings may reflect differences in study design (e.g., exercise intensity, duration and effect of fatigue) and changes in blood volume, as indicated by altered tHb, which were not always reported in full extent.

The mentioned studies selected cadences in a randomised order. In contrast, testing cadences in incremental sequence (i.e. from 60 to 110 rpm) without recovery or rest periods was associated with a decrease in $HbO_2$ and TSI, and an increase in HHb ( Skovereng et al., 2016; Skovereng et al., 2017 and Shastri et al., 2019). It is possible that the changes observed at elevated cadences were the (fatigue) effect of previous exercise at lower cadences.
However, effects of high and low cadences on $m\dot{V}O_2$ values during a combination of moderate and high intensity exercise (i.e. interval training) are not examined yet.

# 5 Aim of the study

For running or walking the relationship between cadences and cardiorespiratory values has been widely studied, often suggesting that the performer spontaneously adopts the pattern of locomotion leading to the lowest energy cost. This does not appear to be the case for cycling. On the one hand the energetically optimal cadence ranges from 40 rpm to 80 rpm in trained or untrained cyclists (Gaesser & Brooks, 1975, Di Prampero, 2000 and Lucía et al., 2001) but on the other hand observations of cyclists often reveal that the freely chosen cadence is significantly higher than the most economical cadence (e.g. Lucía et al., 2001, Ernst A. Hansen et al., 2002, Jacobs et al., 2013 and Hansen & Rønnestad, 2017). Several assumptions have been made to explain this apparent conflict: changes in pedalling forces (Ernst A. Hansen et al., 2002 and Jacobs et al., 2013), neuromuscular activation and less fatigue (T. Takaishi et al., 1996 and Neptune & Hull, 1998), or variation in cardiorespiratory parameters (Hagberg et al., 1981).

Though there are also already studies, that examined pedal cadences on local muscle deoxygenation and pulmonary oxygen uptake during cycling, no study examined the effect of different cadences during a low and high intensity interval training, on m$\dot{V}O_2$, reflecting HHb concentration changes and TSI, as well as cardiorespiratory responses ($\dot{V}O_2$, [[La]], HR) yet.

Therefore, the aim of the present study is to evaluate, if a cycling interval exercise, conducted with high and low cadences, including high and low intensities, shows significant differences in HHb and TSI parameters and cardiorespiratory and metabolic responses in moderatly trained male and females triathletes. Moreover, it should examine, if there is a physiologically meaningful difference between female and male athletes.

# 6 Hypotheses

This aim leads to following hypotheses:

- $H1_0$: There is no significant correlation between cardiorespiratory and metabolic responses and high vs. low intensities.
- $H1_1$: There is a significant correlation between cardiorespiratory and metabolic responses and high vs. low intensities.
- $H2_0$: There is no significant correlation between HHb concentration changes and TSI, and high vs. low intensities.
- $H2_1$: There is a significant correlation between HHb concentration changes and TSI, and high vs. low intensities.
- $H3_0$: There are no significant differences in cardiorespiratory and metabolic responses between high and low cadences.
- $H3_1$: There are significant differences in cardiorespiratory and metabolic responses between high and low cadences.
- $H4_0$: There are no significant differences in HHb concentration changes and TSI, between high and low cadences.
- $H4_1$: There are significant differences in HHb concentration changes and TSI, between high and low cadences.

# 7 Methods

The study was approved by the local ethics committee (#00463) and all subjects signed an informed written consent before participating in the study.

### 7.1.1 Subjects

16 trained subjects (mean ± SD for male: n = 8 age 30.4 ± 4.9 years, $\dot{V}O_{2max}$ 55.3 ± 18.4 $mL \cdot min^{-1} \cdot kg^{-1}$, $P_{max}$ 350 ± 49 W and for female: n = 8 age 28.6 ± 5.17 years, $\dot{V}O_{2max}$ 45.5 ± 3.05 $mL \cdot min^{-1} \cdot kg$-1, $P_{max}$ 256 ± 16 W) participated in the study. All tests were conducted in the laboratory of the Centre for Sport Science and University Sports, Auf der Schmelz 6, 1150 Vienna. The moderately to well trained triathletes visited the laboratory at 3 occasions separated by at least 48 h and maximal 7 days to avoid effects of training and/or detraining. All tests were performed on the subjects' own bicycle.

### 7.1.2 Study design

During visit one, a graded exercise test (GXT) was performed to assess athletes´ $\dot{V}O_2$, AT, RCP and maximal power output ($P_{max}$). This was done in order to compute the intensities for the interval trainings conducted on visit two and three. The same interval training was conducted at different cadences on separated days. The two different cadences, 90 and 60 rpm respectively, were alternatively ordered.

The interval program is designed according to similar study designs (Nimmerichter et al. 2015 and Shastri et al. 2019). The interval program started with a 3-min baseline, after that it includes 4x5 min cycling at ∆25% intensity, following 4x3 min at ∆70% intensity. Whereat ∆25% and ∆70% is defined as VT+[($P_{max}$ - VT)*0.25] and VT+[($P_{max}$ - VT)*0.70]. There was a 3-min rest period at 80 W after each interval. Female athletes cycled 4x3 min at RCP + 5 W instead of the ∆70% intensity. Since pilot testing have shown that female athletes are not able to complete ∆70% intensity. RCP + 5 W is representing a slightly lower power output than ∆70%.

The two workloads are selected to demonstrate different cardiorespiratory and muscular responses. Whereas ∆25% representing a workload between VT and RCP (‘heavy exercise intensity domain’), in which MLSS is present. ∆70% and RCP + 5 W represents a workload between RCP and $P_{max}$, in which [La] accumulates and anaerobic metabolism is present (‘severe exercise intensity domain’).

The interval program is represented in Fig. 1.

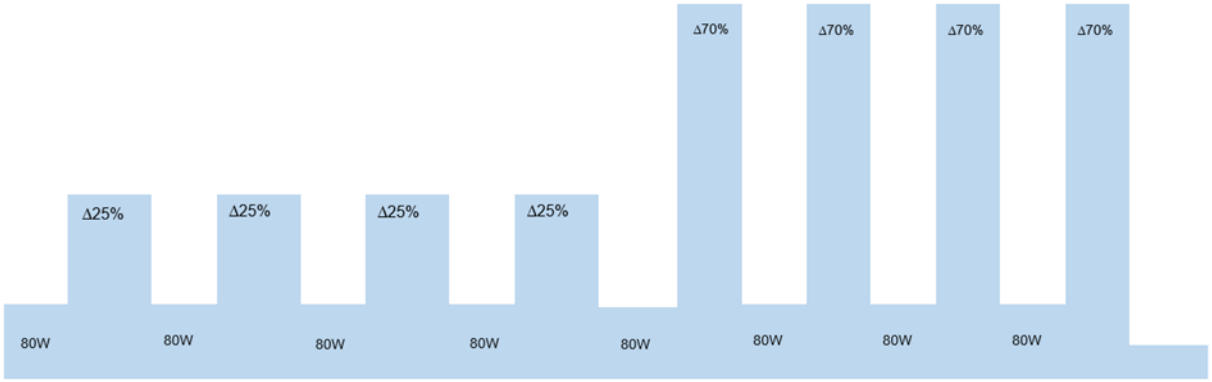



***Figure 1.:*** *Schematic cycling interval program including 4x5 min at ∆25%intensity and 4x3 at ∆70% intensity intervals and 3-minutes recovery periods at 80 W.*

### 7.1.3 Physiological Measurements

Relevant parameters for the study are HR (bpm), [La] (mmol), $\dot{V}O_2$ (ml/min), VE (L/min), RER, HHb (VL(Δμmol)) and TSI (%).

