

# Coordinating arms and legs on a hybrid rehabilitation tricycle: the metabolic benefit of asymmetrical compared to symmetrical arm movements

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## Abstract

**Purpose** The most commonly used propulsion method for handcycling is moving the arms symmetrically. Previous studies indicated that during outdoor handcycling symmetrical arm movements are more efficient. During locomotor movements, however, arm movements are performed asymmetrically in combination with leg movements. We questioned which combination of arm and leg movements is more efficient during combined arm and leg cycling for stationary use.

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**Methods** Twenty-five able-bodied adults performed eight submaximal tests of 6 min on a hybrid handcycle at three incremental gears during four different conditions ('arms only' and 'arms & legs' with arms symmetrical and asymmetrical). Oxygen uptake ( $\text{VO}_2$ ), heart rate (HR) and Borg score (Borg) were assessed.

**Results** Increasing workload resulted in significant increases in  $\text{VO}_2$  (16 W:  $13.0 \pm 2.4 \text{ ml kg}^{-1} \text{ min}^{-1}$ , 31 W:  $14.5 \pm 2.9$ , 49 W:  $15.5 \pm 2.8$ ;  $p < 0.001$ ) and Borg (16 W:  $7.7 \pm 1.7$  points, 31 W:  $8.6 \pm 1.9$ , 49 W:  $9.5 \pm 1.9$ ;  $p < 0.001$ ). During 'arms only', no differences were found in exercise intensity between symmetrical and asymmetrical movements. Contrarily, during 'arms & legs', both  $\text{VO}_2$  ( $p < 0.001$ ) and Borg ( $p = 0.001$ ) were significantly lower for the asymmetrical ( $\text{VO}_2$ :  $13.8 \pm 2.6 \text{ ml kg}^{-1} \text{ min}^{-1}$ , Borg:  $8.1 \pm 1.6$  points) compared to the symmetrical condition ( $\text{VO}_2$ :  $14.9 \pm 2.8$ , Borg:  $9.1 \pm 2.0$ ).

**Conclusions** Results indicated that asymmetrical arm movements, especially in combination with leg movements, represented the most efficient condition on a stationary hybrid handcycle. The current results suggest that neural energy costs are lower when moving in the preferred (asymmetrical) coordination when no steering is required. These findings may have implications for stationary arm & leg cycling rehabilitation and tricycle adaptations in patients with spinal cord injury.

**Keywords** Arm cycling · Metabolics · Spinal cord injury · Central pattern generator · Interlimb · Coordination

## Abbreviations

ANOVA Analysis of variance  
HR Heart rate  
RER Respiratory exchange ratio  
SCI Spinal cord injury  
 $\text{VO}_2$  Oxygen uptake

## Introduction

In spinal cord injury (SCI) patients, coordinating arms in combination with legs has been suggested to promote lower limb movements, and could, therefore, be beneficial for gait rehabilitation (Behrman and Harkema 2000). Furthermore, it has been found that both passively imposed and active arm motion positively influenced the locomotor-like muscle activity in the legs in a group of SCI patients in which the neural connections in the spinal cord between regions controlling upper and lower limbs were (at least partially) preserved (i.e. cervical incomplete SCI) (Kawashima et al. 2008). On the other hand, upper limb movements had no effect when the neural connections in the spinal cord between regions controlling upper and lower limbs were lost (i.e. thoracic complete SCI) (Kawashima et al. 2008). These studies clearly indicate that combining arm and leg movements in rehabilitation of (incomplete) SCI patients could yield additional benefits. This is especially the case since some SCI patients present with residual function of (one of) the legs, and since it is possible to combine arm cycling with functional electrical stimulation of the legs (Heesterbeek et al. 2005).

