The effect of 30-s sprints during prolonged exercise on gross efficiency, electromyography and pedaling technique in elite cyclists

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Original investigation

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Abstract

Background:
Cycling competitions are often of long duration and include repeated high-intensity efforts.

Purpose:
To investigate the effect of repeated maximal sprints during 4 hours of low-intensity cycling on gross efficiency (GE), electromyography patterns (EMG) and pedaling technique compared to work-matched low-intensity cycling in elite cyclists.

Methods:
Twelve elite, male cyclists performed 4 hours of cycling at 50% of VO_{2max} either with 3 sets of 3 x 30-s maximal sprints (*E&S*) during the first 3 h or a work-matched cycling without sprints (*E*) in a randomized order. VO_2, EMG and pedaling technique were recorded throughout the exercises.

Results:
GE was reduced from start to the end of exercise in both conditions (E&S; 19.0±0.2 vs 18.1±0.2, E; 19.1±0.2 vs 18.1±0.2%, both P=0.001), with no difference in change between conditions (condition x time interaction: P=0.8). iEMG increased from start to end of exercise in m.Vastus Lateralis and m.Vastus Medialis (VM; 9.9±2.4, VL; 8.5±4.0 mV, main effect of time: P<0.001 and P=0.03, respectively) and *E&*S increased less than *E* in VM (mean difference -3.3±1.5 mV, main effect of condition: P=0.03, interaction, P=0.06). The mechanical effectiveness only decreased in *E&S* (*E&S;* -2.2±0.7, ES=0.24 vs *E*; -1.3±0.8 percentage points: P=0.04 and P=0.8, respectively). The mean power output during each set of 3x30-s sprints in *E&*S did not differ (P=0.6).

Conclusions:
GE decreases as a function of time during 4 hours of low-intensity cycling. However, the inclusion of maximal repeated sprinting does not affect the GE-changes, and the ability to sprint is maintained throughout the entire session.

Keywords
Elite cyclists
Gross efficiency (GE)
Repeated sprint
Electromyography (EMG)
Pedaling technique

Introduction

90

91

92 The "Classics" in professional cycling are typically ~250 km[1] and performance in these races is
93 mainly determined by maximal oxygen uptake (VO_{2max}), fractional utilization of VO_{2max} and
94 efficiency of movement.[2] However, for tactical reasons, the ability to perform repeated periods of
95 high-intensity efforts are of additional importance.[3] Long-duration sessions with repeated high-
96 intensity efforts are, therefore, important components of cyclists' competitions and training
97 sessions. However, it is currently unknown whether inclusion of sprints during long-duration
98 sessions affect the quality of sprinting or the cost of the session.

99 The majority of time (~70%) during one-day races is spent at an intensity below 70% of VO_{2max}.[4]
100 Hours of cycling at such a low intensity gradually increases VO_2 for the same power output (i.e.
101 reduced GE) in well-trained cyclists.[5,6] This increase in VO_2 seems to be intensity-dependent, where
102 higher intensities lead to a greater drift in VO_2 than lower intensity work.[7-10] It could, therefore, be
103 hypothesized that inclusion of short high-intensity efforts performed during prolonged exercise
104 would reduce GE further.

105 The underlying mechanisms for the observed changes in GE during prolonged exercise, and the
106 possible effects of intensity on this change, are not fully elucidated.[6] Changes in recruitment pattern
107 of motor-units measured by integrated electromyography (iEMG) and median frequency have
108 earlier been reported during high-intensity efforts and prolonged low-intensity cycling, partly
109 explaining the drift in VO_2.[7,11,12] Moreover, changes in pedaling technique might also play a role,
110 since a positive correlation between earlier peak torque during the pedal stroke and improved 40-
111 min TT have been reported.[13] In theory, this could be explained by a reduced time of blood flow
112 obstruction to the working muscles during the downstroke phase, which is highest at peak torque.[14]
113 Furthermore, improved mechanical effectiveness, which means reduced negative torque during the
114 pedaling upstroke, has been reported together with an improved 5-min all-out performance in well-
115 trained cyclists.[15] However, in contrast to would have been expected, reduced negative torque has
116 been associated with increased submaximal VO_2, possibly due to increased hip flexion activity or
117 less efficient flexor muscles in the lower limbs.[15,16] These mechanisms may also be affected by
118 inclusion of high-intensity efforts during long-lasting submaximal work. During the first minutes
119 after high-intensity efforts, the recovery-processes leads to increased energy-consumption, but
120 recovery happens within minutes of rest.[17-20] However, a reduced GE is reported following brief (2
121 km) all-out efforts and is not restored within 30 min of cycling at 55% of VO_{2max}.[8] To restore GE to
122 baseline values, 45 min of absolute rest may be necessary,[21] but this is not possible during races for
123 elite cyclists. It is therefore suggested that decreased GE affects high-intensity performance later in
124 a race,[22] although the effect of repeated maximal sprinting during prolonged exercise on GE and the
125 following recovery hereof has not earlier been investigated. Therefore, the effects of prolonged
126 submaximal cycling with or without repeated sprints on pedaling technique and GE need further
127 elucidation.

128 The primary aim of this study was to investigate the effect of performing repeated 30-s maximal
129 sprints during 4 h of low-intensity endurance cycling on GE, EMG patterns and pedaling technique

130 compared to work-matched low-intensity cycling in elite cyclists. Our secondary aim was to
131 examine how the quality of maximal sprints were affected over time.
132

