MORPHOMETRIC PROFILES OF SOME GRAFTS COMMONLY USED FOR ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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DECLARATION

I, Sabiha Latiff, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science (in Medicine) in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Sabiha Latiff 20th June, 2022

MANUSCRIPTS ARISING FROM THIS DISSERTATION

1) **Latiff S**, Olateju OI. (2022) Quantification and comparison of tenocyte distribution and collagen content in the commonly used autografts for anterior cruciate ligament reconstruction. *Anatomy and Cell Biology. doi: 10.5115/acb.22.005. Epub ahead of print. PMID: 35668478.*

2) **Latiff S**, Olateju OI. Morphometry of the harvestable surface area of quadriceps tendon using a simple tracing method: A common ACL autograft. *European Journal of Anatomy; Manuscript under review.*

DEDICATION

To the Almighty

(For his greatness and for giving me guidance, strength, and blessings),

and

to my families,

‘The AMODs, LATIFFs and GANIEs’

ABSTRACT

The Anterior cruciate ligament (ACL) is a vital knee joint ligament that is commonly damaged in sporting activities. Choice of graft type (allograft or autograft) by surgeons is often based on their training and the observed clinical outcomes and success rates in their practices. The quadriceps (QT), patellar (PT) and semitendinosus (ST) tendons are the commonly used grafts for ACL reconstruction. This study presents the morphometric profiles (e.g. the harvestable surface area, tendon length and widths etc.) of the QT, PT and ST. In addition, the tenocyte distribution and collagen deposition of the tendons were also quantified and compared. A total of 79 adult cadavers of South Africans of European Ancestry were used. Dissections of the anterior and medial aspects of the mid-thigh, knee and mid-leg were conducted to expose these tendons. The tendon morphometries were done *in situ* by tracing the margins and curvatures of the QT or PT on a wax paper. The tracings were scanned and then the morphometries were performed using an ImageJ software. Measurements on the ST were carried out using threads and digital calliper. The length of limb was also measured, and this was used to normalize all the measures. To compare the tendon ultrastructure, tissue samples of the QT, PT and ST were collected from male and female cadavers and then processed for histology. Tenocyte distribution from a H & E– and collagen deposition from a Masson Trichrome– stained sections were analyzed using an ImageJ software. The results showed that all measures produced a high degree of reproducibility using a Lin's concordance test. The limb length for both limbs was similar in the two sexes and the male limbs were significantly longer than the female. For the QT, there was no side or sex difference in all the measures except for the SDW_{QT} that was sexually dimorphic. Some paired parameters showed a strong correlation (e.g. SDW_{QT} vs CDW_{QT} and SA_{QT} vs LOT_{QT}). For the PT, there was also no significant difference in all the measures for both sides or sexes. Also, some paired parameters showed a strong correlation (SA_{PT} vs LOT_{PT}; SPW_{PT} vs CPW_{PT}; SDW_{PT} vs CDW_{PT}) for both sexes and sides. For the ST, there was similarly no significant difference for all the measures for both sides and sexes. Only a paired parameter showed a strong correlation ($C\text{-}MTJ_{ST}$ vs C_{ST}). Finally, tenocyte distributions in the QT and in the ST were significantly higher than in the PT for the female. The male showed no significant difference in the tenocyte distribution across the tendons. Collagen distribution across the tendons was also similar in each sex. Based on the findings of this study, the ST seems to be the preferred graft for an ACL reconstruction. The presented morphometric data on the commonly used grafts for ACL will be useful for preoperative planning of ACL reconstruction surgery and may shed more light into the usability of each graft with respect to healing at the donor site and knee recovery in individuals.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

Introduction

The knee joint is the largest and most complex synovial joint in the human body. It is a weight–bearing joint that is made up of articulations between the femur and tibia. There is also an articulation between the femur and patella where the patella (a sesamoid bone) is acting as a fulcrum for the quadriceps muscle and its tendon over the knee joint. This allows the pull of the quadriceps femoris to be directed anteriorly over the knee to the tibia via the patellar ligament without causing a tendon wear and tear (Drake *et al*., 2015; Van Zyl *et al*., 2016).

