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The effect of pedalling cadence on skeletal muscle oxygenation during cycling

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Key word:	exercise, cycling, cadence, near-infrared spectroscopy, tissue saturation index, muscle
Abstract:	The aim of this study was to assess the changes determined by increased cadence on skeletal muscle oxygenation during cycling at exercise intensity equal to the ventilatory threshold (Tvent). Nine healthy, active individuals, with different levels of cycling experience, exercised at a power output equal to Tvent, pedalling at cadences of 40, 50, 60, 70, 80 and 90 rpm, each for 4 minutes. Cadences were tested in a randomized counterbalanced sequence. Cardiopulmonary and metabolic responses were studied using an ECG for heart rate, and gas calorimetry for pulmonary oxygen uptake and carbon dioxide production. NIRS was used to determine the tissue saturation index (TSI), a measure of vastus lateralis oxygenation. TSI decreased from rest to exercise; the magnitude of this TSI reduction was significantly greater when pedalling at 90rpm (-14±4%), compared to pedalling at 40 (-12±3%) and 50 (-12±3%) rpm (P=0.027 and 0.017, respectively). Albeit small, the significant decrease in ΔTSI at increased cadence recorded in this study suggests that skeletal muscle oxygenation is relatively more affected by high cadence when exercise intensity is close to Tvent.

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The effect of pedalling cadence on skeletal muscle oxygenation during cycling at moderate exercise intensity

Running title: Skeletal muscle oxygenation at different pedalling cadences

Key words: exercise, cycling, cadence, near-infrared spectroscopy, tissue saturation index, muscle, oxygen

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view

Abstract

The aim of this study was to assess the changes determined by increased cadence on skeletal muscle oxygenation during cycling at exercise intensity equal to the ventilatory threshold (T_{vent}).

Nine healthy, active individuals, with different levels of cycling experience, exercised at a power output equal to T_{vent}, pedalling at cadences of 40, 50, 60, 70, 80 and 90 rpm, each for 4 minutes. Cadences were tested in a randomized counterbalanced sequence. Cardiopulmonary and metabolic responses were studied using an ECG for heart rate, and gas calorimetry for pulmonary oxygen uptake and carbon dioxide production. NIRS was used to determine the tissue saturation index (TSI), a measure of vastus lateralis oxygenation.

TSI decreased from rest to exercise; the magnitude of this TSI reduction was significantly greater when pedalling at 90rpm (-14±4%), compared to pedalling at 40 (-12±3%) and 50 (-12±3%) rpm ($P=0.027$ and 0.017, respectively). Albeit small, the significant decrease in ΔTSI at increased cadence recorded in this study suggests that skeletal muscle oxygenation is relatively more affected by high cadence when exercise intensity is close to T_{vent}.

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Introduction

The growing popularity of cycling is stimulating a wealth of research in the field of exercise physiology beyond elite athletes' performance, with several studies investigating the responses to exercise in recreational cyclists. The concurrent advances in technological development allow for a variety of physiological parameters to be studied *in vivo* and non-invasively.

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Changing pedalling cadence during moderate intensity cycling affects a number of physiological responses: at a constant and moderate power output, increasing cadence causes an increase in heart rate (HR), oxygen consumption (VO_2), carbon dioxide production (VCO_2), rate of perceived exertion and lactate [11,16,20,31,32,38]. High pedalling cadences increase skeletal muscle metabolic demand, which up to a point can be matched by a corresponding increase in the cardio-respiratory function that raises the rate of pulmonary oxygen uptake and oxygen delivery at systemic level. In contrast, low pedaling cadences increase intramuscular pressure during the muscular contraction period [19], with a size effect associated with the force generated by the muscular contraction [21]. This phenomenon temporarily reduces or prevents blood perfusion to the contracting muscle and downstream tissues. Inevitably during cycling exercise, low cadences are also associated with proportionally longer muscular relaxation periods, when perfusion is increased. It is currently unclear whether the longer contraction period and greater pedal forces at lower cadence are likely to determine inadequate oxygenation of the exercising muscles [34].

The effect of pedalling cadence on skeletal muscle oxygenation has been rather extensively explored in real time by means of near infrared spectroscopy (NIRS). This technique uses different wavelengths of infra-red light to estimate the haemoglobin and myoglobin in the tissue of interest, measuring their total changes (tHb), as well as the changes in the oxygenated (OxyHb) and deoxygenated forms (HHb). NIRS cannot detect differences between signals from haemoglobin and myoglobin, hence the contribution of myoglobin to the overall signal cannot be completely excluded. However, the hypothesis that most of the NIRS signal is determined by haemoglobin is supported by several observations [8,25,27,28,30,35]. Skeletal muscle oxygenation can then be expressed in terms of tissue saturation index (TSI), the ratio between OxyHb and tHb [9]. TSI provides an overall index of skeletal muscle oxygenation, while OxyHb and HHb estimate oxygen delivery and extraction at the tissue level respectively [14].