Methods

133 ## Methods
134 ### *Participants*
135 Twelve male cyclists, aged 26.2±6.3 years, were recruited for the study. All cyclists had a history of
136 regular endurance training (cycling or running, 54.9±34.6 h recorded 30 d prior to inclusion) and
137 have been competing in cycling for 5.3±4.1 years. The cyclists were categorized as performance
138 level 4-5 according to De Pauw et al.[23] All participants were accustomed to sprinting on the bike,
139 but did not perform specific sprint-training. Physiological parameters are presented in Table 1.
140
141 Before testing, participants were informed of the possible risks and discomforts associated with the
142 study and gave their written, informed consent to participate. The study was approved by the local
143 ethical committee at Lillehammer University College, and performed according to the Declaration
144 of Helsinki.
145 *Insert table 1 here*
146
147
148 ### *Experimental design and procedures*
149 Participants visited the lab on four occasions: (1) to perform an initial measurement of their fitness;
150 (2) for familiarization of the study protocol; (3+4) to undertake two experimental conditions. The
151 initial visit consisted of a 30-s all-out sprint (Wingate), a blood lactate profile and an incremental
152 test until exhaustion to determine VO_{2max}. The familiarization to the experimental protocol
153 consisted of a 4 h bout of endurance exercise combined with series of maximal effort sprints (*E&S*).
154 The participants were instructed to refrain from intense exercise, caffeine, beta-alanine and
155 bicarbonate 24 h prior to all testing. Participants were also instructed to record and duplicate food
156 intake and time of consumption 24 h prior to *E&S* and the work-matched endurance protocol (*E*).
157 All testing was performed 4-9 d apart, starting between 8.00-10.00 AM in a controlled
158 environmental condition (16-21°C and 20-35% relative humidity) with a fan ensuring air
159 circulation.
160
161 ### *Wingate, blood lactate profile and VO_{2max}*
162 Cycling tests were performed on an electromagnetic braked cycle ergometer, measuring power
163 output at 6 Hz (Lode Excalibur Sport, The Netherlands), which was adjusted to the cyclist and
164 replicated throughout all testing. The *Wingate* modus was used for sprints with the resistance set to
165 0.8 nm·kg^{-1} body mass. A standardized 20 min warm-up with 3 x 20-s, non-maximal sprints were
166 performed prior to an all-out 30-s Wingate test. Sprints were started from 80 revolutions per minute
167 (RPM), in a seated position with verbal encouragement throughout. Peak power output (PPO) was
168 defined as the highest power output achieved during the Wingate test and mean power output
169 (P_{mean}) was presented as the 30-s average power output sustained throughout the Wingate test.
170
171 Participants recovered (~30 min) until the blood lactate concentration [BLa$^-$] had returned below
172 1.5 mmol·L^{-1} and thereafter completed a blood lactate profile test as previously described.[24] Blood
173 was sampled from the fingertip and analyzed for [BLa$^-$] using a lactate analyzer (Biosen C line,
174 5214 09 0045, EKF Diagnostic, Germany). After 10 min of recovery, participants completed an
175 incremental test to determine VO_{2max}, starting at 200 W with 25 W increments every minute until

175 exhaustion or RPM <60. VO_{2max} was calculated as the moving average of the 12 highest 5-s VO_2-
176 measurements. VO_2 was measured using a computerized metabolic system with mixing
177 chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany). W_{max} was calculated as the mean
178 power output during the last minute of the incremental test.

179 ***Experimental protocols***
180 The *E&S* protocol consisted of 4 h cycling at a PO equivalent to 50% of VO_{2max} with 3 x 30-s
181 maximal sprints, interspersed by 4 min recovery (1 min completely rest and 3 min cycling at 100
182 W), 41 min into every hour during the first 3 h. No sprinting was performed during the last hour,
183 equivalent to the *E*-protocol (Figure 1). PO at 50% of VO_{2max} was calculated using interpolation
184 from sub-maximal values from the blood lactate profile together with the VO_{2max}. During the
185 familiarization trial, cyclists consumed water, energy drinks and gels without caffeine (Squeezy
186 Sports Nutrition GmbH, Germany) *ad libitum* to prevent dehydration and glycogen depletion.
187 Consumption was recorded during familiarization and replicated on experimental tests. Participants
188 consumed on average 3.2±0.1 L and 3.2±0.1 L of energy drink and water and 277.3±16.5 g and
189 273.6±15.2 g carbohydrate in *E&S* and *E*, respectively. The estimated sweat rate, measured as
190 change in body mass and taking into account water consumption and loss of mass from lavatory-
191 visits, during the 4 h of exercise was 1.7±0.2 L and 1.5±0.2 L in *E&S* and *E*, respectively, with no
192 differences between conditions.

193 *Insert figure 1 here*

194 During experimental visits, participants performed, in a randomized order, *E&S* or *E* (4 h without
195 sprints), separated by 6±2 days. The *E*-protocol was work-matched to *E&S* based on the average
196 power output during the familiarization trial, including power output during sprints and rest periods.
197 Due to the 4 min long recovery periods between sprints in *E&S*, the average PO during steady-state
198 periods had to be somewhat higher in the *E&S*-protocol (*E&S;* 186±5 W vs *E;* 182±4 W) in order
199 to work-match the protocols. The average power output of the protocols was therefore 182±4 W
200 and 182±4 W in *E&S* and *E*, respectively.
201 VO_2, EMG and pedaling technique measurements were recorded from 33rd-35th min and 58th-60th
202 min (6.5 min post sprint) every hour. Participants were instructed to keep the same pedaling
203 frequency during these periods. A 5 min break was allowed every hour for the participants to visit
204 the lavatory and to re-calibrate the metabolic system and the cycle ergometer. The change in VO_2,
205 EMG and pedaling technique measurements were expressed relative to baseline values measured
206 during the first hour from 5th-10th min. Perceived exertion, [BLa-] and HR was registered
207 throughout the experimental protocols (Figure 1).
208
209 ***Pedaling technique***
210 Pedaling technique measurements were recorded using the Lode Ergometry Manager Software
211 (Lode, version 10.4.5, Netherlands). The torque generated perpendicular to the crank axle was
212 recorded at every 2°. Crank angle was referenced to 0° at the top dead center and 180° at the
213 bottom. Angle of peak torque (in degrees) was recorded as the mean of the highest propulsive
214 torque during the downstroke phase. Mechanical effectiveness was defined as mean of the highest
215 resistive torque during the upstroke phase (force acting negatively on the propulsive force)
216 expressed relative to the mean torque (in percentage).
217

Gross Efficiency
Gross efficiency (GE), defined as the ratio between the mechanical power output (PO) and the metabolic power input (PI) was calculated from steady-state periods, using the oxygen equivalent[25] and respiratory exchange ratio (RER) by following equation:[26]

$$PI = VO_2 \; L \cdot s^{-1} \cdot (4{,}840 \; J \cdot L^{-1} \cdot RER + 16{,}890 \; J \cdot L^{-1})$$

EMG
To evaluate muscle fiber recruitment during exercise, EMG measurements via a wireless EMG-module (Ergotest Innovation as, Norway) using MuscleLab system (Pantaray Research Ltd. version 10.5.51.4221, Israel) was performed using surface electrodes (DUO-TRODE, Myotronics Inc, Kent, U.S.A) on m. Vastus Lateralis and m. Vastus Medialis placed as recommended by Konrad 2006.[27] Raw EMG-data were captured at 1000 Hz, and smoothed using a moving average with a 20-sample window width, repeated 20 times. iEMG was calculated as the average of the smoothed EMG data over 60 crank cycles, and expressed relative to the baseline (8th – 9th min). The frequency distribution to obtain median frequency was calculated in Matlab (R2016b) using its PSD routine ('periodogram' function) with default settings with a frequency resolution of 1 Hz.

