Investigations into the Architecture of the Gastrocnemius, Vastus Medialis, and Vastus Lateralis, and Monitoring Changes in Response to Physiotherapy

A commentary on prior publications submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Faculty of Health, Social Care and Education, Kingston University

By

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The research described in this thesis is the summation of many years of research projects carried out by BSc students under my supervision, each one building upon the work that had gone before. They were, in chronological order: Tony Antonios, Emily Skinner, Shendy Engelina, Anastasia Benjafield, Mohammed Khoshkhoo, Abdel Elniel, Harry Arnantha, Zaib Hilal, Jordan Bethel, and Queenie Mae Torrente. My sincere thanks to all: it was a pleasure and a privilege to work with them.

DECLARATION CERTIFICATE

I hereby declare that this PhD thesis entitled:

“Investigations into the Architecture of the Gastrocnemius, Vastus Medialis, and Vastus Lateralis, and Monitoring Changes in Response to Physiotherapy” was carried out by me for the degree of Doctor of Philosophy under the guidance and supervision of **Dr Dimitra Nikoletou and Dr. Agnieszka Lewko,** The Faculty of Health Social Care and Education, Kingston University and St Georges, University of London, United Kingdom. The interpretations put forth are based on my reading and understanding of the original papers published in peer-reviewed journals. The other books, articles, and websites, which I have made use of are acknowledged at the appropriate locations in the text. For the present thesis, which I am submitting to the University, no degree, diploma, or distinction has been conferred on me before, either in this or in any other University.

Date: 28/07/2022 Signature: [Redacted]

Abstract

Background

The vastus medialis (VM) and vastus lateralis (VL) form part of the quadriceps femoris group in the anterior thigh. A balance between these two muscles is key to maintaining normal tracking of the patella in the trochlear groove during flexion and extension of the knee joint, and an imbalance between them is thought to be implicated in the aetiology of patellofemoral pain (PFP). Patellofemoral pain is one of the most common musculoskeletal presenting conditions among young, athletic individuals, and particularly affecting females. First line treatment usually involves physiotherapy, either to strengthen the VM or to stretch the VL. However, there is a lack of evidential data in the literature regarding the effect of these interventions on the architecture of these muscles.

Aims

This thesis, drawing upon a selection of previously published work of the author, aims to review, integrate, and critically appraise these published works.

The body of work presented in this thesis is organised under the following themes:

1. Describe the detailed anatomy of the gastrocnemius and VM by a series of dissection studies and clarify the existence of the proposed subdivisions of the VM: the vastus medialis longus (VML) and the vastus medialis oblique (VMO).

2. Explore the potential of using ultrasound (US) to visualise muscle architecture, first on the gastrocnemius then the VM; validate the method for measuring the VMO fibre angle.

3. Obtain normative values for the pennation angle and insertion level of the VMO in a cohort of young, asymptomatic individuals, and further investigate the dichotomy between active and sedentary individuals.

4. Investigate the effect of physiotherapy on the architecture of the VMO, and how this effect was influenced by the following factors: different exercise techniques, electro-muscular stimulation, and cessation of the physiotherapy.

5. Investigate the effect of stretching exercises and myofascial release on the pennation angle of the VL and VMO.

Methods

Dissection studies were carried out on cadaveric specimens donated for anatomical education and research under the Human Tissue Act (2004). For the ultrasound investigations, young, asymptomatic volunteers were recruited, given an initial ultrasound (US) scan, then scanned again following a physiotherapy programme. Ethical approval was obtained from the host institution, and all volunteers gave informed consent.

Results

The research publications presented here describe the detailed anatomy of the gastrocnemius, VML and VMO, and present normative values for the pennation angle and level of insertion of the VMO in young, asymptomatic individuals. Ultrasound is shown to be a reliable tool for investigating the architecture of the VL and VM *in vivo*, and for monitoring the effects of physiotherapy interventions on these muscles. Furthermore, suitable subjects for such interventions can be identified in clinic by an ultrasound scan.

Conclusions

Gastrocnemius: there was a significant mean difference of 1.74 (±1.43) cm between the medial and lateral bellies in a sample of 84 cadaveric lower limbs.

Vastus medialis and lateralis: physiotherapy interventions to strengthen the VMO, or to stretch the VL, have a measurable effect on the architecture of the muscles, which can be detected using ultrasound. Ultrasound is a safe, non-invasive, inexpensive imaging modality, and has the potential to provide a powerful tool in the clinic to measure initial VL and VM muscle fibre angle in PFP cases, identify suitable patients for this type of treatment, and monitor their progress.

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List of Abbreviations

CCKE	closed chain kinetic exercise
HTA	Human Tissue Authority
HT ACT	Human Tissue Act (2004)
MRI	magnetic resonance imaging
NMES	neuromuscular electrical stimulation
OCKE	open chain kinetic exercise
PFP	patellofemoral pain
PSA	physiological surface area
SGUL	St George's, University of London
SMR	self-myofascial release
VI	vastus intermedius
VL	vastus lateralis
VLL	vastus lateralis longus
VLO	vastus lateralis oblique
VM	vastus medialis
VML	vastus medialis longus
VMO	vastus medialis oblique
US	ultrasound

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1. Introduction

1.1 Background

The study of gross anatomy, i.e., the anatomy that is visible to the naked eye, is an extraordinarily ancient science: written descriptions of human organs are found in the 'Edwin Smith Surgical Papyrus', dating from 1600 BC (van Middendorp et al., 2010). The first recorded school of anatomy, using cadaveric dissection, was founded in Alexandria in the 4th century BC, while the first recorded use of human bodies for anatomical research was by Herophilus and Erasistratus in around 200 BC, who are thought to have carried out vivisection on condemned criminals. Galen, considered by many to be the 'father of medicine and anatomy', taught anatomy and surgery around 200 AD; his many writings on anatomy went unchallenged for 1200 years, despite being based largely on animal dissections (Nutton, 1984). It was not until the Renaissance, and the publication of Vesalius' seminal work, *De Humani Corporis Fabrica* in 1543, that the scientific study of human anatomy, as we understand it today, i.e., the in-depth study of anatomical structure and function, was established.

It might be thought that, nearly two millennia after Galen's work, and half a century after Vesalius published the *Fabrica*, that there would be nothing of significance left to discover about the gross anatomy of the human body, and specifically, in the context of this thesis, the lower limb. Vesalius had accurately depicted the quadriceps femoris muscles in the *Fabrica*, with the superficial group (rectus femoris, vastus lateralis, and vastus medialis), crossed by sartorius, clearly shown (Rifkin et al., 2006), and vastus intermedius revealed by the downward reflection of rectus femoris (*ibid.*)

However, several recent papers have described new findings relating to these muscles. Grob et al. (2016) described a fifth muscle, the 'tensor of vastus medialis', and Olewnik et al. (2021) noted the variable existence of several more proximal attachments (fifth, sixth, seventh, and even eighth heads) and made the case for a new classification based on the number of proximal attachments. They further suggested that the muscle group should no longer be considered as the quadriceps, but, rather, as the “multiceps” femoris.

Another example of a novel finding is the existence of gastrocnemius tertius, discovered during routine dissection coexisting with an accessory soleus muscle (Yildirim et al., 2011). Even the disparity in belly length of the medial and lateral bellies of gastrocnemius, although reported and illustrated without explanation in standard anatomy textbooks (e.g., Standring, 2005), does not appear to have been fully quantified until this author undertook a thorough cadaveric study on 84 lower limbs, combined with an *in vivo* ultrasound investigation (Antonios and Adds, 2008). This study was significant in another way – it was the first attempt by our research group to use ultrasound to investigate the musculoskeletal anatomy of the lower limb.

Progress in science is often associated with the development of new technologies, and anatomy is no exception. The invention of the microscope led to the science of histology, and new imaging techniques, such as MRI, first used on patients in 1977 (APS, 2006) and CT scans, in use from 1971 (Petrik et al., 2006), with the concomitant necessity to interpret cross-sectional images, have furthered our understanding of the body. Ultrasound imaging, which became widely available for medical use in the 1960s, has become a powerful tool for research, diagnosis, and therapeutic interventions. Ultrasound is considered to be safe and non-invasive (WHO, 1998), and the scanners are lightweight and portable, making it an ideal tool for research studies such as those included and described in this thesis. Ultrasound uses the contrast between light and dark images, which enables the orientation of muscle fibres to be visualised, making it possible to research anatomy non-invasively, *in vivo*, using larger samples, and thus making possible the research described here. It is significant that an anatomical variant that was first observed *in vivo* by means of ultrasound was confirmed with a subsequent MRI scan – findings that would have been impossible without access to these new technologies (Benjafield et al., 2014).

These new imaging techniques have necessitated a reappraisal of how anatomists and clinicians view and interpret the body: three-dimensional anatomical knowledge is essential for interpreting the images of the body revealed by endoscopes, X-rays, CT scans, MRI, and ultrasound. Anatomy itself had not changed, but the new ‘inside-out’ approach meant that anatomists and clinicians had to reinterpret the internal anatomy of the body from a new perspective (Standring, 2006; Bazarbashi et al., 2019).

1.2 The Anatomy of the Gastrocnemius

The gastrocnemius is one of three muscles that lie in the superficial part of the posterior compartment of the leg, the others being soleus and plantaris. Gastrocnemius and soleus (known together as the *triceps surae*) converge distally to form the *tendo calcaneus* (calcaneal or Achilles tendon) which inserts into the middle third of the posterior surface of the calcaneus (Standring, 2005). Plantaris is a weak, vestigial muscle absent in approx.10% of subjects (Sinnatamby, 1999), which contributes only slightly to ankle plantarflexion, and may have a role in proprioception of the ankle joint (Moore and Dalley, 2006). Gastrocnemius is a bipennate, biarticular muscle, which consists of two bellies, medial and lateral. The medial belly originates from the posterior aspect of the medial condyle and popliteal surface of the shaft of the femur, while the lateral belly originates from the lateral aspect of the lateral condyle of the femur. Halfway down the leg the broad bellies become aponeurotic and blend with the aponeurosis of the soleus to form the calcaneal tendon (Figure 1). Gastrocnemius acts at both the knee and the ankle joints, whereas soleus is multipennate and monoarticular, acting only on the ankle joint. The main action of the *triceps surae* is plantarflexion of the foot at the ankle joint (Standring, 2005).

Figure 1. A, left leg, posteromedial view of gastrocnemius, L: lateral belly, M: medial belly; B, medial view, g: gastrocnemius, s: soleus, arrowhead: calcaneal tendon

Most standard anatomy textbooks report that the medial belly extends further distally than the lateral belly, with no explanation (Sinnatamby, 1999; Palastanga et al., 2002; Drake et al., 2005; Standring (2005; Moore and Dalley, 2006). Some authors have reported a quantifiable difference in length between MG and LG (Wickiewicz et al., 1983; Huijing, 1985; Kawakami et al., 1998), but these studies were based on small samples and offered no explanation for the disparity.

In the first study described in this thesis (Antonios and Adds, 2008), a dissection study was carried out on a sample of 84 lower limbs to quantify the difference between the medial and lateral bellies, and to carry out a morphometric analysis of the muscle in relation other parameters, including lower limb length and body height.

1.3 The Anatomy of the Vastus Medialis and Vastus Lateralis

The quadriceps femoris, with the sartorius, occupies the anterior compartment of the thigh. The quadriceps are described in standard anatomy texts as comprising the vastus medialis, vastus lateralis, vastus intermedius, and rectus femoris (Standring, 2005) (Figure 2). Vastus medialis is now accepted by many authors as being composed of two distinct parts: the vastus medialis oblique (VMO), and the vastus medialis longus (VML) (Standring, 2005). This separation was first described in Lieb & Perry's (1968) seminal study, and several subsequent studies have confirmed the existence of a fascial plane, or other architectural landmark (such as an abrupt change in fibre angle, or a separate branch of the femoral nerve) between the proximal and distal parts (Weinstabl et al., 1989; Javadpour et al., 1991; Nozic et al., 1997; Hubbard et al., 1997). Other studies, however, failed to find such evidence (Glenn & Samojla, 2002; Peeler et al., 2005; Lefebvre et al., 2006; Waligora et al., 2009) and concluded that there was no such separation. Smith et al., (2009) carried out a systematic review of the evidence on whether the VML and VMO actually exist as separate entities. Twenty-six papers, with data from over a thousand knees, were reviewed for evidence of muscle fibre orientation, the presence of a fibrofascial plane, and separate innervation of the two parts of the VM. Their conclusion was that, while they agreed with Lieb & Perry's (1968) observation of an abrupt change in fibre angle, they found insufficient "good quality evidence" to confirm the existence of the VMO and VML as two separate components, and recommended further investigations, including electromyographic studies.

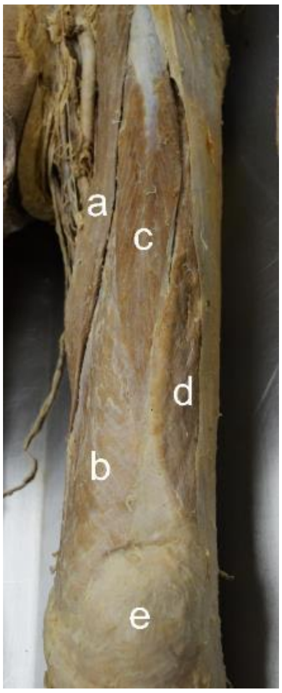

Figure 2. Muscles of the anterior thigh, cadaveric specimen; a: sartorius, b: vastus medialis, c: rectus femoris, d: vastus lateralis, e: patella (consent for images obtained)

A dissection-based investigation was undertaken (see Sections 3.5, 4.3) as the first step in the research programme into the vasti that forms the major part of this thesis (Skinner and Adds, 2012). Tenan et al. (2015) provided clinching evidence that the VML and VMO were neurologically different muscles, and furthermore, that innervation was different in men and women, and Peng et al. (2018) showed that there was differential activation of the vastus medialis oblique from the vastus medialis in various clinical exercise protocols, confirming and expanding Tenan et al.'s (2015) findings. There is, therefore, solid evidence that the VML and VMO exist as separate entities.

There appears to be less controversy regarding the division of the vastus lateralis. Like the vastus medialis, the vastus lateralis has been described as having two distinct heads with different fibre orientations: the vastus lateralis oblique (VLO), and the vastus lateralis longus (VLL). While this terminology is found in research literature (e.g., Weinstabl et al., 1989; Bennett et al., 1993), it is not yet in common anatomical usage.

1.3 The Architecture of the VMO and VML

As shown above, the published studies on the VMO and VML fall broadly into two camps: those confirming their existence as separate entities, and those finding no such evidence. Before embarking on the ultrasound studies, it was important to investigate and define the anatomy. To this end, forty formalin-fixed lower limbs were used in a meticulous dissection study (Skinner and Adds, 2012). By investigating the architecture of the VM in this way, an attempt was made to answer the question of whether the VML and VMO did indeed exist as two separate muscles, and, if so, where, and how, the two parts were separated. The "architecture" of a skeletal muscle includes the arrangement of the muscle fibres in relation to the axis of force (i.e., its pennation angle), as well as other parameters including fibre length, overall muscle length, muscle mass, and the physiological cross-sectional area (Lieber & Fridén, 2000). These factors affect the shape and size of the muscle, as well as its function and efficiency (Nayak et al., 2016). Throughout this thesis, the term "architecture" will be used as a shorthand to denote the fibre angle and insertion level (i.e., the length of the muscle in relation to its insertion site).

1.4 The Use of Ultrasound

Having described and clarified the separation of the VML and VMO (Skinner & Adds, 2012) the next step was to obtain normative data on the architecture of the VM from a young, asymptomatic population. Anatomical data gathered in previous studies, including Skinner and Adds (2012), were largely derived from older populations, and there was a lack of data in the literature from young individuals, i.e., those who are most at risk from patellofemoral pain (PFP). Ultrasound had previously been used to provide *in vivo* data on the architecture of various muscles, including the gastrocnemius (Antonios et al., 2008 - the first of the publications discussed in this thesis), the quadriceps (Blazevich et al., 2006), the triceps brachii (Ikegawa et al., 2008), and the biceps femoris (Freitas et al., 2018). Ultrasound had already been used to investigate parameters of the VMO by Lin et al. (2008) and Jan et al. (2009), however, the value of these studies was limited because firstly, the US method was not backed up with validity data in either study; secondly, Lin et al. (2008) did not report the age of the study cohort (other than being "aged less than 50 years"), or the sex of the subjects; and finally, although Jan et al. (2009) reported the mean age of the cohort as 40.8 ± 9.3 and 40.6 ± 9.6 years, for symptomatic and asymptomatic subjects, respectively, the age range was not stated.

The aim of the first US studies on the VM described in this thesis, therefore, was to address the gap in literature for normative sonographic data on the architecture of the VMO in young, asymptomatic adults. To verify the validity of the method, it was first necessary to assess whether US was a practical, and accurate, imaging modality to use for investigating the architecture of the VMO. To this end, a validation study on soft-fixed cadavers was carried out (Engelina et al., 2014a). By comparing the parameters measured by US with those obtained by direct measurement after dissection, this study showed that the US measurements were accurate and reliable. Having proved that the methodology was sound, an *in vivo* ultrasound study was undertaken out on 40 young, asymptomatic volunteers, in order to measure the fibre angle of the distal VMO, and the insertion ratio (the length of the medial border of the patella into which VMO fibres directly insert, expressed as a percentage of total patella length) (Engelina, et al., 2014b). The methodology employed was based on that used in previous US investigations into muscle architecture; this is discussed in more detail in Section 3.5. The initial investigations not only gave useful baseline values for these parameters, but also indications of a bimodal distribution of the values for fibre angle, depending on the level of physical activity of the volunteers – the more active the volunteer, the greater the VMO fibre angle. The conclusions about the insertion ratio were less clear: there were significant differences between the sexes, with the females having a greater ratio, and, although not statistically significant, there was a trend for the insertion ratio to decrease as the activity level increased. These latter findings seem counterintuitive: if exercise increases the fibre angle (and, also presumably, muscle bulk), it might be expected that the muscle belly would enlarge distally, encroaching further on the border of the patella; also, one might expect that a greater insertion ratio would be protective against patellar maltracking, yet females, with a greater ratio, have a higher incidence of PFP. Subsequent studies would go on to confirm that the situation regarding the insertion ratio remained confusing, whereas data concerning the VMO and VL fibre angles were generally found to be logical and, to a large extent, predictable.