The subjects cycled at baseline for 3 min to record the baseline values of NIRS parameters and respiratory gases. NIRS recording started after one min. Respiratory gas measurements started after two min. This was done to facilitate synchronising of the measurements. Finally, the interval program started after the total baseline of 3 min.

Respiratory gases were measured using a portable gas analyser (MetaMax 3B-R2, Cortex Medical Biophysik GmbH, Leipzig, Germany). Respiratory gases were measured breath-by-breath throughout all tests, except for about 2.5 min at the half time of the interval sessions (after 32 min), where the subjects were allowed to rehydrate.

HR (H7, Polar Electro Oy, Kempele, Finland) was recorded continuously throughout all tests and the heart rate sensor was connect to the gas analyser via Bluetooth. To determine [La] concentration a capillary 20 µL blood sample was obtained from the hyperemic ear lobe after each interval and diluted immediately in 1000 µL glucose system solution. [La] (mmol $L^{-1}$) was measured using an automated lactate analyser (Biosen S_Line, EKF Diagnostics, Barleben, Germany).

To monitor muscle deoxygenation values, a device using a near-infrared technique was placed on the subjects´ right m. vastus lateralis 10 cm (women) and 15 cm (men) above the condylus lateralis.

NIRS was held at a fixed position with black tapes, which minimize interference from ambient light. Transparent film was placed between the skin and the NIRS to protect it from sweat. NIRS was attached at the same position through both test occasions. As muscle $O_2$ consumption can only be calculated reliable with the assumption that tHb remains constant during the measurements, an arterial occlusion should be required (De Blasi 1997). Since arterial occlusion is not part of the method of the present study, it will pay attention to HHb concentration changes and TSI values as an index of $m\dot{V}O_2$ derived from the NIRS. This study did not use the method of arterial occlusion because it is not essential for the present main purpose to detect difference in peripheral circulation and metabolism among pedal cadences during pedalling equivalent to actual competitions, and it causes severe discomfort to the participants. Furthermore, this study uses no total HHb values but HHb concentration changes, as total HHb values are insensitive to increased $O_2$ delivery to the muscle tissue caused by exercise-induced hyperemia (Kime et al. 2013). Since tHb is not stable during exercise, this study uses parameters which have less effects on tHb changes: TSI and HHb concentration changes. NIRS data were sampled at 10 Hz via the online data acquisition system Oxysoft (Artinis Medical Systems, the Netherlands).

[La] was sampled in the last 30 sec of rest and of each cycling bout. Respiratory gases, vastus lateralis muscle oxygenation, and HR were recorded continuously.

### 7.1.4 Statistical analyses

Analyses were performed for cadence, power output, HR, [La], $\dot{V}O_2$, TSI and ΔHHb. ΔHHb was analysed as changes from baseline, TSI as relative change. The normal distribution of variables was checked using the Shapiro-Wilk test. The strength of any relationship between variables was examined using Pearson´s product moment correlations. A paired samples t-test was performed to identify any significant differences in parameters between the Δ25% and the Δ70% conditions for each cadence. Repeated measure ANOVA and Mauchly´s test of sphericity was used to determine time effects of different cadences on the parameters. Bonferroni post-hoc tests were used to identify significant pairs. Differences between male and female parameters were examined using unpaired samples t-tests. All statistical procedures were performed using the software package SPSS

Statistics 21 (IBM Corporation, Armonk, NY, USA). The alpha level of significance was set at $p \leq 0.05$. Descriptive data are presented as mean ± SD.

# 8 Results

All data was normally distributed. Descriptive data is represent in Table 1. Data is represented as mean ± SD.