Statistics
Possible differences in physiological variables within and between conditions were evaluated by a marginal-model approach using the SPSS-software version 23 (SPSS, IBM). Time and condition were specified as fixed effects. Repeated effects were specified by subject. A significant main effect or interaction was further evaluated by a multiple-comparison approach with Sidak adjustment. A significance level of 0.05 was applied and p-values >0.05 and <0.1 were described as tendencies. Hopkins' effect sizes (ES)[28] using pooled SD was calculated to compare the practical significance of differences in changes between conditions. Interpretations of the magnitude of ES were as follows: <0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and 2.0-4.0 very large difference.

Results
Physiological responses and rate of perceived exertion
VO_2 and VE increased from baseline (5-10 min) to the end of exercise (238-240 min) in both conditions, with no difference in relative changes (VO_2: 5±1% vs 6±1%, P=0.4 and VE: 9±2% vs 7±2%, P=0.2 in *E&S* and *E*, respectively; Figure 2A and 2B). Due to the higher PO in steady-state periods during *E&S*, there was an effect of condition, with both VO_2 and VE being higher at all time-points for *E&S* compared to *E* (both P<0.001). No change in RER over time was observed in either condition (P=0.8) but *E&*S was lower compared to *E* (mean difference -0.02±0.01, P=0.01; Figure 2C).

There was an effect of time on RPE, which was increased compared to baseline (10 min) after the first set of sprints (54 min) and remained elevated throughout the exercise in *E&S* (P<0.02), whereas *E* was only increased at the end of exercise (234 min, P=0.002; Figure 2D). No difference was observed in RPE at the beginning or at the end of exercise between conditions (P=0.5 and P=0.7). [BLa⁻] was increased compared to baseline after the first set of sprints in *E&S* (P<0.001) and remained elevated until the last set of sprints (174 min, P<0.001), whereas [BLa⁻] was unchanged in *E* throughout exercise (P=1.0). There were no differences between conditions in [BLa⁻] at the beginning but tended to be higher at the end of exercise (P=1.0 and P=0.08). A significant interaction was observed in $\%HR_{max}$ (P=0.02), which increased after the third set of

265 sprints in *E&S* compared to baseline (P<0.001) and was higher compared to *E* during exercise
266 (P<0.006; Figure 2F). However, there was no difference in change from beginning to end of
267 exercise between conditions (*E&S:* 2.6±0.9 vs *E:* 3.1±1.3 percentage points, P=0.5, respectively).
268
269 *Insert figure 2 here*
270
271 ### Gross efficiency and pedaling frequency
272 GE was in both conditions reduced from baseline (8-10 min) to the end of exercise (238-240 min)
273 (E&S; 19.0±0.2 vs 18.1±0.2, E; 19.1±0.2 vs 18.1±0.2%, pre vs post, respectively, both P=0.001;
274 Figure 3A). There was an overall effect of condition with GE being lower in *E&S* (P=0.002), but
275 there was no interaction between time and condition (P=0.6). Post hoc analysis revealed a
276 difference in GE after the first set of sprints (93-95 min) between *E&S* and *E* (P=0.02). There was
277 no difference in pedaling frequency between conditions in steady-state periods (P=0.2; Figure 3B).
278 During sprints in *E&S* pedaling frequency was increased above baseline (5-10 min) and compared
279 to *E* (P<0.001).
280
281 *Insert figure 3 here*
282
283 ### EMG
284 An overall effect of time was observed in iEMG in VL (P=0.03), post hoc analysis did not reveal
285 significant differences for either condition from baseline (9-10 min) to the end of exercise (238-239
286 min, P=1.0 and P=0.8 in *E&S* and *E* respectively) and there was no effect of condition (P=0.3;
287 Figure 4A). A significant effect of time (P<0.001) and condition (P=0.03) and a tendency for a
288 significant interaction (P=0.06) was observed in VM. Post-hoc analysis revealed a temporary small
289 increase (ES = 0.35) compared to baseline in iEMG after the second set of sprints (118-119 min) in
290 *E&S* (P=0.001) which tended to be greater than for *E* (P=0.053; Figure 4B). iEMG in VM was
291 increased the last hour of exercise (213-239 min) compared to baseline in *E* (P=0.008). This
292 increase was considered small (ES=0.46) but was greater than for *E&S* (P=0.02). Median frequency
293 did not change from baseline to any time point during exercise in either condition in VL (*E&S;* -
294 2.9±4.9, P=1.0 vs *E;* -2.3±5.0 Hz, P=1.0) or VM (*E&S*; -2.7±3.4, P=1.0 vs *E;* -1.3±3.4 Hz, P=1.0)
295 and no difference between conditions was observed (P=0.2).
296
297 *Insert figure 4 here*
298
299 ### Pedaling technique
300 The mechanical effectiveness was decreased by -2.2±0.7 percentage points in *E&S* from baseline
301 (5-10 min) to the end of exercise (238-240 min, P=0.04), while no changes occurred in *E* (-1.3 ± 0.8
302 percentage points, P=0.8: Table 2). This decrease in mechanical effectiveness was greater in *E&S*
303 compared to *E* (P=0.03). The effect of this decrease was small (ES=0.24) and there was no
304 correlation between the reduction in GE and the change in mechanical effectiveness in either *E&S*
305 (*r*=0.08) or *E* (*r*=0.22). There were no changes in angle of peak torque during the pedal stroke in
306 either condition from beginning to end of exercise (P=0.4 and P=0.2 in *E&S* and *E*, respectively).
307 During sprints in *E&S*, mechanical effectiveness higher compared to baseline and compared to *E*
308 (all P<0.001).
309
310 *Insert table 2 here*
311

312 ***Repeated 30-s maximal sprints***
313 The mean power output during each set of 3 x 30 s sprints in *E&*S did not differ (P=0.6). Set 1, 2
314 and 3 was 93±1, 92±1 and 91±1%, respectively compared to an all-out Wingate test (Figure 5).
315
316 *Insert figure 5 here*
317
318