When power output is increased during cycling exercise at a given pedalling cadence, HHb increases and skeletal muscle saturation decreases [3,4,10]. Not as clear is the skeletal muscle oxygenation response to different pedalling cadences at a constant power output. Skovereng et al. [31,32] reported that increasing cadence from 60 to 110 revolutions per minute (rpm), in an incremental sequence at a workload equal to 70% of lactate threshold, decreased skeletal muscle oxygenation. However, pedalling cadence had no significant effect on skeletal muscle oxygenation indexes during cycling, when cadences were tested in a randomised order at power outputs below the ventilatory threshold (T_{vent}). For example, Koulanakis and Geladas [24] reported no change in TSI between 40 and 80 rpm, when cadences were tested in a random sequence at a power output equal to 60% of VO_{2max}. Takaishi et al. [33]

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and Zorgati et al. [39] also reported no clear changes in oxygenation between cadences, when these were tested in a randomized sequence. These studies [24,31-33,39] do differ in terms of experimental design, including power output, cadence ranges and sequence in which they were tested, which may partly explain some differences in their findings. Numerous studies have also been performed to determine the optimal pedalling cadence for efficient cycling performance at a given power output. However, no clear consensus has been reached with some studies favouring a low cadence [23] and others a higher cadence [7], also highlighting the different responses observed between elite and recreational cyclists, where elite cyclists specifically train at high cadence [1,26,37]. Increasing cadence when exercising at T_{vent} may affect skeletal muscle oxygenation [15], yet no study to date has explored the effect of altering cadence on TSI when cycling at T_{vent}.

In this context, the aim of our study was to investigate the effects of different pedalling cadence on the systemic and *vastus lateralis* oxygenation responses to cycling at a constant power output equal to 100% of the T_{vent} in participants with different levels of cycling experience. We hypothesised that skeletal muscle oxygenation would be lower both at the low (40 rpm) and high (90 rpm) cadences, due to the effects of intermittent blood perfusion and insufficient oxygen delivery-to-uptake ratio, respectively.

Materials and Methods

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Participants

The study received ethical approval from the institutional review board of the Nagoya University Graduate School of Medicine (approval no. 2016-0531), and conformed to the standards outlined in the Declaration of Helsinki and to the standards for ethics in sport and exercise science research [18]. Each participant gave her/his informed consent before taking part in the study. Nine healthy participants (male/female = 6/3) were recruited and completed the study. In terms of their activity levels, two participants were triathletes at regional level with three-year experience, six regularly engaged in moderate and vigorous exercise, and one engaged with very light physical activity only occasionally [12]. The participants' age ranged from 21 to 55 years.

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Experimental Protocol

Estimation of ventilatory threshold

The ventilatory threshold for all of the participants was measured with an incremental ramp test. Participants cycled at 60 rpm against an external power output starting at 20 W or 30 W for female and male participants respectively (mean ± SD; starting power output 28 ± 4 W). The external power output increased by 10, 15, 20, 25 W min^{-1} depending on the estimated fitness of the participant tested (rate of external power output increase 20 ± 6 W min^{-1}), aiming for a total duration of the test of around 10 minutes [2,5]. The T_{vent} of each participant was estimated using the V-slope method [22], ventilatory equivalent of oxygen method ($\dot{V}E/\dot{V}O_2$) [36] and ventilatory equivalent of carbon dioxide method ($\dot{V}E/\dot{V}CO_2$) [6]. The mean value is then taken from these four methods and used as an estimation of the participant's

T_{vent}. This approach has been shown to increase the precision of T_{vent} estimations, when compared with using just one of these methods alone [13].

Responses to different cadences

A schematic diagram of the protocol where responses to different cadences were studied is presented in **Figure 1**. After 2 min of rest, participants warmed up for 6 min, pedalling at 60 rpm while external power output increased every 2 min in steps to 25%, 50% and 75% of the power output calculated for T_{vent}. Participants were then asked to cycle at an external power output equal to their T_{vent} at cadences of either 40, 50, 60, 70, 80 or 90 rpm, when real-time cadence was displayed on a digital monitor visible to the participant and a metronome was used in order to help the participants achieve the desired cadence.. Cadences were tested in a randomized, counterbalanced sequence (with 90 rpm always tested last to reduce the potential effect of fatigue). Participants exercised at each cadence for 4 min, immediately followed by 2 minutes of active recovery, cycling at 60 rpm at 25% of T_{vent}. These active recovery periods allowed TSI to return closer to initial values and to reduce the potential effects of fatigue over the course of the experimental protocol.

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Pedalling cadence, expired gases, heart rate and *vastus lateralis* oxygenation were continuously recorded. Blood lactate was recorded in the last 90 s of the initial rest period and of each 4 min bout of cycling exercise at 100% T_{vent}.