1.5 Patellofemoral Pain

Patellofemoral pain (PFP), also known as 'runner's knee', is one of the most common musculoskeletal disorders encountered in clinical practice (Powers et al., 2004; Bhave & Baker 2008). Although its aetiology is acknowledged to be multifactorial, patella maltracking (i.e., where the patella moves medially or laterally in the trochlea groove during knee flexion and extension, causing uneven pressure) is known to be a contributing factor, and the balance between contractions of the vastus medialis and vastus lateralis is key to maintaining normal patellar tracking. Both vastus medialis insufficiency (Lin et al., 2010) and tightness of the vastus lateralis (Bhave & Baker, 2008) have been suggested as significant factors in the development of PFP, and physiotherapy targeting these muscles is often employed as first line treatment. Exercises may be prescribed either to strengthen the vastus medialis, or to stretch (and hence reduce the tone) of the vastus lateralis, depending on the individual patient's presentation (Bhave & Baker, 2008; Al-Hakim et al., 2012).

1.6 VMO Architecture and Activity Level

Although there was now some evidence (from Engelina et al., 2014b) that there is a positive correlation between level of activity and VMO fibre angle, there was a lack of data in the literature on VMO architecture in high-performance athletes, whose increased activity levels, and hence loading of the patellofemoral joint, might be considered to be risk factors for developing PFP (Halabchi et al., 2017). Accordingly, two separate study groups were recruited, one which was largely sedentary, and another composed of volunteers actively engaged in sporting activities. The level of activity was quantified by using the Tegner activity score, a commonly used, validated method of grading a subject's activity level (Tegner and Lysholm, 1985), to group the cohort into two, non-overlapping groups, depending on their level of activity: "athletic" subjects with a high score (active in sports), and "sedentary" subjects with a low score (who did not participate in competitive sports).

It might be assumed that athletic individuals would develop quadriceps hypertrophy, leading to an increase in muscle pennation angle and, therefore, VMO fibre angle. In theory, this should increase medial stability and aid patellar tracking. However, these athletic individuals (and especially female ones) are most at risk for developing PFP. The morphology of the VMO in these individuals had not hitherto been elucidated, so the next study (Benjafield et al., 2015) provided just such data: there was a significant difference in the fibre angle between the athletic and sedentary groups.

1.7 Can VMO Architecture Be Manipulated?

It had been shown that the athletic group had a significantly greater VMO fibre angle than the sedentary group – but was this causation or correlation? To test this hypothesis, the next goal was to investigate whether the architecture of the VMO could be altered by physical exercises, and if this effect could be detected by an US scan. Results showed that a six-week physiotherapy programme targeting the quadriceps resulted in a significant increase in the VMO fibre angle (Khoshkhoo et al., 2016).

The success of this study immediately raised a number of interesting research questions: what type of exercise is most effective, what happens when you stop exercising, how much exercise is needed to maintain the changes achieved during the physiotherapy programme, and what difference, if any, does adding electrical stimulation make to the outcomes? These questions were addressed in the next series of investigations: Elniel et al. (2017), Arnantha et al. (2017), and Hilal et al. (2018).

The focus of the investigations then shifted to the lateral aspect of the distal thigh. Vastus lateralis hypertrophy is a known risk factor in the aetiology of PFP, in which case stretching exercises or myofascial release may be prescribed in order to reduce the tone in the muscles. There was very little literature on the effect of stretching or myofascial release on the fibre angle of the VL, and nothing at all relating to their effect on the VMO. The final two studies described in this thesis addressed this issue by investigating fibre angle changes in both the VL and the VMO, following programmes of stretching (Bethel et al., 2022) and self-myofascial release (Torrente et al., 2022).

1.8 Overall Aims

The aims of the research discussed in this thesis, and detailed in the themes described in Section 2, were as follows:

- Describe and clarify the detailed anatomy of the gastrocnemius and the vastus medialis by dissection studies
- Explore the reliability of ultrasound scanning in analysing muscle architecture in the lower limb
- Obtain normative baseline values for VMO architecture in young, asymptomatic adults, including high-performance athletes
- Investigate whether VMO angle and insertion parameters can be manipulated by physiotherapy, and explore the effects of different types of exercise, electrical stimulation, and the cessation of physiotherapy
- Establish the effects of muscle stretching exercises, and self-myofascial release, on the fibre angles of the VM and VL.

2. Full List of Published Works Presented in this Thesis

The following published peer reviewed papers and online resources indicate how the research progressed from an initial simple anatomical enquiry to embrace research into using ultrasound to investigate, and then to monitor changes in, the architecture of the VM and VL. The papers are listed according to themes and in chronological order.

2.1 Contribution to the Published Papers

The published papers presented in this thesis were based upon research work carried out at St George's, University on London by, or under the direct supervision of, this author (Philip Adds). As P.I., this author was responsible for formulating the initial research questions, writing the research proposal, recruiting and training each student to undertake the research project, and supervising and monitoring the acquisition of data.

All the papers presented were written, edited, and submitted for publication by this author, who was also the corresponding author. Furthermore, as specified by the International Committee of Medical Journal Editors (ICMJE, 2022), this author met all the following criteria:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

2.2 Theme 1

Describe the detailed anatomy of the gastrocnemius and VM by a series of dissection studies, and clarify the existence of the two proposed subdivisions of the VM, the vastus medialis longus (VML) and the vastus medialis oblique (VMO).

Papers under this theme:

- Antonios T, Adds PJ. (2008) The medial and lateral bellies of gastrocnemius: a cadaveric and ultrasound investigation. Clinical Anatomy 21 (1) 66-74. doi 10.1002/ca.20533
- Skinner E, Adds PJ. (2012) Vastus medialis: a reappraisal of VMO and VML. The Journal of Physical Therapy Science 24(6): 475-479 https://doi.org/10.1589/jpts.24.475

2.3 Theme 2

Explore the potential of using ultrasound (US) to visualise muscle architecture, first on the gastrocnemius then the VM; validate the method for measuring the VMO fibre angle.

Papers under this theme:

- Antonios T, Adds PJ. (2008) The medial and lateral bellies of gastrocnemius: a cadaveric and ultrasound investigation. Clinical Anatomy 21 (1) 66-74. doi 10.1002/ca.20533
- Engelina S, Robertson CJ, Moggridge J, Killingback A, Adds PJ. (2014a) Using ultrasound to measure the fibre angle of vastus medialis oblique: A cadaveric validation study. The Knee 21(1): 107-111 DOI:10.1016/j.knee.2012.07.001

2.4 Theme 3

Obtain normative values for the pennation angle and insertion level of the VMO in a cohort of young, asymptomatic individuals, and further investigate the dichotomy between active and sedentary individuals.

Papers under this theme:

- Engelina S, Antonios T, Robertson CJ, Killingback A, Adds PJ. (2014b) Ultrasound investigation of vastus medialis oblique (VMO) muscle architecture: an in-vivo-study. Clinical Anatomy 27(7): 1076-1084 DOI: 10.1002/ca.22413
- Benjafield AJ, Killingback A, Robertson CJ, Adds PJ. (2015) An investigation into the architecture of the vastus medialis oblique muscle in athletic and sedentary individuals: an in-vivo ultrasound study Clinical Anatomy 28(2): 262-268 DOI: 10.1002/ca.22457
- Benjafield A, Howe FA, Killingback A, Adds PJ. (2014) Unusual variant of vastus medialis detected by ultrasound and confirmed by high-resolution MRI. Journal of Physical Therapy Science 26(1): 165-166 https://doi.org/10.1589/jpts.26.165

2.5 Theme 4

Investigate the effect of physiotherapy on the architecture of the VMO, and how this effect was influenced by the following factors: different exercise techniques, electro-muscular stimulation, and cessation of the physiotherapy.

Papers under this theme:

- Khoshkhoo M, Killingback A, Robertson CJ, Adds PJ. (2016) The effect of exercise on vastus medialis oblique muscle architecture: an ultrasound investigation. Clinical Anatomy 29(6): 752-758 DOI: 10.1002/ca.22710

- Elniel A, Robertson CJ, Killingback A, Adds PJ. (2017) Open-chain vs. closed-chain exercise regimes: an ultrasound investigation into the effects of exercise on the vastus medialis oblique. Physical Therapy and Rehabilitation http://www.hoajonline.com/journals/pdf/2055-2386-4-3.pdf DOI:10.7243/2055-2386-4-3
- Arnantha H, Robertson CJ, Killingback A, Adds PJ. (2017) Maintenance of exercise-induced changes in the architecture of the VMO: how much is enough? An in-vivo ultrasound study. Journal of Orthopaedics Spine and Sports Medicine 1:1 003
- Hilal Z, Killingback A, Robertson C, Adds PJ. (2018) The effect of exercise and electrical muscle stimulation on the architecture of the vastus medialis oblique - the 'Empi' electrotherapy system. Global Journal of Orthopedics Research 1(1) DOI: 10.33552/GJOR.2018.01.000503

2.6 Theme 5

Investigate the effect of stretching exercises and myofascial release on the pennation angle of the VL and VMO.

Papers under this theme:

- Bethel J, Robertson CJ, Killingback A, Adds PJ. (2022) The effect of stretching exercises on the fibre angle of the vastus lateralis and vastus medialis oblique: an ultrasound study. The Journal of Physical Therapy Science 34: 161-166
- Torrente QM, Killingback A, Robertson CJ, Adds PJ. (2022) The effect of self-myofascial release on the pennation angle of the vastus medialis oblique and the vastus lateralis in athletic male individuals: an ultrasound investigation. The International Journal of Sports Physical Therapy Published online June 1, 2022. https://doi.org/10.26603/001c.35591.

2.7 Online Resources

Clinical Anatomy. 2015. Video Highlights (https://onlinelibrary.wiley.com/page/journal/10982353/homepage/ca_video_highlights.htm) (accessed 22/4/2022)

3. Research Methodology

3.1 Introduction

This section will outline and discuss the general principles on which the research in this thesis was based. A more detailed description of the ultrasound method is given below (Section 3.5, "Ultrasound Method"), and details of particular investigations are given in Sections 4-7.

The research described in this series of investigations was carried out in two distinct programmes, involving either cadaveric investigations on bodies donated for anatomical teaching and research, or *in-vivo* ultrasound imaging of volunteers. The cadaveric studies firstly investigated the morphology of the gastrocnemius and vastus medialis muscles and their relations with the surrounding anatomical structures, and secondly, validated the ultrasound technique for VMO measurements. The *in-vivo* ultrasound studies investigated the architecture of the VMO, and the effect of various interventions on that architecture, and, in later studies, the architecture of the VL.

3.2 Ethical Considerations

The *in-vivo* studies described here were carried out on student volunteers enrolled in healthcare science courses at St George's, University of London (SGUL) between 2007-2018. Before undertaking the study, guidance was sought from National Research Ethics Service (www.myresearchproject.org.uk). Completion of the flow-chart assessment tool confirmed that the proposed investigations did not require formal NHS REC review (Appendix 11.1: Do I need NHS REC review?) It should be noted, however, that the Integrated Research Application System (IRAS) on-line guide considers only whether NHS REC review is required and does not consider whether other approvals might be needed. It was also, therefore, necessary to check whether any other approvals might be required for the proposed research. To clarify this issue, ethical approval was sought from the SGUL Ethics Committee, with the following outline project proposal: "[The aim of the project is] to collect data on the architecture of the vastus medialis muscles from healthy student volunteers by using ultrasound. This will of course be entirely non-invasive, and the work will be carried out under the supervision of the medical physics department of St George's Hospital NHS Trust, as well as myself (Senior Lecturer in Anatomy, SGUL) and Claire Robertson (Senior Lecturer in Physiotherapy, SGUL/Kingston)".

The Chair of the Committee confirmed that the proposed study did not raise any ethical issues and did not need to be reviewed by the full committee (Appendix 11.2: Email exchange with Professor Christine Heron, Chair of St George's Ethics Committee). However, it was also stipulated that student volunteers would need to receive an information sheet, and a consent form to sign, before taking part in the study.

Informed consent is one of the cornerstones of research ethics, and the volunteers for all the studies described here each received a detailed information sheet, specific to the particular study we were undertaking, with an opportunity to ask questions and the option to withdraw from the study at any time (Xu et al., 2020). Volunteers gave informed, written consent before the initial ultrasound scan. All information gathered was anonymised. Individuals appearing in photographs gave informed consent for images to be taken and used in the study report. Images published in any subsequent papers were edited to ensure that the volunteer was not identifiable. In all our studies, one volunteer was selected at random for the intra-rater reliability study, for which they gave a separate consent. This meant that they did not take part in any of the subsequent physiotherapy activities, but instead were scanned on up to seven separate occasions to provide baseline data.

Cadaveric anatomical investigations and validity studies were carried out on donated bodies in the Dissecting Room at SGUL. Anatomy using human bodies or body parts has been regulated by legislation in the United Kingdom since 1832, when the first Anatomy Act was introduced. The legislation was updated in 1984 with the introduction of the Anatomy Act (1984), which was, in turn, replaced by the Human Tissue Act (2004), which came into force in 2006 (except for Scotland), following the organ retention scandals of 1999-2000. Scotland has retained an updated version of the 1984 Act, the Human Tissue Scotland Act (2006). The Human Tissue Authority (HTA), an executive non-departmental public body of the Department of Health and Social Care, regulates the removal, storage, use and disposal of human bodies, organs and tissue, and issues licences for research, transplantation, education and training, and public display. St Georges, University of London is licensed under the HT Act for carrying out anatomical examinations, and for the storage of bodies and body parts for the purposes of education and research (London Anatomy Office, 2022).

Although under the 1984 Anatomy Act, verbal consent was considered adequate and legal, under the Human Tissue Act (2004), informed consent for whole-body anatomical donations must be obtained before death, and it must be signed and witnessed at the time of signing for it to constitute a legal consent. Consent is given for "anatomical examination" i.e., dissection, and it is made clear in information supplied to potential donors that the donated body may be used for any or all of the following activities: education or training relating to human health, research in connection with disorders or the functioning of the human body, investigations into the structure and biomechanics of the body, variations between individuals and the effects of age and sex on anatomical structures, detailed surgical anatomy of clinically important regions, morphometric analysis of the human body, and validation of research methods.

Consent for use of images is slightly less clear. Under the 1984 Act, consent for images to be taken was assumed, and it was legal to take photographs of bodies or body parts for use in teaching, research, or in publications, provided of course that the images were not offensive, obscene, or identifiable. Compliance with the 1984 Anatomy Act was assessed by HM Inspector of Anatomy, appointed by the Ministry of Health, who issued guidelines on the taking and use of images. Since the initial dissection studies on the gastrocnemius and VM were carried out in 2006-7, and the HT Act (2004) came into force in 2006, the bodies and body parts used in the initial dissection study of the VM would have been donated under the 1984 Anatomy Act, with consent for "macroscopic examination of a body for the purposes of teaching or studying, or training in or researching into, the gross structure of the human body…" (legislation.gov.uk, 2022) and, as has been seen, consent for images.

Images are specifically excluded from the HT Act (2004). Indeed, the Human Tissue Authority's Code of Practice C (Anatomical Examination) states "The making and displaying of images (including photographs, films and electronic images) falls outside the scope of the HT Act" (Human Tissue Authority, 2016, p. 10), however, the consent document sent to donors by the London Anatomy Office, (which co-ordinates anatomical donations for the London and (most) South-East of England Schools of Medicine), includes an option for donors to give, or withhold, consent for images to be taken (it also reassures donors that they would not be identifiable in any images taken) (London Anatomy Office, 2022). The HTA Code of Practice C goes on to state that, despite images falling outwith the 2004 HT Act, "the HTA requires DIs to put systems in place to ensure suitable practices are carried out… [and]… endorses the guidance on images provided by the General Medical Council (GMC) in its publication 'Making and using visual and audio recordings of patients' … [and] … ensuring that the dignity of deceased people is maintained at all times. Therefore, the HTA expects DIs to put systems in place to prevent the inappropriate use of images." (Human Tissue Authority, 2016, p. 10). The "DI" is the "Designated Individual", a senior member of (usually) academic staff who has legal responsibility for ensuring that the conditions of the HT Act are complied with within the anatomy (or research) department.

The gastrocnemius dissection study was carried out at St George's, University of London in 2006 (Antonios & Adds, 2008), and the first dissection study of the vastus medialis was carried out in 2007 (Skinner & Adds, 2012). The donated bodies used in those studies were all donated before the HT Act (2004) came into force on 1st September 2006 (the bodies would have been embalmed prior to September so that they were ready for the new 2006-2007 academic year – a minimum of 6 weeks fixation time is necessary between embalming and dissection), so consent for images would have been assumed. For subsequent anatomical investigations and validity studies (Engelina et al., 2014a), the consents of the donors used in the study were checked to ensure that consent for images was in place before any photographs were taken.

