

**SEX-SPECIFIC EFFECTS OF FATIGUE ON KNEE JOINT MUSCLE  
ACTIVATION AND KINEMATICS DURING A SINGLE-LEG LATERAL  
JUMP LANDING TASK**

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July 2023

A thesis submitted to McGill University in partial fulfilment of the requirements on the degree of  
Master of Science

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## TABLE OF CONTENTS

<b>CONTRIBUTION OF AUTHORS .....</b>	<b>I</b>
<b>ACKNOWLEDGEMENTS .....</b>	<b>II</b>
<b>ABSTRACT.....</b>	<b>IV</b>
<b>RÉSUMÉ .....</b>	<b>V</b>
<b>INTRODUCTION .....</b>	<b>1</b>
<b>LITERATURE REVIEW.....</b>	<b>3</b>
<i>Significance of a single-leg lateral jump-landing task.....</i>	<i>3</i>
<i>Muscle fatigue.....</i>	<i>4</i>
Knee joint kinematics in fatigue.....	5
Electromyography.....	5
<i>Basic sex differences in factors relevant to ACL injury.....</i>	<i>6</i>
<i>Sex differences in biomechanics of jumping.....</i>	<i>7</i>
<i>Sex-specific effects of fatigue.....</i>	<i>8</i>
<i>Knowledge gaps.....</i>	<i>9</i>
<i>Objectives/Hypotheses.....</i>	<i>10</i>
<b>RESEARCH ARTICLE .....</b>	<b>11</b>
<i>Abstract .....</i>	<i>12</i>
1. <i>Introduction .....</i>	<i>13</i>
2. <i>Methods .....</i>	<i>15</i>
2.1. <i>Participants .....</i>	<i>15</i>
2.2. <i>Instrumentation .....</i>	<i>15</i>
2.3. <i>Experimental protocol .....</i>	<i>17</i>
2.3.1. <i>Study design .....</i>	<i>17</i>
2.3.2. <i>Maximal voluntary isometric contraction .....</i>	<i>17</i>
2.3.3. <i>Jump-landing task .....</i>	<i>18</i>
2.3.4. <i>Fatigue protocol .....</i>	<i>19</i>

2.4. Data analysis .....	20
2.4.1. Knee joint kinematics .....	20
2.4.2. Electromyography .....	21
2.4.3. Evidence of fatigue .....	21
2.5. Statistical analysis .....	21
3. <i>Results</i> .....	22
3.1. Baseline measures .....	22
3.2. Knee kinematic measures during landing .....	24
3.3. Electromyographic parameters during landing .....	25
4. <i>Discussion</i> .....	29
4.1. Electromyographic parameters .....	30
4.1.1. Quadriceps activation in females .....	30
4.1.2. Q:H co-activation ratio .....	30
4.1.3. VM:VL co-activation ratio .....	31
4.1.4. VL activation in females .....	32
4.2. Kinematic parameters .....	32
4.3. Limitations .....	33
5. <i>Conclusion</i> .....	34
<b>CONCLUSION</b> .....	<b>35</b>
<b>REFERENCES</b> .....	<b>36</b>
<b>APPENDICES</b> .....	<b>45</b>
<i>Appendix 1. Consent Form</i> .....	45
<i>Appendix 2. Par-Q+</i> .....	51
<i>Appendix 3. International Physical Activity Questionnaire (IPAQ)</i> .....	52
<i>Appendix 4. Borg CR10 Scale (English)</i> .....	58

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Andrew Yoon, M.Sc., assisted with research design and data analysis, and Samuel Lamanuzzi, M.Sc., assisted with data analysis.

## **ACKNOWLEDGEMENTS**

I would first like to thank my supervisor, Dr. Julie Côté, for her guidance and expertise over the years. Julie fosters a culture of resourcefulness and collaboration amongst peers, which are skills I have now built to last a lifetime. I would also like to thank Julie for her flexibility and open mindedness in research, and for giving me the freedom to pursue a topic of great interest to me. Thank you, Julie, for always being ready to extend a helping hand when needed or offer words of advice; I will forever be grateful for my time in your lab.

Without the generous support of the Sylvan Adams Sports Science Institute (SASSI), I would not have been able to comfortably pursue my graduate studies. Thank you to SASSI for their gift and for helping me as a student. Thank you also, to my committee members Dr. Caroline Paquette and Dr. David Pearsall, for their guidance and for helping me fine tune my project.

To my research team – Tailynn Chang, Sophie Pellar and Monica Lubczynski – I could not have done this without you. Thank you for the countless hours spent building and perfecting our protocol, recruiting participants, and collecting data. Thank you for your dedication and support, and for your friendship – I will always be grateful and will miss our chats in the lab. I would especially like to thank Tailynn, who is now my teammate on and off the field, for her immense help on this project – Tay, thank you for everything and best of luck with your future endeavours!

My friend and mentor, Andrew Yoon, has been a role model for me over the years. Andrew is the reason I was considered as a student volunteer in our lab – Biomechanics of Occupation and Sport (BOS) years ago. Over the last 4 years, Andrew has taught me the skills I needed to build my own project and has led by example in his expertise and passion for research. Even though he has long since graduated, he still readily responds to any of my questions or calls for help, which I will always be thankful for. I would also like to thank my friends Erika Renda, Lauren Cederbaum, Sam Lamanuzzi, Yiyang Chen, Malak Selim, and Kerrigan Sunday at BOS lab, as well as my good friend Kemar Harris, who have been there for so many chats and have offered assistance on countless occasions. Thank you for all your help and guidance, for the good conversations, the laughs, and for your friendship.

I would like to also extend my thanks to my parents and my sister. As an adult, I can now appreciate the sacrifices and the hardships my parents overcame, immigrating to Canada to give

my sister and I the opportunities we have now. My mother, who has always excelled at anything she puts her mind to, has shown me the value of hard work, perseverance and resourcefulness that have led me to completion of a Master's degree. Thank you to my family for everything.

Last but foremost, my family at Martlets Rugby have defined my experience at McGill beyond words. My teammates, past and present, have shown me incredible support and companionship over the years and have shaped me into who I am today, particularly while I was recovering and returning to play from my own ACL surgery. I would especially like to thank my teammates who stepped up to help me with this project – I am incredibly grateful for their time and effort. It has been an honour to train and compete alongside my team and I look forward to many more years of friendship and growth.

## **ABSTRACT**

The goal of this Master's project was to compare changes in muscle activation and knee joint angles during a lateral jump-landing task after a high-intensity intermittent fatiguing exercise between the sexes. The motivation for this thesis was to explore sex-specific knee injury mechanisms relevant to sports that involve sudden changes in directions, which show a higher injury prevalence in female athletes. Healthy male and female varsity athletes performed a sideways single-leg jump-landing task before and after a high-intensity cycling fatigue protocol. Changes in muscle activation amplitude of the quadriceps and hamstrings during landing were quantified using surface electromyography. Knee joint angles and displacements during landing were measured using motion capture. Sex differences in fatigue effects were found in quadriceps activation. Females displayed significantly higher quadriceps activation before fatigue than males, but their activation decreased significantly after fatigue to match that of males. Females also exhibited higher activation of the medial quadriceps regardless of fatigue. Finally, males and females showed similar knee joint angles before and after fatigue. Greater quadriceps activity before fatigue in females may reflect a hindered ability to warm up the muscles for high-impact loading, which could potentially help explain females' higher knee injury risk in sporting contexts.

## RÉSUMÉ

Le but de ce projet de maîtrise était de comparer entre les sexes la variation de l'activation musculaire et l'angle du genou lors d'un saut latéral à la suite d'un exercice de fatigue intermittente à haute intensité. L'objectif de cette thèse était d'explorer les mécanismes de blessure aux genoux spécifiques aux sexes et pertinents dans le cadre de sports impliquant des changements de directions brusques, qui sont plus prévalents chez les athlètes féminines. Des athlètes universitaires masculins et féminins en bonne santé ont effectué un saut latéral sur une jambe avant et après un protocole de fatigue induite par une activité de haute intensité sur vélo stationnaire. Les variations de l'amplitude de l'activation musculaire des quadriceps et des ischio-jambiers lors de l'atterrissage ont été quantifiées par électromyographie de surface. Les angles et déplacements du genou lors de l'atterrissage ont été mesurés à l'aide d'un système de caméra à capture du mouvement. Une différence entre les sexes a été répertoriée dans l'effet de la fatigue sur l'activation des muscles du quadriceps. Une activation des quadriceps significativement plus élevée a été observée chez les femmes avant l'induction de fatigue, suivi d'une plus importante diminution après la fatigue comparativement à celle des hommes. Les femmes ont également démontré une activation plus élevée du quadriceps médial indépendamment du niveau de fatigue. Finalement, l'angle du genou avant et après la fatigue s'est avéré similaire chez les hommes et les femmes. L'activité élevée du quadriceps avant la fatigue chez les femmes pourrait refléter une moins bonne capacité à réchauffer les muscles en vue d'une charge à fort impact, ce qui pourrait contribuer à expliquer l'incidence accrue des lésions du genou chez les femmes dans un contexte sportif.



## INTRODUCTION

Knee injuries are a common problem in direction-changing sports, occurring in 23% of Canadian university athletes (Gardiner, 2019). The anterior cruciate ligament (ACL) is made of up connective tissue and is situated in the knee joint, connecting the tibia and the femur. The ACL plays an important role in stabilizing the knee by limiting excessive movement of the tibia from the femur. Injuries to the ACL leave heavy impacts on athletic careers and performance, athlete wellbeing, and the healthcare system (Chia et al., 2022; Ardern et al., 2016).

Non-contact ACL injuries make up most injuries to the ACL. Typically, injuries occur when the foot is planted and the body continues to move relative to the lower leg, causing excessive anterior translation or rotation of the tibia relative to the femur. The most common mechanism of non-contact injury is from one-legged movements, such as landing on one leg or sidestepping in a sporting context (Alsubaie et al., 2021). Currently, single-leg lateral jump landings have been studied as one of the most accurate representations to sideways movements in an actual sporting context (Chinnasee et al., 2018).

Apart from single-leg manoeuvres, neuromuscular fatigue and endurance have been identified as potential risk factors for ACL injury, with higher injury incidence found in later stages of competition (Hiemstra et al., 2001). Fatigue is defined as a task-dependent symptom that often results in decreased performance (Enoka & Duchateau, 2016). However, effects of fatigue on biomechanical parameters pertaining to ACL injury risk show inconclusive results and require further investigation.

Previously, females have shown significantly higher injury rates compared to males in the same sport (Montalvo et al., 2019), despite an overall 7.3% higher participation rate of males in high-risk sports (Eime et al., 2021). For instance, from the years 2004 to 2016, 357 female NCAA athletes reported ACL injuries compared to 172 males across 3 sports: basketball, lacrosse and soccer (Anderson et al., 2019). Sex differences in knee joint laxity, hormone levels, anatomical alignment and muscular strength and power are potential contributors to differences in ACL injury risk (Hiemstra et al., 2001). Furthermore, females exhibit lower type II muscle fiber area, as well as lower muscle fiber size than males (Miller et al., 1993), which may result in less ability to

stabilize the knee upon landing. Thus, it is worth investigating sex differences in biomechanical parameters that contribute to injury risk.

Knee joint kinematics and muscle activation are possible risk factors that contribute to stress on the ACL. When landing, greater movement or displacement of the knee joint, measured through joint kinematics, is often indicative of dynamic instability. Additionally, less activation of the musculature surrounding the knee (quadriceps and hamstrings), as well as imbalances in strength and activation between muscles can add to ACL strain and increase injury risk. However, mixed results have been reported on kinematic and neuromuscular activation parameters during landing. More clarity is needed on sex-specific fatigue effects on these parameters.

Therefore, our objective was to examine the combined effects of sex and fatigue on muscle activation and knee joint kinematics when landing from a single-leg lateral jump among varsity athletes. To do so, participants performed a pre/post fatigue jump-landing task before and after a high-intensity cycling protocol. We hypothesized that with fatigue, athletes would experience less stability of the knee, with more exaggerated changes in knee joint displacement and imbalances in muscle activation. The results of this research will help deepen understanding of the mechanisms by which fatigue and sex can impact knee injury rates, and may contribute to the development of tailored training protocols to reduce injury risk.

## LITERATURE REVIEW

ACL injury mechanism can be classified into 3 categories: non-contact, indirect contact, and direct contact to the knee. Most injuries are considered non-contact in nature, with prevalence rates for this type ranging from 55-80% out of all ACL injuries (Chia et al., 2022; Alsubaie et al., 2021). Non-contact injury mechanisms are influenced by extrinsic and intrinsic factors, where extrinsic factors include the type of playing surface, sport, shoes worn and more (Hiemstra et al., 2001). Intrinsic factors include anatomical structure, neuromuscular control, hormonal influences, muscular power and endurance with fatigue, and the player's sex (Boden et al., 2000). Common sports that are associated with high non-contact ACL injury incidence include team sports involving sidestepping, jumping, or pivoting, such as football, soccer, rugby, basketball, and lacrosse (Chia et al., 2022). Landing is evidently a major risk factor for injury and must be investigated further to observe changes in neuromuscular control of the knee.

