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DOI: 10.1055/a-0835-6286

Document Version Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA):

Formenti, F., Dockerill, C., Dolamulla Hewa Kankanange, L., Zhang, L., Takaishi, T., & Ishida, K. (2019). The Effect of Pedaling Cadence on Skeletal Muscle Oxygenation during Cycling at Moderate Exercise Intensity. *International Journal of Sports Medicine*, *40*(5), 305-311. https://doi.org/10.1055/a-0835-6286

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The effect of pedalling cadence on skeletal muscle oxygenation during cycling

Journal:	International Journal of Sports Medicine
Manuscript ID	IJSM-08-2018-7146-pb.R2
Manuscript Type:	Physiology & Biochemistry
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 ($-14\pm4\%$), compared to pedalling at 40 ($-12\pm3\%$) and 50 ($-12\pm3\%$) rpm (P=0.027 and 0.017, respectively). Albeit small, the significant decrease in Δ TSI 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.

SCHOLARONE[™] Manuscripts

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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 Δ TSI 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	34
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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.

42 Changing pedalling cadence during moderate intensity cycling affects a number of physiological responses: at a constant and moderate power output, increasing 43 cadence causes an increase in heart rate (HR), oxygen consumption (VO_2), carbon 44 45 dioxide production (VCO_2), rate of perceived exertion and lactate [11,16,20,31,32,38]. High pedalling cadences increase skeletal muscle metabolic 46 demand, which up to a point can be matched by a corresponding increase in the 47 cardio-respiratory function that raises the rate of pulmonary oxygen uptake and 48 oxygen delivery at systemic level. In contrast, low pedaling cadences increase 49 intramuscular pressure during the muscular contraction period [19], with a size effect 50 associated with the force generated by the muscular contraction [21]. This 51 52 phenomenon temporarily reduces or prevents blood perfusion to the contracting 53 muscle and downstream tissues. Inevitably during cycling exercise, low cadences are also associated with proportionally longer muscular relaxation periods, when 54 perfusion is increased. It is currently unclear whether the longer contraction period 55 and greater pedal forces at lower cadence are likely to determine inadequate 56 57 oxygenation of the exercising muscles [34]. 58

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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46 47 48	103
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51 52	105
53 54 55	106
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uptake ratio, respectively.

Materials and Methods

84	and Zorgati et al. [39] also reported no clear changes in oxygenation between
85	cadences, when these were tested in a randomized sequence. These studies [24,31
86	33,39] do differ in terms of experimental design, including power output, cadence
87	ranges and sequence in which they were tested, which may partly explain some
88	differences in their findings. Numerous studies have also been performed to
89	determine the optimal pedalling cadence for efficient cycling performance at a given
90	power output. However, no clear consensus has been reached with some studies
91	favouring a low cadence [23] and others a higher cadence [7], also highlighting the
92	different responses observed between elite and recreational cyclists, where elite
93	cyclists specifically train at high cadence [1,26,37]. Increasing cadence when
94	exercising at T _{vent} may affect skeletal muscle oxygenation [15], yet no study to date
95	has explored the effect of altering cadence on TSI when cycling at T_{vent} .
96	
97	In this context, the aim of our study was to investigate the effects of different
98	pedalling cadence on the systemic and vastus lateralis oxygenation responses to
99	cycling at a constant power output equal to 100% of the T _{vent} in participants with
100	different levels of cycling experience. We hypothesised that skeletal muscle

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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-

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. eer pe 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 (VE/ VO₂) [36] and ventilatory equivalent of carbon dioxide method (VE/ VCO₂) [6]. The mean value is then taken from these four methods and used as an estimation of the participant's

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134 T_{vent} . This approach has been shown to increase the precision of T_{vent} estimations,

A schematic diagram of the protocol where responses to different cadences were

135 when compared with using just one of these methods alone [13].