3.3 Cadaveric Investigations

The first studies presented in this thesis aimed to elucidate, by dissection and direct measurement, the detailed morphology of the gastrocnemius (Antonios & Adds, 2008), and the structure of the VM, and the (at that time disputed) existence of separate parts of the VM: the VMO and VML (Skinner & Adds, 2012). The problem that is faced in any such investigation is how many specimens are needed in order to draw any firm conclusions? While observations of a single specimen are usually described as a Case Study, descriptive anatomical studies in the literature range from observations on a single cadaver (e.g., Ono et al., 2005) to hundreds (e.g., Nwoha & Adebisi, 1994). It is not possible to carry out a power calculation for a descriptive study, so time and availability of cadavers are usually the main limiting factors. A study carried out by Tuttle et al. (2013), found that when carrying out an anatomical study, the number of specimens necessary in order to achieve statistical power varies according to the region of the body under investigation. Using published data from muscle fibre length studies they reported an average number of subjects per dissection study of 9 ± 5 (range 1-25).

According to Osuala (2007), “sampling” means taking any portion of a population as a representative of that population, and a “sample” is defined as a set of individuals or participants drawn from a larger population for a particular study (Salant & Dillman, 2004). To ensure that the results are valid and free from selection bias, an adequate sample of the population should be investigated. However, it is difficult to quantify an “adequate” number of cadavers. When considering the sample size necessary for the cadaveric studies, a pragmatic approach was taken, with the aim of utilising the maximum number that were available at that time, given time and access constraints. Cadavers are, of course, a precious resource, and the cadavers that were used for the dissection studies were those allocated for undergraduate teaching, which necessarily limited availability and access. The gastrocnemius study (Antonios & Adds, 2008) involved no destructive dissection other than skin removal, and access was generously given to cadavers at three other Higher Education institutions, as well as those at St George's, University of London, giving a combined total of 84 lower limbs from 45 cadavers.

The investigation into the morphology of the VM (Skinner & Adds, 2012), however, was more complex, and involved further dissection, so was limited to cadavers at St George's, University of London. Time and availability constraints limited the sample size to forty lower limbs from twenty donated cadavers, which is comparable to other published anatomical studies on the VM (and substantially greater than some). It was also all the cadavers that were used for teaching at that institution in the academic year. Since there is no reason to suppose that cadavers differ from year to year, using all the cadavers donated in a single year (i.e., a convenience sample) is a good way to access a representative sample.

Before using ultrasound to measure the VMO fibre angle and insertion length *in-vivo*, the US method was validated on soft-fixed cadavers (Engelina et al., 2014). Ultrasound measurements had previously been validated for measurements on other muscles e.g., the hamstrings, in cadavers (Kellis et al., 2009), but not hitherto for the vasti muscles. Initial trials were carried out on formalin-fixed cadavers, since this would have provided a larger sample, this was, however, unsatisfactory due to the poor echogenicity of the fixed tissue. The technique was then tested on soft-fixed cadavers, which gave much better results. There have been a number of studies in the literature comparing the qualities of "Thiel-embalmed" cadavers with living patients and formalin-fixed cadavers (e.g., Liao et al., 2015), and they are considered to be much more life-like than formalin-fixed cadavers. At St George's, University of London, "soft-fixed" cadavers are produced using a variant of the Thiel method, and, like the Thiel method, these retain much of the flexibility of the living body. The soft-fixed cadavers gave clear US images of the muscle fibres, so were ideal for the validation study, where US measurements were compared to values obtained by direct measurement after dissection from nine lower limbs. The sample size, though small, compares favourably to that of Kellis et al. (2009), who used six. The results obtained showed good agreement between US and direct measurement, with a Pearson coefficient of 0.92 ($p<0.01$); this showed that the method was reliable and could be used with confidence on living subjects.

3.4 Cohort Recruitment

For the initial descriptive studies on the VMO (Engelina at al., 2014b; Benjafield et al., 2015) the aim was to recruit the largest cohort possible within the constraints of the timeframe and availability of scanning facilities. Based on comparable US studies on the VMO, which used study group sizes of 89 knees (Lin et al., 2008) and 54 knees (Jan et al., 2009), it was felt that a target of 80 knees would provide an adequate sample, and this proved achievable within the timeframe; Benjafield et al. actually exceeded the target, with 82 knees.

For the intervention studies, a power calculation was carried out, using data from a pilot study undertaken as part of Benjafield et al.'s (2015) study (unpublished data). For this study, two groups of subjects, "athletic" and "sedentary" were recruited. A small subset of eight individuals from the "sedentary" group consented to take part in the pilot study and undertook a six-week quadriceps-strengthening exercise programme following their initial US scan. Results showed a significant increase in fibre angle, but no significant change in insertion length. Based on these data, the power calculation indicated that a minimum of 23 participants would be needed to ensure statistical significance. To fit with the demographic of PFP, the ideal cohort would have been young, athletic, female volunteers, who form the majority of those experiencing this condition (Boling et al., 2010; Song et al., 2011). To further investigate the observed dichotomy in the fibre angle and insertion level of the VMO in 'active', as opposed to 'sedentary' individuals, an equal cohort of athletic and sedentary volunteers would be required. Volunteers were recruited from among the student population of the Higher Education institution. In an institution with a strong tradition of athletic prowess, particularly in rugby and rowing, it proved easier to recruit male athletic volunteers. Finding a homogenous cohort of willing sedentary subjects, however, was more of a problem. The second challenge was getting volunteers who were also willing to commit to a six- or seven-week programme of US scans and exercises.

In the event, most of the studies discussed in this thesis used male-only study groups. This was a pragmatic decision, influenced by several factors, including the considerations outlined above. Limiting the cohort to males gave us a homogeneous sample for the study and removed the confounding factor of sex difference. Males are also known to have less subcutaneous fat (Eisner et al., 2010), enhancing the echogenicity of the muscle underneath (Reimers et al., 1993), and thus, it was hoped, improving the accuracy of the ultrasound data acquisition. The make-up of the study group was also influenced by the social milieu of the student charged with recruitment. One notable exception to the above rule was the study on electrical muscle stimulation (EMS) by Hilal et al. (2018), for which 25 suitable female subjects were recruited (see Sections 7.3 and 7.3.1).

3.5 Inclusion and Exclusion Criteria

For all *in-vivo* studies, exclusion criteria were as follows: any current or previous knee pain or knee pathology, any current or previous quadriceps pain or pathology, any previous knee surgery, and any lower limb deformities or abnormalities. The age was limited to 18-35 years, which is reflective of the demographic most affected by PFP, and also eliminated any potential outliers. Furthermore, limiting the age to 35 years meant that any potential compounding factors due to the ageing process were eliminated. The Tegner activity score (Tegner & Lysholm, 1985) was also crucial, depending on whether the study aimed to strengthen, or relax, the muscles. The Tegner score is a validated, commonly used scoring system to grade an individual’s level of physical activity (in sports or work), where a higher score indicates increased activity. It was designed to aid the evaluation of patients with knee injuries; the scale goes from level 0 (defined as “Sick leave or disability pension because of knee problems”) to level 10, reserved for elite national or international athletes. In studies investigating the effect of exercise on the architecture of the VMO, the upper limit was set, at least initially, at 3, so that all participants were relatively sedentary, although in one study (Khoshkhoo et al., 2015), it was necessary to relax the criteria for pragmatic reasons, to ensure a sufficiently large cohort.

3.6 Ultrasound Method

Ultrasound probes emit high-frequency sound waves, and the echoes returning to the transducer from underlying tissue are used to create images. Dense tissues, such as bone and tendon, are hyperechoic and create a strong image, seen as light grey on the screen, while soft tissues such as fat are hypoechoic, and create a darker image (Scatliff & Morris, 2014). The method used in the US investigations was based on published research into the VM (Lin et al., 2008; Jan et al., 2009), and later, the hamstrings (Freitas et al., 2018). The same basic method was used in all the published US studies discussed in this thesis. Participants, wearing loose-fitting shorts, lay supine on an examination couch with the lower limbs in full extension. A pillow was placed under the ankles to minimise movement. The superior and inferior borders of the patella were palpated and marked with a fine skin pen. The patella length was measured using digital callipers. The mid-point of the patella was located using the callipers and marked. The ASIS was palpated, and a steel ruler was aligned between the ASIS and the midpoint of the patella. The volunteer was asked to hold the end of the ruler against their ASIS; the edge of the ruler thus equates to the femoral axis (Figure 3). A line approximately 10 cm long was drawn on the skin at the distal end of the femoral axis, to give a reference line.

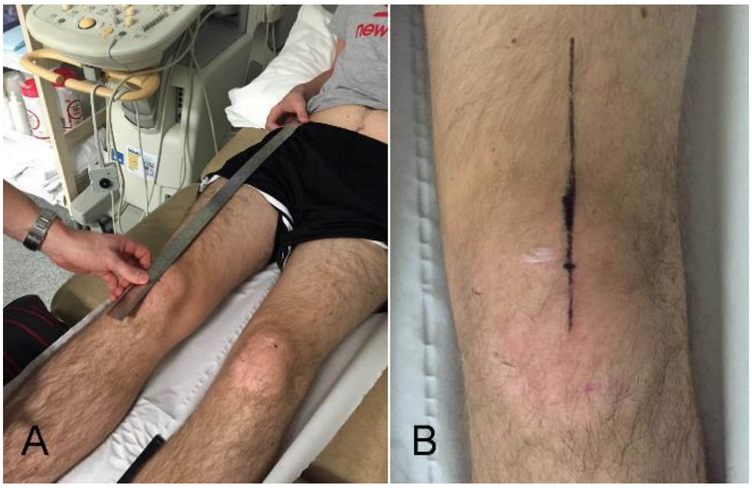

Figure 3. Marking the femoral axis. A: a ruler was placed between the ASIS and the midpoint of the patella; B: the femoral axis was marked on the skin

A Philips iU22 ultrasound machine, L17-5 B-mode (linear array) probe was used to measure the fibre angle and insertion level of both the VMO (and, subsequently, the distal VL) (Figure 4). Ultrasound transmission gel was applied to the probe, which was then positioned on the medial aspect of the thigh, slightly superior to the level of the patella, where the VMO is located.

Figure 4. Scanning set-up with the Philips iU22 ultrasound machine

The probe was rotated slowly until the muscle fibres could be seen running as parallel white lines: the long axis of the probe was then parallel to the muscle fibres (Figure 5). The axis of the probe was marked with two dots on the skin, the probe was removed, and a line was drawn connecting the two dots and intersecting the femoral axis. The angle between the two lines was measured with a clear plastic protractor, giving the VMO fibre angle.

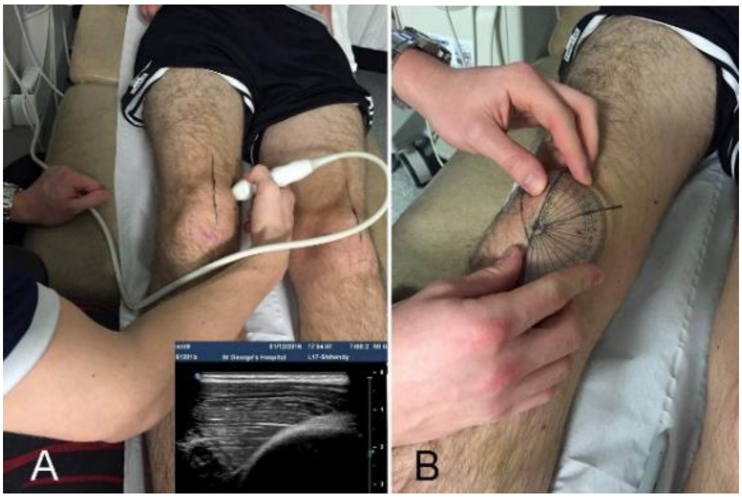

Figure 5. Measuring the fibre angle of the VMO. A: the ultrasound probe was rotated until the muscle fibres were seen as parallel white lines (inset); B: the position of the probe was marked, and the fibre angle was measured with a clear protractor

To identify the insertion level, the US probe was moved distally until muscle fibres were no longer visible – this represented the distal extent of the muscle. The position of the probe was marked on the skin, and the insertion level was measured against the length of the patella (Figure 6). The insertion level was expressed as a percentage of patella length to give the insertion ratio. Essentially the same procedure was used on the lateral side of the thigh to measure the fibre angle of the VL.

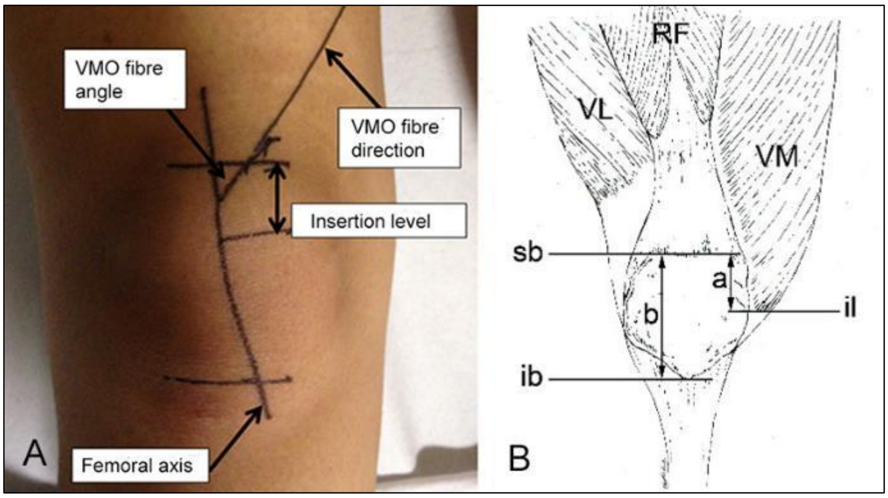

Figure 6. VMO fibre angle and insertion level. A: VMO fibre angle and insertion level were marked on the skin; B VMO insertion level, sb: superior border, ib: inferior border, il: insertion level. Insertion ratio as a percentage given by a/b*100 (drawing by Jennifer Crouch)

To eliminate inter-rater variation and minimise error, all ultrasound scanning was carried out by the same, trained operator, using the same equipment. Ultrasound training was provided by a senior ultrasound physicist from the Clinical Physics Department, who was also present at all subsequent scanning sessions to ensure correct functioning of the equipment. Measurements were taken using digital callipers, a flexible transparent goniometer, and a transparent Perspex protractor. Each measurement was repeated three times, and the mean was then taken.

3.7 Intra-Rater Reliability Study

To assess the reliability of the readings, an intra-rater reliability study was carried out in all our *in-vivo* studies. One individual matching the same exclusion criteria as the study group was randomly selected. This subject did not take part in the physiotherapy programme, and instead, was scanned once a week for a minimum of five weeks. The same measurements of the VMO angle, insertion length and patella length were taken each time. As this individual did not take part in the exercise programme, changes to the architecture of the VMO were not expected. The coefficient of variance (CV) was calculated to give a measure of the reliability of the parameters measured.

3.8 Exercise Programmes

Physiotherapy regimes were devised in collaboration with an experienced clinical physiotherapist who specialises in disorders of the knee. Exercises were chosen that were easy to perform at home, were commonly used in clinical practice, and were aimed to produce the same effect of light fatigue in all study groups. “Fatigue” was described as shaking or a sense of tiring from the muscle as perceived by the subject. All individuals gave informed consent and were given an instruction sheet and demonstrations on how to complete the exercises, from the same clinical physiotherapist. Participants were asked to complete a diary to monitor compliance; this was also intended to improve their compliance, by acting as a reminder. Details of the individual exercise programmes are given in Section 7.

4. Theme 1: Describe the Detailed Anatomy of the Gastrocnemius and Vastus Medialis

The aim of this theme was to describe the detailed anatomy of the gastrocnemius and VM by a series of dissection studies, and to clarify the existence of the two proposed subdivisions of the VM, the vastus medialis longus (VML) and the vastus medialis oblique (VMO).

4.1 Introduction

In this section, a general introduction is given to the anatomy of the skeletal and muscular anatomy of the leg, thigh, and knee joint. The factors affecting the dynamics of the patellofemoral joint are discussed, including the sexual dimorphism of the human skeleton, and its effect on locomotion. The anatomy of quadriceps muscles, and specifically, the vastus medialis and vastus lateralis, is considered in more detail.

4.2 Peer-Reviewed Publications Under This Theme

Antonios T, Adds PJ. 2008. The medial and lateral bellies of gastrocnemius: a cadaveric and ultrasound investigation. Clinical Anatomy 21 (1) 66-74. doi 10.1002/ca.20533

Skinner E, Adds PJ. (2012) Vastus medialis: a reappraisal of VMO and VML. The Journal of Physical Therapy Science 24(6): 475-479 https://doi.org/10.1589/jpts.24.475

4.3 The Anatomy of the Gastrocnemius

The gastrocnemius, soleus, and plantaris occupy the superficial part of the posterior compartment of the leg. Gastrocnemius and soleus (which lies deep to gastrocnemius), known together as the *triceps surae*, converge distally to form the calcaneal (Achilles) tendon which inserts into the calcaneus (Standring, 2005). Plantaris is a weak, vestigial muscle, with a very long, thin, tendon, which is absent in ~10% of subjects (Sinnatamby, 1999) (Figure 7).