### **Significance of a single-leg lateral jump-landing task**

What the aforementioned sports have in common is that they involve jumping in various directions, among other task characteristics. Indeed, the most common mechanisms of non-contact ACL injuries occur when landing from a jump on one leg or from sidestepping manoeuvres, with approximately 64% of cases being from one-legged movements (Alsubaie et al., 2021). Landing can be categorized into 3 distinct stages: pre-contact (flight phase), point of initial contact, and post-contact (landing phase). Most non-contact ACL injuries are expected to occur within the first 61 ms of the landing phase (Bates et al., 2020). A study by Taylor et al. (2016) found that, when compared to double-leg landings, single-leg landings caused more exaggerated changes in knee joint flexion and abduction angles. Additionally, Wang et al. (2011) found that landing from a single-leg jump produced higher ground reaction forces on the knee and higher knee flexion moments. These results suggest that single-leg landing can lead to greater load on the ACL and increased risk of injury. Therefore, a one-legged landing task should be examined over a double-leg task, as an experimental model to better understand ACL injury mechanisms.

In addition to one-legged landing manoeuvres, movements in the lateral direction are also important to examine. Landing in the lateral direction from a jump more closely mimics sidestepping manoeuvres in sport, which seem to be more predictive of ACL injuries than forward

ones (Chinnasee et al., 2018). Furthermore, when compared to forward jump landings, lateral jump landings elicit higher peak knee abduction angles, which is a known contributor to knee injury risk (Taylor et al., 2016). Landing from lateral jumps has also shown significantly different patterns in muscle activation levels compared to forward landings, and may place an important load on the thigh muscles responsible for its mediolateral stability, in a way that can influence injury risk (Sinsurin et al., 2016). Increased activity of lateral musculature has been found to abduct the knee, leaving it more prone to medial knee collapse (Palmieri-Smith et al., 2008). Furthermore, Peel et al. (2021) found that the lateral quadriceps and hamstrings were the greatest contributors to ACL loading, with the lateral quadriceps contributing an additional 89% of body weight to the knee upon landing. Targeting medial components of knee joint musculature could counteract knee abduction and have a protective effect from injury. Thus, it is important to examine jump landings in the lateral direction as a potentially more accurate predictor of injury risk than landings from forward jumps.

### **Muscle fatigue**

Previously, a high injury incidence has been found in team sports during later stages of competition, suggesting that fatigue may be a significant risk factor for knee injury (Hiemstra et al., 2001). Fatigue is defined as a task-dependent disabling symptom that can be classified into performance fatigue and perceived fatigue (Enoka & Duchateau, 2016). Common measures of performance fatigue include declines in pre/post exercise maximal contraction torque and decline in peak power, which are also correlated with increases in perceived physical exertion (Cruz-Montecinos et al., 2019). Neuromuscular fatigue has been found to change contractile properties of muscle and limit neuromuscular control, resulting in an association with increased injury rate (Hiemstra et al., 2001). Indeed, during a fatiguing walking task, knee stiffness has been found to decrease significantly, indicating a hindered ability to resist external pressures on the joint (Shao et al., 2022). Moreover, fatigue has different effects on contractile properties of different muscle fibers. For instance, type I muscle fibers tend to respond better to fatigue and are known to produce lower levels of force, but have greater endurance compared to type II fibers. In contrast, type II muscle fibers can generate higher levels of force and power, which may be beneficial for high-impact tasks such as landing. Furthermore, according to the Henneman principle of motor unit recruitment (Henneman, 1957), type I muscle fibers are activated sooner than type II fibers, with

muscle fiber recruitment gradually increasing from submaximal to maximal effort tasks, as well as during fatigue development. This may influence landing strategies and joint movement in different stages of exertion, such as before and after fatigue.

### *Knee joint kinematics in fatigue*

Joint kinematics provide valuable insight into possible injury mechanisms in sporting contexts. Common kinematic measures include joint angles, displacement, and velocity. Knee joint angles that are often focused on for analysis of jumps are angles at initial contact and peak angles, with joint range of motion (ROM) equaling the difference between peak and initial contact angles. During single-leg jump landings, it has been found that athletes display greater mechanical load on the knee in cases of lesser knee flexion and greater knee abduction angles (i.e. valgus) (Chinnasee et al., 2018). After fatiguing exercise, knee flexion ROM has been shown to decrease, indicating a stiffer landing and malabsorption of ground reaction forces upon landing during a double-leg forward drop jump (Wong et al., 2020). However, other studies have found that peak knee flexion angles increased with fatigue during single-leg drop landing tasks, sidestepping, and cutting manoeuvres (Brazen et al., 2010; Savage et al., 2018). Discrepancies in reported results between studies may be due to differences in jump and fatigue protocols. Furthermore, when examining fatigue effects on knee abduction, Borotikar et al. (2008) found a significant association of fatigue with higher peak knee abduction angles during a variety of sidestepping and lateral landing tasks. However, studies on knee abduction with fatigue during lateral landing tasks are currently lacking and need further examination.

### *Electromyography*

Muscle activation, measured through electromyography (EMG), is another important technique to quantify relationships between muscle fatigue and knee injury risk. EMG readings represent the sum of several motor unit action potentials within a muscle body. EMG can be recorded through surface EMG electrodes, which are placed on the skin superficial to the muscle of interest. Signals can be displayed in two domains: the time and frequency domains. In the time domain, one of the most common methods of presenting EMG is through the root-mean-square (RMS) of a signal, which represents the average amplitude of the signal over a set window of time (Konrad, 2005). Measures of a muscle's activation amplitude, achieved during an experimental task like a jump, are commonly normalized to reference efforts such as maximal voluntary

isometric contractions (MVICs) performed in a standardized way by all subjects, in order to reduce inter-subject variability and allow for comparisons between groups such as sexes (Burden, 2010). Experimental values are then expressed as a percent of the maximum activation amplitude. Previously, central fatigue from high-intensity exercise has been hypothesized to cause a reduction in voluntary drive (Taylor et al., 2006), that can be characterized by a decrease in surface EMG amplitude (Bourne et al., 2019). However, before reaching peak fatigue and exhaustion, to continue accomplishing a submaximal task as fatigue sets in, the opposite is seen, with a gradual increase in EMG RMS with gradual fatigue (Konrad, 2005).

There currently exists a disparity in the literature as to how EMG amplitude of the knee extensors and flexors differ between fatigue conditions during jump tasks. Hamstring activation amplitude has been found to decrease with fatigue during landing (Gehring et al., 2009), with no change in quadriceps activation (Padua et al., 2006). Imbalances of muscle activation across the quadriceps and hamstrings can increase dynamic instability and injury risk (Hiemstra et al., 2001). Main forms of ACL loading during landing include anterior shear force at the proximal tibia and knee extension moment, which are characterized by greater quadriceps activation relative to hamstrings (Q:H), which is defined as the Q:H co-activation ratio for the purposes of this project (Hiemstra et al., 2001; Wojtys et al., 1996). In contrast, hamstring activity can apply a posterior pull on the knee joint to counteract anterior translation caused by the quadriceps, which may reduce injury risk. In fatigue, Q:H co-activation has been shown to increase during landing, posing greater injury risk (Kellis & Kouvelioti, 2009; Padua et al., 2006). It is worth noting that differences in fatigue protocols may influence the measured changes in muscle activation. Thus, the use of a fatigue and jump landing protocol that more closely mimics fatigue generated in high-intensity sporting tasks may more accurately reflect the mechanisms that could link fatigue during sports with the increasing risk of ACL injuries.

### **Basic sex differences in factors relevant to ACL injury**

Over the last two decades, injury incidence has decreased in male athletes but has remained relatively constant in females (Sanders et al., 2016). Females currently exhibit around 3 times greater risk of injury than their male counterparts in the same sport (Montalvo et al., 2019). Physiological sex differences such as muscle size, power and composition can explain the presence of sex differences in sports performance and biomechanics. Typically, females present with lower

muscle size than males, which is associated with lower muscle strength and power production (Bartolomei et al., 2021). Additionally, it has been shown that females have lower type II fiber areas, particularly in the thigh muscles, which would further contribute to lower power output (Miller et al., 1993). Generally speaking, the proportion of muscle fibers also favors type II fibers in males, as compared to females (Miller et al., 1993). The hindered ability to generate more power in females could be detrimental during landing, as muscles are required to produce and absorb forces in a short amount of time to stabilize the knee joint.

### **Sex differences in biomechanics of jump landing**

Studies examining knee joint kinematics during jump tasks have reported differences between the sexes. Chappell et al. (2007) found that females exhibited less knee flexion during a forward bilateral stop jump task. Smaller knee flexion angles exhibited in females suggest a greater risk of injury, as anterior shear forces at the proximal tibia are a major form of ACL loading and tend to increase with less knee flexion (Chappell et al., 2007). In contrast, Gehring et al. (2009) found that females had a slower deceleration from a bilateral drop landing than males, which was represented by greater peak knee flexion angles and greater joint loading. Additionally, females have been found to exhibit significantly greater knee abduction compared to males, regardless of fatigue conditions (Gehring et al., 2009; Pappas et al., 2007; Lessi et al., 2017; Kernozek et al., 2008). This suggests that females have a predisposition to increased injury risk and exhibit greater joint instability in the frontal plane.

In addition to joint kinematics, sex differences have been found in previous studies on thigh EMG during landing. A recent meta-analysis found that females exhibited significantly higher VL activation amplitude than males during various jump landing and sidestepping tasks (Seyedahmadi et al., 2022). However, no significant sex differences were reported in activation of the biceps femoris, semimembranosus, vast or rectus femoris (RF) muscles. In contrast, Ebben et al. (2010) found that males experienced greater hamstring activation than females in the landing phase of sidestepping and jump landing. Additionally, Márquez et al. (2017) found that males had greater activation of the RF when landing from a countermovement jump. Greater levels of activation in males may suggest a greater ability to quickly recruit motor units and generate higher levels of force to counteract extreme loads on the knee upon landing (Márquez et al., 2017). Contrastingly, Hughes and Dally (2015) found that when landing from a one-legged jump or sidestep, females

demonstrated greater activation of the RF. Differences in results may be due to the nature of the jump task, where the first protocol involved a double-leg jump and the second focused more on single-leg movements. Observations on sex differences in the literature on landing tasks are evidently inconsistent and require further examination.

Studies have shown sex differences in muscle co-activation during landing. Previously, females have shown higher levels of Q:H co-activation than males during jump landing or sidestepping (Chappell et al., 2007). Their results suggest that to prepare for landing, females rely on a more quadriceps dominant strategy to stabilize the knee joint than males, which may increase injury risk (Chappell et al., 2007). Furthermore, more reliance on the quadriceps in females shows a hindered ability to decelerate forward progression of the tibia upon landing to stabilize the knee (Landry et al., 2009). Additionally, females have been shown to rely more on the lateral quadriceps and hamstrings in jumping or sidestepping tasks, which may increase load on the ACL (Simonsen et al., 2000). However, medial to lateral co-activation ratios, which play an important role in stabilizing the knee, have not been examined (Kiapour et al., 2016). Furthermore, combined effects of sex and fatigue on co-activation are lacking in the literature and require more research to better understand injury mechanism.

### **Sex-specific effects of fatigue**

In general, females have shown more fatigue resistance than males in a variety of tasks (Hunter, 2016). Mechanisms of sex differences in response to fatigue include aspects ranging from excitability of the motor cortex to impairments in contractile proteins of the muscle, which may vary based on muscle fiber type. However, fatigue resistance is questionable when male and female performance is compared in relative units (Hureau et al., 2021). In fatigue protocols involving multiple sprint exercise, males and females have exhibited similar levels of power reduction when normalized to initial sprint work (Smith & Billaut, 2012). Furthermore, no sex differences were found in a high-intensity cycling protocol in the decrease in MVIC force or average performance level when normalized to peak power (Ansdell et al., 2020). In summary, similar levels of relative fatigability have been reported between the sexes during high-intensity protocols that pertain to intermittent sporting demands. However, some studies have shown different sex-specific effects of fatigue on biomechanical parameters during landing, including joint kinematics and EMG.



