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HAMSTRING MUSCLE ARCHITECTURE PARAMETERS IN STATIC AND DYNAMIC CONDITIONS USING ULTRASOUND IMAGING

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PARÂMETROS DE ARQUITETURA MUSCULAR DO MÚSCULO BÍCEPS FEMORAL EM CONDIÇÕES ESTÁTICAS E DINÂMICAS USANDO IMAGENS DE ULTRASSOM

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Clarissa Müller Brusco

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Dedico à minha família, especialmente ao Thomás,
que sempre me mostram o essencial da vida.

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ABSTRACT

Biceps femoris long head (BFlh) fascicle length (L_f), fascicle angle (FA), and muscle thickness (MT) were assessed at rest and during dynamic contractions using B-mode ultrasound imaging. The assessments were performed in men and women with no previous hamstring strain injury. Volunteers visited the laboratory for two separate data collection sessions (Day 1 and Day 2) relating to two independent projects, seven days apart at the same time of day for test-retest measurements of the static assessments. For the dynamic assessments, familiarization with the isokinetic concentric and eccentric knee flexion exercises were performed on Day 1. On Day 2, BFlh L_f, FA and MT were assessed in real time using two serially aligned ultrasound devices whilst performing consecutive concentric and eccentric knee flexions at 30°/s and 180°/s.

To acquire images at rest, ultrasound imaging was performed using extended field-of-view (EFOV) and static image acquisition sequences of four single images acquired in-series along the muscle. From these images, L_f was assessed using seven methods: EFOV, Collage, manual linear extrapolation, and using four different trigonometric equations, and then FA and MT were measured in EFOV, Collage, and Single images. L_f, FA and MT measured on Days 1 and 2 were not different ($p>0.05$) for any method, reliabilities were very high (ICC: 0.91-0.98), and correlations were strong (≥0.84). Significant correlations ($p<0.05$; $r=0.67$-0.98) were found between EFOV and the other measurement techniques for L_f, FA and MT. The Collage method had the highest reliability for L_f, and highest rank order and correlation with EFOV. The Collage method can therefore be recommended for use if the methodology presented herein is followed.

For assessments during dynamic contractions, participants performed consecutive concentric and eccentric contractions at 30°/s and 180°/s whilst in vivo muscle function was recorded using two in-series transducers. At 30°/s both submaximal (55% maximum) and maximal contractions were performed whilst at 180°/s only maximal contractions were performed both before and after fatiguing exercise. Ultrasound videos were exported and edited to create a single, synchronized video and three fascicles were analyzed through the range of motion. Changes during concentric and eccentric contractions (Δ) in L_f, FA and MT at short (60-90° knee angle; 0° full knee extension) and long (0-30°) muscle lengths and across the full knee flexion range were measured and compared. Comparisons were made within exercises performed with the same contraction velocity. When fascicle behaviors were compared during submaximal and maximal concentric and eccentric contractions at 30°/s, greater ΔL_f was observed at longer muscle length during both eccentric and concentric contractions ($p=0.01$), and this alteration was greater during eccentric contractions ($p=0.02$) at longer muscle lengths ($p<0.001$). Greater ΔFA was observed at long length during eccentric contractions ($p=0.02$). When whole range of motion was analyzed, greater ΔMT was observed ($p=0.03$) in concentric contractions. When fascicle behavior was compared in the maximal contractions at 180°/s before and after fatiguing exercise, greater ΔL_f was observed in the eccentric contraction at the long muscle length ($p = 0.01$), ΔFA was greater at short muscle lengths in the concentric contractions ($p=0.02$) and at long muscle lengths ($p=0.006$) and at full range of motion ($p=0.006$) during eccentric contractions. Less ΔFA was detected across all contractions ($p<0.05$) after the fatiguing exercise when measured through the full range of motion. Greater dynamic torque was observed at long muscle lengths, during eccentric contraction, and before the fatiguing exercise ($p<0.05$).

Although the six different techniques used to estimate L_f during rest provided values similar to EFOV, the Collage sonographic method provided the most accurate and reliable results and is therefore recommended for BFlh architectural analysis when EFOV is not available. Greater ΔL_f and ΔFA were observed for a given joint rotation increment during submaximal and maximal eccentric contractions at long muscle lengths. The increased fascicle strain at long muscle length might contribute to both increased metabolic stress, and therefore longer-term muscle hypertrophy, and to muscle strain injuries.

Key words: ultrasonography; biceps femoris; hamstring strain injury; fascicle length; fascicle angle.

RESUMO

Comprimento de fascículo (L_f), ângulo de fascículo (FA) e espessura muscular (EM) da cabeça longa do bíceps femoral (BFlh) foram avaliados em repouso e durante contrações dinâmicas através de imagens de ultrassom adquiridas em B-modo. Homens e mulheres sem histórico de lesões por estiramento foram avaliados. As avaliações foram realizadas em dois dias (Dia 1 e Dia 2) com pelo menos sete dias de intervalo para teste e reteste das avaliações em repouso e avaliações dinâmicas, resultando em dois projetos independentes. A familiarização com os exercícios de flexão de joelhos concêntrico e excêntrico isocinético para as avaliações em condições dinâmicas foram realizadas no Dia 1. No Dia 2 L_f, FA e EM do BFlh foram avaliadas em tempo real durante as flexões de joelhos concêntricas e excêntricas em 30°/s e 180°/s, usando simultaneamente dois ultrassons alinhados longitudinal ao músculo.

Para a aquisição das imagens em repouso, foram adquiridas imagens de ultrassom usando imagens Panorâmicas e aquisição sequencial de quatro imagens únicas adquiridas em série ao longo do músculo. Dessas imagens, L_f foi mensurado com sete métodos distintos: Panorâmica, Colagem de imagens, extrapolação linear manual, e usando quatro funções trigonométricas distintas, FA e EM foram mensurados nas imagens Panorâmicas, Colagem e nas Imagens únicas. L_f, FA e EM mensurados em repouso nos Dias 1 e 2 não foram diferentes ($p>0.05$) em nenhum dos métodos, reprodutibilidade foi muito alta (ICC: 0.91-0.98), e as correlações foram fortes (≥0.84). Correlações significantes ($p<0.05$; $r=0.67$-0.98) foram observadas entre as imagens Panorâmicas e os outros métodos utilizados para L_f, FA e EM. O método de Colagem teve a maior reprodutibilidade para L_f, e maior coeficiente de correlação de postos e correlação com as

imagens Panorâmicas. O método de Colagem pode ser recomendado se a metodologia utilizada no presente estudo for utilizada.

Para as avaliações durante contrações dinâmicas, foram realizadas contrações concêntricas e excêntricas consecutivas em 30°/s e 180°/s enquanto dois ultrassons foram usados em série para gravação da atividade muscular. Em 30°/s foram realizadas contrações submáximas e máximas e em 180°/s foram realizadas apenas contrações máximas antes e após protocolo de fatiga muscular. Os vídeos de ultrassom foram exportados e editados para criar um único e sincronizado vídeo e três fascículos foram analisados ao longo da amplitude de movimento. Alterações (Δ) L_f, FA e EM durante as contrações concêntricas e excêntricas com o músculo em posição encurtada (60-90° ângulo de joelho; 0° extensão de joelho completa) e alongada (0-30°) e em toda amplitude de realização do movimento (0-90°) foram mensurados e comparados. As comparações foram realizadas separadamente para as contrações realizados em cada uma das velocidades. Quando o comportamento dos fascículos foi comparado nas contrações concêntricas e excêntricas submáximas e máximas realizadas em 30°/s, maior ΔL_f foi observado com o músculo alongado durante as contrações excêntricas e concêntricas ($p=0.01$), e essa alteração foi maior durante as contrações excêntricas ($p=0.02$) na posição alongada ($p<0.001$). Maior ΔFA foi observada com o músculo alongado durante as contrações excêntricas ($p=0.02$). Quando analisada toda amplitude de movimento, maior ΔEM foi observada durante as contrações concêntricas ($p=0.03$). Quando o comportamento de fascículo foi comparado nas contrações máximas antes e após protocolo de fadiga muscular em 180°/s, maior ΔL_f foi observado durante as contrações excêntricas com o músculo alongado ($p=0.01$), maior ΔFA foi observado com o músculo encurtado durante contrações concêntricas ($p=0.02$) e com o músculo alongado ($p=0.006$) e em toda amplitude de movimento ($p=0.006$) durante as contrações excêntricas, menor ΔFA foi em todas as contrações ($p<0.05$) após protocolo de fadiga quando mensurado ao longo de toda amplitude de movimento. Maior torque dinâmico foi observado com o músculo alongado, durante as contrações excêntricas e antes do protocolo de fadiga muscular ($p<0.05$).

Apesar de terem sido observados resultados semelhantes entre os seis diferentes métodos utilizados para estimar L_f em repouso com as imagens Panorâmicas, o método de Colagem de imagens forneceu os valores mais acurados e reprodutíveis, desta forma é recomendado para mensurar a arquitetura muscular do BFlh quando a aquisição de imagens Panorâmicas não está disponível. Maior ΔL_f e ΔFA foram observados determinada rotação articular com o músculo alongado durante as contrações excêntricas submáximas e máximas e após protocolo de fadiga. O maior alongamento de fascículos com o músculo alongado pode contribuir o estresse metabólico, podendo em longo prazo induzir hipertrofia muscular e contribuir para as lesões por estiramento.

Palavras chave: ultrassonografia; bíceps femoral; lesão por estiramento dos isquiotibiais; comprimento de fascículo; ângulo de fascículo.

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF TABLES

TABLE OF CONTENTS

PREFACE

Biceps femoris long head (BFlh) has received significant attention in the literature as strain injuries to the hamstring are the most common is sports and the BFlh is the most affected muscle. Muscle architectural parameters (i.e. fascicle length and fascicle angle) are critical determinants of muscle function and in BFlh both the fascicle length and angle assessed at rest have been associated with the risk of strain injuries. BFlh has long fibers (although shorter than some other hamstrings constituents) and complex fiber arrangement, so questions have been raised regarding the most appropriate methodology to evaluate and estimate fascicle length using ultrasound imaging. In the present study, different methods of ultrasound imaging acquisition used to estimate fascicle lengths were compared. The aim was to compare as many available methods as possible to estimate fascicle lengths and angle to provide information to researchers relating to the quality and reliability of methods to estimate fascicle length.

Nevertheless, assessments of muscle architecture have been performed mostly in resting conditions and, as both training stimuli and muscle strain injuries occur during dynamic conditions, it is necessary to understand fascicle behaviors, including changes in fascicle length and angle, during concentric and eccentric contractions under a variety of contraction conditions. Fascicle behavior is influenced strongly by contraction intensity, as evidenced in *ex vivo* animal studies. However, it is not known whether similar fascicle behaviors are observed *in vivo* in human muscles that work within complex synergistic groups. Importantly, not only might contraction intensity influence fascicle behavior but other factors such as contraction mode, the length at which exercise is performed, and the presence of fatigue may theoretically play a role.

To understand whether these factors influence fascicle behavior might help to reveal aspects related to adaptations to training at different ranges of motion and internal muscle mechanics that may relate to strain injuries. Importantly, our findings regarding the BFlh architecture assessments at rest gave us important information that helped us to determine the methods used to acquire ultrasound images during contraction. Due to the possible errors when extrapolation methods are used to estimate fascicle length, two ultrasound probes were longitudinally aligned to the BFlh to enable the visualization of entire fascicles from the origin (in the mid-aponeurosis) to the insertion into the superficial aponeurosis.

Based on the above ideas, the present thesis was divided into four chapters, developed in a partnership between Universidade Federal do Rio Grande do Sul (UFRGS) and Edith Cowan University (ECU; Australia). The first chapter presents a literature review of the basic concepts of muscle architectural assessments at rest and during dynamic contractions. The second chapter comprises Study 1, entitled: “Reliability and comparison of sonographic methods for in vivo measurement of human biceps femoris long-head architecture”, in which a study of intra- and interrater reliability of the biceps femoris long head architecture parameters obtained by ultrasonography in healthy individuals is presented. Within it, a detailed comparison of different methods to estimate fascicle length, fascicle angle, and muscle thickness is made. The third chapter comprises Study 2, entitled: “Biceps femoris fascicle behavior during submaximal and maximal slow speed contractions”, in which fascicle behaviors were assessed during slow-speed (30°/s) submaximal and maximal concentric and eccentric knee flexion contractions at long and short muscle lengths. The fourth chapter comprises Study 3, entitled: “The effect of fatigue on biceps femoris fascicle behavior during maximal isokinetic contractions”, in which fascicle behaviors were assessed during maximal, moderate-speed (180°/s) concentric and eccentric contractions performed before and after a fatiguing exercise at long and short muscle lengths.

The data obtained in these three studies will serve to better understand the different possibilities available to evaluate BFlh muscle architecture at rest and how fascicles behave during dynamic contractions and its possible relation to training adaptations and strain injuries.

1. CHAPTER 1

LITERATURE REVIEW

1.1 BICEPS FEMORIS LONG HEAD ARCHITECTURAL PARAMETERS AT REST

The hamstrings muscle group are located in the back of the thigh and include the semimembranosus (SM), semitendinosus (ST), and the long and short heads of the biceps femoris (BFlh and BFsh, respectively) (Kellis 2018). SM, ST and BFlh are biarticular muscles that cross the hip and the knee joints, originating at the ischial tuberosity and inserting onto the tibia below the knee joint and act as hip extensors and knee flexors (Beltran et al. 2012). BFsh is the only muscle of the hamstring group that crosses only the knee joint and acts only as a knee flexor (Beltran et al. 2012; Kellis 2018). Despite working as a single unit, the constituent muscles possess different architectural designs that influence their force generating capacities. For example, BFlh and SM have greater fascicle angle and physiological cross-sectional area and thus greater force generation capacity than ST and BFsh. On the other hand, ST and BFsh have greater fascicle lengths and thus greater excursion capacity than BFlh and SM (Kellis 2018).

The architecture of the hamstring muscles seems to predispose BFlh to greater occurrence and recurrence of strain injuries during high-speed running (Huygaerts et al. 2021; Opar et al. 2012; Thelen et al. 2005). When the hamstring is eccentrically contracted, the muscles with shorter fibers are at greater risk of injury than those with longer fibers, which may predispose BFlh and SM to high rates of strain injury (Kellis 2018). Indeed, BFlh is the most injured of the hamstring muscles (Bourne et al. 2018; Opar et al. 2012) and the presence of shorter fascicle lengths (shorter than < 10.56 cm) seem to increase the risk of strain injuries (Timmins et al. 2016a). It is therefore a muscle of great clinical and research importance, and diagnostic imaging techniques are widely used to assess its architectural properties.

BFlh has a complex muscle architecture, with non-uniform and heterogeneous fiber distribution along its length, with lower fascicle angle and greater fascicle length and muscle thickness in the most the proximal region, changing gradually to greater fascicle angle and lower fascicle length and muscle thickness in the distal region (Kellis et al. 2010). In addition, BFlh fascicles follow a non-linear path that can exhibit dual curvature, thus presenting an “S” shape (Pimenta et al. 2018). These variations in muscle architectural parameters can have functional implications for muscle performance as they might indicate that force and excursion capacity differ along the muscle length (Kellis et al. 2010). The unique and complex fiber arrangement of BFlh also imposes difficulties to the in vivo assessment of architectural parameters using ultrasound imaging and require extensive training of the evaluator. Moreover, BFlh has long fascicles that project out of the field of-view of the ultrasound probe (many probes are ~4.5 cm, but sometimes ≤ 10 cm, in length) (Pimenta et al. 2018), so extrapolation methods using different trigonometric functions have been commonly used to estimate fascicle lengths (Alonso-Fernandez et al. 2018; Ribeiro-Alvares et al. 2018; Timmins et al. 2015; Timmins et al. 2016c). However, equations that have been validated in muscles other than BFlh have been used (e.g. in vastus lateralis or gastrocnemius medialis) and their assumptions, such as a lack of fascicle and aponeurosis curvature, introduce estimation errors (Franchi et al. 2020; Pimenta et al. 2018).

Fascicle lengths estimated from single (still-frame) images reveal significant inaccuracies when compared to images obtained using extended field-of-view (EFOV; i.e. panoramic) methodologies. Pimenta et al. (2018) and Franchi et al. (2020) reported that BFlh fascicle length is overestimated when trigonometric functions are used. For example, when fascicle length was estimated using the equation proposed for use in VL by Blazevich et al. (2006), Pimenta et al. (2018) reported an average absolute error of 7.9 mm for fascicle length, and Franchi et al. (2020) reported average absolute biases of 19.1 mm and 5.0 mm with the equation proposed by Finni et al. (2001). If we consider that alterations ranging from 10 - 20 mm have been reported after several weeks of hamstrings eccentric training (Alonso-Fernandez et al. 2018; Pincheira et al. 2021; Presland et al. 2018; Ribeiro-Alvares et al. 2018; Timmins et al. 2016b), the reported errors are considerably large. Nevertheless, Franchi et al. (2020) pointed out that the systematic fascicle length error was not fixed between participants but instead overestimated in some and underestimated in others. This is problematic if it is considered that individuals might be evaluated at different time intervals or in a variety of situations (e.g. pre-season, before and after a training intervention, in the recovery of a strain injury, etc.), and it brings into question the greater increase in fascicle length observed in BFlh when compared to changes in other muscles (Franchi et al. 2020). Considering the possible measurement errors, the use of trigonometric equations to estimate BFlh fascicle length has been discouraged, and instead it is recommended to use EFOV methodologies (Franchi et al. 2020; Pimenta et al. 2018). With EFOV, it is possible to acquire images from a large portion of a muscle, allowing the visualization of entire fascicles and the direct measurement of fascicle length and angle (Noorkoiv et al. 2010). However, the use of

EFOV needs careful training, as BFlh fascicles follow a nonlinear path it is recommended that the transducer also follows a nonlinear path (Pimenta et al. 2018).

Despite the recommendations to use EFOV in order to provide more accurate measurements, this technology has high financial costs so it is not always available in either clinical or research environments. One possible alternative to overcome such limitations is to use a mathematical model to estimate fascicle length from single, still-frame images that consider the architectural complexity of BFlh. Another alternative that has already been explored in some muscles (Kawakami et al. 1998) but not in BFlh is to create a collage of single-frame images. This collage will result in an image that is somewhat similar to an EFOV image and will allow the assessment of whole fascicles. However, these two possibilities have not been explored to date. Considering that BFlh architecture parameters obtained in static conditions are closely linked to injury risk factors, accurate and accessible forms of assessment have to be developed. To explore a variety of methods to assess BFlh muscle fascicle length, fascicle angle, and muscle thickness and to compare with estimates obtained from EFOV images might lead to the development of acceptable methods of assessment for BFlh.

1.2 DYNAMIC ARCHITECTURE ASSESSMENTS

Muscle architecture is a primary determinant of muscle function, hence it has been widely explored using B-mode ultrasonography, mostly in resting conditions (Lieber and Friden 2000; Van Hooren et al. 2020). The influence of muscle architecture measured under static conditions on mechanical output seems to be straightforward, and both fascicle length and angle have even been shown to be statistically associated with the risk of BFlh strain injuries (Timmins et al. 2016a). However, when contracted, fascicles of pennate muscles change in both length and angle, presenting additional challenges for assessment and understanding its impact on force production, training adaptations, and possible injury mechanisms (Eng et al. 2018).

Fibers within pennate muscles change shape when contracted, bulging in both thickness and width directions, prompting fascicle rotation and length change (Roberts et al. 2019). The resulting multidirectional forces generated within the muscle drive complex changes in muscle shape that are constrained by structural and material properties of the surrounding connective tissues, pressures from external tissues, intramuscular pressures, and fascicle geometry, influencing the overall mechanical behavior of skeletal muscles (Raiteri et al. 2016). In the absence of constraints, a wide range of shape changes is possible (Eng et al. 2018).

During concentric contractions fascicles (and their constituent fibers) shorten while tend to rotate to greater fascicle angles, allowing for a greater whole muscle shortening for a given fascicle length change (Brainerd and Azizi 2005; Eng et al. 2018). The magnitude of fascicle rotation is strongly influenced by muscle contraction intensity, in which substantial high fiber rotation is observed in low force, higher-velocity contractions whereas less fiber rotation is observed in high force, slower-velocity contractions (Azizi et al. 2008). This mechanism allows for a greater whole muscle shortening for a given fascicle length change (Brainerd and Azizi 2005) and occurs automatically, shifting from a behavior that favors force to one that favors velocity to match the mechanical conditions of the contraction through dynamic changes in muscle architecture (Azizi et al. 2008; Azizi and Roberts 2014). Fiber rotation during a contraction can also decrease the amount of fascicle length change needed during eccentric contractions (Brainerd and Azizi 2005), which might protect pennate muscle fibers from excessive lengthening. Azizi and Roberts (2014) observed that fascicle length change was less in eccentric than concentric contractions during isotonic contractions of the bullfrog plantaris muscle. This resulted from a relatively large degree of fascicle rotation for a given amount of fascicle shortening and greater lengthening of the muscle-tendon unit, protecting the muscle from eccentric damage (Azizi and Roberts 2014). As this mechanism has been observed in *ex vivo* animal muscles, it seems not to be mediated by the nervous control system but instead results from the complex interaction of intramuscular forces and pressures on connective tissue elements (Azizi et al. 2008; Eng et al. 2018). Muscles are complex structures, and the passive elastic structures that regulate muscle shape during contraction vary between muscles, so a number of intrinsic (e.g. intramuscular pressures, connective tissue properties, stretch of external tendon) and extrinsic (external loading, extramuscular pressures) factors may influence fascicle behavior (Wakeling et al. 2011). Therefore, it might be expected that differences in fascicle behaviors are also observed between contractions performed under different conditions in muscles *in vivo* (Azizi and Roberts 2014).

Indeed, different fascicle behaviors have been reported in human muscles evaluated *in vivo*. Similar to the findings of *ex vivo* animal studies, Ichinose et al. (2000) observed that contraction intensity influenced vastus lateralis fascicle shortening. Ando et al. (2018) also observed that vastus intermedius fascicles were more strained than vastus lateralis during eccentric contractions; the less extensive muscle-tendon unit in vastus intermedius may impose greater mechanical strain to the fascicles during eccentric contractions and fascicles may be forced to lengthen more than in vastus lateralis (Ando et al. 2018). These findings provide evidence of the importance of contraction mode on fascicle behavior. Interestingly, Reeves and Narici (2003) observed a quasi-isometric fascicle behavior in the tibialis anterior during eccentric contractions performed at different velocities. A similar behavior was observed recently in BFlh during the performance of the Nordic hamstring curl exercise (Van Hooren et al. 2022), in which fascicle length remained almost constant during most of the range of motion but then increased markedly near the end of the range of motion. Taken together, these findings provide evidence that fascicle behavior is dependent not only on contraction intensity but also on muscle length and that it may differ between muscles. Therefore, to determine a muscle fascicle behavior, the specific muscle of interest has to be evaluated under specific contraction conditions, and conclusions based on studies of other muscles should be avoided.