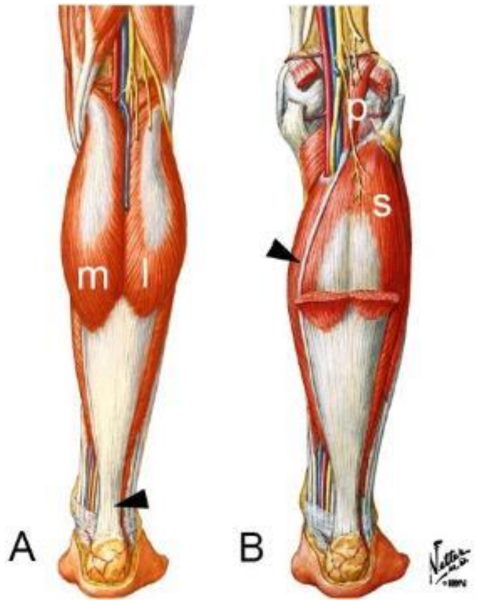

Figure 7. Muscles of the posterior leg; A, superficial view, m: gastrocnemius medial belly, l: gastrocnemius lateral belly, arrowhead: calcaneal tendon; B, deeper view after removal of bellies of gastrocnemius, p: plantaris, s: soleus, arrowhead: tendon of plantaris (image modified from Netter 2001)

Gastrocnemius is a bipennate, biarticular muscle, acting at both the knee and the ankle joints, whereas soleus is multipennate and monoarticular, acting only on the ankle joint. The main action of the *triceps surae* is plantarflexion of the foot at the ankle, though gastrocnemius is also a flexor of the knee (Standring, 2005).

Most standard anatomy textbooks report that the medial belly extends further distally than the lateral belly, though without any explanation (Sinnatamby, 1999; Palastanga et al., 2002; Drake et al., 2005; Standring, 2005; Moore and Dalley, 2006). Some authors have reported a quantifiable difference in length between the medial and lateral bellies (Wickiewicz et al., 1983;

Huijing, 1985; Kawakami et al., 1998), but these studies were based on small samples (3, 8, and 6 legs, respectively), and offered no explanation for the disparity. Wickiewicz et al. (1983) reported that, as well as the difference in length, the two bellies differ in their architecture. In cadaveric studies, they showed that individual fibres of the lateral belly were 46% longer than those of the medial belly and have a smaller angle of pennation (10° vs. 15°). This suggests that speed of contraction is greater in the lateral belly, while force is greater in the medial belly, due to its greater pennation angle, and, therefore, greater physiological cross-sectional area. An investigation into the gastrocnemius was carried out on both cadavers and volunteer subjects (Antonios & Adds, 2008). The aims of this study were (i) to carry out a dissection study to quantify the difference in gastrocnemius belly lengths and relate this difference to leg length and overall body length; and (ii) to analyse the activity patterns of the two bellies *in vivo*, to identify any differences in activity. The dissection study, on 84 lowers limbs, showed that the difference between medial and lateral belly lengths was highly significant in both right and left legs ($P < 0.001$). The overall mean difference was 1.74 cm (±1.43). However, no correlation was found between the difference in muscle belly length and either leg length or total body length. Curiously, in three cases (two females and one male) the medial belly was actually shorter, giving a negative belly length difference.

An *in-vivo* US study was carried out on 5 male volunteer subjects. Five subjects took part, and four of them showed identical patterns of contractions under a range of exercises. It was found that lateral belly contraction preceded medial belly contraction during loaded (heel raise) and unloaded ankle plantarflexion, both with the knee extended and with the knee flexed at 90°. The lateral belly also contracted first during active knee flexion.

Given the greater velocity potential of the lateral belly, it is possible that its rapid initial contraction might give added support to the lateral side of the joint by reinforcing the eversion muscles, as well as stabilising the talocrural articulation during the initial phase of plantarflexion. This could help to reduce the risk of inversion injury, the most common disabling ankle injury (Sugimoto et al., 2003; Beynnon et al., 2005).

While the reason for the difference in belly length may still be unresolved, there is now a better understanding of the specific roles of the two bellies, which can be related to the difference in their architecture. The difference in belly length may indeed be nothing more than a consequence of the difference in architecture, with each belly specialised for its role of velocity (lateral) or power (medial). It is suggestive that the fifth subject in the study, who showed a reversed pattern of contraction to the others (medial belly contracting before the lateral belly), had suffered an inversion injury 7 months prior to the tests. Although based on a single subject, this finding could be of considerable significance to, for example, athletes and physiotherapists.

4.4 The Anatomy of the Vastus Lateralis and Vastus Medialis

4.4.1 Osteology of the Femur, Patella, and Proximal Tibia

The vastus lateralis and vastus medialis are part of the quadriceps femoris, a muscle group that occupies the anterior (extensor) compartment of the thigh. In order to understand the anatomy of these muscles, and their actions, it is necessary first to consider the osteology of the lower limb.

The femur, the longest bone in the body, articulates proximally with the acetabulum of the pelvis, to form the hip joint, a congruent, ball-and-socket synovial joint. From its articulation at the acetabulum, the neck of the femur is angled laterally and slightly inferiorly; from there, the shaft is inclined medially as it proceeds down towards the knee, such that the knees are closer together than the hips (Figure 8). This effect is more marked in females, as the female pelvis is typically wider to accommodate a wider pelvic outlet for giving birth.

Figure 8. Photograph of a human skeleton specimen, SGUL

The close approximation of the knees is advantageous in bipedal locomotion: consider the upright walking of the chimpanzee, where, in the upright stance, the lower limbs do not converge, but rather, appear to run straight down from the hip to give them a wide-legged stance (Figure 9), and, as a consequence, a rather clumsy gait, where the body lurches from side to side as the non-weight-bearing limb is lifted off the ground during the swing phase of the gait cycle. There is clearly an evolutionary advantage in energy-efficient locomotion, and a likely hypothesis for the transition to bipedalism in hominids is that it evolved to reduce locomotor costs, relative to their ape-like evolutionary ancestors (Rodman & McHenry, 1980), although freeing the upper limbs to carry scarce food resources may also have exerted evolutionary pressure (Carvalho et al., 2012). This is borne out by analysis that shows that human walking is approximately 75% more efficient in terms of energy efficiency than both quadrupedal and bipedal walking in chimpanzees (Sockol et al., 2007).

Figure 9. Bipedal locomotion in chimpanzees (chimp.jpg) (bp.blogspot.com) (© Alamy)

While the femur is angled in towards the midline, the tibia descends vertically. The angle formed where the femur meets the tibia is known as the 'Q angle', defined as the angle between the femoral axis and a line passing through the tibial tuberosity and the centre of the patella (Figure 10).

Females typically have a wider pelvis than males, which increases the Q-angle. In males, the Q angle is commonly reported to be around 12°, while in females, it is typically around 18°, although there may be variation in different ethnic groups. A study by Omololu et al. (2009) on a Nigerian population found that, for men, the average Q-angle was 12°, and comparable to figures reported for Caucasian males, while the normal Q-angle in adult Nigerian women ranged from 20° to 28°, significantly higher than in Caucasian women, where a Q-angle greater than 20° is considered abnormal. The reasons for this are not well understood but could be due to a wider pelvis or shorter femurs in Nigerian women (Omololu et al., 2009). Interestingly, however, a study by Grelsamer et al. (2005) found that, in a study of 45 men and 24 women (of unreported ethnicity), there was a mean difference of only 2.3° between the Q angles of men and women, and that the difference between male and female Q angles was due to the fact that men tend to be taller. For whatever reason, the increased Q angle in females, leads, as we have seen, to greater susceptibility to knee pathology; the evolutionary pressure for energy-efficient locomotion is evidenced by the fact that the convergence of the femurs is maintained, despite this. Efficiency in the many outweighs pathology in the few.

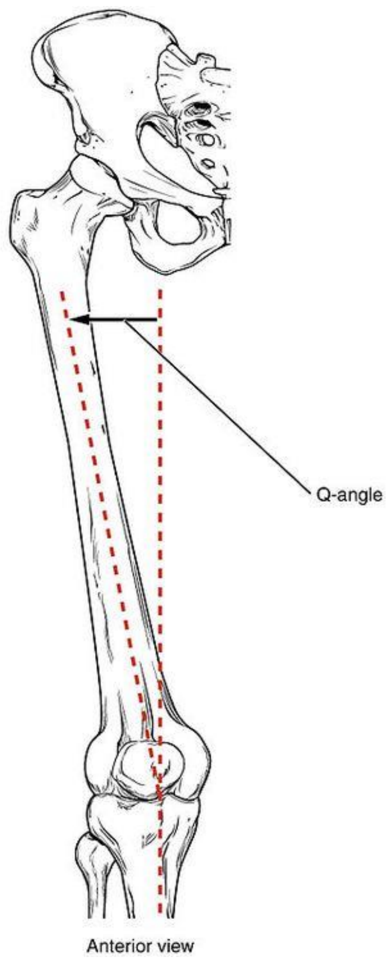

Figure 10. The Q angle (300px-Q_angle_of_knee.jpg (300×743) (physio-pedia.com))

At its proximal end, the femur is marked by three projections: the femoral neck, which terminates with the rounded head, the greater trochanter, and the lesser trochanter. The greater and lesser trochanters are sites of muscle attachment, and are linked on the posterior aspect by the intertrochanteric crest, and by the intertrochanteric line anteriorly (Standring, 2005).

On the posterior of the shaft of the femur is a pronounced, roughened ridge, the linea aspera, which runs for most of the length of the shaft. At its proximal end, the linea aspera is continuous with the intertrochanteric and pectineal lines medially, and the gluteal tuberosity laterally. Distally, the medial and lateral borders of the linea aspera diverge to become the medial and lateral supracondylar lines. The lateral and medial vasti muscles are attached to the lateral and medial borders of the linea aspera, respectively (discussed in more detail below), and hence, their direction of pull, on contraction, is parallel with the shaft of the femur.

On the anterior aspect of the distal femur there is a depression between the medial and lateral condyles, the patella groove or trochlea, which articulates with the deep surface of the patella. The trochlear surface is divided into medial and lateral facets. Proximally, these facets are in continuity with a shallow groove on the anterior aspect of the shaft of the femur, that conforms to the shape of articular surface of the patella. Distally, the trochlea deepens to become the intercondylar notch.

The patella, ensheathed within the quadriceps tendon, is the largest sesamoid bone in the body. It is triangular in shape, with the apex at the inferior margin and a wide upper border. Its anterior surface presents a roughened, rounded, surface, while the posterior surface is marked by two flattened, smooth, articular facets (the lateral and medial condyles), covered in hyaline cartilage, that articulate with the distal femur at the trochlea, to form the patellofemoral joint. In cross-sectional profile, it can be clearly seen that the angulation of both the lateral condyle of the patella and the lateral facet of the trochlea, are more pronounced than those of the medial side (Figure 11), and furthermore, that the lateral facet of the trochlea extends further, and thus presents a more pronounced ridge than does the medial facet.

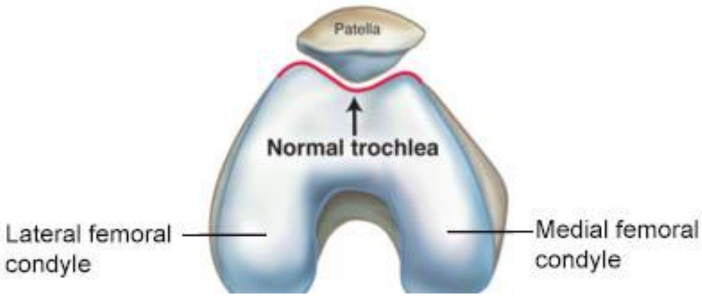

Figure 11. Normal trochlear groove and patella (adapted from Choman & Gilmer, no date)

The proximal tibia articulates with the distal femur to form the tibiofemoral part of the knee joint. The fibula plays no part in the knee joint. The articular surface of the proximal tibia (the tibial plateau) presents two flattened indentations, the medial and lateral condyles, with a crest of bone between them, the intercondylar eminence, which marks the site of attachment of the cruciate ligaments. Anteriorly, just distal to the tibial plateau, is a marked, rounded elevation, the tibial tuberosity, which is the attachment point of the ligamentum patellae (patella tendon), the continuation of the quadriceps tendon.

Because the shaft of the tibia is effectively vertical, while the femur approaches the knee joint at an angle, there is a resultant angulation between the pull of the quadriceps muscles (following the femoral shaft) and the vertical orientation of the patella ligament. A schematic vector diagram of these combined forces is shown in Figure 12, from which it can be see that there is a resultant lateral force that on the patella (there is also, of course, a posterior component, due to the angle of the knee joint in flexion, and the fulcrum provided by the domed anterior face of the patella).

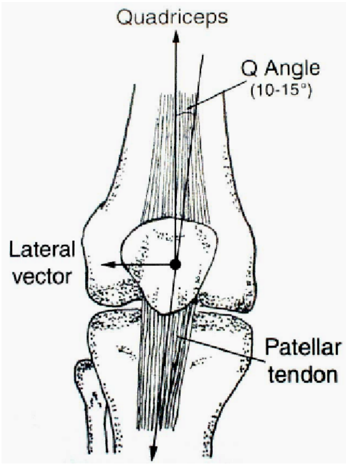

Figure 12. Forces acting on the patella during knee extension (Wong, 2009)

Lateral displacement of the patella during forceful knee extension is, however, resisted by the steeper angulation of the lateral facet of the femoral trochlea, and of the lateral facet of the patella which is congruent with it. Individuals with a flattened or dysplastic trochlea are, therefore, more prone to lateral dislocation of the patella during forced knee extension (Figure 13). Patients suffering recurrent patellar dislocation, a potentially very debilitating condition, may be offered a trochleoplasty, a surgical procedure that creates a deeper groove in the anterior femur in order to stabilise the patella.

Figure 13. Lateral dislocation of the patella (radiograph from the author’s collection)

4.4.2 Muscles of the Anterior Thigh: The Quadriceps Femoris Group

It is generally accepted that the quadriceps femoris muscle group comprises the vastus lateralis, vastus medialis, vastus intermedius, and rectus femoris, though a fifth component, the tensor of the vastus intermedius, was identified by Grob et al. in 2016, and Olewnik et al. (2021) went further, suggesting that the "quadriceps" should be more accurately described as the "multiceps" femoris, to take into account the larger number (up to eight), and variable nature, of the proximal attachments that they identified in their sample of over a hundred lower limbs. Gray's Anatomy (Standring, 2005), however, considers that, as a group, the quadriceps show little variation, and for the purposes of this discussion the standard nomenclature will be used.

The concept of muscle groups can be helpful when considering muscles within a fascial compartment, in this case the anterior compartment of the thigh, and generally, such groups share a common nerve supply and action. It is important to bear in mind, however, that this may be an oversimplification. As a group, the quadriceps muscles act together as powerful extensors of the knee, either in open-chain activities (where the foot is free) such as kicking, or closed-chain (where the foot is fixed in position) such as climbing steps. Individually, however, each muscle may also have a more specialised role, either within the group (i.e., while acting as an extensor), or outwith the group (where a muscle may have an additional mode of action).

The quadriceps femoris muscles, then, occupy most of the anterior compartment of the thigh, and act together to extend the knee. They all receive innervation from the femoral nerve (L2,3,4), which enters the thigh deep to the inguinal ligament, at the lateral margin of the femoral triangle, before splitting into multiple strands and entering the muscle bellies. The innervation of vastus medialis will be considered in more detail later. All the component muscles of the group are attached proximally to the femur, and distally to the tibial tuberosity via the ligamentum patellae, a continuation of the quadriceps tendon, which encloses the patella.

4.4.3 Rectus Femoris

Let us now consider some of the constituent muscles in more detail. As mentioned above, there are often exceptions to the concept of the "muscle group", and this is indeed the case in the quadriceps, as rectus femoris, uniquely among this group, arises from the pelvis, rather than the shaft of the femur via two heads: a straight head that attaches to the anterior inferior iliac spine, and a reflected head, which attaches to the superior margin of the acetabulum. As this muscle crosses both the knee and hip joints, it also has a dual role, and acts as an extensor of the knee (together with the other muscles of the quadriceps group), and as a flexor of the hip (Standring, 2005).

4.4.4 Vastus Lateralis

Vastus lateralis and vastus medialis both arise from the shaft of the femur, and both act as extensors of the knee, however, their role as extensors is more complex than that, as they both also have an important role in stabilising the patella during powered extension of the knee. Due to their influence on patellar tracking, both vastus medialis and vastus lateralis have been the subject of a great deal of research interest.

Vastus lateralis, the bulkiest muscle of the group, arises from the upper part of the intertrochanteric line, the greater trochanter, the outer border of the gluteal tuberosity, and the lateral lip of the linea aspera. Distally, it attaches to the lateral side of the quadriceps tendon, where it unites with the tendon of rectus femoris (Figure 14).

Figure 14. Anatomy of the anterior thigh; a, vastus lateralis; b, vastus medialis longus; c, vastus medialis oblique; d, quadriceps tendon; e, patellar ligament (image modified from Netter, 2001)

Like the vastus medialis, the vastus lateralis has been described as having two distinct heads with different fibre orientations: the vastus lateralis oblique (VLO) (short head), inserting onto the lateral margin of the patella, and the vastus lateralis longus (VLL) (long head) inserting onto the lateral part of the base of the patella via the aponeurosis of the quadriceps tendon (Weinstabl et al., 1989; Bennett et al., 1993). While this terminology is common in research literature, it has not yet been adopted by standard anatomy texts; while Gray's Anatomy (Standring, 2005) acknowledges that some authors distinguish the distal, more horizontal fibres of vastus medialis as the vastus medialis obliquus, no mention is made of the different parts of vastus lateralis. As an indication of its popularity in the anatomical lexicon, a PubMed search for the terms ("vastus lateralis oblique" AND "VLO" AND "anatomy") yielded two results, whereas a search for the equivalent terms for vastus medialis oblique yielded twenty-one.