Few studies have compared how males and females respond to fatigue during tasks relevant to ACL injury risk. A study by Kernozek et al. (2008) examined one-legged forward drop jumps and found that females exhibited lower knee flexion angles than males after fatigue generated from submaximal squatting. Similarly, Gehring et al. (2009) found a more exaggerated increase in peak knee flexion in males during a bilateral forward drop landing after fatigue generated from submaximal leg press. These findings suggest that males are more effective in shock absorption and reducing the amount of anterior shear force on the knee when landing in fatigued states. Despite this, conclusive results of sex differences on knee abduction under fatigue are currently lacking. This may be due to the nature of the jump tasks employed, which have mostly been focused more on forwards movements rather than sideways.

Additionally, combined effects of sex and fatigue on EMG during landing have been reported, with some existing studies reporting null results (Gehring et al., 2009; Padua et al., 2006). Existing studies examining sex-specific effects of fatigue have focused mostly on lateral muscle activation. Lessi et al. (2017) found that during a one-legged jump landing, males stayed constant in lateral hamstring activation, whereas females had an increase in activation with fatigue from submaximal squatting. In contrast, Daniusevičiūtė et al. (2013) found that after fatigue induced by repetitive bilateral drop jump landings, females experienced a greater drop in activation of the lateral quadriceps and hamstrings during the landing phase. Conflicting results in the literature may be due to the type of jump task involved and the nature of the fatiguing task, where one study focused on fatigue generated from repetitive squatting, and the other focused more on fatigue from repetitive landing and shock absorption. Furthermore, although the lateral thigh muscles in these studies were examined, overall thigh muscle activation amplitude and co-activation ratios with the medial musculature were not. It would be beneficial to compare changes in both sets of muscles relative to each other to identify possible imbalances in activation. In summary, studies have found conflicting results on sex-specific effects of fatigue on co-activation ratios of muscles responsible for frontal plane movement stabilization. More research is needed on knee EMG during landing to gain a deeper understanding of injury mechanism.

### **Knowledge gaps**

Lateral single-leg landings mimic sidestepping manoeuvres in a real-life sporting context, but a lack of literature currently exists on combined sex and fatigue effects on knee biomechanics

during landing. Furthermore, null results on sex-specific effects of fatigue on variables such as muscle co-activation and knee abduction angles in the sagittal plane may have different outcomes when tested in the frontal plane. Inconsistencies in the literature in sex and fatigue differences in muscle co-activation, overall thigh EMG, and changes in knee flexion and abduction angles must also be addressed.

### **Objectives/hypotheses**

The objective of the current study was to examine the sex-specific effects of fatigue on knee joint kinematics and muscle activation during a single-leg lateral jump-landing task. Specific aims of the study were to 1) quantify sex-specific changes in activation amplitude of the thigh muscles, 2) examine changes in muscle coordination ratios between the anterior and posterior thigh muscles as well as between the medial and lateral thigh muscles, and 3) measure knee joint kinematics during a jump-landing task and calculate joint displacement and angles. We hypothesized that with fatigue, both sexes would have similar decreases in activation amplitude, but females would have a greater increase in muscle coordination ratios, with greater lateral activation relative to medial and greater quadriceps activation relative to hamstrings. Finally, we hypothesized that females would have greater knee joint ROM in fatigue in knee flexion and abduction.

## RESEARCH ARTICLE

Sex-specific effects of fatigue on muscle activation and knee joint kinematics during single-leg lateral jump landing

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## Abstract

Anterior cruciate ligament (ACL) injuries are common in direction-changing sports, with higher injury rates reported in later stages of competition with fatigue. In addition, the literature reports that females exhibit around 3 times greater ACL injury incidence than males. Knee joint displacement and imbalances in muscle activation are thought to be risk factors for ACL rupture. However, it is unclear if fatigue affects these parameters in the same way for males and females, and whether sex-specific fatigue mechanisms would underlie this sex difference in injury prevalence. Thus, the study aimed to examine sex-specific effects of fatigue on thigh muscle electromyography (EMG) and knee joint kinematics during a single-leg lateral jump-landing task. Fifteen female ( $22.0 \pm 2.0$  years) and 10 male ( $21.1 \pm 1.5$  years) healthy varsity athletes performed single-leg lateral jump landings before and after a 16.5 min high-intensity fatigue protocol consisting of repeated cycling sprints performed with a cycle ergometer. Surface EMG of the vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), biceps femoris and semitendinosus muscles, as well as motion capture-based kinematics of the knee joint were recorded during jump landings before and immediately after the fatiguing task. EMG root-mean-square amplitude of individual muscles, as well as activation ratios within the quadriceps muscles (VM:VL) and between the quadriceps and the hamstring (Q:H) muscles were used to assess muscle activity. Knee joint angles and range of motion were used to assess knee joint landing kinematics. Sex x Fatigue interactions were observed in the VL ( $P = 0.002$ ) and RF ( $P < 0.001$ ) and in the Q:H activation ratio ( $P = 0.003$ ). Females had significantly higher quadriceps activation pre-fatigue but there was no sex difference in the post-fatigue condition. VM:VL activation ratio was higher in females ( $P = 0.039$ ) and in the post-fatigue condition ( $P < 0.001$ ). No significant differences were found in knee flexion and abduction angles. Results show that males and females experience different changes in muscle activation with fatigue. Results suggest that females may have greater injury risk before fatigue, which may be related to sex differences in muscle priming and the need for more extensive warm-ups, especially in females.

## 1. Introduction

Knee injuries in team sports requiring rapid changes of directions are a common problem, with a prevalence of 23% in Canadian university athletes (Gardiner, 2019). The anterior cruciate ligament (ACL) is a poorly vascularized tissue located within the knee joint connecting the femur and the tibia, whose main role is to stabilize the knee by protecting against sudden forces that cause anterior tibial translation and, especially, tibial rotation. ACL injuries are a serious form of knee injury and have a large impact on athletic careers, resulting in temporary or permanent retirement from competition, as well as psychological and financial burdens (Zhang et al., 2020).

In sports, injuries to the ACL often result from one-legged manoeuvres such as landing or sidestepping (Alsubaie et al., 2021). When compared to double-leg landings, single-leg landings display greater magnitude of knee joint angular displacements and muscle activation levels that are associated with higher injury risk (Taylor et al., 2016; Sinsurin et al., 2016). However, previous studies lack conclusive results on changes in knee neuromuscular activation patterns during single-leg lateral tasks. Given the high injury risk associated with this manoeuvre, more studies are needed to more precisely characterize how it affects structures of the knee, including changes in parameters indicative of stress on the ACL, like muscle activation and knee range of motion (ROM).

Even though there are known risk factors such as biological sex, anatomical characteristics, neuromuscular control, and muscular power and endurance, in sports practice, it is difficult to predict and prevent the occurrence of ACL injuries. As one of the main suspected risk factors, muscle fatigue is defined as a task-dependent disabling symptom that often results in inferior performance (Enoka & Duchateau, 2016). Fatigue has previously been identified as a potential risk factor for ACL injury, with higher injury incidence found in later stages of competition (Hiemstra et al., 2001). However, the literature on the fatigue effects on neuromuscular control and joint positioning during lateral landings show conflicting results and require further investigation. Knee motion, particularly with fatigue, is indicative of instability while landing. Previous studies have shown that after fatigue, athletes display greater load on the ACL with higher knee abduction and lower knee flexion angles (Borotikar et al., 2008; Wong et al., 2020). Furthermore, mixed results on muscle activation, measured through electromyography (EMG), have been found after fatigue. The maximally fatigued state at the end of high-intensity exercise

can be characterized by a decrease in EMG amplitude (Bourne et al., 2019). However, changes in thigh muscle activation differ between different types of fatigue protocols (Kellis & Kouvelioti, 2009). Moreover, quadriceps activation relative to hamstrings activation (Q:H co-activation) has been shown to increase with fatigue (Padua et al., 2006), although it is unclear if this is due more to the quadriceps or the hamstrings when landing.

Despite similar levels of fatigability between the sexes during high-intensity exercise (Ansdell et al., 2020), statistics show that females exhibit approximately 3 times higher injury occurrence than males in the same sport (Montalvo et al., 2019). Previous studies have referred to differences in anatomy such as the knee's Q-angle, in strength, and in hormones between male and female athletes to explain this higher ACL injury likelihood in female athletes (Khasawneh et al., 2019; Stearns et al., 2013; Wojtys et al., 2002). However, much of the mechanistic origin for this sex difference in ACL injury rates remains poorly understood. For instance, sex differences in biomechanical parameters during landing have been found in previous studies. Generally, lower knee flexion angles (Chappell et al., 2007), and greater knee abduction angles and mediolateral displacement have been reported in females upon landing (Weeks et al., 2015). Furthermore, sex differences in EMG have been reported, with lower hamstring activation amplitude found in females (Ebben et al., 2010), along with higher Q:H co-activation (Chappell et al., 2007). Females have also shown more reliance on the lateral quadriceps during landing (Seyedahmadi et al., 2022), but studies investigating co-activation ratios between muscles responsible for medial and lateral knee stabilization are lacking. Despite evident differences between males and females, a majority of studies investigating sex differences during landing do not explore sex-specific effects of fatigue. In summary, more research is needed to build a deeper understanding of sex and fatigue effects on knee joint kinematics and muscle activation patterns during landing.

Therefore, the present study aimed to quantify sex-specific changes in 1) quadriceps and hamstrings activation amplitude, 2) muscle co-activation of knee medial and lateral stabilizers, and 3) knee joint abduction and flexion angles, occurring with fatigue, during a unilateral jump-landing task. We hypothesized that: 1) males and females would have similar decreases in activation amplitude with fatigue, 2) females would have greater increases in co-activation ratios with fatigue, and 3) females would have greater knee joint range of motion or displacement across the frontal and sagittal planes with fatigue.

## 2. Methods

### 2.1. Participants

Sample size was determined through G\*Power using a priori power analysis with repeated measures ANOVA with within and between interactions. The primary outcome was identified as EMG root-mean-square (RMS), as a function of sex and fatigue. To achieve statistical power of 0.80 (Hintze, 2008) with alpha set to 0.05, and an effect size of 0.28, a total of 28 participants (14 F) was required. Effect size was calculated using a study performed by Lessi et al. (2017) that investigated sex-specific changes in lower body muscle activation with fatigue during a drop jump task. Group means for effect size calculation were determined from pre/post fatigue vastus lateralis RMS. The group mean of males was  $1.9 \pm 2.5$  (pre-fatigue RMS =  $62.2 \pm 6.7$  % maximal voluntary isometric contraction (MVIC), post-fatigue RMS =  $64.1 \pm 9.2$  % MVIC), and the group mean of females was  $0.6 \pm 2.1$  (pre-fatigue RMS =  $63.6 \pm 6.6$  % MVIC, post-fatigue RMS =  $64.2 \pm 8.7$  % MVIC), where group means equalled the post-fatigue values subtracted by the pre-fatigue values.

Through convenience sampling, 15 females (mean  $\pm$  SD age:  $22.0 \pm 2.0$  years, height:  $165.5 \pm 4.2$  cm, mass:  $66.3 \pm 5.3$  kg) and 10 males (age:  $21.1 \pm 1.5$  years, height:  $177.5 \pm 5.7$  cm, mass:  $79.9 \pm 9.2$  kg) agreed to participate in the study and provided the data presented in this thesis. Full sample size was not reached due to limited recruitment opportunities and time constraint to fit within the scope of a 2-yr Master's thesis. Participant criteria included: 1) no history of musculoskeletal or neuromuscular pathologies, 2) no injury to the lower extremity within 6 months of data collection (Walsh et al., 2012), and 3) participating in jumping or cutting team sports at least 3 times per week (Herman & Barth, 2016) at the university varsity level. Participants were instructed to refrain from alcohol consumption or vigorous exercise 24 h prior to the testing session, with no caffeine or heavy food consumption up to 2 h before the testing session. Upon arrival, the participant completed a written consent form (Appendix 1), the PAR-Q+ (Appendix 2) (Warburton et al., 2011) and the International Physical Activity Questionnaire (IPAQ) (Appendix 3). The study received institutional ethical approval from the McGill University Faculty of Medicine Institutional Review Board (IRB study number = A02-B93-20B (20-11-041)).

### 2.2. Instrumentation

Wireless surface EMG sensors (Trigno Avanti, Delsys, Natick, MA, USA; double-differential bar (Ag) electrodes; 2.7 cm x 3.7 cm; common-mode rejection ratio = 80 dB) were

placed on the semitendinosus (ST), the long head of the biceps femoris (BF), vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM) thigh muscles. Sensors were placed approximately halfway between the ischial tuberosity and the medial tibial condyle for the ST, halfway between the ischial tuberosity and the lateral tibial condyle for the BF, and halfway between the anterior superior iliac spine (ASIS) and the superior border of the patella over the muscle belly of the RF. Vastus sensors were placed approximately 2/3 of the distance from the ASIS to the superolateral border of the patella for the VL, and 4/5 of the distance from the ASIS to the joint space just anterior to the medial collateral ligament of the knee for the VM, according to SENIAM guidelines (Hermens et al., 1999). All sensor placements were verified to be over the respective muscle belly by asking the participant to contract each muscle and verifying with online feedback that activation bursts were elicited. Prior to electrode placement, the skin was shaven and abraded with alcohol. Sensors were placed running parallel to muscle fibers and secured with tape on the participant's dominant leg, which was defined as the leg used to kick a ball to maximum distance (Aizawa et al., 2016; McLean et al., 2007; Walsh et al., 2012). EMG data were sampled at 2000 Hz using Vicon Nexus software (Vicon Nexus 2.8.0, VICON Motion Systems Ltd., Oxford, United Kingdom). Additionally, a HR monitor (Polar H10, Polar Electro, Kempele, Finland) was fitted on the participant's chest.