Equipment

Cycle ergometer and pedal force measurements

An electronically braked cycle ergometer (Aerobike 75XL, Combi, Tokyo, Japan) was used for all experiments. The external power output could be set to the nearest 1 W, using personalized, pre-programmed protocols.

Pedal force was recorded using three miniature force transducers (LM-50KA, Kyowa Dengyo, Tokyo, Japan) on the pedal and a DC amplifier (DPM-601A, Kyowa Dengyo, Tokyo, Japan). Three force signals were converged to one signal and calculated the pedal force perpendicular to the pedal. Peak force was calculated for each cycle. Pedal cadences were calculated using the principle of electromagnetic induction by four small magnets on the gear and coil. The system generated four peak voltage signals at each pedal revolution, so that cadence can be precisely calculated.

We recorded pedal force and, importantly, pedalling cadence during each experiment in order to establish participants' protocol adherence or deviations from the expected cadence.

Cardiopulmonary responses and rate of perceived exertion measurements

Heart rates were measured continuously during all stages of the trials by means of a three-lead electrocardiogram (AB-621G, Nihon Kohden, Tokyo, Japan) connected using gel electrodes applied to the skin. All analyzed data were linearly interpolated between each cycle or heart beat to yield a data point at each 1 s interval.

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Respiratory and metabolic data were recorded with the ARCO-2000 (Arco System Inc., Chiba, Japan) with a mass spectrometer and a Fleisch pneumotachometer. Participants wore a facemask (7450, Hans-Rudolph Inc., MO, USA) with dead space of ~100 ml.

Participant's rate of perceived exertion was recorded on a standard Borg scale table just after the end of each exercise bout (Borg, 1982).

Blood lactate concentration

Blood lactate concentration values were recorded using the Lactate Pro 2® analyser (HaB International Ltd., England). Before taking a reading, the finger was cleaned with an alcohol swab (70% Isopropyl alcohol) and wiped with a tissue to avoid alcohol contamination of the sample.

Skeletal muscle (vastus lateralis) oxygenation

Participants' muscle oxygenation values (OxyHb, HHb, tHb, TSI) were sampled at 10 Hz using the PortaMon® (Artinis Medical Systems, Einsteinweg, The Netherlands) [29]. Briefly, the NIRS device was positioned on the participant's skin over the muscle belly of the right *vastus lateralis*, along the main axis of the thigh, approximately 16 cm from the knee joint. The device was secured using a Velcro strap to prevent the device from moving during the experiment and to cover the sensors, ensuring no ambient light contaminated the NIRS signal.

Data Analysis

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Analyses were performed for peak pedal force, pedalling cadence, heart rate, blood lactate, RPE, $\dot{V}O_2$, $\dot{V}CO_2$, OxyHb, HHb, tHb and TSI. Mean ± standard deviation values at each cadence during the 100% T_{vent} tests were calculated from the last 60 s of each cycling bout in Microsoft Excel (Version 15.25.1, Microsoft Corporation, California, USA).

SigmaPlot (13.0.0.83. Systat Software, Inc., San Jose, California, USA) was used for statistical analysis. The Shapiro-Wilk test was used to check for normal distribution of the data. The Brown-Forsythe test was conducted to test for equal variance. Data for physiological variables at different cadences were analysed using a One Way Repeated Measures Analysis of Variance (ANOVA), if they passed the normality tests. A Bonferroni pairwise multiple comparison procedure was used as a post-hoc test to compare the means of each cadence.

RPE and $\dot{V}O_2$ data did not pass the Shapiro-Wilk and Brown-Forsythe tests, so a Friedman's one way repeated measures ANOVA based on ranks and Tukey's post hoc test were performed to test for differences between responses at each cadence. Results are presented as mean ± standard deviation unless otherwise stated. Statistical significance was set at $P < 0.05$ for all tests.

Results

Participants' characteristics and protocol adherence

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Six male and three female participants took part in this study. The characteristics of these participants are presented in **table 1**. The recorded cadences matched the required cadences well, as presented in **table 2**.

Changes in cardiorespiratory and metabolic function, perceived exertion and pedal force at different pedalling cadences

Figure 2 shows the physiological, metabolic, rate of perceived exertion and peak pedal force values at the different pedalling cadences recorded at at 100% T_{vent}. HR (Figure 2A), VO_2 (Figure 2C), VCO_2 (Figure 2D) and peak pedal force (Figure 2F) changed significantly at the higher pedalling cadences when compared to the lower pedalling cadences ($P < 0.05$). The respiratory rate did not increase significantly between 40 and 90 rpm (30 ± 5 and 31 ± 4 breaths per minute respectively, $p = 0.09$), unlike tidal volume and ventilation that increased respectively from 1.7 ± 0.5 L to 2.0 ± 0.5 L ($p = 0.0001$) and from 50 ± 17 L/min to 62 ± 21 L/min ($p = 0.0002$). A significant but small increase in blood lactate concentration was recorded at 60 rpm (Figure 2B). No significant or marked changes were seen in RPE at the different pedalling cadences (Figure 2E).