The varied degree of fiber length alteration for a given whole muscle length change during contraction (i.e. the muscle's "gear") should have important functional implications. Greater fascicle rotation should reduce fascicle lengthening, thus protecting from excessive strain (Azizi and Roberts 2014), but it may also speculatively attenuate the hypertrophic stimulus (McMahon et al. 2014b; Noorkoiv et al. 2014; Pedrosa et al. 2021). Training at long muscle lengths has been shown to promote greater metabolic stress, IGF-1 release, and activation of proteins and Akt/mTOR (McMahon et al. 2014a; Rindom et al. 2019; Russ 2008), thus resulting in increased muscle hypertrophy after a period of training (McMahon et al. 2014b; Noorkoiv et al. 2014; Pedrosa et al. 2021). As increased hypertrophy has also been observed after both dynamic (McMahon et al. 2014b; Pedrosa et al. 2021) and isometric training (Noorkoiv et al. 2014), the increased hypertrophic stimulus seems to be primarily related to the joint angle (i.e. muscle length) used in training rather than the contraction mode. The muscle length during contraction affects pressure within the muscles but also from the synergistic muscles, which in turn might influence fascicle rotation and lengthening according to muscle length. If fascicles are more strained at long muscles lengths, it might negatively influence acute damage as well as positively affect longer-term hypertrophic responses.

As suggested above, contraction mode is a possibly important factor that might influence fascicle behavior. Greater peak forces are possible during eccentric contractions than in concentric or isometric contractions (Huxley and Simmons 1971) and this should directly affect fascicle behavior (Azizi and Roberts 2014; Brainerd and Azizi 2005). Azizi and Roberts (2014) observed greater fascicle rotation during maximal eccentric contractions, minimizing fascicle strain and thus potentially protecting from excessive fiber strain. Nevertheless, strain injuries still occur in human pennate muscles, mostly during eccentric contractions, despite the possible protection offered by fascicle rotation. Therefore, it is questioned whether fascicle strain and rotation differ between concentric and eccentric contractions. Speculatively, it is possible that fascicle behavior differs between maximal concentric and eccentric contractions due to the different forces produced but also the different muscle activation patterns used and different the intra- and inter-muscular pressures produced. Thus, to determine whether contraction mode itself plays a role in fascicle behavior *in vivo*, it is necessary to specifically study behaviors under specific test conditions.

Hamstring strain injuries are the most common in sports, affecting mostly the BFlh (Ekstrand et al. 2011), so this muscle has received great attention in the scientific literature. Understanding the possible mechanisms underpinning strain injuries might help in the development of training strategies to minimize injury occurrence. It is accepted that muscle strain injuries occur while the muscle is activated during lengthening contractions (Lieber and Friden 2002). Indeed, strain injuries to BFlh occur during the late swing phase of high-speed running (Askling et al. 2007; Koulouris and Connell 2003; Opar et al. 2012), when the muscle-tendon unit is lengthened while receiving significant activation from the central nervous system (Freckleton and Pizzari 2013; Thelen et al. 2005). Nevertheless, whole-muscle strain is not reflective of specific fiber (or fascicle) strains within the muscle, so despite it being accepted that BFlh is stretched at the point of strain injury, it is not possible to affirm that fibers themselves are more strained. Thus, it is not explicitly known whether strain and rotation at the fascicle level are substantial, and thus influence strain injury risk during contractions *in vivo*. Nevertheless, if fibers are working at longer average lengths or produce greater strain whilst activated, greater risk of strain or damage is expected. BFlh fascicle behavior remains poorly understood; thus, no conclusions or recommendations regarding the possible contribution of fascicle strain on injury can be drawn.

Another important factor that may critically affect strain injury risk and might also influence fascicle behavior is fatigue (Huygaerts et al. 2020). Fatigue may conceivably influence fascicle behaviors through fatigue-induced reductions in force capacity, muscle and fiber-specific swelling, or alterations in relative muscle activation (i.e. between muscles within a synergistic group). Contraction intensity has been shown to influence fascicle rotation and strain during eccentric contractions in *ex vivo* preparations (Azizi and Roberts 2014), so it is possible that, due to the decreased force produced when the muscle is fatigued, fascicle behavior might be affected. However, fatigue-induced alterations might also result from muscle swelling. Increased swelling increases intramuscular pressures (Ploutz-Snyder et al. 1995; Ploutz-Snyder et al. 1997), which might affect muscle shape change and thus fascicle behavior. As fatigue may vary between muscles within a synergistic group, alterations both within and between muscles may affect muscle shape change and thus fascicle behaviors in ways that are not currently possible to predict. Finally, alterations in relative activations of muscles within a synergistic group can occur with fatiguing exercise (Baumert et al. 2021; Huygaerts et al. 2020). This will alter the force and velocity characteristics of muscles (independently of muscle-specific swelling) and thus influence fascicle behaviors. Cumulatively, these complex effects on muscles in vivo make it impossible to predict fascicle behaviors, so direct measurements of fascicle behaviors after fatiguing exercise are needed.

Muscles are complex tissues that are influenced by a number intrinsic and extrinsic factors. Learning how fascicles behave under a variety of conditions may help to understand possible mechanisms related both training adaptations and muscle strain injury.

1.3 SUMMARY

This review provides evidence relating to the difficulties of BFlh fascicle length assessments of at rest using ultrasound imaging, and the importance of using adequate and accurate methods of image acquisition and analyses. In addition, evidence is also provided for the importance of assessing fascicle rotation and length during dynamic contractions *in vivo*, as it may relate to training adaptations and to fiber strain and injury. Some factors that theoretically influence fascicle behavior include the contraction intensity, contraction mode, muscle length, and the presence of fatigue. However, BFlh fascicle behavior is still poorly understood. It is therefore necessary to investigate fascicle behavior during dynamic contractions and the possible factors that might influence it.

1.4 AIM OF THE THESIS

The major aims of the present thesis are to (i) develop a method to estimate biceps femoris long head fascicle length using ultrasound imaging during rest that has a high correlation to extended field of view imaging and presents a low measurement error; (ii) to examine whether contraction mode, contraction intensity, and muscle length, either independently or cumulatively, influence BFlh fascicle behaviors during slow-speed (30°/s) dynamic contractions; and (iii) to examine whether contraction mode and muscle length, independently or cumulatively, influence BFlh fascicle behavior during higher-speed (180°/s) dynamic contractions performed before and after fatiguing exercise.

2. CHAPTER 2

STUDY 1: RELIABILITY AND COMPARISON OF SONOGRAPHIC METHODS FOR IN VIVO MEASUREMENT OF HUMAN BICEPS FEMORIS LONG-HEAD ARCHITECTURE

2.1 INTRODUCTION

The hamstring muscle group, comprising the biceps femoris short and long heads (BFsh and BFlh, respectively), semimembranosus (SM), semitendinosus (ST), act to extend the hip and flex the knee (Kellis 2018). Despite working as a single unit, the constituent muscles possess architectural designs that may influence their force generating capacities as well as temporal responses to repeated, external loading, i.e. physical training (Kellis 2018). Anatomical, and especially architectural, differences speculatively predispose BFlh to greater occurrence and recurrence of strain injuries during high-speed running (Huygaerts et al. 2021; Opar et al. 2012; Thelen et al. 2005). Indeed, BFlh is the most injured of the hamstring muscles (Bourne et al. 2018; Opar et al. 2012) and both the lengths and angles of its constituent fascicles have been statistically associated with both force generation capacity and injury risk (Lieber and Friden 2000; Timmins et al. 2015). It is therefore a muscle of great clinical and research importance, and diagnostic imaging techniques are therefore widely used to assess its architectural properties.

Ultrasound imaging is the most ubiquitous method used to characterize hamstring muscle architecture, especially in BFlh, however such evaluation is fraught with methodological difficulty. BFlh has a complex architecture with variability in fiber arrangement along its length, including longer fascicle length (L_f) and greater fascicle angle (FA) proximally than distally (Franchi et al. 2020; Huygaerts et al. 2021; Kellis et al. 2010). In addition, BFlh fascicles follow a nonlinear path, and the length of its fascicles ensures that the fascicles project out of the field of-view of the ultrasound probe (many probes are ~4.5 cm, but sometimes ≤10 cm, in length) (Pimenta et al. 2018). Extrapolation methods using different trigonometric functions are therefore applied to estimate fascicle lengths. However, these methods have been validated in muscle groups other than the hamstrings (e.g. in vastus lateralis or gastrocnemius medialis) and carry assumptions such as a lack of fascicle and aponeurosis curvature that introduce estimation errors (Franchi et al. 2020; Pimenta et al. 2018; Sarto et al. 2021). Indeed, comparisons between estimations obtained from single (still-frame) images and extended field-of-view (EFOV; i.e. panoramic) images reveal significant inaccuracies in single-image measurements, especially for fascicle length. For example, Pimenta et al. (2018) reported an average absolute error of 7.9 mm for fascicle length while Franchi et al. (2020) reported average absolute biases of 19.1 mm with the equation proposed by Blazevich et al. (2006) and 5.0 mm with the equation proposed by Finni et al (2001), which are large in comparison to the 10-20 mm changes often reported after several weeks of hamstrings strength training (Alonso-Fernandez et al. 2018; Pincheira et al. 2021; Presland et al. 2018; Ribeiro-Alvares et al. 2018; Timmins et al. 2016b). Both, Pimenta et al. (2018) and Franchi et al. (2020), reported L_f overestimation when using extrapolation methods, and Franchi et al (2020) pointed out that the systematic L_f error was not fixed between participants but instead overestimated in some and underestimated in others. Therefore, extrapolation of L_f

from single images may be problematic and the use of EFOV is therefore preferred (Franchi et al. 2020; Pimenta et al. 2018).

The advantage of EFOV methodology is that it provides a panoramic picture of a large section of the muscle, enabling visualization of entire fascicles and thus allowing direct measurement despite their substantial length and curvature (Noorkoiv et al. 2010). However, EFOV technology is not always available in either clinical or research environments, largely due to the financial costs of the technology. One potential alternative is to establish better mathematical methods of estimating L_f from single, still-frame images. This can be done by rigorously testing both previously published and potentially new estimation equations. An alternative might be to create a collage of single-frame images, as has been done for BFlh (Chleboun et al. 2001) and other muscles (Kawakami et al. 1998), however the validity and reliability of this method has not been tested for BFlh. Both of these opportunities are yet to be fully explored, although some investigations have commenced the search (Franchi et al. 2020; Pimenta et al. 2018). In the present study, biceps femoris long head (BFlh) fascicle length, fascicle angle and muscle thickness estimates obtained from EFOV images were compared to those measured from a collage of single ultrasound images as well as a range of geometric equations and extrapolation methods on single images to test both validity and inter-day reliability performed by a trained rater. The hypotheses were tested that all the methods could be used to provide reliable estimates but that the trigonometric extrapolation equations would overestimate L_f.

2.2 METHODS

2.2.1 Type of study

To achieve the study purposes, the diagnostic accuracy and reliability of a collage of ultrasound images, trigonometric equations, and extrapolation methods were compared to measurements obtained using the extended field-of-view method, with testing and retesting completed on different days.

2.2.2 Participants

Twenty healthy adults (10 men and 10 women; age, 26.5 ± 3.7 years; height, 171.3 ± 6.8 cm; body mass, 70.7 ± 8.4 kg) without history of right hamstring strain injury volunteered for the study. The experimental protocol was approved by the Human Research Ethics Committee of Edith Cowan University (22326) and written informed consent was given by all participants prior to participation.

2.2.3 Experimental design

To compare biceps femoris long head (BFlh) fascicle length (L_f), fascicle angle (FA) and muscle thickness (MT) using different assessment methods, ultrasound imaging was performed using (i) EFOV image acquisition, in which a panoramic image is acquired axially along the muscle, and (ii) static image acquisition, in which four single images were acquired in series along the muscle (Figure 1); five images were taken for one participant whose fascicles tended to extend past the 4-image distance. From these images, L_f was assessed using seven methods: (i) EFOV, in which the fascicles were directly measured between origin and insertion on EFOV images; (ii)

Collage, in which the static images were arranged serially (in panorama) to form a single image and fascicles were then measured between origin and insertion on that image; (iii) manual linear extrapolation (MLE), in which L_f was estimated as a straight line between aponeuroses using an MLE method; (iv) Equation A, in which L_f was estimated using the equation of Stavnsbo and colleagues (the equation was originally developed by Anders Stavnsbo; see Acknowledgements); (v) Equation B, in which L_f was estimated using the equation of Abe and colleagues (Abe et al. 2000); (vi) Equation C, in which L_f was estimated using the equation first described by Finni and colleagues (Finni et al. 2001); and (vii) Equation D, in which L_f was estimated using the equation of Blazevich and colleagues (Blazevich et al. 2006). FA and MT were also measured in the EFOV panoramic images, collage images, and single images. To achieve these aims, the participants visited the laboratory on two occasions (Day 1 and Day 2), seven days apart at the same time of day, and the same assessments were performed on both days. All participants were asked not to perform exhaustive or unaccustomed lower-limb exercise for 48 h before each testing session and to log their physical activity in order to repeat, as best as possible, the activity completed before Day 1 testing.

2.2.4 Ultrasound imaging

Ultrasound images were acquired with an ultrasound device (Prosound F75, Aloka Co Ltd., Japan) in B-mode using a linear probe with 50-mm field of view (operating at 13 kHz, gain 75 and 60-mm depth, Aloka Co Ltd, Japan) by an operator who had completed >6 months of sonographic training and a minimum of 150 practice scans for each scan type. The same probe positioning and skin landmarks were used for ultrasound imaging acquisition using EFOV and the static image methods (but removed and reestablished between test days), as described below. For all scans, the ultrasound probe was coated with water-soluble gel to improve acoustic contact between the probe and skin, allowing for a higher image quality and for minimal pressure to be applied to the skin.

In both sessions, the participants were positioned in ventral decubitus with the knees fully extended and asked to completely relax during the sonographic scans. After 10 min in a lying position to allow fluid redistribution (Lopez et al. 2019) BFlh anatomical location was identified by moving the ultrasound transducer aligned in the transverse plane from the proximal to distal myotendinous junction to show an axial section. The probe path was then marked on the skin with a pen. To determine the region of interest (ROI) for image acquisition, a point 50% of the distance from the greater trochanter to femoral lateral condyle was identified. Image acquisition started with the probe edge ~1-2 cm outside the fascicle origin to ensure fascicle origin from the mid-muscle aponeurosis at this region could be identified. The transducer was aligned to the fascicle plane where both superficial and mid (mid-belly/intramuscular) aponeuroses were as close to parallel as could be identified, the muscle thickness (i.e., perpendicular distance between superficial and mid aponeuroses) on the image was greatest, and the hyperechoic lines delineating the BFlh fascicles (i.e., perimysial membranes) permitted a clear visualization of the fascicles inserting onto the mid-aponeurosis (Figure 1A). Pilot scans of the muscle were then performed in which the muscle was scanned proximo-distally with the transducer positioned axially (longitudinally) and following the muscle's fascicle direction while taking care to produce images in which the fascicles remained continuous and visible as possible. Once the optimum scanning path was identified, this path was marked on the skin to guide image acquisition for both the extended field-of-view and static image recordings. In addition, three points each 2 cm apart were marked on the skin from the most proximal probe position along the ultrasound path to mark the four locations for static ultrasound acquisitions. These specific distance points were also used to facilitate construction of the Collage images (see Figure 1B for example). To achieve the best fascicle visualization, the position of the transducer was altered along the path during the pilot scans, and the optimum transducer orientation at each point along the path was replicated as closely as possible during the acquisition scans.

During extended field-of-view image acquisition the transducer was slowly and continuously moved proximo-distally along the marked path with constant pressure and care to maintain the transducer in the fascicle plane. Image acquisition was repeated if the center of the longitudinal axis of the probe moved off the marked path, if fascicles did not remain continuous and visible, or the aponeuroses did not remain approximately parallel in the formed image. Three acceptable images were thus obtained (see Figure 1C for example). For static image acquisition, three images were acquired at each of the four points marked along the imaging path (Figure 2) for subsequent construction of a collage as well as single image assessments. To build the Collage, Camtasia Techsmith (Version 2019.0.0) software was used (Supplementary File 1 at Appendix 5). Images were carefully positioned with their proximal edges 2 cm apart and aligned by visually locating similar anatomical landmarks (i.e. connective tissue, subcutaneous adipose tissue) between the images as well as with consideration of fascicle alignment. In each session, extended field-of-view images were always acquired before static images. During testing on Day 2, images obtained on Day 1 were also consulted to ensure images as similar as possible could be obtained on both days. Muscle and adipose tissue were checked for similarity in both testing days (Blazevich et al. 2012).

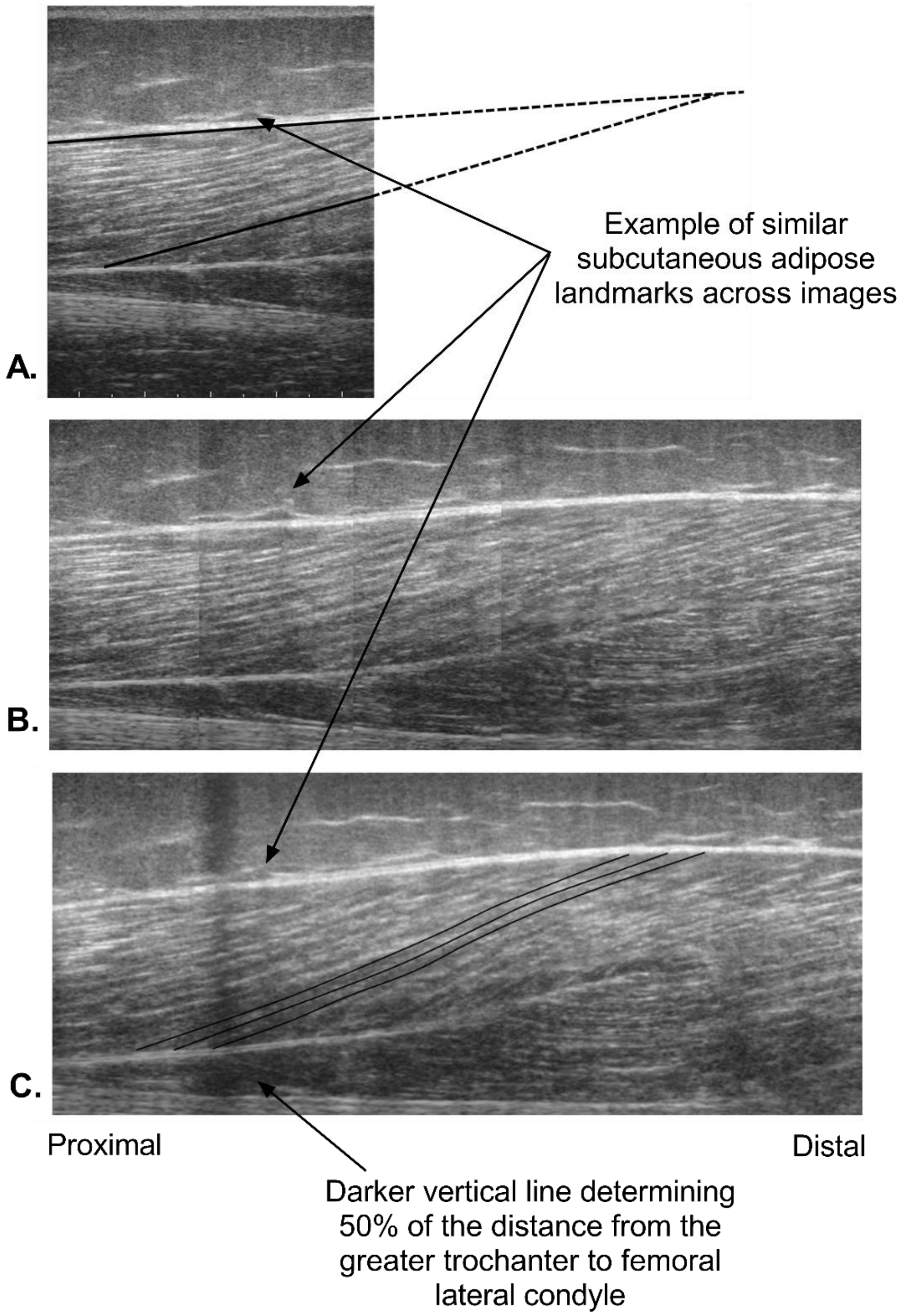

Figure 1. Examples of a static BFlh image (A), a collage constructed using a series of static images (B), and an EFOV image (C). Image A was used to analyze muscle architecture parameters using manual linear extrapolation (reference lines), and Equations A, B, C and D (an example of manual extrapolation is shown). Care was taken to locate subcutaneous adipose and intramuscular anatomical landmarks in each image, which helped to ensure that image location and transducer pitch, roll, and yaw angles were similar between acquisitions.

Figure 2. Example of transducer path marked on the skin for ultrasound image acquisition. Note that the path is non-linear; transducer pitch, roll, and yaw was altered along the path during EFOV and Collage image acquisition in order to follow the fascicular pathway as closely as possible.

2.2.5 Muscle (BFlh) architecture assessments

All images were digitized using an image-processing program (Tracker 5.0.7, https://physlets.org/tracker/), with fascicle length (L_f), fascicle angle (FA) and muscle thickness (MT) being measured in each image. All fascicles analyzed were located at around 50% of femur length for all image acquisition methods. The mean of three images was analyzed for Days 1 and 2, whereas three fascicles were analyzed in each image. As extended field-of-view and static images were obtained using the same transducer positioning, care was taken to identify the same fascicles (or as close to be the same as possible) for analysis in each image of the methods used. the most proximal image used to build the Collage, or the second most proximal image (depending on which image gave a better view of fascicles), were used for Static image L_f analyses using the different methods.

For EFOV and Collage, L_f was determined from the mid-aponeurosis origin to insertion to the superficial aponeurosis using the segmented line tool. A fascicle was considered appropriate for analysis when a clear visualization of the insertion into the mid-aponeurosis (proximal) was possible and a clear fascicular pathway was observed to the more distal end.

It is not currently known whether BFlh L_f can be accurately estimated from single ultrasound images; the ability to accurately estimate the non-visible portion of a fascicle (as it extends off the image) is the critical step that would allow accurate estimation. In the present study, five estimation methods (manual linear extrapolation [MLE] and four different trigonometric equations) and a collage of images were compared for accuracy relative to the direct measurements on EFOV images. MLE was used, as described by Franchi and colleagues (Franchi et al. 2020), in which the visible fascicles were extrapolated using straight lines over the non-visible portion of the muscle until the linearly-projected intersection point with the superficial aponeurosis of the muscle. The superficial aponeurosis was projected as a line crossing both ends of the visible portion of the superficial aponeurosis and was projected to the non-visible portion.

The equation proposed by Stavnsbo and colleagues, referred to here as Equation A, was used in which:

Equation A:

$$L_f = L_{vf} + \frac{sin(180 - b).A}{sin(b - c)}$$

where, as shown in Figure 3, L_{vf} is the visible, measured portion of the fascicle, *A* is the vertical distance from the point at which the fascicle ends at the right side of the image to the superficial aponeurosis, *b* is the inferior angle between the superficial aponeurosis and the vertical line created by the image edge, and *c* is the inferior angle between the fascicle and the vertical line created by the image edge.