A Vicon 8-motion capture infrared camera system (Vero VICON, Oxford Metrics Ltd., Oxford, UK) at 250 Hz was used to capture kinematic data. Twelve retro-reflective, spherical markers (diameter = 14.0 mm) were placed on the following sites, following guidelines based on the Vicon Plug-in Gait Reference Guide (Vicon Motion System Ltd., 2018): left/right ASIS and posterior superior iliac spine, knee joint axis centre (JAC) on the lateral side of the knee, lateral thigh along the line between the greater trochanter and the knee JAC, lateral malleolus of the ankle, lateral tibia along the line between the knee JAC and the lateral malleolus, heel, and the metatarsal of the 2<sup>nd</sup> toe on the participant's dominant leg. Markers were placed on the participant's skin and shoes with double-sided tape. A static calibration trial was performed with the participant standing still for 5 s, with arms raised to the sides. This was followed by a dynamic calibration trial where the participant performed 5 squats with arms placed across the chest.

A Monark 894E (Varberg, Sweden) mechanically braked cycle ergometer was used to complete the repeated high-intensity cycle procedure. Seat height was adjusted at the height of the

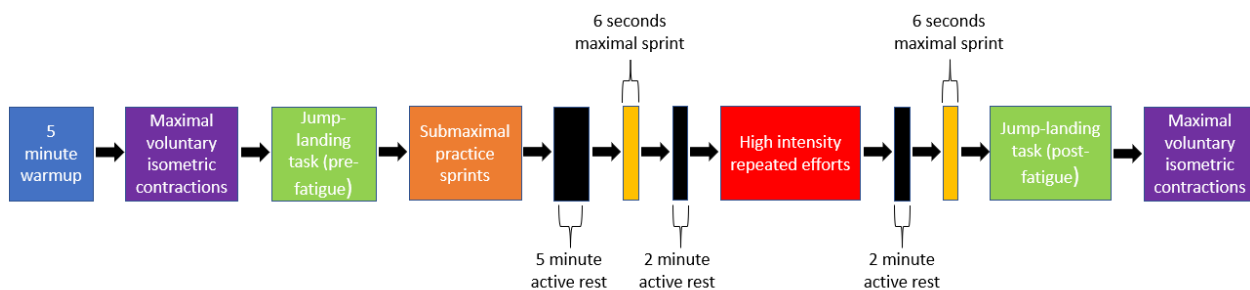


greater trochanter, with approximately 5° knee flexion with the pedal positioned at the lowest point of each cycle (Harvey et al., 2017). Handlebars were adjusted to participant preference. The participant was instructed to maintain grip on the handlebars and remain seated during the cycling protocol. Monark Anaerobic Test Software (Monark Anaerobic Test Software 3.0, Monark Exercise AB, Vansbro, Sweden) was used to record the raw cycle ergometer data to be analyzed offline.

## 2.3. Experimental protocol

### 2.3.1. Study design

The study followed a repeated pre/post within-subject design, involving a single-leg lateral jump-landing task before and after a fatigue protocol on the cycle ergometer (Figure 1). Prior to testing, the participant was instructed to wear their own athletic shirt, shorts, and flat-soled shoes (Aizawa et al., 2016). Before warming up, the jump-landing task was explained, and 5 practice trials were given for familiarization purposes. A standardized 5-minute warm-up was then completed on the cycle ergometer at a power output of 60-80 W for females and 80-100 W for males, and continued along the sequence described in Figure 1 and below.



**Figure 1.** Flowchart of the experimental protocol

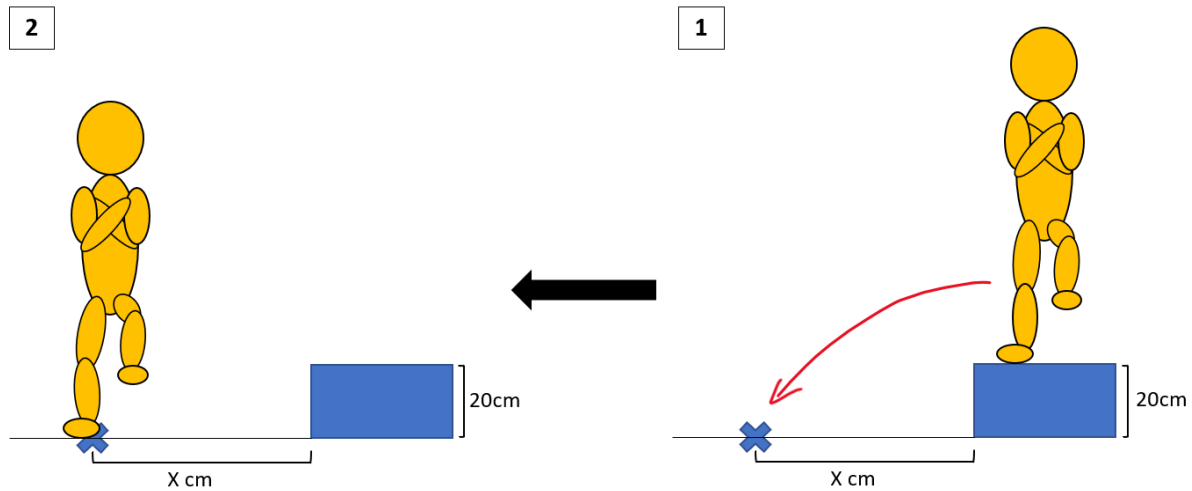
### 2.3.2. Maximal voluntary isometric contraction task

To normalize EMG data and quantify fatigue, a series of MVICs was performed on an isokinetic dynamometer (CON-TREX MultiJoint, Physiomed Elektromedizin AG, Schnaittach, Germany). Two types of knee flexion trials were assessed, the first with the participant lying in prone position, and the second with the participant in seated position. Placement of hamstring (BF and ST) EMG sensors was done prior to the prone MVIC trials. Prone knee flexion trials were

only taken before fatigue for EMG normalization purposes, as the hamstring sensors in the seated position were compressed between the participant's thigh and the seat of the dynamometer, which affected the quality of the EMG signal. For prone trials, the participant was instructed to let their arms hang off the sides of the table. Next, quadriceps (VL, RF and VM) EMG sensors were placed on the participant prior to seated MVIC trials. Seated knee extension trials were then performed for EMG normalization and baseline MVIC torque measurement for fatigue analysis. Then, seated knee flexion trials were performed for baseline MVIC torque measurement, in order to stay consistent with seated extension trials. Seated trials were performed with the participant sitting upright on the dynamometer, with their hips and shoulders strapped to the seat and hands gripping the edges of the seat by their sides. All seated MVIC trials were performed with the knee fixed at 90° knee flexion. For all MVIC trials, two trials were performed in each position, and the participant was instructed to pull or push as hard as they could, with a 2 s gradual increase in force, 3 s at 100%, and 2 s gradual decrease.

### *2.3.3. Jump-landing task*

Following the MVICs, the participant completed a pre-fatigue jump task which was based on the task described by Aizawa et al. (2016), the details of which are provided below. The task was performed using the participant's dominant leg. A 20 cm-high step was placed at a distance equal to the participant's leg length from the center of a mark on the floor (Figure 2). Leg length was measured as the distance from the ASIS to the medial malleolus. The participant was then instructed to stand on the step, facing forward, on their dominant leg, with the opposite knee bent. They were then instructed to keep their arms crossed across their chest with hands placed in the opposite axillae for the duration of the jump task, to eliminate effects of arm motion. Finally, they were instructed to jump sideways to land with their dominant foot on the mark as naturally as possible, with no intended upwards motion, and to maintain balance for 5 s upon landing. A successful trial was considered having full foot contact on the marked area, with no sliding or moving of the foot upon landing. The sole of the opposite foot must also have not touched the floor. Three successful trials were taken for data analysis. Jump trials were performed consecutively until 3 successful trials were achieved. The participant was not informed of the criteria of a successful trial and did not receive any specific coaching prior to or during trials, to keep landing techniques as natural as possible. Failed trials were determined visually by the tester.



**Figure 2.** A frontal view of the jump-landing task as described by Aizawa et al., 2016.

#### 2.3.4. *Fatigue protocol*

Prior to the fatigue protocol, 4 sets of 30 s practice cycling bouts were performed, as well as a pre-fatigue 6 s sprint. Practice cycling bouts were interspersed by 90 s of active rest. Active rest periods were unloaded, and the participant was instructed to maintain a power output of 60-80 W for females, and 80-100 W for males. After the practice bouts, the participant received 5 mins of active rest before the baseline 6 s sprint. The pre-fatigue sprint was then followed by 2 mins of active rest before the start of the fatigue protocol.

The fatigue protocol consisted of 9 sets of 30 s high-intensity cycling, with 90 s rest between each 30 s high-intensity bout. This high-intensity training protocol was selected as it is a common fitness test used in training by professional cycling teams (Colorni et al., submitted), and mimics high-intensity demands of sports requiring rapid changes of direction. Cycling bouts were performed at a fixed load of 0.005 kg per kg of the participant's body mass. The participant was instructed to pace themselves in order to accumulate the highest total distance they possibly could, summed across all 9 sets. Sets were interspersed with 90 s of active rest. Ratings of perceived exertion (RPE) were asked and verbally provided by the participant at the end of each set using the Borg CR-10 scale (Appendix 4) (Borg, 1982). Specifically, the participant was asked to "rate how hard, heavy and strenuous was the preceding [cycle]" (Hureau et al., 2016). A warning was given 30 s and 15 s before the start of the next set, along with a 5 s countdown to the start of the set. Additionally, the participant was instructed to take the last 2 s of the countdown to accelerate

to their desired pace. A whiteboard marking the number of sets completed was left visible to the participant during the task.

Upon completion of the last bout of high intensity cycling, a 2 min active rest period was performed, followed by a final 6 s maximal sprint. Directly after the final 6 s sprint, the participant completed the jump-landing task as previously described for post-fatigue measurements. In order to minimize recovery effects, the participant was given a maximum of 2 mins after completion of the final sprint to complete the post-fatigue jump task (3 successful trials). Any marked successful jump trial completed within 2 mins of the final sprint was taken for analysis, even if 3 successful trials were not achieved. On average, participants took  $1.5 \pm 0.7$  mins from the end of the final sprint to the completion of the final jump task. Additionally, participants took on average  $5.5 \pm 1.8$  jumps to achieve 3 successful trials post-fatigue. Following the post-fatigue jumps, the participant completed post-fatigue extension and flexion MVIC trials.

## *2.4. Data analysis*

### *2.4.1. Knee joint kinematics*

All kinematic data were processed using a custom written Matlab script (MATLAB R2021b; MathWorks, Natick, MA). Joint angles were calculated during the landing phase, which was defined as the point of initial contact (IC) to 300 ms after IC (Sinsurin et al., 2013). IC was defined as the point in time the velocity of the marker on the 2<sup>nd</sup> toe reached zero (Lessi et al., 2017). Position data of the lateral thigh, knee joint centre and lateral tibia markers were gathered from the raw kinematic data and filtered using a 4<sup>th</sup> order, zero-lag Butterworth low-pass filter at a cut-off frequency of 10 Hz.

Knee joint angles were calculated as the tibia movement relative to the thigh, with the jump being performed in the XZ (frontal) plane. The thigh segment (upper vector) was defined as the line from the lateral thigh marker to the knee JAC, and the tibia segment (lower vector) was defined as the line from the knee JAC to the lateral tibia marker. Joint angles of interest were calculated as the angle between the upper and lower vectors and included knee flexion and knee abduction at the point of IC, as well as the peak values during the landing phase. Knee flexion was calculated as the knee angle projected onto the YZ (sagittal) plane, and knee abduction was calculated as the knee angle projected onto the XZ plane. Finally, knee joint ROM was calculated in each plane as

the joint angle at IC subtracted from the peak joint angle. ROM angles were taken for statistical analysis to express joint displacement in fatigue.

