THESIS

THE INTERACTION BETWEEN CYCLING CADENCE AND SUBSTRATE UTILIZATION IN TRAINED CYCLISTS

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ABSTRACT

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Currently, the optimal pedaling rate for road cycling endurance performance is not very well understood. It is known that muscle fiber recruitment patterns vary between low and high cadence rates. However, it is unclear whether different muscle fiber recruitment patterns stimulate different substrate utilization patterns between low and high cycling cadences. PURPOSE: We investigated if pedaling at a higher cadence at a submaximal work level results in a higher proportion of fat oxidation compared to cycling at the same intensity at a low cadence. In addition, we aimed at studying the optimal cadence for endurance road cycling and why well-trained and professional cyclists tend to pedal at higher rates. METHODS: Participants were trained, competitive cyclists and/or triathletes (VO₂ max $60.4 \pm 7.1 \text{ ml/kg/min}$, aged $24 \pm 2.5 \text{ years}$, n = 11) living in Fort Collins, CO. All were training at least 8 hours per week and had participated in a competitive event in the past two years. Baseline testing consisted of a maximal consumption test (VO_2 max test) that started at a low work level (50 – 100 watts) and increased by 25 watts every three minutes until exhaustion after which a verification bout was performed. From the VO_2 max test, the first ventilatory threshold (VT1) was determined for each participant and served as the power output used during the cadence protocol that followed on a separate day. The cadence protocol entailed seven stages each lasting six minutes in length with a fourminute recovery period in between. Work rate remained constant during the cadence protocol while a different cadence was assigned randomly to each stage (60, 70, 80, 90, 100, 110 and freely chosen cadence (FCC)). **RESULTS:** Cadence had a significant effect on HR (estimated slope = 0.2634, SE = 0.032, p < 0.001), VO₂ (estimated slope = 0.098, SE = 0.012, p < 0.001), RER (estimated slope = 0.0007, SE = 0.0001, p < 0.001, as well as absolute (estimated slope = 0.012, SE = 0.001, p < 0.001) and relative

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percentage of CHO utilization (estimated slope = 0.2696, SE = 0.053, p < 0.001. Cadence did not have a significant effect on absolute fat utilization, but it did have a significant effect on the relative percentage of fat utilization (estimated slope = -0.2283, SE = 0.053, p < 0.001. VO₂, HR, and VE were minimized at 70 rpm while carbohydrate utilization was minimized at 60 rpm. FCC was found to be 89.8 rpm. Pairwise comparisons with FCC showed significant mean differences with respect to HR and VO₂ between FCC and 70, 100, and 110 rpm as well as significant mean differences with respect to CHO utilization between FCC and 110 rpm. **CONCLUSION**: The increase in energy expenditure at higher cadences is not counterbalanced by a significant increase in fat oxidation, thereby resulting in a carbohydrate penalty at higher cadences. FCC is not solely chosen to limit metabolic cost or optimize substrate utilization. An optimal pedaling cadence may be one that allows the cyclists to maintain the highest wattage desired without a considerable amount of muscular fatigue while minimizing the consequent increased metabolic cost and CHO penalty.

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CHAPTER 1 – LITERATURE REVIEW

Introduction

Every summer, the top cyclists in the world battle it out for the coveted yellow leader's Jersey, the maillot jaune, at cycling's most iconic race, the Tour de France. With advanced research in optimal training, new bicycle technologies and the talent of riders, the sport is more impressive and evolving faster than ever before. Cyclists have endless choices of gearing, wheels, and nutrition to optimize their performance for any kind of race conditions. However, despite all these advancements, the pedaling rate at which one should pedal at for optimal performance is still not very well understood.

Today, well-trained and professional cyclists tend to pedal at a rate of 90-95 revolutions per minute (rpm) or higher. However, this was not always the case. High cadence work seemed to dramatically increase during the 1992 edition of the Tour de France when Lance Armstrong first came onto the professional cycling scene and adopted a very high (110+ rpm) pedaling rate, even when climbing. Pedaling at high cadences seems to be counterintuitive as cadence rates of 40-60 rpm have been shown to elicit the lowest metabolic and oxygen cost at low workloads and the most metabolic economical cadence will increase with increasing workloads [1, 2]. So there exists a discrepancy between a cyclist's freely chosen cadence and the cadence that is most metabolically optimal for road cycling.

It has been established that pedaling at different cadence rates has different physiological and biomechanical tradeoffs. Pedaling at a higher cadence at a high but submaximal effort (> 65% VO₂ max) as well as lower workloads (45 - 65% VO₂ max) will invoke a greater oxygen cost and heart rate response than lower or moderate cadence rates [1-7]. Research has shown that pedaling at higher cadence requires less muscle activation and innervation as measured by integrated myography (iEMG) data [8-

10], with greater recruitment coming from slow twitch muscle fibers [11, 12]. This leads to less neuromuscular fatigue and torque required per pedal stroke. Pedaling at lower cadences requires greater muscle activation and recruitment, with a greater proportion of muscle activation from fasttwitch muscle fibers, especially at high power outputs [13]. This translates to a greater torque per pedal stroke which might lead to greater muscular fatigue and lactate production [11, 13, 14]. Bieuzen et al., observed differences in the energetically optimal cadence (most metabolically economical) and the most neuromuscular optimal cadence. These cadences were 63.5 and 93.5 rpm respectively [15].

Currently, therefore, the optimal pedaling cadence for overall endurance performance in road cycling remains elusive. It likely involves a complex interaction of multiple variables. Optimizing one variable, muscular fatigue for instance, may hinder another variable, such as oxygen cost. The aim of this review is to explain what is known about the interplay of factors that may determine optimal pedaling rate.

Muscle Fiber Recruitment and Cadence

Recruitment and contraction of muscle fibers repeated over and over allows movement to occur except for repeated isometric contractions. This process is important for daily activities as well as structured exercise, such as cycling. The type of muscle fibers recruited depends on the task at hand. Slow twitch fibers, also known as type I fibers, typically have a higher mitochondrial density than their Fast twitch counterparts, and therefore have superior oxidative capacities. Type I muscle fibers have a lower force production but are far slower to fatigue. Fast twitch muscle fibers, also known as Type II muscle fibers, which can be further categorized into Type IIa and Type IIx, often rely on glycolytic and anaerobic pathways, making them less efficient and more prone to fatigue. However, type II fibers can generate a higher force and power than slow twitch fibers, making them effective for intense activities.

Recruitment of muscle fibers for endurance exercise, such as cycling, begins with anaerobic metabolism and makes a seamless transition to reliance on aerobic metabolism. For instance, by the 3rd

minute of exercise, 60% of energy production originates from aerobic metabolism. Since type II fibers fatigue more quickly, exercise bouts of one to four hours are heavily reliant on aerobic energy production (Type I muscle fibers) with less contribution from anaerobic metabolism (Type II muscle fibers). The intensity of the exercise will dictate muscle fiber recruitment which affects how long the chosen intensity can be sustained. In addition to duration and intensity of the exercise, muscle fiber recruitment while cycling is also dependent on a few other variables including the phase in the pedal cycle, the position of the cyclist (seated or standing) and pedaling rate.

The pedal cycle has two main phases: the push power phase and the pull power phase. The push power phase occurs when the crank position is between 0° (12 o'clock position) to 180° (6 o'clock position), and the pull power phase is from 180° to 360° [16].

Seating and standing positions on the bicycle will produce different combinations of pedal forces, crank torque, muscle recruitment pattern and joint movement profiles. For example, when standing, the vastus lateralis, an important knee extensor, is recruited earlier in the pull power phase and remains engaged for a longer duration of the push power phase. Furthermore, Li and Caldwell demonstrated that when climbing in the standing position, the gluteus maximus is further engaged compared to a seated position. They determined that the further engagement of the muscle allowed for increased stabilization while standing, figure 1 shown below [17]. This trend of greater and longer recruitment patterns can be seen for most lower limb muscles in the standing position compared to the seated position. Despite the increased muscle recruitment and force while standing, Millet et al., (2002) concluded that level-seated, uphill seated, or standing cycling positions produce similar external economy and efficiency in trained cyclists at submaximal intensity [18]. In addition, it was included that heart rate (HR) was significantly higher in a standing position (p < 0.05), and while cycling uphill, ventilation (VE) was higher in a standing position compared to a seated position (p < 0.05)[18].

Cycling cadence can alter the pattern of muscle fiber recruitment. Ahlquist et al., (1992) found that glycogen depletion was different between slow-twitch and fast-twitch muscle fibers at different cadences. As shown in Figure 2, glycogen content of the individual fiber types was quantified as an optical density (*D*) of the periodic acid-Schiff (PAS) stain. The glycogen depletion data for type I fibers indicated that there were no significant differences between 50 and 100 rpm. However, the glycogen depletion data for type II fibers indicated that pedaling at 50 rpm resulted in a significantly greater (*p* < 0.05) amount of glycogen depletion compared to the 100 rpm exercise bout [12]. From this, the authors concluded that fewer fast-twitch fibers, compared with slow-twitch fibers, are recruited when pedaling cadence is increased from 50 to 100 rpm, shown in figure 2[12]. This recruitment pattern is in response to the reduced muscle fiber force and tension required per pedal revolution at a higher cadence.



Fig. 2. Ensemble curves of linear envelope EMG for 6 muscles for all conditions. LS, level seated; US, uphill seated; ST, uphill standing. All curves in 1 panel here used same arbitrary units on vertical axes. Scales of vertical axes used in different panels may be different. Horizontal axes are labeled by corresponding crank angle (in degrees). One complete cycle = top dead center (TDC) from 0° to TDC 360°.