Trigonometric linear extrapolation used the equation proposed by Abe and colleagues (Abe et al. 2000), referred to as Equation B, in which the fascicle projection angle (α) and vertical (transverse) distance between superficial and mid-aponeuroses (muscle thickness; MT) were measured and L_f estimated:

Equation B:

$$L_f = MT\ /sin\alpha$$

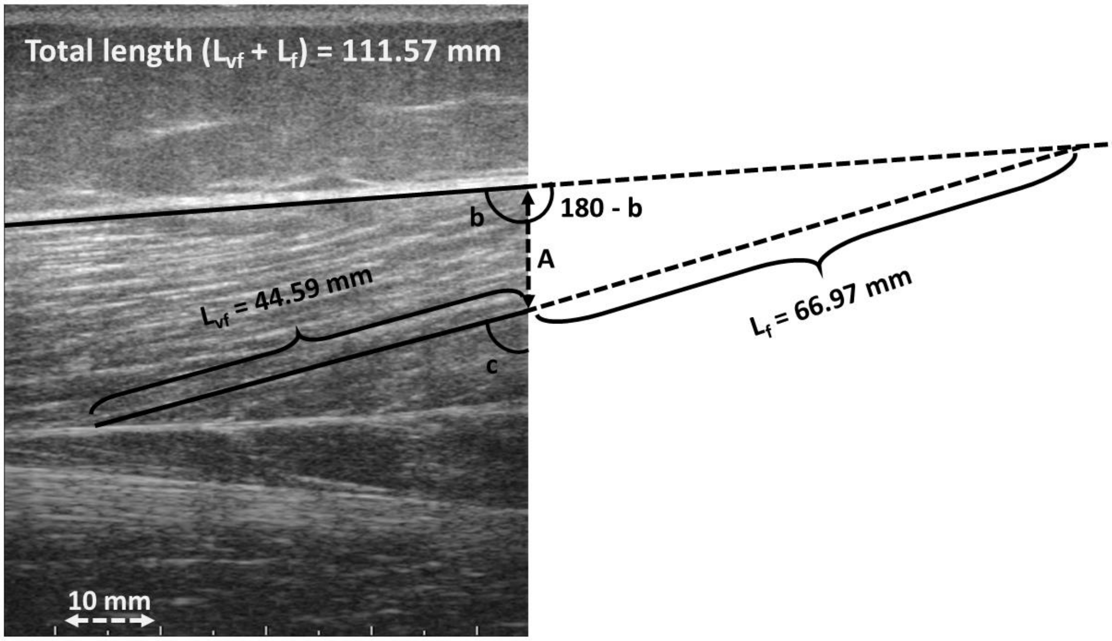

Figure 3. Trigonometric extrapolation technique proposed by Stavnsbo and colleagues. L_{vf} refers to the visible part of the fascicle directly measured, A is the height from the fascicle's intersection with the proximal border of ROI to the aponeurosis' intersection with the proximal border of ROI, L_{vf} corresponds to the extrapolated part of the fascicle by trigonometric equation. A is the height from the fascicle's intersection with the proximal border of ROI to the aponeurosis' intersection with the proximal border of ROI. b and c correspond to the angles measured to allow construction of the artificial triangle to allow the trigonometric extrapolation.

The equation used by Finni and colleagues (2001) and others (Abellaneda et al. 2009; Avela et al. 2004; Geremia et al. 2019), referred to here as Equation C, uses the equation:

Equation C:

$$L_f = L_{vf} + (h/sin\alpha)$$

where L_{vf} is the visible, measured portion of the fascicle (Finni et al. 2001), *h* is the perpendicular distance between the superficial aponeurosis and the fascicle's visible distal end point, and α is the angle between the fascicle (drawn linearly to the most distal point) and the superficial aponeurosis.

The equation used by Blazevich and colleagues (2006), referred to here as Equation D, uses the equation:

Equation D:

$$L_f = \frac{\sin(y + 90°) \,.\, MT}{\sin(180° - (y + 180° - \alpha)}$$

where y is the angle between the superficial and mid-aponeurosis, MT is the muscle thickness calculated as the mean of the vertical distance between the mid-aponeurosis measured in both (right and left) ends of the image, and α is the fascicle angle.

In all images, FA was measured as the angle between the mid (mid-muscle) aponeurosis and a point along the fascicle at 50% of the distance from the mid to the superficial aponeurosis. If the fascicle projected off the image at <50% distance from mid-aponeurosis, as it was the case in some static images, FA was instead measured to the point at which the fascicle projected off the image. MT was measured as the vertical distance from the fascicle origin on the mid-aponeurosis to the superficial aponeurosis. Therefore, assessments of MT were performed only in the most proximal ending of the image where fascicles were inserted.

2.2.6 Statistical analysis

2.2.6.1 Test-retest repeatability

Data analysis was performed in SPSS software (v20, Chicago, USA). Test–retest reliability for each technique was determined by calculating the intraclass correlation coefficient ($ICC_{3,1}$), Pearson's correlation coefficient (r), coefficient of variation (CV) and standard error of measurement (SEM) (Hopkins 2000). A paired sample t test was performed to determine whether the measurements differed systematically between sessions (Table 1). Intraday reliabilities were classified as little (ICC <0.25), low (0.26–0.49), moderate (0.50–0.69), high (0.70–0.89), and very high (>0.90) (Domholdt 1993).

2.2.6.2 Technique comparisons

Normality of data was confirmed using the Shapiro-Wilk test. Comparisons of L_f between the seven techniques were made using one-way analyses of variance (ANOVA). FA and MT measured on extended field-of-view, collage and static images were also made using one-way analyses of variance (ANOVA). *Post-hoc* analyses were performed using Bonferroni tests.

Pearson's correlation coefficients (r) were computed between values obtained by EFOV (considered the reference method) and other methods to verify the linearity between the techniques. The agreements for L_f between EFOV and both Collage, Extrapolation, Equation A, Equation B, Equation C and Equation D, and for FA between EFOV and both the collage and static images were determined using Bland-Altman analysis (Giavarina 2015). Additionally, the Spearman's rank-order correlation coefficient (ρ) was calculated for L_f and FA to determine whether individuals were ranked similarly within the cohort when the variables were calculated using different methods of analysis. Spearman's and Pearson's coefficients were classified as weak (<0.3), moderate (0.3 - 0.7) and strong (>0.7) (Sheskin 2000). The α level was set at <0.05 for all tests.

2.3 RESULTS

2.3.1 Test-retest repeatability

BFlh architecture reliability outcomes using the seven methods are shown in Table 1. The t-tests revealed no statistical differences ($p > 0.05$) in L_f, FA or MT measured at Day 1 and Day 2 for any method. L_f, FA and MT reliabilities were very high for EFOV, Collage, MLE, Equation A, Equation B, Equation C, and Equation D, and correlations between measurements made on Days 1 and 2 for L_f, FA and MT were strong (≥ 0.84).

2.3.2 Comparison between techniques

No significant main effect of technique was found for L_f (p=0.75), FA (p=0.48), or MT (p=0.99). Significant correlations were found between L_f measured using EFOV and those measured using Collage (r=0.98, p<0.001), MLE (r=0.81, p<0.001), Equation A (r=0.77, p<0.001), Equation B (r=0.67, p=0.001), Equation C (r=0.77, p<0.001), and Equation D (r=0.72, p<0.001). Significant correlations were also found between FA measured using EFOV and Collage (r=0.87, p<0.001) and between EFOV and static image (r=0.91, p<0.001). In addition, significant correlations were observed for MT measured using EFOV and Collage (r=0.99, p<0.001) as well as EFOV and static image ($r=0.98$, $p<0.001$). The linear regression (A) and Bland-Altman analysis (B) plots are shown in Figure 4 for L_f and Figure 5 for FA.

A strong Spearman's correlation coefficient was found between L_f measured using EFOV vs. Collage ($\rho=0.98$, $p<0.001$), MLE ($\rho=0.73$, $p<0.001$), Equation B ($\rho=0.71$, $p=0.001$) and Equation C ($\rho=0.75$, $p<0.001$), whereas moderate coefficients were found between L_f measured using EFOV vs. Equation A ($\rho=0.64$, $p<0.001$) and Equation D ($\rho=0.63$, $p<0.001$). Strong Spearman's coefficients were found between FA measured using EFOV vs. Collage ($\rho=0.84$, $p<0.001$) and static images ($\rho=0.90$, $p<0.001$).

Using Static images, 51.6 ± 8.90% of L_f was estimated using the Manual linear extrapolation, 50.7 ± 8.50% of L_f was estimated using Equation A, and 49.7 ± 7.8% of L_f was estimated using Equation C, with the remainder of the length measured in the visible part of the ultrasound image.

Table 1. Intra-day repeatability of the assessments of the BFlh fascicle length, fascicle angle and muscle thickness using EFOV, collage, MLE, and trigonometric equations.

Outcome	Technique	Day 1	Day 2	ICC [95% CI]	r	SEM	p	CV	d
Fascicle length (mm)	EFOV	87.0 ± 9.4	87.1 ± 9.7	0.95 [0.90-0.98]	0.92	2.61	0.926	2.22 ± 2.03	0.01
	Collage	87.6 ± 9.1	88.5 ± 9.6	0.95 [0.88-0.98]	0.91	2.78	0.348	2.47 ± 2.11	0.09
	MLE	93.2 ± 17.5	96.5 ± 21.3	0.93 [0.83-0.97]	0.89	6.40	0.141	5.21 ± 4.72	0.17
	Equation A	90.5 ± 15.5	92.2 ± 18.5	0.91 [0.78-0.97]	0.85	6.44	0.466	4.71 ± 4.97	0.09
	Equation B	87.7 ± 14.5	88.1 ± 20.5	0.94 [0.85-0.98]	0.94	4.23	0.864	4.74 ± 3.34	0.02
	Equation C	87.7 ± 13.5	89.6 ± 18.6	0.95 [0.86-0.98]	0.94	3.86	0.280	3.86 ± 3.51	0.11
	Equation D	86.5 ± 16.3	89.7 ± 20.2	0.96 [0.90-0.98]	0.95	4.02	0.054	3.53 ± 3.71	0.17
Fascicle angle (°)	EFOV	15.7 ± 2.8	15.8 ± 2.6	0.91 [0.77-0.96]	0.84	1.07	0.790	5.94 ± 3.84	0.05
	Collage	16.4 ± 2.4	16.5 ± 2.6	0.93 [0.82-0.97]	0.87	0.88	0.712	4.78 ± 3.15	0.03
	Static image	15.5 ± 2.3	15.6 ± 2.7	0.92 [0.80-0.97]	0.86	0.91	0.977	5.20 ± 3.67	0.00
Muscle thickness (mm)	EFOV	23.1 ± 3.2	22.6 ± 3.3	0.95 [0.88-0.98]	0.91	0.96	0.391	2.91 ± 3.19	0.14
	Collage	23.0 ± 3.4	22.8 ± 3.3	0.98 [0.94-0.99]	0.96	0.71	0.156	2.51 ± 1.93	0.06
	Static image	22.9 ± 3.3	22.8 ± 3.3	0.97 [0.92-0.99]	0.94	0.82	0.582	2.98 ± 2.32	0.05

MLE, manual linear extrapolation, r, Pearson's correlation coefficient; d, Cohen's effect size; SEM, standard error of measurement, CV, coefficient of variation of day 1 and day 2.

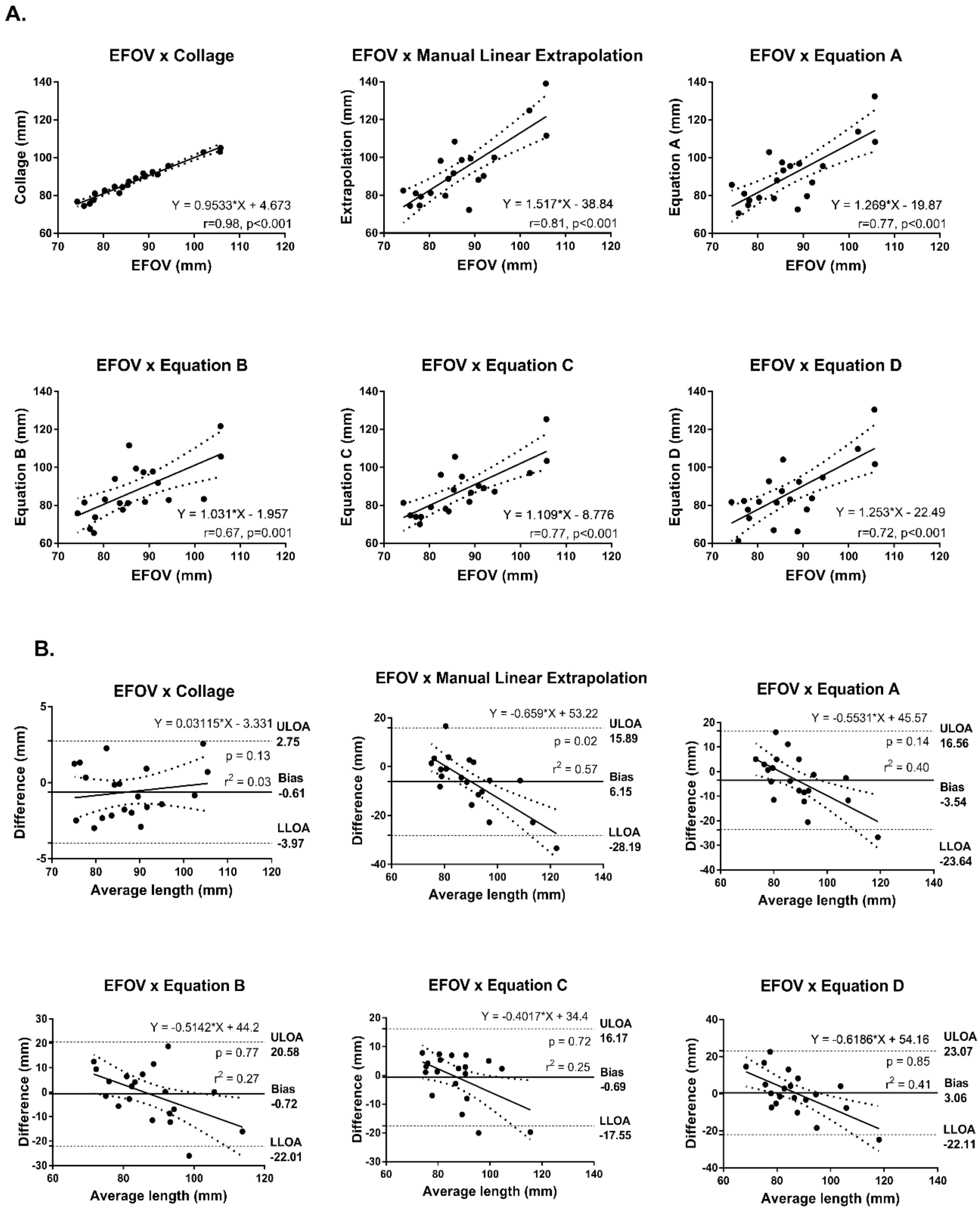

Figure 4. Agreement of L_f measurements between EFOV and Collage, manual linear extrapolation (MLE), and Equations A, B, C, and D: linear regression (A) and Bland-Altman analyses (B) showing absolute differences with respect to the average L_f obtained between methodologies. ULOA = upper limit of agreement, LLOA = lower limit of agreement.

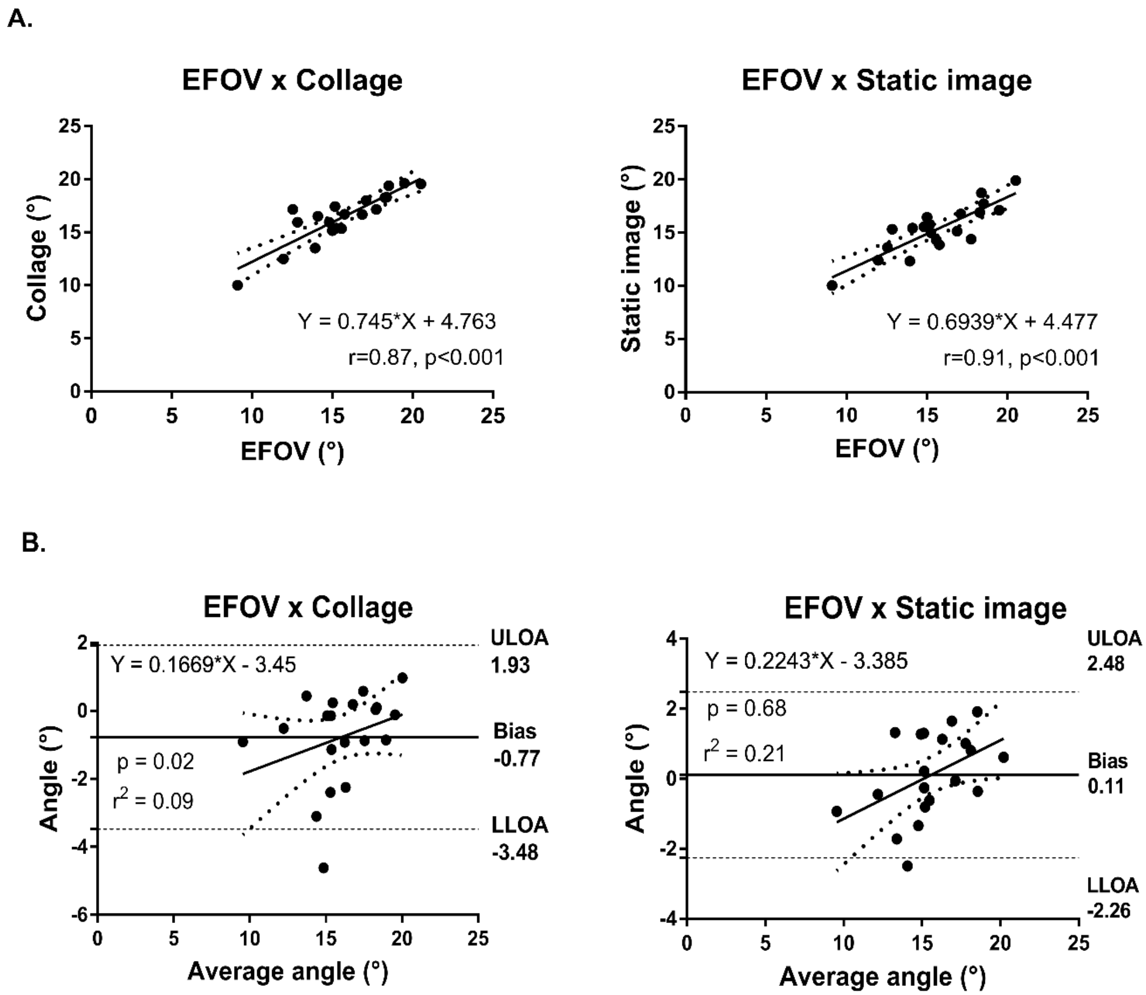

Figure 5. Agreement of FA measurements between EFOV, Collage, and Static image: linear regression (A) and Bland-Altman analyses (B) showing absolute differences with respect to the average FA obtained between methodologies. ULOA = upper limit of agreement, LLOA = lower limit of agreement.

2.4 DISCUSSION

The present study compared a variety of methods of BFlh muscle architecture parameter estimation, including EFOV, Collage of images, manual linear extrapolation (MLE), and four trigonometric equations. The main findings were that (a) all techniques showed acceptable between-day reliability for L_f, FA and MT assessment; (b) L_f measured with Collage, MLE, and Equations A-D were not statistically different from EFOV; (c) FA and MT measured with three different techniques (EFOV, Collage, Static image) resulted in similar outcomes. Our hypotheses that the different techniques would provide reliable estimates but that trigonometric extrapolation equations would overestimate L_f were partially confirmed. While reliability was very high between measurements made on Days 1 and 2 for L_f, FA and MT, systematic L_f overestimation was not observed for any trigonometric extrapolation equation. Given the high L_f reliability and both rank order and Pearson's correlations with EFOV (when using the methods presented herein), the Collage method can be recommended for use when extended field-of-view technology is not available.

2.4.1 Measurement reliability

Measurement reliabilities of L_f, FA and MT across Days 1 and 2 were very high (i.e. ICC ≥ 0.91) and the correlations between measurements were strong (i.e. r ≥ 0.84) across the seven methods used for L_f and three used for FA and MT estimation, in accordance with previous studies (Franchi et al. 2020; Pimenta et al. 2018). The high reliability found in all L_f measurements might be attributed to the digitization procedure adopted in the present study, which was similar for all methods. A segmented line tool was used in all L_f measurements, following the recommendations of Pimenta and colleagues (Franchi et al. 2020; Pimenta et al. 2018; Sarto et al. 2021) who reported higher reliability with the *segmented-line* tool than the *straight-line* tool. In addition, care was taken that all measurements were performed using the same probe pitch, roll and yaw orientations along the fascicle plane and therefore high reliability would be expected in all methods. Additionally, exceptional care was taken to locate the same anatomical site for imaging between days (see Methods for details) and to find the optimum probe angles to visualize both the fascicles and aponeuroses with high resolution. Although such details are not clearly described in previous studies, it may be that the high reliability and the close between-method agreement obtained in the present study is partly attributable to the attention paid to these details. We recommend that researchers pay close attention to these issues in future studies.

We should highlight the good reliability of Collage and Equation A methods, due to the novelty of these techniques. Collage has previously been used to diagnose musculoskeletal disorders (Kane et al. 2004) as well as to assess quadriceps muscle cross-sectional areas (Damas et al. 2016; Lixandrao et al. 2014). However, to the best of our knowledge, the present study is the first to use Collage to build an image of the BFlh to evaluate architectural parameters. Alternatively, Equation A used in the present study was first proposed by Stavnsbo and colleagues in his Master's thesis in 2011 and it was not previously published as a peer-reviewed paper. In that study, Stavnsbo and colleagues validated vastus lateralis L_f measurements and showed acceptable inter-rater and inter-session reliabilities. The use of the two aforementioned techniques is only possible if good reliability can be achieved, which we observed presently.

In case that the Collage is used to estimate L_f, extensive training and practice are recommended for both image acquisition and collage construction accuracy and precision (see Supplementary video data). To guide image acquisition, skin marks with a 2-cm distance were made on the participants' thighs to indicate the probe position for acquisition of each image. This distance was based on pilot testing in which we observed that this distance reduced the total number of images when compared to a closer image spacing yet led to less probe misalignment and improved image fitting (collage construction) when compared to larger image distances. Other researchers might find that different probe distances are more suitable for their image acquisition and collage construction, but regardless, this distance has to be carefully considered when building the Collage. In the present study, the 2-cm distance was indicated by using marks at the bottom of the images when exported from the ultrasound system. Attention was also given to subcutaneous fat and muscle aponeurosis markings, as shown in Figure 1. The proper alignment of these landmarks facilitated both image acquisition and collage construction. Considering the good between-day reliability of Collage, and the possibility to trace whole BFlh fascicles without the use of EFOV ultrasound, future studies might use this technique to evaluate BFlh architecture given the impossibility of using EFOV. Of note, although four consecutive images were used to build collage images, additional images may be needed in some cases in which individuals have longer fascicles (or fewer may be needed for shorter fascicles) as was the case for one participant in the present study.