#### *2.4.2. Electromyography*

All EMG data were processed using Matlab. Raw EMG signals were filtered (4<sup>th</sup> order, zero-lag Butterworth band-pass filter at 20-400 Hz), rectified and smoothed by an RMS 20 ms moving window. Filtered data was normalized to pre-fatigue MVIC (prone knee flexion and seated knee extension) and expressed as a percent MVIC for analysis. The normalized RMS (nRMS) of each muscle during the landing phase was taken for analysis. To examine differences in medial to lateral activation, the ST:BF ratio was calculated as the nRMS of the ST divided by the nRMS of the BF, and the VM:VL ratio was calculated as the nRMS of VM divided by the nRMS of the VL during the landing phase (IC to 300 ms later). To represent general quadriceps behaviour during the landing phase, the nRMS of the VL, RF and VM were averaged. Similarly, the nRMS of the ST and BF were averaged to represent the general hamstrings behaviour (Chappell et al., 2007). Finally, the averaged quadriceps nRMS was divided by the averaged hamstrings nRMS to represent differences in anteroposterior activation. Non-normalized RMS data of each muscle was also analysed during landing and taken for statistical analysis. This was performed to check for consistencies with nRMS data and identify possible sex-based impacts on EMG data from normalizing to MVIC. The average of 3 successful jump trials in the pre- and post-fatigue conditions was taken for statistical analysis of all kinematic and EMG variables. If 3 successful trials were not achieved in the post-fatigue condition, the average of the successful recorded trials was taken for analysis (could vary between 1 to 2 jumps).

#### *2.4.3. Evidence of Fatigue*

Evidence of fatigue induced by the cycling protocol was confirmed through RPE, sprint, and MVIC data. Sprint power output and MVIC torque data were processed using custom written Matlab script. The peak value of each MVIC trial (pre/post-fatigue) was extracted and taken for statistical analysis for both torque and EMG RMS data.

#### *2.5. Statistical analysis*

Statistical analyses were conducted using SPSS statistical software (version 27.0; IBM Corp; Armonk, NY). All data were analyzed using general estimating equations (GEE) and expressed as

mean  $\pm$  SE. Normal distribution of the data was confirmed via Shapiro-Wilk test after inspection of outliers. All data were then analyzed with GEE by a two-way (Sex x Fatigue) design, with  $\alpha$  set at  $P < 0.05$ . GEE was conducted using gamma probability distribution and a log link function. Kinematic and EMG outcomes were considered dependent variables, with fatigue set as a within-subject factor. When significance was found, post-hoc sequential Bonferroni pairwise comparisons were performed. Data were analyzed using GEE to provide greater statistical robustness over general linear models.

### **3. Results**

#### *3.1. Baseline measures*

Participant demographics and fatigue outcomes are reported in Tables 1 and 2, respectively. From Table 1, there were significant group (i.e. sex) differences in height and mass, and amount of minutes spent performing vigorous sports-related exercise the week prior, with the majority of participants being recruited from varsity rugby teams. Table 2 provides evidence that the high-intensity task was effective at inducing fatigue. According to a main Fatigue effect, RPE increased significantly from the first ( $4.2 \pm 0.3$ ) to the last ( $8.0 \pm 0.3$ ) bout. However, sprint power output remained similar in both sexes before and after fatigue. In contrast, peak torque during knee extension MVICs decreased significantly with fatigue (main Time effect,  $P < 0.001$ ), with no significant changes in MVIC knee flexion torque. Finally, EMG RMS values of the VL and VM during the knee extension MVIC differed significantly between fatigue conditions (VL,  $P < 0.001$ ; VM,  $P < 0.001$ ). It should also be noted that main effects of sex in those baseline, pre- vs post-fatigue measures, were also observed, in MVIC extension torque, MVIC flexion torque, and VL and VM EMG RMS during the knee extension torque, with a Sex x Fatigue interaction observed in VM EMG RMS during MVIC knee extension torque.

**Table 1.** Participant demographics reported in mean and standard deviation.

	Female	Male	<i>P</i> -value
<i>N</i>	15	10	NA
Age (yrs)	22.0 ± 2.0	21.2 ± 1.5	.285
Height (cm)	165.5 ± 4.2	177.5 ± 5.7	< . <b>.001</b>
Mass (kg)	66.3 ± 5.3	79.9 ± 9.2	< . <b>.001</b>
<b>IPAQ (Physical activity in the last 7 days)</b>			
Work (mins)	196.0 ± 442.3	150.0 ± 474.3	.807
Transportation (mins)	309.3 ± 230.1	311.0 ± 236.4	.986
Household (mins)	92.7 ± 119.1	27.5 ± 39.1	.065
Sport – vigorous (mins)	246.0 ± 174.4	478.5 ± 248.6	<b>.011</b>
Sport – moderate (mins)	49.0 ± 49.6	88.0 ± 110.6	.316
Hours spent sitting	40.5 ± 9.7	33.5 ± 13.2	.137
<b>Participants by sport (<i>N</i>)</b>			<b>Total (<i>N</i>)</b>
Rugby	11	4	15
Soccer	1	3	4
Hockey	1	1	2
Lacrosse	1	2	3
Field Hockey	1	0	1

**Table 2.** Sex and Fatigue effects on baseline measures, reported in mean and standard error.

	Sex x Fatigue					<i>P</i>	Sex			Fatigue		
	Female		Male		<i>P</i>		Female		<i>P</i>	Pre	Post	<i>P</i>
	Pre	Post	Pre	Post			Female	Male				
RPE (/10)	4.2 ± 0.4	8.2 ± 0.4	4.3 ± 0.5	7.9 ± 0.6	.642	5.9 ± 0.4	5.8 ± 0.5	.860	4.2 ± 0.3	8.0 ± 0.3	< .001	
Sprint power output (W/BW)	46.2 ± 1.2	45.1 ± 1.7	47.4 ± 1.8	47.0 ± 1.8	.642	45.6 ± 1.4	47.2 ± 1.7	.480	46.8 ± 1.1	46.0 ± 1.2	.364	
MVIC extension torque (Nm)	143.9 ± 8.9	123.8 ± 8.4	222.9 ± 20.3	184.3 ± 16.0	.416	133.5 ± 8.5	202.7 ± 17.5	< .001	179.1 ± 9.9	151.1 ± 8.3	< .001	
MVIC flexion torque (Nm)	66.7 ± 3.8	68.3 ± 3.9	100.9 ± 8.0	102.5 ± 6.9	.867	67.5 ± 3.6	101.7 ± 7.4	< .001	82.1 ± 4.0	83.7 ± 3.7	.377	
MVIC EMG RMS of VL (μV)	274.2 ± 20.2	120.8 ± 17.5	451.0 ± 42.2	153.8 ± 27.8	.293	182.1± 15.3	263.4 ± 28.8	.007	351.7 ± 21.0	136.3 ± 15.8	< .001	
MVIC EMG RMS of VM (μV)	378.0 ± 49.9	224.7 ± 28.6	790.0 ± 143.6	263.7 ± 53.3	.010	291.4 ± 34.5	456.4 ± 75.4	.027	546.4 ± 61.4	243.4 ± 29.1	< .001	

RPE: Rating of perceived exertion; W/BW: Watts (W) per body weight (BW); Nm: Newton meters; μV: microvolts.

### 3.2. Knee kinematic measures during landing

The kinematic results of knee motion during the jump-landing task are displayed in Table 3. No significant main or interaction effects were found in any of the knee kinematic outcomes. However, females showed slightly higher knee flexion and knee abduction angles at IC, as well as greater peak angles and ROM than males. Additionally, females exhibited a trend towards significance of greater knee abduction ROM than males ( $F = 7.7 \pm 1.2$ ,  $M = 4.8 \pm 1.1$ ,  $P = 0.093$ ). With regards to fatigue, knee flexion angles tended to decrease slightly after fatigue, with a trend towards significance at IC (pre =  $26.3 \pm 1.1$ , post =  $25.2 \pm 1.45$ ,  $P = 0.053$ ). Knee abduction angles increased slightly with fatigue at both IC (pre =  $16.5 \pm 0.9$ , post =  $16.9 \pm 0.9$ ) and at peak values (pre =  $21.9 \pm 1.6$ , post =  $23.1 \pm 1.4$ ). Furthermore, knee ROM increased slightly for both knee flexion (pre =  $28.0 \pm 1.0$ , post =  $29.1 \pm 0.9$ ) and abduction (pre =  $5.7 \pm 1.1$ , post =  $6.4 \pm 1.1$ ) post-fatigue, showing more joint displacement across both sexes. Interestingly, females showed a slight decrease in knee abduction ROM post-fatigue (pre =  $8.0 \pm 1.3$ , post =  $7.4 \pm 1.5$ ), whereas males showed a slight increase with fatigue (pre =  $4.1 \pm 1.4$ , post =  $5.6 \pm 1.5$ ), although, again, no significant interaction or main effects were found on any of those kinematic measures.



**Table 3.** Mean and standard error (degrees) for kinematic data.

Kinematic data at IC											
	Sex x Fatigue					Sex			Fatigue		
	Female		Male		<i>P</i>	Female	Male	<i>P</i>	Pre	Post	<i>P</i>
	Pre	Post	Pre	Post							
Knee flexion	26.3 ± 1.5	25.6 ± 1.9	26.3 ± 1.6	24.8 ± 2.2	.495	25.9 ± 1.7	25.5 ± 1.9	.869	26.3 ± 1.1	25.2 ± 1.45	.053
Knee abduction	16.7 ± 0.8	17.2 ± 0.9	16.3 ± 1.6	16.5 ± 1.5	.606	17.0 ± 0.9	16.4 ± 1.6	.729	16.5 ± 0.9	16.9 ± 0.9	.169
Peak kinematic data											
	Sex x Fatigue					Sex			Fatigue		
	Female		Male		<i>P</i>	Female	Male	<i>P</i>	Pre	Post	<i>P</i>
	Pre	Post	Pre	Post							
Knee flexion	55.8 ± 1.2	54.8 ± 1.9	53.5 ± 2.2	53.7 ± 2.7	.553	55.3 ± 1.4	53.6 ± 2.4	.537	54.6 ± 1.3	54.2 ± 1.7	.731
Knee abduction	24.2 ± 2.0	24.6 ± 2.1	19.7 ± 2.3	21.7 ± 1.8	.429	24.4 ± 1.9	20.7 ± 1.9	.170	21.9 ± 1.6	23.1 ± 1.4	.277
Kinematic ROM data											
	Sex x Fatigue					Sex			Fatigue		
	Female		Male		<i>P</i>	Female	Male	<i>P</i>	Pre	Post	<i>P</i>
	Pre	Post	Pre	Post							
Knee flexion	28.9 ± 1.1	29.2 ± 1.4	27.2 ± 1.7	28.9 ± 1.1	.403	29.1 ± 1.0	28.0 ± 1.4	.555	28.0 ± 1.0	29.1 ± 0.9	.228
Knee abduction	8.0 ± 1.3	7.4 ± 1.5	4.1 ± 1.4	5.6 ± 1.5	.396	7.7 ± 1.2	4.8 ± 1.1	.093	5.7 ± 1.1	6.4 ± 1.1	.619

IC: Initial Contact; ROM: Range of motion

### 3.3. Electromyographic parameters during landing

EMG RMS results of the thigh muscles during landing are displayed in Table 4. Muscle co-activation ratios during landing are displayed in Table 5. Sex x Fatigue interactions were observed in EMG RMS of the VL ( $P = 0.002$ ) and the RF ( $P < 0.001$ ), as well as in the co-activation ratio between the quadriceps and the hamstrings ( $P = 0.003$ ) during landing (Figure 3). Females had significantly higher activation of the VL and RF (VL:  $61.3 \pm 9.5$ , RF:  $119.0 \pm 25.4$  % MVIC) than males (VL:  $27.9 \pm 5.3$ , RF:  $26.4 \pm 7.0$ ) before fatigue (Figure 4). Females also had significantly lower activation of the VL and RF post-fatigue (VL:  $28.1 \pm 4.5$ , RF:  $33.4 \pm 6.0$ ) compared to pre-fatigue. No significant difference in activation was found between sexes post-fatigue. With regards to Q:H co-activation, females had a larger Q:H co-activation ratio ( $6.5 \pm 1.1$ ) than males ( $1.7 \pm 0.4$ ) pre-fatigue. Females also had significantly lower Q:H co-activation post-fatigue ( $2.8 \pm 0.5$ ) compared to pre-fatigue. No significant difference was observed in Q:H co-

activation between females and males post-fatigue. No other significant interaction effects were found in any of the other variables reported in Tables 4 and 5.