Fig. 2. Cumulative frequency distribution for periodic acid-Schiff stain intensity of individual type II fibers prior to (REST) and post-exercise (EX) for the 50 and 100 rev-min⁻¹ conditions. $-\cdots$ REST 50; — REST 100; … EX 50; — EX 100

Figure 2. Adapted from Ahlquist et al. 1992

Ahlquist et al. further concluded that the force demands of pedaling, not the velocity of contraction is what determines the muscle fiber recruitment pattern [12]. In addition, Gollnick et al., (1974a) have shown that isometric contractions greater than 20% of maximal voluntary contractile strength (MVC) result in greater glycogen depletion in type II fibers compared to type I fibers. Furthermore, contractions of less than 20% MVC result in preferential glycogen depletion in type I fibers [19]. Other studies produced similar findings [13, 14, 20].

Efficiency and Cadence

Mechanical efficiency can be defined as the ratio of mechanical work done to the metabolic energy expended to do that work [21]. It is a measure of effective mechanical work accomplished and is expressed as the percentage of total energy expenditure that produces external work. External work is the application of force by the muscles through a distance [21, 22]. The external work produced when cycling can be calculated by the displacement of the flywheel and resistance opposing to the displacement of the flywheel. Energy expenditure is calculated based upon oxygen uptake (VO₂) and the corresponding respiratory exchange ratio (RER) [4, 23]. Cycling efficiency has been reported to be in the range of 10-25%. Therefore, this implies that 75-90% of total energy utilized to maintain equilibrium is obtained from ATP hydrolysis and released as heat [4]. The mechanical efficiency value obtained refers to the gross efficiency (GE) index, as first proposed by Gaesser and Brooks (1975). Gross efficiency can be expressed as a percentage of the external work accomplished divided by the total energy consumed $(GE = \frac{W_{ext}}{E_{rot}} \times 100$)[4].

Gross efficiency could be considered one the most vital functional abilities of a cyclist as it determines the power that a cyclist can produce for a given oxygen cost and level of energy expenditure [24]. In addition, it has been suggested that other methods of calculating efficiency, such as net, work and delta efficiency, have all been suggested to be conceptually flawed [25, 26]. For instance, net efficiency involves a baseline subtraction of all processes that are not part of the energy flow through the system of interest, such as resting metabolic rate [4, 23]. By doing so, this then assumes that the processes related to resting metabolism are independent, isolated, and running in parallel from the process of doing work. However, there is evidence that particularly at high work rates, various processes are affected (e.g., gastrointestinal, splanchnic metabolism, etc.); therefore, it is hard to argue that the working system and the resting metabolism are completely isolated [4, 23]. Work and delta efficiency have similar flaws. With work efficiency, the negative component is often removed from calculations (by taking the absolute value) [27]. This approach suggests that negative work is always an energy loss and never converted to external work. Finally, an advantage of delta efficiency is that knowledge of the resting metabolic rate is not required and will be less affected by changes in the baseline energy cost caused by work rate. However, the issue is that it assumes the energy flow for the change in power production is independent of the energy flow for the first amount of power produced. In other words, when applying delta efficiency as a measure for muscular efficiency the assumption is made that efficiency is independent of work rate, and furthermore, not a true measure of efficiency [26]. Therefore, it seems prudent to focus on gross efficiency as the primary outcome variable for efficiency for whole body exercise, particularly in cyclists.

Gross efficiency has been shown to be influenced by a number of factors including body mass [28], cycling position [29], pedaling technique [30], muscle fiber type [31], training status [32-34], and cadence [5, 35] and power output [13]. Furthermore, regardless of pedaling rate and other factors, multiple studies have demonstrated GE significantly improves with an increase in power output in both lower limb [4] and upper limb exercise [36].

Gross efficiency can also be influenced by cadence [5, 35]. Lucia et al., (2004) conducted a study that analyzed the effect of pedaling rates on gross efficiency and other physiological variables on elite and professional cyclists at high power outputs. Cadences of 60, 80, and 100 rpm were chosen for the study. Results showed that GE was highest (24.2 +/- 2.0%) at 100 rpm compared to 60 and 80 rpm.

Additionally, mean values of oxygen consumption (VO₂), HR, rating of perceived exertion (RPE), lactate and EMG data all decreased at increasing cadences [13]. It was concluded that professional cyclists working at a high-power output are more efficient at higher cadences (100 rpm) compared to lower cadences (80 rpm). On the contrary, studies involving nonprofessional cyclists found that GE actually decreases at higher cadences [13].

There are many factors that may contribute to greater efficiency at higher cadences. One contribution to the greater efficiency of high pedaling rates in trained cyclists is that the working muscles are contracting closer to the speed of shortening that maximized their efficiency [37]. Heglund and Cavagna (1987) also demonstrated this concept in an *in vitro* study. They found the efficiency of contraction of mammalian and frog skeletal muscle increases with the speed of contraction until reaching a maximum, which corresponds to the optimal velocity of shortening [38].

Improving Gross Efficiency

Multiple studies have demonstrated the ability to improve efficiency during cycling through high intensity cycling intervals [33] and heavy strength training [39, 40]. However, improving efficiency might be dictated by muscle fiber composition as suggested by Hansen [41] who demonstrated that cyclists with a greater proportion of type I muscle fibers have greater potential to increase their efficiency.

Hopker et al. (2010) studied the effects of incorporating two high-intensity training sessions per week for 6 weeks in improving gross efficiency in cyclists. It was found that gross efficiency increased significantly between pre and post-high-intensity training in Group A ($1.6 \pm 1.4\%$; p < 0.05), while there was no evidence of change in GE in Group B (control group) ($0.1 \pm 0.7\%$; p > 0.05) [34]. A 1.6% increase in GE signified those cyclists in Group A increased their GE from an average of 19.9% to 21.5%. This indicates that the cyclists were able to work at the same power output at a lower oxygen cost after the 6 weeks of specific training. Additional studies by Hopker et al., found that although high-intensity training

has been shown to improve gross efficiency, it did not lead to any changes in VO₂ max [25, 32-34, 42, 43].

Another way of improving gross efficiency in cycling is through heavy strength training. Ronnestad et al., (2011) demonstrated that heavy strength training can improve gross efficiency during the last hour of a 3-hour bout of submaximal cycling [39]. In addition to cyclists having improved efficiency, they also had lower blood lactate concentrations and reported lower RPE values at the same work rate. The improvements in gross efficiency could be from improvements in the cyclists' maximal strength and force. Therefore, the peak force required per pedal stroke is now a lower percentage of maximal force. This improvement in strength could potentially lead to greater recruitment of more efficient and highly oxidative slow twitch muscle fibers.

Economy and Cadence

Economy is related to the oxygen consumption (VO₂) required to maintain a certain power output, and it can be expressed by the equation, $T = P_{out}/VO_2$, where T is economy, VO₂ is the oxygen consumption required, and P_{out} is the power output. Economy is an indicator the metabolic cost of a certain activity at a particular power output. The lower the VO₂ value, the lower the metabolic or energy cost resulting in greater economy.

Cadence has been shown to affect metabolic economy [1-4, 6]. Therefore, at a fixed power output, economy will either increase or decrease based on the pedaling rate of the cyclist. Minimizing oxygen consumption and conserving energy is particularly important for cycling races, triathlons, and training. The cadence at which oxygen consumption and metabolic cost is minimized is referred to as the energetically optimal cadence (EOC).

Multiple studies have demonstrated that when cycling at a submaximal power output, lower oxygen cost is seen when pedaling at 50-70 rpm compared to higher cadences of 90 rpm or higher. [1,

35]. This indicates a lower metabolic cost and an improvement in economy at lower cadences [35, 44]. Specifically, a study by Bieuzen et al. found that the most energetically optimal cadence was 64.5 rpm, while a study from Buśko (2004) showed that oxygen cost was minimized at 60 rpm (see Figure 3) [15, 45].

Similar to the relationship between GE, power output, and cadence, multiple studies have demonstrated that the most energetically optimal cadence is increased as power output is increased in well-trained and professional cyclists [1, 2, 13, 46]. Coast and Welch (1985) found that when welltrained cyclists maintained a power output of 100 watts, the most economical cadence was 50 rpm whereas when working at 300 watts, the most economical cadence increased to 80 rpm, see figure 4 below [2]. Perhaps it is not surprising to see an increase in pedaling rate in accordance with work rate. Producing a power output at 300 watts while pedaling at 50 rpm will greatly increase the force required to turn the pedals compared to pedaling at 80 rpm. This suggests that limiting neuromuscular force and fatigue may play a large role in determining the optimal cadence rate.



Fig. 1

Exemplary dependence of $\rm VO_{2net}-pedalling$ rate of one examined subject. Arrows determine the optimal pedalling speed and the minimal oxygen intake during effort with load equal 250 W





Fig. 1. \dot{V}_{O_2} and pedal rate. Each line represents one power output. Points are mean \pm SE of five values. \dot{V}_{O_2} was significantly different between pedal rates at each power output. Arrow indicates optimum pedalling rate at each power output

Figure 4. Adapted from Coast and Welch 1985

Neuromuscular Effects of Cadence

In the previous section, it was discussed that the metabolic economy at submaximal power outputs is optimized at cadences 50 and 70 rpm with the most metabolically economical cadence increasing with increasing power output. Therefore, while pedaling at low cadences elicits the lowest oxygen cost, greater muscles forces and torques are required per pedal stroke to maintain the desired power output [14, 47]. Lower cadences require greater muscle activation and recruitment. MacIntosh et al., (2000) determined there is a unique cadence to minimize muscle activation at a given power output, with the unique cadence increasing with increasing power output.