Changes in skeletal muscle oxygenation at different pedalling cadences

Figure 3 shows the changes in skeletal muscle oxygenation in the *vastus lateralis* muscle at different pedalling cadences. OxyHb and TSI decreased from resting levels (Figure 3A and 3D), while HHb and tHb levels increased from their resting values (Figure 3B and 3C). TSI was not different in the 30 s preceding each cadence

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test (*p* = 0.86), with SD values ~1% for each individual. The magnitude of the TSI reduction was significantly greater when pedalling at 90 rpm (-14.6% ± 4), compared to pedalling at 40 (12.3% ± 3) and 50 (-12.2% ± 3) rpm (P = 0.027 and 0.017, respectively).

Discussion

In our study of participants with different cycling expertise, pulmonary oxygen uptake recorded at the highest cadence of 90 rpm was greater than at lower cadences during exercise at 100% T_{vent}. This greater pulmonary oxygen uptake was associated with a 3% greater TSI decrease at high cadence of 90 rpm compared with low cadences of 40 and 50 rpm.

Increased pedalling cadence at constant power output of 100% T_{vent} resulted in a greater cardiorespiratory response

Both the cardiovascular and respiratory systems' function increased at the higher cadence of 90 rpm, in order to meet the increased metabolic demands of the exercising muscles. These cardiopulmonary results are in agreement with previous findings and suggest that skeletal muscle oxygenation may also be affected at the high cadence. The extra work at higher cadence is associated with a greater oxygen demand (extraction); when this oxygen demand exceeds oxygen supply (delivery) beyond a given threshold, TSI may decrease, as observed at high cadences in our study.

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Skeletal muscle oxygenation at high cadence when pedalling at constant power output

Changes in HHb are considered a good indicator of skeletal muscle oxygen extraction because the HHb signal is not affected by an increase in oxygenated blood to the skin for thermoregulation [14]. HHb tended to increase from baseline levels during cycling at 100% T_{vent}, indicating a moderate increase in fractional oxygen extraction in the exercising muscles, achieved via an increase in cardiac output and/or a reduction in the peripheral vascular resistance at the exercise intensity tested.

Despite these changes from baseline and a trend for an increase in HHb and tHb at high cadence, there was no significant change in these skeletal muscle oxygenation parameters between the pedalling cadences. These findings are in agreement with previous studies, which reported that cadence had no clear effect on OxyHb, HHb and tHb in conditions similar to those tested here [24,39].

TSI is an overall indicator of skeletal muscle oxygenation [14,17]. TSI significantly decreased from baseline during cycling exercise at 100% T_{vent}, and from 40 and 50 rpm to 90 rpm (Figure 3D). The significant changes in TSI observed at higher pedalling cadences, which we tested in a randomized sequence at 100% T_{vent}, are in agreement and strengthen the findings from Skovereng et al. [31,32]. These results are supported by previous observations at a relatively lower power output equal to 60% of VO_2max, where skeletal muscle oxygenation was not different at the onset of

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cycling exercise at either 40 or 100 rpm [24], confirming our results in an acute exercise context.

It is likely that the effect of intramuscular pressure on TSI is associated with the absolute pressures generated during the contraction. Given the higher external power output at which elite cyclists exercise (for a similar relative exercise intensity, e. g. 100% T_{vent}), these absolute intramuscular pressures are likely to be greater in elite than in recreational cyclists. This is a putative mechanism that could explain the difference in our findings with those reported in trained cyclists by Skovereng et al., where TSI decreased at high cadence even at a lower relative external power output corresponding to 75% of the participants' lactate threshold [31,32].

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The group of participants studied was limited to nine individuals and rather heterogeneous in terms of age, exercise capacity and cycling expertise. Given the limited sample size considered in this study, we acknowledge that this finding needs confirmation on a larger scale.

A limitation of our study is that the T_{vent} was estimated at one pedalling cadence only. It is possible that estimating T_{vent} at higher or lower pedalling cadence could have affected the estimated T_{vent}. For the incremental test, we chose a cadence that all participants could exercise at comfortably, and that has been used in several published studies before, making our results comparable with those presented in the literature. In addition, there is often a degree of error in the estimation of T_{vent}, so we think that the estimated T_{vent} would have only varied significantly if cadence had markedly been reduced or increased from 60 rpm. An additional limitation is in the choice of testing the highest cadence (i. e. 90 rpm) always last, where it cannot be entirely excluded that the results associated with the 90 rpm conditions are in part determined by the preceding exercise. However, TSI was not different (within participant) between rest and the final part of each recovery period, so the likelihood of TSI decrease observed at 90 rpm being determined by the preceding exercise appears limited.