***Table 1**.: Descriptive data of all measured physiological parameters. Data is presented as mean ± SD.*

| | PO (W) | | $\dot{V}O_2$ (mL/min) | | RER | | VE (L/min) | | HR (bpm) | | [La] ($mmol.L^{-1}$) | | HHb VL (Δμmol) | | TSI % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | **Low intensity** | | | | | | | | | |
| | | | | | | | **60 rpm** | | | | | | | | | |
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** |
| **Interval 1** | 211 ± 21 | 167 ± 14 | 2658 ± 183 | 2121 ± 222 | 0.92 ± 0.03 | 0.94 ± 0.04 | 65.2 ± 5.7 | 59.4 ± 9.3 | 132 ± 8 | 146 ± 9 | 2.2 ± 0.4 | 3.0 ± 0.9 | 7.05 ± 3.08 | 2.22 ± 3.19 | 57.1 ± 3.0 | 75.0 ± 12.0 |
| **Interval 2** | | | 2620 ± 205 | 2109 ± 272 | 0.90 ± 0.03 | 0.93 ± 0.03 | 65.9 ± 7.1 | 60.5 ± 10.7 | 138 ± 9 | 152 ± 9 | 1.9 ± 0.5 | 2.7 ± 0.9 | 8.32 ± 2.39 | 2.42 ± 3.68 | 56.4 ± 3.9 | 73.9 ± 10.2 |
| **Interval 3** | | | 2687 ± 202 | 2116 ± 231 | 0.92 ± 0.02 | 0.92 ± 0.04 | 69.6 ± 6.9 | 60.1 ± 9.8 | 141 ± 10 | 154 ± 8 | 1.9 ± 0.5 | 2.5 ± 0.7 | 8.15 ± 2.74 | 2.09 ± 3.68 | 56.9 ± 3.7 | 74.6 ± 9.8 |
| **Interval 4** | | | 2701 ± 199 | 2205 ± 185 | 0.89 ± 0.03 | 0.92 ± 0.03 | 69.1 ± 6.9 | 64.3 ± 8.4 | 143 ± 10 | 156 ± 8 | 1.9 ± 0.5 | 2.6 ± 0.8 | 8.94 ± 2.75 | 1.77 ± 3.80 | 56.5 ± 3.5 | 74.7 ± 9.3 |
| | | | | | | | **90 rpm** | | | | | | | | | |
| **Interval 1** | | | 3026 ± 286 | 2310 ± 240 | 0.92 ± 0.02 | 0.98 ± 0.03 | 80.3 ± 12.3 | 68.0 ± 10.0 | 144 ± 11 | 157 ± 11 | 3.3 ± 0.7 | 4.5 ± 1.1 | 7.77 ± 3.20 | 1.99 ± 1.46 | 57.2 ± 5.5 | 73.8 ± 9.2 |
| **Interval 2** | | | 3063 ± 285 | 2302 ± 283 | 0.92 ± 0.01 | 0.97 ± 0.03 | 84.4 ± 11.4 | 71.0 ± 10.8 | 150 ± 10 | 160 ± 12 | 3.3 ± 0.7 | 4.3 ± 1.2 | 7.32 ± 3.84 | 1.68 ± 1.84 | 57.1 ± 5.4 | 73.6 ± 6.8 |
| **Interval 3** | | | 3109 ± 313 | 2330 ± 375 | 0.91 ± 0.02 | 0.97 ± 0.03 | 86.2 ± 11.6 | 75.4 ± 12.9 | 153 ± 10 | 163 ± 13 | 3.0 ± 1.0 | 4.4. ± 1.3 | 6.67 ± 4.24 | 1.51 ± 2.13 | 57.7 ± 5.1 | 73.2 ± 4.9 |
| **Interval 4** | | | 3136 ± 290 | 2322 ± 309 | 0.90 ± 0.02 | 0.97 ± 0.04 | 89.0 ± 11.3 | 76.0 ± 13.0 | 155 ± 11 | 165 ± 12 | 3.1 ± 0.7 | 4.5 ± 1.3 | 7.15 ± 3.63 | 1.34 ± 2.01 | 57.7 ± 5.2 | 72.8 ± 4.1 |
| | | | | | | | **High intensity** | | | | | | | | | |
| | | | | | | | **60 rpm** | | | | | | | | | |
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** |
| **Interval 1** | | | 3224 ± 341 | 2386 ± 200 | 1.03 ± 0.07 | 1.01 ± 0.04 | 101.1 ± 18.3 | 75.8 ± 9.9 | 159 ± 10 | 163 ± 9 | 4.4 ± 1.2 | 4.7 ± 1.5 | 10.45 ± 4.23 | 1.44 ± 4.11 | 55.9 ± 4.4 | 75.1 ± 11.5 |
| **Interval 2** | | | 3232 ± 396 | 2420 ± 204 | 1.00 ± 0.04 | 1.00 ± 0.04 | 103.2 ± 20.4 | 80.7 ± 12.5 | 161 ± 11 | 167 ± 9 | 5.8 ± 1.3 | 5.5 ± 1.9 | 10.49 ± 4.35 | 1.72 ± 4.12 | 56.3 ± 4.2 | 74.6 ± 10.8 |
| **Interval 3** | 294 ± 37 | 212 ± 22 | 3321 ± 372 | 2437 ± 221 | 1.02 ± 0.02 | 1.00 ± 0.03 | 113.0 ± 20.4 | 83.2 ± 10.5 | 164 ± 14 | 169 ± 8 | 6.3 ± 1.3 | 5.8 ± 2.1 | 10.75 ± 4.83 | 1.69 ± 4.16 | 56.1 ± 4.1 | 75.3 ± 10.3 |
| **Interval 4** | | | 3356 ± 277 | 2427 ± 227 | 1.01 ± 0.01 | 0.99 ± 0.04 | 118.0 ± 15.5 | 85.1 ± 13.0 | 167 ± 10 | 169 ± 11 | 6.7 ± 1.2 | 6.2 ± 2.6 | 10.74 ± 4.97 | 1.65 ± 4.28 | 56.2 ± 3.9 | 76.0 ± 11.6 |
| | | | | | | | **90 rpm** | | | | | | | | | |
| **Interval 1** | | | 3552 ± 387 | 2281 ± 456 | 1.04 ± 0.05 | 1.08 ± 0.07 | 129.7 ± 21.3 | 83.5 ± 15.4 | 168 ± 9 | 170 ± 10 | 5.9 ± 1.5 | 6.8 ± 1.8 | 7.39 ± 4.13 | 0.93 ± 2.29 | 57.7 ± 5.2 | 73.2 ± 4.4 |
| **Interval 2** | | | 3636 ± 437 | 2476 ± 397 | 1.04 ± 0.05 | 1.04 ± 0.07 | 139.0 ± 26.4 | 90.0 ± 13.4 | 172 ± 9 | 176 ± 9 | 7.8 ± 1.6 | 8.2 ± 2.8 | 8.15 ± 4.25 | 0.79 ± 2.40 | 57.8 ± 4.9 | 73.9 ± 6.0 |
| **Interval 3** | | | 3675 ± 379 | 2435 ± 415 | 1.04 ± 0.05 | 1.03 ± 0.06 | 150.5 ± 22.1 | 90.2 ± 12.1 | 173 ± 9 | 174 ± 14 | 9.2 ± 1.6 | 9.3 ± 3.1 | 9.13 ± 3.88 | 0.91 ± 2.28 | 57.1 ± 4.8 | 72.7 ± 3.6 |
| **Interval 4** | | | 3757 ± 328 | 2395 ± 357 | 1.04 ± 0.03 | 1.01 ± 0.08 | 158.8 ± 18.2 | 99.2 ± 10.6 | 175 ± 9 | 177 ± 11 | 10.3 ± 1.7 | 9.2 ± 2.6 | 9.00 ± 4.07 | 1.14 ± 2.87 | 56.9 ± 5.4 | 70.6 ± 3.2 |

PO = power output; $\dot{V}O_2$ = oxygen uptake; RER = respiratory quotient; VE = ventilation; HR = heart rate; [La] = blood lactate concentration; HHb = deoxygenated haemoglobin; TSI = tissue saturation index; m = male; f = female

## 8.1 Differences between cadences

Results obtained from the t-test to identify the differences between high and low cadences (60 vs. 90 rpm) are represented in Table 2. HR, [La] and VE values show significant differences between the high and low cadences in both intensities. ($p < 0.001$). RER values show significant differences between the high and low cadences in low intensities ($p = 0.015$). However, during high intensity interval 3 and 4 no significant difference in RER and high and low cadences was found ($p = 0.089$ for interval 3 and $p = 0.148$ for interval 4). $\dot{V}O_2$ values show significant differences between the high and low cadences in low intensities ($p < 0.000$). For high intensities differences were found in the last 3 intervals ($p = 0.008$ for interval 2; $p = 0.024$ for interval 3 and $p = 0.048$ for interval 4). No difference was found in the first high intensity interval ($p = 0.300$). $\dot{V}O_2$ dynamics during the interval test are represent in Figure 2. HHb values show no significant differences between the two cadences in low intensities ($p = 0.297$). Differences in high intensities occur only in interval 1 and 2 ($p = 0.043$ for high intensity interval 1 and $p = 0.024$ for high intensity interval 2). HHb dynamics are represent in Figure 3. TSI values show no significant differences between high and low cadences in both intensities ($p = 0.853$ for low intensities and $p = 0.571$ for high intensities). TSI dynamics are represent in Figure 4.