**Table 4.** Mean and standard error EMG RMS during landing expressed as % MVIC.

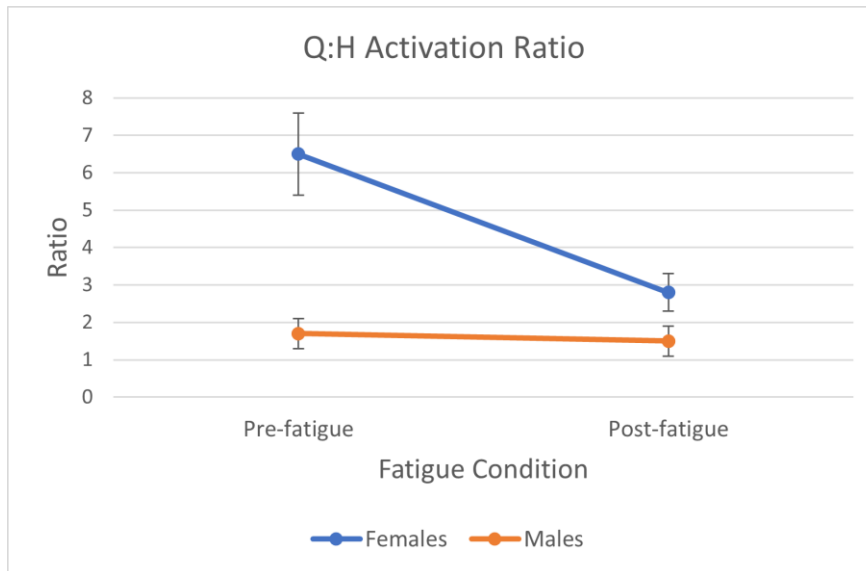
	Sex x Fatigue					<i>P</i>	Sex			Fatigue		
	Female		Male		<i>P</i>		Female	Male	<i>P</i>	Pre	Post	<i>P</i>
	Pre	Post	Pre	Post								
BF	18.2 ± 1.8	18.7 ± 2.9	17.5 ± 3.9	15.1 ± 3.2	.222	18.5 ± 2.0	16.2 ± 3.4	.592	17.9 ± 2.1	16.8 ± 2.2	.407	
ST	31.8 ± 4.6	33.6 ± 8.4	31.5 ± 9.4	40.1± 13.0	.628	32.7 ± 5.9	35.5 ± 9.4	.795	31.6 ± 5.2	36.7 ± 7.5	.440	
VL	61.3 ± 9.5	28.1 ± 4.5	27.9 ± 5.3	33.1 ± 10.2	<b>.002</b>	41.5 ± 5.3	30.4 ± 6.8	.229	41.4 ± 5.1	30.5 ± 5.3	<b>.045</b>	
RF	119.0 ± 25.4	33.4 ± 6.0	26.4 ± 7.0	26.9 ± 6.9	<b>&lt;.001</b>	63.0 ± 10.1	26.7 ± 6.5	<b>.003</b>	56.0 ± 9.6	30.0 ± 4.7	<b>&lt;.001</b>	
VM	85.2 ± 14.4	66.1 ± 11.5	28.7 ± 6.3	27.9 ± 6.9	.352	75.0 ± 11.3	28.3 ± 6.2	<b>&lt;.001</b>	49.4 ± 6.9	42.9 ± 6.5	.242	

BF: Biceps femoris; ST: Semitendinosus, VL: Vastus lateralis, RF: Rectus femoris; VM: Vastus medialis

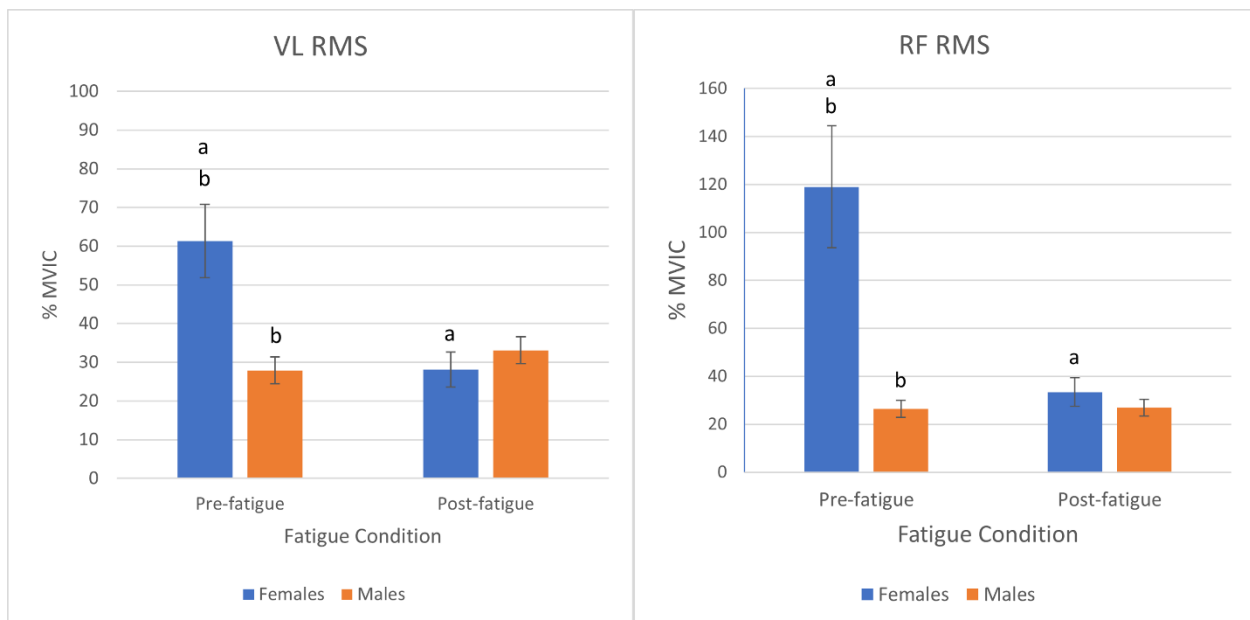
**Table 5.** Mean and standard error muscle activation ratios during landing.

	Sex x Fatigue				<i>P</i>	Sex			Fatigue			
	Female		Male			<i>P</i>	Female	Male	<i>P</i>	Pre	Post	<i>P</i>
	Pre	Post	Pre	Post								
VM:VL	1.3 ± 0.2	2.0 ± 0.3	0.9 ± 0.1	1.4 ± 0.3	.882	1.6 ± 0.2	1.1 ± 0.1	<b>.039</b>	1.1 ± 0.1	1.7 ± 0.2	<b>&lt;.001</b>	
ST:BF	2.1 ± 0.3	2.2 ± 0.6	2.3 ± 0.4	2.2 ± 0.5	.679	2.2 ± 0.4	2.2 ± 0.4	.912	2.2 ± 0.3	2.2 ± 0.4	0.945	
Q:H	6.5 ± 1.1	2.8 ± 0.5	1.7 ± 0.4	1.5 ± 0.4	<b>.003</b>	4.3 ± 0.6	1.6 ± 0.4	<b>&lt;.001</b>	3.3 ± 0.5	2.1 ± 0.3	<b>&lt;.001</b>	

VM:VL: Vastus medialis (VM) activation relative to Vastus lateralis (VL) activation; ST:BF: Semitendinosus (ST) activation relative to Biceps femoris (BF) activation; Q:H: Quadriceps (Q) activation relative to hamstrings (H), where Q activation equals the average activation of the vastus lateralis, rectus femoris, and vastus medialis, and H activation equals the average activation of biceps femoris and semitendinosus root mean square.



**Figure 3.** Quadriceps to Hamstrings (Q:H) activation ratio across fatigue conditions, where quadriceps activation equals the average activation of the vastus lateralis, rectus femoris, and vastus medialis, and hamstrings activation equals the average activation of biceps femoris and semitendinosus root mean square. Error bars indicate standard error for each group.



**Figure 4.** Vastus lateralis (VL) and rectus femoris (RF) root mean square expressed as % maximal voluntary isometric contraction (MVIC) across fatigue conditions. <sup>a</sup> Denotes significant differences between pre/post-fatigue conditions within females. <sup>b</sup> Denotes significant differences between sexes pre-fatigue. Error bars indicate standard error for each group.

In addition to Sex x Fatigue interactions, some main effects were observed. Main effects of sex were observed in VM activation ( $P < 0.001$ ) and in VM activation relative to VL (VM:VL) ( $P = 0.039$ ). Additionally, main effects of fatigue were observed in VM:VL co-activation during landing ( $P < 0.001$ ). Females ( $75.0 \pm 11.3$ ) had significantly higher activation of the VM compared to males ( $28.3 \pm 6.2$ ), as well as a higher VM:VL co-activation ratio. Furthermore, VM:VL activation was higher post-fatigue ( $1.7 \pm 0.2$ ) compared to the pre-fatigue condition ( $1.1 \pm 0.1$ ) across both sexes. A main effect of fatigue was also observed in VL activation during landing ( $P = 0.045$ ), where VL activation significantly decreased in the post-fatigue condition ( $30.5 \pm 5.3$ ) compared to the pre-fatigue condition ( $41.4 \pm 5.1$ ).

Finally, we performed follow-up analyses on jump landing EMG data to determine whether normalizing the EMG data to the pre-fatigue MVIC values (displayed in Table 2) had an impact on sex-specific results. Non-normalized EMG RMS data of the thigh muscles are displayed in Table 6, with non-normalized calculated ratios displayed in Table 7. These analyses showed that in contrast to results from normalized data, no Sex x Fatigue interaction was observed in non-normalized VL activation during landing. Females displayed similar levels of activation in VL to males in the pre-fatigue condition, with equal level of activation post-fatigue. Females also displayed a smaller decrease in VL activation post-fatigue than compared to the normalized data. Additionally, no Sex main effect was observed in VM:VL co-activation with non-normalized data. No other significant differences between normalized and non-normalized data were observed.

**Table 6.** Mean and standard error of non-normalized EMG RMS during landing expressed in microvolts ( $\mu\text{V}$ ).

	Sex x Fatigue					Sex			Fatigue		
	Female		Male		<i>P</i>	Female	Male	<i>P</i>	Pre	Post	<i>P</i>
	Pre	Post	Pre	Post							
BF	41.8 ±	39.1	47.0 ±	50.0 ±	.554	40.4 ±	48.4 ±	.418	44.3 ±	44.2	.977
	3.8	± 5.3	11.5	9.6		4.0	9.7		9.7	± 5.2	
ST	72.7 ±	75.4	49.6 ±	92.1 ±	.168	74.1 ±	67.6 ±	.697	60.0 ±	83.4	.120
	7.6	±	8.7	29.9		10.1	12.8		6.2	±	
VL	180.1	98.8	141.7	104.8	.139 <sup>a</sup>	133.4	121.9	.737	159.8	101.7	<.001
	± 29.3	±	± 31.2	± 24.8		± 22.1	± 25.5		± 21.9	±	
RF	284.3 ±	96.2	90.9 ±	98.2 ±	<.001	165.4 ±	94.5 ±	.078	160.7 ±	97.2	.001
	54.8	±	25.4	28.9		28.2	25.3		27.3	±	
VM	261.8 ±	218.5	189.8 ±	132.6 ±	.555	239.1 ±	158.7 ±	.023	222.9 ±	170.2	.073
	31.7	±	33.5	25.6		25.0	23.5		23.9	±	

BF: Biceps femoris; ST: Semitendinosus, VL: Vastus lateralis, RF: Rectus femoris; VM: Vastus medialis. <sup>a</sup>Significance differs from normalized data.

**Table 7.** Mean and standard error non-normalized muscle activation ratios during landing.

	Sex x Fatigue					<i>P</i>	Sex			Fatigue			
	Female		Male		<i>P</i>		Female	Male	<i>P</i>	Pre	Post	<i>P</i>	
	Pre	Post	Pre	Post									
VM:VL	1.5 ± 0.2	2.2 ± 0.3	1.6 ± 0.3	2.3 ± 0.4	.899	1.8 ± 0.2	1.9 ± 0.3	.735 <sup>a</sup>	1.5 ± 0.2	2.2 ± 0.2	.001		
ST:BF	1.8 ± 0.2	1.9 ± 0.4	2.1 ± 0.5	1.7 ± 0.5	.378	1.9 ± 0.3	1.9 ± 0.4	.885	1.9 ± 0.2	1.8 ± 0.3	.759		
Q:H	7.7 ± 1.3	3.7 ± 0.7	2.9 ± 0.7	2.7 ± 0.6	.007	5.3 ± 0.8	2.8 ± 0.6	.014	4.8 ± 0.7	3.2 ± 0.5	.001		

VM:VL: Vastus medialis (VM) activation relative to Vastus lateralis (VL) activation; ST:BF: Semitendinosus (ST) activation relative to Biceps femoris (BF) activation; Q:H: Quadriceps (Q) activation relative to hamstrings (H), where Q activation equals the average activation of the vastus lateralis, rectus femoris, and vastus medialis, and H activation equals the average activation of biceps femoris and semitendinosus root mean square. <sup>a</sup>Significance differs from normalized.