Takaishi et al., studied neuromuscular recruitment and cadence extensively [10, 11, 14]. One technique employed was using integrated electromyography (iEMG) slope data, defined as the changes in iEMG as a function of time during exercise, as a technique to measure fatigue across different cadence rates. In their earlier studies, they were able to conclude that the cadence at which neuromuscular fatigue is minimized coincides with the cyclists' preferred cadence, not the most economical cadence. Takaishi et al. reported that iEMG signals were minimized when well-trained cyclists pedaled at 80 and 90 rpm at a moderate intensity [14]. However, this was not true for untrained cyclists as the iEMG signals were minimized at 70 rpm, signifying the point of minimal neuromuscular fatigue [11, 14]. This would suggest that through the process of training, cyclists are able to shift the cadence that minimizes neuromuscular fatigue to a higher one.

Furthermore, in a 1998 study, the research team again measured iEMG data on 3 leg muscles (biceps femoris (BF), vastus lateralis (VL), and vastus medialis (VM)) on cyclists and noncyclists that had similar aerobic capacities. All participants pedaled at 5 different cadences (45, 60, 75, 90, & 105 rpm) at 150 and 200 watts. As shown in Figure 5 below, the iEMG data for VL and VM for noncyclists was minimized at 60 rpm and abruptly rose for all higher cadences whereas iEMG data for VL and VM for

cyclists stayed consistently low until the 105-rpm bout. The BF data for cyclists showed a dramatic increase after 60 rpm. In addition, all but one cyclist preferred to pedal at 90 rpm while over half of the untrained cyclists preferred pedaling at 60 rpm [10] This again demonstrates that trained cyclists are able to ward off neuromuscular fatigue at higher cadences compared to untrained or noncyclists, indicating a positive training adaptation.



Figure 5. Adapted from Takaishi et al. 1998

Neptune and Hull (1998) had similar results with well-trained cyclists. In their study, subjects pedaled at a submaximal work level (265 watts) at 3 different pedaling rates: 75, 90, and 105 rpm. EMG data was collected at fourteen different sites on the working muscles and summed together to get a total muscle activation for each of the three cadences. Results showed the total muscle activation and muscle force quantities were minimized at 90 rpm compared to 75 and 105 rpm respectively [48]. In addition, Bieuzen et al. concluded that the neuromuscular optimal cadence was 93.5 in well-trained cyclists [15].

These results align with the idea that if muscle fibers are recruited in cycling based on force requirements (rather than speed of pedaling), the lower force requirement of 90 rpm would indicate a

lower proportion of fast twitch muscle fibers recruited and a larger proportion of slow fast twitch muscle fiber recruitment [12]. Slow twitch muscle fibers are known for their low recruitment threshold [49], higher oxidative capacity and greater efficiency [31, 50-52].

Given that most well-trained and elite cyclists tend to pedal at cadences of 90 rpm or higher, perhaps minimizing neuromuscular fatigue, and not oxygen consumption, might be an important deciding factor for choosing the optimal cadence rate.

Freely Chosen Cadence

A cyclist's preferred pedaling rate can be referred to as their freely chosen cadence (FCC). FCC varies between professional/elite cyclists and recreational and untrained cyclists. A study by Marsh et al. found rating of perceived exertion (RPE) values could be a critical component in cadence selection [53]. Multiple studies have concluded that professional/elite cyclists tend to naturally select pedaling rates between 85 and 95 rpm or higher [46, 54] compared to recreational and untrained cyclists that seem to prefer pedaling rates as low as 60 rpm [3, 55]. Terrain and other environmental conditions can greatly impact cadence selection; therefore, depending on the terrain (flat, mountain or descent) most professional cyclists will choose a cadence between 80 and 126 rpm. From this, it is clear that there exists a large discrepancy between the most economical cadence and one's freely chosen cadence. In addition, Takaishi et al. demonstrated that the torque required to maintain the same power output will decrease going from a lower cadence to a higher cadence [14]. This suggests that cyclists prefer a pedaling rate that decreases neuromuscular fatigue, but it may not be minimized at their FCC. Therefore, it stands to reason that minimizing metabolic cost and oxygen consumption may not be the main or only driving force of FCC, and other factors such as minimizing neuromuscular fatigue likely play an important role.

Benefits of having a high FCC

Despite the increased oxygen consumption for a given power output (due to increase in internal work for repetitive limb movements) [1, 3, 4], pedaling at a higher rate may confer multiple advantages. For instance, higher pedaling rates elicit less force per pedal revolution and shorten muscle fiber contraction time, contracting with a greater velocity. Shortening of muscle fiber contraction time has been shown to encourage blood flow to the muscle. Gotshall et al., (1996) demonstrated that higher pedaling cadences enhanced cardiac output in excess of the increased oxygen consumption which potentially leads to a more effective skeletal muscle pump allowing for greater muscle blood flow and venous return [56]. In addition, high cadences could have positive metabolic and substrate usage consequences, as they are associated with a minimization of type II muscle fiber recruitment, which would be expected to result in less lactate accumulation (metabolic acidosis) [57] and potentially a more optimal substrate utilization by sparing glycogen. It has been shown that the respiratory exchange ratio (RER) for 90 rpm is significantly lower than for 60 rpm [58]. This again suggests a greater reliance on slow twitch muscle fibers, which are highly oxidative and more efficient, at higher pedaling rates [56, 58].

High cadences reduce neuromuscular fatigue, promote better blood flow through the working muscles and potentially have better substrate usage outcomes as shown by RER. Therefore, high cadences can prove to be advantageous despite the reduced economy.

Too high of a cadence? Implications for going above and beyond high cadence

It has been speculated that there is an upper limit to having a high cadence [59]. One of the main factors that influences the upper limit is referred to as negative muscle work. Negative muscle work occurs when the cadence rate is exceptionally high. The starting point varies per individual but starts to occur around 105+ rpm. At these exceptionally high pedaling rates, it becomes more difficult to have effective muscle coordination because of activation dynamics and the increased need for

movement control and stabilization, such as to prevent knee hyperextension. The result is cocontraction. Co-contraction occurs when both the agonist (e.g. quadriceps) and antagonist (e.g. hamstrings) muscles are producing force at the same time, akin to Lombard's Paradox [60]. Cocontraction causes negative muscle work as extra oxygen is being utilized by the working muscles without any extra work being produced. Although negative muscle work occurs at lower cadences, such as 90 and 100 rpm, it is typically overcome and becomes negligent with additional positive work by the opposite muscle group to maintain the power output. However, when agonist and antagonist muscles are producing force at the same time, negative muscle work cannot be overcome [59].

Neptune and Herzog conducted a study analyzing cadence and crank torque to measure the amount of negative work being performed at each cadence. Five different cadence rates were tested (60, 75, 90, 105, 120 rpm). They found that there was no negative muscular crank torque generated at 60 rpm and negligible amounts produced at 75 and 90 rpm. Negative work significantly increased at 105 and 120 rpm. They determined that the critical pedaling rate, the highest pedaling rate before negative muscle work increases substantially, was 90 rpm. Furthermore, they concluded that the critical pedaling rate was highly correlated to the cyclists' preferred pedaling rate [59].

Cadence and Cycling Performance

As previously described in this review, well-trained and professional cyclists tend to pedal at higher cadences compared to novice and amateur cyclists. This section aims at discussing cadence rates of professional cyclists and potential reasons why well-trained and professional cyclists tend to pedal at higher rates compared to amateur cyclists.

Adopting a higher cadence rate was first brought into professional cycling by 5-time Tour de France (TDF) winner Miguel Indurain (1991-1995). Higher cadences in the professional cycling scene

were made popular by 7-time TDF winner¹, Lance Armstrong (1999-2005). In the tour, Armstrong often maintained a cadence of 90+ rpm regardless of whether he was on a climb or riding on flat road. During the 2001 edition of the TDF, Armstrong ascended the famous Alpe d' Huez averaging 100 rpm and an estimated power output of 450 watts [13].

Lucia et al., (2001) compiled data from the three grand tours in men's professional cycling: Giro d'Italia, Tour de France, and Vuelta a Espana. On mountain stages, the average cadence and speed of the riders were lower as riders ascended climbs averaging 7.2% while averaging 70 rpm but ranging from 60 – 80 rpm [20]. While ascending, riders will switch between a seated and a standing position on the bike. Although standing on the bike is a less economical position, cyclists are able to push more force through the pedals as the cyclists' entire body weight is being used to produce power. In addition, standing could potentially increase blood flow through iliac artery which is partly occluded over the psoas muscle during a seated position [61]. On long, flat stages, cadence was much higher with an average 90 rpm with some riders averaging 126 rpm during sprints. Cadences during the individual time trials (ITT) can be much higher, with the best time trialists averaging 96 rpm during their effort [20].

The cadence that leads to the best performances on the track seem to be even higher than pedaling cadences on the road. The 1-hour record on the velodrome has been routinely set with the cyclists maintaining a high cadence of over 100 rpm [62, 63]. The current 1-hour record holder, Victor Campenaerts set the record (in 2019) covering 55.089 kilometers (34.23 miles). He aimed for an average of 105 rpm for the effort [64].

In regard to time trials (TT) performed in a laboratory setting, Foss and Hallen conducted a cadence and performance study on fourteen elite male cyclists. Cyclists completed 30-minute time trials at different cadences (60, 80, 100, 120, FCC) while having the ability to adjust the workload throughout

¹ Titles were stripped in 2012 due to doping violations.

the effort. Compared to 80 rpm, finishing times at 60, 100, and 120 rpm were 3.5, 1.7 and 10.2% slower (*P*<0.05)[65]. Finishing time at FCC (mean rpm of 90) was undifferentiated from 80 and 100 rpm. The maximal energy turnover rate was 1.7% higher at 100 rpm compared to 80 rpm. The authors concluded that pedaling at more efficient cadences or a cyclist's FCC can lead to better performances of this length[65], although it is unclear whether longer TT performances, such as a 40k TT, would lead to the same conclusions.