***Table 2**.: P-values for the differences between cadences obtained from the t-test.*

| | **$\dot{V}O_2$** | **RER** | **VE** | **HR** | **[La]** | **HHb** | **TSI** |
|---|---|---|---|---|---|---|---|
| **Low intensity** | | | | | | | |
| Interval 1 | <0.001* | 0.027* | <0.001* | <0.001* | <0.001* | 0.653 | 0.887 |
| Interval 2 | <0.001* | 0.017* | <0.001* | <0.001* | <0.001* | 0.219 | 0.801 |
| Interval 3 | <0.001* | 0.014* | <0.001* | <0.001* | <0.001* | 0.161 | 0.862 |
| Interval 4 | <0.001* | 0.002* | <0.001* | <0.001* | <0.001* | 0.154 | 0.861 |
| **High intensity** | | | | | | | |
| Interval 1 | 0.300 | 0.002* | <0.001* | <0.001* | <0.001* | 0.043* | 0.980 |
| Interval 2 | 0.008* | 0.021* | <0.001* | <0.001* | <0.001* | 0.024* | 0.250 |
| Interval 3 | 0.024* | 0.089 | <0.001* | 0.002* | <0.001* | 0.066 | 0.451 |
| Interval 4 | 0.048* | 0.148 | <0.001* | 0.001* | <0.001* | 0.071 | 0.604 |

* identifies a significant difference ($p < 0.05$)

$\dot{V}O2$ = oxygen uptake; RER = respiratory quotient; VE = ventilation; HR = heart rate; [La] = blood lactate concentration; HHb = deoxygenated haemoglobin; TSI = tissue saturation index

