The Interaction of Fatigue and Potentiation Following an Acute Bout of Unilateral Squats

Samantha K. Andrews, Jesse M. Horodyski, Daniel A. MacLeod, Joseph Whitten and David G. Behm

School of Human Kinetics and Recreation, Memorial University, St. John’s, Newfoundland, Canada

Abstract

A prior conditioning resistance exercise can augment subsequent performance of the affected muscles due to the effects of post-activation potentiation (PAP). The non-local muscle fatigue literature has illustrated the global neural effects of unilateral fatigue. However, no studies have examined the possibility of acute non-local performance enhancements. The objective of the study was to provide a conditioning stimulus in an attempt to potentiate the subsequent jump performance of the affected limb and determine if there were performance changes in the contra-lateral limb. Using a randomized allocation, 14 subjects (6 females, 8 males) completed three conditions on separate days: 1) unilateral, dominant leg, Bulgarian split squat protocol with testing of the exercised leg, 2) unilateral, dominant leg, Bulgarian split squat protocol with testing of the contra-lateral, non-exercised leg and 3) control session with testing of the non-dominant leg. Pre- and post-testing consisted of countermovement (CMJ) and drop jumps (DJ). The exercised leg exhibited CMJ height increases of 3.5% (p = 0.008; d = 0.28), 4.0% (p = 0.011; d = 0.33) and 3.2% (p = 0.013; d = 0.26) at 1, 5, and 10 min post-intervention respectively. The contra-lateral CMJ height had 2.0% (p = 0.034; d = 0.18), 1.2% (p = 0.2; d = 0.12), and 2.1% (p = 0.05; d = 0.17) deficits at 1, 5, and 10 min post-intervention respectively. Similar relative results were found for DJ measures or control CMJ measures. The findings suggest that PAP effects were likely predominant for the exercised leg whereas the conditioning exercise provided trivial magnitude although statistically significant neural impairments for the contra-lateral limb.

Key words: Cross-education, post-activation potentiation, countermovement jump, drop jump.

Introduction

Cross-education, which is the occurrence of positive training adaptations in an untrained limb following training of the contra-lateral limb was first reported by Scripture et al. in 1894 (Scripture, 1894). Whereas the cross-education literature is extensive and relatively consistent (Hellebrandt, 1951; Munn et al., 2004; Lee et al., 2007), the acute effects of crossover fatigue or non-local muscle fatigue (NLMF) lacks such a broad base of literature and presents more inconsistent findings (Halperin et al., 2015). Halperin et al. (2015) in their NLMF review reported that NLMF occurs more consistently with the lower rather than the upper body, isometric and cyclical fatiguing protocols provide a greater incidence and magnitude of NLMF compared to stretch-shortening cycle type activities, and single discrete contractions were less likely to present NLMF deficits than repetitive or prolonged contractions. Multi-joint global tasks such as balance also demonstrated mixed results with both no NLMF effects (Arora et al., 2015) as well as impairments (Lima et al., 2014; Paillard et al., 2010). Thus there is considerable conflict in the NLMF literature and further insight is needed. Fatigue measures have not been the only variable examined with crossover or non-local type studies.

Non-local effects have also been reported with stretching, with increased range of motion (ROM) of contralateral quadriceps following unilateral quadriceps stretching (Chaouachi et al., 2015) and enhanced ROM of the upper (shoulder flexion) and lower (hip flexion) limbs after stretching the lower and upper body respectively (Behm et al., 2016). Studies investigating non-local effects of stretching have reported either no effect (Behm et al., 2016; Chaouachi et al., 2015) or impairments (Lima et al., 2014; Marchetti et al., 2014) in the force or power performance of a non-stretched muscle group. The increased ROM has been attributed to muscle afferents that inhibit corticospinal motor pathways (Amann et al., 2013), diminishing central drive to the working muscle and potentially to the non-exercised muscles as well (Amann et al., 2013), resulting in a more relaxed non-local muscle. Hence, publications examining non-local muscle effects (fatigue and stretching) have primarily focused on the possibility of reduced central drive or force and power impairments. However, is it possible for non-local muscle transfer effects to provide acute facilitation of muscle performance?