We conclude that increasing cadence beyond a given threshold at moderate exercise intensity close to the T_{vent} is less energetically efficient (as confirmed by the higher $\dot{V}O_2$ and $\dot{V}CO_2$ recorded for a given power output here [Fig. 2]) and that high cadence may compromise skeletal muscle oxygenation during cycling exercise.

Disclosure of interest: The authors report no conflict of interest.

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References

1. Ansley L, Cangley P. Determinants of optimal cadence during cycling. European journal of sport science 2009; 9: 61-85
2. Belardinelli R, Barstow TJ, Porszasz J, Wasserman K. Changes in skeletal muscle oxygenation during incremental exercise measured with near infrared spectroscopy. Eur J Appl Physiol Occup Physiol 1995; 70: 487-492
3. Boone J, Barstow TJ, Celie B, Prieur F, Bourgois J. The impact of pedal rate on muscle oxygenation, muscle activation and whole-body VO(2) during ramp exercise in healthy subjects. Eur J Appl Physiol 2015; 115: 57-70
4. Boone J, Barstow TJ, Celie B, Prieur F, Bourgois J. The interrelationship between muscle oxygenation, muscle activation, and pulmonary oxygen uptake to incremental ramp exercise: influence of aerobic fitness. Appl Physiol Nutr Metab 2016; 41: 55-62
5. Buchfuhrer MJ, Hansen JE, Robinson TE, Sue DY, Wasserman K, Whipp BJ. Optimizing the exercise protocol for cardiopulmonary assessment. Journal of applied physiology: respiratory, environmental and exercise physiology 1983; 55: 1558-1564
6. Caiozzo VJ, Davis JA, Ellis JF, Azus JL, Vandagriff R, Prietto CA, Mcmaster WC. A Comparison of Gas-Exchange Indexes Used to Detect the Anaerobic Threshold. J Appl Physiol Respir Environ Exerc Physiol~~Journal of applied physiology: respiratory, environmental and exercise physiology~~ 1982; 53: 1184-1189
7. Chavarren J, Calbet JA. Cycling efficiency and pedalling frequency in road cyclists. Eur J Appl Physiol Occup Physiol 1999; 80: 555-563

For Peer Review

8. Davis ML, Barstow TJ. Estimated contribution of hemoglobin and myoglobin to near infrared spectroscopy. Respir Physiol Neurobiol 2013; 186: 180-187

9. Ferrari M, Mottola L, Quaresima V. Principles, techniques, and limitations of near infrared spectroscopy. Can J Appl Physiol 2004; 29: 463-487

10. Ferreira LF, Lutjemeier BJ, Townsend DK, Barstow TJ. Effects of pedal frequency on estimated muscle microvascular O_2 extraction. Eur J Appl Physiol 2006; 96: 558-563

11. Formenti F, Minetti AE, Borrani F. Pedaling rate is an important determinant of human oxygen uptake during exercise on the cycle ergometer. Physiological reports 2015; 3: e12500

12. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC, Swain DP, Med ACS. Quantity and Quality of Exercise for Developing and Maintaining Cardiorespiratory, Musculoskeletal, and Neuromotor Fitness in Apparently Healthy Adults: Guidance for Prescribing Exercise. Med Sci Sports Exerc~~Medicine and science in sports and exercise~~ 2011; 43: 1334-1359

13. Gaskill SE, Ruby BC, Walker AJ, Sanchez OA, Serfass RC, Leon AS. Validity and reliability of combining three methods to determine ventilatory threshold. Med Sci Sports Exerc~~Medicine and science in sports and exercise~~ 2001; 33: 1841-1848

14. Grassi B, Quaresima V. Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. J Biomed Opt 2016; 21: 091313

15. Grassi B, Quaresima V, Marconi C, Ferrari M, Cerretelli P. Blood lactate accumulation and muscle deoxygenation during incremental exercise. J Appl Physiol 1999; 87: 348-355

For Peer Review

16. Hagberg JM, Mullin JP, Giese MD, Spitznagel E. Effect of pedaling rate on submaximal exercise responses of competitive cyclists. J Appl Physiol Respir Environ Exerc Physiol~~Journal of applied physiology: respiratory, environmental and exercise physiology~~ 1981; 51: 447-451

17. Hamaoka T, McCully KK, Niwayama M, Chance B. The use of muscle near-infrared spectroscopy in sport, health and medical sciences: recent developments. Philos T R Soc A 2011; 369: 4591-4604

18. Harriss D, MacSween A, Atkinson G. Standards for ethics in sport and exercise science research: 2018 update. Int J Sports Med 2017; 38: 1126-1131

19. Hill AV. The pressure developed in muscle during contraction. J Physiol 1948; 107: 518-526

20. Hirano M, Shindo M, Mishima S, Morimura K, Higuchi Y, Yamada Y, Higaki Y, Kiyonaga A. Effects of 2 weeks of low-intensity cycle training with different pedaling rates on the work rate at lactate threshold. Eur J Appl Physiol 2015; 115: 1005-1013