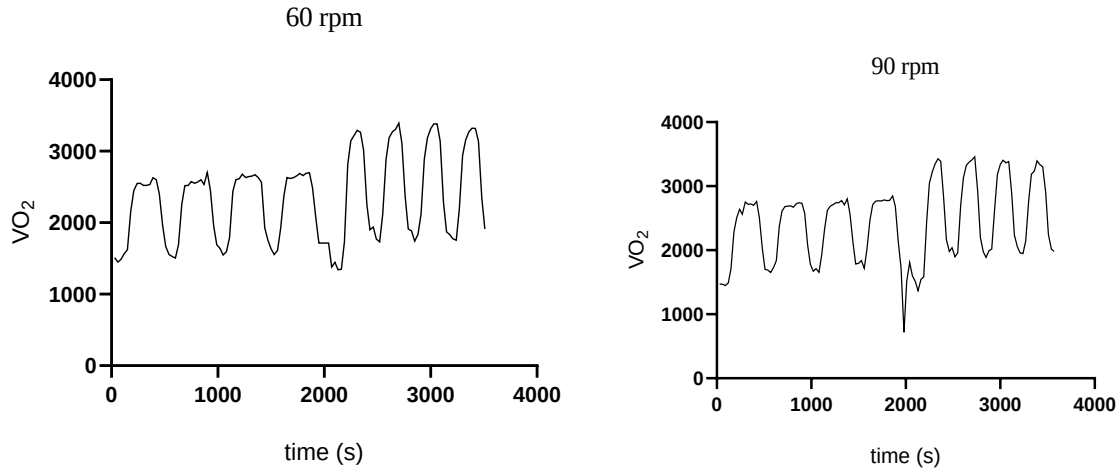



***Figure 2**.: Oxygen uptake of a representative subject during the interval exercise.*

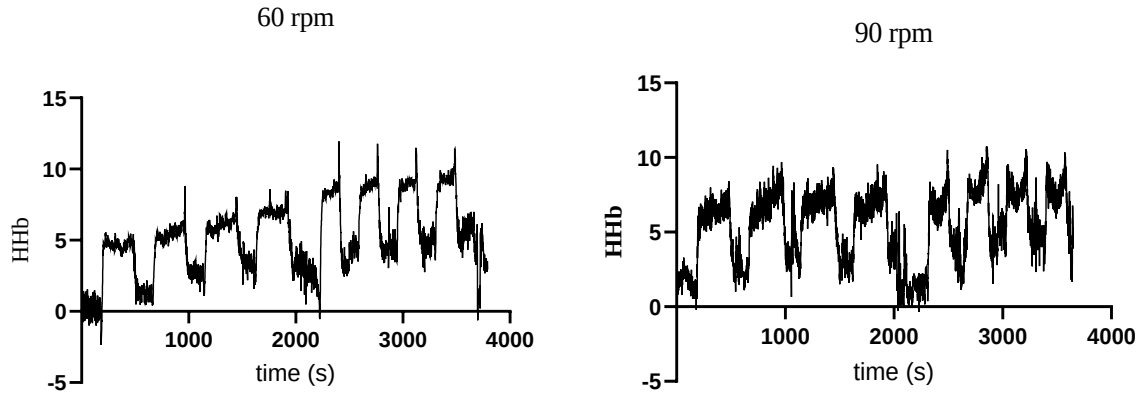



***Figure 3**.: HHb concentration changes of a representative subject during the interval exercise.*

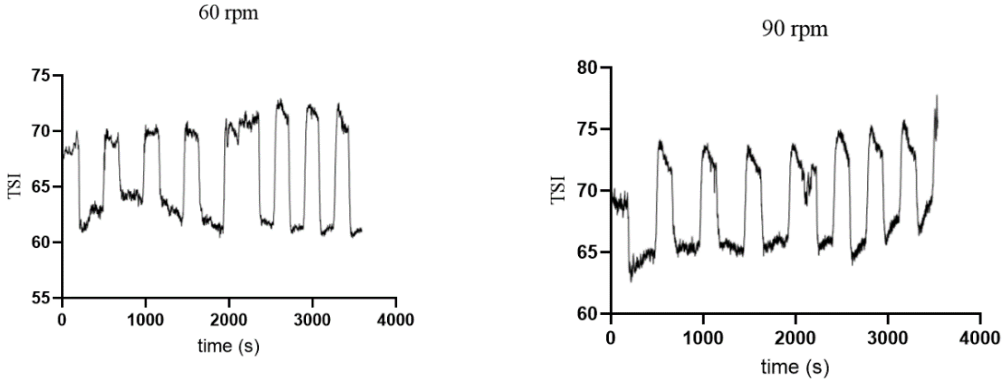
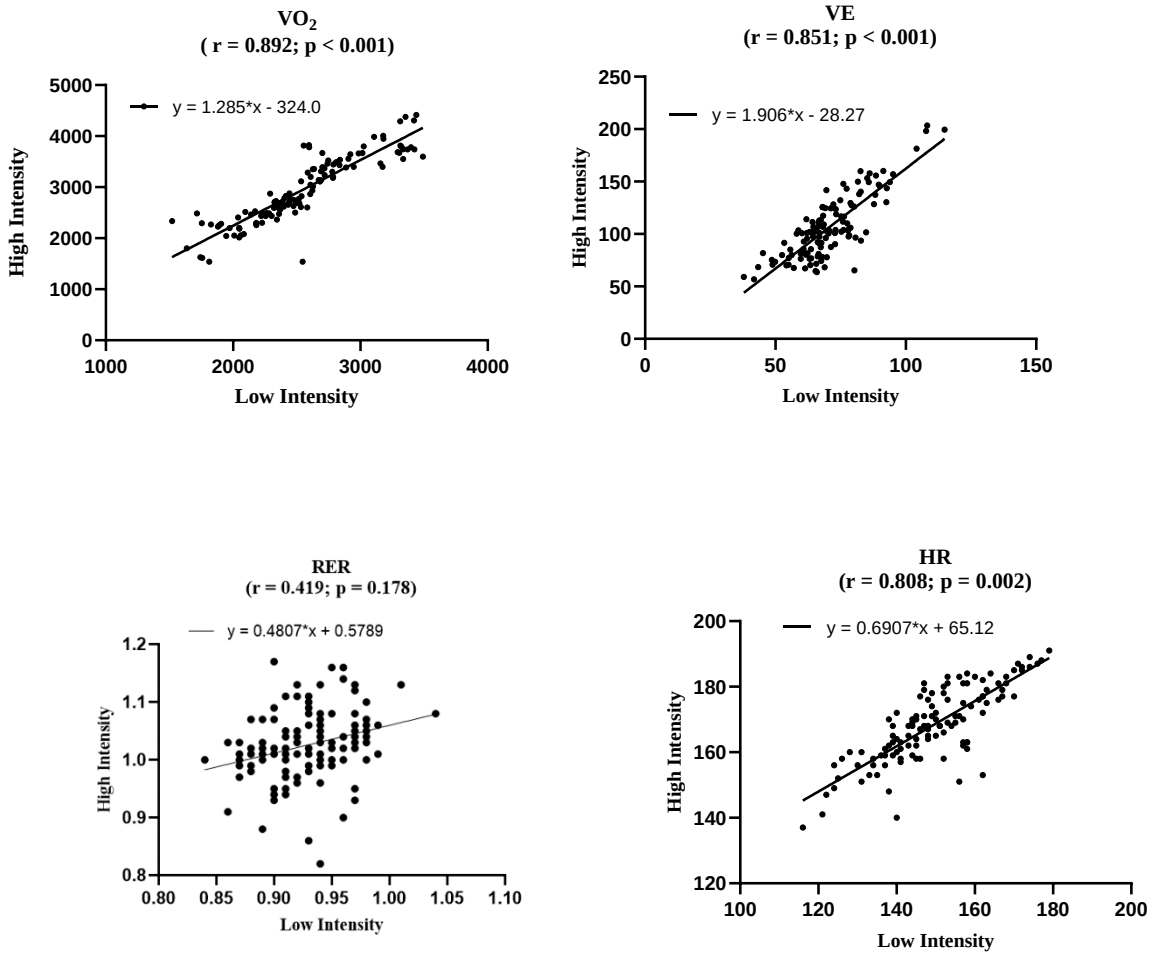



***Figure 4**.: TSI parameters of a representative subject during the interval exercise.*

## 8.2 Correlations

The Pearson´s product moment correlations values (r) between high and low intensities and cardiorespiratory as well as m$\dot{V}O_2$ values were determined. All measures, except RER, were significantly correlated between high and low intensities, respectively. Scatterplots are represented in Figure 5.



