

# Muscle activation patterns in paralympic and novices hand cycling during incremental test

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## ABSTRACT

The few studies on muscle timing in hand cycling are difficult to classify in the motor skill acquirement due to their variety of methods and content. This study aims to replicate two existing studies and thus to extend and connect the current state of research concerning the muscle timing during synchronous pedalling. The activity on- and offset of biceps brachii, triceps brachii, posterior deltoid, anterior deltoid, upper trapezius, and pectoralis major were identified during an incremental test of an elite and three novice athletes. The results showed differences in inter-muscular coordination between the elite and novice hand cyclists. Although the distinction between active and inactive phases was already evident in novice data, the activation pattern of the elite athlete showed an even more precise differentiation between these two phases. These time windows remained stable even with increasing load accompanied only by changes in all signal amplitude, except for deltoid activity, which showed a later onset and offset with increasing load. Thus, in training of hand cyclist novices, the muscle timing concerning the duration and the crank position should be considered. In future studies, the effect of handicap severity and crank frequency must be studied in greater detail.

**Keywords:** Inter-muscle coordination; Electromyography; Motor control; Paralympic sports.

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## INTRODUCTION

The optimal timing of muscle activation in cycling movements is a crucial determinant to maintain an efficient ratio between the mechanical work and the metabolic cost (Blake, Champoux, & Wakeling, 2012; Hug, Bendahan, Le Fur, Cozzone, & Grélot, 2004). In particular, the activation and deactivation of muscles along a 360° synchronous pedal cycle concerning its antagonist's activity determine the economical transfer of muscle work into propulsive power. Here, the development of antagonistic dominance and the precise activation and deactivation of propulsive muscle work within a cyclic movement has been identified as a small-sized indicator of motor skill acquirement (Brueckner, Kiss, & Muehlbauer, 2018). Thus, the analysis of inter-muscular coordination in hand cycling is important to understand the development of motor skills and to develop effective motor control strategies in the range from Paralympic athletic elite performance (Perry & Burnfield, 2010) to able-bodied recreational sports (Quittmann, Abel, Albracht, & Strüder, 2019).

Science in hand cycling has to challenge the scarce availability of athletes, who usually vary, if handicapped, in classification type and performance level. Both variables impact the power output and motor control (Morriën, Taylor, & Hettinga, 2017). Previous research addressed this limitation by studying able-bodied participants (Litzenberger, Mally, & Sabo, 2015; Quittmann et al., 2018; Quittmann et al., 2019). Although able-bodied athletes are known to activate lower limb muscles during pedalling and, therefore, use different muscle activation strategies (Kouwijzer, Nooijen, van Breukelen, Janssen, & Groot, 2018), these findings contributed to the understanding of the requirements in para-cycling. Additionally, the few existing studies of muscle activity during hand cycling (Faupin, Gorce, Watelain, Meyer, & Thevenon, 2010; Litzenberger et al., 2015; Quittmann et al., 2018; Stone, Mason, Warner, & Goosey-Tolfrey, 2019) also vary in bike geometry, investigated muscles, workload protocol, post-processing, and individual research interests. Thus, generalization of the results as well as a direct comparison between the studies is often not possible. Here, this study aimed to bridge two similar studies to enable an evaluation of muscle activation strategies of elite cyclists against an extract of able-bodied subject data.

Lindschulten (2008) and Litzenberger (2015) analysed the electromyographic activity patterns of hand cycling. Lindschulten studied able-bodied participants, who were unfamiliar with hand cycling. The results included the onset, offset, and the range of activation of six main power-producing muscles. Lindschulten concluded that there is a need for reference data from top-level athletes to understand his results concerning the motor learning process since it remained unclear to which extent the activity of the biceps brachii during elbow extension is used to stabilize the arm or whether it represents an uneconomical activity at an early state of motor learning (Chapman, Vicenzino, Blanch, & Hodges, 2008; Milner, 2004; Sigward & Powers, 2006). Litzenberger et al. (2015) tried to clear this uncertainty. They examined the inter-muscular coordination of an elite athlete while varying the crank height and seat incline and found the supposed co-activation of the pectoralis major and the biceps brachii seems to result from an ineffective cranking. Nonetheless, the existing data from a single case study remains insufficient to provide reliable recommendations for defining motion guidance.