Coyle et al., found certain physiological and biomechanical attributes that professional cyclists possess that could make them exceptionally efficient and primed for adopting high cadence rates [50]. Coyle et al., (1991) categorized a group of cyclists as either "elite-national" or "good-state" cyclists. During a 1-hour effort, elite-national cyclists were able to generate 11% more power and were able to work at a higher percentage of their VO₂ max compared to the good-state cyclists, 90 +/- 1% versus 86 +/- 2% respectively. They found that the elite-national cyclists were able to generate higher peak torques and push power through the downstroke phase earlier compared to the good-state cyclists. They concluded that it was the higher percentage of type I muscle fibers and possessing a 23% greater muscle capillary density found in the elite cyclists that may contribute to the greater capabilities for performance [50]. Furthermore, another study by Rodriquez et al. (2002) concluded that professional cyclists have a higher percentage of type I muscle fibers (64%), mitochondrial volume (4.3%) and capillary density [66]. Therefore, it seems through years of endurance training, professional cyclists are able to optimize their muscle fiber type and composition to be able to perform well riding at cadences of 90 rpm or above.

Substrate Utilization and Cadence

Many factors can contribute to how the body utilizes carbohydrates and fats for energy. Factors such as age, sex, training status, and diet can change the ratios of substrate utilization both at rest and

while exercising [67]. At rest skeletal muscle accounts for approximately 20% of the body's total energy requirements [68], and about 80% of the muscles' energy while at rest is derived from fat [69].

The human body has a much greater capacity to store and reserve fats for energy compared to glycogen. The largest energy reserve in the body is composed of endogenous triacylglycerols and is 60 times greater than the amount of energy stored as glycogen. As glycogen depletion is an important component to fatigue in prolonged exercise [70], different strategies of glycogen sparing can be crucial to improving endurance performance. A study by Coyle et al., (1986) found that when given a carbohydrate solution, participants were able to exercise at 71% of their VO₂ max for an additional hour compared to the placebo ($3.02 \pm 0.19 \text{ h}$) vs. ($4.02 \pm 0.33 \text{ h}$)[71]. Given that the rate of glucose oxidation did not drop and there was very little reliance on muscle glycogen in the final hour with the carbohydrate solution trial, the participants were able to oxidize carbohydrates at a high rate from other sources than muscle glycogen and allowing them to perform longer [71].

The intensity of exercise can change the proportions of carbohydrate and fat is used for energy. Romijn et al., (1993) determined that fat is the predominant source of fuel for the muscles during prolonged low and moderate-intensity exercise (up to about 65% of VO₂ max). A study from that group analyzed substrate oxidation rates at three different intensities: 25%, 65%, and 85% of VO₂ max. Respiratory exchange ratios (RER) were significantly different and increased with each intensity (0.73 ± 0.01, 0.83 ± 0.02, and 0.91 ± 0.01 respectively). Fat oxidation rates were significantly higher at 65% VO₂ max compared to 25 and 85% VO₂ max. In addition, whole body fat oxidation was significantly higher than free fatty acid (FFA) uptake by the muscle at 65 and 85% VO₂ max [72]. Carbohydrate oxidation did significantly increase with each exercise intensity. This manifests that energy derived from carbohydrate oxidation eventually becomes the main fueling source as intensity and energy expenditure increase.

Saltin and Karlsson (1971) conducted a study looking specifically at glycogen depletion rates at various percentages of VO₂ max [73]. Figure 6 shows the mean glycogen depletion rates on workloads from 30% to 120% of VO₂ max. The steepness of the curves is related to the relative workloads. They found that at lower intensities (30% and 60% of VO₂ max), participants were able to exercise for the entire 120 minutes without much glycogen depletion. At intensities of 70-80% VO₂ max, it was found that exhaustion coincided with muscle glycogen stores approaching zero (0). They concluded that workloads above 50% VO₂ max, carbohydrates are the dominant fuel source, but a reduced absolute amount of fat oxidation is not found until workloads of 70-80% VO₂ max. Therefore, it could be concluded that at intensities between 65 and 75% of VO₂ max, glycogen depletion is the limiting factor to performance as the point of exhaustion coincided with very low muscle glycogen values.



during bicycle exercise of different intensities. The results are from ref. 7, 9, and unpublished materials.

Figure 6. Adapted from Saltin & Karlsson 1971

Despite the fact that skeletal muscle is heavily reliant on carbohydrate oxidation for fuel, there is evidence that endurance training increases muscle oxidative capacity and fat oxidation in submaximal exercise without an increase in the rate of lipolysis of adipose tissue triacylglycerols [74]. Instead, other factors such as increased mitochondrial density [75] and a proliferation of capillaries within the skeletal muscle [76] contribute to the greater capacity for fat oxidation. This could lead to sparing of glycogen and improved performance. Furthermore, predominance of muscle fiber type might also be an important determinant of substrate metabolism during exercise as mitochondrial density is greater in slow-twitch fibers compared to fast-twitch fibers [77]. Given that higher cadences have been shown to utilize a greater proportion of slow-twitch muscle fibers, pedaling at higher cadences during submaximal cycling could lead to greater fat oxidation usage for energy.

As described elsewhere in this review, higher pedaling rates utilize a greater proportion of slowtwitch muscle fibers compared to lower cadences. Since slow-twitch muscle fibers often rely on fat oxidation, substrate utilization may be optimized at higher cadences while cycling. Ahlquist et al., found that prolonged cycling at 85% VO₂ max at 50 rpm resulted in greater fast-twitch muscle fiber glycogen depletion compared to pedaling at 100 rpm. This result indicates that more fast twitch muscle fibers are recruited at lower cadences. The required force and torque output derived from changing the pedaling rate might change the recruitment of muscle fibers; therefore, leading to influence substrate metabolism. Preferential use of fat as a fuel substrate at higher pedaling cadences has been reaffirmed by research demonstrating RER to be lower when pedaling at 90 rpm compared to 60 rpm [58]. Thus, if higher pedaling cadences utilize more fat, they might be expected to improve cycling performance through glycogen sparing [50, 78].

Summary

Despite many technological advancements in cycling, the optimal cadence at which a rider should pedal at for the best road endurance performance has not been determined. Furthermore, the relationship between cadence and potential differences in substrate utilization is not well understood. However, it has been confirmed by research that there are different muscle fiber recruiting patterns between low and high cadence rates. Since research suggests that higher cadence rates recruit more slow twitch muscle fibers, it is likely that high pedaling rates preferentially utilize fat as an energy

source. This would be expected to be advantageous for cycling performance through glycogen sparing or at the very least, allowing the optimization of other factors such as decreasing neuromuscular fatigue without a large metabolic penalty. The potential negative consequence that high cadence rates have a higher metabolic cost may be outweighed by the excess energy requirement being met by fat oxidation, of which everyone has an endless supply.

CHAPTER 2 – INTRODUCTION

Cycling is a popular sport and activity not only in the United States but worldwide as well. For some, riding a bike is a form of transportation or a leisure activity. However, for others cycling is a competitive sport, requiring hours of training for athletes to be able to perform at their best in the world most prestigious races, such as the Tour de France. With the introduction of disc brakes, electronic and wireless shifting, and ultra-light carbon frames, professional cyclists are able to go faster than ever. In addition, advanced research in training methods, sports performance, and nutrition has allowed athletes to train and recover more effectively. Yet, despite all these advancements, there is little consensus as to what the optimal pedaling cadence is to maximize endurance performance on the road. Well-trained and professional cyclists tend to pedal at a rate of 90 rpm or higher. This seems counterintuitive as cadence rates of 50 to 60 rpm tend to elicit the lowest metabolic and oxygen cost. However, higher cadences result in recruitment of a higher proportion of slow twitch muscle fibers and less neuromuscular fatigue. It is known that muscle fiber recruitment and potentially substrate utilization is different between low and high cadences. Very little research has been conducted on the effects of cadence and substrate utilization. Therefore, if a higher metabolic cost is related to higher cadences, it would be inconsequential if it came from fat oxidation. If this were true, cyclists would have the benefits of higher cadences with little negative effect on the metabolic cost of cycling. The present study aims to characterize the effects of cycling cadence on substrate utilization in trained competitive cyclists in order to maximize endurance performance.

Statement of the Problem

It is presently unclear whether pedaling at a high cadence rate while cycling elicits a greater fat oxidation rate compared to pedaling at a lower cadence.

Aims and Hypotheses

The first aim of this study is 1. to characterize the effect of pedaling cadence on fat oxidation in trained cyclists at a submaximal workload, and 2. to determine the cycling cadence that maximizes fat oxidation in trained cyclists at a submaximal workload. Hypothesis: Pedaling at a moderate cadence (80-100 rpm) will result in a higher proportion of energy coming from fat oxidation compared to pedaling at a low cadence (60-70 rpm). The second aim is to characterize the effect of pedaling cadence on carbohydrate oxidation in trained cyclists at a submaximal workload. Hypothesis: Pedaling at a moderate cadence will not differ in carbohydrate utilization compared to pedaling at a low cadence. Finally, the third aim is to characterize the relationship between self-selected cadence and fat oxidation, carbohydrate oxidation, and metabolic efficiency in trained cyclists at submaximal workload. Hypothesis: Self-selected cadence will occur at a pedaling rate that generates maximal fat oxidation and elicit a higher metabolic cost than the most efficient cadence.

CHAPTER 3 – METHODS

Research environment

Prior to recruitment of subjects, the research team obtained approval to conduct the study by the Institutional Review Board (IRB) at Colorado State University in Fort Collins, Colorado. Once selected, subjects provided informed consent to all protocol methods and procedures as explained by a research team member at the Human Performance Clinical Research Laboratory (HPCRL) at Colorado State University. All study visits were performed in the Human Performance Clinical Research Laboratory at Colorado State University under the supervision of Dr. Fahrner with IRB approval for Kuali protocol #1765.