The repeatability of FA and MT assessments were very high (ICC $\geq$ 0.91) and correlations between Day 1 and Day 2 measurements were also very high ($r \geq 0.84$). These results were expected as different methods of assessment should not influence FA or MT outcomes. The lack of between-method difference in MT indicates that the pressure and tilt of the probe on the muscle was minimal and consistent between the methods of assessment.

2.4.2 EFOV versus Collage of images and Extrapolation methods

The lack of differences in BFlh FA and MT estimates in the present study is consistent with previous studies (Franchi et al. 2020; Pimenta et al. 2018), and are somewhat expected considering that (i) the measurements were made in the proximal part of the images where the fascicles insert into the mid-belly aponeurosis, and (ii) EFOV, Collage and Single images were acquired with the same probe positioning.

Contrary to our hypotheses, however, we observed a lack of difference in L_f between Collage, MLE, Equations A, B, C, and D, and EFOV, despite slightly higher values being observed in MLE and Equation A. This finding contrasts that of previous studies (Franchi et al. 2020; Pimenta et al. 2018; Sarto et al. 2021). Pimenta and colleagues (Pimenta et al. 2018) reported longer L_f using the equation proposed by Blazevich and colleagues (Blazevich et al. 2006), referred to here as Equation D, than EFOV, and Franchi and colleagues (Franchi et al. 2020) reported longer L_f measured using the trigonometric equations proposed by Blazevich and colleagues (Blazevich et al. 2006) and Finni and colleagues (Finni et al. 2001), referred to here as Equations D and C, respectively. BFlh has a complex architecture with fascicles following nonlinear paths and, as previously described, possibly exhibiting both convex and concave curvatures (forming an “S” shape). Thus, the use of trigonometric equations has previously been discouraged. The lack of statistical difference in our study may be explained by the high measurement variability when using trigonometric equations. For an example, L_f measured using MLE or Equations A, B, C and D (see Table 1) had high standard deviations that were ~1.5 times the standard deviation for EFOV. The high variability in trigonometric estimates were also reported by Franchi et al

(Franchi et al. 2020) and by Sarto et al (Sarto et al. 2021) who also highlight that, for some individuals, Equation D underestimated whilst other equations overestimated L_f when compared to EFOV. In the present study, it is possible to observe in Figure 4B that for those with longer L_f, overestimation tended to happen using MLE and Equations A-D. For an example, when looking at individual cases, the same participant showed 33-mm and up to 26-mm L_f overestimations using MLE and the trigonometric equations, while L_f in another participant was underestimated by up to 22 mm compared to EFOV. This high variability underpins the different Spearman's rank-order correlations, the lower Pearson's correlation (r), and the greater data dispersion between techniques when compared to EFOV. The rank order differed most from EFOV for Equations A and D (ρ=0.64 and ρ=0.63, respectively), while a near-perfect rank-order correlation (ρ=0.98) was observed for Collage. Thus, a participant may be ranked differently within a cohort depending on the method used, and this issue needs to be considered in future research; comparisons between studies using different techniques may also be problematic.

The proportion of each fascicle that needed to be extrapolated in the present study were higher than previous studies in which 35.4 ± 7.0% was estimated using a 6-cm width probe (Pimenta et al. 2018). In the present study a 5-cm width probe was used, resulting in decreased field-of view and the necessity to estimate more of the fascicle's length. The amount of fascicle estimated in the present study is consistent with the suggestion of Pimenta et al (Pimenta et al. 2018) that each 1 cm decrease in ultrasound probe field of view will require approximately 11% more of the fascicle length to be estimated.

To make choices regarding L_f estimation, it is necessary to consider the muscle under study and the methods' specifications. BFlh fascicles are curved and have different fascicle length and MT along the muscle length (Kellis et al. 2010; Pimenta et al. 2018). These characteristics must be considered when using extrapolation techniques. For example, MLE and Equation D account for aponeuroses angle, which may induce error or increase L_f variability depending on the fascicle and aponeuroses curvatures outside the field of view. In addition, if for any reason (i.e. probe position, individuals' muscle specifications) the aponeuroses are non-parallel then estimation errors will result in under or overestimation of L_f, depending on the direction of the angle. This possible error due to aponeurosis angle was well explored by Sarto et al (Sarto et al. 2021), who reported that even a small change in the angle between aponeuroses can result in considerable change in L_f estimates in the absence of considerable changes in MT and FA. To decrease this error, drawing of a horizontal line crossing the distal end may be better than drawing a line crossing both visible ends of the visible aponeurosis. However, this measurement was not performed in the present study, so future studies could investigate this possibility. Despite the possible estimation error, no significant differences were observed when compared to EFOV; instead we observed high estimation variability, which may be particularly problematic for studies evaluating longitudinal changes in muscle architectural parameters. Indeed, Sarto et al (Sarto et al. 2021) observed that, after 10 days of bed rest, no significant alteration was observed in BFlh L_f using the equation proposed by Blazevich and colleagues (Blazevich et al. 2006) (named here as Equation D) but a significant decrease in L_f was observed when using EFOV, highlighting that the method used to estimate L_f may influence the findings of longitudinal studies.

2.4.3 Conclusions

Although no statistical differences were observed in L_f estimates between the seven methods, the use of trigonometric equations is discouraged for L_f estimation due to the higher between-subject measurement variability, resulting in under- and overestimation of L_f within individuals. This variability may not significantly affect single (acute) assessments but may cause issues with between-subject comparisons as well as accuracy of estimates of temporal L_f changes. Nonetheless, in addition to L_f measurements being very similar to EFOV, Collage produced the lowest L_f variability and the highest rank-order and Pearson's correlations with EFOV, possibly because Collage allows visualization of entire fascicles along their lengths, thus allowing their direct measurement. If care is taken, therefore, Collage may be the most suitable method for BFlh architectural assessment when EFOV technology is not available.

2.5 ACKNOWLEDGMENTS

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3. CHAPTER 3

STUDY 2: BICEPS FEMORIS FASCICLE BEHAVIOR DURING SUBMAXIMAL AND MAXIMAL SLOW SPEED CONTRACTIONS

3.1 INTRODUCTION

Fibers within pennate muscles change length and rotate during contraction. Fiber rotation contributes to whole muscle length change, thus influencing both the magnitude and velocity of fiber strain during dynamic contractions (Brainerd and Azizi 2005; Eng et al. 2018). The decrease in fiber length change for a given whole muscle length change during contraction should have important functional implications. For example, reduced fascicle length change should limit exercise-induced damage and thus minimize muscle fiber functional decline during repetitive contractions (Azizi and Roberts 2014). Alternatively, the reduced fiber length change, and thus the mean fiber length during contraction, might also speculatively attenuate the hypertrophic stimulus (McMahon et al. 2014b; Noorkoiv et al. 2014; Pedrosa et al. 2021).

Multiple factors influence the degree of fiber rotation. For example, reduced fiber rotation has been observed in contractions performed with heavier loads, thus increasing the requirement for muscle fiber length change (Brainerd and Azizi 2005). By contrast, increased fiber rotation is observed in contractions performed with lower loads, decreasing relative muscle fiber length change. Additionally, the total contribution of rotation to muscle length change has been shown to be greater in eccentric than concentric contractions (Azizi and Roberts 2014), which may offer a protective effect against damage or injury for the hamstring muscles by reducing negative fiber strain. Nonetheless, fiber behaviors are often studied in isolated muscle preparations (Azizi et al. 2008) and, although fascicle behavior (a proxy for fiber behavior) has been investigated *in vivo* in humans under some contraction conditions (Ando et al. 2018; Dick and Wakeling 2017; Maganaris et al. 1998; Randhawa et al. 2013; Wakeling et al. 2011), the effects of load on human muscles *in vivo* has yet to be studied in detail across a broad range of contraction conditions. Considering that length-dependent muscle (and intra-muscular) activation variations will affect fascicle behaviors (Butterfield and Herzog 2006), *in vivo* behaviors might also be expected to differ from those measured *ex vivo* because of the many factors influencing muscle function during dynamic contractions. Thus, it is of great interest to further our understanding of *in vivo* fascicle behaviors in work-producing skeletal muscles under differing contraction intensities and modes.

Eccentric contractions are unique in both their capacity to generate force, to stimulate muscle hypertrophy, and to evoke muscle damage (Franchi et al. 2017; Hody et al. 2019; Huxley and Simmons 1971). The greater force produced in maximal eccentric than isometric or concentric contractions should directly affect fascicle behavior (Azizi and Roberts 2014; Brainerd and Azizi 2005). However, additional intrinsic (within fiber; e.g. non-linear passive force-elongation properties) and extrinsic (e.g. extra-muscular pressures) factors should also influence *in vivo* fascicle behavior in muscles operating within synergistic groups (Eng et al. 2018). In addition, a muscle’s length will affect intramuscular pressure as well as passive forces, thus allowing fascicle behavior to vary according to muscle length. The possibility exists, for example, that fascicle strains may be greater over some whole-muscle length ranges, which might then influence both acute damage and longer-term hypertrophic outcomes. Because all muscles vary in their architecture, play different roles across tasks, and are submitted to different external forces from neighboring tissues, one might expect that fascicle behavior will not only vary between muscles (Ando et al. 2018; Azizi and Roberts 2014; Maganaris et al. 1998) but also vary according to muscle length.

One muscle that has been the target of a substantial recent research effort is the biceps femoris long head (BFlh), as it is the most susceptible muscle to strain injury during running (Ekstrand et al. 2011). Strain injuries occur when the hamstring muscle-tendon unit lengthens whilst receiving substantial activation from the central nervous system (Freckleton and Pizzari 2013; Thelen et al. 2005). Fiber (or fascicle) strain within BFlh is unlikely to mirror whole-muscle strain, yet the extent of BFlh fascicle rotation and length change during dynamic contractions remains unknown. It is also not known whether fascicle length change and rotation differ between concentric and eccentric contractions and whether this difference is muscle length dependent. This information may further our understanding of the conditions under which strain injuries occur and provide a platform to align fascicle behaviors with injury risk.

Given the above, the aim of the present study was to compare biceps femoris long head fascicle strain and rotation at long and short lengths between submaximal and maximal concentric and eccentric contractions. We tested the hypotheses that fascicle behavior would differ by both contraction mode and intensity and that these effects would be muscle length dependent.

3.2 METHODS

3.2.1 Participants

Twenty-two healthy physically active adults without history of right hamstring strain injury participated in the study. Due to probe-fascicle misalignments fascicles visible in one ultrasound field could not be confidently identified in the second ultrasound field, preventing accurate whole-fascicle length assessment, for this reason images of three participants had to be excluded. Additionally, one participant did not move through the full knee joint range of motion during data collection. Therefore, 18 participants (10 men and 8 women; age, 26.5 ± 4.1 years; height, 171.1 ± 6.9 cm; body mass, 70.4 ± 8.6 kg) were included in the analysis. The experimental protocol was approved by the Human Research Ethics Committee of Edith Cowan University (22326), written informed consent was given by all participants before participation.

3.2.2 Experimental design

To assess BFlh fascicle length (L_f), fascicle angle (FA) and muscle thickness (MT) changes during dynamic knee flexion contractions, two ultrasound systems were used simultaneously to visualize entire fascicles (or their majority), as done previously (Ando et al. 2018). To achieve these aims, the participants visited the laboratory on two occasions, seven days apart at the same time of day. In the first visit, participants were familiarized with the isokinetic, concentric-eccentric knee flexion exercise protocol to be performed in the subsequent testing session. Two to three attempts of each exercise were performed; however, if the participant reported not be confident with any task, then further attempts were performed until complete confidence was expressed. In the second visit, concentric and eccentric contractions performed at 30°/s were evaluated with simultaneous BFlh ultrasound assessment at submaximal (maintaining torque between 50% and 60% of maximal isometric contraction torque) and maximal intensities. All participants were asked not to perform exhaustive or unaccustomed lower-limb exercise for 48 h before each testing session.

3.2.3 Exercise protocol

Dynamic concentric and eccentric knee flexions were performed with the right limb on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical Systems, Shirley, NY). The participants laid prone with the hip placed on a large triangular pad to maintain a flexed position at ~45°. The dynamometer's axis of rotation was aligned with the lateral femoral condyle of the tested limb while straps were secured across the torso to restrict compensatory movements (Figure 1). A warm-up consisting of 10 submaximal, concentric, and eccentric contractions at 60% of self-controlled perceived maximum effort was performed at 60°/s; the slightly faster speed used in warm-up (compared to 30°/s in testing) was chosen to minimize time under tension and thus risk of fatigue. The exercise test protocol consisted of isometric and dynamic knee flexions performed in the following order: maximal isometric knee flexion; consecutive submaximal concentric and eccentric knee flexions at 30°/s; and maximal concentric and eccentric knee flexions at 30°/s (see below). BFlh ultrasound images were recorded during concentric and eccentric phases.

Before the dynamic test contractions were performed, two maximal isometric knee flexions were performed with the knee flexed at 40° (0° = full extension) with 3 min rest between trials, although a third trial was performed if >10% difference in peak torque was observed between the two trials. Then, during the submaximal concentric and eccentric test contractions at 30°/s, the participants were instructed to flex their knee whilst maintaining a torque level between 50% and 60% of their maximal isometric contraction torque, and as close as possible to the center of those lines (i.e. 55% torque), through a joint range of motion of 0 - 90° (contractions were checked to ensure that a full ROM was completed). A large monitor was placed in front of the participants showing the 50% and 60% target boundaries in real time along with the torque trace. The participants were verbally and visually stimulated to sustain the target torque. It was determined *a priori* that if a participant was unable to sustain the target torque (i.e. torque was visually observed to deviate outside the target lines) then a new trial would be allowed; however, no participant needed to perform additional trials. The contraction in which the participant maintained torque closest to 55% of maximum with the least calculated absolute error of the target torque through the range of motion was selected for analysis, whilst for maximal trials the concentric and eccentric repetition in which the greatest torque was produced was selected. For both submaximal and maximal dynamic contractions, one attempt of four consecutive concentric-eccentric repetitions was performed at each intensity. The first (concentric) and last (eccentric) contractions were subsequently removed from analysis, leaving three concentric and eccentric contractions for interrogation. Unfiltered torque and joint position data (analog-digital conversion rate = 2000 Hz) obtained during the isokinetic contractions were time synchronized to the ultrasound systems using a digital trigger signal fed from the computer running LabChart DAQ software (ADInstruments; Sydney, Australia) to both ultrasound systems.

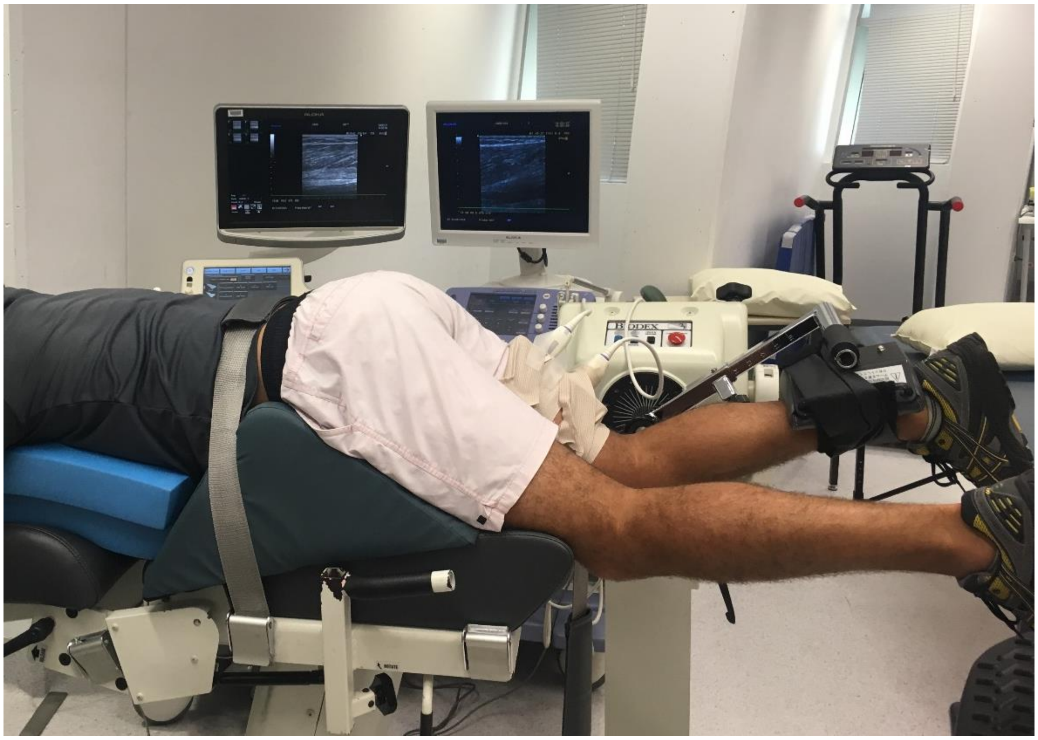

Figure 6. Participant positioning in the isokinetic dynamometer for strength assessments with concomitant ultrasound image acquisition.

3.2.4 Ultrasound imaging

Ultrasound images were acquired with two ultrasound devices (Prosound F75 and SSD, Aloka Co Ltd., Japan) in B-mode using linear probes with 50-mm fields of view (operating at 13 MHz, and 60-mm depth, Aloka Co Ltd, Japan) by an operator who had completed >12 months of sonographic training and had >300 practice scans. Two probes were used to avoid the need to use extrapolation methods to estimate Lf, as previous studies have reported estimation errors when using various trigonometric equations (see Study 1 in Chapter 2; Franchi et al. 2020; Pimenta et al. 2018). For all assessments, the ultrasound probes were coated with water-soluble gel to improve acoustic contact between the probe and skin, allowing for a higher image quality and for minimal pressure to be applied to the skin. Ultrasound images were captured at 30 Hz during concentric and eccentric knee flexion contractions, as described above.

To determine the region of interest (ROI) for image acquisition, a point 50% of the distance from the greater trochanter to femoral lateral condyle was identified. After the participant was placed in the prone position on the isokinetic dynamometer, the two transducers were longitudinally aligned in the fascicle plane with both superficial and deep (mid-belly/intramuscular) aponeuroses as close to parallel as could be identified and the hyperechoic lines delineating the BFlh fascicles (i.e., perimysial membranes) permitted a clear visualization of the fascicles inserting onto the deep aponeurosis. To determine the ROI, the participant's knee was first kept in a flexed position ~20° and, once a possible ROI was determined, the participant was asked to freely move the lower limb into knee flexion then extension. They were then also asked to flex the knee in a "mild isometric contraction" to ensure muscle visualization was clear during active muscle shortening/lengthening while taking care to produce images in which the fascicles remained continuous and visible, and the aponeuroses remained as parallel as possible. Extensive piloting indicated that this procedure resulted in the capture of the most useable images during subsequent testing (see Figure 2 for example images). Once the optimum ROI was identified, the two ultrasound probes were positioned longitudinally over BFlh with 1-cm distance between image windows; this distance was perfectly obtained when the probes sat together in series and did not need to be measured. The probes were placed within a custom-constructed device to improve probe stability and ensure that perpendicular positioning to the muscle was maintained. The devices were strapped to the leg using elastic bandage (Elastoplast

Sport, Elastoplast) to maintain probe position during the testing at a sampling rate of 25 (Prosound α10, Aloka, Japan) and 30 MHz (Prosound F75, Aloka, Japan).

3.2.5 Ultrasound image analysis

Videos from both ultrasounds were exported to an external computer and synchronized with the data exported from the dynamometer. Before analyses, videos were arranged in a collage matrix using Camtasia Techsmith software (Version 2019.0.0) to create a single, synchronized video of the whole ultrasound recording area during dynamic contraction. Care was taken to align the superficial and deep aponeuroses between videos and to accurately replicate the 1-cm spacing between the video edges. As the isokinetic dynamometer and ultrasound systems were synchronized, it was possible to specify in the videos the time at which each contraction was performed and the specific ROM reached; therefore, marks specifying the frames corresponding to every 10° of range of motion from 0 to 90° were inserted in the videos to guide and facilitate subsequent analysis, which were performed in one frame at each ROM. Videos were exported from Camtasia Techsmith to Fiji software (National Institute of Health, Betheseda, USA, Version 1.53) for analyses, as described below.

3.2.6 Muscle (BFlh) architecture assessments

BFlh fascicle length (L_f), fascicle angle (FA) and muscle thickness (MT) of three clearly observable fascicles were analyzed every 10° of knee range of motion from 0 to 90° of knee flexion, and the mean of the three fascicles was used for analysis. Fascicle length (L_f) was measured using the segmented line tool to follow the line of the fascicle as it curved, and L_f was measured from the mid-aponeurosis origin to its insertion onto the superficial aponeurosis. A fascicle was considered to be appropriate for analysis when a clear visualization of the insertion onto the mid-aponeurosis was possible, a clear fascicular pathway was observed from the proximal to the distal end through the images obtained by the ultrasound systems, and the fascicle insertion was clear throughout the full range of motion, as shown in Figure 2. FA was measured as the angle between the deep (mid-muscle) aponeurosis to the superficial aponeurosis along the fascicle. MT was measured as the vertical distance on the image from each fascicle insertion onto the deep aponeurosis to the superficial aponeurosis, and the mean value of the three fascicles was used for analysis. After determining the fascicles to be measured, the same three fascicles were tracked every 10° of ROM along the whole ROM. Post-hoc, changes (Δ) in L_f, FA and MT over the ROMs 0°-30° (long muscle length), 60°-90° (short muscle length) and 0°-90° (full length range) were calculated as fascicle shortening during concentric contractions and as fascicle lengthening during eccentric contractions, these values were submitted to statistical analysis. An example of muscle architecture assessment can be found at Figure 7.

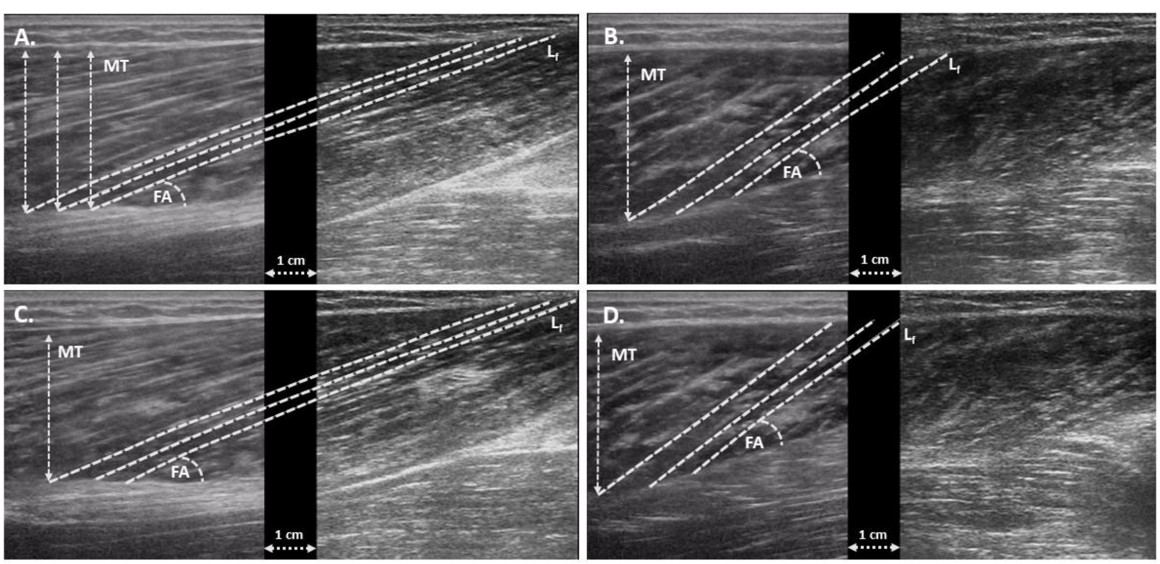

Figure 7. Example of fascicle length (L_f), fascicle angle (FA) and muscle thickness (MT) analyses during maximal submaximal (A and B) and maximal (C and D) eccentric contractions with the knee at 0° (A and C) and 90° (B and D) of flexion.