Additionally, analysing more elite athletes would have the potential to optimize movement and to develop more effective training strategies (Lindschulten, 2008). Thus, the study aimed to analyse the muscle activity timing during elite hand cycling. An additional analysis of able-bodied novices in this study should allow a comparison to Lindschulten's results (2008). Since there were already remarkable differences in the duration of activity between these studies, we hypothesized that there would be differences in onset, offset, and duration of muscle activity between elite and novice hand cyclists.

## MATERIALS AND METHODS

The study design and protocol were closely aligned with those of Lindschulten (2008) and Litzenberger (2015) to allow an integration of this study's results into the existing findings.

### Participants

One elite athlete and three novices were included in this study and were informed about the risks associated with the state of maximal exhaustion. The institute's local ethics committee previously approved the non-invasive study, according to the Declaration of Helsinki. All participants gave their written consent to participate.

The novice group consisted of three subjects (N1-3, Table 1) with no experience in hand cycling. The inclusion criteria included (1) the regular exercise of a top body demanding sport and (2) anthropometry that fits the bike. Persons with acute arm or shoulder injuries or similar complaints which would have prevented them from pedalling without symptoms were excluded. A test day at least three days before the study allowed all novice subjects (N1-3) to get familiarized with the handling of the hand bike. The athletic history of all subjects contained competitive, upper-body demanding sports such as handball and swimming.

Table 1. Overview of participant's data.

Participants	Age [years]	Weight [kg]	Power output in incremental test [P (W/kg BW)]	Rating of max perceived Exertion [6-20]	Cadence [rpm]
Novice 1 (N1)	23	60	100 (1.67)	19	67
Novice 2 (N2)	24	70	100 (1.43)	19	52
Novice 3 (N3)	22	73	100 (1.37)	20	66
Elite (E100)	52 ( $\pm 2$ )*	70	100 (1.43)	7	94
(E220)			220 (3.27)	19	85

Note.\* Due to the existing involvement of the athlete in international championships, the age of the subject at the time of the measurement was stated in only a defined range).

At the time of the study, the elite athlete (E1) was a member of the German Paralympic Team. He is classified as paraplegic with impairments corresponding to a complete lesion from T11 or below and no lower limb function (H4 class, (Union cycliste internationale [UCI], 2015)). The training volume was approximately 20.000 km/year. The maximal power output during the incremental test was 220 W (3.27 W/kg BW).

### Procedures

The study bike was a customized recumbent arm power bike (AP-bike) equipped with parallel cranks (length 170 mm) with a neutral grip position (Bressel, Bressel, Marquez, & Heise, 2001). The upper body position corresponded to the UCI requirements allowing "[...] a clear vision. As such, the horizontal [of his] eye line must be above the crank housing/crank set, when he is sitting with his hands on the handlebars facing forward at full extent, the tip of his shoulder blades in contact with the backrest and his head in contact with the headrest, when applicable" (UCI, 2015). This seating position corresponded to a racing position (Litzenberger et al., 2015) and was flatter than in Lindschulten's study.

The bike was mounted on a Cyclus2 ergometer (RBM electronic, Leipzig, Germany). The test protocol included an initial load of 40 W and a stepwise increase of 20 W every 5 min. Since, the novices were not able to maintain the cadence of about 90 rpm of the elite hand cyclist, they were allowed to choose their

frequency. Novices in Lindschulten's study, pedalled at a cadence of approximately 86 rpm, whereas Litzenberger et al. (Litzenberger et al., 2015) investigated 40 s trials at 130, 160 and 190W at 85 rpm. The participants were fixed to the seat with a hip belt, while the feet were not fixed on the tray to limit the power generating use of the lower extremities of the able-bodied athletes (Kouwijzer et al., 2018). Athletes were asked for their rating of perceived exertion (RPE) after 4 min within each step using Borg RPE 15-scale (Borg, 1998). The study protocol included a 10-minute warm-up prior test at a self-selected load.



Figure 1. The test set-up with the recumbent arm power bike mounted on the Cyclus2 ergometer. The crank position was defined along the horizontal 0-180° axis. A hip belt was used to stabilize the participants on the bike.