21. Hirvonen L, Sonnenschein RR. Relation between Blood Flow and Contraction Force in Active Skeletal Muscle. Circ~~ulation~~ ~~r~~Res~~earch~~ 1962; 10: 94-&

22. Hoogeveen AR, Hoogsteen GS. The ventilatory threshold, heart rate, and endurance performance: relationships in elite cyclists. Int J Sports Med 1999; 20: 114-117

23. Jacobs RD, Berg EK, Slivka DR, Noble JM. The effect of cadence on cycling efficiency and local tissue oxygenation. J Strength Cond Res 2013; 27: 637-642

24. Kounalakis SN, Geladas ND. Cardiovascular drift and cerebral and muscle tissue oxygenation during prolonged cycling at different pedalling cadences. Appl Physiol Nutr Metab 2012; 37: 407-417

For Peer Review

25. Lai N, Zhou HY, Saidel GM, Wolf M, McCully K, Gladden LB, Cabrera ME. Modeling oxygenation in venous blood and skeletal muscle in response to exercise using near-infrared spectroscopy. J Appl Physiol 2009; 106: 1858-1874

26. Lucia A, Hoyos J, Chicharro JL. Preferred pedalling cadence in professional cycling. Med Sci Sports Exerc~~Medicine and science in sports and exercise~~ 2001; 33: 1361-1366

27. Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, Wilson JR. Validation of near-infrared spectroscopy in humans. J Appl Physiol 1994; 77: 2740-2747

28. Marcinek DJ, Amara CE, Matz K, Conley KE, Schenkman KA. Wavelength shift analysis: a simple method to determine the contribution of hemoglobin and myoglobin to in vivo optical spectra. Appl~~ied~~ spectrosc~~opy~~ 2007; 61: 665-669

29. McManus CJ, Collison J, Cooper CE. Performance comparison of the MOXY and PortaMon near-infrared spectroscopy muscle oximeters at rest and during exercise. J Biomed Opt 2018; 23: 1-14

30. Seiyama A, Hazeki O, Tamura M. Noninvasive quantitative analysis of blood oxygenation in rat skeletal muscle. J~~ournal of biochemistry~~ Biochem 1988; 103: 419-424

31. Skovereng K, Ettema G, van Beekvelt M. The Effect of Cadence on Shank Muscle Oxygen Consumption and Deoxygenation in Relation to Joint Specific Power and Cycling Kinematics. PLoS One 2017; 12: e0169573

32. Skovereng K, Ettema G, van Beekvelt MC. Oxygenation, local muscle oxygen consumption and joint specific power in cycling: the effect of cadence at a constant external work rate. Eur J Appl Physiol 2016; 116: 1207-1217

For Peer Review

33. Takaishi T, Ishida K, Katayama K, Yamazaki K, Yamamoto T, Moritani T. Effect of cycling experience and pedal cadence on the near-infrared spectroscopy parameters. Med Sci Sports Exerc~~Medicine and science in sports and exercise~~ 2002; 34: 2062-2071

34. Takaishi T, Sugiura T, Katayama K, Sato Y, Shima N, Yamamoto T, Moritani T. Changes in blood volume and oxygenation level in a working muscle during a crank cycle. Med Sci Sports Exerc~~Medicine and science in sports and exercise~~ 2002; 34: 520-528; discussion 529

35. Tran TK, Sailasuta N, Kreutzer U, Hurd R, Chung Y, Mole P, Kuno S, Jue T. Comparative analysis of NMR and NIRS measurements of intracellular PO2 in human skeletal muscle. Am J Physiol 1999; 276: R1682-1690

36. Urhausen A, Coen B, Weiler B, Kindermann W. Individual anaerobic threshold and maximum lactate steady state. Int J Sports Med 1993; 14: 134-139

37. Whitty AG, Murphy AJ, Coutts AJ, Watsford ML. Factors associated with the selection of the freely chosen cadence in non-cyclists. Eur J Appl Physiol 2009; 106: 705-712

38. Zoladz JA, Rademaker AC, Sargeant AJ. Human muscle power generating capability during cycling at different pedalling rates. Exp Physiol 2000; 85: 117-124

39. Zorgati H, Collomp K, Amiot V, Prieur F. Effect of pedal cadence on the heterogeneity of muscle deoxygenation during moderate exercise. Appl Physiol Nutr Metab 2013; 38: 1206-1210

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Figure captions

Figure 1. Schematic representation of the experimental protocol. Participants pedalled at 60 rpm during the warm-up and 2 min active recovery periods. **A**: Rest period, **B:** warm-Up period (6 min), **C**: 100% T_{vent} exercise bout at a given cadence (4 min), **D**: active recovery period (2 min). T_{vent}: ventilatory threshold; rpm: revolutions per minute; min: minutes.

Figure 2: Physiological responses to cycling exercise at different pedalling cadences.