3.2.7 Statistical analysis

Normality of data was confirmed using the Shapiro-Wilk test. To examine fascicle behavior at long (Δ0-30°) and short (Δ60-90°) muscle lengths during submaximal and maximal concentric and eccentric contractions, three-way analyses of variance (ANOVA) with repeated measures (position [long vs. short] × contraction intensity [submaximal vs. maximal] × contraction mode [concentric vs. eccentric]) were used. To examine fascicle behavior from long to short muscle length (Δ0-90) two-way analyses of variance (ANOVA) with repeated measures (contraction intensity [submaximal vs maximal] × contraction mode [concentric vs eccentric]) were used. Torques produced during submaximal contractions at long and short lengths were compared using two-way analyses of variance (ANOVA) with repeated measures (position [long vs. short] × contraction mode [concentric vs. eccentric]). In addition, the mean torques produced at long and short muscle lengths during submaximal and maximal contractions were compared using three-way analyses of variance (ANOVA) with repeated measures (position [long vs. short] × contraction intensity [submaximal vs. maximal] × contraction mode [concentric vs. eccentric]). Separate analyses were performed for L_f, FA and MT. Significance level was set at $\alpha \leq 0.05$. All analyses were performed with SPSS software 20.0 (IBM, Somers, NY, USA), and results are reported as mean ± SD. To calculate the % of alteration of L_f, FA and MT in long muscle lengths and full length ranges, values at 0° were used as the reference, whereas in short muscle lengths, values at 60° were used as reference for both concentric and eccentric contractions.

3.3 RESULTS

Absolute changes (mean and SD) of ΔL_f, ΔFA and ΔMT at long and short muscle lengths and over the whole range of motion (0° to 90°) during submaximal and maximal concentric and eccentric knee flexions are shown in Table 2 and Figure 8. For detail of L_f, FA and MT at each angle (mean ± SD), see Appendix 6, Table 4.

ΔL_f during submaximal concentric and eccentric contractions were -14.1 ± 9.3% and 18.8 ± 6.1% at long muscle lengths (0°-30°), -10.5 ± 7.1% and 8.8 ± 7.5% at short muscle lengths (60°-90°), and -38.9 ± 7.8% and 40.6 ± 7.3% over the full length range (0°- 90°), respectively. During maximal concentric and eccentric contractions, ΔL_f were -15.6 ± 10.7% and 22.3 ± 7.7% at long muscle lengths (0°- 30°), -11.2 ± 5.2% and 8.5 ± 6.8% at short muscle length (60°- 90°), and -38.3 ± 6.5% and 39.9 ± 7.1% through the full length range (0°- 90°), respectively. A significant main effect of muscle length ($p < 0.001$) was observed in which greater ΔL_f was observed at longer muscle lengths (0°-30°). In addition, a significant main effect of contraction mode was observed ($p = 0.01$) in which greater ΔL_f was observed in eccentric contractions. The trend towards an effect of contraction intensity did not reach statistical significance ($p = 0.06$). These effects were accompanied by a significant interaction effect between muscle length and contraction mode ($p = 0.009$), with greater ΔL_f observed during both concentric and eccentric contractions at longer muscle lengths ($p = 0.02$, $p < 0.001$, respectively), but with this alteration being greater during eccentric contractions at the longer muscle length ($p = 0.003$). No significant muscle length ×

intensity ($p = 0.18$), contraction intensity × contraction mode ($p = 0.71$), or muscle length × contraction intensity × contraction mode ($p = 0.51$) interactions were observed; thus, the greater ΔL_f detected at longer muscle lengths during eccentric contractions was consistent between contraction intensities. When ΔL_f was analyzed through the whole ROM, no significant main effects of contraction intensity ($p = 0.92$) or contraction mode ($p = 0.22$), and no main significant contraction intensity × contraction mode interactions, were observed ($p = 0.92$).

ΔFA during submaximal concentric and eccentric contractions were 9.6 ± 17.3% and 16.1 ± 12.2% at long muscle lengths, 13.7 ± 16.1 % and 11.5 ± 14.9% at short muscle lengths, and 59.5 ± 42.2% and 63.1 ± 45.3% through the full length range, respectively. During maximal concentric and eccentric contractions, ΔFA were 11.6 ± 15.8% and 25.4 ± 25.0% at long muscle lengths, 11.5 ± 12.2% and 8.7 ± 15.5% at short muscle lengths, and 51.1 ± 41.8% and 64.7 ± 55.3% through the full length range, respectively. No significant main effects of muscle length ($p = 0.99$), contraction intensity ($p = 0.93$), or contraction mode ($p = 0.14$) were observed; i.e. no consistent differences in ΔFA were observed between conditions. However, a significant muscle length × contraction mode interaction ($p = 0.05$) was observed, with greater ΔFA at longer muscle lengths during the eccentric than concentric contractions ($p = 0.02$). No muscle length × intensity ($p = 0.13$), contraction intensity × contraction mode ($p = 0.72$), or muscle length × contraction intensity × contraction mode ($p = 0.56$) interactions were detected; thus, the greater ΔFA detected at longer muscle lengths during eccentric contractions was consistent between contraction intensities. When ΔFA was analysed through the whole ROM, no significant main effects of contraction intensity ($p = 0.20$) or contraction mode (although $p = 0.06$), and no main significant contraction intensity × contraction mode interactions, were observed ($p = 0.33$).

ΔMT during submaximal concentric and eccentric contractions were 0.5 ± 4.7% and 0.8 ± 4.3% at long muscle lengths, -1.5 ± 3.1% and 0.1 ± 5.3% at short muscle lengths, and -0.9 ± 7.8% and 1.1 ± 8.1% through the full length range, respectively. During maximal concentric and eccentric contractions, ΔMT were -0.5 ± 4.6% and 0.3 ± 5.4% at long muscle lengths, 0.7 ± 4.6% and 2.5 ± 3.6% at short muscle length, and -2.3 ± 8.6% and 1.2 ± 9.4%, through the full length range, respectively. No significant main effects of muscle length ($p = 0.07$), contraction intensity ($p = 0.17$) or contraction mode ($p = 0.54$) were observed. Further, no muscle length × intensity ($p = 0.46$), muscle length × contraction mode ($p = 0.67$), contraction intensity × contraction mode ($p = 0.07$) or muscle length × contraction intensity × contraction mode ($p = 0.50$) interactions were detected. When ΔMT was analysed through the whole ROM, no significant main effect of contraction intensity was observed MT ($p = 0.13$), however a significant main effect of contraction mode was observed ($p = 0.03$) in which a greater ΔMT was detected in concentric contractions. No main significant contraction intensity × contraction mode interactions were observed ($p = 0.48$).

Peak torques produced during the maximal concentric and eccentric contractions were 90.2 ± 22.3 Nm and 101.6 ± 25.0 Nm, respectively. Mean torques produced during submaximal concentric contractions were 56.2 ± 2.2 Nm and 58.8 ± 3.2 Nm at long muscle lengths, 50.9 ± 1.3 Nm and 57.5 ± 1.2 Nm at short muscle lengths, and 53.4 ± 2.9 Nm and 58.7 ± 3.2 Nm through the full ROM, respectively. Additionally, mean torques produced during maximal concentric and eccentric contractions were 72.5 ± 9.6 Nm and 76.6 ± 0.9 Nm at long muscle lengths, 54.8 ± 5.3 Nm and 68.3 ± 4.8 Nm at short muscle lengths, and 64.5 ± 10.6 Nm and 75.2 ± 11.7 Nm when analyzed through the full ROM. For Δtorque, significant main effects of muscle length ($p = 0.04$), contraction mode ($p = 0.004$), and contraction intensity ($p = 0.001$) were observed, such that greater torque was produced (i) at longer muscle lengths, (ii) during the eccentric contractions, and (ii) during the maximal intensity contractions. A significant muscle length × contraction intensity ($p = 0.03$) interaction subsequently indicated that while greater torque was produced in maximal contractions at both shorter ($p = 0.005$) and longer ($p = 0.001$) muscle lengths, the greater torque at longer lengths resulted from the increase during maximal rather than submaximal contractions ($p = 0.04$); torque in submaximal contractions did not differ between muscle lengths. No significant contraction mode × muscle length ($p = 0.55$), contraction mode × contraction intensity ($p = 0.10$), or contraction mode × muscle length × contraction intensity ($p = 0.74$) interactions were observed.

Table 2. Absolute change of length fascicle (ΔL_f), fascicle angle (ΔFA) and muscle thickness (ΔMT) at long (Δ 0 - 30°) and short (Δ60 - 90°) muscle lengths as well as through the full range of motion (Δ0 - 90°) during submaximal and maximal concentric and eccentric knee flexions.

		Submaximal		Maximal	
		Concentric	Eccentric	Concentric	Eccentric
Fascicle length change (mm)	Long	12.7 ± 8.1*	-16.9 ± 5.5*†	14.4 ± 10.1*	-20.4 ± 7.4*†
	Short	7.0 ± 5.1	-5.4 ± 4.6	7.6 ± 3.8	-5.4 ± 4.6
	Total	35.5 ± 7.6	-36.7 ± 8.0	35.6 ± 7.0	-36.9 ± 8.8
Fascicle angle change (°)	Long	-1.2 ± 2.2	2.0 ± 1.6†	-1.5 ± 2.1	2.9 ± 2.8†
	Short	-2.3 ± 2.8	1.2 ± 4.9	-1.7 ± 2.0	1.4 ± 2.8
	Total	-7.1 ± 4.9	6.2 ± 6.6	-5.7 ± 4.8	7.0 ± 5.5
Muscle thickness change (mm)	Long	-0.1 ± 1.2	0.2 ± 1.1	-0.1 ± 1.1	0.0 ± 1.4
	Short	0.4 ± 0.8	0.0 ± 1.2	0.5 ± 0.9	-0.6 ± 0.9
	Total	0.3 ± 1.9‡	0.2 ± 2.0	0.7 ± 2.1‡	-0.5 ± 2.4

*$p < 0.05$, significant main effect of ΔL_f muscle length, with greater ΔL_f at the longer lengths and †$p < 0.05$, significant main interaction of contraction mode and position for ΔL_f and ΔFA (separately), with greater ΔL_f and ΔFA observed during eccentric contractions at long muscle lengths; ‡$p < 0.05$ significant effect of ΔMT contraction mode, with greater ΔMT observed during concentric contractions when whole length changes were analyzed.

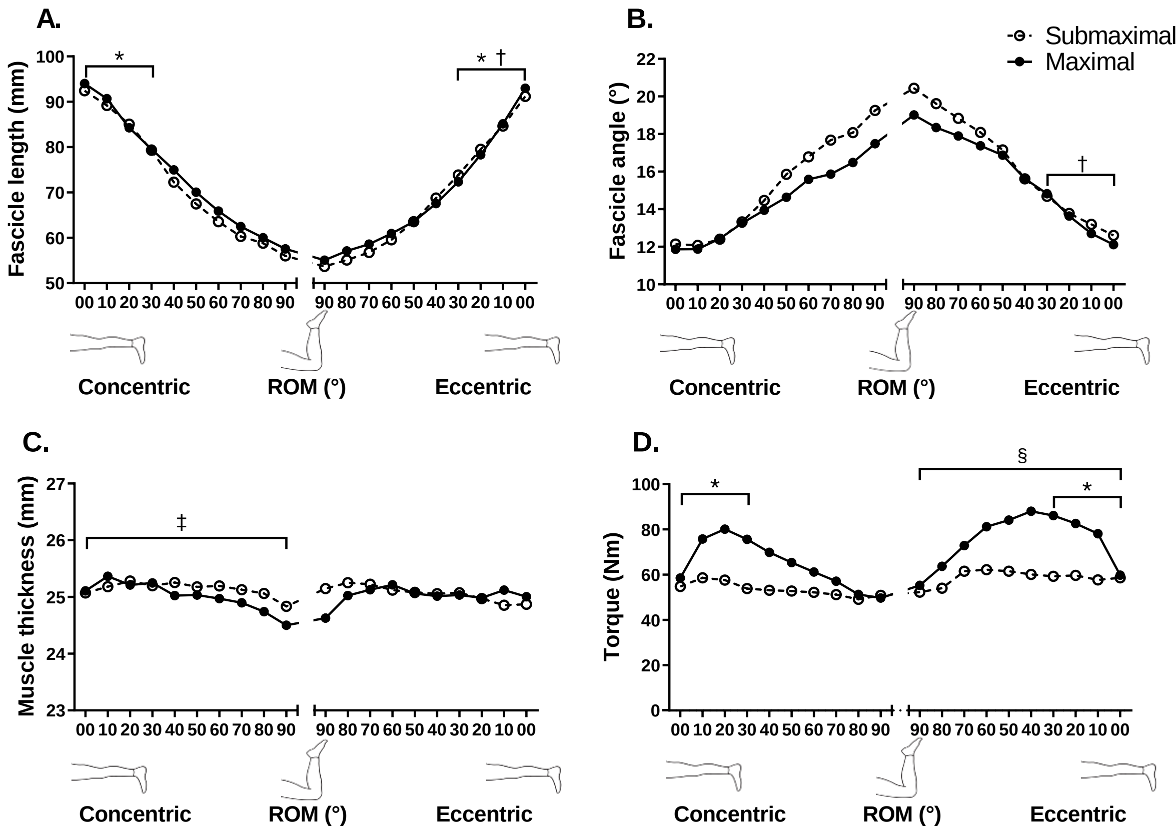

Figure 8. Fascicle length (A), fascicle angle (B), muscle thickness (C), and torque (D) during submaximal and maximal concentric and eccentric contractions.
*p < 0.05, greater change in fascicle length (A) and torque (D) were at the longer lengths; †p < 0.05, greater change in fascicle lengths (A) and fascicle angle (D) was observed during eccentric contractions at long muscle lengths; ‡p < 0.05, greater change in muscle thickness (C) was observed in whole length range during concentric contractions; § p < 0.05, greater change in torque (D) was observed during the eccentric contractions and during the maximal intensity contractions (open vs. closed circles in D).

3.4 DISCUSSION

The main findings of the present study were that (i) greater ΔL_f was observed at longer than shorter muscle lengths in both concentric and eccentric contractions, with this difference being larger in eccentric contractions, (ii) greater ΔFA was observed at longer muscle lengths during eccentric than concentric contractions, (iii) in both submaximal and maximal intensity contractions, ΔL_f and ΔFA were similar between concentric and eccentric contractions when analyzed though the whole ROM (from 0° to 90°), validating the analysis of non-consecutive repetitions.

The first major finding of the study, that a greater fascicle length change (ΔL_f) in BFlh was observed when the knee was at more extended joint angles (and thus when the hamstrings were at longer lengths), especially during eccentric contractions (about four times greater at long than short muscle lengths) is similar to the findings of Van Hooren et al (Van Hooren et al. 2022) who found that BFlh fascicle length did not change substantially until the end of the range of motion (i.e. long muscle length) during the Nordic hamstring curl and Roman chair hold, and has several potential practical implications. In relation to intra-cellular signaling, increased fiber strain during strength training has been shown to promote both a robust protein synthesis signaling response (e.g. Akt/mTOR and p70S6k signaling; Rindom et al. 2019; Russ 2008) as well as a greater resulting whole-muscle hypertrophy after a period of training (McMahon et al. 2014b; Noorkoiv et al. 2014; Pedrosa et al. 2021). Thus, the greater fascicle strain under eccentric conditions might partly explain the greater muscle hypertrophic responses observed when eccentric contractions are used in training, either in isolation (Farthing and Chilibeck 2003; Higbie et al. 1996) or during consecutive concentric-eccentric contraction repetitions (McMahon et al. 2014b; Pedrosa et al. 2021). Conversely, increased fiber strain during contraction has also been shown to induce greater fiber injury, increasing the potential for either or both exercise-induced muscle damage or acute muscle injury (Askling et al. 2008; Nosaka and Sakamoto 2001). It will be of future interest to determine whether individuals who show greater resistance to acute damage or injury show a reduced fascicle strain during eccentric contractions, and whether acute interventions such as local muscle fatigue or chronic interventions such as strength or flexibility training alter the fascicle strain response during eccentric contractions.

A greater fascicle rotation (ΔFA) was also observed at longer muscle lengths during submaximal and maximal eccentric contractions, which should theoretically have contributed to overall muscle lengthening and thus reduced the need for fascicle length change (Azizi and Roberts 2014). Fascicle rotation should therefore be considered as a strategy leading to reduced injury susceptibility. Nonetheless, while fascicle rotation increased at longer lengths, it did not offset the requirement for fascicle lengthening, which was also greater at long muscle lengths. Regardless, the possibility exists that individuals who show a greater fascicle rotation might possess greater resilience to damaging processes during eccentric contractions, and this is an interesting hypothesis to test in future studies.

Cumulatively, the findings of both greater elongation and rotation at longer muscle lengths during eccentric contractions indicate that greater whole BFlh muscle lengthening must have occurred when the knee extended nearer to anatomical position, and that both ΔL_f and ΔFA are thus strongly dependent on muscle length *in vivo*. One might speculate that changes in muscle moment arms contributed to a greater muscle length change at more extended angles; that is, an increasing moment arm would have required greater muscle length change to achieve a given joint angle rotation. However, this is neither consistent with published data showing a decreased BFlh (and general hamstrings) moment arm at more extended knee angles (Buford et al. 1997; Herzog and Read 1993) nor with the contrasting fascicle length change and rotation behavior observed in concentric contractions through the same range of motion in the present study. An alternative explanation is that early joint rotation is accompanied by relatively greater stretch in series elastic components during isokinetic eccentric contractions, with later fascicle lengthening and rotation resulting in greater muscle length change. Conversely, concentric contractions might instead be performed with the series components stretched as force increased at contraction initiation, so the majority of the range of motion would require relatively constant changes in muscle length. Evidence against this hypothesis includes that these eccentric fascicle behaviors were also observed in the submaximal contractions, in which torque was relatively constant and the increasing moment arm during knee extension should have increased the muscle force requirement, subsequently stretching series elastic components and thus reducing the need for fascicle lengthening. Possibly, a combination of factors explains the finding, including alterations in intra- and inter-muscle activation and both intrinsic and extrinsic muscle pressures (impacting muscle shape change and thus fascicle behaviour; Wakeling and Randhawa 2014), and it will be of great interest to determine whether similar fascicle behaviors are observed in future experiments in isotonic or isoinertial contractions.

The fascicle lengthening and rotation behaviors observed under the conditions of the present study differ from previous studies using *ex vivo*, single-muscle models that reported a reduced fascicle lengthening, or a quasi-isometric fascicle behavior, during eccentric contractions; i.e. the muscle operated in relatively high gear when contracting eccentrically (Azizi and Roberts 2014). Such fascicle behavior should protect the muscle from excessive fascicular strain, indicating that both fascicle rotation and series elastic elongation act as mechanical buffers during active lengthening (Azizi and Roberts 2014; Reeves and Narici 2003). However, considerable differences in study designs likely underpin the between-study differences. Primarily, Azizi and Roberts (2014) analyzed behaviors *in vitro* during isotonic phases of contractions so that tendon and changes in muscle force could not influence fascicle behavior. However, in the present study the participants either varied their torque, and thus muscle force, greatly (e.g. during maximal contractions; see Figure 3.3) or maintained relatively constant torque (e.g. during submaximal contractions) in which case muscle forces would have been relatively small and result only from a changing moment arm. Instead, the joint rotation amplitude and velocity were held constant in the present study, and thus the muscle length change would have varied between conditions as the muscle forces varied. This allowed, for example, greater fascicle length change at the longer than shorter muscle lengths despite the same angular rotation amplitude being imposed. In the present study, BFlh fascicle behavior was tested *in vivo* so numerous synergists possibly contributed to muscle length change, to force production, and ultimately to fascicle behavior. Cumulatively, these substantial methodological differences (and others not listed herein), likely explain the differing results between studies and indicate that in vivo behaviors may be difficult to predict from data obtained *ex vivo*.

The greater ΔFA observed at longer muscle lengths during maximal eccentric contractions was accompanied by greater Δtorque with the muscle at longer length during the maximal contractions. This result is not consistent with the lesser fascicle rotation reported with increasing contraction force in previous studies (Azizi and Roberts 2014; Reeves and Narici 2003), although the present results seem to be reasonable from a muscle mechanical viewpoint. Fibers of pennate muscles are arranged at an angle relative to the muscle's axis of force transmission (Eng et al. 2018), and only the component of fiber force that is in line with the muscle axis of force transmission will contribute to muscle force. The greater ΔFA during maximal eccentric contractions means that the fascicles were less angled relative to the aponeurosis later in the contraction (and, presumably, the tendon), favoring force transmission to the tendon. This should allow greater force transmission from the muscle, as required during high force contractions. The greater rotation might therefore assist force production at longer lengths, shifting the torque-angle curve, or at least changing its shape to extend the plateau region and reduce the slope of the descending limb.

A similar magnitude of fascicle lengthening (ΔL_f) and rotation (ΔFA) were observed in submaximal and maximal contractions when whole ROM (from 90° to 0°) was analyzed, which was expected given that the same range of motion was used; this result is, however, important for study validation since the fascicle analyses were not necessarily performed on consecutive contractions (Table 3.1, Figure 3.3). Previous observations indicate that the greater fascicle rotation during low force contractions should be accompanied by an increased thickness (Azizi et al. 2008; Wakeling and Randhawa 2014). However, no evidence was found for this in the contractions performed in the present study. As MT was not a primary outcome, the probe was primarily aligned to achieve the most accurate L_f and FA measurements, so we cannot exclude the possibility that, during contraction, the ultrasound transducer was not perfectly aligned to accurately capture MT. Regardless, the lack of detection of changes in MT probably indicate that any real changes that occurred would have been relatively small. Therefore, significant changes in muscle width likely occurred in order to maintain volume as the muscle length changed; this shape change can be more specifically examined in future studies. Also worthy of consideration is that BFlh has a heterogeneous fiber architecture along its length (e.g. greater fascicle length and lower fascicle angle proximally; Kellis et al. 2010), allowing fascicle behavior to vary intramuscularly. So fascicle behaviors in other BFlh regions might differ to those observed in the central region studied presently.