The muscle timing of biceps brachii (BIC), triceps brachii (TRI), posterior deltoid (DEP), anterior deltoid (DEA), upper trapezius (TRD) and pectoralis major (PEC) were analysed using electromyography (EMG, myon 320, prophysics, SUI; sample rate 1000Hz). The skin preparation and electrode placement (Ambu, Sensor N, Ag/AgCl) were done according to the SENIAM guidelines (Hermens, 1999). Data was recorded from the first 10 s of the last minute of each incremental step. Four magnetic switches detected the crank position at the positions 0° (foremost position), 90°, 180° and 270° (Figure 1). Intermediate crank positions were calculated using linear interpolation. The 0°-180° axis corresponded to the horizontal position of the bike. The post-processing of the EMG data included 10-500Hz bandpass filtering, rectification, and smoothing by using a moving average filter with a window size of 100ms (ProEMG V.2.1.3.4., prophysics, SUI). The signal-to-noise ratio was estimated to be > 35dB. According to Lindschulten (2008), muscle activity onset was defined at an onset threshold of > 15% of each pedal cycle's maximum muscle activity. Signals below this 15% threshold were defined as inactive phases. Deviations to Litenberger et al. (2008) who applied a > 30% threshold had to be accepted. The effect of the difference on the comparison between the studies will be discussed later. The analysis of at least 6 averaged pedal cycles from hand cycling data is reported to have a good reliability (interclass coefficient > 0.8) (Quittmann et al., 2019). Standard deviations of the individual activity durations and the relative duration of activity within the cycle were calculated. Additionally, the average root mean square (RMS) was calculated for all cycles within each trial. The variance of the RMS

is considered as a measure of movement variability. In this study, the standard deviation was calculated from 6 to 13 cycles, depending on the athletes' cadence.

EMG signals at a workload of 100 W were analysed for elite and novice cyclists. In Lindschulter's study, as well as in this study, this workload corresponded to the maximum novices' power output. For the elite cyclist, the analysis at 220 W was also performed to allow a comparison of activity at the maximal novices' exertion level (RPE 19-20) and to the results of Litzenberger et al. (2015), were the elite hand cyclists at a maximum workload of 190 W.

Inter-individual differences between activity and the duration of activities were tested using the non-parametric Kruskal-Wallis test and multiple comparisons with Bonferroni corrections. Effect sizes were calculated (Cohens  $d$ ) and categorized into strong ( $d \geq 0.8$ ), medium ( $0.8 < d \leq 0.5$ ) and weak effects ( $0.5 > d \geq 0.2$ ) (Cohen, 2013).

## RESULTS

Due to a technical defect of the EMG sensor at the DEP, the data was excluded from data analysis. Descriptive data of the analysed trials are listed in Table I, which includes the cadence, the maximal power output, and the maximal perceived exertion level of the athletes. Figure 2 shows the EMG signals from this study in the context of the current state of research, including the onset, offset, and range of muscle activation. The range of muscle activation is presented relative to the crank position.

Considering the activity level of the analysed muscles, down- and upward phases can be distinguished for both, elite and novice athletes. Downward rotation was accompanied by high activity of TRD and BIC and low activity of TRI, starting at approximately  $40^\circ$  before horizontal crank position ( $0^\circ$ ) and ending at about  $90^\circ$  crank position, where upward rotation begins. This second phase is marked by high activity of DEA and PEC, while the TRD and PEC activity declines. The activity overlap was less than  $5^\circ$  of BIC and TRI, and PEC and DEA activity at the shift from downward to upward crank rotation.

The visual comparison of elite and novice activation patterns revealed a shift of muscle activation relative to the crank position. Novices' muscle activation of TRD tended to start  $90^\circ$  later, whereas DEA was activated about  $45^\circ$  earlier (Figure 2). All novices showed a significantly more extended activation within the pedal cycles for TRI, DEA, and PEC, and in some cases, also for BIC and TRD when comparing with the elite athlete at the same workload of 100 W (Table II). The relative duration of activation was 26% (PEC, TRI) to 113% (DEA) longer for novices than for the elite athlete. The activity of the elite hand cyclist was therefore characterized by shorter activation phases, which resulted in a more precise separation of active and passive phases.