The VLO and VLL muscle fibres generate their own respective force vectors which act on the knee in different ways. While the VLL acts mainly as an extensor of the leg, the VLO influences the alignment of the patella in a comparable role to the VMO (Weinstabl et al., 1989; Waryasz & McDermott, 2008). The VLO pulls the patella laterally, thereby counteracting the medial pull of the VMO (Figure 15). Both the VL and the VM also act together to pull the patella posteriorly into the trochlear groove (Amis, 2007).

The angle of insertion of the VLO muscle fibres into the quadriceps tendon is between 26° and 41° with respect to the femoral axis, with the angle of insertion for the VLL being between 10° and 17° (Weinstabl et al., 1989; Amis, 2007). The angle of the VLO is more horizontal in males (44.91° mean) compared with females (35.82° mean) (Bennett et al., 1993). This makes sense when one considers the greater Q angle, and hence greater lateralising forces acting on the patella in females – a reduced VLO angle would help to lessen the lateral force.

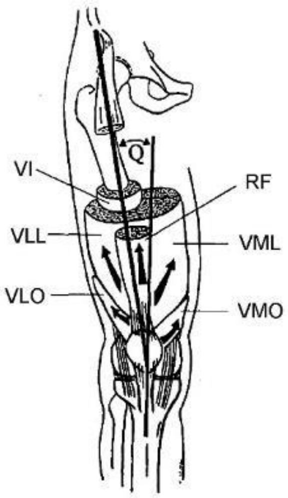

Figure 15. Lines of action of the quadriceps muscle group; RF, rectus femoris; VI, vastus intermedius; VLL, vastus lateralis longus; VLO, vastus lateralis oblique; VML, vastus medialis longus; VMO, vastus medialis oblique (image from Weinstabl et al., 1989)

4.4.5 Vastus Medialis

The anatomy of the vastus medialis (VM) has been extensively investigated over the past fifty years, with an on-going controversy over whether it should be considered as a single muscle or as two independent constituents, the vastus medialis oblique (VMO) and the vastus medialis longus (VML). Some authors have gone even further, and suggested that, based on its pattern of innervation, the VM should be considered, at least functionally, as a tripartite structure (Thiranagama, 1990; Lefebvre et al., 2006).

The distinction between the VML and VMO was first described in Lieb and Perry's (1968) seminal cadaveric investigation (albeit on a sample size of only six specimens), and there have been many studies and review articles since then. In their 2009 review, Smith et al. asked "Do the vastus medialis obliquus and vastus medialis longus really exist?" To which they concluded that the separation was not obvious (there was "insufficient good quality evidence"), and more research was needed. Anecdotally, there are experienced anatomy academics, who, based on their observations from many years teaching in the Dissecting Room, refused to believe in the separate existence of the VMO and VML. There have, however, been many more studies in the literature since Smith et al.'s 2009 review, and I think it can be agreed that the current consensus is that they do indeed exist as separate entities. Although the presence of a distinct fascial separation, and an abrupt change in fibre angle may not always be obvious, clinching evidence was provided by Tenan et al. (2015) who showed that the VMO and VML are separately innervated: the VML and VMO, they concluded, were neurologically different muscles, as each had a neuronal origin at different cortical levels. Furthermore, the inclusion of the VMO in Gray's Anatomy, as we have seen, suggests that the concept has now become accepted fact, and much research effort has been directed at the association between the VMO and the occurrence, and aetiology, of patellofemoral pain.

Anatomically, the vastus medialis lies medial to rectus femoris, and generally extends further distally than does the vastus lateralis, forming a distinct bulge medial to the patella in athletic individuals. The muscle arises from the distal third of the intertrochanteric line and the medial lip of the linea aspera, running down to the medial supracondylar line. Its distal attachment is to the medial side of the quadriceps tendon and from there into the tibial tuberosity via the patellar ligament. The more distal VMO arises from the adductor magnus and from the tendon of the adductor longus and inserts onto the medial border of the patellar retinaculum (an expansion of the fibrous capsule of the knee joint) (Figure 14), which effectively gives the VMO direct attachment to the medial border of the patella. The insertion level of the distal attachment of the VMO onto the medial patella (i.e., how far the VMO descends) varies between individuals. The insertion level can be expressed as a percentage of total patella length, referred to as the insertion ratio (Figure 16).

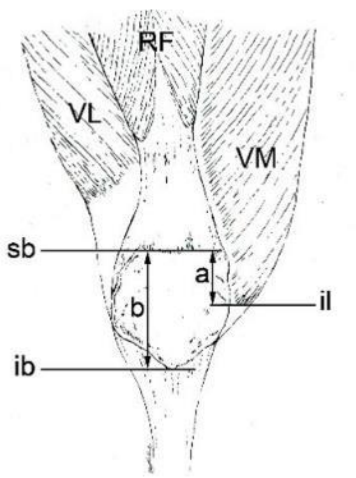

Figure 16. Insertion ratio of the vastus medialis oblique; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; ib, inferior border of patella; sb, superior border of patella; a, insertion length of VM; b, total patella length; insertion ratio (%) given by a/b*100 (drawing by Jennifer Crouch)

Intuitively, it might be expected that athletic individuals, with a well-developed VMO, would have a concomitantly greater insertion ratio, and that this would then be protective against patellar mal-tracking, and hence, against PFP. The situation in reality, however, is not so clear (*vide infra*).

4.5 The VMO and VML

This section will show how light was shed on VMO and VML structure, morphology, and variant anatomy through the dissection study described by Skinner & Adds (2012).

The VMO, then, forms the distal part of the VM, and can be distinguished by its more angled muscle fibres compared to the VML, and, sometimes, by a distinct fascial separation and/or a separate branch of the femoral nerve. Historically, much of the data on the VMO were gathered from dissection studies, meaning that the data came from a predominantly (if not exclusively) aged population.

When undertaking this investigation into the VM, the first priority was to go back to first principles and begin with a dissection study, in order to gain a thorough understanding of the anatomy. To this end, careful dissection was carried out on forty formalin-fixed lower limbs, from bodies donated for anatomical examination under the 1984 Anatomy Act (Skinner & Adds, 2012). The main objective of the study was to observe if there was an abrupt change in muscle fibre angle along the length of the muscles, and, if so, to measure the lengths of the proximal and distal parts of the muscles and the fibre angles on each side of the VMO/VML divide. The branches of the femoral nerve were also carefully followed to ascertain whether there was separate innervation of the VMO and VML (Figure 17).

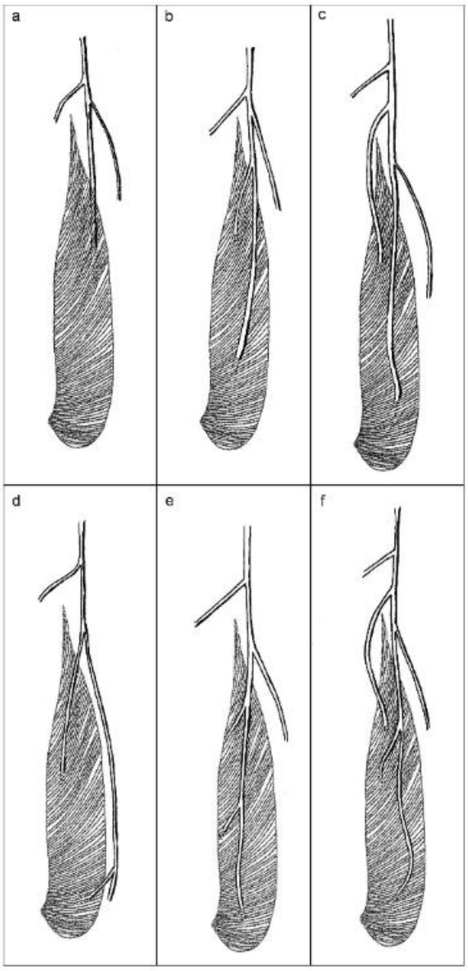

Figure 17. Variations in innervation of the VMO (drawing by Emily Skinner, from Skinner & Adds, 2012)

The abrupt change in angle that defines the VMO was by no means omnipresent, and in fact was only observed in 22 of the 40 limbs analysed (55% of the sample). Structural features observed at the VML/VMO interface included a fibro-fascial plane (12 specimens), a branch of the femoral nerve (11 specimens), and vasculature (3 specimens), although some of the limbs exhibited more than one of the structural features, and none of the features was seen in limbs where there was no abrupt change in fibre angle. Other dissection studies have described variable findings. In a large-scale study on 374 lower limbs, Hubbard et al. (1997) reported a gradual increase in fibre angle from proximal to distal in all the limbs studied, but did not report any abrupt change, and found no anatomical structure separating the VM into two independent structures, firmly refuting any suggestion that the VMO and VML might exist as separate anatomical entities. A notable difference between our study (Skinner & Adds, 2012), and others in literature, concerns the actual position of the VMO/VML interface, in specimens where a distinct change in angle was seen. While some authors (e.g., Hubbard et al., 1997) recorded a gradual change, some have given a specific location of the division. Ono et al. (2005), found it to be at the level of the adductor hiatus (admittedly, in a sample of only two lower limbs), Peeler et al. (2005) (32 limbs) described the VMO as being below the uppermost point of muscle fibre insertion into the quadriceps tendon, while Bose et al. (1980) described the VMO as originating from the tendon of the adductor magnus. All these levels are noticeably more distal than the level of separation that we found, which was, on average, in the proximal third of the VM (Figure 18).

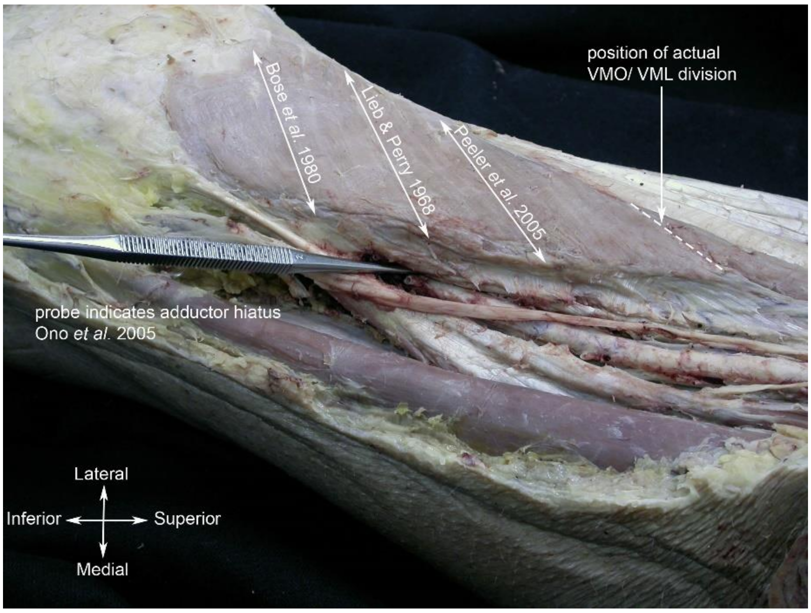

Figure 18. Levels of the VMO/VML separation; dashed line indicates location identified on this specimen (from Skinner & Adds, 2012)

Why the discrepancy? Could the vastus intermedius have been mis-identified as the VML, and what was thought to be the VMO/VML interface was in fact the separation of VM and VI? To clarify this, deep dissections were performed on four limbs from separate cadavers. All the muscles of the thigh were removed except for the vastus medialis and vastus intermedius, so that the origins of these two muscles could be observed. It was found that although the VI was closely related to the VM, the two muscles had distinctly different origins, and this confirmed the initial identification. However, the location was troubling when compared to other studies in the literature. Comparison of the fibre angles of the VMO and VML reported in this study (Skinner & Adds, 2012), and other studies in the literature, was intriguing, and furnished a possible explanation for the difference. In the Skinner & Adds study, the mean angle of the distal fibres of VMO was 52°, very similar to other studies (Peeler et al., 2005; Hubbard et al., 1997; Nozic et al., 1997). However, the mean VML fibre angle was found to be 5° (range -10° to +12°), whereas numerous other studies reported this angle to be 15–18° (Lieb & Perry, 1968; Peeler et al., 2005; Hubbard et al., 1997; Ono et al., 2005). It was striking that this was almost exactly the angle of the VMO that was found at the division between the VMO and VML (i.e., 16.4°) (Skinner & Adds, 2012). One possible explanation for this is that what some previous investigators regarded as the separation between the VM and VI was in fact the division of the VML and VMO, so they looked more distally for the VMO/VML divide. Interestingly, none of the reports cited here describe carrying out deep dissections to confirm their identification. The literature describing the VMO as a separate entity ascribes to it the role of medial patella stabilisation, so the separation of the VMO and VML that we found in the proximal third of the muscle might seem counterintuitive. Peeler et al. (2005) defined the VMO as being the part of the VM “below the uppermost point of muscle fiber (*sic*) insertion into the quadriceps tendon”. They then subdivided the VMO into two parts, a superior part inserting into the quadriceps tendon, and an inferior section that inserted directly into the medial side of the patella, making the VM in effect a tripartite structure: indeed, Thiranagama (1990) also concluded that the VM should be divided into 3 functional compartments because of the changes in fibre angle: the upper third consisting of fibres with angles ≤10°, the middle third

15°-35° and the lower third 40°-50°. The fibre angles that reported here (Skinner & Adds, 2012) tend to support this tripartite hypothesis: the fact that the VMO comprised nearly 70% of the VM, with only 7.2% inserted directly into the patella, suggests that a reappraisal of VMO and VML might be called for. While there seems to be no doubt that the distal fibres of the VM do indeed bring about medial stabilisation of the patella, and have been shown to be separately innervated (Tenan et al., 2015), and the fibres of the VML contribute to knee extension, should it perhaps be accepted that there is a central region of the muscle, contributing to both, with fibre angles between approximately 15°-40°, delineated proximally by an abrupt change in fibre angle (with or without an intervening structure - nerve/artery/fascial plane), and distally, by their separate innervation *sensu* Tenan et al. (2015), and again, with or without a structural marker?

While Tenan et al. (2015) have shown that the proximal and distal parts of the VM are indeed capable of functioning as independent units, can they be truly considered to be separate muscles? In their study of 374 lower limbs, Hubbard et al. (1997) found no evidence of an abrupt change in fibre angle, and referred to a description of skeletal muscles in Grant's Method of Anatomy (Basmajian, 1980), in which a skeletal muscle was defined thus: it must have distinct points of origin and insertion, a distinct nerve supply allowing it to be activated independently from the surrounding muscles, and it needs to be encompassed by a fascial plane so that when it contracts, it can do so independently of related muscles. According to Basmajian (1980), all these conditions need to be met to permit a distinct muscle to have a unique function (Basmajian, 1980 cited by Hubbard et al. 1997). On that basis, it is highly unlikely that VMO and VML could be considered as separate muscles. From the dissection studies carried out by Skinner & Adds (2012) it was clear that, while there were separate nerve branches to proximal and distal parts of the VM in 43.6% of specimens examined, the innervation was derived solely from a single branch of the femoral nerve to the VM. Furthermore, evidence of an abrupt change in fibre angle, with an intervening structural feature, was seen in only 55% of the sample. While there is good evidence from the analysis of fibre angles that the muscle should perhaps be considered as a bipartite, or even tripartite, structure, evidence for its existence as two separate muscles is weak. Nonetheless, it is clear that VMO and VML exist as functional subunits of the VM and can be activated independently; whether or not they exist as two separate muscles is largely irrelevant.

4.5.1 A Rare Anatomical Variant of the VMO

An interesting and unexpected anatomical variant described in the dissection study by Skinner & Adds (2012) was that in five limbs, the VMO was present as a superficial layer covering the VML, with the longitudinal fibres of the VML continuing deep to the VMO until approximately 2 cm above the patella, where the fibres of the VML and VMO fused (Figure 19). This rare variant anatomy had been previously reported by Barbaix & Pouders (2006) in three out of "more than a hundred" dissected lower limbs, and by Nwoha & Adebisi (1994), bilaterally in one out of a sample of 200 Nigerian cadavers, which the authors called an "accessory quadriceps femoris muscle". Prior knowledge of this variant was helpful when interpreting apparently anomalous ultrasound images that were encountered later on in this research (see Section 6.4).

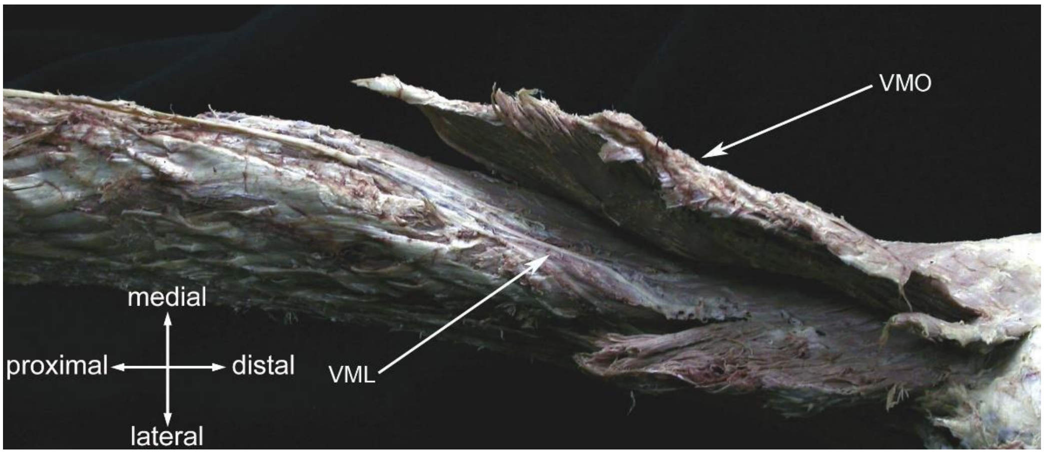

Figure 19. Rare anatomical variant: VMO present as a superficial layer over the distal VML (VMO shown reflected)

5. Theme 2: Explore the Potential of Using Ultrasound to Visualise Muscle Architecture

The aim of this theme was to explore the potential of using ultrasound (US) to visualise muscle architecture, first on the gastrocnemius then the VM; and to validate the method for measuring the VMO fibre angle.