Discussion

320 The main finding of this study was that including repeated 30-s maximal sprints during 4 h low-
321 intensity cycling did not affect the reduction in GE from the start to the end of the session,
322 compared to a work-matched constant load cycling in elite cyclists. However, a temporary increase
323 in energy expenditure and a reduction in GE was evident after the first set of sprints in *E&S,*
324 although this temporary decrease in GE diminished and did not affect repeated sprint-ability later
325 during exercise.
326
327 GE was reduced from ~19 to ~18% in both conditions, indicating that duration of exercise is mainly
328 responsible for the reduced GE during long-lasting events. This is supported by the findings of
329 earlier studies where prolonged low-intensity exercise (2-3 h) increases VO_2 in untrained to highly
330 trained subjects.[5,6,29] Together with this gradually declining GE, we found an increased VE and
331 RPE during exercise in both conditions, whereas no changes in RER, as an indicator of substrate
332 oxidation, occurred. There are likely multiple explanatory factors for our findings; Increased VE
333 has earlier been calculated to account for a small fraction (12-18%) of the variance in GE[30] and
334 does not fully explain the change in GE found here. Furthermore, an overall effect of time, with
335 increasing iEMG in VL and VM, was found, and indicate a gradual recruitment of additional motor-
336 units simultaneously as there was a reduction in GE. The increasing iEMG may indicate a
337 decreasing efficiency of already recruited fibers, as reported earlier during both low-intensity[12] and
338 supramaximal intensities[7], without indication of change in fiber type recruitment (i.e. increased
339 mean power frequency). However, in our study the maximal effort sprinting only temporarily
340 increased iEMG while the effect on iEMG was small and patterns returned to baseline prior to the
341 next set of sprints. It could be speculated that the short breaks after sprinting (1 min passive rest and
342 3 min at 100 W) during *E&S* was sufficient to recover the muscles, and therefore demonstrated no
343 effect of time on GE compared to work-matched low-intensity work.
344
345 The acute metabolic stress response during and after maximal sprint exercise was evident by the
346 drastically increased [Bla⁻] and RPE, which is previously shown to momentarily decrease muscle
347 efficiency[31]. Consequently, energy-consumption in the recovering process increases due to active
348 transportation by Na^+/K^+-ATPase pumps, SERCA-pumps and recovery of metabolic products,[17-19]
349 which may be indicated by the slightly increased HR in this study. This temporary change in
350 homeostasis and consequently increased energy expenditure seems to account for the greater VO_2,
351 VE and HR in *E&S* compared to *E* during this time-period. However, during the ~1 h cycling
352 between sprint-sets, both RPE and [BLa⁻] were restored to the same levels as *E*. Despite the rather
353 long recovery between sprints, GE was not restored to baseline levels. The latter is in agreement
354 with the findings in trained cyclists by Groot et al. who showed a reduced GE 30 min after all-out
355 exercise.[8] In line with the present findings, reduced GE has earlier been observed not to affect 30-s
356 sprint performance in competitive cyclists.[22] The present study supports this notion since repeated
357 sprint performance did not seem affected by a reduced GE. Hence, performing sprints early during a
358 prolonged low-intensity exercise does not negatively affect the quality of repeated sprints

359 performed later during the same session.[32] We therefore speculate that including sprints in
360 prolonged low-intensity exercise could benefit both moderately[33] and highly trained cyclists.
361
362 As expected, pedaling technique was drastically changed during repeated 30-s maximal sprints.
363 Specifically, RPM was increased and mechanical effectiveness was improved during sprinting
364 compared to low-intensity steady state cycling. Improved mechanical effectiveness has earlier been
365 reported together with an improved 5-min all-out performance in well-trained cyclists.[15] However,
366 in the current study, mechanical effectiveness did not change during submaximal exercise in *E*
367 which is in contrast to previous findings.[34] In the study by Sanderson and Black, competitive
368 cyclists rode on a relative higher power output (80% of maximum power output) to exhaustion,
369 which might explain the differences to our study. Although not different from *E*, the *E&S* group in
370 the current study experienced a slight decrease in mechanical effectiveness from baseline to the end
371 of exercise and temporal increases in energy consumption due to increased RPM.[35] This could in
372 theory contribute to explain the reduced GE, but since there were no differences between
373 conditions, the ES of adding sprints was small and an earlier observation that mechanical
374 effectiveness was not indicative of GE,[16] we find it difficult to relate pedaling technique to the
375 observed reduced GE in *E&S*. A study on combined strength and endurance training in highly-
376 trained cyclists have shown correlations between an earlier occurrence of peak torque during the
377 pedal stroke and improved 40 min TT.[13] An earlier peak torque could hypothetically reduce the
378 time of blood flow obstruction to the working muscle during the downstroke phase, which is
379 highest at peak torque.[14] In the present study, mean angle of peak torque during the down stroke
380 phase did not change during prolonged cycling and did therefore not seem to affect GE. Hence,
381 changes in pedaling technique does not seem to explain the reduction in GE seen during prolonged
382 low-intensity exercise.
383

Practical applications

385 Compared to work-matched low-intensity cycling, repeated sprinting does not negatively affect the
386 decrease in GE from the start to the end of 4 hours of low-intensity cycling in elite cyclists.
387 Furthermore, the repeated sprint-ability is not negatively affected by the decreased GE, implying
388 that cyclists can include repeated sprints in their long-duration sessions without interfering the
389 quality of sprinting and without a greater accumulation of fatigue compared to low-intensity
390 cycling. Thus, repeated sprint exercise included in long-duration sessions could be an effective tool
391 for concurrent development of both sprint-ability and endurance performance that should be further
392 explored.[30] In addition, the general reduction in GE over time found here indicates that elite cyclists
393 and coaches should explore the potential for developing training regimes or technical solutions to
394 better maintain GE over time.
395
396

Conclusion

398 GE decreases as a function of time during 4 hours of low-intensity cycling. However, the inclusion
399 of maximal repeated sprinting does not affect the GE-changes compared to work-matched low-
400 intensity cycling. The temporal increases in [BLa$^-$], as well as the major changes in pedaling
401 technique and muscle activity patterns during and directly after sprints led to a temporarily reduced
402 GE after the initial sets of sprints. However, this did not negatively affect the subsequent repeated
403 sprint performance in elite cyclists.
404
405