At maximum power output (RPE 19-20), novices' muscle activity lasted 7% (TRI) to 53% (DEA) longer than the elite athlete's activity (Figure 3). Only a few cases of individual differences between the participant's inactive part of the cycle were significant (Table II). While the novice reached their maximum workload at 100W, the elite athlete achieved at the maximal RPE a higher power output (220 W) accompanied by shorter activation of his muscles.

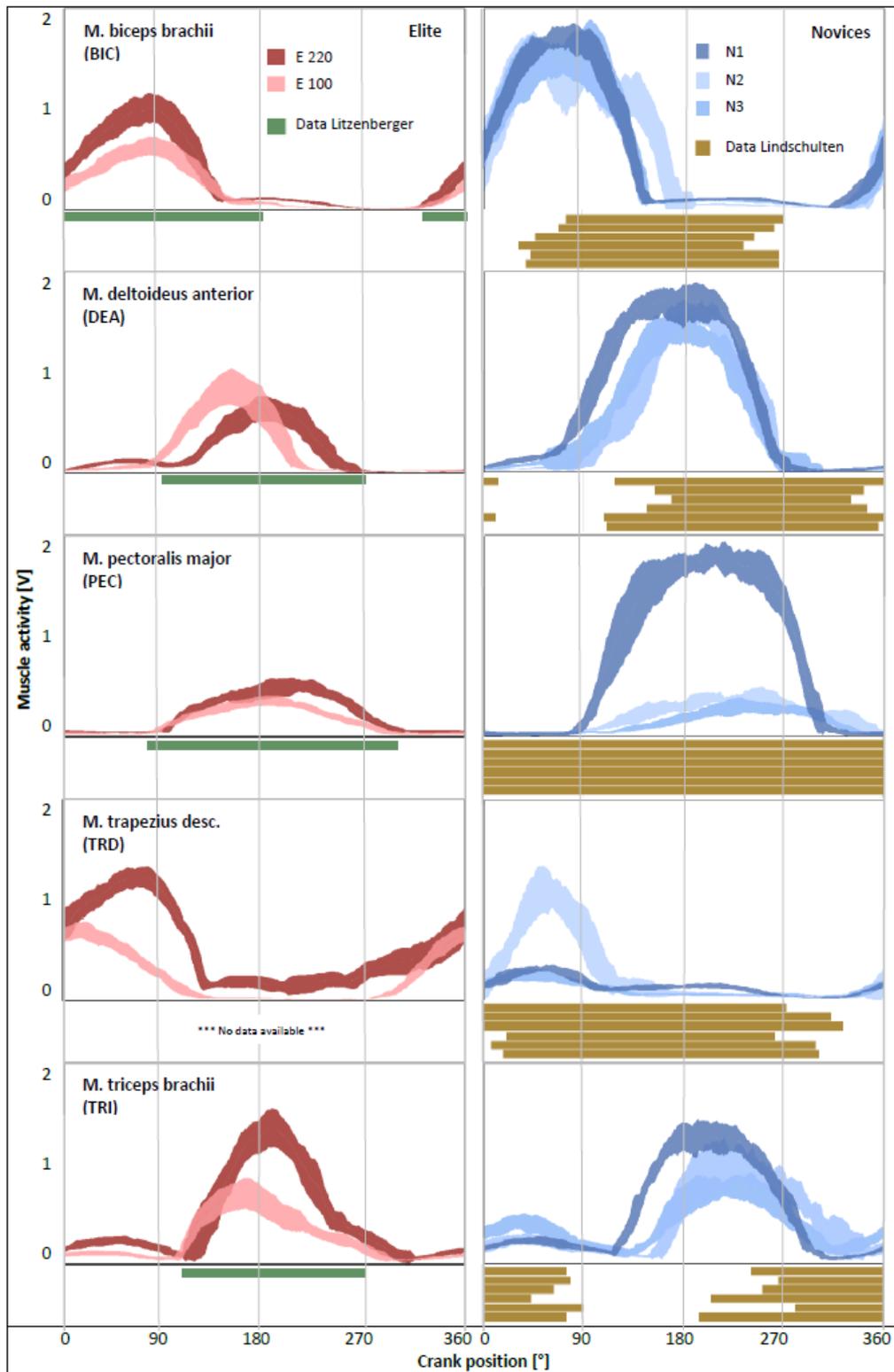


Figure 2. EMG Signals from elite hand cyclist at 100 and 220 W (E100, E220) and three novices (N1-N3) at 100 W each. Signals are normalized to the crank position and averaged over 6-12 cycles depending on cadence. For details see Table I. Horizontal bars present the results of Litzenberger (2015) and Lindschulten (2008) who analysed similar athletes.

Table 2. Overview of the analysed cycles during the incremental test of the elite hand cyclist at 100 and 220 W (E100 and E220) and the three novices (N1-N3) at 100 W each.

	Athlete	Cycles	Average RMS <sup>1</sup>		Total cycle duration [s]	Active part of total cycle				
			RMS ± SD [V]	[%SD]		[%]	Cohen's d for difference to <sup>2</sup>			
							E100	E220	N1	N2
BIC	E100	13	0.51 ± 0.07	7	0.64 ± 0.01	34 ± 3				
	E220	12	0.77 ± 0.08	8	0.70 ± 0.01	39 ± 2	1.96			
	N1	8	1.20 ± 0.13	13	0.89 ± 0.02	37 ± 3	1.00	-0.78		
	N2	7	1.18 ± 0.12	12	1.16 ± 0.02	47 ± 4	3.68*	2.53	2.83*	