137 Responses to different cadences

studied is presented in Figure 1. After 2 min of rest, participants warmed up for 6 9 0 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 1 asked to cycle at an external power output equal to their T_{vent} at cadences of either 2 40, 50, 60, 70, 80 or 90 rpm, when real-time cadence was displayed on a digital 3 monitor visible to the participant and a metronome was used in order to help the 4 participants achieve the desired cadence.. Cadences were tested in a randomized, 5 6 counterbalanced sequence (with 90 rpm always tested last to reduce the potential effect of fatigue). Participants exercised at each cadence for 4 min, immediately 7 8 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 9 the potential effects of fatigue over the course of the experimental protocol. 0 Pedalling cadence, expired gases, heart rate and vastus lateralis oxygenation were 2 continuously recorded. Blood lactate was recorded in the last 90 s of the initial rest 3

154 period and of each 4 min bout of cycling exercise at 100% T_{vent} .

Equipment

1 2		
3 4 5 6 7 8	159	Cycle ergometer and pedal force measurements
	160	An electronically braked cycle ergometer (Aerobike 75XL, Combi, Tokyo, Japan) was
	161	used for all experiments. The external power output could be set to the nearest 1 W,
9 10 11	162	using personalized, pre-programmed protocols.
12 13	163	
14 15	164	Pedal force was recorded using three miniature force transducers (LM-50KA, Kyowa
16 17 18	165	Dengyo, Tokyo, Japan) on the pedal and a DC amplifier (DPM-601A, Kyowa
19 20	166	Dengyo, Tokyo, Japan). Three force signals were converged to one signal and
21 22	167	calculated the pedal force perpendicular to the pedal. Peak force was calculated for
23 24 25	168	each cycle. Pedal cadences were calculated using the principle of electromagnetic
25 26 27	169	induction by four small magnets on the gear and coil. The system generated four
28 29 30 31 32	170	peak voltage signals at each pedal revolution, so that cadence can be precisely
	171	calculated.
32 33 34	172	
35 36	173	We recorded pedal force and, importantly, pedalling cadence during each
37 38	174	experiment in order to establish participants' protocol adherence or deviations from
39 40 41	175	the expected cadence.
42 43	176	
44 45	177	Cardiopulmonary responses and rate of perceived exertion measurements
46 47 48	178	Heart rates were measured continuously during all stages of the trials by means of a
48 49 50	179	three-lead electrocardiogram (AB-621G, Nihon Kohden, Tokyo, Japan) connected
51 52	180	using gel electrodes applied to the skin. All analyzed data were linearly interpolated
53 54	181	between each cycle or heart beat to yield a data point at each 1 s interval.
55 56 57 58 59 60	182	

1 2		
2 3 4	183	Respiratory and metabolic data were recorded with the ARCO-2000 (Arco System
5 6	184	Inc., Chiba, Japan) with a mass spectrometer and a Fleisch pneumotachometer.
/ 8 9	185	Participants wore a facemask (7450, Hans-Rudolph Inc., MO, USA) with dead space
10 11	186	of ~100 ml.
12 13	187	
14 15 16	188	Participant's rate of perceived exertion was recorded on a standard Borg scale table
17 18	189	just after the end of each exercise bout (Borg, 1982).
19 20	190	
21 22 23	191	Blood lactate concentration
24 25	192	Blood lactate concentration values were recorded using the Lactate Pro 2 [®] analyser
26 27	193	(HaB International Ltd., England). Before taking a reading, the finger was cleaned
28 29 20	194	with an alcohol swab (70% Isopropyl alcohol) and wiped with a tissue to avoid
30 31 32	195	alcohol contamination of the sample.
33 34	196	
35 36	197	Skeletal muscle (vastus lateralis) oxygenation
37 38 39	198	Participants' muscle oxygenation values (OxyHb, HHb, tHb, TSI) were sampled at 10
40 41	199	Hz using the PortaMon ${ m I\!R}$ (Artinis Medical Systems, Einsteinweg, The Netherlands)
42 43	200	[29]. Briefly, the NIRS device was positioned on the participant's skin over the
44 45 46	201	muscle belly of the right vastus lateralis, along the main axis of the thigh,
40 47 48	202	approximately 16 cm from the knee joint. The device was secured using a Velcro
49 50	203	strap to prevent the device from moving during the experiment and to cover the
51 52	204	sensors, ensuring no ambient light contaminated the NIRS signal.
53 54 55	205	
55 56 57	206	
58 59 60	207	Data Analysis