Values for (A) heart rate (bpm), (B) lactate concentration (mM), (C) $\dot{V}O_2$ (ml/kg/min), (D) $\dot{V}CO_2$ (ml/kg/min), (E) RPE and (F) peak pedal force (N) for each cadence at 100% T_{vent} (N = 9). Lactate concentrations greater than 8 mM (n = 3 out of 63) were considered as technical errors and excluded from the analysis.

a, b, c, d, e: $P < 0.05$ when compared to 40, 50, 60, 70 and 80 rpm respectively, at the same T_{vent}. min: minutes; bpm: beats per minute; rpm: revolutions per minute; T_{vent}: ventilatory threshold; $\dot{V}O_2$: pulmonary oxygen uptake; $\dot{V}CO_2$: carbon dioxide output; RPE: rate of perceived exertion; AU: arbitrary units.

Figure 3: Skeletal muscle oxygenation responses to cycling exercise at different cadences. Results are of changes from rest for (A) OxyHb, (B) HHb, (C) tHb and (D) TSI for each cadence performed at 100% T_{vent}. For OxyHb, HHb and

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tHb (A, B and C) N = 8 for changes from baseline (due to one missing baseline data set). For each 90 rpm data set N = 7 (due to one missing data set at this cadence). a, b: $P < 0.05$ when compared to 40 and 50 rpm respectively, at the same T_{vent}. min: minutes; AU: arbitrary units; TSI: tissue saturation index; OxyHb: oxygenated haemoglobin; HHb: deoxygenated haemoglobin; tHb: total haemoglobin; T_{vent}: ventilatory threshold; rpm: revolutions per minute.

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Parameter	N = 9
Age (years)	29 ± 11
Height (m)	1.70 ± 0.07
Weight (kg)	62 ± 10
BMI (kg m^2)	21.5 ± 2.5
Power output at T_{vent} (W)	125 ± 44
VO_2 at T_{vent} (ml/kg/min)	25 ± 9
Baseline TSI (%)	72 ± 5

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Table 1. Participants' demographic data. The large standard deviation value for the power output at T_{vent} (range from 80 to 200 W) indicates a wide variety of exercise capacity across the participants' group. TSI: tissue saturation index; T_{vent}: ventilatory threshold.

Required cadence (rpm)	Recorded cadence (rpm)
40	41 ± 2
50	50 ± 2
60	60 ± 1
70	70 ± 2
80	79 ± 3
90	89 ± 3

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Table 2. Required and recorded cadences. The participants were instructed to cycle at cadences of 40, 50, 60, 70, 80 and 90 rpm for 4 min bouts during the trial. The table shows the required cadence and cadence recorded during each exercise bout. rpm: revolutions per minute.

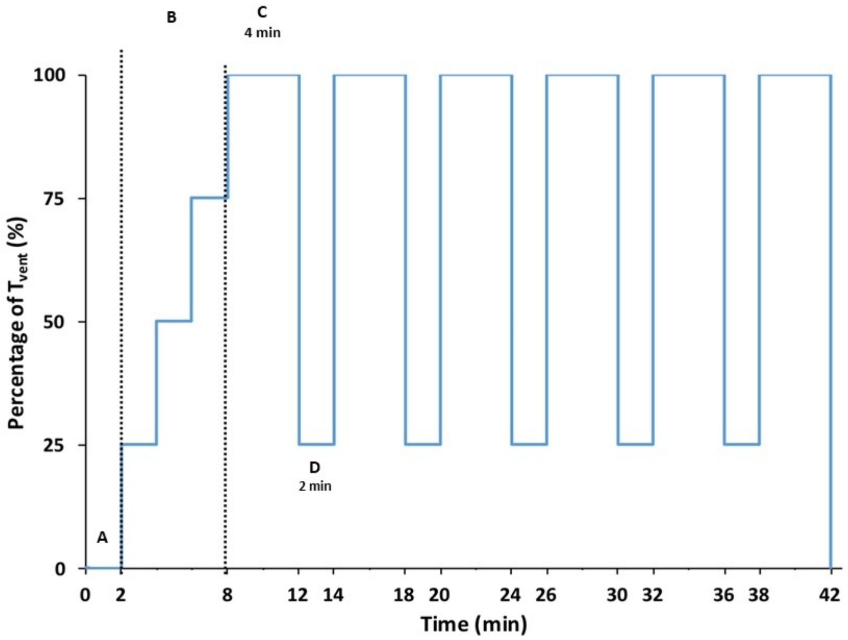

Figure 1: Schematic representation of the experimental protocol. Participants pedalled at 60 rpm during the warm-up and 2 min active recovery periods. A: Rest period, B: warm-Up period (6 min), C: 100% Tvent exercise bout at a given cadence (4 min), D: active recovery period (2 min). Tvent: ventilatory threshold; rpm: revolutions per minute; min: minutes.