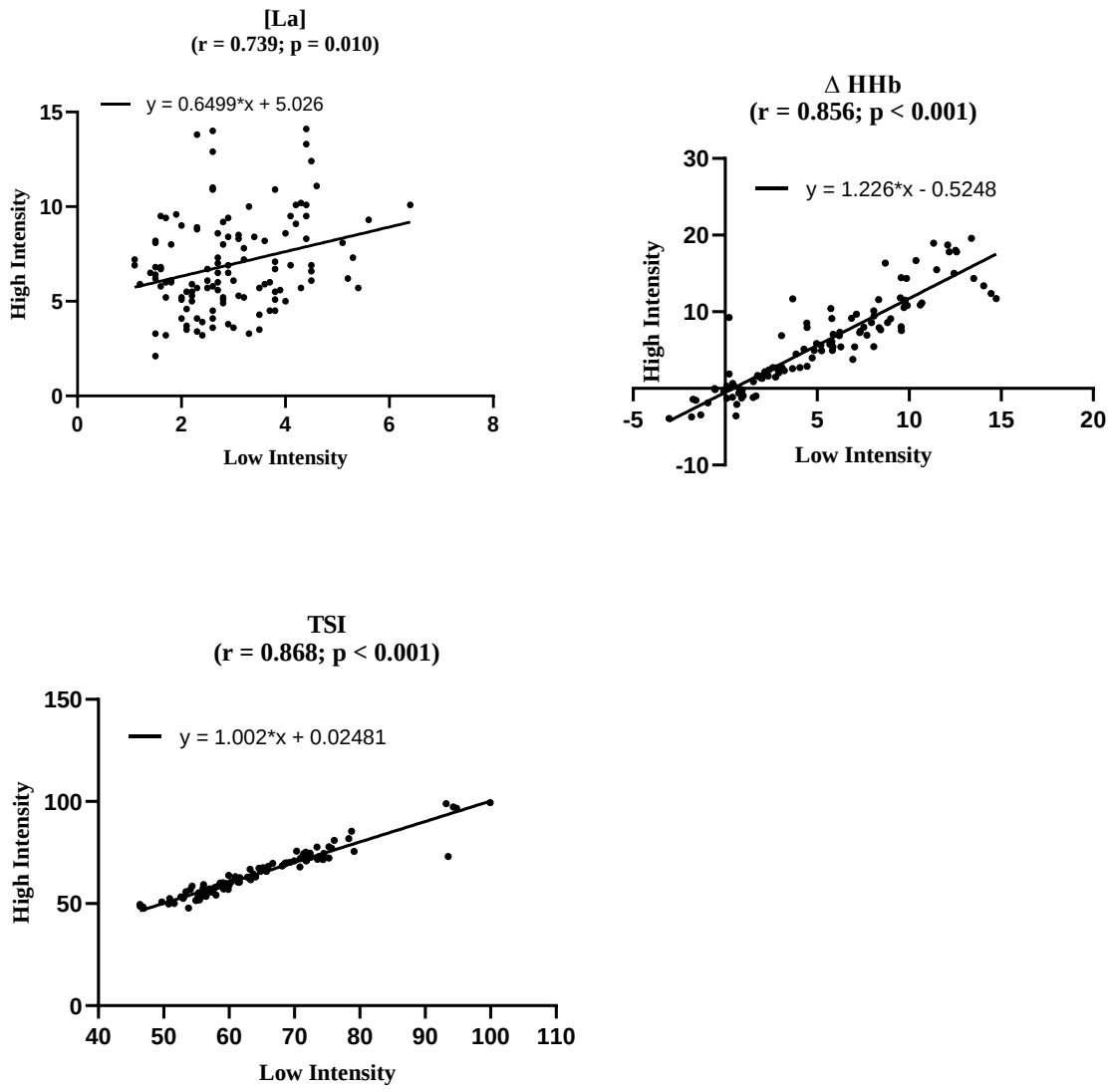



***Figure 5.:*** *Scatterplots of all determined parameters for low and high intensities.*

## 8.3 Time effects

An overview of detected time effects is represented in Table 3. No significant time effect was revealed for HHb and TSI for both cadences (p = 0.140-0.598 for HHb and p = 0.340-0.743) or TSI). However, a significant time effect was found for HR in both cadences. Bonferroni post hoc tests show significant differences during low intensities and 60 rpm as well as 90 rpm (p = 0.001). Furthermore, during high intensities significant time effects were found in 60 rpm (p = 0.006) and 90 rpm (p = 0.029). [La] values show a significant time effect in low intensity intervals during 60 rpm (p = 0.001), whereas no time effect was found during 90 rpm and low intensity intervals (p = 0.614). Post hoc test showed that the significant pairs are spread randomly through the 60 rpm low intensity intervals. During high intensities ANOVA shows a

significant time effect in both cadences for [La] (p = 0.001). Bonferroni post hoc test showed time effects all pairs. There is a significant time effect for RER in low intensity intervals in both cadences (p = 0.001 and p = 0.033). According to the Bonferroni post hoc test, time effects occurred in all pairs during low intensity intervals. However, no significant time effect in high intensities has been found (p = 0.147 for 60 rpm and p = 0.477 for 90 rpm). VE values show a significant time effect in both cadences and in low as well as in high intensity intervals (p = 0.001). Post hoc tests show, that the effect occurs during high cadences. $\dot{V}O_2$ values show a significant time effect in low and high intensities but only during 90 rpm (p = 0.026 for low intensities and p = 0.033 for high intensities). However, Bonferroni post hoc test showed no significant pairs. During 60 rpm no significant time effect has been found (p = 0.075 for low intensities and p = 0.033 for high intensities).

***Table 3**.: Time effect statistics: p-values derived from a repeated measures ANOVA*

| | $\dot{V}O_2$ | RER | VE | HR | [La] | HHb | TSI |
|---|---|---|---|---|---|---|---|
| **Low intensity** | | | | | | | |
| **60 rpm** | 0.075 | 0.001* | 0.001* | 0.001* | 0.001* | 0.140 | 0.340 |
| **90 rpm** | 0.026* | 0.033* | 0.001* | 0.001* | 0.614 | 0.152 | 0.743 |
| **High intensity** | | | | | | | |
| **60 rpm** | 0.052 | 0.147 | 0.001* | 0.006* | 0.001* | 0.598 | 0.466 |
| **90 rpm** | 0.033* | 0.477 | 0.001* | 0.029* | 0.001* | 0.154 | 0.467 |

* identifies a significant time effect (p < 0.05)

$\dot{V}O_2$ = oxygen uptake; RER = respiratory quotient; VE = ventilation; HR = heart rate; [La] = blood lactate concentration; HHb = deoxygenated haemoglobin; TSI = tissue saturation index

## 8.4 Gender differences

Mean p-values of detected gender differences are presented in Table 4.

A t-test to identify gender differences shows significant differences in HHb, TSI and $\dot{V}O_2$ in all intervals (p < 0.05). There are significant gender differences for HR, but only during 60 rpm - low intensity intervals. Significant differences between male and female athletes in VE have been observed. However, the differences are only present in high intensities. There are significant gender differences for [La], but only during 90 rpm and low intensities. However,

there are no significant differences in high intensities. There are significant gender differences for RER, but only in low intensities and only during 90 rpm.

***Table 4**.: Gender statistics: Mean p-values derived from the t-test.*

| | $\dot{V}O_2$ | RER | VE | HR | [La] | HHb | TSI |
|---|---|---|---|---|---|---|---|
| **Low intensity** | | | | | | | |
| **60 rpm** | 0.001* | 0.120 | 0.201 | 0.017* | 0.196 | 0.006* | 0.002* |
| **90 rpm** | 0.001* | 0.001* | 0.071 | 0.128 | 0.046* | 0.007* | 0.001* |
| **High intensity** | | | | | | | |
| **60 rpm** | 0.001* | 0.528 | 0.009* | 0.438 | 0.676 | 0.003* | 0.002* |
| **90 rpm** | 0.001* | 0.817 | 0.000* | 0.718 | 0.635 | 0.005* | 0.002* |

* identifies a significant gender difference ($p < 0.05$)

$\dot{V}O_2$ = oxygen uptake; RER = respiratory quotient; VE = ventilation; HR = heart rate; [La] = blood lactate concentration; HHb = deoxygenated haemoglobin; TSI = tissue saturation index

# 9 Discussion

The purpose of this study was to examine if a cycling interval exercise, conducted with high and low cadences, including high and low intensities, shows significant differences in HHb and TSI parameters and cardiorespiratory as well as metabolic responses in trained male and female triathletes. Moreover, the study examined, whether there is a physiologically meaningful difference between female and male athletes.

The results of this study showed correlations between exercise intensities (∆25% and ∆70% intensity) and all measured physiological responses. With an exception of RER, which showed no significant correlation regarding the two exercise intensities ($p > 0.05$). A possible explanation for that finding could be a good training status of the subjects, who conduct cycling trainings regulary for at least 1 year. Trained subjects have a better ability to exhale $CO_2$ and inhale $O_2$ even at high intensities. This could explain the lack of correlation for RER between low and high intensities. Another explanation for similar RER values throughout the interval exercise could be different responses to same relative intensity presecription. For some subjects the ∆70%-intensity could be not that hard than for others, reflecting lower RER values even in the high intensity interval.

Significant differences ($p < 0.05$) between all physiological parameters in high and low intensities and both cadences (60 and 90 rpm) were found, except for TSI. For [La], HR, VE and $\dot{V}O_2$ higher values were found during the high intensity. In addition, higher [La], HR, VE and $\dot{V}O_2$ were found in the 90-rpm cadence. Higher RER were found during 90 rpm in the low intensity-intervals. During high intensities differences between the cadences were only found for interval 1 and interval 2, also reflecting higher values during 90 rpm than during 60 rpm. For HHb higher values were found in higher intensities, whereas no differences were found between the two cadences, except for high intensity interval 1 and 2.

Significant time effects were found HR and VE during all conditions, indicating increasing values from interval 1 to interval 4. In $\dot{V}O_2$ and [La], time effects occurred only partial: For [La], time effects were found at the high intensity at both cadence conditions, whereas at the low intensity time effects were found only during 60 rpm. The dedicated [La] time effect showed an increase with increasing number of conducted intervals. For $\dot{V}O_2$, time effects were found for

both intensities but only at 90 rpm, also indicating an increase from interval 1 to interval 4. For $m\dot{V}O_2$ parameters, HHb and TSI, no significant time effects were found.