Conventional rehabilitation after SCI is primarily focused on regaining functional independence, often by increasing muscle strength above the level of the lesion. To reach this goal, upper limb muscle strength in SCI patients is mostly trained by arm cranking (on a fixed stationary upper limb ergometer), handcycling (on an outdoor handcycle) or wheelchair training (Bougenot et al. 2003; Jacobs 2009; Valent et al. 2008, 2010). Previous studies on upper limb cranking and cycling in SCI patients have focused on the differences between symmetrical (i.e. synchronous) and asymmetrical (i.e. asynchronous) arm movements. When moving the arms symmetrically, left and right arms move in flexion and extension in unison, while for asymmetrical movements, one arm is in flexion and the other is in extension. Results in arm cycling studies should be divided into studies using arm cranks and studies using a handcycle, since these tasks differ on an essential element, i.e. steering. Studies on arm cranking usually report asymmetrical arm movements to be both physiologically and mechanically more efficient compared to symmetrical arm movements (Goosey-Tolfrey and Sindall 2007; Mossberg et al. 1999; Hopman et al. 1995). This means that oxygen uptake was found to be lower, while gross mechanical efficiency was higher at specific workload intensities for asymmetrical arm movements. On the other hand, in studies using a handcycle, symmetrical arm cycling was reported to be physiologically and mechanically more efficient than asymmetrical cycling (Abel et al. 2003; van der Woude et al. 2000, 2008; Dallmeijer et al. 2004; Bafghi et al. 2008). It was suggested in previous research that the advantage of

symmetrical handcycling is caused by the effective use of trunk movements, i.e. moving the trunk forward and backward to increase power generation through the upper limbs, while this is not effective during asymmetrical handcycling (van der Woude et al. 2000; Dallmeijer et al. 2004). However, the results of a study by Faupin et al. (2011) do not agree with this suggestion, since they did not find a difference of flexion–extension of the trunk between asymmetrical and symmetrical handcycling (in wheelchairs with backrest angle of 45° or 85°) (Faupin et al. 2011). The differences in results between arm cranking and handcycling studies are obviously related to the fact that during arm cranking no steering is required, in contrast to handcycling, i.e. it is easier to steer when moving the arms symmetrically (van der Woude et al. 2000). Related is also that trunk muscle activity is increased and more continuous during asymmetrical compared to symmetrical handcycling, resulting in higher rotation and lateral flexion of the trunk to propel and stabilize the participant and the steering (Bafghi et al. 2008; Faupin et al. 2011). This increase in muscle activity to stabilize steering direction appears to increase the energy cost during asymmetrical handcycling (Dallmeijer et al. 2004; van der Woude et al. 2008). A possible explanation for the preference for asymmetrical arm movements, in situations where steering and trunk movements are not required or allowed, is that for locomotor movements, such as walking and crawling, the arms usually move alternately as well (Wannier et al. 2001). This preference for asymmetrical coordination for locomotion is a trait that humans have in common with (quadrupedal) animals (Duysens and Van de Crommert 1998; Dietz 2003; Zehr et al. 2009). It has been suggested in literature that the similarity in inter-limb coordination during locomotion between these species is apparent due to a similar neural locomotor network, possibly because of a shared ancestral lineage (Dominici et al. 2011; Dietz 2002; Zehr and Duysens 2004). An interesting characteristic of our neural network for locomotion is that the movements of the limbs of the upper and lower girdle influence each other. For instance, it was found that reflexes elicited by stimulating nerves in the leg evoked reflex responses in arm muscles (Dietz et al. 2001). More importantly, these responses were more pronounced during gait as compared to standing and sitting. Other reflex studies reported that passive flexion–extension movements at the elbow were sufficient to augment soleus H-reflex amplitudes (Hiraoka and Nagata 1999), while, in contrast, active arm swing or arm cycling reduced them (Hiraoka and Iwata 2006; Knikou 2007). These (reflex) studies clearly indicate that leg movements and arm movements directly influence each other at remote girdles, which strongly suggests that the interconnections between girdles are important, especially during locomotion (Zehr and Duysens 2004). Additionally, several studies on neurologically intact

persons have indicated the potential benefit of combining upper limb movements with lower limbs movements on a recumbent stepper, a stepping machine that couples asymmetrical arm movements to asymmetrical leg movements (resembling human locomotor movements) (Huang and Ferris 2004; Kao and Ferris 2005; Ferris et al. 2006). It was demonstrated that when upper limb exertion increased, lower limb muscle activation increased proportionally, even though lower limbs were relaxing (both when the asymmetrical arms movements were coupled or uncoupled to the leg movements) (Huang and Ferris 2004). Furthermore, when increasing the frequency of the upper limb movements, lower limb muscle activation increased as well (Kao and Ferris 2005). These studies clearly indicate the facilitating effect of upper limb movements on neuromuscular recruitment of the lower limbs, and highlight the possibility of reduced neural energy costs when activating the preferred asymmetrical pattern (possibly due to reduced active inhibition at spinal cord level). However, the effect of the type of arm coordination in combination with leg movements on oxygen uptake and exertion has not yet been explored.