3.4.1 Limitations

A possible limitation of the present study is that dynamic contractions induce variable changes across the muscle-tendon unit and, although great effort was made to correctly align the transducer to the fascicle plane, we cannot exclude the possibility that fascicles moved out of the recording plane during contraction. Our main concerns were to capture fascicle length and angle in the correct plane across the whole contraction range of motion, and the simultaneous use of two ultrasound probes allowed us to obtain a greater window width to capture the full length of fascicles as well as to manipulate the orientation of each probe independently. In the case that fascicles moved out of window view, a degree of error is induced. However, this effect of this error is reduced by using the same ultrasound placements across conditions, reducing within-subject error.

3.4.2 Conclusion

We observed greater ΔL_f and ΔFA at longer BFlh muscle lengths (i.e. as the knee progressed towards extension), with this effect being greater during eccentric than concentric contractions. Thus, both contraction mode and muscle length affected BFlh fascicle behavior *in vivo* although the effects of joint torque (and thus muscle force) were less substantive. The increased fascicle strain observed at long muscle lengths might be expected to promote acute molecular signaling and subsequently enhance hypertrophic responses to continued exercise training, but might also potentially acutely increase fiber damage (or injury) risk.

4. CHAPTER 4

STUDY 3: THE EFFECT OF FATIGUE ON BICEPS FEMORIS FASCICLE BEHAVIOR DURING MAXIMAL ISOKINETIC CONTRACTIONS

4.1 INTRODUCTION

All pennate muscles share a capacity to change length by altering fascicle length, angle (fascicle rotation), or both, under varying contractile conditions. During concentric contractions, both *in vivo* and *in vitro* studies show that fascicles (and their constituent fibers) shorten while simultaneously rotating to greater angles, allowing a greater whole muscle shortening for a given fascicle length change (Brainerd and Azizi 2005). On the other hand, studies in *ex vivo* muscle preparations show that fascicles rotate substantially whilst lengthening during eccentric contractions (Azizi and Roberts 2014), minimizing fascicle strain and thus potentially reducing subsequent muscle damage. Notwithstanding, strain injuries still occur in pennate muscles despite the protection offered by fascicle rotation.

Muscles are complex structures, and their function is influenced by numerous intrinsic (e.g. intramuscular pressures, connective tissue properties, stretch of external tendon) and extrinsic (external loading, extramuscular pressures) factors that should ultimately influence fascicle behavior (Wakeling et al. 2011). In a recent *in vivo* study, biceps femoris long head (BFlh) fascicle length and angle changes were not detectibly altered by modest changes in concentric or eccentric contraction intensity, despite previous evidence indicating that it should have been the case (Azizi & Roberts, 2014), although fascicle lengthening was found to be greater at longer muscle lengths during eccentric than concentric contractions (Study 2, Chapter 3). Nonetheless, it is possible that factors other than contraction intensity, including changes in intermuscular coordination, intramuscular pressures, or fiber-specific forces, might influence fascicle behavior in muscles that operate within synergistic groups (Wakeling et al. 2011). Thus, an examination of fascicle length and angle changes under a variety of conditions is needed to further our understanding of human skeletal muscle operation.

A critical effect of fascicle strain is that it imposes a strain injury risk on muscle fibers (Lieber and Friden 2002). In humans, strain injuries to the hamstring muscles are the most common and predominately affect the biceps femoris long head (BFlh) (Crema et al. 2018; Ekstrand et al. 2011). These injuries often occur during the late swing phase of high-speed running (Askling et al. 2007; Koulouris and Connell 2003; Opar et al. 2012), possibly because the hamstring muscle-tendon unit is strained considerably whilst receiving significant activation from the central nervous system (Freckleton and Pizzari 2013; Thelen et al. 2005). As the amount of fascicle strain during this phase differs from whole muscle-tendon unit length change (e.g. due to fascicle rotation, tendon length change, etc.), it is questionable whether strain and rotation at the fascicle level are substantial, and thus influence strain injury risk during contractions *in vivo*. However, it is expected that risk would be increased if fibers worked at longer average lengths or produced greater strain whilst activated (Butterfield and Herzog 2005). As BFlh fascicle behaviors during contraction have not yet been fully described, no conclusions or recommendations regarding the possible contribution of fascicle strain on injury can be drawn.

Alongside a number of risk factors, muscle fatigue is speculated to influence strain injury risk (Huygaerts et al. 2020; McCall et al. 2015a; McCall et al. 2015b). The performance of repeated sprint running efforts for example, as required in many sports, induces greater and more prolonged strength loss (i.e. fatigue) in the hamstrings than the quadriceps muscles (Baumert et al. 2021). As a consequence, decreases in muscle force capacity, muscle swelling, and hip knee kinematic muscle activation alterations (or coordination) are observed immediately after repeated sprint efforts, which may increase strain injury risk (Baumert et al. 2021). The fatigue-induced decrease in force capacity might be expected to directly influence fascicle behaviors given that contraction intensity itself has been shown to influence fascicle rotation and strain during eccentric contractions in *ex vivo* preparations (Azizi and Roberts 2014), despite the lack of effect of contraction intensity observed in Study 2 (Chapter 3) suggesting that intensity-dependent effects *in vivo* may be small, or that effects might only be observed between contractions of substantially different intensities. Nonetheless, fatigue also exacerbates muscle swelling, increasing fluid pressures both within BFlh and surrounding muscles. Swelling would be expected to impact muscle shape changes during dynamic contractions, which should consequently affect fascicle rotation and length change (Eng et al. 2018). Additionally, alterations in relative activation of muscles within a synergist group may also occur with fatigue (Lepers et al. 2002), influencing factors such as intra- and inter-muscular forces and therefore influencing fascicle strain and rotation patterns. Thus, fatigue-induced fluid pressure and activation changes might speculatively alter fascicle behaviors and consequently influence injury risk, although this hypothesis has yet to be explicitly tested *in vivo* muscles.

The circumstances that place BFlh at greater injury risk are complex and multifactorial and include contraction mode, contraction intensity, and instantaneous muscle length. Moreover, it is not yet clear whether *in vivo* fascicle behaviors are a causal factor of injury. A first, important, step towards gaining a more complete understanding of injury mechanisms is to describe fascicle behavior in BFlh under controlled contractile conditions both with and without the presence of fatigue. Hence, the aim of the present study was to compare biceps femoris long head fascicle length change and rotation at long and short muscles lengths between concentric and eccentric contractions and to determine the specific, immediate effects of fatiguing exercise on these behaviors. We hypothesized that fascicle behavior would differ between contraction modes, and that these differences would both vary with muscle length and be acutely affected by fatiguing exercise.

4.2 METHODS

4.2.1 Participants

Twenty-two healthy physically active adults without history of right hamstring strain injury participated in the study. Due to misalignments, videos of three participants could not be analyzed and one participant did not reach the whole range of motion during data collection, therefore 18 participants (10 men and 8 women; age, 26.5 ± 4.1 years; height, 171.1 ± 6.9 cm; body mass, 70.4 ± 8.6 kg) were used for analysis. The experimental protocol was approved by the Human Research Ethics Committee of Edith Cowan University (22326), written informed consent was given by all participants before participation.

4.2.2 Experimental design

Changes of BFlh fascicle length (L_f), fascicle angle (FA) and muscle thickness (MT) during dynamic knee flexion contractions before and after fatiguing exercise, were assessed with two ultrasound systems used simultaneously to visualize entire fascicles. For this purpose, two visits to the laboratory at the same time of day with at least seven days' interval were required. Familiarization with the isokinetic knee flexion exercises was performed in the first visit, each participant executed two to three attempts of concentric and eccentric knee flexion and additional trials were allowed if necessary for complete understanding. In the second visit, concentric and eccentric knee flexion contractions were evaluated at 180°/s before and after fatiguing exercise with simultaneous BFlh ultrasound assessment. Although vigorous non-isokinetic movements are performed at higher speeds than those selected in the present study, contractions were performed at 180°/s as fewer frames of high-resolution ultrasound imaging data can be captured at faster speeds, compromising L_f, FA, MT analyses. Also, contractions in the current study did not include a stretch-shorten cycle action, which is predominant in faster human movements. Participants were asked not to perform exhaustive or unaccustomed lower-limb exercise for 48 h before each testing session. Figure 9 details the experimental design.

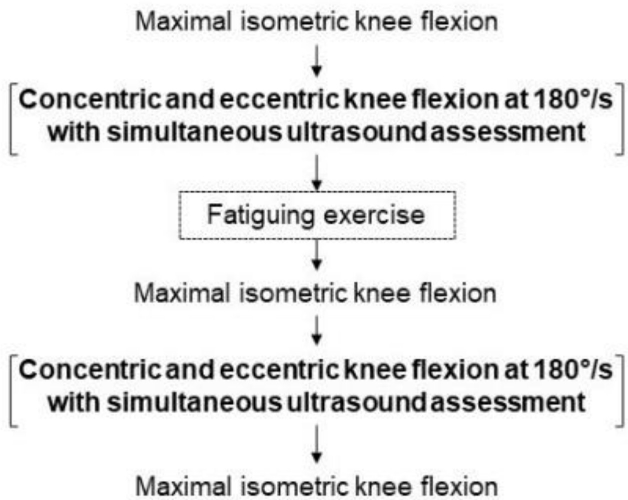

Figure 9. Experimental design

4.2.3 Test protocol

Dynamic concentric and eccentric knee flexions were performed on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical Systems, Shirley, NY) with the participants laying in the prone position. A supporting pad was used to maintain hip flexion at ~45° while also aligning the dynamometer's axis with the lateral femoral condyle of the tested limb. Inelastic straps were placed across the torso to restrict compensatory movements. The exercise test protocol consisted of isometric and dynamic knee flexions performed before and after fatiguing exercise (described below). Tests were preceded by a warm-up of 10 concentric and eccentric submaximal dynamic contractions (at 60% of their perceived maximum effort) performed at 60°/s.

Maximal isometric knee flexions were performed before the first series of dynamic test contractions, immediately after the fatiguing exercise, and immediately after the post-exercise dynamic test contractions. This protocol was chosen to confirm the presence of fatigue after the fatiguing exercise. Before the fatiguing exercise, two maximal isometric knee flexions were performed with the knee flexed at 40° (0° = full extension) with 3 min rest between trials; a third trial was performed if more than 10% difference in peak torque was observed between the two trials (this was eventually offered to 5 participants). After the fatiguing exercise only one attempt was performed at each time point.

Maximal concentric and eccentric knee flexions at 180°/s were performed before and after the fatiguing exercise, with one attempt of four consecutive concentric and eccentric contractions performed at each time point. Contractions started with the knee fully extended so the first contraction was concentric and was followed immediately by eccentric knee flexion. The fatiguing exercise protocol consisted of 50 consecutive maximal concentric knee flexions at 180°/s. Concentric contractions were performed to disturb the metabolic homeostasis to induce force decline without inducing any muscle damage. Participants were encouraged to always perform the contractions with maximal effort, and visual feedback was provided in real time by a screen placed in from of them. A 3-min rest was allowed between trials for tests performed before fatiguing exercise (isometric and dynamic contractions), but no rest Excepting several seconds to prepare for the test) was allowed between trials after the fatiguing exercise. Concentric and eccentric contractions with the highest peak torque performed before and after the fatiguing exercise were separately selected for analysis.

BFlh ultrasound images were recorded during both concentric and eccentric knee flexions before and after the fatiguing exercise. Unfiltered torque and joint position data (1000-Hz analog-to-digital conversion rate) obtained during the isokinetic contractions were time synchronized to the ultrasound systems using a digital trigger signal fed from the computer running LabChart DAQ software (ADInstruments; Sydney, Australia) to both ultrasound systems.

4.2.4 Ultrasound imaging

Two ultrasound devices (Prosound F75 and SSD, Aloka Co Ltd., Japan) were used to simultaneously capture images in B-mode. Linear probes with 50-mm fields of view (operating at 13 MHz and 60-mm depth; Aloka Co Ltd, Japan) were positioned longitudinally over the muscle in a custom-constructed device that improved probe stability and ensured that perpendicular positioning to the muscle was maintained. The distance between probes was as minimal as possible, with 1-cm distance between image windows. This distance was possible by placing the probes in series immediately adjacent to each other. Images were acquired by an operator who had completed >12 months of sonographic training and had >300 practice scans. For all assessments, the ultrasound probes were coated with enough water-soluble gel to allow acoustic contact between the probe and skin whilst applying minimal pressure to the skin. Ultrasound images were captured at 30 Hz during the dynamic knee flexion contractions, as described above.

To determine probe placement on the thigh for BFlh image acquisition, the region of interest (ROI) was identified at a point 50% of the distance from the greater trochanter to femoral lateral condyle. The ROI was specified as the position at which both BFlh superficial and deep (mid-belly/intramuscular) aponeuroses were as close to parallel as possible, the hyperechoic lines delineating the BFlh fascicles (i.e., perimysial membranes) permitted a clear visualization of the fascicles inserting onto the deep aponeurosis, and the fascicles were well visualized for the maximum of their lengths. The ROI was first determined in the most proximal transducer, then

ROI of the distal transducer was determined by maintaining alignment of the transducers to follow the fascicular path. To determine the ROI, the participant was placed in the prone position on the isokinetic dynamometer and the knee was flexed at ~20°. When a possible ROI was determined, the participant was asked to freely move the lower limb into knee flexion then extension and also to produce a mild isometric contraction. These tasks were performed several times to ensure optimal transducer placement. When a clear visualization of the fascicles was possible in both ultrasound fields of view, considering that the fascicles should remain continuous and visible across both ultrasounds and the aponeuroses should remain quasi-parallel, the probes were positioned in a custom-constructed device and fixed onto the thigh with elastic bandage (Elastoplast Sport, Elastoplast) to maintain probe position during the whole testing session.

4.2.5 Ultrasound image analysis

Videos from both ultrasound systems and the dynamometer data were synchronously exported to an external computer. A collage of videos from both ultrasounds was created to build a single, synchronized video of the muscle for each trial using Camtasia Techsmith software (Version 2019.0.0). Videos were built with a 1-cm spacing between edges to respect ultrasound distance, and superficial and deep aponeuroses were aligned in the videos. Also, marks were placed in the videos specifying the frames corresponding to every 10° of range of motion from 0 to 90°, which were made to allow analyses over specific knee joint ranges of motion. Analyses were performed on the collage videos, which were exported from Camtasia Techsmith to ImageJ software (National Institute of Health, Betheseda, USA, Version 1.53) analyses.

4.2.6 Muscle (BFlh) architecture assessments

BFlh fascicle length (L_f), fascicle angle (FA) and muscle thickness (MT) of three clearly observable fascicles were analyzed every 10° of knee range of motion from 0 to 90° of knee flexion, with the mean of the three fascicles being used for analysis. Fascicle length (L_f) was measured using the segmented line tool to follow the line of the fascicle as it curved, and L_f was measured from the mid-aponeurosis origin to its insertion onto the superficial aponeurosis. A fascicle was considered to be appropriate for analysis when a clear visualization of the insertion onto the mid-aponeurosis was possible, a clear fascicular pathway was observed from the proximal to the distal end through the images obtained by the ultrasound systems, and the fascicle insertion was clear throughout the full range of motion. FA was measured as the angle between the deep (mid-muscle) aponeurosis to the superficial aponeurosis along the fascicle. MT was measured as the vertical distance on the image from each fascicle insertion onto the deep aponeurosis to the superficial aponeurosis, and the mean value of the three fascicles was used for analysis. After determining the fascicles to be measured, the same three fascicles were tracked every 10° of ROM along the whole ROM. Post-hoc, changes (Δ) in L_f, FA and MT over the ROMs 0°-30° (long muscle length), 60°-90° (short muscle length), and 0°-90° (full length range) were calculated as fascicle shortening during concentric contractions and as fascicle lengthening during eccentric contractions, and these values submitted to statistical analysis.

4.2.7 Torque

The mean torque produced at long (Δ0-30) and short (Δ60-90) muscle lengths and over the whole range of motion (Δ0-90) were used for analyses. Peak isometric torque produced before and after fatiguing exercises were used for analyses.

4.2.8 Statistical analysis

Normality of data was tested using the Shapiro-Wilk test after determining that the sample was normally distributed. To examine fascicle behavior and torque at long (Δ0-30) and short (Δ60-90) muscle lengths during concentric and eccentric contractions performed before and after fatiguing exercise, separate three-way analyses of variance (ANOVA) with repeated measures (position [long vs. short] × contraction mode [concentric vs. eccentric] × fatigue [before vs. after fatiguing exercise) were used for ΔL_f, ΔFA, ΔMT and Δtorque. To examine fascicle behavior and torque across the whole range of motion (Δ0-90), a separate two-way analysis of variance (ANOVA) with repeated measures (contraction mode [concentric vs. eccentric] × fatigue [before vs. after fatiguing exercise) was used. Separate analyses were performed for ΔL_f, ΔFA, ΔMT and Δtorque. Mean fascicle length (L_f) before and after fatiguing exercise was also compared using paired sample t-tests for concentric and eccentric contractions separately. Peak isometric torque was compared over time using one-way analyses of variance (ANOVA). Significance level was set at $\alpha \leq 0.05$. All analyses were performed with SPSS software 20.0 (IBM, Somers, NY, USA), and results are reported as mean ± SD.

4.3 RESULTS

ΔL_f, ΔFA and ΔMT at long (Δ0-30) and short (Δ60-90) muscle lengths and over the whole range of motion (0° to 90°) during maximal concentric and eccentric knee flexions performed before and after fatiguing exercise are shown in Table 3. For detail of L_f, FA and MT at each angle (mean ± SD), see Appendix 7, Table 5.

ΔL_f during concentric and eccentric contractions before fatiguing exercise were 8.4 ± 7.6% and 16.0 ± 11.6% at long muscle lengths (0°-30°), 12.9 ± 7.8% and 8.7 ± 8.6% at short muscle lengths (60°-90°), and 31.3 ± 8.5% and 36.3 ± 7.6% over the full length range (0°-90°), respectively. During concentric and eccentric contractions after fatiguing exercise, ΔL_f were 7.1 ± 7.6% and 13.7 ± 7.6% at long muscle lengths (0°- 30°), 11.9 ± 8.5% and 6.8 ± 9.2% at short muscle length (60°-90°), and 29.3 ± 7.7% and 31.1 ± 10.0% through the full length range (0°-90°), respectively. No significant effects of muscle length ($p = 0.08$), contraction mode ($p = 0.19$) or fatigue ($p = 0.14$) were observed for ΔL_f. A significant muscle length × contraction mode ($p = 0.02$) interaction was observed in which greater ΔL_f was detected in eccentric contractions at long muscle lengths ($p = 0.01$). No significant muscle length × fatigue ($p = 0.87$), contraction mode × fatigue ($p = 0.79$), or muscle length × contraction mode × fatigue ($p = 0.83$) interactions were observed for ΔL_f. When ΔL_f was analyzed across the whole ROM, no significant effects of contraction mode ($p = 0.12$), fatigue ($p = 0.18$), or a contraction mode × fatigue ($p = 0.43$) interaction were observed. Mean ΔL_f during concentric contractions were 72.9 ± 9.6 mm and 75.8 ± 9.9 mm before and after fatiguing exercise; and during eccentric contractions ΔL_f were 67.6 ± 11.1 mm and 71.6 ± 9.5 mm before and after fatiguing exercise. Paired samples t-test revealed that mean BFlh L_f was significantly longer during both concentric ($p < 0.001$) and eccentric contractions ($p < 0.001$) after fatiguing exercise.

ΔFA during concentric and eccentric contractions before fatiguing exercise were 10.6 ± 23.3% and 22.0 ± 23.6% at long muscle lengths (0°-30°), 16.4 ± 20.5% and 12.0 ± 14.5% at short muscle lengths (60°-90°), and 47.0 ± 34.5% and 65.2 ± 39.1% over the full length range (0°- 90°), respectively. During concentric and eccentric contractions after fatiguing exercise, ΔFA were 0.3 ± 12.0% and 19.0 ± 17.8% at long muscle lengths (0°- 30°), 14.4 ± 22.1% and 6.5 ± 16.2% at short muscle length (60°- 90°), and 36.4 ± 37.7% and 45.3 ± 25.7% through the full length range (0°- 90°), respectively. A significant main effect of fatigue ($p = 0.04$) was observed in which a smaller ΔFA was observed after the fatiguing exercise. No significant effects of muscle length ($p = 0.74$) or contraction mode ($p = 0.11$) were observed. A significant muscle length × contraction mode ($p = 0.02$) interaction was observed in which greater ΔFA was observed at short muscle lengths during concentric contractions ($p = 0.02$). In addition, greater ΔFA was observed in the eccentric contractions at long muscle lengths ($p = 0.006$). No significant muscle length × fatigue ($p = 0.86$), contraction mode × fatigue ($p=0.48$), or muscle length × contraction mode × fatigue ($p = 0.26$) interactions were observed. When ΔFA was analyzed through the whole ROM, significant main effects of contraction mode ($p = 0.006$) and fatigue ($p = 0.01$) were observed, in which greater ΔFA was detected in eccentric contractions and before the fatiguing exercise. No significant contraction mode × fatigue ($p = 0.86$) interaction was observed.

ΔMT during concentric and eccentric contractions before fatiguing exercise were 0.1 ± 4.7% and 2.3 ± 8.9% at long muscle lengths (0°-30°), 1.9 ± 4.8% and 2.4 ± 3.6% at short muscle lengths (60°-90°), and 1.3 ± 10.2% and 1.1 ± 8.7% over the full length range (0°- 90°), respectively. During concentric and eccentric contractions after fatiguing exercise, ΔMT were 1.6 ± 7.5% and -2.2 ± 4.1% at long muscle lengths (0°- 30°), 0.7 ± 4.9% and 1.0 ± 4.3% at short muscle length (60°-90°), and 0.1 ± 9.8% and 0.5 ± 7.4% through the full length range (0°- 90°), respectively. No significant effects of muscle length ($p = 0.08$), contraction mode ($p = 0.35$), or fatigue ($p = 0.20$) were observed. In addition, no significant muscle length × contraction mode ($p=0.41$), muscle length × fatigue ($p=0.93$), contraction mode × fatigue ($p=0.89$), or muscle length × contraction mode × fatigue ($p=0.59$) interactions were observed for ΔMT. When ΔMT was analyzed through the whole ROM, no significant effects of contraction mode ($p = 0.68$) or fatigue ($p = 0.53$) were observed. In addition, no significant contraction mode × fatigue ($p = 0.77$) interaction was observed.