5.1 Peer-Reviewed Publications Under This Theme:

- Antonios T, Adds PJ. (2008) The medial and lateral bellies of gastrocnemius: a cadaveric and ultrasound investigation. Clinical Anatomy 21 (1) 66-74. doi 10.1002/ca.20533
- Engelina S, Robertson CJ, Moggridge J, Killingback A, Adds PJ. (2014a) Using ultrasound to measure the fibre angle of vastus medialis oblique: A cadaveric validation study. The Knee 21(1): 107-111 DOI:10.1016/j.knee.2012.07.001

5.2 Exploring the Potential Use of Ultrasound in Muscle Studies

The study carried out on the muscle bellies of gastrocnemius (Antonios & Adds, 2008) demonstrated the potential of US to visualise the architecture of skeletal muscle. In order to better understand the normal anatomy of the VMO, it was necessary to establish normative values from a young, asymptomatic population, and US presented the ideal imaging modality to achieve this. Previously published fibre angle data had been derived from dissection studies, whereas PFP predominantly affects younger individuals. Although various studies on the VM and other muscles had previously shown that ultrasound could be used to measure muscle pennation angles *in vivo* (e.g., Blazevich et al., 2006; Ikegawa et al., 2008; Jan et al., 2009) it was necessary first to validate the technique for the VM, by comparing values for VMO fibre angle from US measurement, with values obtained by direct measurement following dissection of a series of soft-fixed cadavers (Engelina et al., 2014a).

6. Theme 3: Obtain Normative Values

The aim of this theme was to obtain normative values for the pennation angle and insertion level of the VMO in a cohort of young, asymptomatic individuals, and further investigate the dichotomy between active and sedentary individuals.

6.1 Peer-Reviewed Publications Under This Theme

- Engelina S, Antonios T, Robertson CJ, Killingback A, Adds PJ. (2014b) Ultrasound investigation of vastus medialis oblique (VMO) muscle architecture: an in-vivo-study. Clinical Anatomy 27(7): 1076-1084 DOI: 10.1002/ca.22413
- Benjafield AJ, Killingback A, Robertson CJ, Adds PJ. (2015) An investigation into the architecture of the vastus medialis oblique muscle in athletic and sedentary individuals: an in-vivo ultrasound study Clinical Anatomy 28(2): 262-268 DOI: 10.1002/ca.22457
- Benjafield A, Howe FA, Killingback A, Adds PJ. (2014) Unusual variant of vastus medialis detected by ultrasound and confirmed by high-resolution MRI. Journal of Physical Therapy Science 26(1): 165-166 https://doi.org/10.1589/jpts.26.165

6.2 Obtain Normative Values for the VMO

Once it had been established that the methodology was robust and reliable, forty young, asymptomatic volunteers were recruited (males and females, all undergraduate students of healthcare sciences) in order to gather normative data in a sample representative of the age group most affected by PFP.

The subject's age, height, weight and Tegner score were recorded prior to US measurement. The Tegner score is a validated, commonly used scoring system to grade an individual's level of physical activity (in sports or work), where a higher score indicates increased activity (Tegner and Lysholm, 1985).

There were found to be no significant differences in VMO angle between sex and ethnicity, and some evidence of a trend in female subjects whereby higher levels of exercise correlate with increased VMO angles, although this finding was not statistically significant (P=0.08) (Engelina et al., 2014b). In males there was no such correlation. Although the uneven distribution of Tegner scores (the majority of subjects reported a score of 3) may have skewed the results, this was of sufficient interest to warrant further investigation.

6.3 VMO Morphology in Active and Sedentary Individuals

It might be expected that there would be an increased fibre angle in more active individuals. Participants in athletic sports would be expected to have a high degree of quadriceps muscle hypertrophy, leading to an increase in muscle pennation angle and, therefore, VMO fibre angle. Theoretically, this should increase medial stability and aid patellar tracking. However, PFP has a high incidence in athletic individuals - their increased activity levels, and hence loading of the patellofemoral joint, are risk factors for the development of knee pathologies (Halabchi et al., 2017). There was, however, a gap in the literature regarding the detailed morphology of the VMO in high-performance athletes.

The aim of the next study in this series of investigations (Benjafield et al., 2015), therefore, was to investigate and compare the muscle architecture of the VMO in two groups of healthy, asymptomatic volunteers: an athletic group, and a sedentary group. Volunteers were again recruited from among enrolled healthcare science students, but for this study, recruitment specifically targeted those who were actively engaged in sports, as well as those who actively avoided it. For this study, Benjafield et al. (2015) used ultrasound to assess eighty-two knees, from 26 athletic male volunteers and 15 sedentary male volunteers (aged 20-28 years). Limiting the cohort to males gave a homogeneous sample for the study and removed the confounding factor of sex difference. Males are also known to have less subcutaneous fat (Eisner et al., 2010), enhancing the echogenicity of the muscle underneath (Reimers et al., 1993) due to reduced attenuation of the sound waves, and thus, it was hoped, improving the accuracy of the ultrasound data acquisition. Their level of activity was defined using the Tegner scoring system (Tegner & Lysholm, 1985). The Tegner scale was designed to aid the evaluation of patients with knee injuries; the scale goes from "level 0" (defined as "Sick leave or disability pension because of knee problems") to "level 10", reserved for elite national or international athletes. In this study, the 'active' study group had a mean reported Tegner score of 7.7 (±0.7), with a range of 7-9. A score of 7 is defined as "Competitive sports: tennis, athletics (running), motorcars speedway; or Recreational sports: soccer, ice-hockey, squash, basketball, or rugby". The 'sedentary' group had a mean Tegner score of 2.5 (±0.7), range 1-3. A "level 3" score is defined as "Work: light labor (*sic*) (nursing, etc.), Recreational sports: swimming", so the level of activity of the 'sedentary' group would have been no more than that, while the 'active' group were representing their university in competitive sports such as football, rugby, and rowing. Unsurprisingly, with two such distinctly different groups, there was a significant difference in the mean VMO fibre angle: 67.8° for the 'active' group, and 53.6° for the 'sedentary' group ($P<0.001$). There was a trend for the insertion ratio to be higher the athletic group (43.0%), than in the sedentary group (39.5%), though this difference fell just outside significance ($P=0.06$). A video summary of this study is available on Clinical Anatomy's 'CA Video Highlights' web page (Clinical Anatomy, no date).

6.4 An *in-vivo* Example of a Rare Anatomical Variant

While carrying out US examination on one of the volunteers for the previous study, a puzzling image appeared, that was notably different from all the others that had been seen thus far (Figure 20). The unusual morphology was present bilaterally.

Figure 20. Unusual VM presentation a) two-layered presentation of VM seen on the US monitor; b) control subject showing usual morphology (from Benjafield et al., 2014)

It occurred to this author that it could be an example of the anatomical variant had been observed in five of the lower limbs that had been dissected previously (Skinner & Adds, 2012, see Section 4.4). To verify this, informed consent was obtained from the volunteer to perform an MRI scan of her knees. The resulting images were compared to images from an anonymised patient, matched for sex and age, who had previously undergone an MRI scan for an unrelated condition. The images demonstrated clearly that this was indeed an example of the VM being present as two layers, with the VML continuing deep to the VMO, which was present as a superficial layer (Figure 21) (Benjafield et al., 2014).

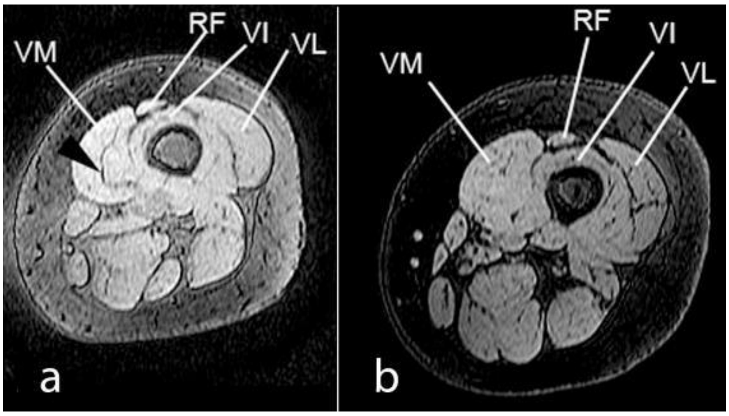

Figure 21. Axial MRI of left thigh; a) arrowhead indicates plane of separation between VMO (superficial) and VML (deep); b) control subject, no plane of separation visible in VM at the corresponding axial level. RF rectus femoris, VI vastus intermedius, VL vastus lateralis, VM vastus medialis (from Benjafield et al., 2014)

This appears to be only the second time that this variant anatomy has been recorded bilaterally, and the first ever report of it *in vivo*. The biomechanical implications of this arrangement remain unclear, but it is possible that the thin, superficial VMO lamina reduces its physiological cross-sectional area and hence its effectiveness as a medial stabiliser of the patella. The fact that this individual suffered from recurrent patellar dislocations tends to add weight to this argument, though more work is needed to establish if this type of VMO morphology is more common among patients presenting with recurrent patellar dislocations or patellar instability.

This completed the first phase of the investigation. Having validated the methodology and established baseline, normative data for the architecture of the VM in cadaveric specimens as well as in young, asymptomatic subjects; and, furthermore, having found a significant difference in the VMO fibre angle between active and inactive individuals, the focus of the research now switched to investigating whether the characteristics of the VMO could be manipulated by targeted interventions.

7. Theme 4: Investigate the Effect of Physiotherapy on the Architecture of the VMO

The aim of this theme was to investigate the effect of physiotherapy on the architecture of the VMO and if this effect was influenced by the following factors: different exercise techniques, electro-muscular stimulation, and cessation of the physiotherapy.

7.1 Peer-Reviewed Publications Under This Theme

- Khoshkhoo M, Killingback A, Robertson CJ, Adds PJ. (2016) The effect of exercise on vastus medialis oblique muscle architecture: an ultrasound investigation. Clinical Anatomy 29(6): 752-758 DOI: 10.1002/ca.22710
- Elniel A, Robertson CJ, Killingback A, Adds PJ. (2017) Open-chain vs. closed-chain exercise regimes: an ultrasound investigation into the effects of exercise on the vastus medialis oblique. Physical Therapy and Rehabilitation http://www.hoajonline.com/journals/pdf/2055-2386-4-3.pdf DOI:10.7243/2055-2386-4-3
- Arnantha H, Robertson CJ, Killingback A, Adds PJ. (2017) Maintenance of exercise-induced changes in the architecture of the VMO: how much is enough? An in-vivo ultrasound study. Journal of Orthopaedics Spine and Sports Medicine 1:1 003
- Hilal Z, Killingback A, Robertson C, Adds PJ. (2018) The effect of exercise and electrical muscle stimulation on the architecture of the vastus medialis oblique - the ‘Empi’ electrotherapy system Global Journal of Orthopedics Research 1(1) DOI: 10.33552/GJOR.2018.01.000503

7.2 Introduction

Having established a positive correlation between an individual's activity level and their VMO pennation angle, the next step in the investigation was to investigate whether, and how, the fibre angle could be manipulated. Physiotherapy is currently the first line of treatment for PFP, and often concentrates on exercises to strengthen the quadriceps, with special emphasis on the VM, if VMO insufficiency is thought to be implicated in the aetiology of the patient's condition. Physical therapy of this sort has been shown to lead to a significant degree of pain reduction (Crossley et al., 2002), although the evidence to support this approach is limited, and it is mainly based on the theory that quadriceps strengthening exercises will lead to changes in the architecture of the VMO, which will in turn increase its force, preventing maltracking, and thus reducing the anterior knee pain (Chiu et al., 2012). This is a long chain of suppositions, for which there has hitherto been little, if any, empirical evidence.

For the intervention studies described in this section, subjects undertook a six-week physiotherapy programme. This was felt to be the optimum period given constraints such as subjects' availability and other commitments, and is also commonly used in clinics, and in other PFP research programmes in the literature (Crossley et al., 2002).

7.3 The Effect of Exercise on the Architecture of the VMO

The rationale for the first intervention study (Khoshkhoo et al., 2015) was quite simple: what effect does exercise actually have on the architecture of the VMO? There was a gap in the literature for empirical evidence, and a compelling need to provide hard data. The first line of enquiry, then, was to recruit a cohort of relatively sedentary volunteers and measure their VMO fibre angle and insertion ratio before, and after, a six-week programme of physiotherapy in order to quantify any changes in fibre angle and insertion level.

Twenty-four subjects were recruited for the study. One subject dropped out, and one was used for the intra-rater study, so did not participate in the physiotherapy. The upper limit of the Tegner score was set at 5, and the mean Tegner score of the cohort was 3. 19 (a score of three is defined as "light labor (*sic*), e.g., nursing; competitive and recreational sports, e.g., swimming; walking in forest is possible") (Tegner and Lysholm, 1985). This is slightly higher than in the sedentary group in the previous comparison of athletic and sedentary individuals (Benjafield et al., 2015) (see Section 6.3, VMO morphology in active and sedentary individuals) which was 2.5, however, it was necessary to relax the inclusion criteria slightly in order to recruit a sufficiently large group, as students in this institution tend to be quite active in sports.

The volunteers were given an initial ultrasound scan to measure the fibre angle and insertion level of the VMO, before starting a six-week programme to strengthen the quadriceps femoris. The US scan and measurements were then repeated. The exercises were a simple combination of knee extensions and isometric quadriceps contractions (i.e., open-chain kinetic exercises (OCKE)) to be carried out every second day for six weeks. Details of the exercise protocols are given in Khoshkhoo et al. (2015). Results were clear: there was a mean increase of 5.24° in VMO fibre angle ($P<0.001$), and mean increase of 2.69 mm in the insertion length ($P<0.001$).

Further statistical analysis of the data revealed two further potentially useful insights: there was a moderate negative correlation between initial fibre angle and fibre angle change, and a highly significant positive correlation between compliance (as recorded by each volunteer in a "compliance diary") and changes in VMO fibre angle ($R^2= 0.796$). This provides useful information that could be used to guide interventions in the clinic. Patients who would be benefit most from this type of physiotherapy i.e., those presenting with a low VMO fibre angle (say, under 55°) could be identified by a simple US scan. Also, the knowledge that the level of compliance is an important factor in the success of the treatment could be used to motivate the patient's engagement with the treatment programme.

7.4 Open-Chain or Closed-Chain Kinetic Exercises?

Having established the effect of an exercise programme on the VMO, the next study to be undertaken investigated which type of exercise would be most effective (Elniel et al., 2017). There has been discussion in the literature as to which type of exercise is more effective: "closed-chain" kinetic exercises (CCKE) with the foot on the ground, or "open-chain" (OCKE), where the foot is free to move (Irish et al., 2010). Studies have generally shown an improvement in symptoms and improved functionality in PFP sufferers following both OCKE and CCKE exercises, though there have been differing reports as to which was the more effective in the relief of anterior knee pain. While the clinical effectiveness of the two different approaches has been compared, there was no information in the literature regarding the effect of these exercise types on the muscles of the thigh. The next study, therefore, aimed to fill the gap in the literature by comparing the impact of open-chain and closed-chain exercise regimes on the architecture of the vastus medialis oblique.

A cohort of 23 relatively sedentary male volunteers was recruited (the mean Tegner score was 2.05). Exclusion criteria included a Tegner activity score >3, or gym training once a week or more. The volunteers were given an initial US scan of their VMO fibre angle and insertion level, and then divided into two groups – matched, as far as was possible, by height and BMI, and assigned to one of two groups: OCKE or CCKE. Each group was given a choice of two exercises to carry out (it was thought that giving participants a choice of exercises would encourage them to engage with the study, and hence improve compliance) and asked to carry out the exercises every other day for six weeks. Details of the exercise protocols are given in Elniel et al. (2017). Results from both groups showed a significant increase ($p < 0.001$) in both fibre angle (4.65° and 5.73° for OCKE and CCKE, respectively), and insertion length (4.31 mm and 4.63 mm, respectively). There was, however, no significant difference between the two interventions ($p = 0.82$), so both types of exercise can be prescribed with confidence, if the aim is to increase VMO pennation and bulk.

Again, there was an inverse relationship between initial fibre angle and fibre angle change, in both groups, showing that subjects with a low initial fibre angle showed the greatest amount of change. This was also true of the insertion length, though the correlation was less marked. The mean self-reported compliance, (as recorded by the participants in a "compliance diary"), was 67% and 68% respectively for OCKE and CCKE. Interestingly, this is almost exactly the same as in the previous study (Khoshkhoo et al., 2015), where the compliance was 67.7, though the latter figure was slightly distorted as one volunteer recorded 150% compliance. Removing the outlier from the Khoshkhoo et al. (2015) data brings the average for that study down to 65%, so it is arguable if the strategy to increase compliance was successful.