Therefore, it is of great clinical interest to examine the differential effect of two types of arm coordination during combined arm and leg cycling on exercise intensity, since it may lead to adaptations of the crank setup of hybrid rehabilitation tricycles used by persons with neuromotor disorders (such as multiple sclerosis, amyotrophic lateral sclerosis, and SCI) depending on their active control of the upper and lower limbs. Based on previous literature concerning neural locomotor control, we hypothesized that an additional facilitating effect would be apparent when the arms and legs are coordinated in the same asymmetrical way as during normal walking. Therefore, in this initial study, we investigated whether, in able-bodied participants, asymmetrical arm cycling is more efficient compared to symmetrical arm cycling in conditions with and without asymmetrical leg cycling.

## Methods

### Participants

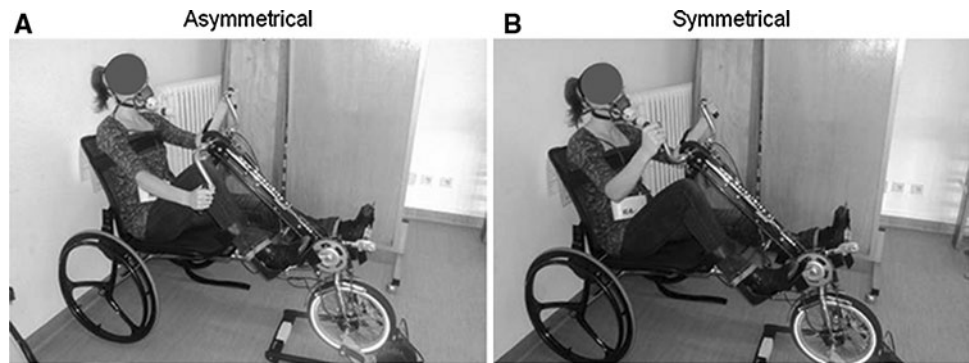
A total of 25 able-bodied participants (male/female 11/14; age  $30.5 \pm 12.1$  years; height  $1.76 \pm 0.10$  m; weight  $69.0 \pm 9.5$  kg) with no reported history of any musculoskeletal or neurological impairments were included in the study. None of the participants had previous experience with handcycling. All experiments were approved by the local ethical committee (“Commissie Medische Ethiek van de Universitaire Ziekenhuizen Leuven”) and were performed with the written informed consent of the participants in accordance with the Declaration of Helsinki.

### Experimental setup and protocol

The hybrid handcycle used in this study was a prototype of the BerkelBike (BerkelBike BV, The Netherlands). It is a tricycle that can be used for outdoor activities as well as stationary training, and is propelled by the arms, the legs or by the combination of arms and legs. The movements of the legs were mechanically coupled to the movements of the arms, i.e. when cycling with the arms, the legs automatically follow (while this was not true for the opposite; i.e. the legs did not drive the arms). Both arms and legs, however, contributed to the externally achieved workload. To use the BerkelBike in a controlled laboratory setting, the front wheel of the tricycle was mounted on an air-braked ergometer and the steering mechanism was fixed (preventing sideways movements). This prototype of the BerkelBike allowed changing the arm crank from a symmetrical position to an asymmetrical position (Fig. 1). Gear diameters (and tooth counts) for the arm driven gear and leg driven gear were 7 cm (17), and 7 cm (17), respectively. These gears result in a 1:1 arm to leg revolution frequency. The crank length for the arms was 15 cm, while that of the legs was 12.5 cm. The transmission from the leg cranks to the wheel hub was 40:16. Three gear ratios of the additional hub gear were used (0.64, 0.75, and 0.85) for the three levels of resistance used in the protocol (see below; 16, 31, and 49 W, respectively). The hybrid handcycle was individually adjusted to have an appropriate distance between the arm cranks and the trunk, and to provide a comfortable seating position. The foot rests, seat and arm crank handlebar could be adjusted in both height and distance. To counteract trunk movements as much as possible, the participant’s trunk was fixed to the back of the hybrid cycle using a Velcro belt.