#### 4. Discussion

The present study aimed to quantify sex-specific changes with fatigue in muscle activation and joint kinematics during a lateral jump-landing task. Our main findings revealed that 1) males and females displayed different responses to fatigue in quadriceps (VL and RF) activation amplitude, 2) females displayed a decrease in Q:H co-activation ratio with fatigue, resulting in a similar Q:H ratio after fatigue compared to males, 3) VM:VL co-activation differed between sexes regardless of fatigue conditions, 4) normalization of EMG data resulted in significant effects in

VL activation and VM:VL co-activation that were not reported in non-normalized data, and 5) knee flexion and abduction ranges of motion were similar between both sexes and fatigue conditions.

#### *4.1. Electromyographic parameters*

##### *4.1.1. Quadriceps activation in females*

Quadriceps fatigue differed significantly between sexes during landing. Specifically, females displayed higher VL and RF activation than males before fatigue, with a significant decrease in quadriceps activation after fatigue. These results suggest that females rely more on the quadriceps than males to stabilize their knee joint when landing, at least in the absence of fatigue. This is consistent with previous studies that have found higher VL and RF activation in females during various jump-landing tasks (Seydahmadi et al., 2022; Hughes & Dally, 2015). Although this can seem like a beneficial joint stabilization strategy, in addition to anterior shear force at the proximal tibia, higher quadriceps activation adds compressive force to the knee joint. This can lead to increased strain to the ACL and may reflect an inability to absorb energy upon landing by the quadriceps (Meyer & Haut, 2005). Quadriceps dominance in females may also explain the greater drop in VL and RF activation after fatigue, where the VL and RF experienced more usage and thus, more fatigue than other muscles.

##### *4.1.2. Q:H co-activation ratio*

Amongst our main results, we also showed that females exhibited a higher Q:H co-activation ratio than males before fatigue, with a significant decrease after fatigue. However, the Q:H co-activation ratio was highly influenced by changes in quadriceps activity, as the hamstrings did not demonstrate significant differences between sexes or fatigue conditions. This suggests that during landing before fatigue, the quadriceps served as the main contributor to possible injury risk in females, regardless of hamstring behaviour. This is consistent with previous findings that demonstrated that the quadriceps may play a larger role in knee stability than hamstrings during a single-leg drop landing (Kellis & Kouvelioti, 2009). Furthermore, studies have found a relation between ACL strain and higher quadriceps activity, even at knee angles where the quadriceps produced posterior force (Boden et al., 2010). Posterior force also produced by the hamstrings is thought to counter anterior shear force that contributes to ACL strain. However, in accordance

with our findings, the hamstrings have been found to exhibit low activation at landing, particularly in females, which suggests a hindered force production and less ability to stabilize the knee (Simonsen et al., 2000). Thus, our results, combined with the understanding of ACL injury mechanisms, support that it may be beneficial to focus on training different landing patterns and quadriceps control in ACL injury prevention, and especially in females.

Interestingly, Q:H co-activation in females decreased to resemble that of males after fatigue. This suggests that females had a significantly higher risk of injury before fatigue but were less at-risk after fatigue, at least from the perspective of the Q:H co-activation ratio. This differs from previous studies that have found an increase in Q:H co-activation ratio with fatigue, although either no sex-specific effects of fatigue were reported (Padua et al., 2006) or the study did not perform a sex-stratified analysis of results (Kellis & Kouvelioti, 2009). A possible explanation for the greater Q:H co-activation ratio that was only seen in females before fatigue could be that males are more effective at priming or warming up their muscles to an optimal muscle temperature for high performance and injury prevention. As ACL injuries tend to occur almost immediately after impact, fast-twitch muscle fibers are needed to resist sudden, quick motions and high forces. As males are known to have a higher composition of type II muscle fibers (Miller et al., 1993), it may be easier for them to prime the muscle for sudden activation compared to females. Thus, inadequate warm-up may play a similarly important role in knee injury prevalence as fatigue. Furthermore, previous studies have found similar ACL injury occurrence between the first and second halves of competition (Anderson et al., 2019; Doyle et al., 2018). Additionally, possible sex differences in pre-fatigue maximal contraction ability may support the hypothesis that females may show difficulties in optimally activating the muscles before exercise. This can be tested by comparing non-normalized data to results that are normalized to MVIC, which indeed shows an effect of sex in our post-hoc analyses. However, more research is needed to examine sex differences in muscle priming ability to support this explanation.

#### *4.1.3. VM:VL co-activation ratio*

VM:VL co-activation was significantly greater in females across both fatigue conditions. However, because higher VL activation has been shown to add strain to the ACL (Peel et al., 2021), a larger VM:VL ratio in females could suggest that the VM worked harder to counteract effects of the VL, such as lateral compression of the knee joint. Lateral forces on the knee joint

could further be related to sex-specific anatomical characteristics, such as greater Q angle and knee abduction known to occur in females (Khasawneh et al., 2019; Loudon, 2016). This could lead to a protective effort from the VM to attempt to stabilize the knee laterally in females. Additionally, previous findings reported that increased medial activation during forward hopping helped decrease knee abduction angles (Palmieri-Smith et al., 2008). Thus, targeting training of the VM specifically could improve females' knee stability during landing. More research is required to find an optimal activation ratio between the VM and VL for injury prevention in females.

#### *4.1.4. VL activation in females*

When compared to results from data normalized to MVIC, non-normalized results differed in VL activation and VM:VL activation ratio. Specifically, when results were not normalized to VL MVIC, males and females behaved similarly in VL activation and VM:VL co-activation during landing. However, when VL values were normalized, females showed a significantly higher VL activation, which is reflective of a hindered ability to maximally activate the VL when performing a MVIC. The ability to suddenly activate the VL could play an important role in shock absorption during landing, as the VL is a large contributor to ACL loading compared to other quadriceps muscles. Furthermore, it is well known that females have greater subcutaneous fat than males (Westerbacka et al., 2004), such that the results of sex differences in non-normalized data could reflect the greater attenuation of the high-frequency EMG content due to subcutaneous fat in females. It has previously been shown that different normalization methods have influenced sex-specific comparisons under fatigue (Cid et al., 2020). Therefore, future studies comparing muscle activation patterns of males and females in different fatigue or injury states should be mindful of the potential impact of the EMG normalization method, and should consider interpreting their group difference results with caution and consideration for this important methodological step of sex differences studies.

#### *4.2. Kinematic parameters*

A tertiary objective of the current study was to examine sex-specific changes in knee flexion and abduction angles and landing motion amplitude with fatigue. Our results show that males and females displayed similar levels of knee joint displacement in flexion and abduction. These results contrast with previous studies that have found both Sex and Fatigue effects in knee flexion and abduction angles during landing or single-leg tasks (Kernozek et al., 2008; Weeks et



al., 2015). Differences between results may be due to different fatigue protocols and participant populations, where the implementation of a high-intensity intermittent cycling task in trained varsity athletes may have had less influence on knee kinematics. However, we did observe a trend towards greater knee abduction displacement in females, which suggests greater frontal plane displacement and joint instability during landing. This trend is consistent with findings of greater mediolateral displacement in females during a single-leg squat (Weeks et al., 2015). A trend towards significance was also observed on knee flexion angles at IC with fatigue, which decreased in both sexes. This suggests a decreased ability to absorb higher ground reaction forces on impact and a more upright landing, which could add load to the ACL (Aizawa et al., 2016). Nevertheless, we conclude that there were no significant effects on any of our kinematic parameters. Overall, implementation of different fatigue protocols and a variety of landing tasks across the frontal plane could provide more insight into sex-specific effects of fatigue on knee joint kinematics.

#### *4.3. Limitations*

A relatively small sample size should be considered during interpretation of the results. The calculated sample size of 28 participants was not met, with only 25 total participants recruited. Additionally, the measurement of knee joint kinematics during a high-impact loading task using skin-based retroreflective markers may pose as a limitation in reporting accurate changes in joint angles. The use of skin-mounted markers requires a gross estimation of the knee joint centre of rotation, that may exhibit a sex-based bias due to physiological differences. On another note, one of the most common methods of EMG normalization for a jump task is normalizing data to MVIC trials. However, different methods of normalization should be explored in future, as our results prove that there were inconsistencies between significance reported in normalized data versus non-normalized data. The menstrual cycle in female athletes was also not considered for our study. Our study was part of a larger scale study that required participants to partake in 2 testing sessions spaced 3-7 days apart (with the other session testing another type of fatigue protocol, and the order of sessions randomized), which caused scheduling difficulties with menstrual cycle timing. It is noted that some studies have reported that the menstrual cycle has an impact on knee injuries in female athletes (Herzberg et al., 2017). However, others have found no significant differences in ACL laxity and injury risk across the menstrual cycle (Shafiei et al., 2016), with a recent meta-analysis reporting inconclusive results on the impact of the menstrual cycle on ACL injury

(Dos'Santos et al., 2023). Finally, differences between sports and training schedules could influence jumping and landing patterns. Despite recruiting participants from sports that required similar movements, different training intensities amongst athletes may influence fatigue effects.

## **5. Conclusion**

The present study is the first to examine sex-specific effects of fatigue on muscle activation patterns and knee joint kinematics during a single-leg lateral landing task. We found that females demonstrated a higher risk of injury from high Q:H activity pre-fatigue, with a greater drop in quadriceps control than males. We also found that females displayed higher VM:VL co-activation, and that both sexes exhibited similar knee joint displacement across the frontal and sagittal planes. These results show that neuromuscular differences exist between the sexes, and suggest that the ability to efficiently warm up the muscles, in addition to fatigue, could play a role in injury risk, especially in females. Furthermore, these results can help clinicians and coaches better understand and adapt warm-up approaches, as well as target training of certain muscle components to help with injury prevention and rehabilitation. Future studies should investigate sex differences in a wider variety of sports and movements that are associated with injury risk. Additionally, studies should consider more sports-specific fatigue protocols, along with additional methods of movement analysis. Taken together, ours and future results of follow-up studies can help better understand effects of sex and fatigue on lower extremity muscle activation and movement patterns during sporting manoeuvres.

## **Acknowledgements**

The authors would like to thank the Sylvan Adams Sports Science Institute (SASSI) for supporting the project by a graduate student award. The authors would also like to thank the pilot subjects and participants for their contributions to the project.

## **Disclosure Statement**

The authors report no potential conflicts of interest.

## **Funding**

The study was funded by the Sylvan Adams Sports Science Institute (SASSI) through a graduate student award.

## CONCLUSION

The goal of this thesis was to investigate possible interactions of sex and fatigue on muscle activity and knee joint angles during lateral landings. Surface electromyography and joint kinematics were used to measure muscle activity and joint positioning respectively, in male and female varsity athletes during a single-leg lateral jump task. Our results indicated that during landing, males and females had different responses to fatigue in their patterns of muscle activation, despite showing similar knee joint angles. Overall, our findings show the importance of examining sex differences in muscle coordination patterns during lateral movements after fatigue as well as the impact of electromyography normalization methods on results of sex differences. It should be noted that the current project was limited by a small sample size. Future research should include a larger participant pool, as well as fatigue protocols that are more ecological to the individual sports of participants. Additionally, measurements taken during real-life practices or matches, as well as during sudden, reactive, knee stabilization tasks should also be assessed to further dive into muscle priming mechanisms and to optimize ecological validity. Furthermore, future studies should also consider examining additional muscles and joints, such as the hip and ankle, to broaden our understanding of movement patterns in direction-changing manoeuvres. Deepening knowledge on neuromuscular control during sideways movements can help build sex-specific training strategies and rehabilitation programs to prevent future ACL injuries.

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## APPENDICES

### Appendix 1. Consent Form



**McGill**

Faculty of  
Education

Department of  
Kinesiology and Physical Education

#### Consent form

##### Title

Sex differences in central and peripheral fatigue induced by repeated sprint exercise

##### Researcher in charge of project

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##### Research trainee

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##### Source of funding

Julie Côté

- NSERC Discovery operating grant (#RGPIN-2015-05111)
- McGill Sport Science operating grant (internal)
- Canada Foundation for Innovation equipment and infrastructure grant (#36715)

SangHoon Yoon

- NSERC Canada Graduate Scholarships – Master's

##### Conflicts of Interest

There are no known conflicts of interest among the researchers, institutions, and sponsors involved in this project.

##### Introduction

We are inviting you to take part in a research study that aims to advance our knowledge on exercise physiology. Before agreeing to participate in this project, please take the time to read and carefully consider the following information.

This consent form explains the aims of this study, the procedures, advantages, risks, and inconveniences as well as the persons to contact, if necessary.

This consent form may contain words that you do not understand. We invite you to ask any questions that you deem useful to the researcher and the other members of the staff assigned to the research project, and ask them to explain any words or information that are not clear to you.