Prior conditioning exercises with appropriate intensities and volumes can induce post-activation potentiation (PAP). PAP involves both peripheral (muscle) (Sale, 2002; Sweeney et al., 1993; Szczesna et al., 2002; Tillin and Bishop, 2009) and central (neural) (Behm, 2004; Sale, 2002; Tillin and Bishop, 2009) mechanisms. Whereas the PAP muscle component induces myosin light chain (MLC) phosphorylation (Sweeney et al., 1993) increasing the rate constant for crossbridge attachment (Houston et al., 1990), the PAP neural component can facilitate the increased recruitment of higher threshold motor units (Behm, 2004; Sale, 2002) and reduce transmission failure (Tillin and Bishop, 2009). For example, cortical motor-evoked potential facilitation has been reported following short duration (5 s), submaximal intensity contractions (50%) of the thenar muscles (Balbi et al., 2002). At the spinal level, Gullich and Schmidtbileicher (1996) attributed the short-term increase in explosive force following a few maximal voluntary contractions (MVC) to an improved neuromuscular activation. Prior contractions may

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provide motoneuron facilitation from both supraspinal and afferent input contributing to improved force production (Behm, 2004; Behm et al., 2004). Studies examining evidence for PAP have only investigated the exercised muscle groups (Hodgson et al., 2005; Sale, 2002). It is as yet unknown whether a unilateral conditioning exercise can induce performance enhancements of the contralateral limb.

Hence the objective of the study was to investigate if a unilateral, resistance training type, conditioning exercise session would elicit a non-local (crossover) facilitation of jump performance. It was hypothesized that performing a non-fatiguing single leg squat protocol with the dominant leg would result in a transient increase in vertical jump performance in the exercised and contralateral non-exercised leg.

**Methods**

**Participants**

Eight male (21.3 ± 1.8 years, 80.4 ± 11.8 kg, 1.77 ± 0.05 m) and six female university athletes (21.2 ± 0.4 years, 63.8 ± 3.1 kg, 1.68 ± 0.08 m) volunteered for this study. All participants were recruited through convenience sampling, targeting sports that require explosive lower limb capabilities (i.e. basketball, volleyball, soccer). The athletes had at least two years of resistance training experience and were familiar with the exercise equipment used during the study. Participants were required to be free of injury at the time of the study and have had no prior lower limb surgery. They were asked to refrain from training for 24 hours prior to testing and not indulge in alcohol, nicotine or caffeinated drinks at least 3 hours prior to testing. All participants were informed of the procedures and risks involved in the study, and a written consent was obtained before commencing the study. The Health Research Ethics Authority (HREA #16.003) approved the study.

**Experimental design**

Using a randomized allocation, participants completed three conditions on separate days: 1) unilateral, dominant leg, Bulgarian split squat protocol with testing of the exercised leg, 2) unilateral, dominant leg, Bulgarian split squat protocol with testing of the contralateral, non-exercised (non-dominant) leg and 3) control session with testing of the non-dominant leg. Pre- and post-testing consisted of countermovement jump (CMJ) height and drop jumps (DJ) with variables including DJ height, contact time and reactive strength index.

**Familiarization Session**

The familiarization session involved informing participants about the procedures, equipment, experimental protocols and obtaining consent. Background (i.e. previous injuries, years of resistance training experience and sport involvement) and anthropometric information was then collected from each subject. The dominant leg was ascertained by determining the leg used to kick a soccer ball. The technique and mechanics of a rear foot elevated, split squat (Bulgarian split squat) was reviewed. To ensure a level of standardization, participants rested their non-dominant leg (back foot) on an elevated step. The base of their patella determined the height of the step. A piece of clear hospital tape was placed on the ground, orthogonal to the step platform, to provide a guide for a narrow base for each participant. The participant then measured three-foot lengths away from the platform to standardize the placement of their dominant leg (front foot). A second piece of tape was placed on the ground to mark this spot. A complete squat was characterized by a 90° angle at both knee joints in the down phase of the squat (Figure 1). Each squat repetition (eccentric and concentric phases) was a duration of 5s. Participant 1 repetition maximum (RM) for this exercise was then estimated based on repetitions of submaximal resistance (LeSuer 1997). Participants did not perform more than 3 sets to estimate their 1RM. This predicted 1RM was then used to determine their 50%, 70%, and 90% 1RM weights.