References

1. Lucia A, Hoyos J, Chicharro JL. Physiology of professional road cycling. *Sports Med.* 2001;31(5):325-337.
2. Coyle EF. Integration of the physiological factors determining endurance performance ability. *Exerc Sport Sci Rev.* 1995;23:25-63.
3. Sanders D, van Erp T, de Koning JJ. Intensity and Load Characteristics of Professional Road Cycling: Differences between Men's and Women's Races. *Int J Sports Physiol Perform.* 2018:1-23.
4. Fernandez-Garcia B, Perez-Landaluce J, Rodriguez-Alonso M, Terrados N. Intensity of exercise during road race pro-cycling competition. *Med Sci Sports Exerc.* 2000;32(5):1002-1006.
5. Ronnestad BR, Hansen EA, Raastad T. Strength training improves 5-min all-out performance following 185 min of cycling. *Scand J Med Sci Sports.* 2011;21(2):250-259.
6. Hopker JG, O'Grady C, Pageaux B. Prolonged constant load cycling exercise is associated with reduced gross efficiency and increased muscle oxygen uptake. *Scand J Med Sci Sports.* 2017;27(4):408-417.
7. Vanhatalo A, Poole DC, DiMenna FJ, Bailey SJ, Jones AM. Muscle fiber recruitment and the slow component of O2 uptake: constant work rate vs. all-out sprint exercise. *Am J Physiol Regul Integr Comp Physiol.* 2011;300(3):R700-707.

451 8. Groot S, van de Westelaken LH, Noordhof DA, Levels K, de Koning JJ. Recovery of Cycling
452 Gross Efficiency After Time-Trial Exercise. *Int J Sports Physiol Perform.* 2018:1-21.
453 9. Noordhof DA, Mulder RC, Malterer KR, Foster C, de Koning JJ. The decline in gross
454 efficiency in relation to cycling time-trial length. *Int J Sports Physiol Perform.* 2015;10(1):64-
455 70.
456 10. Gaesser GA, Poole DC. The slow component of oxygen uptake kinetics in humans. *Exerc*
457 *Sport Sci Rev.* 1996;24:35-71.
458 11. Krustrup P, Secher NH, Relu MU, Hellsten Y, Soderlund K, Bangsbo J. Neuromuscular
459 blockade of slow twitch muscle fibres elevates muscle oxygen uptake and energy turnover
460 during submaximal exercise in humans. *J Physiol.* 2008;586(24):6037-6048.
461 12. Hausswirth C, Argentin S, Bieuzen F, Le Meur Y, Couturier A, Brisswalter J. Endurance and
462 strength training effects on physiological and muscular parameters during prolonged
463 cycling. *J Electromyogr Kinesiol.* 2010;20(2):330-339.
464 13. Ronnestad BR, Hansen J, Hollan I, Ellefsen S. Strength training improves performance and
465 pedaling characteristics in elite cyclists. *Scand J Med Sci Sports.* 2015;25(1):e89-98.
466 14. Takaishi T, Sugiura T, Katayama K, et al. Changes in blood volume and oxygenation level in
467 a working muscle during a crank cycle. *Med Sci Sports Exerc.* 2002;34(3):520-528;
468 discussion 529.
469 15. Hansen EA, Ronnestad BR, Vegge G, Raastad T. Cyclists' improvement of pedaling efficacy
470 and performance after heavy strength training. *Int J Sports Physiol Perform.* 2012;7(4):313-
471 321.
472 16. Korff T, Romer LM, Mayhew I, Martin JC. Effect of pedaling technique on mechanical
473 effectiveness and efficiency in cyclists. *Med Sci Sports Exerc.* 2007;39(6):991-995.
474 17. Barclay CJ, Woledge RC, Curtin NA. Energy turnover for Ca2+ cycling in skeletal muscle. *J*
475 *Muscle Res Cell Motil.* 2007;28(4-5):259-274.
476 18. Walsh B, Howlett RA, Stary CM, Kindig CA, Hogan MC. Measurement of activation energy
477 and oxidative phosphorylation onset kinetics in isolated muscle fibers in the absence of
478 cross-bridge cycling. *Am J Physiol Regul Integr Comp Physiol.* 2006;290(6):R1707-1713.
479 19. Gunnarsson TP, Christensen PM, Thomassen M, Nielsen LR, Bangsbo J. Effect of intensified
480 training on muscle ion kinetics, fatigue development, and repeated short-term
481 performance in endurance-trained cyclists. *Am J Physiol Regul Integr Comp Physiol.*
482 2013;305(7):R811-821.
483 20. Glaister M, Stone MH, Stewart AM, Hughes M, Moir GL. The influence of recovery duration
484 on multiple sprint cycling performance. *J Strength Cond Res.* 2005;19(4):831-837.
485 21. Burnley M, Doust JH, Jones AM. Time required for the restoration of normal heavy exercise
486 VO2 kinetics following prior heavy exercise. *J Appl Physiol (1985).* 2006;101(5):1320-1327.
487 22. Passfield L, Doust JH. Changes in cycling efficiency and performance after endurance
488 exercise. *Med Sci Sports Exerc.* 2000;32(11):1935-1941.
489 23. De Pauw K, Roelands B, Cheung SS, de Geus B, Rietjens G, Meeusen R. Guidelines to classify
490 subject groups in sport-science research. *Int J Sports Physiol Perform.* 2013;8(2):111-122.
491 24. Ronnestad BR, Kojedal O, Losnegard T, Kvamme B, Raastad T. Effect of heavy strength
492 training on muscle thickness, strength, jump performance, and endurance performance in
493 well-trained Nordic Combined athletes. *Eur J Appl Physiol.* 2012;112(6):2341-2352.
494 25. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. *Can J Sport*
495 *Sci.* 1991;16(1):23-29.