Subject Selection

Young, healthy, competitive cyclists and/or triathletes aged 18 and over of both genders and all races and ethnic backgrounds were recruited from Fort Collins and the surrounding communities. Exclusion criteria included less than 18 years old, presence of cardiovascular, renal, pulmonary, or metabolic condition, use of a medication that alters normal heart rate responses, and pregnancy or trying to become pregnant. Trained cyclists and triathletes were chosen to be subjects for this study as cyclists would provide the most relevancy to the data and results. Additionally, a trained cyclist would be expected to be able to complete the testing protocol in its entirety, whereas a healthy, fit person (but someone who does not regularly cycle) might struggle to complete the cadence protocol due to the unfamiliarity of riding a bike on a trainer and the length of the cadence protocol. Trained cyclists were defined as an individual who trains for at least eight (8) hours per week or rides 200 miles or more per week. In addition, all participants were required to have competed in cycling and/or triathlon competitions during the past 2 years. Twelve cyclists/triathletes were recruited for the study with one

cyclist dropping out due to injury. Eleven cyclists/triathletes (VO₂ max 60.4 ± 7.1 ml/kg/min; Range 50.5-73.2 ml/kg/min) completed the study. Subjects consisted of six males and five females (aged 24 ± 3 years; Range 22-31 years) all residing in Fort Collins, CO.

Pre-visit Assessments

Subjects completed a general health history questionnaire on the REDCap data collecting software. Information gathered from this questionnaire included any current health conditions, injuries, medications and supplements, and the details of the bike (brake type, cassette, crank arm length, etc.) that they would be bringing for both study visits.

Visit 1 – VO₂ Max Test

Participants arrived at the lab with having eaten their last large meal at least 2 hours beforehand. Participants could eat a small meal/snack that was relatively low in protein, fat, and fiber within that time window if desired. Participants abstained from caffeine and strenuous exercise for the previous 24-hours. Following the consenting process, participants underwent various anthropometric assessments upon arrival to their first visit. Height and weight measurements were recorded. In addition, participants' body composition was measured by using a dual X-ray absorptiometry (DEXA). Finally, resting heart rate and blood pressure were obtained.

Participants performed a VO₂ max test that measured their maximal oxygen uptake. VO2 max assessments were administered using each participant's road bike, a Wahoo Kickr Direct Drive Smart Trainer© in Erg mode, and a Parvo Medics TrueOne 2400 Metabolic Measuring System (Sandy, Utah). The VO₂ max test involved 3-minute stages on the bike starting at 50, 75 or 100 watts (depending on level of talent of each cyclist) and increased by 25 watts every stage. All participants progressed through the stages until exhaustion with the duration ranging from 25-39 minutes. Blood pressure was taken during the last minute of each stage. Heart rate, ventilation, and gas exchange were also measured.

Following a 10-minute rest and recovery period, participants performed a VO₂ verification bout. Given the relatively long duration of the first VO₂ max test protocol, this second bout was performed to confirm the maximal rate of oxygen consumption. During this verification bout, participants pedaled at the power output equal to either the power output of the final interval of the VO₂ max test, or 25 watts higher if the subject was over halfway through the stage when test was ceased. Participants pedaled at this power output until exhaustion, and the duration ranged from 1 to 8 minutes. The same variables listed above were recorded every minute during the verification bout. The criteria used to determine that VO₂ max was achieved are listed below. Two out of three criteria had to be met to consider it a successful test.

VO₂ max test criteria:

- Heart rate (HR) within 10 beats of participants' HR max. If participants did not know their maximal HR, it was calculated using age-predicted max HR (APMHR = 220 – age).
- Plateau in VO₂ despite increasing workload.
- RER > 1.05

The first ventilatory threshold was determined by the following:

• VT1 = first increase in V_E/VO₂ with a concomitant increase in PETO₂, while no increase in V_E/VCO₂

Visit 2 – Cadence Protocol

Participants arrived at the lab following a 12-hour abstention from alcohol and food, a 24-hour abstention from caffeine, and an 18-hour abstention from exercise. A fast was chosen for this protocol as it has been shown that food consumed within 4 hours of exercise can affect substrate utilization. Resting heart rate was recorded for all participants.

Following a brief warm-up, participants completed seven (7) different stages that were six (6) minutes in length. Six-minute stages were chosen to ensure participants reached a steady state, and

data were collected during the last two minutes of each stage. Participants pedaled at pre-determined cadence for each stage selected in a randomized order (60, 70, 80, 90, 100, 110, and self-selected cadence). The power output (watts) remained consistent throughout all stages. The power output corresponded to the power output that each participant was working at when ventilatory threshold one (VT1) was achieved during the VO₂ max test and ranged from 100 to 250 watts. This power output was chosen as it allowed participants to reach a steady state without the accumulation of excess lactate or excessive muscular fatigue compounding from subsequent stages. Power output remained constant between all cadences due to the ERG mode available on the Wahoo Kickr.

Each stage was followed by a 4-minute active recovery/ washout period where participants pedaled at a very easy work rate (50 or 75 watts) with no set cadence. Cyclists whose VT1 occurred at less than 150 watts recovered at 50 watts, while cyclists whose VT1 was 150 watts or greater recovered at 75 watts.

Equipment

Cadence protocols were administered using each participants' road bike, a Wahoo Kickr Direct Drive Smart Trainer©, and a Parvo Medics TrueOne 2400 Metabolic Measuring System (Sandy, Utah).

Measurements

Heart rate and gas exchange measurements were recorded and averaged during the last 2 minutes of each stage. The rate of fat and carbohydrate (CHO) oxidation was determined from the respiratory exchange ratio (RER) and is automatically generated by the Parvo using the non-protein respiratory quotient [79]. The equations are:

- CHO oxidation rate = 4.585 VCO₂ 3.226 VO₂
- Fat oxidation rate = 1.695 VO₂ 1.701 VCO₂

- VO₂ and VCO₂ expressed in L/min
- Oxidation rates expressed in g/min

Data Analysis and Statistics

Three different statistical models were used to appropriately analyze the data. First, a mixed model with cadence (rpm) as a numeric, continuous variable was fit as a covariate for each response variable separately (ex: RER, %CHO, etc.). Cadence (rpm) was included as a fixed effect. Subject was included as a random effect to account for repeated measures on subjects. To account for potential differences in sex, a separate analysis was run in which cadence (rpm), sex (M, F) and cadence*sex interaction were included as fixed effects. Subject was included as random effect to account for repeated measures on subjects to account for repeated measures on subjects to account for repeated measures on subjects. This model allows the effect of cadence to vary by sex. In other words, this allows different slope estimates for each sex. However, we did not find evidence of sex or sex*cadence interaction for any response variables. Finally, to compare response variables on FCC against all other cadence stages, a mixed model with cadence (rpm) treated as a categorical variable was performed. Cadence as a categorical variable allows mean comparison. CadenceCAT (rpm) was a fixed effect and subject as a random effect. Pairwise comparisons comparing mean differences of FCC against all other cadences were run using Bonferroni as post hoc analysis. All data analyses were run on SPSS version 27. Statistical significance was established at p < 0.05 for all analyses.

Subject Characteristics

Table 1 depicts subject characteristics. The average VO₂ max was 60.4 ± 7.1 ml/kg/min; Range 50.5-73.2 ml/kg/min, indicating sample of very highly trained cyclists/triathletes.

Tab	le	1:	Sul	bject	С	har	act	er	istics
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Subject	Sex	Age (years)	Height (cm)	Weight (kg)	VO₂ Max (ml/kg/min)
1	F	24	173.0	63.0	60.6
2	Μ	22	179.8	68.7	73.2
3	М	24	176.5	80.9	65.0
4	F	24	175.9	66.7	50.5
5	F	25	155.7	48.0	52.7
7	М	31	185.4	76.1	66.3
8	М	23	170.0	66.3	65.9
9	F	26	165.1	54.9	54.9
10	F	23	173.0	67.0	52.1
11	Μ	25	170.2	64.6	62.0
12	М	22	185.0	87.9	60.7

Freely Chosen Cadence (FCC) and Work Rate for all Stages

Table 2 represents the FCC for each subject as well as the work rate, determined from their VT1, at which they pedaled at for all seven stages of the cadence protocol. One of the seven stages of the cadence protocol had no set cadence for the subjects to maintain. Instead, the cadence number was hidden from view, and subjects pedaled at their freely chosen cadence (FCC). The average FCC was 89.8 \pm 5.0 rpm, ranging from 84 to 100 rpm.

From the model treating cadence as categorical (7 levels), pairwise comparisons with all other cadence stages against FCC showed evidence of statistically significant differences in heart rate (bpm), oxygen cost (VO₂), and carbohydrate utilization (g/min). Average heart rate and substrate utilization data for each cadence can be found in Table 3 and in Table 4 for specific sex differences.