7.5 Maintenance of the Changes Achieved by Physiotherapy

Having established that we can increase the fibre angle and insertion level by six weeks of physiotherapy using either OCKE or CCKE exercises, the next research question to be tackled was: what happens to the changes after the end of the programme of therapy? And how much exercise is needed to maintain the gains achieved (Arnantha et al., 2017)?

This was a challenging study to carry out, as it was necessary to recruit a sedentary cohort who were willing to commit for a minimum 12-week period – an initial six weeks of exercises for all (Phase One), then a further six weeks on a schedule of either the same, or a reduced programme of exercises (Phase Two). In the event, a convenience sample was recruited of 16 eligible volunteers, 10 females and six males, with a mean Tegner score of 3.35.

At the completion of Phase One, volunteers were randomly assigned to one of four different groups and asked to continue the exercises once a week, twice a week, three times a week, or not at all. Volunteers were given an initial US scan, then re-scanned at the end of phase one, and again at the end of Phase Two. While the low number of subjects meant that this study was not statistically powerful, the results nonetheless showed a clear trend. There was a significant increase in VMO fibre angle of 4.41° after Phase One ($p<0.05$). At the end of Phase Two, there was a mean decrease in fibre angle of 5.88° with no exercise; a decrease of 2.5° with exercises once a week; a gain of 0.75° with exercises twice a week; and a gain of 3.12° with exercises 3 times a week ($p<0.001$ for all changes in Phase Two). There were no statistically significant changes in the insertion level of the VMO, either in Phase One or Phase Two.

Possibly partly due to the low numbers of volunteers in the study, the self-reported compliance was high. The mean compliance for phase one was 91.25%, and for phase two, the mean compliance was 94% (unsurprisingly, the compliance for the group with no exercises was 100%). There was found to be a very strong correlation ($R^2 = 0.994$) between VMO angle change and the number of days of exercise per week.

Therefore, not only has it now been shown that the physiotherapy for PFP has a positive effect on the VMO fibre angle, but there is also some evidence that it is necessary to continue the exercises twice a week after the initial exercise period, in order to maintain the initial gains.

7.6 Neuro-Muscular Electrical Stimulation (NMES)

The next question to be addressed was whether, and to what extent, could the effects of exercise be enhanced by using electrical stimulation? Neuro-muscular electrical stimulation (NMES) is a form of treatment that uses small electrical impulses to repair tissue and stimulate muscles, to increase sensation and muscle strength (Insausti-Delgado et al., 2021). Electrical stimulation is being increasingly used to aid musculoskeletal rehabilitation following injury or surgery, and for other immobile patients groups, e.g., on ITU, acutely unwell, with chronic disease, or following stroke (Hill et al., 2018; Silva et al., 2019). Evidence has shown it to be effective in treating PFP (Bily et al., 2008), giving relief from symptoms (Callaghan et al., 2004), as well as increasing the strength of the muscle (Garcia et al., 2010); NMES alongside physical therapy is now encouraged in first line management of PFP (Dos Santos et al., 2013). However, although NMES is being increasingly prescribed, there was a notable lack in the literature of empirical data on its effects on the targeted muscles. The next study to be undertaken, therefore, aimed to explore the effects of NMES on the VMO.

For the next study, Hilal et al. (2018) were able to recruit an all-female, sedentary cohort of 25 volunteers, all of whom had a Tegner score ≤3. This investigation basically re-created the earlier study (Khoshkhoo et al., 2016), with participants carrying out a six-week exercise programme. However, this time, neuro-muscular electrical stimulation (NMES) was applied to one lower limb using the ‘Empi’ NMES system. The contralateral limb, therefore, acted as each participant’s own control. The ‘Empi’ muscle stimulators for the study were kindly loaned by Donjoy Ltd.

The results were striking: without NMES, the mean increase in VMO fibre angle was 3.95°, and this is broadly in line with the results of our previous studies, however, with NMES, the mean increase was nearly double, at 7.33°. The change in insertion length was less clear. Although there was a greater increase in the stimulated limb (7.4 mm compared 3.8 mm in the unstimulated limb), the difference was not statistically significant (P=0.231).

Of course, these results must be looked at with the *caveat* that the subjects were all females, whereas all the previous interventions had been carried out with male subjects. The reasons for this are discussed in Section 3, Research Methodology. However, bearing in mind that PFP is more common in females, this study provided convincing and conclusive evidence of the potential benefits of NMES combined with a programme of physiotherapy.

7.6.1 All-Male vs All-Female Cohort Comparison

It is interesting to compare the results from Khoshkhoo et al. (2016) (six-week exercise programme with all-male subjects) with the results from Hilal et al. (2018) without NMES (all females, an identical six-week exercise programme). Are the results comparable? While the fibre angle increase was greater in the Khoshkhoo study (5.24° vs 3.95°), the initial fibre angle was lower (62.24° vs 71.0°), so this was perhaps not surprising. The standard deviation was also higher in the Khoshkhoo study (±5.72 for initial fibre angle measurements vs ±4.3) suggesting that perhaps the expected difficulties in US imaging of the VM in females may have been over-estimated.

8. Theme 5: Investigate the Effect of Stretching Exercises

The aim of this theme was to investigate the effect of stretching exercises and myofascial release on the pennation angle of the VL and VMO. A seven-week exercise period was chosen for these studies in order as far as possible to match studies already in the literature. Although e Lima et al. (2015) used a programme of stretches over eight weeks, it was not possible exactly to match this due to the time constraints. However, the frequency of exercises was increased, and it was felt that this would provide sufficient time for the exercises to take effect.

8.1 Peer-Reviewed Publications Under This Theme

- Bethel J, Robertson CJ, Killingback A, Adds PJ. (2022) The effect of stretching exercises on the fibre angle of the vastus lateralis and vastus medialis oblique: an ultrasound study. The Journal of Physical Therapy Science 34: 161-166
- Torrente QM, Killingback A, Robertson CJ, Adds PJ. (2022) The effect of self-myofascial release on the pennation angle of the vastus medialis oblique and the vastus lateralis in athletic male individuals: an ultrasound investigation. The International Journal of Sports Physical Therapy Published online June 1 2022. https://doi.org/10.26603/001c.35591.

8.2 Lateral Thinking - the Vastus Lateralis

At this point, it was felt that the investigations into the VMO in asymptomatic individuals had gone about as far as possible, and that the gaps in the literature that had been identified had been satisfactorily filled. Up to this point, the VMO had been considered in isolation. The next phase of the investigation widened to include the lateral aspect of the distal thigh, and, specifically, the vastus lateralis. It is known that the balance between contractions of the vastus medialis and vastus lateralis is key to maintaining normal patellar tracking, and can be adversely affected both on the medial side, by insufficiency of the VMO, or on the lateral side, either by tightness in the tensor fasciae latae and iliotibial band (ITB), or by hypertrophy of the vastus lateralis (Bhave & Baker, 2008; Al-Hakim et al., 2012). Treatment for PFP, therefore, can include stretching exercises, either in addition to, or as an alternative to, quadriceps strengthening.

While there has been previous research into the architecture and effect of exercises on the VMO, no studies could be found investigating the effect of exercise on the muscle fibre angle of the VL. Equally, no studies could be found on the effect of stretching exercises on the fibre angle of the VMO, although e Lima et al. (2015) investigated the effect of stretching on the VL; they found no significant change in VL fibre orientation after a stretching programme. However, e Lima et al.'s (2015) study was based on measurements from a small sample of only twelve lower limbs, so it seemed appropriate to repeat the study with a larger cohort, before drawing any firm conclusions. Accordingly, for the next study (Bethel et al., 2022), twenty-seven athletic male participants were recruited, with a Tegner score ≥4. These are the sort of individuals with well-developed thigh muscles, who might well be prescribed stretching exercises if they presented with PFP, if VL hypertrophy was suspected to be an aetiological factor.

All participants were given an initial US scan to record the baseline fibre angles of the VL and VMO. They then undertook a seven-week quadriceps stretching programme, three times a week on three separate days, after which the US scans were repeated. The results showed a significant mean reduction of 7.73° ($p<0.001$) in the fibre angle of the VL, and a mean reduction of 4.9° in the VMO angle. Although the absolute values were similar, in percentage terms the mean reduction in the VL was more than double (-16.23%) than the reduction in the VMO (-7.12%).

If the success of exercises to strengthen a muscle can be quantified by measuring the increase in pennation angle, and hence, the physiological surface area (PSA) of the muscle, by inference, a decrease in the pennation angle (and PSA) could equally be applied as a measure of the *loss* *of strength* of the muscle. If PFP was being caused by a hypertrophied VL, then reducing its strength (or at least, its tone) could be considered to be beneficial. The risk is, of course, that the reduction in tone might not be limited to the VL, and might also affect the other muscles of the quadriceps, and particularly, the VM. If the VMO is weakened, it could have an adverse effect on patellar tracking, and so negate the gains achieved by the stretching of the VL. These are considerations that will need to be borne in mind when treatment for PFP is being prescribed. At least information is now available on which to base such clinical judgements.

8.3 Self-Myofascial Release

An alternative approach to VL hypertrophy is to use self-myofascial release to increase muscle flexibility (Cornell & Ebersole, 2020). Self-myofascial release (SMR) is a technique to reduce fascial tightening by using a foam roller to stretch it, by applying sustained pressure on the surface of the muscle. Studies on SMR have shown that foam rolling can increase joint range of motion and reduce delayed onset muscle soreness before and after exercise (MacDonald et al., 2013; Cheatham et al., 2015). Hitherto, however, there has been no information in the literature describing the effect of SMR on muscle architecture. Indeed, Cornell & Ebersoll (2020) even recommend using ultrasound to further investigate the changes brought about by an acute bout of SMR. The aim of the next study, therefore (Torrente et al., 2020), was to further our understanding of the effects of SMR by again investigating changes in the fibre angles of the VMO and VL after a seven-week programme of SMR using a foam roller.

Twenty-five young, athletic male participants were recruited to use a foam roller, on both thighs, for 7 weeks (Figure 22). As in the previous study, ultrasound was used to determine the initial and final VMO and VL pennation angles on both limbs. Participants were then scanned again on completion of the SMR programme.

Figure 22. Roller method for self-myofascial release: the subject moved himself backwards and forwards on his elbows for one minute on each lower limb (from Torrente et al., 2022)

Results showed a significant (P < 0.001) mean decrease in the pennation angles of the VL (-6.65°) and VMO (-7.65°). Again, although the absolute values were similar, in percentage terms the reduction in the VL was greater (-18% mean change) than in the VMO (-11.5% mean change). There was a weak negative correlation between initial VMO fibre angle and angle change, and a moderate correlation for the effect in the VL. The risk of applying SMR for hypertrophy or tightness of the VL is, of course, that it might also affect the VM, leading to a concomitant reduction in the tone or fibre angle of the VMO, and hence, adversely affecting the ability of the VMO to counteract the lateralising force of the VL. However, from the data shown here, the effect on the VL is greater (in terms of percentage change), and also, there is only a weak correlation between the initial VMO angle and fibre angle change. It would appear, then, that SMR can be prescribed with confidence to suitable patients, safe in the knowledge that the effect on the VL will be greater than the effect on the VM, though monitoring VMO fibre angle during the course of treatment might be a sensible precaution.

The SMR study concluded the ultrasound investigations into the VM and VL.

9. Discussion: Reflections and Afterword

9.1 The Journey

In August 2006, while driving along a country road in Essex, I happened to get stuck behind a group of cyclists, proceeding at a smooth eighteen miles an hour. It was a twisty road with few opportunities to overtake, so, while following the cyclists for some distance, I could not fail to notice how well-developed their calf muscles were, and furthermore, that the medial and lateral bellies of their gastrocnemii were of unequal length: the medial belly extended further distally than the lateral belly. I observed that this occurred bilaterally and appeared to be common to all the cyclists in the group. I started to wonder why, and whether this was a constant anatomical feature, and, if so, what was the significance, and how could it best be investigated?

On carrying out a review of the literature, I found that most standard anatomy textbooks reported that the medial belly extends further distally than the lateral belly (Williams and Warwick, 1980; Sinnatamby, 1999; Palastanga et al., 2002; Drake et al., 2005; Moore and Dalley, 2006), with no explanation of the functional significance. There were some studies in the literature (Wickiewicz et al., 1983; Huijing, 1985; Kawakami et al., 1998), but these were based on small samples (3, 8, and 6 lower limbs, respectively). Wickiewicz et al. (1983) also reported differences in the architecture of the two bellies. Kawakami et al. (1998) used ultrasound to measure fascicle length and angle – which immediately suggested a possible mode of further investigation.

While exploring the possibilities of collaboration to investigate this further, I established links with the Clinical Physics team of St George's Hospital NHS Trust, and this led to our first publication, "The medial and lateral bellies of gastrocnemius: a cadaveric and ultrasound investigation" (Antonios et al., 2008). In many ways, this first study can be seen as a paradigm for the subsequent investigations into the quadriceps: it began with a cadaveric study to revise and define the anatomy, then moved on to an ultrasound investigation with student volunteers undertaking exercises while undergoing real-time ultrasound imaging.

The experience that I gained during this study paved the way for further collaborations with the Medical Physics Department at St George's University Hospitals NHS Foundation Trust and with Physiotherapy practitioners, which led to us undertaking many more ultrasound-based investigations, most of which were centred on the lower limb. The five-mile trip that I set out on back in 2006, and the hold-up behind the cyclists, led me on a journey of discovery that lasted fourteen years, included fifteen further separate investigations, and generated fifteen peer-reviewed publications, fourteen national or international conference presentations (seven of which won a prize for best oral or poster presentation), and fourteen published abstracts.

9.2 Impact Assessment

9.2.1 Publication Metrics

Based on data from ResearchGate, Web of Science, and Google Scholar, the papers that we have published have amassed a total of 6404 reads, and 119 citations in other publications. Our work has had an impact on ultrasound methodology, and knowledge progression in lower limb anatomy: three publications (Antonios & Adds, 2008; Benjafield et al., 2015, and Engelina et al., 2015) are cited in a book chapter "Automatic and Quantitative Methods for Sonomyography" (Zhou & Zheng, 2021); and Engelina et al. (2014) is cited in Tenan et al.'s (2016) important paper showing that the VMO and VML were separately innervated, and hence finally settling the controversy over whether or not they exist as separate entities.

The older papers, in general, have been cited more often than the more recent ones, with Engelina et al. (2014a; 2014b) achieving a combined total of 46 citations, and Antonios et al. (2008) having 24 (Google Scholar, 16/2/22). It is gratifying to see that the majority of these citations appear in papers concerning injury, rehabilitation, and clinical or surgical application, indicating that our work is helping to inform and shape patient care in the clinic. The two Engelina et al. papers from 2014 cited above, plus Benjafield et al. (2015) have a combined total of 62 citations; fourteen of these citations are directly related to the investigation and/or treatment of patellofemoral pathology. Benjafield et al. (2015) is cited in a 2022 review of management options for patellofemoral joint degeneration (Kamat et al., 2022) demonstrating that our work is continuing to provide the evidence base for PFP treatment. Furthermore, Khoshkhoo et al. (2016) is cited in a PhD thesis entitled “Scientific underpinnings of the clinical assessment of patellofemoral alignment” (Campbell-Karn, 2018), indicating that our work is being used to further the evidence base for the investigation and treatment of patellofemoral pathology.

9.2.2 Video Highlights

In 2013, the journal ‘Clinical Anatomy’ incorporated a new feature on their web site, “Video Highlights: summaries of recently published CA articles created by the articles' authors”, and in 2015, I was invited by the Editor-in-Chief of ‘Clinical Anatomy’ to submit a video summary of our paper “An investigation into the architecture of the vastus medialis oblique muscle in athletic and sedentary individuals: an in-vivo ultrasound study” (Benjafield et al., 2015). The video is available on the Clinical Anatomy website, and can be viewed on YouTube, where it has received 889 views (Clinical Anatomy, 2015), bringing our work much wider recognition. The video itself was cited as an example of best practice for subsequent videos, by the Editor-in-Chief, Prof Shane Tubbs.

9.2.3 Clinical Impact

The collaboration that I have established with a clinical physiotherapist specialising in disorders of the knee has also meant that insights from the studies described here have fed back directly into clinical practice at The Wimbledon Clinics, and has been presented at international physiotherapy meetings, including the 2022 Biennial Physiotherapy Conference in the UAE, and the 2022 British Orthopaedic Association Annual Congress, helping to inform the knowledge base on which treatment for PFP is based. For example, the study on the use of foam rollers for self-myofascial release (Torrente et al., 2022) has provided much-needed evidential data for both clinicians and patients (Robertson, 2022).

9.2.4 Lack of Impact?

I have been disappointed by the fact that two papers have achieved only one citation each: Arnantha et al. (2017) and Hilal et al. (2018) (although this may be due, at least in part, to the fact that these publications are relatively recent). In Arnantha et al. "Maintenance of exercise-induced changes in the architecture of the VMO: how much is enough? An in-vivo ultrasound study" we investigated the effect of stopping the exercises, and how much further exercise was necessary to maintain the gains achieved after a six-week physiotherapy regime. This, I would have thought, is exactly the sort of information needed to inform an evidence-based treatment regime, and I am disappointed it has not been more widely shared. However, although the results were convincing (R^2= 0.994 for the correlation between the number of exercises per week and fibre angle change), the study itself was limited by the small sample size, with only 4 participants in each of the study groups. Furthermore, it appears that *The Journal of Orthopaedics Spine and Sports Medicine*, in which it was published, is no longer available, which may explain the paper's lack of visibility.