	N3	9	1.43 ± 0.10	10	0.91 ± 0.01	47 ± 3	4.33*	3.14	3.33*	0.00
TRI	E100	13	0.53 ± 0.10	10	0.64 ± 0.01	34 ± 3				
	E220	12	0.95 ± 0.13	13	0.70 ± 0.01	40 ± 5	1.46			
	N1	8	0.61 ± 0.06	6	0.89 ± 0.02	41 ± 2	2.75*	0.26		
	N2	7	0.81 ± 0.07	7	1.16 ± 0.02	43 ± 5	2.18*	0.60	0.53	
	N3	9	1.05 ± 0.07	7	0.91 ± 0.01	43 ± 3	3.00*	0.73	0.78	0.00
DEA	E100	13	0.77 ± 0.12	12	0.64 ± 0.01	23 ± 3				
	E220	12	0.53 ± 0.09	9	0.70 ± 0.01	30 ± 4	1.98			
	N1	8	1.03 ± 0.08	8	0.89 ± 0.02	46 ± 4	6.51*	4.00*		
	N2	6	1.19 ± 0.06	6	1.16 ± 0.02	41 ± 2	7.06*	3.48	-1.58	
	N3	10	1.44 ± 0.10	10	0.91 ± 0.01	51 ± 4	7.92*	5.25*	1.25	3.16
TRD	E100	12	0.56 ± 0.05	5	0.64 ± 0.01	35 ± 3				
	E220	11	0.80 ± 0.10	10	0.70 ± 0.01	61 ± 6	5.48*			
	N1	8	0.21 ± 0.03	3	0.89 ± 0.02	75 ± 4	11.31*	2.75		
	N2	7	0.80 ± 0.12	12	1.16 ± 0.02	29 ± 8	-0.99	-4.53*	-7.27*	
	N3	8	0.20 ± 0.01	1	0.91 ± 0.01	37 ± 2	0.78	-5.37	-12.02*	1.37
PEC	E100	12	0.29 ± 0.03	3	0.64 ± 0.01	44 ± 2				
	E220	13	0.40 ± 0.05	5	0.70 ± 0.01	50 ± 2	3.00*			
	N1	8	0.23 ± 0.03	3	0.89 ± 0.02	51 ± 3	2.75*	0.39		
	N2	7	0.29 ± 0.03	3	1.16 ± 0.02	64 ± 6	4.47*	3.13*	2.74	
	N3	9	1.54 ± 0.05	5	0.91 ± 0.01	52 ± 5	2.10*	0.53	0.24	-2.17

Note. <sup>1</sup>RMS values show the variance over the analysed cycles. <sup>2</sup>Differences between active part of cycles were tested by Kruskal-Wallis test and multiple comparisons with Bonferroni corrections \*:  $p < .05$ .