Table 2: Work rate and FCC

		Freely Chosen
	Work Rate	Cadence (FCC)
Subject	(watts)	(rpm)
1	175	97
2	250	91
3	200	91
4	175	87
5	100	87
7	250	84
8	225	85
9	150	90
10	150	100
11	175	90
12	225	86
AVG	186.6 ± 46.6	89.8 ± 5.0

Table 3. Cardiometabolic Responses

Cadence (rpm)	60	70	80	FCC	90	100	110
Heart Rate (bpm)	139 ± 13	135 ± 10	141 ± 13	140 ± 12	140 ± 12	146 ± 13	151 ± 14
VO2 (ml/kg/min)	38.39 ± 5.87	37.23 ± 6.30	37.97 ± 6.36	38.89 ± 6.31	39.16 ± 6.64	40.64 ± 6.56	43.21 ± 6.82
RER	0.84 ± 0.03	0.86 ± 0.03	0.86 ± 0.03	0.86 ± 0.03	0.87 ± 0.04	0.86 ± 0.04	0.88 ± 0.03
CHO (g/min)	1.34 ± 0.39	1.55 ± 0.50	1.56 ± 0.49	1.60 ± 0.41	1.70 ± 0.59	1.71 ± 0.49	2.09 ± 0.56
Fat (g/min)	0.73 ± 0.26	0.61 ± 0.21	0.63 ± 0.21	0.65 ± 0.29	0.62 ± 0.35	0.66 ± 0.35	0.59 ± 0.22
%сно	44.9 ± 12.4	53.9 ± 12.3	53.0 ± 10.3	54.1 ± 11.3	56.6 ± 14.6	55.5 ± 13.3	62.0 ± 8.2
%Fat	51.9 ± 11.8	46.8 ± 12.1	47.6 ± 10.2	46.5 ± 11.4	44.0 ± 14.6	45.1 ± 13.1	37.6 ± 7.5

Table 4. Sex Differences in Cardiometabolic Responses

Cadence										
Stage (rpm)	Heart Rate (bpm)		VO2 (ml/kg/min)		RER		CHO (g/min)		Fat (g/min)	
	м	F	м	F	м	F	м	F	м	F
60	140 ± 12	137 ± 14	42.04 ± 4.25	34.01 ± 4.43	0.83 ± 0.04	0.84 ± 0.04	1.52 ± 0.41	1.12 ± 0.24	0.88 ± 0.22	0.56 ± 0.19
70	137 ± 10	132 ± 11	41.14 ± 4.79	32.53 ± 4.49	0.86 ± 0.03	0.87 ± 0.05	1.81 ± 0.48	1.24 ± 0.35	0.72 ± 0.11	0.46 ± 0.21
80	144 ± 13	137 ± 13	41.48 ± 5.58	33.77 ± 4.65	0.86 ± 0.04	0.85 ± 0.02	1.88 ± 0.42	1.18 ± 0.21	0.71 ± 0.22	0.53 ± 0.16
FCC	143 ± 13	137 ± 10	42.87 ± 4.81	34.11 ± 4.30	0.84 ± 0.04	0.88 ± 0.02	1.73 ± 0.45	1.43 ± 0.34	0.82 ± 0.27	0.43 ± 0.12
90	142 ± 11	137 ± 13	43.47 ± 4.82	33.97 ± 4.47	0.86 ± 0.06	0.87 ± 0.01	1.97 ± 0.69	1.38 ± 0.21	0.76 ± 0.43	0.46 ± 0.13
100	149 ± 14	143 ± 11	45.28 ± 4.50	35.08 ± 3.38	0.85 ± 0.04	0.88 ± 0.03	1.92 ± 0.54	1.47 ± 0.31	0.84 ± 0.36	0.45 ± 0.16
110	153 ± 17	148 ± 11	47.58 ± 5.16	37.96 ± 4.46	0.88 ± 0.03	0.89 ± 0.02	2.40 ± 0.48	1.72 ± 0.43	0.72 ± 0.19	0.42 ± 0.12
AVG	143 ± 13	138 ± 12	43.41 ± 4.98	34.49 ± 4.24	0.85 ± 0.04	0.86 ± 0.03	1.89 ± 0.53	1.36 ± 0.34	0.78 ± 0.26	0.47 ± 0.15

Heart Rate (bpm) and VO₂ (ml/kg/min)

Figure 7 represents the average heart rate for each cadence. The average heart rate across all cadences was 142 ± 13 bpm. There was evidence of a positive association between cadence and heart rate (estimated slope = 0.2634, SE = 0.032, p < 0.001). For every 10 rpm increase in cadence, heart rate can be expected to increase by an average of 2.6 ± 0.32 bpm. Regarding oxygen cost, the average VO₂ was 39.36 ± 6.44 ml/kg/min. Figure 8 represents the average VO₂ for each cadence. There was evidence of a positive association between cadence and VO₂ (estimated slope = 0.098, SE = 0.012, p < 0.001). For every 10 rpm increase in cadence above 70 rpm, VO₂ can be expected to increase an average of 0.99 ± 0.12 ml/kg/min. This equated to subjects working on average at $65 \pm 7\%$ of their VO₂ max. The average intensity for each cadence is show in Figure 9.

From the model treating cadence as categorical (7 levels), pairwise comparisons showed evidence that heart rate was significantly lower at 70 rpm (MD = -5.618, SE = 1.584, p = 0.005) and significantly higher at 100 and 110 rpm compared to FCC (MD = 5.527 and 10.600, SE = 1.584, p = 0.005 and 0.001 respectively). In addition, pairwise comparisons showed that oxygen cost (VO₂) was



Figure 7. Average heart rate (bpm) for each cadence (rpm) stage.

significantly lower at 70 rpm (MD = -1.662, SE = 0.536, p = 0.018) and significantly higher at 100 and 110 rpm compared to FCC (MD = 1.753 and 4.319, SE = 0.536, p = 0.011 and 0.001 respectively).



Figure 8. Average VO₂ (ml/kg/min) and Cadence



Figure 9. Work rate as a percentage of VO₂ max for each cadence (rpm) stage.

The respiratory exchange ratio (RER) was measured across all cadence stages and is represented in Figure 10. The average RER was 0.86 ± 0.04 . Analyses showed a positive association between cadence and RER (estimated slope = 0.0007, SE = 0.0001, p < 0.001). Therefore, for every 10 rpm increase in cadence, RER can be expected to increase by an average of 0.007 ± 0.001 . Furthermore, the average RER for females was slightly higher compared to males (0.87 ± 0.03 vs. 0.85 ± 0.04).



Figure 10. Average Respiratory Exchange Ratio (RER) for each cadence (rpm)

Figure 11 represents the rate of carbohydrates (g/min) utilized while Figure 12 shows the relative contribution (as a percentage) that carbohydrate utilization provides for total substrate utilization across all cadences. The average absolute rate of carbohydrates (g/min) utilized was 1.65 ± 0.52 g/min across all subjects. From the model treating cadence as numeric (rpm), there was evidence of a positive association between cadence and absolute carbohydrate utilization (g/min) (estimated slope = 0.012, SE = 0.001, p < 0.001) and relative carbohydrate utilization (estimated slope = 0.270, SE = 0.053, p < 0.001). For every 10 rpm increase in cadence, absolute carbohydrate utilization can be

expected to increase an average of 0.12 ± 0.01 grams per minute, while the relative percentage of carbohydrate utilization can be expected to increase by 2.7%.

From the model treating cadence as categorical (7 levels), pairwise comparisons of all cadence stages versus FCC showed that carbohydrate utilization was significantly higher at 110 rpm compared to FCC (MD = 0.497, SE = 0.098, p = 0.001).



Figure 11. Carbohydrate utilization (g/min) for each cadence (rpm) stage.



Figure 12. Average carbohydrate utilization as a percentage for each cadence (rpm) stage.

The absolute ate of fat (g/min) utilized for each cadence is depicted in Figure 13. The relative contribution (as a percentage) that fat utilization provides for total substrate utilization across all cadences is represented in Figure 14. The average rate of fat utilized was 0.64 ± 0.27 g/min across all subjects. From the model treating cadence as numeric (rpm), there was no evidence of an association between cadence and fat utilization, (estimated slope = -0.002, SE = 0.0009, *p* = 0.06). However, there



Figure 13. Average fat utilization (g/min) for each cadence (rpm) stage.



Figure 14. Average fat utilization as a percentage for each cadence (rpm) stage.

was a negative association between the relative rate of fat utilization and cadence, (estimated slope = - 0.228, SE = 0.053, p < 0.001). For every 10 rpm increase in cadence, the relative percentage of fat utilization can be expected to decrease by 2.3%.

Figure 15 represents the number of calories expended from carbohydrates versus fat across all the cadences. From the model treating cadence as numeric (rpm), there was evidence of a positive association between cadence and calories expended from carbohydrates (estimated slope = 0.049, SE = 0.007, p < 0.001). Furthermore, there was no evidence of an association between cadence and calories expended from fat (estimated slope = -0.015, SE = 0.008, p = 0.06). For every 10 rpm increase in cadence the average calories per minute of carbohydrate expended can be expected to increase by 0.49 ± 0.07 kcals/min, while the average calories per minute fat can be expected to decrease by 0.15 ± 0.08 kcals/min.



Figure 15. Average calorie expenditure as a function of carbohydrate versus fat utilization.

CHAPTER 5 – DISCUSSION

Introduction

The present study tested the hypothesis that substrate utilization is optimized towards a greater reliance on fat oxidation at higher cadences while pedaling at a fixed submaximal work rate. In addition, this study also sought to determine if highly trained cyclists inherently select a cadence that is more metabolically costly, but one in which the additional energy requirements are provided predominantly by fat oxidation and therefore would allow the cyclist to take advantage of the benefits of pedaling faster (e.g., decreased muscular fatigue) while minimizing the effect of a metabolic penalty. The primary findings of the study demonstrate a greater reliance on carbohydrate oxidation as pedaling rate is increased. In addition, HR, oxygen consumption and minute ventilation were minimized at 70 rpm.