The participants performed a total of eight submaximal tests, with each test taking 6 min (Hol et al. 2007). Six tests were performed using both the arms and legs for propulsion (‘arms & legs’), and the remaining two tests using only the arms for propulsion (‘arms only’; with the legs disconnected from the tricycle and the feet placed on the floor). During ‘arms only’ cycling, the participants were asked to place the feet on the floor since this resembles handcycling and arm cranking the most (i.e. in literature, participants were sitting in wheelchairs with their feet on the rests). These tests allowed for the comparison of measures of exercise intensity between symmetrical and asymmetrical arm movements to propel the BerkelBike, and the effect of the combination of using the arms and legs together. The six ‘arms and legs’ tests comprised three levels of resistance [16 W (gear 2), 31 W (gear 3), and 49 W (gear 4)] for the two hand positions (symmetrical and asymmetrical; Fig. 1). The two ‘arms only’ tests were performed at 49 W (gear 4), either with symmetrical

**Fig. 1** Experimental setup. Experimental setup of stationary ‘arms & legs’ cycling with the asymmetrical (a) and symmetrical (b) hand position



or asymmetrical arm movements. The order of the eight tests was pseudo-randomized (i.e. counterbalanced model) to control for carry-over and learning effects. The participants were required to follow a cadence of 63 revolutions per minute in all tests indicated by a metronome. Before each test, all participants were allowed to practice cycling to get acquainted with the required resistance and cadence. The resting heart rate of each participant was measured in a seated position before the start of the tests. In between two tests, there was a period of rest of about 15 min. The participants were allowed to proceed with the next test if their heart rate, measured after the 15 min of rest, was less than ten percent above their resting heart rate.

#### Outcome measures

The participants' heart rate (HR) was measured with a polar heart rate monitor (Kempele, Finland). Oxygen uptake [ $\text{VO}_2$  ( $\text{ml kg}^{-1} \text{min}^{-1}$ )] and the respiratory exchange ratio (RER) were measured during each test with a breath by breath, portable gas analysis system (K4b2; Cosmed, Rome, Italy). The mean  $\text{VO}_2$  and RER were calculated over the last 3 min of each 6-min test to assure physiological steady-state. After each test, the participants were asked to rate their perceived exertion of the test with the Borg scale. The 15-grade Borg scale is a tool that helps the participant to indicate fatigue by giving a score from 6 to 20 after each test (Borg 1982).

#### Statistics

A repeated measures ANOVA was used to compare the outcome parameters ( $\text{VO}_2$ , HR, and Borg) between symmetrical and asymmetrical ‘arms & legs’ cycling. Repeated measures factors included ‘Phase’ (symmetrical or asymmetrical) and ‘Resistance level’ (16, 31, 49 W). Similarly, for ‘arms only’ cycling, symmetrical and asymmetrical arm cycling were compared using a repeated measures ANOVA with ‘Phase’ as a factor. Tukey's post hoc comparisons

were systematically applied, and  $\alpha = 0.05$  was used to establish statistical significance. Statistica 8.0 (StatSoft Inc., OK, USA) was used for the statistical analyses.