Throughout the muscle activity of the novices, a plateau at the highest level of activation is consistently visible for all muscles and athletes. This prolonged activation resulted in significantly longer activation phases compared to the elite hand cyclist. Due to the lack of amplitude normalization, a direct comparison of the activity levels is not possible. The standard variation of the RMS values of the cycles within each trial ranged from 1-13%.

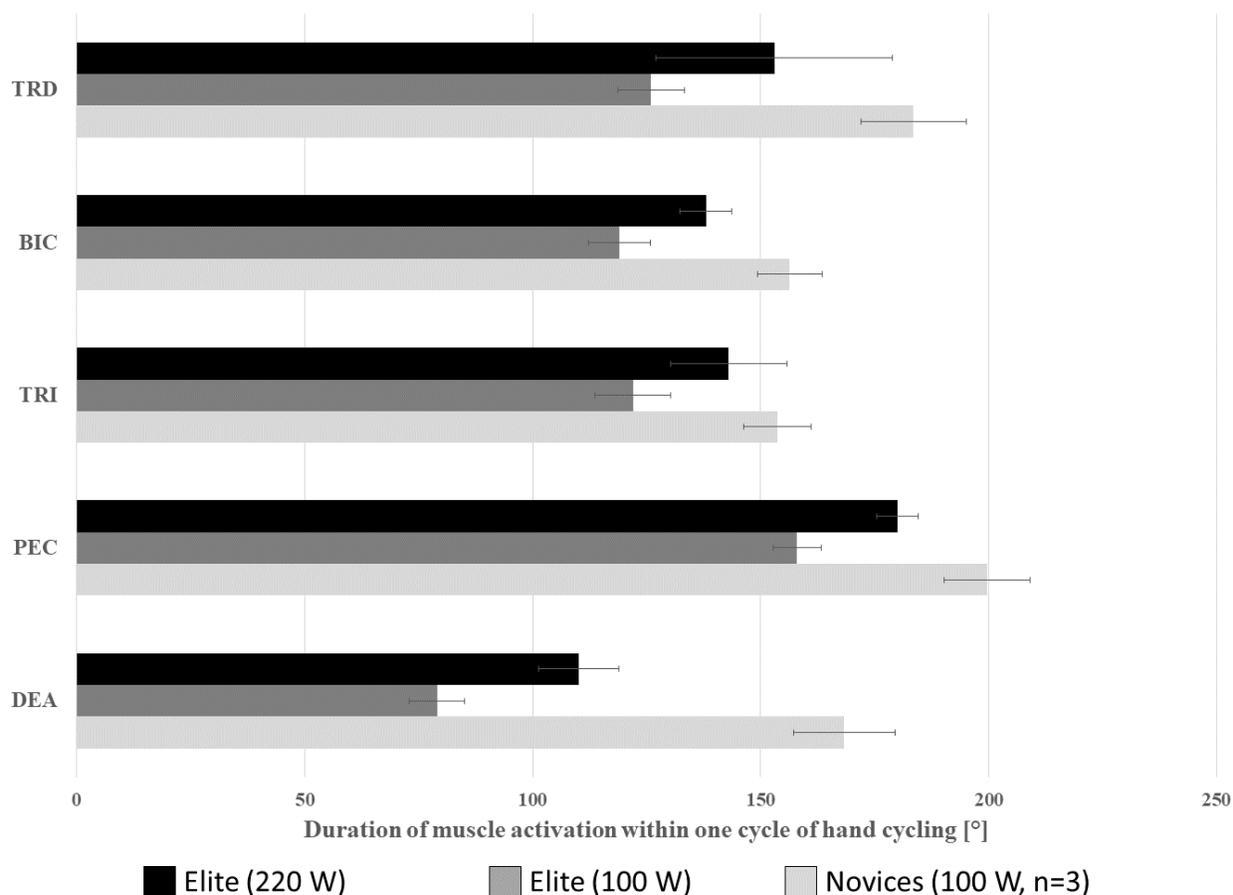


Figure 3. Duration of muscle activation of five muscles in relation to 360°-full pedal cycle (see Figure 1). Presented are average sizes (M) and standard deviation (SD) of cycle segments with activated muscle of 6-13 cycles.