VO₂, VE, HR and Cadence

It was hypothesized that oxygen consumption (VO₂), ventilation (VE), and heart rate (HR) would be minimized at low cadences, indicating optimal metabolic economy at low cadences. Results for oxygen VO₂, VE, and HR demonstrated the same patterns across cadences as they all increased with an increase in pedaling rate from 70-110 rpm at a submaximal work level. Furthermore, oxygen consumption VO₂, VE, and HR were all lower at 70 rpm compared to 60 rpm (37.23 \pm 6.30 vs. 38.39 \pm 5.87 ml/kg/min), (64.10 \pm 16.45 vs. 65.84 \pm 16.55 L/min), (135 \pm 10 vs. 139 \pm 13 bpm). These findings were similar to previous studies that demonstrated pedaling at a higher cadence will elicit a higher oxygen cost than lower or moderate cadence rates [1-6]. The current study, along with previous studies, has also shown that oxygen cost, HR and VE are all minimized between 50 and 70 rpm with slight variations depending on the power output and that pedaling rates greater than this becomes progressively more costly. Buśko showed that oxygen cost was minimized at 60 rpm while Bieuzen

found it was 64.5 rpm [15, 45]. Slight differences between the most metabolically efficient cadence might exist due to subjects' familiarity with pedaling at low cadences, differences in intensity, and power output.

Substrate Utilization and Cadence

Substrate utilization was one of the main outcome variables in this study to be able to characterize its relationship to pedaling rate at a submaximal work level. It was hypothesized that pedaling at moderate cadences (80-100 rpm) would result in a higher proportion of energy originating from fat oxidation compared to pedaling at low (60-70 rpm) or high cadences (> 100 rpm) based on previous research that demonstrated glycogen depletion was greater at lower cadences [12], and the fact that a greater proportion of efficient, oxidative slow-twitch muscle fibers are recruited at higher cadences. However, results demonstrated that the relative proportion of energy originating from fat oxidation decreases as cadence rate increases. This was shown through both RER and individual substrate utilizations (g/min).

RER is an indicator of substrate utilization with a value of 0.70 representing a predominance of fat combustion as an energy source, 1.00 representing carbohydrates as the sole fuel source, and values in-between representing a mixture of fuel sources. It was found that RER increased with a subsequent increase in pedaling rate. Additionally, both relative (%fat) values of fat oxidation significantly decreased as pedaling rate increased. Therefore, from this study, it can be concluded that fat oxidation is maximized at lower cadences. These findings are contrary to the findings of Hagan who demonstrated RER was lower at 90 rpm compared to 60 rpm. In Hagan's study, however, subjects were pedaling at much lower workloads (127 and 166 watts) compared to subjects in the present study [58]. These low workloads could have accounted for the differences in RER data compared to the present study. For instance, at lower workloads, RER values can be expected to be lower in general. Whereas, in the

present study, the subjects pedaled at a power output equivalent to their first ventilatory threshold VT1. This equated to subjects working at an average of 60% of VO₂ max. The workload at VT1, and not an exact percentage of VO₂ max, was used in an effort to select an intensity that could more closely represent an average power output used in a prolonged (several hour) cycling race. Choosing a lower work rate, therefore, might have led to different substrate utilization patterns, such as a greater reliance on fat oxidation, but would not have been realistic compared to the work rate required during a long elite-level cycling race. Furthermore, using the power output at each cyclist's VT1 represented a specific physiologic point rather than an arbitrary percentage of VO₂ max which may actually represent different relative intensities for each cyclist.

A second hypothesis was that carbohydrate utilization would remain the same for low and moderate cadences but would increase at higher cadences. Results demonstrated that both relative and absolute carbohydrate oxidation increased with an increase in pedaling rate from low to moderate to high cadences. Although glycogen depletion was not measured in this study, this would contradict Ahlquist's study in which he concluded that glycogen depletion for type II muscle fibers was greater at 50 rpm compared to 100 rpm while pedaling at 85% of VO₂ max [12]. It is unclear whether the subjects in that study were fasted before the exercise bouts. This is an important note because if any source of carbohydrates was ingested within a few hours prior to exercise, this could preferentially favor the utilization of carbohydrates over fat.

These findings indicate that although muscle fiber recruitment patterns may be different at different pedaling rates [11-14], substrate utilization may be more determined by the workload, HR, and oxygen cost than the actual cadence, as different intensities will require the recruitment of more or less fast twitch muscle fibers, which will most likely affect substrate utilization.

Freely Chosen Cadence (FCC)

Subjects' self-selected cadence was measured and analyzed for a few different reasons. Firstly, we wanted to determine the FCC in well-trained and professional cyclists and confirm the consistency with previous studies. Secondly, we wanted analyze metrics at FCC to in an effort to determine how and why cyclists choose a specific cadence. For instance, if metabolic cost was higher but carbohydrate utilization was minimized at FCC (i.e., a greater contribution from fat oxidation at FCC), cyclists would be able to take advantage of the benefits of pedaling faster while minimizing the effect of a metabolic penalty.

It was hypothesized that the FCC would not occur at the most metabolically efficient cadence. FCC was found to occur at 89.8 rpm, ranging from 84 to 100 rpm, – a pedaling rate significantly higher than where VO₂ and HR were minimized. This demonstrates that cyclists do not inherently pedal at the most metabolically optimal cadence, which was an expected finding given that previous studies have determined that highly trained cyclists tend to adopt a pedaling rate of 90-95 rpm or higher and metabolic cost is minimized at 60 rpm. In the present study, only two participants pedaled greater than 95 rpm for their FCC. Coyle et al., found a strong relationship (r = 0.75; p<0.001) between years of endurance training and percentage of type I muscle fibers. Type I muscle fibers are known for their efficiency and are the predominant muscle fiber recruited at higher cadences. In addition, the present study had both cyclists and triathletes as subjects whereas other studies typically were studying only cyclists. Given that a triathlete's training revolves around three sports instead of just one, the average triathlete most likely does not spend the amount of time training on the bike as a pure cyclist does. This could lead to differences in recruiting specific muscles throughout the pedal stroke (pedaling skill and efficiency), FCC, and efficiency on the bike.

In terms of substrate utilization, it was hypothesized that FCC would occur at a pedaling rate that generates maximal fat oxidation. Yet, this was not the case. Fat oxidation at FCC was 0.65 ± 0.29 g/min, while carbohydrate oxidation was 1.60 ± 0.41 g/min. FCC was determined to be 30 rpm greater than the most substrate optimal (i.e., predominantly fat utilization) cadence and 20 rpm greater than the most economical (i.e., lowest metabolic cost) cadence. Although not statistically significant, it is important to note that the carbohydrate utilization rate at FCC was less than the rate at 90 rpm (p =0.20), and the fat utilization rate was greater at FCC than 80 and 90 rpm (p = 0.75 and 0.55) respectively. It can be concluded that from this study that in well-trained cyclists and triathletes, FCC is not chosen solely to limit metabolic cost or optimize substrate utilization. Additional factors must therefore play a role in the FCC that is naturally selected. For example, the FCC could be influenced by an effort to limit muscle activation and fatigue as well as selecting the cadence that subjectively feels the most comfortable and easiest, as measured by RPE. As discussed in the literature review, choosing a FCC that is higher could have other potential benefits as well such as improved blood flow and minimizing negative muscle work. Importantly, it has been shown that cyclists can be successful pedaling at higher cadences despite the greater metabolic cost. Lance Armstrong, for instance, arguably one of the top cyclists in the world, would often ascend the highest mountaintops of the Tour de France pedaling at 100 rpm. Additionally, the 1-hour cycling track record is routinely set with the cyclist maintaining a cadence over 100 rpm.

The Carbohydrate Penalty

Although cyclists have repeatedly shown extraordinary success pedaling at high cadences, it comes at a cost of more carbohydrate utilization – demonstrating less-than-optimal substrate utilization for endurance performance. In the present study, 60 rpm was found to utilize the lowest amount of carbohydrates compared to all other cadences with a utilization of 1.34 ± 0.39 g/min. Therefore, anything above that rate would be considered a carbohydrate penalty (CHO Penalty). CHO Penalty =

CHO oxidation rate @ (70, 80, FCC, 90, 100, 110) – CHO oxidation rate @ 60 rpm. Therefore, 60 rpm has a carbohydrate penalty of zero (0). By converting the carbohydrate utilization from grams per minute to grams per hour, the carbohydrate penalty at other cadences was determined.

Figure 16 shows the carbohydrate penalty at varying cadences. For instance, if a cyclist were to pedal at 110 rpm for one hour, they would utilize 45 more grams of carbohydrate compared to pedaling at 60 rpm which equates to 180 more kcals expended during that hour. Pedaling between 90 and 100 rpm for an hour would have a cyclist on average utilizing 21.6 – 22.2 more grams of carbohydrate compared to baseline (60 rpm). This equates to 88 more kcals expended in the hour. While this might not seem drastic for just one hour, it could add up to be quite significant over a 4–5-hour ride, requiring the ingestion of a large amount of extra carbohydrates and increasing the risk of glycogen depletion resulting in significant fatigue and deteriorating performance. Although fueling needs vary from person to person, 40-80 grams of carbohydrates per hour must be ingested to avoid glycogen depletion, depending on the intensity while cycling.



Figure 16. Carbohydrate penalty (g/hour) at each cadence with 60 rpm as the baseline of zero (0) penalty.

Interestingly, the CHO penalty for FCC is 6 grams lower compared to 90 rpm, even though the values are close together. One potential explanation for this is that cyclists have trained their muscles to utilize substrates more optimally at a very specific cadence.

Unexpected Results

Although not statistically significant, results showed differences in substrate utilization between males and females in the study. Males had a higher rate of fat oxidation compared to females across all cadences (0.77 \pm 0.26 vs. 0.47 \pm 0.15 g/min; *p* = 0.465). This is surprising as there is research that demonstrates females have a greater ability for fat oxidation, mainly due to hormonal differences, such as estrogens [80]. A study of 300 men and premenopausal women showed the energy contribution of fat was significantly higher in women vs. men at all exercise intensities ranging from 41 – 61% VO₂ max [81]. In the present study, all female subjects were premenopausal. Estrogen levels are known to fluctuate throughout the menstrual cycle, usually being higher during the luteal phase (LP) compared to the follicular phase (FP). There is mixed evidence of the impact of different hormone concentrations affecting substrate utilization. Studies have shown no differences fat oxidation between phases [80, 84]. For the present study, a larger sample size would likely be needed to determine the true trend of fat oxidation and cadence between different genders.