## 9.1 Intensities and physiological responses

The detected correlations between physiological parameters and intensities indicate, that cardiorespiratory ($\dot{V}O_2$, VE, RER and HR), metabolic ([La]) and $m\dot{V}O_2$ (HHb and TSI) responses increase with increasing power output (from ∆25% to ∆70%).

These greater cardiorespiratory and metabolic responses associated with an increase in power output at a constant cadence are in agreement with previous findings (Gaesser & Brooks, 1975; Seabury et al., 1977; Hagberg et al., 1981; Grassi & Quaresima, 2016), and are particularly evident during incremental exercise (Zoladz et al., 1998; Ferreira et al., 2006 and Boone et al., 2015). It is generally accepted, that $\dot{V}O_2$ increases when exercise intensity increases (Moore et al., 2008). In addition, when exercise intensity increases so is VE, in order to buffer acidosis (Davis, 1985). RER represents the ratio between $CO_2$ and oxygen used during exercise, however no correlation between high and low intensities were found for RER.

Considering that $\dot{V}O_2$ is limited by the cardiac output, it becomes clear that $\dot{V}O_2$ and HR are parallel processes and influence each other. At the present study $\dot{V}O_2$ evinced a $\dot{V}O_2$ slow component that increased systematically during the high intensity intervals. HR increased with increasing $\dot{V}O_2$, whereas the initial increase in HR is generally faster than that of pulmonary $\dot{V}O_2$ (Poole & Jones, 2012), indicating that cardiac work is not the demanding factor in high intensity exercise. In addition, when cycling at higher intensity, [La] increases in the muscles (Faria et al., 2005). Higher intensities create a higher cardiorespiratory work, which as a results causes the accumulation of [La] in the muscle. When oxygen is non-limiting, the higher the exercise intensity the higher the rate of muscle production and release of [La]. Furthermore, the slow phase of $\dot{V}O_2$ is linearly correlated with the increase in [La].

Besides the cardiorespiratory values, also $m\dot{V}O_2$ values were correlated with exercise intensity, indicating that $m\dot{V}O_2$ increases with increasing intensity. This finding is in agreement with previous finings of Lucero et al. (2018), who stated that mean muscle blood flow was correlated linearly with exercise intensity and was directly proportional to $m\dot{V}O_2$. However, other authors found sigmoid HHb pattern when exercise intensity increases linear (Ferreira et al., 2007 and Boone et al., 2010). TSI does not directly reflect $m\dot{V}O_2$ but can identify the rate of oxidative metabolism. It can signal the balance between muscle oxygen supply and demand. As TSI is calculated as the ratio $HbO_2$ / tHb this value is influenced by muscle blood flow

(Grassi & Quaresima, 2016). Considering this, the difference in TSI between the intensities could be due to a change muscle blood flow.

## 9.2 Cadences and physiological responses

*Pulmonary responses*

In the present study differences in $\dot{V}O_2$ between the two cadences during low and high intensities were found. The results indicate higher $\dot{V}O_2$ values during the higher cadence of 90 rpm than the lower 60 rpm cadence. In addition, VE and RER were significantly different during both intensities, also indicating higher values during 90 rpm.

These findings are in agreement with the findings of other similar studies (Chavarren & Calbet, 1999; Mora-Rodriguez & Aguado-Jimenez, 2006; Jacobs et al., 2013; Hirano et al., 2015, Nimmerichter et al., 2015; Graham et al., 2018 and Shastri et al., 2019). Where the pulmonary responses ($\dot{V}O_2$, VE and RER) increased at higher cadences compared to lower cadences. Therefore, it is generally accepted that higher cadences generate greater respiratory work due to the increased mechanical internal work.

However, Takaishi et al. (1996) found that at higher cadences (85 – 96 rpm) a minimal neuromuscular fatigue takes place. That could be one reason why FCC are generally higher, although higher cadences create a higher respiratory work. Another reason for higher FCC could be the more effective skeletal-muscle pump which increases muscle blood flow and venous return (Gotshall et al., 1996). Furthermore, the higher internal work results in a reduced external work (force on the pedal) (Takaishi et al., 2002). The “optimal” cadence (lowest point of $\dot{V}O_2$) has shown to increase linearly with power output (Coast & Welch, 1985). However, it has been found that the effect of cadence on $\dot{V}O_2$ is gradually reduced when increasing intensity (Sidossis et al., 1992; Boone et al., 2015 and Zorgati et al., 2015). That fact is supported by Zorgati et al. (2015), who found no significant difference in $\dot{V}O_2$ during an cycling exercise test until exhaustion at 90% $\dot{V}O_{2max}$.

*Cardiac and metabolic responses*

For HR and [La], significant differences between the cadences were found for low and high intensities. Similar to respiratory parameters, HR and [La] have been shown to increase more

at high cadences than at low cadences (Coast & Welch, 1985; Gotshall et al., 1996; Jacobs et al., 2013; Hirano et al., 2015; Nimmerichter et al., 2015; Graham et al., 2018 and Shastri et al., 2019).

*Muscle oxygen responses*

For HHb, no significant differences between the cadences were found in the low intensity condition. During the high intensity, significant differences in HHb concentration changes were only found in the first two intervals. It is possible that the detected differences in cadences observed at elevated intensity were the effect of the previous exercise as an effect of fatigue through the first part of the interval training (4x5 min at ∆25%). In the study by Shastri et al. (2019) HR and TSI before the beginning of each exercise bout returned to values not different from resting levels, suggesting that participants were in similar conditions before the beginning of each exercise bout. The rest period of 3 min at 80 W during the interval training of the present study may have been to short for a full muscular recovery, indicating that no HHb concentration difference between high and low cadences was found. A further possible explanation for no significant differences in HHb in both intensity conditions could be the training status of the subjects. Lucía et al. (2001) described that trained cyclists have a higher preferred cadence (> 85 rpm). Indeed, considering that the preferred cadence in cyclists and runners are also higher than in less-trained subjects (Marsh & Martin, 1997) a pedalling cadence of 90 rpm could be likely closer to the preferred cadence of cyclists and triathletes than that of untrained subjects. On the other hand, during uphill cycling, trained cyclists tend to slow their preferred cadence down to decrease the cardiorespiratory work. Indicating, that trained subjects have a better skill to pedal at various cadence without major changes in HHb concentration. These findings are in agreement to Shastri et al. (2019) who found no differences at all between different cadences (30-110 rpm) in high and low intensities. An important methodological aspect of this previous study is that cadences were tested in incremental sequence.