**Interventions**

The three aforementioned sessions (1. unilateral, dominant leg, Bulgarian split squat protocol with testing of the exercised leg, 2. unilateral, dominant leg, Bulgarian split squat protocol with testing of the contralateral, non-exercised (non-dominant) leg and 3. control session with testing of the non-dominant leg) were performed with at least 48 hours of recovery between each session. Participants began each session with a standardized 5 min aerobic warm-up on a cycle ergometer at 1kp and maintaining a pace of 60-70 rpm. The subjects then completed the unilateral squat protocol intervention, consisting of various repetitions of Bulgarian split squats performed with the dominant leg. Initially, participants completed 5 sequential squats at 50% 1RM, followed by a 3 min rest period. The participant then performed 2 sequential squats at 70% 1RM, followed by a 3 min rest period. Finally, the participant completed 1 squat at 90% 1RM. The higher
intensity of the conditioning squats was chosen as more strenuous and high muscle activation is required to elicit neural potentiation compared to muscular potentiation (Hodgson et al. 2005). The 3 min rest periods were provided to minimize the possible effects of fatigue (Behm 2004; Behm et al. 2004). The control session involved the same aerobic warm-up followed by sitting for a comparable period (8 min) as the experimental intervention time.

**Testing measures**

Pre-intervention testing commenced 1 min after the warm-up. Testing included two unilateral DJ and two CMJ on the leg to be tested in that session. The order of the DJ and CMJ testing was randomized. The same tests were implemented post-test at 1, 5, and 10 min following the final squat in the intervention. With the unilateral CMJ, participants were allowed to use arm movements to aid coordination, balance, and maintain familiarity and consistency with their typical CMJ routine. Unilateral DJ was performed akimbo from a standardized 21 cm high platform. Participants were instructed to perform each jump with explosive intent (i.e. to jump as high and fast as possible).

Jump height and contact time were monitored with a force plate (AMTI, Watertown, MA, USA). Data were collected at a sampling rate of 2000 Hz. All ground reaction force data were processed using custom-designed software (MATLAB 2013a; Visual Basic 6.0). The jump performances with the greatest jump height (flight time) were used for statistical analysis. Reactive strength indices (RSI) for DJ were calculated from a ratio (flight time / contact time), which gives an indication of neural efficiency (Young et al. 2003; Flanagan et al. 2008).

**Statistical analysis**

CMJ and DJ height, DJ contact time, and RSI data were analyzed separately with a 2-way analysis of variance (2 x 4) with repeated measures to determine if there were significant main effects for the condition (control, exercised leg, contralateral leg) and testing intervals (pre-intervention; 1, 5, and 10 min post-intervention.) Significance was set at \( p \leq 0.05 \). If significant main effects were found, a Bonferroni post-hoc analysis was performed and Cohen’s effect sizes (ES) were calculated. ES were used to provide qualitative descriptors of the magnitude of the standardized effects using the following criteria: trivial: < 0.2, small: 0.2 - 0.5, moderate: 0.5 - 0.8, and large: > 0.8 (Cohen 1988).

**Results**

All reported percentage changes at 1, 5, and 10 min are in comparison to pre-test values. There were no significant main effects for DJ contact time, DJ RSI or control CMJ Height.

There was a significant \( (p = 0.003) \) main effect for time for the CMJ height trials. A condition x time interaction indicated that the exercised leg exhibited significant but small magnitude jump height increases of 3.5% \( (p = 0.008; d = 0.28) \), 4.0% \( (p = 0.011; d = 0.33) \) and 3.2% \( (p = 0.013; d = 0.26) \) at 1, 5, and 10 min post-intervention respectively. On the contrary, the contralateral CMJ had trivial magnitude; 2.0% \( (p = 0.034; d = 0.18) \), 1.2% \( (p = 0.2; d = 0.12) \), and 2.1% \( (p = 0.05; d = 0.17) \) deficits at 1, 5, and 10 min post-intervention respectively (Figure 2).