Optimal Pedaling Cadence

Two of the objectives of this present study were to analyze the effect of cadence on substrate utilization to determine optimal cadence and how highly trained cyclists may inherently select a pedaling rate. Results of the current study showed that cyclists do not routinely pedal at a metabolically optimal cadence nor a cadence that utilizes the least amount of carbohydrates. So, do they naturally choose a less-than-optimal cadence? Although glycogen sparing is an important concept for endurance

performance, it may not be the only factor that determines what is optimal, and this study has demonstrated that it is not the main factor driving natural cadence selection. Perhaps then, an optimal cadence for cycling endurance performance is a balance between conflicting factors. An optimal cadence may be one that allows the cyclist to maintain the highest wattage desired without a considerable accumulation of muscular fatigue while minimizing the consequent increased metabolic cost and "CHO penalty." Of note, the optimal cadence will also vary depending on the power output and terrain (climbing vs. flat) and potentially the task at hand (time-trialing vs. sprinting) as discussed in the literature review.

Highly trained cyclists inherently select a cadence that is more metabolically costly, but *perhaps* it is one that takes advantage of some of the benefits of a higher cadence (e.g., decrease muscular fatigue) while minimizing the effect of a metabolic penalty. Therefore, maybe no one factor is truly optimized, but there is an optimal combination of all the factors. The ultimate test for the "optimal" pedaling cadence would be to simulate actual road race conditions (e.g., a lengthy time trial or an actual road race – which may have different optimal pedaling cadences) and have cyclists pedal at different cadences and characterize the effect on elapsed time or overall performance.

CHAPTER 6 – SUMMARY AND CONCLUSIONS

Cycling is becoming an increasingly popular sport both in the United States and around the world. With new technology such as disc brakes, electronic and wireless shifting, and ultra-light carbon frames, both professional cyclists and recreational athletes are able to go faster than ever. Despite the advancements, the optimal pedaling rate at which one should pedal at to maximize endurance performance is still not very well understood. It is known that metabolic cost is minimized when pedaling between 50 and 70 rpm; however, professional cyclists pedal between 90 to 95 rpm. Pedaling at that rate elicits a higher oxygen and energy cost. The relationship between cadence rate and substrate utilization has not been well established. Therefore, the aim of the present study was to examine substrate utilization rates and ratios at different cadences in well-trained cyclists and triathletes. Another key objective was to observe the cadence that these subjects inherently select and examine the potential consequences and benefits of that pedaling rate. Of note, this is one of the first studies studying the relationship between cadence rates and its effect on substrate utilization.

To examine the questions of interest, we implemented a seven-stage cadence protocol where each stage was a different cadence (60, 70, 80, 90, 100, 110, FCC) performed in a randomized order. Prior to this study visit, subjects completed a VO₂ max test to: 1. Assess their maximal aerobic capacity, 2. determine the power output of the first ventilatory threshold that each subject would work at for the cadence protocol. During the cadence protocol, cadence, HR, VO₂, RER, substrate utilization markers (CHO & fat (g/min)) were assessed during the last two minutes of each stage.

We were able to observe HR, RER, VO₂, and CHO utilization values increase to with increasing cadence, which all proved to be statistically significant. The average FCC was found to be 89.8 rpm, which is higher than the metabolically and substrate optimal cadence. Therefore, it can be concluded

that higher cadences do not minimize CHO utilization nor maximize fat utilization and so FCC was not chosen to optimize metabolic cost or substrate utilization. It would be reasonable to speculate that this allows cyclists to take into account neuromuscular and biomechanical benefits.

Delimitations, Limitations and Assumptions

The current study presented a couple of limitations which compromise the ability to extrapolate the findings to a wide range of athletes. First, a sample size of 11 subjects (6 males, 5 females) reduced the statistical power; however, this sample size is comparable to other cycling and cadence studies. Secondly, this study included a combination of males and females. Previous studies involving cycling performance and cadence typically only involve male subjects. With this current study, females' menstrual cycles were not tracked or accounted for. Although there is mixed evidence on whether the phase of the menstrual cycle impacts substrate utilization, it would be beneficial to account for it in a future study. Additionally, although this current study did include a 12-hour fast before the cadence visit, it did not include a control of diet 24 hours before the visit. Finally, another potential limitation is the total weekly training volume. For instance, a minimum training volume of 8 hours was set to be eligible to participate in the study. Furthermore, years of cycling was not accounted for either. We assume that all subjects were all rested and fully hydrated going into each study visit.

Future Directions

First and foremost, additional subjects should be added to see substrate utilization trends across a larger sample size. In addition, a future study should investigate the effect that gender can have not only on substrate utilization at different cadences, but how gender may affect FCC as well. Considering that women on average had a higher FCC than men in this study, it would be interesting to investigate this further. Regarding substrate utilization, women on average utilized more carbohydrates compared to men at every cadence. A future study might involve investigating this further.

The ultimate test for the "optimal" pedaling cadence would be to simulate actual road race conditions (e.g., a lengthy time trial or an actual road race – which may have different optimal pedaling cadences) and have cyclists pedal at different cadences and see its effect on elapsed time or overall performance.

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APPENDIX

HEALTH HISTORY QUESTIONNAIRE

Study Title: The interaction between cycling cadence and fat oxidation rates in trained cyclists Study Number: 1765

The purpose of this form is to ensure you do not have any exclusion criteria that would make you ineligible to participate in the study. You may refuse to answer any of the following questions. However, please be aware that your refusal may prevent researchers from safely assessing your suitability for participation in this investigation. If in the case an exclusion criterion does apply to you, your form and all information given will be shredded.

CONTACT INFORMATION:

1. What is your name?

- 2. What is your telephone number?
- 3. What is your email address?

4. Please provide the name and contact information (telephone number) of your Emergency Contact. What is your relationship to your Emergency Contact (e.g. spouse, parent, child, sibling, etc.)?

PERTINENT MEDICAL INFORMATION:

1. What is your date of birth?

2. What biological sex was assigned to you at birth (e.g. male, female)?

3. Do you have any cardiovascular, kidney, metabolic (like diabetes), or respiratory conditions? If yes, please explain.

4. Do you use tobacco products? If yes, provide more details (e.g. what do you use, how often do you use tobacco products, etc.). If no, please provide more details (e.g. have you ever used tobacco products, what did you use, when did you stop, etc.).

5. Do you have any current injuries that may limit your exercise performance (e.g. back pain, knee pain, sore feet, etc.)?

6. Are you currently taking any prescription medications? If yes, please list all medications, dosage, frequency, and reason for taking them.

7. Are you currently on a beta-blocker medication or any other medication that may alter normal heart rate or respiratory responses?

8. Women only: To the best of your knowledge, are your currently pregnant or trying to become pregnant?

GENERAL QUESTIONS RELATED TO RESEARCH STUDY:

1. Are you willing and able to perform difficult exercise on a stationary bike or your bicycle?

2. Do you have your own road or mountain bike that you are willing and able bring and use for the study visits? If yes, please provide, make/model of bike, crank length, rear cassette cogs (ex. 11-25), if your wheels are quick-release or thru-axle, and if it is a disc or rim brake bike.

If it is determined that your bike is not suitable for the Kickr, an electrically braked stationary bike will be used.

FASTING & FEEDING INSTRUCTIONS

Study Title: The interaction between cycling cadence and fat oxidation rates in trained cyclists Study Number: 1765

Visit 1

A fast is NOT REQUIRED prior to the VO2max test. However, it is highly recommended that your last meal is eaten at least 2 hours prior to your study visit to minimize the chance of stomach and/or gastrointestinal distress. Food can be consumed within 2 hours of your study visit, but it is recommended that foods higher in protein, fiber, and fat be avoided. Drinking plenty of fluids and staying hydrated is highly recommended.

Caffeine is to be abstained from 24 HOURS PRIOR to the start of your scheduled study visit. This includes caffeine from any sources such as coffee, energy drinks, teas, soda, 5-hour energy, etc.

Upon arrival for your visit, you will be asked if you have abstained from caffeine for 24 hours and provide a signature to confirm. Your appointment will be rescheduled for another day if instructions were not followed.

Visit 2

A 12-hour fast IS REQUIRED for the cadence protocol visit. Depending on the time of your study visit, you will be asked to stop eating 12 hours prior. For example, if your study visit is scheduled for 7am, you will be asked to stop eating at 7 pm the night before. This includes any liquid calories such as juice, sports drink, or soda. Water is permitted to be consumed without any restriction and is highly recommended to stay hydrated. In addition, caffeine is to be abstained from 24 HOURS PRIOR to the start of your scheduled study visit. This includes caffeine from any sources such as coffee, energy drinks, teas, soda, 5-hour energy, etc.

Upon arrival for your visit, you will be asked if you are at least 12 hours fasted and 24 hours without caffeine. You will provide a signature to confirm. Your appointment will be rescheduled for another day if instructions were not followed.

If you have any questions regarding the fasting and feeding instructions, please reach out to Victoria Dippold -> <u>victoria.dippold@colostate.edu</u> | 814-594-9671.

Visit 1

_____(participant name printed), can confirm I have abstained from caffeine l, ____ for at least 24 hours prior to the time of my study visit ______ (insert time of visit).

Visit 2

I, ______(participant name printed), can confirm I have fasted (food and liquid calories for at least 12 hours prior to my study visit and have abstained from caffeine for at least 24 hours prior to the time of my study visit ______ (insert time of visit).

(signature of participant)

(signature of research team member)

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(date)

(date)