For TSI no significant difference between the two cadences were found, wheatear during high nor during low intensity exercise. One would expect higher $StO_2$ oscillation at lower cadences due to greater intramuscular pressure during contraction as well as longer time of contraction. As TSI describes the index of $HbO_2$ and tHb, one would expect lower TSI values during high cadences and high intensities. In addition, during moderate intensity exercise (∆25%), an increase in cardiac output and decrease in systemic vascular resistance occurs (Gotshall et al., 1996), which would indicate higher TSI. However, in the present study no changes in TSI were found. This finding is in agreement with those by Shastri et al. (2019), who found no

differences of TSI values between cadences and two different exercise intensities. Furthermore, Kounalakis & Geladas (2012) also found no differences in the $StO_2$ parameter during 2 h moderate exercise at high and low cadences. However, Skovereng et al. (2016) and Skovereng et al. (2017) found, that $StO_2$ decreased with increasing cadence. An explanation for these rather outstanding finding may be the relative low exercise intensity (75% LT) selected in these studies. It can therefore be concluded that an increase in cadence (= increase in intramuscular work rate) is not an important determinant of the physiological response at muscular level. The result found in the present study indicates that TSI is not affected by intramuscular pressure or muscle fiber contraction speeds. Furthermore, this would lead to the conclusion that the local muscle metabolism is not linked with the cardiorespiratory metabolism.

## 9.3 Time effects

Significant time effects occurred in the cardiorespiratory parameters, which may be the effect of fatigue throughout the interval exercise. It is known that during high intensity $\dot{V}O_2$ shows a continuous exponential increase until the end of the exercise (i.e. $\dot{V}O_2$ slow component). A $\dot{V}O_2$ slow component was found in the present study, showing greater $\dot{V}O_2$ demands at the higher intensity. It is also known that during prolonged exercise duration a progressive rise in HR occurs (i.e. cardiac dift). A cardiac drift was also found in the present study. So the examined $\dot{V}O_2$ slow component and cardiac drift are two results of fatigue during the interval exercise test. These findings are in agreement with the subjective feeling of fatigue reported from the subjects through the prolonged interval test. However, no time effects for $m\dot{V}O_2$ parameters have been found in the present study. In other words, HHb and TSI values for the first and the last interval of the same intensity interval showed no significant differences. These outstanding results would lead to the assumption that no fatigue occurs in the working muscles, but in the whole cardiorespiratory system. The findings could indicate a fatigue effect of breathing muscles, whereas an increase in cardiac work has to happen, to accomplish the exercise intensity.

No significant time effect indicates that no effect of fatigue was found for local $m\dot{V}O_2$ parameters. Although subjects reported fatigue in the legs during the interval test. This is to best knowledge the first study examining this condition. These findings are very interesting, since they contradict the assumption, that there is a higher local deoxygenation as the cycling test prolongs. This disproved assumption would be supported by the fact, that the other physiological parameters ($\dot{V}O_2$ and HR) showed significant time effects. This finding suggests

that $m\dot{V}O_2$ parameters are not affected during prolonged cycling and are no determinants of fatigue during cycling exercise. However, these findings are in contrast to the findings of Hopker et al. (2017), who found increased $m\dot{V}O_2$, indicating higher HHb, during 2h of prolonged constant load cycling. Therefore, it could be suggested that there are different $m\dot{V}O_2$ responses for different exercise conditions (constant load vs. interval exercise).

## 9.4 Gender differences

Differences between male and female subjects were found in HHb, TSI and $\dot{V}O_2$. Only few authors conducted cycling studies with female subjects or explored differences between male and female cyclists. It has generally been found that professional female cyclists prefer higher cadences (Impellizzeri et al., 2008), which is in agreement with previous findings in male professional cyclists (Lucía et al., 2001). Furthermore, femal physiological characteristics in cycling are similar to those of male cyclists (Impellizzeri et al., 2008). Absolute values of $\dot{V}O_2$ have already been shown to be greater in male than in female cyclists ($p < 0.05$) (Kang et al., 2006). A significant difference between $\dot{V}O_2$ values for male and female subjects was also found in the present study.

[La] and RER gender differences occurred only at 90 rpm low intensity intervals. VE differences were found only during high intensities. However, to the best knowledge, this is the first study to examine differences in $m\dot{V}O_2$ parameters, HHb and TSI, between male and female subjects. The detected gender differences in HHb concentration changes, TSI and $\dot{V}O_2$ could be due to the different body composition in female and male athletes (i.e. muscle fiber composition) or higher subcutaneous fat percentage. Considering that muscle fiber type composition influences muscle blood flow powerful, and muscle blood flow influences $HbO_2$ and tHb (and therefore TSI), it can be suggested that different fiber composition results in different TSI values. Furthermore, NIR light has to cross the skin and subcutaneous fat in order to reach the muscle. A higher percentage of subcutaneous fat in female individuals could cause a sensitivity problem for NIRS measurement or could be one reason for the differences in the $m\dot{V}O_2$ values.

Based on these results, gender differences in HHb, TSI and $\dot{V}O_2$ are present in cycling exercise, especially at high intensity exercises. $\dot{V}O_2$ has already been shown to rise higher in male than in female cyclists. That could be due to the different body composition, since men have a greater thorax and therefore greater lungs. This is supported by the fact, that VE was higher in men during high intensities. The differences in $m\dot{V}O_2$ parameters could also be

explained by different muscle fibre distribution and a different hormone status as well as higher subcutaneous fat in female athletes. Male subjects had much higher ΔHHb than female subjects, a finding which supports the theory that this is because of a different body composition (i.e. higher muscle ratio in male).

# 10 Limitations

It should be noted that there are some methodological limitations that have to be considered. While measuring $m\dot{V}O_2$ parameters, no arterial occlusion was conducted. However, because of this methodological fact, no attention was paid on $HbO_2$ values. It would have been advantageous to collect RPE data during the interval test. Furthermore, an FCC test trial would have given further possibility to compare and interpret the collected parameters. RPEs and FCC trial combined could have been used to discuss the individually perceived effort during high and low cadences, respectively.

# 11 Conclusion

The present study shows that cardiorespiratory ($\dot{V}O_2$, VE, RER and HR), metabolic ([La]) and local deoxygenation (HHb and TSI) responses increase with increasing power output. However, the main focus of this study was on the effect of different cadences and different intensities in the presence of an interval exercise. The higher $\dot{V}O_2$, HR and [La] found at high cadences could be an important finding for cadence specific training application, whereas it must be taken into account that the effect of cadences on $\dot{V}O_2$ decreases with increasing intensity. Furthermore, higher preferred cadence could be established by a minimization of neuromuscular fatigue. The measured physiological responses were lower at lower cadences, whereas other studies have shown that low cadences could be used for cycling specific strength training due to higher force application on the pedals. The lack of difference in HHb and TSI between the two different cadences indicate that those m$\dot{V}O_2$ parameters may not be important indicators in cycling exercise. This is supported by the fact, that no time effect (and therefore no effect of fatigue) was found for those m$\dot{V}O_2$ parameters, although effects of fatigue were found for the other cardiorespiratory parameters. This finding is important for further cycling training interventions and studies examining local muscle deoxygenation.

# Tables

# Figures

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