## DISCUSSION

### *Muscle activation in novices' hand cycling*

In this study, the analysis of three hand cycling novices resulted in muscle activity patterns that showed a clear identification of active and passive phases consistently, allowing a separation of push and pull movements along the 360° pedal cycle. The duration of the activation conformed to the activity of only TRI, DEA and BIC analysed by Lindschulten, while all five analysed muscles' activity duration confirm novice's EMG analyses of Faupin et al. (2010) and Quittmann et al. (2019). However, the onset timing agreed only with Quittmann et al. (2019), whereas the comparison to Faupin et al. (2010) and Lindschulten (2008) revealed an onset shifted towards the 0° position. This result indicates and confirms that the more upright backrest position of 45° on the bike (Faupin et al., 2010) and the test apparatus (Lindschulten, 2008) leads to a different muscle timing. For recumbent racing bikes, the activation time windows, including onset, offset, and duration of activation, can therefore, be confirmed for biceps brachii, triceps brachii, anterior deltoid, upper trapezius and pectoralis major in novices' hand cycling as presented in Figure 2.

Lindschulten reported a permanent activity of the PEC. He assumed that the novices could have stabilized their position in the bike by exerting pressure along the axis of rotation. Indeed, this would result in a

permanent activation of the PEC and confirm the findings of Zerby et al. (1994) regarding muscle co-activation during hand cycling as well. In this study, a stabilizing effect of the PEC, as described by Zerby et al. (1994), Marciello et al. (1995) and Lindschulten (2008), could not be confirmed in this study. These results suggest that the co-activation is more likely to be the result of ineffective movement at a very early state of specific motor learning.

### ***Muscle activation in elite hand cycling***

The inter-muscular coordination in elite hand cycling showed a further shortening of the active phase and, thus, a more prolonged recovery phase when compared to the novice cyclists. The athlete responded to the increased load with increased activation, a lower cadence, and longer muscle activity durations. This increase, however, still happened within a shorter time window than in the novice hand cyclists (Figure 3). Although, due to the lack of amplitude normalization to a maximum muscle effort, the increase of the muscular action potential (MAP) corresponds to the results of Quittmann et al. (2019), who found a correlation between increased MAP and muscle efforts. Although the activation levels during the inactive phase remained below 15% of the maximum activation, the muscles in the inactive phase show a significantly increased level of activation.

Furthermore, the lack of co-activation of the antagonist muscles, shows the ability of the athlete to control unnecessary degrees of freedom of movement (Bernstein, 1984) even under higher loads. This improved muscle economy led the athlete not to waste energy by activating muscles that are not required for propulsive efforts. Contrary, DEA activity showed a later onset, while peak muscle activity decreased. Since fatigue can prevent the athlete from explosive muscle activation during cyclic motion (O'Bryan, Brown, Billaut, & Rouffet, 2014), it could explain why the athlete switched from an explosive drive to a continuous drive. Here, further analysis needs to study the loading rate of amplitude-normalized EMG data.

Comparing the results of Litzenberger et al. (2015) with this study, both, differences and similarities in the muscle activity of BIC, TRI, PEC, and TRD were found: The average duration of the active phases is significantly shorter for the athlete in this study (BIC =  $-14^\circ$ , TRI =  $-33^\circ$ , TRD =  $-22^\circ$  and PEC =  $-24^\circ$ ). Considering the standard deviation from the individual cycles of the athlete in this study (SD range =  $6-25^\circ$ , Figure 3), the meaning of this average shortening seems less significant. Unfortunately, a comparison with our data was not possible, since variances from Litzenberger's study were not available. The average onsets were all detected earlier in this study (BIC =  $-71^\circ$ , TRI =  $-27^\circ$ , TRD =  $-61^\circ$ , and PEC =  $-50^\circ$ ). One reason is certainly that Litzenberger et al. (2015) have defined the onset threshold as 30% of the maximum muscle activity. Similar to Lindschulten's study (2008), an 15% threshold was used in this study. This lower threshold leads to an earlier onset and a later offset. The magnitude of this shift depends on the incline in activity and the maximum amplitude during the cycle. According to studies that compared different methods for onset detection (Bonato, D'Alessio, & Knaflitz, 1998; DeLuca, 1997; Merlo, Farina, & Merletti, 2003) in cyclic movements, a clockwise shift between  $27^\circ$  and  $71^\circ$  cannot be explained by this methodological difference alone, especially since the offset was also detected earlier in this study and thus led to a shortening of the activity duration. Another explanation could be the different classifications of the athletes (H3 vs. H4), the crank frequency, or seat tilt. These parameters can only be listed speculatively, since the details of the athlete in the Litzenberger study are not available for a systematic comparison.