### **Project description and objectives**

We are kindly inviting you to participate in our study that investigates why and how humans fatigue during repeated sprint exercise, and how males and females differ in these fatigue responses. Further, it also aims to see the effects of self-determining the rest duration in-between the sprints compared to a standardized rest duration (i.e., pre-determined).

The practical outcome of this project is to enhance athletic training and injury prevention by making it more personalized, tailored to the athlete's sex and allowing for greater autonomy by the athletes regarding their training configuration (e.g., choosing their work-to-rest ratio).

Thirty-six healthy subjects will be recruited for this project and will perform a laboratory assessment protocol. Results from this project will be disseminated in the forms of a master's thesis, peer-reviewed conference presentations, and manuscripts.

### **Study Procedures**

#### **COVID-19 safety protocol**

Please note that the following health and safety protocols have been implemented to minimize the risk of transmission of COVID-19 during your study participation. These measures have been devised from current federal and provincial public health, and McGill University directives:

- 2-meter distancing will be respected whenever possible.
- When 2-meter distancing is not possible (e.g., during placements of electrodes), the researcher will wear a face shield in addition to face mask (only one researcher will be within 2-meters for appropriate electrode placements and testing).
- Every person in the laboratory will wear a face mask, except for yourself when you perform the exercise protocol.
- Hand washing and sanitization will be done before and after study participation.
- All surfaces that have been in contact with or in close proximity to lab staff and study participants will be disinfected after each lab visit and between users.

Our laboratory members have training on prevention of infection spread and all McGill students and employees are required to fill out a self-assessment health questionnaire prior to every lab visit. This questionnaire asks if the individuals have any symptoms of COVID-19, have been in contact with anyone who has or has had COVID-19, and if they travelled out of the country within the last two weeks. Participation will be cancelled or postponed if any of these screening questions is responded with a 'yes'. Wearing a mask

that covers both the mouth and nose is mandatory inside all McGill buildings, in accordance with Québec public health regulations.

By agreeing to participate in this study, you acknowledge that you have been informed of the health and safety procedures in place and agree to follow them. Please be reminded that participation is voluntary, and you may decline or postpone participation at any time.

### **Experimental protocol**

The experimental procedure will take place at the Biomechanics of Occupation and Sport (BOS) Laboratory, located in Currie Gymnasium of McGill University in Montreal. You are asked to participate in ~~three~~ experimental sessions that will last from 1.5 to 2 hours each. Each session will involve three phases: Phase 1: preparation (30 minutes), Phase 2: pre-fatigue tests (45 minutes), Phase 3: fatigue protocol (15 minutes).

During Phase 1, you will be asked to fill out questionnaires, and the locations of surface electrodes and optodes will be marked on your skin using a make-up pen. ~~Ultrasound will be used to measure tissue thickness at these locations.~~ Then, the electrodes/optodes will be applied on the skin over your dominant lower limb muscles in order to measure their activity, ~~blood flow, and oxygenation.~~ None of these procedures are invasive and you will be asked to simply sit or stand during the placements of electrodes/optodes.

~~During Phase 2, an electrical stimulator will be used to stimulate the nerve that is connected to and controls your quadriceps muscles. The cathode and anode (small electrode pads) will be placed over the proximal region of your thigh and below your buttock, respectively. A maximal stimulation will be used during the protocol to measure neuromuscular fatigue, which requires that we first determine the stimulation intensity that evokes the greatest knee extension force. To do this, stimulation intensity will be increased gradually until the knee extension force plateaus.~~

Following this, baseline strength, blood flow, oxygenation, and lactate will be measured. ~~The lactate measurements will be invasive as the earlobe will be pricked with a sterile lancet to obtain a drop of blood.~~

During Phase 3, depending on the session, you will be asked to complete a fatiguing protocol on a stationary bicycle which will include 10 bouts of either:

- 10s sprints interspersed by 30s of rest (session 1),
- 30s sprints interspersed by 60s, or a self-determined rest duration (session 2 & 3).

The order of sessions 2 and 3 will be randomized using a simple computer (Excel) function. In both of these sessions, during the rest period, you will be asked to remain still on the stationary bicycle while blood flow and oxygenation are recorded, ~~as well as lactate via earlobe prick.~~ Prior and following the fatiguing exercise, you will be asked to perform maximal knee extension on a dynamometer ~~with electrical stimulations.~~ In addition, ~~pressure pain threshold will be measured, where you will be asked to press a signal button at the moment that a pressure sensation applied on your leg from the padded plate of an electronic algometer first starts to become painful.~~

## **Benefits**

You should not expect to benefit from your participation in this research. However, we hope to learn more about the fundamental science of human physiology, biomechanics, and applied knowledge in sport science.

## **Risks**

~~As the lactate measurements require pricking the earlobe, there are risks of infection. However, the researchers will take cautionary procedures by using sanitized gloves and only new sterile lancets. None of the other measurements represents any medical risk as they are non-invasive, including electrical stimulation of the leg and pressure pain threshold measurements. However, the electrical stimulation may feel uncomfortable, although it is very short in duration.~~

Also, you will experience some fatigue towards the end of the protocol which may cause some tenderness, stiffness, and/or pain in the lower limb area during and/or following the session. These symptoms should dissipate within 48 hours following the completion of the protocol.

In case of adverse events, emergency protocols are in place to contact emergency personnel (911, McGill security, Sports Medicine and Winsor Clinics).

## **Personal inconvenience**

The duration of each experimental session (approximately 1.5-2 hours) may represent an inconvenience for you. The possibility that some small areas (4, about 3x3 cm each) of the skin over your thigh muscles have to be shaved before placing the electrodes may also represent an inconvenience for you. Although it is hypo-allergenic, the adhesive tape used to fix the electrodes on your skin may occasionally produce some slight skin irritation. Should this happen, a hypo-allergic lotion will be applied on your skin to relieve skin irritation.

## **Compensation**

You will be compensated for your time of participation. This compensation will be prorated even if you opt to withdraw from the study prior to completion of the third session, such that you receive \$15.00 per session, to a maximum amount of \$45.00 upon completion of the third session. Transport costs as a result of your participation in this research can be reimbursed upon request and upon receipt of appropriate documentation.

## **Subject Rights**

You have the right to ask questions at any time. Your participation in this study is completely voluntary and you can opt not to participate or to withdraw at any time. There



are no penalties or repercussions if you choose not to participate in the study or if you choose to withdraw from the study after it has started. Your decision to participate or to withdraw will not affect your ability to access any services or resources to which you are otherwise entitled. If you choose to withdraw before completing the study, any information collected from you will be destroyed at your request.

### **Confidentiality**

All the personal information collected for this study will be codified with numbers and/or letters to ensure confidentiality. Physical data will be stored in a locked cabinet accessible only by the researcher's keys. Digital data will be encrypted and stored in a password-protected folder on a lab desktop computer.

Identifiable data will only be accessible by the researcher in charge and research trainee identified on this consent form on page 1, while other researchers involved in the project will only have access to the codified data. Other lab personnel may conduct further data analysis for future publications and conference presentations but will only have access to the de-identified data. If the results of this research project are presented or published, nothing will allow your identification. A member of the McGill Institutional Review Board, or a person designated by this Board or by McGill University may access the study data and records to assess the ethical conduct of this study.

The data will be kept for a period of 7 years from the date of publication. After this period, the data will be destroyed. The hard copy information will be shredded, and digital information will be erased. Lastly, no access to your medical file is required for this study.

### **Contact**

If you need to ask questions about the project, signal an adverse effect and/or an incident, you can contact Julie Côté, Ph.D., or SangHoon (Andrew) Yoon, B.Sc., at any time at the numbers indicated on the 1<sup>st</sup> page.

If you have any questions or concerns regarding your rights or welfare as a participant in this research study, you can contact the McGill Ethics Officer at 514-398-8302 or [ilde.lepore@mcgill.ca](mailto:ilde.lepore@mcgill.ca).

### DECLARATION OF CONSENT

**STUDY: Sex differences in central and peripheral fatigue induced by repeated sprint exercise**

I declare to have read and understood the project, the nature and the extent of the project, as well as the risks and inconveniences I am exposed to as described in the present document. I had the opportunity to ask all my questions concerning the different aspects of the study and to receive explanations to my satisfaction.

I do not give up any of my legal rights by signing this consent form. I do not free the researchers, or the institutions involved from their legal and professional obligations.

I, undersigned, voluntarily accept to participate in this study. I can withdraw at any time without any prejudice. I certify that I have received enough time to take my decision.

A signed copy of this information and consent form will be given to me. NAME OF PARTICIPANT (print): \_\_\_\_\_ SIGNATURE OF PARTICIPANT: \_\_\_\_\_ SIGNED IN \_\_\_\_\_, on \_\_\_\_\_, 20\_\_\_\_.

### COMMITMENT OF RESEARCHER

I, undersigned, \_\_\_\_\_, certify

- (a) having explained to the signatory the terms of the present form;
- (b) having answered all questions he/she asked concerning the study;
- (c) having clearly told him/her that he/she is at any moment free to withdraw from the research project described above; and
- (d) that I will give him/her a signed and dated copy of the present document.

\_\_\_\_\_  
Signature of person in charge of the project or representative

SIGNED IN \_\_\_\_\_, on \_\_\_\_\_ 20\_\_\_\_.

## Appendix 2. Par-Q+

Physical Activity Readiness  
Questionnaire - PAR-Q  
(revised 2002)

# PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If  
you  
answered

### YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

### NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

#### DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

**Informed Use of the PAR-Q:** The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

**No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.**

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME \_\_\_\_\_

SIGNATURE \_\_\_\_\_

DATE \_\_\_\_\_

SIGNATURE OF PARENT  
or GUARDIAN (for participants under the age of majority) \_\_\_\_\_

WITNESS \_\_\_\_\_

**Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.**



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## Appendix 3. International Physical Activity Questionnaire (IPAQ)

### INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

#### LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

##### FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

##### **Background on IPAQ**

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

##### **Using IPAQ**

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

##### **Translation from English and Cultural Adaptation**

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at [www.ipaq.ki.se](http://www.ipaq.ki.se). If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

##### **Further Developments of IPAQ**

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

##### **More Information**

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at [www.ipaq.ki.se](http://www.ipaq.ki.se) and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

## INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

### PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No



**Skip to PART 2: TRANSPORTATION**

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

\_\_\_\_\_ **days per week**

☐

No vigorous job-related physical activity



**Skip to question 4**

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

\_\_\_\_\_ **hours per day**

\_\_\_\_\_ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

\_\_\_\_\_ **days per week**

☐

No moderate job-related physical activity



**Skip to question 6**

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?
- \_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**
6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.
- \_\_\_\_\_ **days per week**
- ☐ No job-related walking → **Skip to PART 2: TRANSPORTATION**
7. How much time did you usually spend on one of those days **walking** as part of your work?
- \_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

## **PART 2: TRANSPORTATION PHYSICAL ACTIVITY**

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?
- \_\_\_\_\_ **days per week**
- ☐ No traveling in a motor vehicle → **Skip to question 10**
9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?
- \_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?
- \_\_\_\_\_ **days per week**
- ☐ No bicycling from place to place → **Skip to question 12**



11. How much time did you usually spend on one of those days to **bicycle** from place to place?
- \_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**
12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?
- \_\_\_\_\_ **days per week**
- ☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***
13. How much time did you usually spend on one of those days **walking** from place to place?
- \_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

### ***PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?
- \_\_\_\_\_ **days per week**
- ☐ No vigorous activity in garden or yard → ***Skip to question 16***
15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?
- \_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**
16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?
- \_\_\_\_\_ **days per week**
- ☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

\_\_\_\_\_ **days per week**

☐

No moderate activity inside home



***Skip to PART 4: RECREATION,  
SPORT AND LEISURE-TIME  
PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

#### ***PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY***

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

\_\_\_\_\_ **days per week**

☐

No walking in leisure time



***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

\_\_\_\_\_ **days per week**

☐

No vigorous activity in leisure time



***Skip to question 24***



23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?
- \_\_\_\_\_ **hours per day**  
 \_\_\_\_\_ **minutes per day**
24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?
- \_\_\_\_\_ **days per week**
- ☐ No moderate activity in leisure time      ➔ **Skip to PART 5: TIME SPENT SITTING**
25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
- \_\_\_\_\_ **hours per day**  
 \_\_\_\_\_ **minutes per day**

#### **PART 5: TIME SPENT SITTING**

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?
- \_\_\_\_\_ **hours per day**  
 \_\_\_\_\_ **minutes per day**
27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?
- \_\_\_\_\_ **hours per day**  
 \_\_\_\_\_ **minutes per day**

**This is the end of the questionnaire, thank you for participating.**

#### Appendix 4. Borg CR10 Scale (English)

0	Nothing at all
0.5	Very, very light
1	Very light
2	Light
3	Moderate
4	Somewhat hard
5	Hard
6	
7	Very hard
8	
9	
10	Maximal