Despite the complexity of the knee joint, it is a stable joint. The structural design of the joint, the ligaments and tendons surrounding the joint contribute to its stability. There are two fibro-cartilaginous menisci (c-shaped intra-articular cartilage attaching to the tibial condyles) that help to improve knee joint congruency by aligning the femoral and tibial condyles during joint movements. They also act as shock absorbers and play a vital role in the synovial fluid distribution. Ligaments also contribute to the knee function and stability. These ligaments are often categorized as extracapsular (e.g. patellar ligament, medial collateral ligament, lateral collateral ligament, oblique popliteal ligament and arcuate popliteal ligament) and intra-articular (e.g. anterior and posterior cruciate ligaments, posterior meniscofemoral ligament) ligaments (Drake *et al*., 2015).

The anterior cruciate ligament (ACL) and the posterior cruciate ligament (PCL) are important and strong intra-articular ligaments of the knee which interconnect the adjacent articulating ends of the femur and the tibia. The PCL runs across the ACL from the posterior intercondylar area to the lateral surface of the medial femoral condyle (Drake *et al*., 2015). The

PCL contributes to the stability of the knee by preventing posterior displacement of the tibia on the femur (Drake *et al*., 2015). ACL on the other hand runs from the posteromedial wall of the lateral femoral condyle to the centre of the intercondylar area of the tibia plateau (Petersen and Tillmann, 2002; Markatos *et al*., 2013; Drake *et al*., 2015). Like the PCL, the ACL prevents anterior displacement of the tibia on the femur (Zhu *et al*., 2012; Markatos *et al*., 2013; Cerulli *et al*., 2013).

The ACL fibres are arranged in unique patterns to become taut during flexion or extension thus preventing anterior displacements of the tibia, knee hyperextension (Zhu *et al*., 2012; Markatos *et al*., 2013; Cerulli *et al*., 2013) and resisting secondary valgus and varus forces (Quatman and Hewette, 2009). The synovial membrane of the knee joint attaches to the margins of the articular surfaces and to the superior and inferior outer margins of the menisci thus places the cruciate ligaments outside the articular cavity (Drake *et al*., 2015). Since the ACL lies outside the articular cavity but enclosed within the fibrous membrane, the ACL is prone to injuries during rotational movement of the knee (Van Zyl *et al*., 2016). In a situation where the tibia moves excessively anterior that exceeds the load of the ACL, the ligament tears (Shultz *et al*., 2015). ACL injury may be due to a non-contact deceleration or sudden drastic change in direction while a resultant injury may be due to physical impact in some contact sports e.g. rugby and football (Corry and Webb, 2000; Hurley *et al*., 2018).

ACL injury is commoner in the females than in males. This is due to anatomical, hormonal, biomechanical and neuromuscular control factors (Casado *et al*., 2019). Structurally, females have ‘hypermobile’ joints that are highly flexible (Harmon and Ireland, 2000). The cross-sectional area of ACL, intercondylar ridge and ACL volume are smaller in the female than in the male (Fayad *et al*., 2008; Casado *et al*., 2019). In addition, during menstrual cycle, ovulatory phase or pregnancy the resulting hormone imbalance or surges cause laxity of the ACL ligaments and knee stiffness (Harmon and Ireland, 2000; Park *et al*., 2009; Csintalan et al., 2008; Shultz, 2015). However, contact-related ACL injuries are commoner in the male than in the female (Peck *et al*., 2013; Lin *et al*., 2018). Interestingly, more severe contact-related injuries of the knee and ankle ligaments are more frequent in the female which also indicate the influence of underlying factors such as anatomical and hormonal differences in the two sexes (Larruskain *et al*., 2018; Schilaty *et al*., 2018; Casado *et al*., 2019; Boguszewski *et al*., 2015; Park *et al*., 2009; Dugan, 2005). Biomechanical factor arising from imbalance of neuromuscular control is another important factor that explains the prevalence of ACL injury in the female than in the male. As explained by Casado *et al*. (2019), in a scenario where a female athlete stops in her tracks after running a distance, there may be "a delay in the activation of the hamstrings compared to the quadriceps". This delay may result in a greater anterior displacement of the tibia and a pronounced valgus in the knees which introduces increased stress and damage to the ACL (Casado *et al*., 2019).