**CMJ Power**

There was a significant \( (p = 0.04) \) main effect for time for the CMJ power trials. A condition x time interaction indicated that the exercised leg exhibited trivial to small

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**Figure 2.** Figure illustrates countermovement jump (CMJ) height (meters) changes with the exercised leg (circles), contralateral leg (triangles) and control (squares) condition at pre-intervention and 1, 5, and 10 min post-intervention. The \( p \) values indicate significant \( (p \leq 0.05) \) or non-significant differences from pre-intervention results. Vertical bars represent standard deviation.
Crossover fatigue vs. potentiation

Figure 3. Figure illustrates countermovement jump (CMJ) power (Newtons) changes with the exercised leg (circles), contralateral leg (triangles) and control (squares) condition at pre-intervention and 1, 5, and 10 min post-intervention. The p values indicate significant (p ≤ 0.05) or non-significant differences from pre-intervention results. Vertical bars represent standard deviation.

Magnitude jump height increases of 4.0% (p = 0.02; d = 0.36), 0.9% (p = 0.06; d = 0.08) and 1.6% (p = 0.04; d = 0.15) at 1, 5, and 10 min post-intervention respectively. The contralateral CMJ had trivial magnitude; 1.3% (p = 0.23; d = 0.12), 0.9% (p = 0.09; d = 0.10), and 1.7% (p = 0.03; d = 0.19) deficits at 1, 5, and 10 min post-intervention respectively (Figure 3).

**DJ**

There was a significant main effect for DJ height with a 2.8% (p = 0.039; d = 0.21) increase overall (data combined over all conditions and time).

**Discussion**

The most important findings in the present study were the contrasting effects of the unilateral Bulgarian split squat on the exercised and contralateral non-exercised leg. The prior conditioning squat exercise increased the CMJ height of the exercised leg but provoked statistically significant CMJ height impairments in the contralateral leg. The results illustrate how PAP and voluntary force decrements can occur concurrently (Behm et al., 2004). The ability to produce or sustain forces can be viewed as a balance between fatigue-induced impairments and neuromuscular strategies to augment performance (Behm, 2004).

The augmentation of CMJ height is not consistent in the literature. CMJ height increases have been reported following sets of 3RM (Esformes et al., 2013; Kilduff et al., 2007) and 5RM (Boulossa et al., 2013; McCann et al., 2010; Mitchell et al., 2011; Young et al., 1998) squats. Lower et al. (2012) used resistance trained individuals and reported no PAP effect with 5 repetitions of 56% 1RM squats but did find CMJ facilitation with 4 repetitions of 70% 1RM and 3 repetitions of 93% 1RM squats from 4-8 minutes post-conditioning stimulus. However, CMJ deficits were experienced when tested 1 min after the squats. Two sets of 5 repetitions of either 25-35% 1RM or 45-65% 1RM squats both improved CMJ performance (3.5%) of athletes 3 min after the intervention (Sotiropoulos et al., 2010). Two repetitions each of 20, 40, 60, 80 and 90% of 1RM squats stimulated a 2.4% increase in CMJ height at 5 min post-conditioning stimulus in physically active men (Gourgoulis et al., 2003). Although recreationally active individuals did not show jump squat increases following 5 sets of 90% 1RM squats, athletes in sports requiring explosive strength did experience improvements (Chiu et al., 2003). Similarly, the present study also used university athletes from explosive sports. Fletcher (2013) incorporated a conditioning squat protocol somewhat similar to the present study using 3 repetitions at 30% and 70% 1RM, as well as 2 repetitions at 90% 1RM with collegiate athletes and found 8.7-10.4% increases in CMJ, DJ and squat jump height, 4 minutes after the squat protocol. The 3-4% increases in CMJ height following 5 repetitions of 50% 1RM, 3 repetitions of 70% 1RM and 1 repetition of 90% 1RM squats utilized in the present study was lower than the findings of Fletcher (2013) but similar to others (Gourgoulis et al., 2003; Sotiropoulos et al. 2010). A factor contributing to the small magnitude CMJ height increases and lack of change with DJ measures in the present study could be the increased coordination and balance challenges associated with performing a unilateral jump.