## Results

### Symmetrical versus asymmetrical ‘arms and legs’ cycling

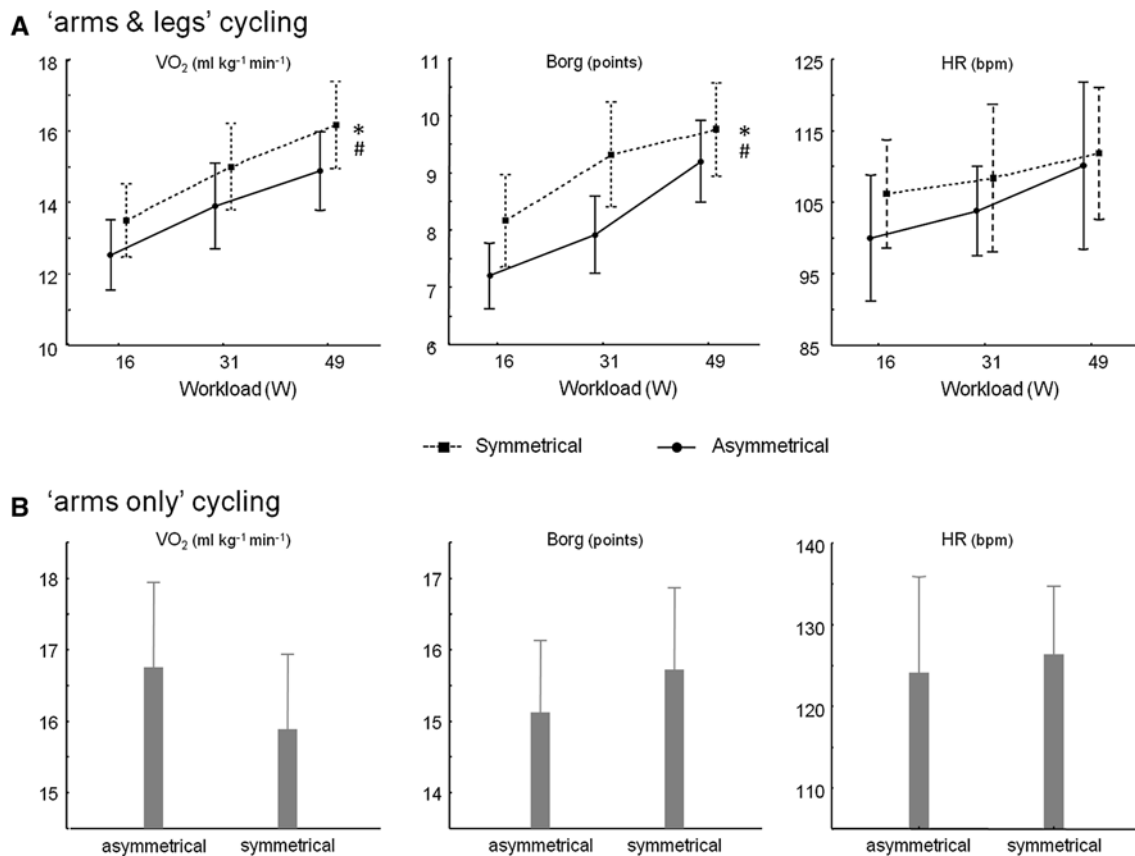
A significant main effect of Phase was found for  $\text{VO}_2$  ( $F = 17.64$ ,  $p = 0.0003$ ) and Borg ( $F = 13.12$ ,  $p = 0.0014$ ). A lower  $\text{VO}_2$  and Borg scores were found for the asymmetrical compared to the symmetrical hand position (see Fig. 2a). This was confirmed by the post hoc analysis ( $\text{VO}_2$ :  $p = 0.0005$  and Borg:  $p = 0.0015$ ). No significant difference in HR between the two conditions was found ( $F = 1.05$ ,  $p = 0.32$ ).

As expected, when the level of resistance increased, all measures of exercise intensity increased for the symmetrical as well as for the asymmetrical hand position (see Fig. 2a). This was revealed by a significant main effect of resistance level for both  $\text{VO}_2$  ( $F = 64.25$ ,  $p < 0.0001$ ) and Borg scores ( $F = 33.96$ ,  $p < 0.0001$ ), while the main effect of resistance level did not reach significance for HR ( $F = 2.53$ ,  $p = 0.11$ ). Tukey's post hoc analysis revealed that  $\text{VO}_2$  and Borg scores were significantly lower for 16 W versus 31 W ( $\text{VO}_2$ :  $p = 0.0001$  and Borg:  $p = 0.0004$ ), and 31 W versus 49 W ( $\text{VO}_2$ :  $p = 0.0001$  and Borg:  $p = 0.0009$ ).

No significant Phase \* Resistance level interaction was found for any of the variables ( $\text{VO}_2$ :  $F = 0.25$ ,  $p = 0.78$ ; Borg:  $F = 2.49$ ,  $p = 0.094$ ; HR:  $F = 0.23$ ,  $p = 0.80$ ).

### Symmetrical versus asymmetrical ‘arms only’ cycling

No significant differences between the symmetrical and asymmetrical hand position were found for any measure of exercise intensity (Borg:  $F = 2.84$ ,  $p = 0.10$ ;  $\text{VO}_2$ :  $F = 2.71$ ,  $p = 0.11$ ; HR:  $F = 0.17$ ,  $p = 0.69$ ; see Fig. 2b).