Data analysis shows that only evaluating muscle activity levels above a predefined threshold (Figure 3) is not enough to understand the intra-muscular coordination during hand cycling adequately. Figure 2 shows that with increasing workload, the amplitude of the muscle signal increases. This increase is not detected due to the threshold of 15% of the local maximum, but is subject to a noticeable change, which could indicate

the loss of relaxation during the passive phases. The choice of the threshold concept should be questioned in further studies, even if this, in turn, leads to restrictions in the comparability with these data.

### ***Muscle activity in a motor learning process***

The assumption that the similar activation patterns of elite athletes from Litzenberger et al. (2015) and this study represent the optimal interaction of the biceps brachii, triceps brachii, anterior deltoid, upper trapezius and pectoralis major in synchronous pedalling indeed corresponds to a substantial reduction in the complexity of the biomechanical aspects of this exercise. Nevertheless, by further classifying the activity patterns of the novices into an early stage of motor learning, some features of the learning process can already be derived.

The passive phase and thus, the recovery phase within the cycle can be achieved by avoiding extended phases of maximum activation. The training must prepare TRD, BIC, and TRI to maintain the main propulsive efforts with increasing workload. Due to the lack of normalization of the amplitudes, this statement cannot be derived directly from the results of this study. However, the development of the MAP in the incremental test shows a similar increase in MAP, as in the study of Quittmann et al. (2019).

The analysis of the standard deviation of the RMS does not seem to be a clear indicator of an advanced learning process. Here the magnitudes of the standard deviations could not be systematically assigned to the elite athlete or the novices. However, the question of whether a low or pronounced movement variability would be optimal.

### ***Limitations of the study***

The authors are aware of some limitations of this study. According to Litzenberger (2015) and Lindschulten (2008), the lower backrest incline has a negligible effect on muscle activation timing. Instead, it led to a phase shift of the bottom centre position towards 0°. The results of Arnet et al. (2014) support this correlation, although they did not analyse EMG data. Their analysis of different seat positions during hand cycling showed no significant effects of backrest incline on cycling efficacy measured with power output. Reviewed by Hug and Dorel (2009), it is additionally known that the myo-electrical signal depends strongly on the cadence. Although this was standardized within a range at 90 rpm for the elite athlete, there is no information about what the athlete's self-selected frequency would have been. A further limitation involves the comparison of a spinal cord injured person with healthy athletes. It is known that the use of the lower extremities leads to a gain in power (Kouwijzer et al., 2018) and, thus, can lead to a performance advantage. It is unknown to what extent this ability has an effect on the muscle timing of the muscles, and it cannot be answered in this study. In this study, the fixation of the feet was omitted to limit this uncertainty.

## **CONCLUSIONS**

The results indicate a clear difference in inter-muscular coordination between the elite and novice hand cyclists. Although drive phases are already evident in the novices' analysis, the activation pattern of the elite athletes shows an even more precise delineation of the active and inactive phases. These time windows remained stable even with increasing load, and the only changes observed were in signal amplitude, except for DEA activity. Here, a clear right shift was shown with increasing load. Thus, the training of novice hand cyclists should consider the length and location of active time windows to achieve a more efficient propulsion.

## AUTHOR CONTRIBUTIONS

SK contributed significantly to the derivation of the question and drafted the manuscript. Both authors were equally involved in the development of the research design, the data collection and the evaluation of the results.

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## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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