496   26.   Noordhof DA, Skiba PF, de Koning JJ. Determining anaerobic capacity in sporting activities.
497   *Int J Sports Physiol Perform.* 2013;8(5):475-482.
498   27.   Konrad P. The ABC of EMG. Vol 1.4: Noraxon INC; 2006.
499   28.   Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in
500   sports medicine and exercise science. *Med Sci Sports Exerc.* 2009;41(1):3-13.
501   29.   Mullins AK, Annett LE, Drain JR, Kemp JG, Clark RA, Whyte DG. Lower limb kinematics and
502   physiological responses to prolonged load carriage in untrained individuals. *Ergonomics.*
503   2015;58(5):770-780.
504   30.   Hopker JG, Coleman DA, Gregson HC, et al. The influence of training status, age, and
505   muscle fiber type on cycling efficiency and endurance performance. *J Appl Physiol (1985).*
506   2013;115(5):723-729.
507   31.   Vanhatalo A, Black MI, DiMenna FJ, et al. The mechanistic bases of the power-time
508   relationship: muscle metabolic responses and relationships to muscle fibre type. *J Physiol.*
509   2016;594(15):4407-4423.
510   32.   Glaister M. Multiple sprint work : physiological responses, mechanisms of fatigue and the
511   influence of aerobic fitness. *Sports Med.* 2005;35(9):757-777.
512   33.   Gunnarsson TP, Brandt N, Fiorenza M, Hostrup M, Pilegaard H, Bangsbo J. Inclusion of
513   sprints in moderate intensity continuous training leads to muscle oxidative adaptations in
514   trained individuals. *Physiol Rep.* 2019;7(4):e13976.
515   34.   Sanderson DJ, Black A. The effect of prolonged cycling on pedal forces. *J Sports Sci.*
516   2003;21(3):191-199.
517   35.   Ettema G, Loras HW. Efficiency in cycling: a review. *Eur J Appl Physiol.* 2009;106(1):1-14.
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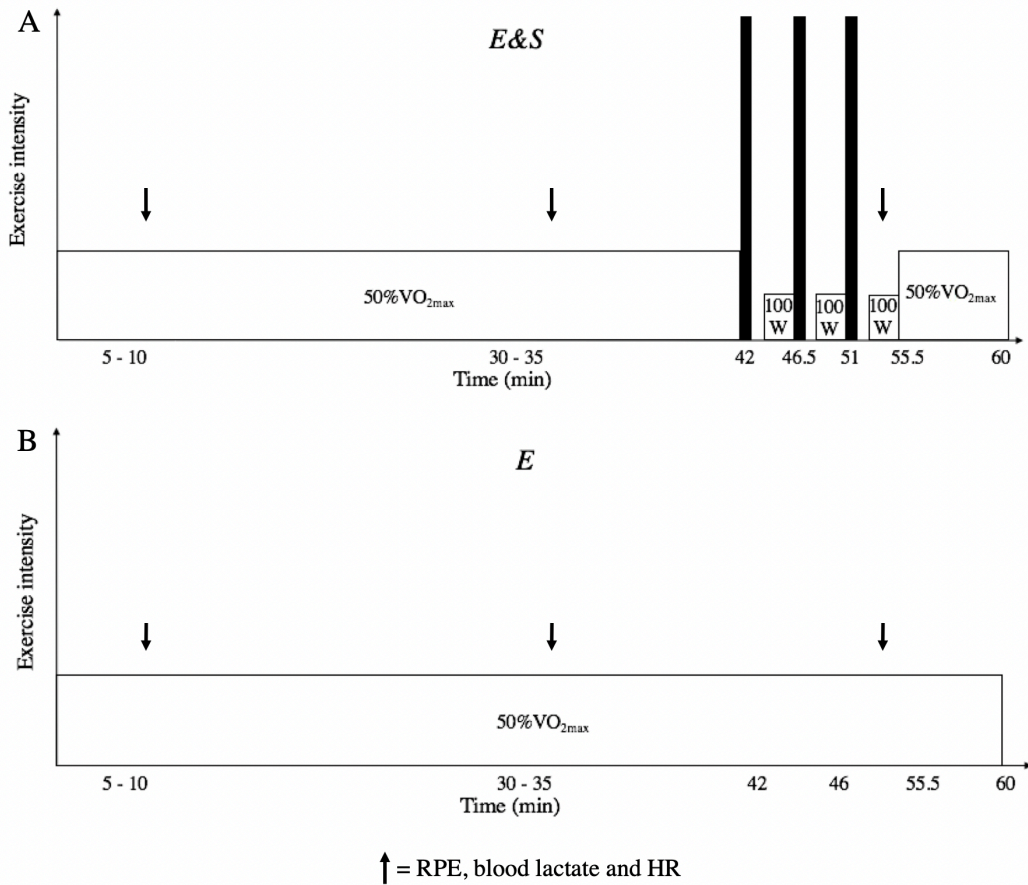
Figures