254x190mm (96 x 96 DPI)

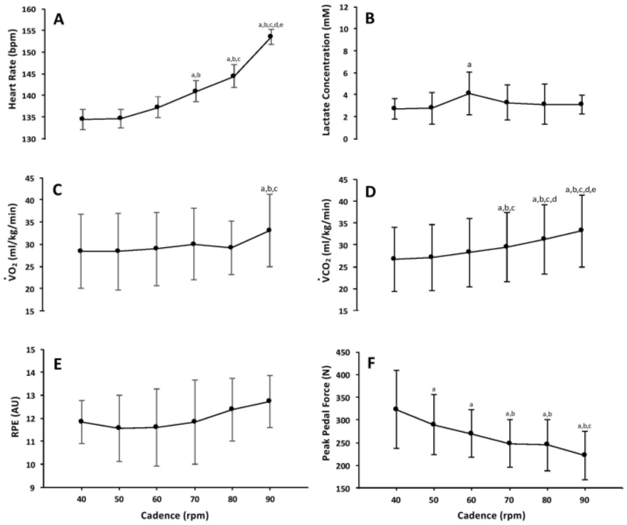

Figure 2: Physiological responses to cycling exercise at different pedalling cadences. Values for (A) heart rate (bpm), (B) lactate concentration (mM), (C) VO2 (ml/kg/min), (CD) VCO2 (ml/kg/min), (D) lactate concentration (mM), (E) RPE and (F) peak pedal force (N) for each cadence at 100% Tvent (N = 9). Lactate concentrations greater than 8 mM (n = 3 out of 63) were considered as technical errors and excluded from the analysis.
a, b, c, d, e: $P < 0.05$ when compared to 40, 50, 60, 70 and 80 rpm respectively, at the same Tvent. min: minutes; bpm: beats per minute; rpm: revolutions per minute; Tvent: ventilatory threshold; VO2: pulmonary oxygen uptake; VCO2: carbon dioxide output; RPE: rate of perceived exertion; AU: arbitrary units.

297x209mm (300 x 300 DPI)

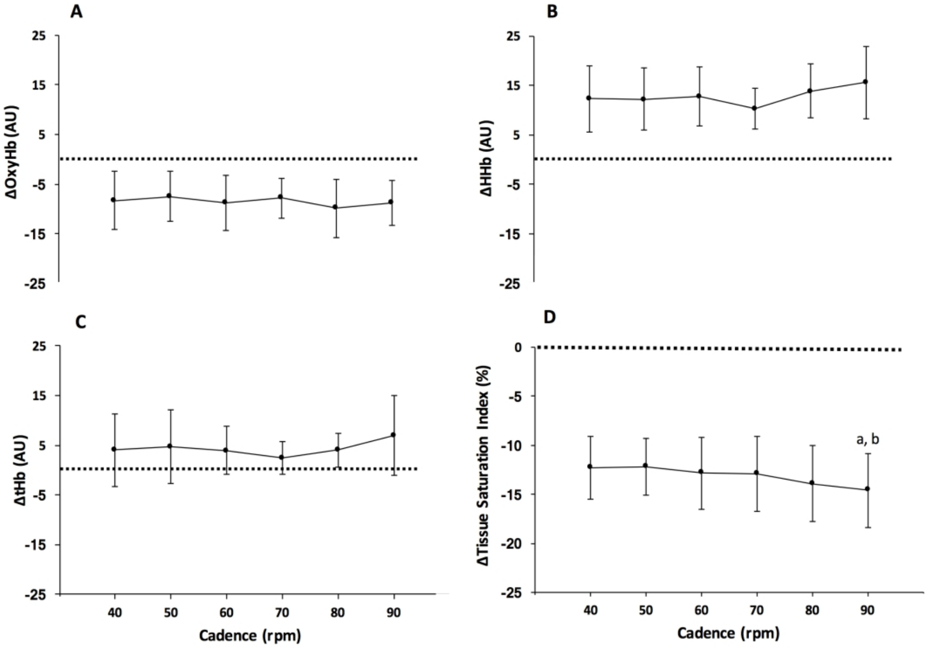

Figure 3: Skeletal muscle oxygenation responses to cycling exercise at different cadences. Results are of changes from rest for (A) OxyHb, (B) HHb, (C) tHb and (D) TSI for each cadence performed at 100% Tvent. For OxyHb, HHb and tHb (A, B and C) N = 8 for changes from baseline (due to one missing baseline data set). For each 90 rpm data set N = 7 (due to one missing data set at this cadence).
a, b: $P < 0.05$ when compared to 40 and 50 rpm respectively, at the same Tvent. min: minutes; AU: arbitrary units; TSI: tissue saturation index; OxyHb: oxygenated haemoglobin; HHb: deoxygenated haemoglobin; tHb: total haemoglobin; Tvent: ventilatory threshold; rpm: revolutions per minute.

207x142mm (300 x 300 DPI)