On the contrary CMJ increases with a mixed group of young adult athletes and recreationally active individuals did not occur following 3 squat repetitions at 70% 1RM, however large variations were reported with some individuals experiencing substantial improvements (Witmer et al., 2010). Hanson et al. (2007) attributed the lack of jump augmentation with their resistance-trained subjects to an insufficient squat conditioning stimulus (either 8 repetitions at 40% 1RM or 4 repetitions at 80% 1RM). Recreationally active subjects did not display CMJ height increases following separate sessions of 2, 3, 4, or...
5 squat repetitions of 85% 1RM (Khamoui et al., 2009). CMJ height of university volleyball players was not improved two minutes after either 3 squat repetitions of 90% 1RM or 12 repetitions using 37% 1RM (Moir et al., 2009). The lack of CMJ improvements in these studies might be attributed to a number of factors including subjects who were not accustomed to actions involving explosive strength, insufficient conditioning exercise work volume or testing too soon after the conditioning exercise. A number of studies have illustrated deficits when the testing was soon after the conditioning stimulus (i.e. 15s; Kilduff et al., 2007) followed by increases when the testing was conducted later (i.e. 12 min (Kilduff et al., 2007). Since fatigue and PAP have differing time courses (Behm, 2004) an early testing period may be more prone to exhibit the effects of fatigue rather than PAP. However in the present study, statistically significant CMJ height increases were evident from 1-10 minutes after the intervention with the conditioned leg. Figure 2 illustrates that the standard deviations for CMJ height results were not excessive representing only 11% of the means. The use of participants (athletes) who had at least two years of resistance training experience probably contributed to the lower variance and increased the probability of achieving significance with this population.

An appropriate conditioning stimulus may improve subsequent performance by eliciting PAP. A primary mechanism for PAP involves myosin light chain (MLC) phosphorylation (Sweeney et al. 1993). MLC phosphorylation induces an increase in calcium sensitivity of the thin actin filament regulated ATPase activity (Szczesna et al., 2002), increasing the rate constant for crossbridge attachment (Houston et al., 1990). The phosphorylation of the MLC also potentiates contractions by altering the myosin head structure by moving it away from the thick myosin filament backbone and moving closer to the actin filament (Tillin and Bishop, 2009). Hence with explosive movements such as the CMJ, such physiological responses at the level of the muscle would contribute to an enhanced rate of force development and power. CMJ power measures followed the same trend as the CMJ height measures (Figures 2 and 3)

An additional component would be the possible increase in muscle temperature. Increased muscle temperature has been documented to increase instantaneous power output (4%/ Celsius increase) (Bergh and Ekblom, 1979) and maximal sprint cycling power output (2-10% / Celsius increase) (Sargent, 1987). These performance increases are likely attributed to increased muscle temperature-related increases in ATP turnover rate (Gray et al., 2006), positive shifts in the force velocity curve (deRuiter et al. 2000), increased enzymatic cell reactions and nerve conduction velocity (Bishop, 2003). Since the contralateral leg was inactive it could not have benefited from a conditioning exercise-induced increase in muscle temperature. However, the contralateral leg was pre-tested after a 5 min cycle warm-up and not tested again for 8 minutes (duration of intervention). It could be possible that the contralateral leg was warmed for the pre-tests and cooled appreciably for the post-intervention tests, contributing to the deficits. However, the influence of increased muscle temperature on the exercised leg might be limited as it can take 15 minutes of an active warm-up to increase muscle temperature 30 Celsius (Bishop, 2003) and muscle temperature only reaches equilibrium after 10-20 minutes of exercise (Saltin et al., 1968). In the present study, the intervention was approximately 8 minutes in duration with two; 3 minute rest periods between sets and thus may not have provided a sufficient duration to adequately increase muscle temperature.

Potentiating effects can also occur at the level of the central nervous system. Appropriate conditioning stimuli can elevate excitation potentials across synaptic junctions at the spinal cord contributing to the increased recruitment of higher threshold (fast twitch) motor units (Tillin and Bishop, 2009). Synaptic transmission failures commonly occur acting as a neurotransmitter activation reserve (Tillin and Bishop, 2009). However, prior contractions can reduce this transmission failure by increasing the quantity and efficacy of neurotransmitter release, or a reduction in axonal branch-point failure along the afferent fibres (Tillin and Bishop, 2009). Hence both peripheral (muscle) and central (neural) PAP mechanisms can contribute to improving the development of explosive force.