Mean torques produced at long (Δ0-30) and short (Δ60-90) muscle lengths and over the whole range of motion (0° to 90°) during maximal concentric and eccentric knee flexions performed before and after fatiguing exercise are shown in Table 1. The torques produced at each range of motion (from 0° to 90°) during concentric and eccentric contractions before and after fatiguing exercise are shown at Figure 1. Peak torque produced during concentric and eccentric contractions before fatiguing exercise were 99.0 ± 26.5 Nm and 117.2 ± 28.5 Nm, respectively, and after fatiguing exercise were 90.4 ± 31.3 Nm and 103.1 ± 31.9 Nm, for concentric and eccentric contractions, respectively. Dynamic concentric and eccentric torques decreased -9.4 ± 16.9% and -12.6 ± 12.2% after fatiguing exercise, respectively. Significant main effects of muscle length ($p = 0.03$), contraction mode ($p = 0.04$), and fatigue ($p = 0.001$) were observed for torque, in which greater torque was observed at long muscle lengths, during eccentric contraction, and before the fatiguing exercise. No significant muscle length × contraction mode ($p = 0.42$), muscle length × fatigue ($p = 0.10$), contraction mode × fatigue ($p = 0.87$) or muscle length × contraction mode × fatigue ($p = 0.77$) interactions were observed. Peak isometric torque was 109.6 ± 26.5 Nm before the fatiguing exercise, and 84.9 ± 21.0 Nm and 78.3 ± 16.6 Nm, respectively, after the fatiguing exercise and then after the post-exercise test contractions. Peak isometric torque was significantly higher before than after the fatiguing exercise both before ($p = 0.004$) and after the dynamic test contractions ($p < 0.001$), whereas no differences were detected in the peak isometric torque from before to after the post-exercise test contractions ($p = 1.00$).

Table 3. Changes in fascicle length (L_f), fascicle angle (FA), and muscle thickness (MT) at long (Δ0° - 30°) and short (Δ60° - 90°) muscle lengths as well as through the full range of motion (Δ0° - 90°) during maximal concentric and eccentric knee flexions performed before and after fatiguing exercise.

		Before		After	
		Concentric	Eccentric	Concentric	Eccentric
Fascicle length change (mm)	Long	7.2 ± 6.4	-13.9 ± 10.7*	6.3 ± 6.6	-11.7 ± 6.3*
	Short	9.4 ± 6.5	-5.7 ± 6.6	8.8 ± 7.0	-4.6 ± 6.9
	Total	27.3 ± 9.3	-31.5 ± 8.4	26.1 ± 8.9	-27.1 ± 8.8
Fascicle angle change (°)	Long	-1.3 ± 2.5	2.5 ± 2.7 [c]	0.1 ± 1.4	2.4 ± 2.7[c]
	Short	-2.2 ± 2.5 [b]	2.1 ± 2.2 [a]	-2.1 ± 3.0[b]	1.0 ± 2.3
	Total	-5.7 ± 4.1 [a]	8.0 ± 4.8 [ad]	-4.3 ± 4.5	5.9 ± 3.8[d]
Muscle thickness change (mm)	Long	0.0 ± 1.2	0.4 ± 1.8	-0.3 ± 1.9	0.5 ± 1.0
	Short	0.5 ± 1.2	-0.6 ± 0.9	0.2 ± 1.2	-0.3 ± 1.1
	Total	0.5 ± 2.7	-0.4 ± 2.1	0.2 ± 2.7	0.0 ± 1.9
Torque (Nm)	Long	74.5 ± 10.1[eg]	97.8 ± 4.1[ef]	61.9 ± 12.7	84.8 ± 3.4[f]
	Short	52.6 ± 3.6[g]	78.7 ± 2.4[fg]	35.8 ± 2.7	63.3 ± 3.0[f]
	Total	62.9 ± 12.1[g]	89.2 ± 13.5[fg]	47.8 ± 14.6	74.8 ± 13.9[f]

*$p < 0.05$, significant muscle length × contraction mode interaction for ΔL_f, with greater change in fascicle length observed in eccentric contractions at the longer muscle length; [a] lower ΔFA after fatiguing exercise, [b] greater ΔFA at short muscle lengths during concentric contractions, [c] greater ΔFA at long muscle lengths during eccentric contractions, and [d] greater ΔFA during eccentric contractions through the whole range of motion; [e] greater torque at long muscle lengths, [f] during eccentric contractions and [g] before fatiguing exercise. Long and short muscle lengths were compared in a separate analysis that that assessing total length range.

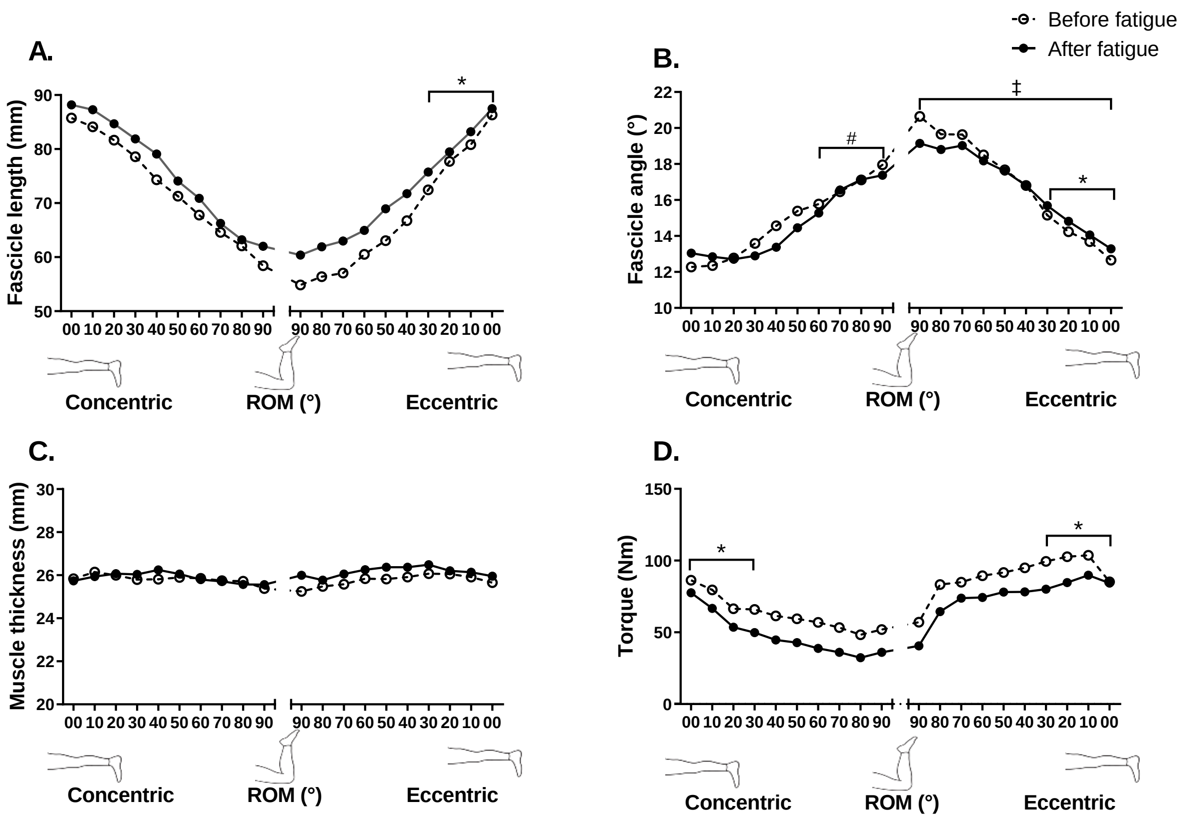

Figure 10. Absolute fascicle length (A), fascicle angle (B), muscle thickness (C), and torque (D) during concentric and eccentric contractions before and after fatiguing exercise.
*$p < 0.05$, greater change in fascicle length (A) and fascicle angle (B) were observed at long muscle length during eccentric contractions and also in torque (D) during both concentric and eccentric contractions; # $p < 0.05$ greater change in fascicle angle (B) was at short muscle lengths during concentric contractions and ‡at full length range during eccentric contractions. Lower change in fascicle angle was observed after fatiguing exercise for both concentric and eccentric contractions (open vs. closed circles in B); greater change in torque (D) was observed during eccentric contractions (concentric vs. eccentric in D) and before fatiguing exercise (open vs. closed circles in D).

4.4 DISCUSSION

The main findings of the present study were that (i) greater ΔL_f and ΔFA were observed at longer than shorter muscle lengths during eccentric contractions, (ii) contractions were performed with greater mean fascicle length after fatiguing exercise, but no effect on ΔL_f was observed, (iii) a greater ΔFA was observed at shorter than longer lengths in concentric contractions, and (iv) a greater ΔFA was observed before fatiguing exercise and in eccentric contractions when analyzed though the full ROM (from 0° to 90°). Thus, a unique fascicle behavior, including greater ΔL_f and ΔFA, was observed in relatively high speed (180°/s) isokinetic eccentric contractions. However, whilst the fatiguing exercise tended to increase mean L_f and reduce ΔFA (especially in eccentric contractions), the exercise did not alter the magnitude of fascicle length change.

Greater ΔL_f was observed during eccentric contractions when the muscle was at longer than shorter length (the knee more extended) both before and after fatiguing exercise. This finding is consistent with the observations at a slower movement speed (30°/s) described in Study 2 (Chapter 3), in which greater BFlh fascicle lengthening was observed at long muscle lengths during submaximal and maximal isokinetic eccentric knee flexions. It is also consistent with the findings of Van Hooren et al (2022), who observed the greatest fascicle lengthening later in the eccentric phase with the knee near full extension during the performance of Nordic hamstring curl and Roman chair exercises (Van Hooren et al. 2022); the fascicle behavior observed in the present study using the two-probe technique, allowing more accurate fascicle length analysis, therefore partly validate the behaviors identified with a single (50-mm field of view) probe used in that study. Nonetheless, a greater fascicle lengthening at longer lengths was not observed when using the same techniques during the multi-joint, single-leg deadlift (Van Hooren et al. 2022), indicating that fascicle lengthening during contractions may be exercise-dependent. Nonetheless, the findings of the present study indicate that, regardless of the presence of fatigue, BFlh fascicles undergo greater strain at longer muscle lengths during eccentric contractions. When considered alongside the findings of Study 2, it may be concluded that *in-vivo* BFlh fascicle strain patterns are strongly influenced by contraction mode but are less markedly influenced by contraction intensity or the presence of fatigue, at least in single-joint tasks.

In contrast to fascicle length results, the presence of fatigue significantly influenced ΔFA, with less fascicle rotation observed during both concentric and eccentric contractions after fatigue. The lower muscle force produced during the contractions after fatigue might be expected to partly explain this reduction, however the results of Study 2 within the current thesis do not substantiate this hypothesis since BFlh ΔFA was found to be similar during submaximal and maximal contractions in that study. Another factor that might influence fascicle rotation is the magnitude of osmotic fluid shift leading to muscle "swelling" after the fatiguing exercise. Increased muscle swelling might be expected to affect muscle shape change (Eng et al. 2018) and thus influence BFlh fascicle rotation (Roberts et al. 2019). The fatigue induced by the concentric knee flexion exercise would probably have increased muscle swelling not only in BFlh but also in the synergistic muscles, increasing pressures exerted onto BFlh simultaneously with pressures changing within the muscle. It is also possible that the exercise altered the relative activation of muscles within the synergistic group (Wakeling et al. 2011), directly influencing fascicle behaviors and also acting in concert with fluid shifts to alter intermuscular pressures. Together, such effects might lead to complex shape changes and thus to fascicle behaviors that are not easily predicted from *ex vivo* observations in isolated muscles. The present results highlight the need for *in vivo* experiments in determining fascicle behaviors, even during relatively simple tasks such as the knee flexion task studied herein.

It would be expected that the muscle swelling due to fatigue would increase muscle thickness, however this was not the case, as ΔMT was not significantly altered with fatigue, or affected by contraction mode or muscle length. Early studies theorized that muscle thickness might remain constant during contraction to maintain muscle volume, however later *in vivo* studies have observed that a wide range of shape changes is possible during contraction (2018). Some studies have reported increase in MT (Maganaris et al. 1998; Randhawa et al. 2013; Wakeling et al. 2011), others have reported decreases in MT (Randhawa et al. 2013) and a lack of change in muscle thickness has also been observed during contraction (Maganaris et al. 1998), as observed in the present study. The lack of MT change does not indicate that muscle shape remained constant, as alterations in muscle shape to maintain volume during contraction might have occurred in the width-wise rather than thickness direction.

The lesser fascicle rotation observed after fatigue should mean that fascicles needed to exhibit greater strain to accommodate the same joint range of motion as before fatigue. Indeed, it worth mentioning that fascicles operated at a longer mean length after the fatiguing exercise (see Figure 10 and Supplementary Table 5 at Appendix 7). This increase in mean fascicle length should have at least partly accommodated the longer series elastic component length associated with the reduced muscle force (assumed from the reduced joint torques) after fatiguing exercise. Thus, although knee joint range of motion, and thus muscle-tendon unit length change, was constant across conditions, the longer L_f observed in the fatigued state might theoretically place BFlh at greater risk of strain injury (Butterfield and Herzog 2006). In contrast, it is also possible that the greater fascicle lengths also influence intramuscular hypertrophic signaling, speculatively indicating a unique, fascicle length-dependent role for fatigue in the hypertrophic process.

Interestingly, fascicle rotation was statistically influenced by both contraction modes in a muscle length-dependent manner, as greater ΔFA was observed at long muscle lengths during eccentric contractions but at short muscle lengths during concentric contractions. Of interest was the dissociation between changes in fascicle angle and fascicle length indicating that, at least under the current conditions, changes in fascicle length were not strongly dependent upon fascicle rotation. A question remains, then, as to the primary importance of fascicle rotation. One possibility is that the additional rotation at long lengths during eccentric contractions might have helped to minimize overall fascicle lengthening and thus reduced injury risk, even though the change in fascicle length remained greater within this muscle length range. Thus, rotation may play an injury minimization role at muscle lengths in which fascicle lengthening is greatest. However, an alternative possibility is that fascicle rotation is accompanied by inter-fascicular (and possibly inter-fiber) shear, and that greater rotation at longer lengths may promote shear-based injury risk. In eccentric contractions in particular, where fewer motor units are believed to be active for a given level of muscle force (Hody et al. 2019), inter-fiber variations in contraction strength and stiffness may provide an added source of shear force and speculatively contribute to injury risk. Nonetheless, reduced fascicle rotation was observed after the fatiguing exercise, which is not consistent with the propensity for injury in fatigued muscles. Thus, the immediate implications of the greater ΔFA at longer lengths in eccentric contractions, and lesser ΔFA after fatiguing exercise, are yet to be elucidated. Regardless, the architectural characteristics of BFlh include a relatively short fascicle length, and thus lower excursion capacity, when compared to the other hamstring muscles. Fiber strain is considered a primary determinant of muscle injury, so a muscle with low excursion capacity and subsequently greater fiber strain for a given muscle lengthening distance might be at greater risk of injury (Kellis 2018; Kumazaki et al. 2012; Ward et al. 2009). It will be of great future interest to compare fascicle behaviors during contraction between individuals with and without a history of hamstring strain injury, or to perform prospective studies assessing the associations between fascicle behaviors and future injury.

4.4.1 Limitations

A possible limitation of the present study is that we could only measure fascicles within a single BFlh region. Fascicle lengthening and rotation measured in the present study may not represent fascicle behavior from other parts of the muscle as fibers within BFlh are longer and have greater fascicle angle proximally than distally (Kellis 2018); thus, different fascicle behavior might be expected. If images from other muscle regions were acquired using the tools available, different ultrasound positioning would be necessary, probably inducing error if ultrasound transducer was to be repositioned. We chose to evaluate BFlh at its mid portion as (i) this is a highly studied region of the muscle in previous injury-related studies (Alonso-Fernandez et al. 2018; Timmins et al. 2016a), and (ii) it was possible to clearly visualize fascicles inserting into the mid- and superficial aponeuroses.

4.4.2 Conclusion

We observed that fatigue predominately affected BFlh fascicle rotation rather than length change, as less ΔFA was observed in all conditions after fatiguing exercise but, in contrast to our hypothesis, no effect of fatigue on ΔL_f was observed. Nonetheless, a greater ΔL_f was observed at longer BFlh muscle lengths during eccentric than concentric contractions regardless of fatigue state, and the mean fascicle length was greater after fatiguing exercise in both concentric and eccentric contractions. When considered alongside the findings of reduced fascicle rotation after fatiguing exercise, the longer mean fascicle length and reduced rotation at long muscle lengths after fatiguing exercise coupled with generally increased fascicle strain during eccentric contractions might place BFlh at increased risk of strain injury. By contrast, the greater mean fascicle lengths observed after fatiguing exercise might be expected to influence intramuscular hypertrophic signaling, speculatively providing a unique, fascicle length-dependent role for fatigue in the hypertrophic process. Future research is required to determine whether fatigue-induced fascicle behaviors might differ between previously injured and non-injured individuals, or whether fascicle behaviors are associated prospectively with injury risk.

FINAL CONSIDERATIONS

The different methods used to estimate L_f, FA and MT during rest in Study 1 presented high reliability and strong correlation between measurements across two different days. Estimations of fascicle length were similar between the seven compared methods, nevertheless high between-subject measurement variability was observed with the trigonometric equations and its use may induce to error. Thus, the use of trigonometric equations for biceps femoris long head fascicle length estimation is discouraged. If acquisition of panoramic images is not available, the use of a Collage of images may be a good strategy to evaluate fascicle lengths as it allows visualization of entire fascicles along their lengths, thus allowing direct measurement.

During submaximal and maximal contractions performed at 30°/s in Study 2, a greater alteration in fascicle length (i.e. greater strain) was observed at long muscle lengths during eccentric contractions, and this was accompanied by greater fascicle rotation at long muscle lengths. The data therefore show that contraction mode influences BFlh fascicle behavior in a length-dependent manner. The greater strain at long muscle lengths may be an important stimulus for muscle hypertrophy through upregulation of anabolic signaling pathways, but may acutely leave the fibers susceptible to damage. Similarly, greater alteration in fascicle length was observed at long muscle lengths during eccentric contractions performed at 180°/s both before and after fatiguing exercise in Study 3. However, fatiguing exercise did not affect fascicle lengthening behavior, despite a trend towards the fascicles working at longer average lengths through the range of motion. The exercise did, however, result in a reduced fascicle rotation. The increased fascicle lengthening observed at long muscle lengths combined with the lesser post-exercise fascicle rotation may theoretically increase strain injury risk; this should also be considered alongside the trend towards greater average fascicle lengths, indicating that some individuals within the cohort may show a substantial shift in mean fascicle length after exercise. It will be of interest in future research to prospectively study the association between fatigue-induced changes in mean fascicle length and reductions in fascicle rotation with injury risk or overall hypertrophic outcomes.

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APPENDIX

APPENDIX 1. ETHICAL APPROVAL (STUDIES 1, 2 AND 3)

AUSTRALIA ECU EDITH COWAN UNIVERSITY

STREAM

System for Tracking Research Ethics Applications and Monitoring

APPLICATION FOR RESEARCH INVOLVING HUMAN PARTICIPANTS

SECTION 1 – PROJECT DETAILS

1.1 TITLE	
PROJECT NUMBER	22326
PROJECT NAME	Biceps femoris long head fascicle behaviour during muscle actions at different speeds
PROJECT SHORT NAME	Muscle behaviour in different speed muscle actions
PROJECT STATUS	Approved
VERSION	2

APPENDIX 2. INFORMATION LETTER TO PARTICIPANTS (STUDIES 1, 2 AND 3)

School of Medical and Health Sciences

JOONDALUP CAMPUS
270 Joondalup Drive, Joondalup
Western Australia 6027
☎ 134 328
www.ecu.edu.au
ABN 54 361 485 361 CRICOS IPC 00279B

INFORMATION LETTER TO ADULT PARTICIPANTS

BICEPS FEMORIS LONG HEAD FASCICLE BEHAVIOUR DURING MUSCLE ACTIONS AT DIFFERENT SPEEDS

Thank you for expressing your interest in this project. This document explains the methods, benefits, and risks involved with participation in the study in order that you may make a fully informed decision as to whether to participate. Please read this document carefully, and do not hesitate to ask any questions.

Researchers contact and details:

This is an international research project conducted by the chief investigator during her stay abroad in the Centre for Exercise and Sports Science Research at Edith Cowan University (ECU). The researchers for this project are:

Chief investigator: Ms Clarissa Müller Brusco, MSc (c.brusco@ecu.edu.au)
Supervisor: Prof. Anthony J. Blazevich (a.blazevich@ecu.edu.au)
Associate investigator: Mr Sergio Maroto-Izquierdo (s.marotoizquierdo@ecu.edu.au)

Background

Injuries to the muscles at the back of the thigh (hamstrings) often occur in the biceps femoris. They are most common when the muscle is at longer lengths or lengthening, such as in the late swing phase in high-speed running when they act to slow the forward movement of the leg and reaccelerate it back towards the ground to produce propulsive force. Also, the muscle fatigue that develops during intense physical activity or through repeated sports practice during a season also appears to strongly influence injury risk. However, there is still little information regarding how the hamstring muscle fibres, or fibre bundles (fascicles), operate during movements where the muscles are lengthening, and considering that reductions in injury risk in professional and amateur athletes is of great importance, it is necessary to obtain more information about how the fibres/fascicles behave during these high-risk activities in both men and women.

Differences in muscle fibre/fascicle length changes have been reported between low- and high-force muscle contractions and between active muscle shortening and lengthening. Because differences in muscle force and the type of muscle contraction influence how the muscle operates, and considering that knowledge regarding how hamstring muscle lengthening occurs might be critical for injury protection in many sports, it is of great importance to describe 'normal' muscle function during a variety of muscle contractions, with a future aim of detecting 'abnormal' function. In the present research, the first experiments will be performed to observe muscles (using ultrasound imaging) during contractions to describe how fascicle lengths change (and also their degree of rotation) during tasks that require muscle shortening versus lengthening at different speeds and through different knee joint ranges of motion. A comparison between men and women will also be performed to determine whether sex differences exist in muscle function.

Objective of the study

The aim of the present study is to evaluate length changes and rotation of the biceps femoris (long head section) fascicles during shortening and lengthening actions of different speeds and through different knee joint ranges of motion before and after a fatiguing exercise protocol in men and women.

Eligibility

You will be eligible for this study if:

- You are a male or female between 18 and 40 years old;
- You are moderately active and healthy person, engaged in 5–10 hours of recreational physical activity per week;
- You have never experienced neuromuscular injuries in the hamstring and are free of

any medical contraindications;

- You are not engaged in any physical activity (e.g. eccentric training) that could potentially influence the outcome measurements.

Research outline

In order to participate in this study, you will be informed of the possible risks and discomforts associated with the study procedures and, if you agree to be a participant and you meet the eligibility criteria, you will then be asked to sign a Consent to Participate form, and complete a Pre-exercise Medical Questionnaire. If a contraindication to participation is detected, all information provided by you will be immediately deleted from our database. However, if you are deemed able to safely perform the tests, you will be included in the study. As a participant in this study, you will be asked to refrain from performing sports or strenuous exercise (for 72 hours) prior to the testing sessions. All testing will take place in the Exercise Physiology Laboratory (Building 19, Room 19.150) at ECU Joondalup campus over two sessions, separated by at least one week.

This study requires you to visit the laboratory on two occasions. The first session will last around 1 h, it will be used to familiarise you with the experimental procedures, collect your personal and anthropometric data (height, weight, etc.), test hip joint range of motion, and to practice active stiffness measurements, and isometric and dynamic (eccentric-only and concentric-eccentric) knee flexion exercises in the dynamometer at different speeds. In this session, you will be fully familiarised with the testing procedures.