**Fig. 2** Comparison of the outcome measures between the conditions. **a** The mean values and standard deviations are provided for oxygen uptake (VO<sub>2</sub>; **a left**), Borg score (Borg; **a middle**) and heart rate (HR; **a right**) in able-bodied participants ( $n = 25$ ) during 'arms & legs' cycling with the asymmetrical hand position (full line and circles) and symmetrical hand position (dotted line and squares) for the different resistance levels. A significant effect of Phase (\*) and resistance level (#) was found for VO<sub>2</sub> and Borg. **b** The mean values and stand-

ard deviations are shown for oxygen uptake (VO<sub>2</sub>; **b left**), Borg score (Borg; **b middle**) and heart rate (HR; **b right**) in able-bodied participants ( $n = 25$ ) during 'arms only' cycling with the asymmetrical hand position and symmetrical hand position at the same resistance level. Although VO<sub>2</sub> seemed to be higher for the asymmetrical hand position, and conversely the Borg score appeared higher for the symmetrical hand position, no statistically significant differences were found

## Discussion

The current study demonstrates that, in healthy adults, the asymmetrical hand position results in more attenuated oxygen uptake than the symmetrical hand position during stationary 'arms & legs' cycling. Measures of exercise intensity (i.e. oxygen uptake and level of perceived exertion) were significantly lower for asymmetrical arm cycling combined with leg cycling compared to symmetrical arm cycling combined with leg cycling (for the different levels of resistance). This suggests that, with the same amount of effort, people will be able to ride further distances (i.e. for a longer period) when they are 'arms & legs' cycling stationary using the asymmetrical hand position.

In 'arms only' cycling, we did not find any significant differences in measures of exercise intensity between symmetrical and asymmetrical hand positions. In contrast, previous arm cranking studies observed higher energy

demands in symmetrical than in asymmetrical arm movements (Goosey-Tolfrey and Sindall 2007; Mossberg et al. 1999; Hopman et al. 1995). In these previous arm cranking studies, different protocols with different power output levels and durations were applied, which hinder direct comparisons, but, in general, reported energy demands in those studies were lower (Goosey-Tolfrey and Sindall 2007; Mossberg et al. 1999). Additionally, the differences in results could be due to the different setup, i.e. in the current study a hybrid handcycle was adapted for an experimental laboratory protocol, while other studies used arm crank ergometers. Furthermore, in the current study, the participant's trunk was stabilized to the back of the chair to control for trunk movements.

In the current study, the beneficial (metabolic) effect of asymmetrical hand position was only manifested when arm cycling was combined with leg cycling (i.e. oxygen uptake was significantly reduced during 'arms & legs' cycling



compared to ‘arms only’ cycling with the asymmetrical hand position; see Fig. 2b). This clear beneficial effect was not apparent for the symmetrical hand position. These results can be explained by the proposition that asymmetrical arm (and leg) movements are the preferred type of coordination for locomotion in humans. The increase in oxygen uptake due to ‘arms & legs’ cycling with asymmetrical arm movements could be caused by a physiological mechanism underlying neural interlimb coordination (e.g. neural energy costs are lower when moving in the preferred coordination), or an increase in biomechanical efficiency (e.g. less co-contraction needed in the trunk muscles), or a combination. The current results strengthen observations from previous studies that suggest that there is a facilitating coupling between upper and lower limb locomotor movements (Ferris et al. 2006; Huang and Ferris 2004; Kao and Ferris 2005). A recent study by de Kam et al. (2013) confirmed this notion since they found that active arm movements increased leg muscle activity during recumbent stepping (de Kam et al. 2013). The authors indicate that this facilitating effect was most likely of neuromuscular origin, since the arms are not needed for postural control or weight-bearing during recumbent stepping. In this respect, their study is in agreement with the current study, except that de Kam et al. (2013) did not find a significant difference in facilitating effect on leg muscle activation between symmetrical and asymmetrical arm movements. The authors did indicate that some trends were apparent in their Arms\*Condition interactions, and the lack of significance is likely caused by their small sample size ( $n = 10$ ). Nevertheless, combined with results of previous studies in able-bodied participants (Huang and Ferris 2004; Kao and Ferris 2005; Ferris et al. 2006), the results of the current study clearly indicate a preference for combining arm movements with leg movements and an advantage for using the asymmetrical arm coordination. Neurophysiological results from other motor control literature support the contention that the preferred coordination pattern is expected using reciprocating arm action. For instance, in a study by Carroll et al. (2005), an enhanced pattern of cutaneous reflex modulation was shown during asymmetrical arm cycling compared to symmetrical arm cycling, which suggests that the pattern generating activity is enhanced when the arms are moving asymmetrically (Carroll et al. 2005).