An injured or torn ACL often produces excruciating pain and a complete loss of knee function which necessitates surgical repair (Sayampanathan *et al*., 2017). ACL tears also negatively affect posture and balance (Sayampanathan *et al*., 2017). The severity of an ACL injury would mean "the beginning of the end for the knee" according to Torg *et al*. (1976). However, in present times with improved diagnostic imaging equipment and advanced surgical techniques, the goal of achieving and maintaining a long-term knee health and stability after an injury is realisable. The surgical procedure is not an easy task as patients still require post-surgery rehabilitation. Successful surgical intervention and well-programmed rehabilitation regimes have allowed most sports men and women to return to their intense sports (Sayampanathan *et al*., 2017). Unfortunately, about 30% of athletes do not return to their sporting careers between 2 – 7 years post-surgery (Ardern *et al*., 2012; Shultz, 2015). About 70% of patients develop osteoarthritis within 10 – 15 years after ACL injuries because the long-term consequence of ACL injury is osteoarthritis in the tibiofemoral joint (Oiestad *et al*., 2010;

Shultz, 2015). This is also supported by Sayampanathan *et al*. (2017) that the risk of early onset of premature osteoarthritis remains high for persons who undergo ACL surgical repair.

The ACL is indeed a complex structure that can provide varying tensile resistances in response to the stresses experienced on multiaxial planes (Strocchi *et al*., 1992). Microstructurally, it is composed of thick wavy bundles of collagen fibres in a loose connective tissue (Strocchi *et al*., 1992) which are interspersed along its length by elongated or flat tenocytes. The tenocytes in the ACL are either spindle– or round–shaped which resemble the fibrochondrocytes of bone lacunae. This means that the ACL microstructure is histologically different from the tendon microstructure (Zhu *et al*., 2012) but tenocytes are the predominant cells in both ACL and tendons. The tenocytes react to mechanical stimuli applied to the ACL and tendon and are also responsible for collagen Type 1 production (Hadjicostas *et al*., 2007b; Zhu *et al*., 2012) while the collagen of the extracellular matrix is responsible for the tensile strength of the ACL and tendons. Through a process of mechanical transmission, the tensile loads in the ACL act as a signal for collagen production by the tenocytes (Zhu *et al*., 2012). Macrostructurally, the ACL is also different from other tendons and ligaments (Strocchi *et al*., 1992) and it is more complex than any of the hamstring tendons (gracilis or semitendinosus) which are the commonly used grafts in ACL reconstruction surgeries (Zhu *et al*., 2012).

In the event of an ACL injury, it is necessary to eliminate instability and prevent any further damage to the menisci by performing a reconstruction surgery using a graft (Hurley *et al*., 2018). However, the decision to use a graft depends on factors such as the dimension of harvestable graft, location and post-surgery recovery etc. (Hamada *et al*., 1998; Shelton and Fagan., 2011; Reboonlap *et al*., 2012; Mehran *et al*., 2013; Janssen *et al*., 2013; Sun *et al*., 2020). This means that the graft to be harvested must closely match the size and strength of an ACL (Shelton and Fagan., 2011). Thus, surgeons are often faced with the choice of using a synthetic graft, an allograft, or an autograft for ACL reconstruction (Romanini *et al*., 2010; Mall *et al*., 2012; Mehran *et al*., 2013; Sun *et al*., 2020).

A synthetic graft is a graft with artificial materials. It was introduced in 1980 and it became popular due to its lack of donor morbidity, its abundant supply and significant strength, its instantaneous loading capability, and its reduced post-operative rehabilitation (Legnani *et al*., 2010). However, its popularity as a graft decreased significantly due to the nature of the synthetic materials (e.g. carbon fibers and polyester) in the graft which posed serious setbacks such as knee joint instability, chronic effusions, immunological responses and cross-infections etc. (Legnani *et al*., 2010; Romanini *et al*., 2010; Dhammi *et al*., 2015; Hurley *et al*., 2018). Synthetic graft is less commonly used because of its high post-surgery complications and a high failure rate (Hospodar and Miller, 2009).