Only one testing session will then follow (visit 2) lasting approximately 2 h. In visit 2, you will perform an active muscle stiffness measurement, and isometric and dynamic knee flexion at two different speeds (slow and fast) and at two ranges of motion (full and partial range of motion). In addition, these measurements will be performed one more time/s after a muscle fatigue protocol performed in the dynamometer. A total of 12 sets of exercise will be performed (including the isometric and dynamic contractions).

You will be instructed to start each dynamic attempt with a concentric (muscle shortening) action and, after the determined range of motion is reached, to perform an eccentric muscle action in the specified velocity. A 2-minute recovery period will be given after the testing trials before the fatigue test, and a 1-minute recovery will be given after the fatiguing exercise before the final tests. Data describing your force producing ability will be recorded with simultaneous monitoring of your hamstring muscle length changes and rotations during exercise using ultrasonography; an ultrasound probe will be attached to the back of your thigh while you are executing the movements.

Risks

- You will be interviewed, and then complete a Pre-exercise Medical Questionnaire to ensure you are free from known medical and musculoskeletal conditions that would prevent you from safely performing the proposed tests. You will be allowed to participate in this study only after completing the questionnaire.
- Risks identified with maximal isokinetic muscle force measurements:
    - You will be asked to perform sets of maximal muscular contractions of the knee flexors muscles, thus there is a very small risk of musculoskeletal injury. However, this risk will be minimised through the use of a standardised warm-up and test familiarisation;
    - Maximal voluntary effort, even for brief periods of time, could potentially result in fainting, severe exhaustion or cardiac events though these side effects are very rare within the healthy population.
- Risks identified with ultrasonography:
    - Ultrasound is considered safe and has no major risks associated with the imaging modality;
    - A water-based gel will be applied on your skin in order to obtain ultrasound images. This gel is accredited and certified for use on human skin and no previous allergic reactions have been reported. However a reaction to a substance applied on the skin may occur on rare occasions. In order to reduce this small risk, paper towel will be used to wipe the gel down from the skin, and alcohol wipes will be applied to clear any gel residue.

Benefits

- You will know your maximum hip flexion range of motion;
- You will have the opportunity to see some of the most advanced research techniques used to measure muscle activation, muscle gearing mechanics, and maximum voluntary muscle force;
- You will know your maximum strength in isometric, concentric and eccentric actions;
- This study might help you in understanding mechanisms of musculoskeletal behaviour;
- You will have the opportunity to access your personal results, which may assist you to design your personal training programmes.

Confidentiality of information

All information collected during this research is private and confidential. Your data will be de-identified and your anonymity will, at all times, be safeguarded. Your contact information will only be accessible by the chief investigator during the period of the study and only the investigators will have access to the raw data collected in this study. Data will be stored on a password-protected computer and is only available to the investigators. Hard copy data will only be kept in the researcher's office and locked in a specific drawer/filling cabinet. All data will be stored according to ECU policy and regulations following the completion of the study, and it will be removed 7 years after publications have been finalised.

Results of research study

The results of this study may be presented at conferences/seminars and published in peer- reviewed journals, as journal articles, as an online article or part of a book section or report. Published results will not contain information that can be used to identify participants unless specific consent for this has been obtained. A copy of published results can be obtained from the investigator upon request.

Voluntary participation

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you do not want to participate or continue to participate will not disadvantage you or involve any penalty.

You are free to withdraw your consent to further involvement in this project at any time. You also have the right to withdraw any personal information that has been collected during the research.

Questions and further information:

If you have any questions or require any further information about the research project, please do not hesitate to contact:

Chief investigator:
Clarissa Müller Brusco (MSc)
School of Medical and Health Sciences
Centre for Exercise and Sports Science Research
Edith Cowan University
270 Joondalup Drive, Joondalup WA 6027
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Principal supervisor:
Anthony J. Blazevich (Professor)
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270 Joondalup Drive, Joondalup WA 6027
Phone: (08) 6304 5472
Email: a.blazevich@ecu.edu.au

If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

Research Ethics Officer:
Edith Cowan University
270 Joondalup Drive, Joondalup WA 6027
Phone: (08) 6304 2170
Email: research.ethics@ecu.edu.au

This project has been approved by the ECU Human Research Ethics Committee

Figure 1 Ultrasound assessment to identify the biceps femoris location along the back of the thigh.

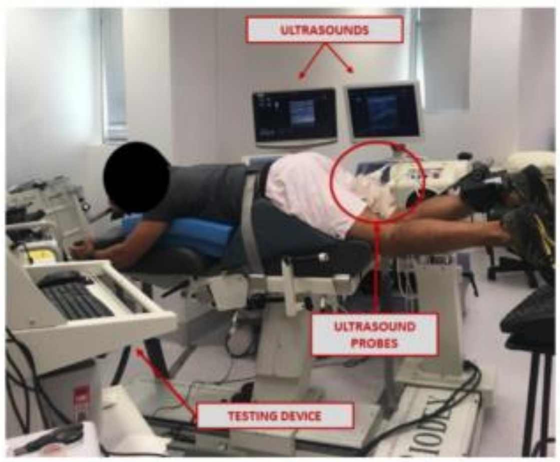

Figure 2. Positioning of the participant in the isokinetic dynamometer and of the ultrasound probes during the dynamic evaluations.

APPENDIX 3. CONSENT FORM (STUDIES 1, 2 AND 3)

Edith Cowan University
School of Medical & Health Sciences

CONSENT TO PARTICIPATE IN RESEARCH

BICEPS FEMORIS LONG HEAD FASCICLE BEHAVIOUR DURING MUSCLE ACTIONS AT DIFFERENT SPEEDS

I, ____, have read the information letter provided and attended an information session outlining the procedures listed below. Any questions I have asked have been answered to my satisfaction. I thereby agree to volunteer in this activity, realising that I may withdraw at any time without reason and without prejudice.

The investigation, and my part in the investigation, have been outlined and explained to me in detail and I understand the explanation. I understand that the tests will be conducted by Clarissa Brusco and I consent to the tests being conducted by this researcher. I received a copy of the procedures, and a description of any risks and discomforts has been provided to me and discussed in detail with me.

I, as a volunteer in this study, understand that participating in the research project will involve:

1) Hamstring flexibility test;
2) Active muscle stiffness assessment;
3) Maximal isometric, dynamic (concentric and eccentric) knee flexion contraction test (isokinetic);
4) Performance of a fatigue protocol, in which dynamic concentric contractions will be performed;
5) Ultrasonography measurements of the hamstring (biceps femoris long head) during the testing attempts;

I also understand that as part of the testing I may feel:

1) Discomfort during testing tests performed;
2) Low levels of muscle soreness if I am unaccustomed to resistance training;
3) Low levels of muscular fatigue after testing.

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I hereby certify that, to the best of my knowledge and belief, I have no physical condition that would increase the risk to me of participating in this investigation.

I understand that all information provided will be treated as strictly confidential and will not be released by the investigator unless required to by law. I have been advised with regards to the purpose of the research, the type of data that will be collected, and the way the data will be used upon completion of the research.

I agree that any research data gathered for the study may be published, provided my name or other identifying information is not used.

Participant signature ________________________________ **Date** ______________________

Emergency contact details:

Name: _____________________________

Contact number: _________________________

Chief investigator:

Clarissa Müller Brusco (MSc)

School of Medical and Health Sciences

Centre for Exercise and Sports Science Research

Edith Cowan University

270 Joondalup Drive, Joondalup WA 6027

Mobile phone: 0491 065 029

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Principal supervisor:

Anthony J. Blazevich (Professor)

Centre for Exercise and Sports Science Research

Edith Cowan University

270 Joondalup Drive, Joondalup WA 6027

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APPENDIX 4. PRE-EXERCISE MEDICAL QUESTIONNAIRE

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Pre-exercise Medical Questionnaire

The following questionnaire is designed to establish a background of your medical history, and identify any injury and/ or illness that may influence your testing and performance. If you are under 18 then a parent or guardian should complete the questionnaire on your behalf or check your answers and then sign in the appropriate section to verify that they are satisfied the answers to all questions are correct to the best of their knowledge.

Please answer all questions as accurately as possible, and if you are unsure about anything please ask for clarification. All information provided is strictly confidential.

Personal Details

Name:____

Date of Birth (DD/MM/YYYY):__________________ Gender: Female/ Male

PART A

1. Are you a male over 45 yr, or female over 55 yr or who has had a hysterectomy or are postmenopausal?

Yes No If YES, please provide details

2. Are you a regular smoker or have you quit in the last 6 months? Y N _______________

3. Did a close family member have heart disease or surgery, or stroke before the age of 60 years? Y N Unsure _______________

4. Do you have, or have you ever been told you have blood pressure above 140/90 mmHg, or do you current take blood pressure medication? Y N Unsure _______________

5. Do you have, or have you ever been told you have, a total cholesterol level above 5.2 mmol/L (200 mg/dL)? Y N Unsure _______________

6. Is your BMI (weight/height^2) greater than 30 kg/m^2? Y N Unsure ____________________

PART B

1. Have you ever had a serious asthma attack during exercise? Y N ____________________

2. Do you have asthma that requires medication? Y N ____________________

3. Have you had an epileptic seizure in the last 5 years? Y N ____________________

4. Do you have any moderate or severe allergies? Y N ____________________

5. Do you, or could you reasonably, have an infectious disease? Y N ____________________

6. Do you, or could you reasonably, have an infection or disease that might be aggravated by exercise? Y N ____________________

7. Are you, or could you reasonably be, pregnant? Y N ____________________

PART C

1. Are you currently taking any prescribed or non-prescribed medications?
Y N ____________________

2. Have you had, or do you currently have, any of the following?

If YES, please provide details

Rheumatic fever	Y	N	___________________
Heart abnormalities	Y	N	___________________
Diabetes	Y	N	___________________
Epilepsy	Y	N	___________________
Recurring back pain that would make exercise problematic, or where exercise may aggravate the pain	Y	N	___________________

PART C cont'd

Recurring neck pain that would make exercise problematic, or where exercise may aggravate the pain	Y	N	___________________
Any neurological disorders that would make exercise problematic, or where exercise may aggravate the condition	Y	N	___________________
Any neuromuscular disorders that would make exercise problematic, or where exercise may aggravate the condition	Y	N	___________________
Recurring muscle or joint injuries that would make exercise problematic, or where exercise may aggravate the condition	Y	N	___________________
A burning or cramping sensation in your legs when walking short distances	Y	N	___________________
Chest discomfort, unreasonable breathlessness, dizziness or fainting, or blackouts during exercise	Y	N	___________________

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PART D

Have you had flu in the last week? Y N ____________________

Do you currently have an injury that might affect, or be affected by, exercise? Y N ____________________

*Is there any other condition not previously mentioned that may affect your ability to participate in this study?

Y N ____

Declaration (to be signed in the presence of the researcher)

I acknowledge that the information provided on this form, is to the best of my knowledge, a true and accurate indication of my current state of health.

Participant

Name:______________________ Date (DD/MM/YYYY):______________

Signature:__________________________

Researcher:

Signature:___________________________

Date (DD/MM/YYYY):________________

APPENDIX 5. SUPPLEMENTARY FILE 1, CHAPTER 2, STUDY 1

How to construct the Collage:

Single BFlh images were acquired proximo-distally at 2-cm intervals along the thigh with the same probe orientation at each point along the thigh as used during EFOV image capture. After acquiring four images at each determined capture site (or five in one participant whose fascicles could not be captured fully within 4 frames), this process was repeated two more times (i.e. obtaining the necessary images three times to build 3 muscle collages), and the data were exported from the ultrasound system to a personal computer.

The Collage was constructed using Camtasia Techsmith to Fiji software (National Institute of Health, Betheseda, USA, Version 1.53) software. The borders of the images were cropped and sized to fit into the software canvas. After piloting, a specific scale for the images was determined, so calibration was constant across all images, the same specifications were used for images of all participants. The most BFlh proximal image (named here as Image 1) was first positioned to the left side of the software canvas, and thereafter the other images (i.e. Images 2, 3 and 4) were positioned one-by-one in front of the previous image. The left margin of Image 2 was then placed 2 cm to the right of the left margin of Image 1 (following the ruler at the bottom of the image; see Figure 11), Image 3 was placed 2 cm to the right of Image 2, and Image 4 was placed 2 cm to the right of Image 3. Then, anatomical landmarks such as subcutaneous adipose tissue, connective tissue and fascicles that should be continuous from one image to the other were located and adjustments were made to fit the images as best as possible. The proper location of the anatomical landmarks was very important, as it allowed images to be fitted together so that fascicles could be delineated from one image to the next, as shown in Figure 11 below.

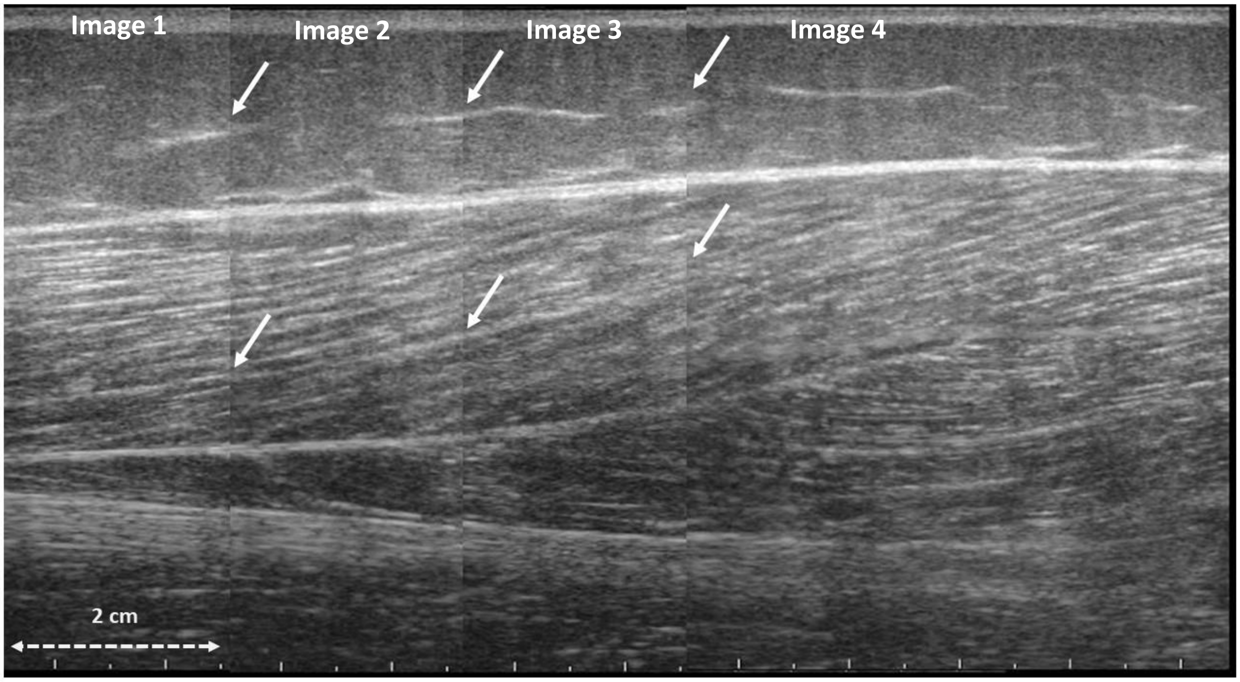

Figure 11. Example of a Collage with marked subcutaneous adipose and connective tissues continuous from one image to the next, and the ruler at the bottom marking the images 2 cm apart before final, small movements were made to create the final Collage.

APPENDIX 6. SUPPLEMENTARY TABLE 4, CHAPTER 3, STUDY 2

Table 4. Mean ± SD of length of fascicle, fascicle angle and muscle thickness in each angle analyzed from 0° to 90° of knee flexion.

		Submaximal		Maximal	
	Muscle length	Concentric	Eccentric	Concentric	Eccentric
Length of fascicle (mm)	0°	91.5 ± 10.5	90.4 ± 11.2	93.1 ± 9.7	92.0 ± 11.8
	10°	88.5 ± 11.1	84.0 ± 11.2	89.9 ± 10.3	84.4 ± 11.2
	20°	84.4 ± 11.6	79.3 ± 11.3	83.6 ± 12.4	77.7 ± 11.0
	30°	78.9 ± 13.6	73.5 ± 10.9	78.8 ± 13.4	71.6 ± 12.1
	40°	71.9 ± 13.3	68.3 ± 10.8	74.3 ± 12.9	66.7 ± 10.5
	50°	67.0 ± 13.1	63.2 ± 11.1	69.3 ± 12.1	62.9 ± 10.4
	60°	63.0 ± 13.2	59.1 ± 10.7	65.2 ± 11.3	60.4 ± 9.8
	70°	59.7 ± 12.2	56.3 ± 10.2	61.8 ± 9.9	58.0 ± 9.4
	80°	58.0 ± 11.4	54.6 ± 9.8	59.3 ± 9.4	56.4 ± 9.2
	90°	56.0 ± 10.6	53.7 ± 10.0	57.5 ± 8.8	55.1 ± 8.7
Fascicle angle (°)	0°	12.2 ± 2.3	12.8 ± 2.4	11.8 ± 2.9	12.1 ± 3.2
	10°	12.1 ± 2.6	13.3 ± 3.0	11.9 ± 3.3	12.8 ± 3.3
	20°	12.4 ± 3.0	13.8 ± 3.2	12.4 ± 3.8	13.7 ± 3.3
	30°	13.4 ± 3.2	14.8 ± 3.3	13.3 ± 4.3	15.0 ± 4.5
	40°	14.6 ± 3.2	15.8 ± 3.5	14.0 ± 4.5	15.8 ± 4.7
	50°	16.0 ± 3.7	17.3 ± 4.2	14.8 ± 4.9	17.1 ± 5.0
	60°	17.0 ± 4.7	18.3 ± 4.5	15.7 ± 4.6	17.6 ± 4.9
	70°	17.9 ± 5.2	19.1 ± 5.4	16.0 ± 4.8	18.2 ± 5.4
	80°	18.4 ± 5.3	19.9 ± 6.0	16.7 ± 5.2	18.6 ± 5.6
	90°	19.3 ± 5.6	20.4 ± 5.9	17.5 ± 5.3	19.0 ± 5.7
Muscle thickness (mm)	0°	25.1 ± 2.5	24.9 ± 2.5	25.2 ± 2.8	25.1 ± 2.8
	10°	25.3 ± 2.7	24.9 ± 2.7	25.4 ± 2.6	25.2 ± 2.7
	20°	25.4 ± 2.5	25.0 ± 2.6	25.3 ± 2.6	25.0 ± 2.4
	30°	25.3 ± 2.6	25.1 ± 2.6	25.3 ± 2.7	25.1 ± 2.4
	40°	25.3 ± 2.5	25.1 ± 2.8	25.1 ± 2.6	25.1 ± 2.3
	50°	25.2 ± 2.4	25.1 ± 2.6	25.1 ± 2.4	25.1 ± 2.3
	60°	25.2 ± 2.3	25.2 ± 2.6	25.0 ± 2.3	25.3 ± 2.3
	70°	25.1 ± 2.2	25.2 ± 2.4	24.9 ± 2.3	25.2 ± 2.3
	80°	25.1 ± 2.2	25.3 ± 2.4	24.7 ± 2.3	25.0 ± 2.5
	90°	24.8 ± 2.2	25.1 ± 2.5	24.5 ± 2.4	24.6 ± 2.5

APPENDIX 7. SUPPLEMENTARY TABLE 5, CHAPTER 4, STUDY 3

Table 5. Mean ± SD of length of fascicle, fascicle angle and muscle thickness in each angle analyzed from 0° to 90° of knee flexion.

		Before fatigue		After fatigue	
	Muscle length	Concentric	Eccentric	Concentric	Eccentric
Length of fascicle (mm)	0°	85.7 ± 10.8	86.3 ± 10.4	88.2 ± 11.2	87.5 ± 10.0
	10°	84.1 ± 12.1	80.8 ± 12.9	87.3 ± 11.6	83.2 ± 11.7
	20°	81.7 ± 12.8	77.7 ± 13.2	84.7 ± 11.5	79.5 ± 12.9
	30°	78.6 ± 11.8	72.5 ± 13.3	81.9 ± 11.5	75.8 ± 12.1
	40°	74.3 ± 12.6	66.8 ± 12.2	79.1 ± 12.3	71.7 ± 12.2
	50°	71.3 ± 12.7	63.1 ± 11.1	74.1 ± 10.9	68.9 ± 12.3
	60°	67.8 ± 11.8	60.5 ± 10.7	70.9 ± 10.0	65.0 ± 11.9
	70°	64.6 ± 10.5	57.1 ± 9.0	66.3 ± 8.8	63.0 ± 10.4
	80°	62.1 ± 9.1	56.4 ± 9.1	63.2 ± 8.1	61.9 ± 9.8
	90°	58.4 ± 7.2	54.9 ± 8.5	62.0 ± 7.7	60.4 ± 11.2
Fascicle angle (°)	0°	12.3 ± 2.8	12.7 ± 3.1	13.0 ± 3.1	13.3 ± 3.1
	10°	12.4 ± 2.8	13.7 ± 2.9	12.8 ± 3.0	14.1 ± 3.7
	20°	12.8 ± 2.8	14.2 ± 3.1	12.7 ± 2.7	14.8 ± 4.1
	30°	13.6 ± 3.9	15.2 ± 3.5	12.9 ± 2.9	15.7 ± 4.0
	40°	14.6 ± 4.7	16.8 ± 4.1	13.4 ± 3.6	16.8 ± 4.1
	50°	15.4 ± 4.8	17.7 ± 4.5	14.5 ± 4.1	17.6 ± 4.5
	60°	15.8 ± 5.0	18.5 ± 5.3	15.3 ± 4.3	18.2 ± 5.3
	70°	16.5 ± 5.4	19.6 ± 5.5	16.6 ± 4.5	19.0 ± 5.5
	80°	17.1 ± 5.5	19.7 ± 5.9	17.1 ± 5.1	18.8 ± 5.7
	90°	18.0 ± 5.5	20.6 ± 6.0	17.4 ± 5.2	19.1 ± 5.5
Muscle thickness (mm)	0°	25.8 ± 2.4	25.7 ± 2.9	25.7 ± 2.8	26.0 ± 2.2
	10°	26.1 ± 2.3	25.9 ± 2.6	25.9 ± 2.2	26.1 ± 2.6
	20°	26.0 ± 2.1	26.1 ± 2.4	26.1 ± 2.3	26.2 ± 2.2
	30°	25.8 ± 1.9	26.1 ± 2.3	26.0 ± 2.3	26.5 ± 2.1
	40°	25.8 ± 1.9	25.9 ± 2.2	26.2 ± 2.2	26.4 ± 2.1
	50°	25.9 ± 1.8	25.8 ± 2.2	26.1 ± 2.1	26.4 ± 1.9
	60°	25.9 ± 1.9	25.8 ± 2.3	25.8 ± 1.9	26.3 ± 1.7
	70°	25.7 ± 2.0	25.6 ± 2.4	25.7 ± 1.7	26.1 ± 1.6
	80°	25.7 ± 2.5	25.5 ± 2.5	25.6 ± 1.6	25.8 ± 1.7
	90°	25.4 ± 2.5	25.2 ± 2.8	25.6 ± 1.6	26.0 ± 1.9