Figure 1: Experimental protocols. Panel A shows the endurance and sprint protocol (E&S) which was repeated three times for the first 3 h followed by 1 h of the E-protocol (panel B). Panel B show the endurance protocol (E) which was a work-matched endurance exercise for 4 h with no sprinting. Oxygen uptake (VO$_2$) and electromyography (EMG) was recorded for three periods during each hour (5-10 min, 30-35 min and 58-60 min, respectively). Black arrows indicate the time point at which rate of perceived exertion (RPE), blood lactate concentration [BLa⁻] and heart rate (HR) was registered.
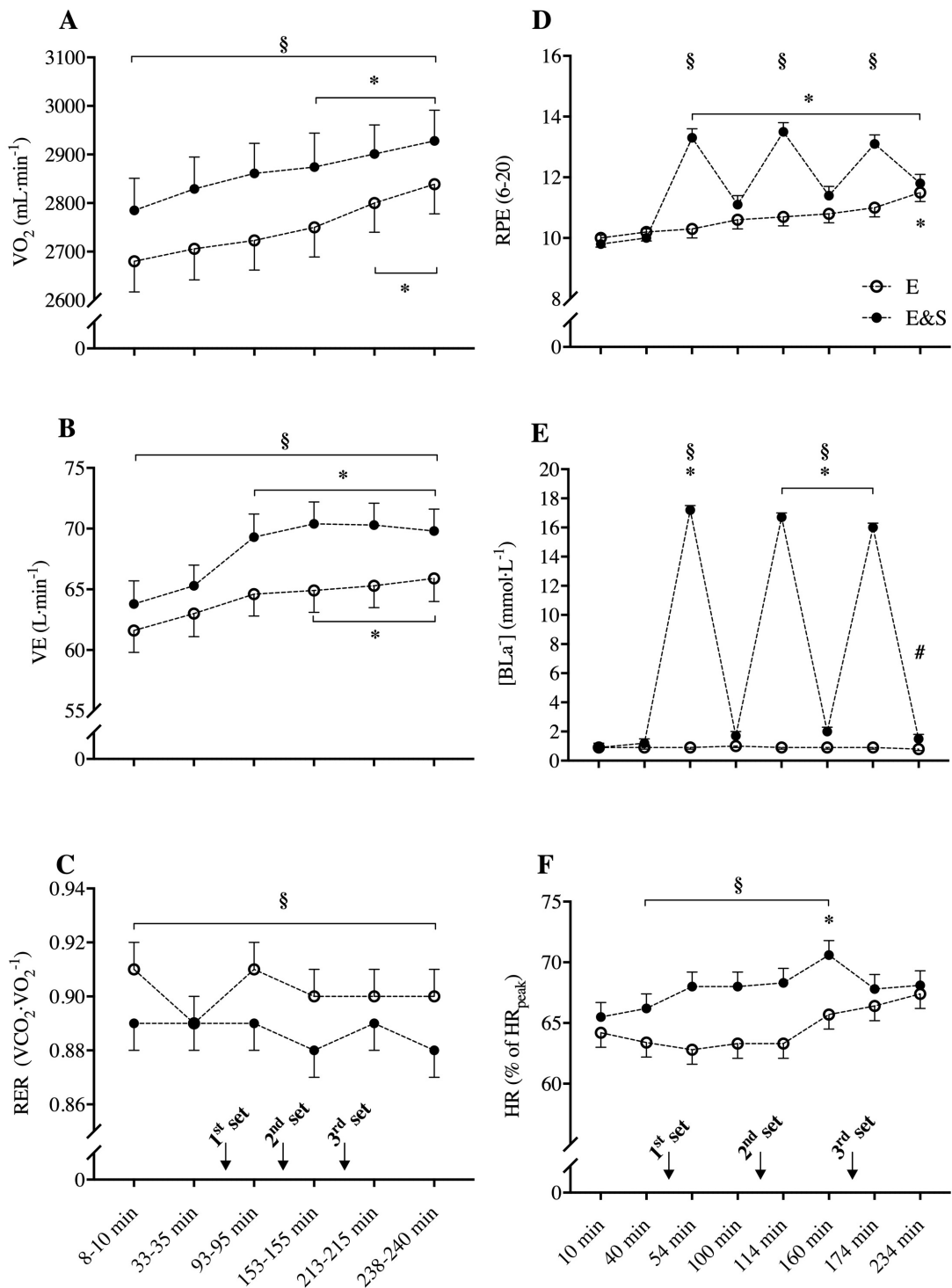
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*Figure 2: Panel A: Changes in oxygen consumption (VO₂), panel B: Respiratory exchange ratio
(RER), panel C: Ventilation (VE), panel D: Rate of perceived exertion (RPE) on a scale from 6-20,
panel E: Blood lactate concentration [BLa⁻] and panel F: Relative heart rate (%HR$_{peak}$) during 4 h
of exercise with 9 x 30 s sprint (E&S; •) or without sprints (E; o). Mean ± SE, n = 12, * indicates
significantly different (P<0.05) from baseline (1st h, 8-10 min), § indicates significant difference
P<0.05 between conditions, # indicates tendency to difference (P<0.1) between conditions.*

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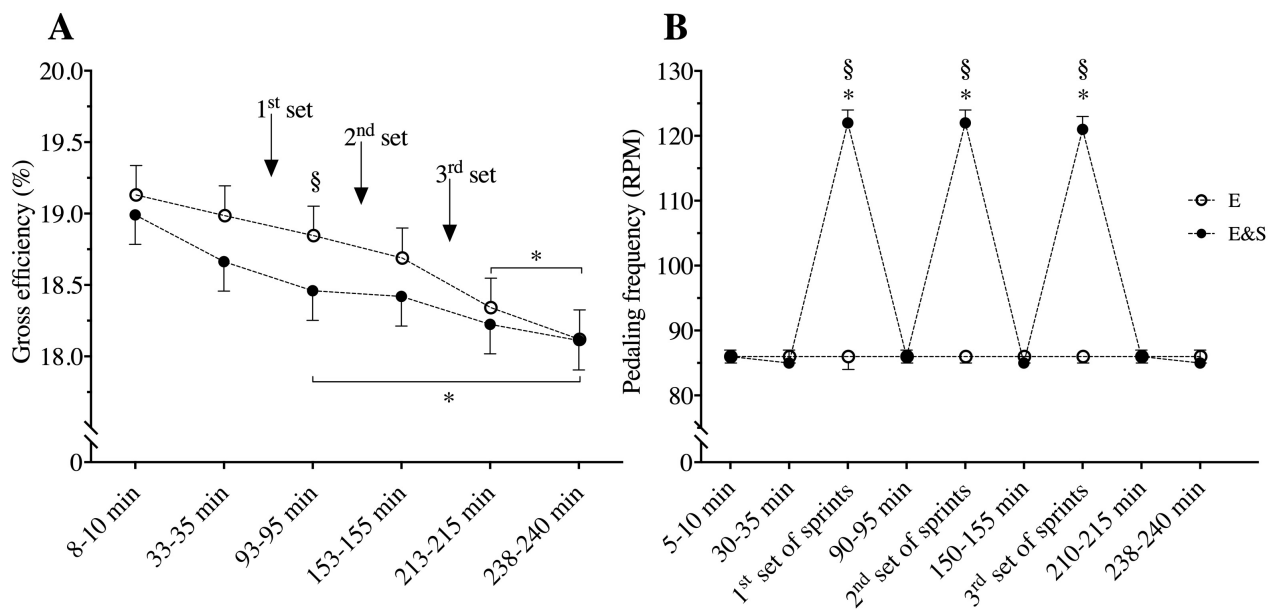
Figure 3: Panel A: Changes in gross efficiency measured in steady-state periods during 4 h of exercise with 9 x 30 s sprint (E&S; •) or without sprints (E; o). Arrows indicate time of 3 x 30 s sprint during E&S. Panel B: Pedaling frequency (RPM) in steady-state periods and during each set of 3 x 30 s sprints). Mean ± SE, n = 12, * indicates significantly different (P<0.05) from baseline (1st hr 8-10 min), § indicates significant difference (P<0.05) between conditions.
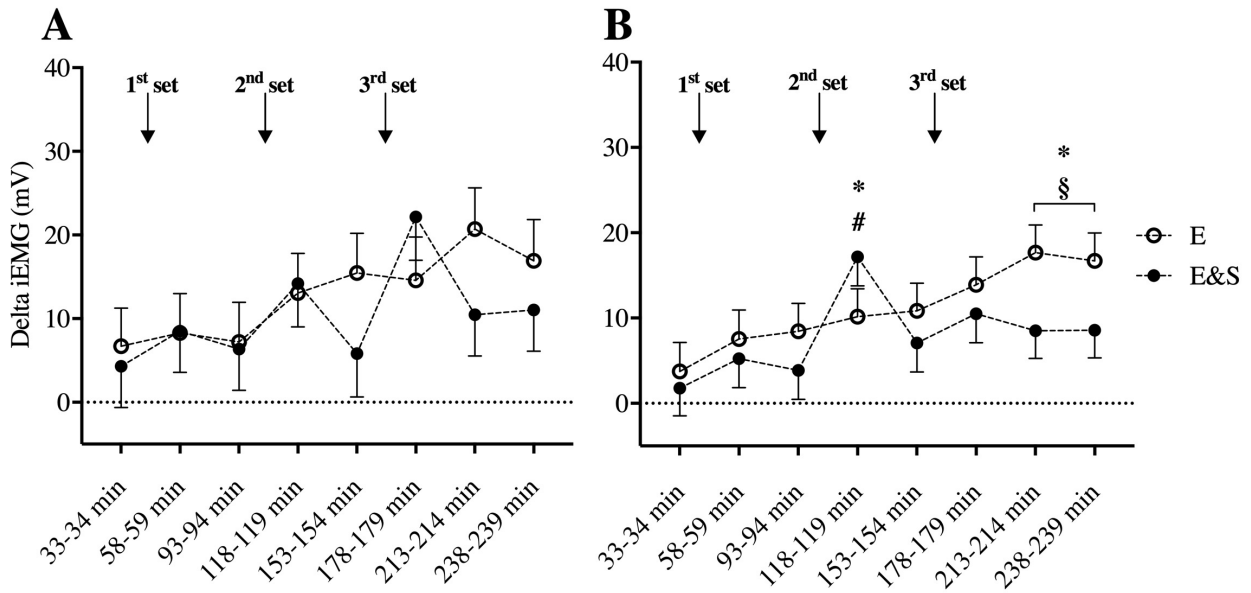
Figure 4: Absolute changes in integrated electromyography (iEMG) (mV) from baseline (1st h, 9-10 min) during 4 h of exercise with 9 x 30 s sprint (E&S; •) or without sprints (E; o) in A; Vastus Lateralis and B; Vastus Medialis. Filled markers represent E&S, open markers represent E. Arrows indicate time of sprint during E&S. Mean ± SE, n = 12, * indicates significantly different (P<0.05) from baseline (1st h, 9-10 min), § indicates significant difference (P<0.05) between conditions, # indicates tendency (P<0.1) to difference between conditions.
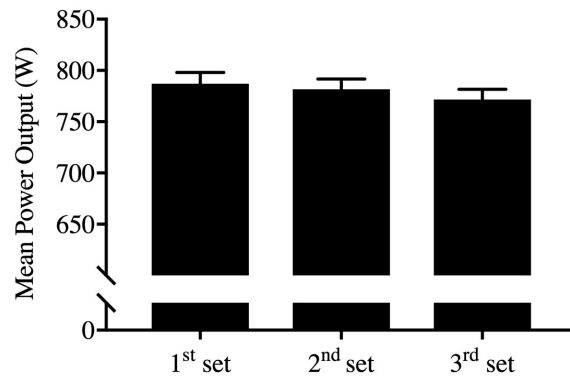
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627 *Figure 5: Mean power output of 3 sets of 3 repeated maximal 30-s sprints performed during E&S-*
628 *protocol. Each set was separated by 1 h of low-intensity cycling at a power equivalent to*
629 *~50%VO$_{2max}$ and each sprint was separated by 4 min recovery. All sprinting was started with a*
630 *pedaling frequency of 80 RPM. Mean ± SE, n = 12.*
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Tables