2 3	208	Analyses were performed for peak pedal force, pedalling cadence, heart rate, blood
4 5	209	lactate RPE VO ₂ VCO ₂ OxyHb HHb tHb and TSI. Mean + standard deviation
6 7	205	
8 9	210	values at each cadence during the 100% T _{vent} tests were calculated from the last 60
10 11	211	s of each cycling bout in Microsoft Excel (Version 15.25.1, Microsoft Corporation,
12 13	212	California, USA).
14 15	213	
10 17 18	214	SigmaPlot (13.0.0.83. Systat Software, Inc., San Jose, California, USA) was used for
19 20	215	statistical analysis. The Shapiro-Wilk test was used to check for normal distribution
21 22	216	of the data. The Brown-Forsythe test was conducted to test for equal variance. Data
23 24 25	217	for physiological variables at different cadences were analysed using a One Way
25 26 27	218	Repeated Measures Analysis of Variance (ANOVA), if they passed the normality
28 29	219	tests. A Bonferroni pairwise multiple comparison procedure was used as a post-hoc
30 31 32	220	test to compare the means of each cadence.
32 33 34	221	RPE and VO $_2$ data did not pass the Shapiro-Wilk and Brown-Forsythe tests, so a
35 36	222	Friedman's one way repeated measures ANOVA based on ranks and Tukey's post
37 38	223	hoc test were performed to test for differences between responses at each cadence.
39 40 41	224	Results are presented as mean ± standard deviation unless otherwise stated.
42 43	225	Statistical significance was set at P < 0.05 for all tests.
44 45	226	
46 47	227	
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51 52	229	Results
53 54	230	
55 56 57 58 59 60	231	Participants' characteristics and protocol adherence

2 3 4 5 6 7 8 9 10 11 12 13	232	Six male and three female participants took part in this study. The characteristics of
	233	these participants are presented in table 1. The recorded cadences matched the
	234	required cadences well, as presented in table 2.
	235	
	236	
14 15	237	Changes in cardiorespiratory and metabolic function, perceived exertion and
16 17 18	238	pedal force at different pedalling cadences
19 20	239	Figure 2 shows the physiological, metabolic, rate of perceived exertion and peak
21 22	240	pedal force values at the different pedalling cadences recorded at at 100% $T_{vent}.$ HR
23 24 25	241	(Figure 2A), VO_2 (Figure 2C), VCO_2 (Figure 2D) and peak pedal force (Figure 2F)
26 27	242	changed significantly at the higher pedalling cadences when compared to the lower
28 29	243	pedalling cadences ($P < 0.05$). The respiratory rate did not increase significantly
30 31 32 33 34 35 36 37 38	244	between 40 and 90 rpm (30 \pm 5 and 31 \pm 4 breaths per minute respectively, p =
	245	0.09), unlike tidal volume and ventilation that increased respectively from 1.7 \pm 0.5 L
	246	to 2.0 \pm 0.5 L (p = 0.0001) and from 50 \pm 17 L/min to 62 \pm 21 L/min (p = 0.0002). A
	247	significant but small increase in blood lactate concentration was recorded at 60 rpm
39 40 41	248	(Figure 2B). No significant or marked changes were seen in RPE at the different
42 43	249	pedalling cadences (Figure 2E).
44 45 46 47 48	250	
	251	
49 50	252	Changes in skeletal muscle oxygenation at different pedalling cadences
51 52	253	Figure 3 shows the changes in skeletal muscle oxygenation in the vastus lateralis
53 54	254	muscle at different pedalling cadences. OxyHb and TSI decreased from resting
56 57	255	levels (Figure 3A and 3D), while HHb and tHb levels increased from their resting
57 58 59 60	256	values (Figure 3B and 3C). TSI was not different in the 30 s preceding each cadence