In Hilal et al. (2018) we showed that the 'Empi' electrotherapy system nearly doubled the effect of an exercise programme, which again provides a solid evidence base for recommending a particular treatment regime, in this case using electrical muscle stimulation in conjunction with physiotherapy (see Section 5.5). I was surprised that the suppliers showed no interest in our results. Although I felt that it was a strong study, the journal in which it was published, *The*

Global Journal of Orthopedics Research, clearly has a limited distribution and little impact. It is perhaps telling that the only citations for this study, and for Arnantha et al., both occur in the same paper, Hosseini & Bagheri (2021), which is from Iran, and appears to be only available in Farsi script.

While it is perhaps not immediately visible, the work described here is having an impact on clinical physiotherapy practice, particularly in the training of post-graduate physiotherapists specialising in disorders of the knee. Previously, an ‘umbrella’ approach was used for PFP patient treatment, which included strengthening exercises for the gluteal and quadriceps muscles, and stretches for the vastus lateralis and iliotibial band. Now, it is being increasingly recognised that there is little point in prescribing quadriceps-strengthening exercises for young, athletic patients who already have a well-developed VMO, and US is being increasing utilised in physiotherapy clinics to aid in making such judgements. Treatment being targeted to suit the individual needs of the patients, and data presented in this series of studies aids clinicians in making treatment decisions (see Section 9.4).

9.3 *Quo Vadis*?

“*There is nothing new to be discovered in physics now. All that remains is more and more precise measurement*” (Lord Kelvin, 1900).

As for the direction of future work, there remains much to be discovered. Of course, the Covid pandemic severely impacted anatomical teaching and research from early 2020, but, given time and resources there is much more I would like to do.

9.3.1 The Unanswered Questions

There remain some questions that have arisen from the work discussed here, and some questions that remain unanswered. The following proposed studies would build on and extend this work.

1. The Arnantha et al. (2017) study on maintaining the changes achieved, should be repeated, but with a much larger cohort to give it statistical power. This would add important information to the evidence base on which physiotherapy muscle strengthening programmes are based.

2. There is a gap in the literature about the effects of quadriceps strengthening exercises on the VL. We know that targeted exercises will strengthen the VM, but what about their effect on the VL? If VMO insufficiency is implicated, the VM, but not the VL, needs to be strengthened. A review of electromyographic studies by Smith et al. (2009) concluded that it is not possible to preferentially activate the VMO, so there was no point in clinicians focusing on VMO strengthening. While we have already established that stretching and SMR have a greater impact on the VL than VM in terms of percentage change, there are currently no equivalent data in the literature on the effects of exercise. With hindsight, this is something that should have been added to the research goals from the beginning, but at the time the focus was on the VM. Also, experience showed that measuring the fibre angle of the VL is technically more challenging that the VM, so perhaps it was sensible to leave that aspect until the art had been mastered.

3. The data that have been gathered from these studies on the insertion level and insertion ratio of the VMO remain confusing. A meta-analysis of all the 538 knees scanned in this series of studies is ongoing, in an attempt to unpick the relationship between the VMO angle and insertion level.

4. This line of research has already been expanded to include ultrasound assessment of patellar position, where lateral movement or patellar tilt may be suspected. Following a cadaveric validation study (Kwan et al., 2022), US scans were successfully used to detect lateral patellar shift on adduction of the hip in asymptomatic volunteers (Kwan et al., 2021). This extends the

potential use of US scans in physiotherapy clinics, though further research on symptomatic patients is needed.

5. Blood flow restriction (BFR) training is becoming popular as a method of increasing the benefits of exercise for patients who cannot tolerate heavy loading (Erickson et al., 2019). There is, however, little in the literature on the effects of this type of training on the architecture of the muscles. Research into the effects of BFR training on the VM would help to provide an evidence base for clinical evaluation of this treatment.

9.4 Applications to Symptomatic Patients in Clinic

The groundwork that has been laid here has clearly shown the potential for ultrasound to be applied clinically. From the work that has been described in this thesis, it is clear that US can be used as a screening tool for patients presenting with PFP. Initially, US scans can identify those patients with a low initial VMO fibre angle, who might benefit most from exercises to strengthen the VM. In patients who present with a high initial VMO angle, little change would be expected would be expected, whereas in patients presenting with a low initial fibre angle, a significant increase would be expected. The results from the study of athletic and sedentary individuals (Benjafield et al., 2015), indicate that an appropriate cut-off point for treatment would be 60°, so patients with an initial VMO fibre angle of 60° or below would benefit most from muscle strengthening exercises. Furthermore, if therapists were keen to potentiate the effects of the exercises, addition of neuromuscular electrical stimulation (NMES) has the potential to double the increase in fibre angle, as shown in the 2018 study (Hilal et al., 2018). The progress of the prescribed physiotherapy can be easily monitored in clinic by periodical repeat scans. On completion of the exercise programme, I have shown that it is necessary to carry on with the exercises twice a week in order to maintain the gains achieved (Arnantha et al., 2017). Taken as a whole, I believe that the research described in this thesis has given clinicians a valuable toolkit for assessment and treatment of patients presenting with PFP.

9.5 Future Directions

Mentoring, teaching, and research student supervision: on a personal level, the experience that I have gained while carrying out this series of research studies, and subsequently, while critically appraising and reviewing it while writing this thesis, has given me valuable insights that I am keen to share with students and early-career researchers in the future. With suitable further training, I believe I would be in a good position to help to supervise future PhD students researching into musculoskeletal anatomy.

I have also been involved over the last five years in writing an anatomy textbook, with a former colleague from SGUL. The book, "Anatomy: Clinical, Surgical, and Applied" (Baker & Adds, 2023), has been accepted for publication by the CRC Press, Taylor & Francis Group. The knowledge and skills that I have acquired while carrying out the research programme described in this thesis, and writing and publishing the associated papers, have informed my approach to writing and editing the 550 pages of this book, and, in particular, the musculoskeletal anatomy of the lower limb.

Producing the video for Clinical Anatomy has also given me experience in creating online resources – experience that proved valuable when making resources for blended learning during lockdown. I feel I still have much to offer in the way of teaching and research and am looking forward to new opportunities and further challenges.

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11. Appendices

11.1 Do I Need NHS REC Review?

Go straight to content.

Medical Research Council

NHS Health Research Authority

Do I need NHS REC review?

To print your result with title and IRAS Project ID please enter your details below:

Title of your research:

Ultrasound investigations into quadriceps muscle architecture and response to physiotherapy

IRAS Project ID (if available): N/A

Your answers to the following questions indicate that **you do not need NHS REC review for sites in England.**

This tool only considers whether NHS REC review is required, it does not consider whether other approvals are needed. You should check what other approvals are required for your research.

You have answered **'YES'** to: Is your study research?

You answered **'NO'** to all of these questions:

Question Set 1

- Is your study a clinical trial of an investigational medicinal product?
- Is your study one or more of the following: A non-CE marked medical device, or a device which has been modified or is being used outside of its CE mark intended purpose, and the study is conducted by or with the support of the manufacturer or another commercial company (including university spin-out company) to provide data for CE marking purposes?
- Does your study involve exposure to any ionising radiation?
- Does your study involve the processing of disclosable protected information on the Register of the Human Fertilisation and Embryology Authority by researchers, without consent?

Question Set 2

- Will your study involve potential research participants identified in the context of, or in connection with, their past or present use of services (NHS and adult social care), including participants recruited through these services as healthy controls?
- Will your research involve prospective collection of tissue (i.e. any material consisting of or including human cells)

from any past or present users of these services (NHS and adult social care)?
- Will your research involve prospective collection of information from any past or present users of these services (NHS and adult social care)?
- Will your research involve the use of previously collected tissue and/or information from which individual past or present users of these services (NHS and adult social care), are likely to be identified by the researchers either directly from that tissue or information, or from its combination with other tissue or information likely to come into their possession?
- Will your research involve potential research participants identified because of their status as relatives or carers of past or present users of these services (NHS and adult social care)?

Question Set 3

- Will your research involve the storage of relevant material from the living or the deceased on premises in England, Wales or Northern Ireland without a storage licence from the Human Tissue Authority (HTA)?
- Will your research involve storage or use of relevant material from the living, collected on or after 1st September 2006, and the research is not within the terms of consent for research from the donors?
- Will your research involve the analysis of human DNA in cellular material (relevant material), collected on or after 1st September 2006, and this analysis is not within the terms of consent for research from the donor? And/or: Will your research involve the analysis of human DNA from materials that do not contain cells (for example: serum or processed bodily fluids such as plasma and semen) and this analysis is not within the terms of consent for research from the donor?

Question Set 4

- Will your research involve at any stage procedures (including use of identifiable tissue samples or personal information) involving adults who lack capacity to consent for themselves, including participants retained in study following the loss of capacity?
- Is your research health-related and involving offenders?
- Does your research involve xenotransplantation?
- Is your research a social care project funded by the Department of Health and Social Care (England)?
- Will the research involve processing confidential information of patients or service users outside of the care team without consent? And/ or: Does your research have Section 251 Support or will you be making an application to the Confidentiality Advisory Committee (CAG) for Section 251 Support?

If your research extends beyond **England** find out if you need NHS REC review by selecting the 'OTHER UK COUNTRIES' button below.

OTHER UK COUNTRIES

If, after visiting all relevant UK countries, this decision tool suggests that you do not require NHS REC review follow this link for final

confirmation and further information.

Print This Page

NOTE: If using Internet Explorer please use browser print function.

11.2 Email Exchange with Prof Christine Heron, Chair, SGUL Ethics Committee

On 05/01/11, **Christine Heron** < [Redacted] > wrote:

Dear Philip,

Thank you for sending this to me. I do not think that this raises any ethical issues and it will not need to be reviewed by a full committee however, the medical students will need to receive an information sheet to read and a consent form to sign before they undergo the ultrasound scan. This does not need to be too lengthy but you can get an idea of what is needed from the NRES website - www.myresearchproject.org.uk

Best wishes

Christine

From: Philip Adds [mailto: [Redacted]]

Sent: 04 January 2011 15:40

To: Christine Heron

Subject: Fwd: Re: ethics

Dear Christine,

As you can see from the forwarded emails, I am just wondering if I can run this past you. I have attached an outline proposal, but basically it is a student BSc project to collect data on the architecture of the vastus medialis muscles from healthy student volunteers by using ultrasound. This will be of course be entirely non-invasive, and the work will be carried out under the supervision of the medical physics department as well as myself and Claire Robertson, a Senior Lecturer in Physiotherapy.

If you require any more information do let me know,

Regards,

Phil Adds

Philip J Adds

Senior Lecturer in Anatomy

Basic Medical Sciences (Anatomy)

St George's University of London

[Redacted]

11.3 Publication Metrics

Authors	Publication	Researchgate		Google Scholar
		Reads	Citations	Citations
Antonios T, Adds PJ	Clin Anat 21 (1) 66-74	89	17	24
Skinner E, Adds PJ	JPTS 24(6): 475-479	1080	12	12
Engelina S, Robertson CJ, Moggridge J, Killingback A, Adds PJ	Knee 21(1): 107-111	306	21	25
Engelina S, T. Antonios, CJ Robertson, A Killingback, Adds PJ	Clin Anat 27(7): 1076-1084	620	20	21
Benjafield AJ, Killingback A, Robertson CJ, Adds PJ	Clin Anat 28(2): 262-268	133	16	16
Benjafield A, Howe FA, Killingback A, Adds PJ	JPTS 26(1): 165-166	298	1	1
Khoshkhoo M, Killingback A, Robertson CJ, Adds PJ	Clin Anat 29(6): 752-758	1072	12	16
Elniel A, Robertson CJ, Killingback A, Adds PJ.	Physical Therapy and Rehabilitation http://www.hoajonline.com/journals/pdf/2055-2386-4-3.pdf	1169	2	4
Arnantha H, Robertson CJ, Killingback A, Adds PJ	JOSS Med 1:1 003	713	1	
Hilal Z, Killingback A, Robertson C, Adds PJ	GJ Orthop Res 1(1)	924	1	

Bethel J, Robertson CJ, Killingback A, Adds PJ	JPTS 34: 161–166			
Torrente QM, Killingback A, Robertson CJ, Adds PJ	Int J Sport Physl Ther			
		6404	103	11 9

11.4 Full List of Published Papers Discussed in This Thesis

Antonios T, Adds PJ. (2008) The medial and lateral bellies of gastrocnemius: a cadaveric and ultrasound investigation. Clinical Anatomy 21 (1) 66-74. doi 10.1002/ca.20533

Skinner E, Adds PJ. (2012) Vastus medialis: a reappraisal of VMO and VML. The Journal of Physical Therapy Science 24(6): 475-479 https://doi.org/10.1589/jpts.24.475

Antonios T, Adds PJ. (2008) The medial and lateral bellies of gastrocnemius: a cadaveric and ultrasound investigation. Clinical Anatomy 21 (1) 66-74. doi 10.1002/ca.20533

Engelina S, Robertson CJ, Moggridge J, Killingback A, Adds PJ. (2014a) Using ultrasound to measure the fibre angle of vastus medialis oblique: A cadaveric validation study. The Knee 21(1): 107-111 DOI:10.1016/j.knee.2012.07.001

Engelina S, Antonios T, Robertson CJ, Killingback A, Adds PJ. (2014b) Ultrasound investigation of vastus medialis oblique (VMO) muscle architecture: an in-vivo- study. Clinical Anatomy 27(7): 1076-1084 DOI: 10.1002/ca.22413

Benjafield AJ, Killingback A, Robertson CJ, Adds PJ. (2015) An investigation into the architecture of the vastus medialis oblique muscle in athletic and sedentary individuals: an in-vivo ultrasound study Clinical Anatomy 28(2): 262-268 DOI: 10.1002/ca.22457

Benjafield A, Howe FA, Killingback A, Adds PJ. (2014) Unusual variant of vastus medialis detected by ultrasound and confirmed by high-resolution MRI. Journal of Physical Therapy Science 26(1): 165-166 https://doi.org/10.1589/jpts.26.165

Khoshkhoo M, Killingback A, Robertson CJ, Adds PJ. (2016) The effect of exercise on vastus medialis oblique muscle architecture: an ultrasound investigation. Clinical Anatomy 29(6): 752-758 DOI: 10.1002/ca.22710

Elniel A, Robertson CJ, Killingback A, Adds PJ. (2017) Open-chain vs. closed-chain exercise regimes: an ultrasound investigation into the effects of exercise on the vastus medialis oblique. Physical Therapy and Rehabilitation http://www.hoajonline.com/journals/pdf/2055-2386-4-3.pdf DOI:10.7243/2055-2386-4-3

Arnantha H, Robertson CJ, Killingback A, Adds PJ. (2017) Maintenance of exercise-induced changes in the architecture of the VMO: how much is enough? An in-vivo ultrasound study. Journal of Orthopaedics Spine and Sports Medicine 1:1 003

Hilal Z, Killingback A, Robertson C, Adds PJ. (2018) The effect of exercise and electrical muscle stimulation on the architecture of the vastus medialis oblique - the 'Empi' electrotherapy system. Global Journal of Orthopedics Research 1(1) DOI: 10.33552/GJOR.2018.01.000503

Bethel J, Robertson CJ, Killingback A, Adds PJ. (2022) The effect of stretching exercises on the fibre angle of the vastus lateralis and vastus medialis oblique: an ultrasound study. The Journal of Physical Therapy Science 34: 161-166

Torrente QM, Killingback A, Robertson CJ, Adds PJ. (2022) The effect of self-myofascial release on the pennation angle of the vastus medialis oblique and the vastus lateralis in athletic male individuals: an ultrasound investigation. The International Journal of Sports Physical Therapy Published online June 1, 2022. https://doi.org/10.26603/001c.35591.

11.4.1 Full texts of the following open-access papers are publicly available

Skinner E, Adds PJ. 2012. Vastus medialis: a reappraisal of VMO and VML. The Journal of Physical Therapy Science 24(6): 475-479 https://doi.org/10.1589/jpts.24.475

Benjafield A, Howe FA, Killingback A, Adds PJ. 2014. Unusual variant of vastus medialis detected by ultrasound and confirmed by high-resolution MRI. Journal of Physical Therapy Science 26(1): 165-166 https://doi.org/10.1589/jpts.26.165

Elniel A, Robertson CJ, Killingback A, Adds PJ. 2017. Open-chain vs. closed-chain exercise regimes: an ultrasound investigation into the effects of exercise on the vastus medialis oblique. Physical Therapy and Rehabilitation http://www.hoajonline.com/journals/pdf/2055-2386-4-3.pdf DOI:10.7243/2055-2386-4-3

Arnantha H, Robertson CJ, Killingback A, Adds PJ. 2017. Maintenance of exercise-induced changes in the architecture of the VMO: how much is enough? An in-vivo ultrasound study. Journal of Orthopaedics Spine and Sports Medicine 1:1 003

Hilal Z, Killingback A, Robertson C, Adds PJ. 2018. The effect of exercise and electrical muscle stimulation on the architecture of the vastus medialis oblique - the 'Empi' electrotherapy system Global Journal of Orthopedics Research 1(1) DOI: 10.33552/GJOR.2018.01.000503

Bethel J, Robertson CJ, Killingback A, Adds PJ. 2022. The effect of stretching exercises on the fibre angle of the vastus lateralis and vastus medialis oblique: an ultrasound study. The Journal of Physical Therapy Science 34: 161-166

Torrente QM, Killingback A, Robertson CJ, Adds PJ. 2022. The effect of self-myofascial release on the pennation angle of the vastus medialis oblique and the vastus lateralis in athletic male individuals: an ultrasound investigation. The International Journal of Sports Physical Therapy Published online June 1 2022. https://doi.org/10.26603/001c.35591.