The trivial magnitude effect sizes but statistically significant decreases in the contralateral CMJ height reveals which mechanism was most predominant with the improvement of the exercised leg CMJ height. Prior research has demonstrated the global or non-local neural effects on fatigue (Aboodarda et al. 2015a; 2015b; 2015c; Halperin et al., 2014a; 2014b; 2015), pain (Aboodarda et al., 2015d), flexibility (Behm et al., 2016), and balance (Arora et al., 2015; Paillard et al. 2010). The lack of CMJ height augmentation with the contralateral limb highlights the lack of a PAP neural component, emphasizing MLC phosphorylation and possibly increased muscle temperature as the primary mechanisms contributing to the exercised leg CMJ height increase. Furthermore the statistically significant decrease in contralateral CMJ height suggests the possibility of central nervous system fatigue that was compensated by PAP with the exercised leg. Both Aboodarda et al. (2015b) and Sambaher et al. (2016) reported diminished cortical excitability of the non-exercised limb after fatiguing contractions of the contralateral limb. Furthermore spinal excitability of a non-exercised limb has also been decreased after fatiguing contractions of the contralateral limb (Aboodarda et al., 2015a). Although the present conditioning exercise was similar to prior potentiating protocols (Chiu et al., 2003; Gourgoulis et al., 2003; Fletcher, 2013), it was considered to have a relatively low volume of work and sufficient rest periods for an athletic population; however, the intensity of the intervention may have contributed to neural fatigue.

DJ measures were not affected by the intervention in the present study. It is possible that possible PAP effects may have been masked by the increased difficulty of maintaining balance and coordination upon landing and taking off on one leg. A further consideration is that the squat repetitions were performed at a 5s cadence (slow and controlled), whereas each DJ involved a rapid and
explosive stretch-shortening cycle (SSC) type contraction. The rapid SSC of the DJ increases muscle spindle reflex activity with high motor unit firing frequencies (Behm, 1993; 1995; Behm and Sale, 1993). This training specificity discordance (Behm et al., 1993) between the slow SSC intervention and the rapid SSC DJ testing may have contributed to the small and trivial magnitude changes of the exercised and contralateral limbs respectively.

Conclusion

External manifestations of movement are an intricate interplay of internal facilitatory and inhibitory influences (Behm, 2004). Whether we can optimize or augment a performance is a balance between fatiguing and PAP factors (Behm, 2004; Behm et al., 2004). The increased CMJ height of the exercised leg contrasting with the trivial magnitude but statistically significant decrease of the contralateral leg illustrates these competing influences. In the present study, it is proposed that myosin phosphorylation of the exercised leg played a more predominant role than PAP neural influences. However neither component was directly measured. Another possible mediating factor could be changes in muscle temperature with the conditioning exercise (exercised leg) versus cooling effects of the contralateral limb played a more predominant role. Individuals who are competing or training must optimize training volumes, intensities and rest periods to induce PAP in the target muscles while being cognizant of the possible transfer of dissimilar effects to non-local muscle groups.

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**Key points**

- Post-activation potentiation of unilateral CMJ height was achieved following 5 sequential squats at 50% 1RM, 2 squats at 70% 1RM, 1 squat at 90% 1RM with 3 min rest periods.

- The conditioning exercises did not elicit significant drop jump improvements, likely due to balance challenges.

- In contrast to the potentiation of the affected leg, there were statistically significant impairments of contralateral CMJ height suggesting the co-existence of post-activation potentiation (affected limb) and crossover neural fatigue.

**AUTHOR BIOGRAPHY**

Samantha K. ANDREWS

**Employment**

Student

**Degree**

Bachelor of Kinesiology

**Research interests**

Applied Neuromuscular physiology and exercise/sport science

Jesse M. HORODYSKI

**Employment**

Student

**Degree**

Bachelor of Kinesiology

**Research interests**

Applied Neuromuscular physiology and exercise/sport science
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<th>Name</th>
<th>Employment</th>
<th>Degree</th>
<th>Research interests</th>
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<tr>
<td>Daniel A. MacLEOD</td>
<td>Student</td>
<td>Bachelor of Kinesiology</td>
<td>Applied Neuromuscular physiology and exercise/sport science</td>
</tr>
<tr>
<td>Joseph WHITTEN</td>
<td>Student</td>
<td>Master of Science (Kinesiology)</td>
<td>Applied Neuromuscular physiology and exercise/sport science</td>
</tr>
<tr>
<td>David G. BEHM</td>
<td>University Research professor</td>
<td>PhD</td>
<td>Applied Neuromuscular physiology and exercise/sport science</td>
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Dr. David G. Behm  
School of Human Kinetics and Recreation, Memorial University, St. John’s, Newfoundland, Canada, A1C 5S7