Table 1: Subject characteristics and physiological parameters of 12 elite male cyclists determined during a Wingate test, incremental lactate profile and incremental maximal exercise test. Values are mean ± SD

Body mass (kg)	76.1 ± 3.2
Height (cm)	183 ± 5
VO_{2max} (L·min^{-1})	5.57 ± 0.35
W_{max} (W)	477 ± 29
Peak power output (W)	1610 ± 235
Mean power output (W)	851 ± 64
Power output at 4 mmol·L^{-1} [BLa^{-1}] (W)	322 ± 40
VO_{2max} (mL·kg^{-1}·min^{-1})	73.4 ± 4.0
W_{max} (W·kg^{-1})	6.3 ± 0.3

VO_{2max}; Maximal oxygen consumption, W_{max}; Maximal power produced the last minute during incremental maximal test, PPO; Peak Power Output during a 30s all-out test.

702 *Table 2: Mechanical effectiveness and angle at which peak torque is obtained during a revolution*
703 *(degrees °) during 4 h of exercise with 9 x 30 s sprint (E&S) or without sprints (E) in steady-state*
704 *periods and during sprints. Power output was kept constant in E during the equivalent "sprint*
705 *period" in E&S, where a mean of the three 30-s sprints was calculated. Mean ± SE, n = 10, **
706 *indicates significantly different P<0.05 from baseline (1st h, 5-10min), § indicates significant*
707 *difference P<0.05 between conditions.*

		5-10 min	*30-35 min*	*Mean of sprint 1-3 /control*	*90-95 min*	*Mean of sprint 4-7 /control*	*150-155 min*	*Mean of sprint 8-10 /control*	*210-215 min*	*238-240 min*
E&S	Mechanical effectiveness (%)	73.7 ± 1.3	73.0 ± 1.0	96.4 ± 0.7 *§	72.5 ± 1.0	96.8 ± 0.6 *§	72.5 ± 1.1	96.6 ± 0.7 *§	71.5 ± 1.1	71.5 ± 1.0 *
	Angle of peak torque (degrees)	91.9 ± 1.2	92.1 ± 1.2	93.8 ± 3.3	91.5 ± 1.2	91.2 ± 3.2	91.9 ± 1.1	91.1 ± 2.6	91.6 ± 1.0	91.3 ± 0.9
E	Mechanical effectiveness (%)	72.9 ± 1.1	72.4 ± 1.0	72.5 ± 1.0	72.1 ± 1.1	71.8 ± 1.0	71.5 ± 1.0	71.3 ± 1.0	70.9 ± 1.0	71.5 ± 1.1
	Angle of peak torque (degrees)	93.1 ± 1.2	93.1 ± 1.1	92.7 ± 1.1	92.7 ± 1.1	92.2 ± 1.2	92.7 ± 1.0	92.7 ± 1.1	93.1 ± 1.3	92.2 ± 1.3

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