3 4	257	test ($p = 0.86$), with SD values ~1% for each individual. The magnitude of the TSI
5 6 7	258	reduction was significantly greater when pedalling at 90 rpm (-14.6% \pm 4), compared
7 8 9	259	to pedalling at 40 (12.3% \pm 3) and 50 (-12.2% \pm 3) rpm (P = 0.027 and 0.017,
10 11	260	respectively).
12 13	261	
14 15 16	262	
17 18	263	
19 20	264	Discussion
21 22 23	265	In our study of participants with different cycling expertise, pulmonary oxygen uptake
24 25	266	recorded at the highest cadence of 90 rpm was greater than at lower cadences
26 27	267	during exercise at 100% T_{vent} . This greater pulmonary oxygen uptake was
28 29 30	268	associated with a 3% greater TSI decrease at high cadence of 90 rpm compared
31 32	269	with low cadences of 40 and 50 rpm.
33 34	270	
35 36 37	271	
38		
39	272	Increased pedalling cadence at constant power output of 100% T _{vent} resulted in
39 40 41	272 273	Increased pedalling cadence at constant power output of 100% T _{vent} resulted in a greater cardiorespiratory response
39 40 41 42 43	272 273 274	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
39 40 41 42 43 44 45 46	272 273 274 275	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
39 40 41 42 43 44 45 46 47 48	272 273 274 275 276	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
39 40 41 42 43 44 45 46 47 48 49 50	272 273 274 275 276 277	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
39 40 41 42 43 44 45 46 47 48 49 50 51 52 53	272 273 274 275 276 277 278	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
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39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57	272 273 274 275 276 277 278 279 280	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

1 2		
2 3 4	282	
5 6	283	
7 8 9 10 11 12 13 14 15	284	Skeletal muscle oxygenation at high cadence when pedalling at constant
	285	power output
	286	Changes in HHb are considered a good indicator of skeletal muscle oxygen
	287	extraction because the HHb signal is not affected by an increase in oxygenated
16 17 18	288	blood to the skin for thermoregulation [14]. HHb tended to increase from baseline
19 20	289	levels during cycling at 100% T_{vent} , indicating a moderate increase in fractional
21 22	290	oxygen extraction in the exercising muscles, achieved via an increase in cardiac
23 24 25	291	output and/or a reduction in the peripheral vascular resistance at the exercise
25 26 27	292	intensity tested.
27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43	293	
	294	Despite these changes from baseline and a trend for an increase in HHb and tHb at
	295	high cadence, there was no significant change in these skeletal muscle oxygenation
	296	parameters between the pedalling cadences. These findings are in agreement with
	297	previous studies, which reported that cadence had no clear effect on OxyHb, HHb
	298	and tHb in conditions similar to those tested here [24,39].
	299	
44 45	300	TSI is an overall indicator of skeletal muscle oxygenation [14,17]. TSI significantly
46 47	301	decreased from baseline during cycling exercise at 100% T_{vent} and from 40 and 50
48 49 50	302	rpm to 90 rpm (Figure 3D). The significant changes in TSI observed at higher
51 52	303	pedalling cadences, which we tested in a randomized sequence at 100% T_{vent} , are in
53 54	304	agreement and strengthen the findings from Skovereng et al. [31,32]. These results
55 56 57	305	are supported by previous observations at a relatively lower power output equal to
58 59 60	306	60% of VO ₂ max, where skeletal muscle oxygenation was not different at the onset of