This neural interlimb facilitation might have implications for locomotor rehabilitation programs in persons with neuromotor disorders (such as multiple sclerosis, amyotrophic lateral sclerosis, and SCI) and might lead to adaptations to their outdoor tricycles. A previous study already indicated that arm movements positively influenced leg muscle activity in SCI patients in which the neural connections between regions controlling upper and lower limbs were preserved (Kawashima et al. 2008). Therefore, it

seems preferential to choose the type of tricycle and stationary ergometer rehabilitation in function of the type/height of the lesion. For instance, a person with a cervical incomplete SCI could benefit from the asymmetrical arm cycling combined with leg cycling (especially if there is some residual function in the legs). On the other hand, a person with a thoracic complete SCI might benefit more from using symmetrical arm cycling (possibly combined with leg cycling using functional electrical stimulation), since then trunk movements could improve their symmetrical arm movement performance (van der Woude et al. 2000).

A limitation that should be taken into account is that asymmetrical arm cycling hinders steering (van der Woude et al. 2000, 2008; Bafghi et al. 2008). It is expected that the hindering of steering for asymmetrical arm movements [as found for handcycling (i.e. ‘arms only’ cycling)] would also affect the combined ‘arms & legs’ cycling. Therefore, asymmetrical arm movements could be encouraged for indoor (stationary) but not necessarily for outdoor tricycle training. In addition, it should be further examined whether the results in able-bodied participants can be reproduced in persons with varying types of SCI or other neuromotor disorders. In persons with neuromotor disorders, it would be of particular interest to differentiate between the power produced by the legs and the arms as well. Another limitation to keep in mind when interpreting the current results is that energy supply during some exercise conditions might have been partially anaerobic. During ‘arms & legs’ cycling, this is very unlikely since we found low RER values ( $\leq 0.91$ ). For ‘arms only’ cycling, we cannot exclude this possibility completely since RER values above 1 and Borg scores above 15 were observed in some participants. We, therefore, did not compare the  $\text{VO}_2$  of ‘arms & legs’ cycling with the  $\text{VO}_2$  of ‘arms only’ cycling. Nevertheless, this does not influence the interpretation of our results that there is no difference between asymmetrical and symmetrical hand positions for stationary ‘arms only’ cycling, while there is a difference for stationary ‘arms & legs’ cycling. This is especially the case since we observed similar RER values in the symmetrical and asymmetrical hand positions for each condition (suggesting that the anaerobic component is comparable for both hand positions,  $p = 0.21$  and  $p = 0.15$ ). Furthermore, even though stationary ‘arms only’ cycling appeared as a more exerting exercise, all participants were able to maintain it for 6 min. The RER may have been influenced by hyperventilation, especially when breathing frequency would have been coupled to arm frequency. Such coupling is theoretically more likely to occur during symmetrical than during asymmetrical arm movements. However, we only found a small difference in breathing frequency between the two phases (symmetrical 25.92 breaths/min  $\pm$  5.18 versus asymmetrical

$25.16 \pm 5.34$ ,  $p = 0.42$ ). Moreover, 79.5 % of the cases showed a ratio of ‘arm frequency/breathing frequency’ that deviated more than 5 % from the nearest integer. This suggests that breathing frequency was not coupled to arm frequency for both conditions, and has not influenced  $\text{VO}_2$ .

## Conclusions

The combination of the asymmetrical hand position with stationary ‘arms & legs’ cycling was found to be more efficient in terms of measures of exercise intensity compared to symmetrical stationary ‘arms & legs’ cycling. These results are in agreement with the hypothesis of a facilitating effect between locomotor lower limb and upper limb movements resulting from neural intergirdle coupling pathways. Thus, it is potentially valuable to integrate asymmetrical arm movements when combined with leg cycling in the stationary locomotor rehabilitation and handcycle ergometry, as well as to account for choosing the most appropriate tri-cycle adjustments in persons with neuromotor pathology (for instance in patients with SCI: depending on the type and level of SCI).

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**Conflict of interest** The authors declare that they have no conflict of interest.

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