3 4	307	cycling exercise at either 40 or 100 rpm [24], confirming our results in an acute
5 6 7	308	exercise context.
, 8 9	309	
10 11	310	
12 13	311	It is likely that the effect of intramuscular pressure on TSI is associated with the
14 15 16	312	absolute pressures generated during the contraction. Given the higher external
17 18	313	power output at which elite cyclists exercise (for a similar relative exercise intensity,
19 20 21	314	e. g. 100% T_{vent}), these absolute intramuscular pressures are likely to be greater in
21 22 23	315	elite than in recreational cyclists. This is a putative mechanism that could explain the
24 25	316	difference in our findings with those reported in trained cyclists by Skovereng et al.,
26 27	317	where TSI decreased at high cadence even at a lower relative external power output
28 29 30	318	corresponding to 75% of the participants' lactate threshold [31,32].
31 32	319	
33 34	320	
35 36 37	321	The group of participants studied was limited to nine individuals and rather
37 38 39	322	heterogeneous in terms of age, exercise capacity and cycling expertise. Given the
40 41	323	limited sample size considered in this study, we acknowledge that this finding needs
42 43	324	confirmation on a larger scale.
44 45 46	325	A limitation of our study is that the T_{vent} was estimated at one pedalling cadence
47 48	326	only. It is possible that estimating T_{vent} at higher or lower pedalling cadence could
49 50	327	have affected the estimated $T_{vent}.$ For the incremental test, we chose a cadence that
51 52	328	all participants could exercise at comfortably, and that has been used in several
55 54 55	329	published studies before, making our results comparable with those presented in the
56 57	330	literature. In addition, there is often a degree of error in the estimation of T_{vent} , so we
58 59	331	think that the estimated T _{vent} would have only varied significantly if cadence had

1 ว		
2 3 4	332	markedly been reduced or increased from 60 rpm. An additional limitation is in the
5 6 7	333	choice of testing the highest cadence (i. e. 90 rpm) always last, where it cannot be
7 8 9	334	entirely excluded that the results associated with the 90 rpm conditions are in part
10 11	335	determined by the preceding exercise. However, TSI was not different (within
12 13	336	participant) between rest and the final part of each recovery period, so the likelihood
14 15 16	337	of TSI decrease observed at 90 rpm being determined by the preceding exercise
10 17 18	338	appears limited.
19 20	339	
21 22 23	340	We conclude that increasing cadence beyond a given threshold at moderate
23 24 25	341	exercise intensity close to the T_{vent} is less energetically efficient (as confirmed by the
26 27	342	higher VO_2 and VCO_2 recorded for a given power output here [Fig. 2]) and that high
28 29 20	343	cadence may compromise skeletal muscle oxygenation during cycling exercise.
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37 38	347	Disclosure of interest: The authors report no conflict of interest.
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2 3 4	462	Figure captions				
5 6 7	463					
8 9 10	464	Figure 1. Schematic representation of the experimental protocol. Participants				
11 12	465	pedalled at 60 rpm during the warm-up and 2 min active recovery periods. A: Rest				
13 14	466	period, B: warm-Up period (6 min), C : 100% T_{vent} exercise bout at a given cadence				
15 16 17	467	(4 min), D : active recovery period (2 min). T_{vent} : ventilatory threshold; rpm:				
18 19	468	revolutions per minute; min: minutes.				
20 21	469					
22 23 24	470					
25 26	471	Figure 2: Physiological responses to cycling exercise at different pedalling				
27 28 29	472	cadences.				
30 31	473	Values for (A) heart rate (bpm), (B) lactate concentration (mM), (C) VO ₂ (ml/kg/min),				
32 33	474	(D) VCO_2 (ml/kg/min), (E) RPE and (F) peak pedal force (N) for each cadence at				
34 35 26	475	100% T_{vent} (N = 9). Lactate concentrations greater than 8 mM (n = 3 out of 63) were				
30 37 38	476	considered as technical errors and excluded from the analysis.				
39 40	477	a, b, c, d, e: P < 0.05 when compared to 40, 50, 60, 70 and 80 rpm respectively, at				
41 42	478	the same T _{vent} . min: minutes; bpm: beats per minute; rpm: revolutions per minute;				
43 44 45	479	T_{vent} : ventilatory threshold; VO ₂ : pulmonary oxygen uptake; VCO ₂ : carbon dioxide				
46 47	480	output; RPE: rate of perceived exertion; AU: arbitrary units.				
48 49	481					
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53 54	483	Figure 3: Skeletal muscle oxygenation responses to cycling exercise at				
55 56 57	484	different cadences. Results are of changes from rest for (A) OxyHb, (B) HHb, (C)				
58 59 60	485	tHb and (D) TSI for each cadence performed at 100% $T_{vent}.$ 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 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

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

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

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.

24 26

Time (min)

30 32

36 38









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.

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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.

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