On the other hand, an allograft is taking a graft tissue from a donor (e.g. cadaver) for use in a recipient and it is considered an effective and reliable method for ACL reconstruction (Macaulay *et al*., 2012). The two types of allografts used for ACL reconstruction are bone–tendon–bone and soft tissue allografts (Hulet *et al*., 2019). The soft tissue allografts are harvested from the tendons of tibialis anterior, tibialis posterior, semitendinosus, gracilis, fibularis tendons and iliotibial band of a donor (Hulet *et al*., 2019). The bone–tendon–bone allograft has an advantage in that there is a bone–to–bone healing on both ends of the femur and tibia in the joint space. Whereas quadriceps or achilles tendon would only have a single bone block thus providing a tendon–to–bone healing (Hulet *et al*., 2019). Some of the other advantages of using allografts are that it requires small incisions, reduced time of procedure, no harvest site morbidity, reduced kneeling pain, and a low risk of patellar fracture (Hospodar and Miller, 2009; Mall *et al*., 2012; Cerulli *et al*., 2013). It is however less desirable due to a very high risk of tissue rejection by the host as well as a delayed and a less favourable healing arising from a compromised remodelling and integration processes of healing. It also has a higher risk of failure in young athletes which significantly increases the cost of surgery i.e. surgical correction of a procedure (Mall *et al*., 2012; Cerulli *et al*., 2013).

Unlike an allograft, an autograft is a graft tissue harvested from the same individual requiring a graft (Mehran *et al*., 2013). The commonest tendons used as autograft are the hamstring and patellar tendons. Prior to the surgery, the injured knee is examined under anaesthesia to understand the extent of the ACL damage (Frank *et al*., 2017). If a semitendinosus tendon is desired as an autograft, it is harvested by making a 2 – 3 cm long incision along the pes anserinus (which is easily palpable) just midway between the tibial tuberosity and the posteromedial border of the tibia (Frank *et al*., 2017). The fasciae and fat around the semitendinosus are also cleared until the desired tendon is visible. The tendon is carefully stripped and then the graft is prepared for ACL reconstruction using a surgical technique preferred by the orthopaedic surgeon (Frank *et al*., 2017). It is a common practice that one or two hamstring tendons (semitendinosus and/or gracilis) may be harvested. Where a patellar tendon is desired, an incision on the anterior surface of the knee is made to enable easy stripping of this tendon. Including a bone block on either end is advantageous for the healing process (Mall *et al*., 2012). In the case of the quadriceps tendon, an incision is made on the anterior knee superior to the patella to expose the tendon. The graft is then removed and prepared for ACL reconstruction (Emerson *et al*., 2019). Despite the popularity of ACL reconstruction surgeries, the choice of graft remains a preference of surgeons (Macaulay *et al*., 2012) as there is no structured ‘scale’ that speaks to the suitability of a graft source in an individual in order to guide the choice of graft (Romanini *et al*., 2010; Mehran *et al*., 2013; Sun *et al*., 2020). As it stands, many surgeons rely on their surgical training and the success rates in their practices when choosing a graft (Mall *et al*., 2012).

From an anatomical point of view, the decision to use a graft should also be based on the graft morphometries in addition to using the patient’s age, body size, activity level, concomitant injuries, donor-site morbidity, and return to athleticism (Mall *et al*., 2012; Macaulay *et al*., 2012; Hurley *et al*., 2018). More so, there are morphological differences and variations between individuals of different sexes, race and ethnicity (Xerogeanes *et al*., 2013; Van Zyl *et al*., 2016; Gupta *et al*., 2017; Vadgaonkar *et al*., 2018). This therefore means that graft preference based on a surgeon's experience may not be entirely adequate for deciding a graft to harvest and use in surgery in an individual because each graft has its own advantages and disadvantages (Dhammi *et al*., 2015; Romanini *et al*., 2010; Cerulli *et al*., 2013). The present study further explored the morphologies of the commonly used grafts with the intention of presenting data that could be useful to surgeons on deciding which graft to choose. The most important factor in ACL reconstruction is to restore the native anatomical footprint which entails the graft being biomechanically similar to the ACL (Iriuchishima *et al*., 2013). In addition, the grafts must be easily harvestable, can be easily secured surgically, have a rapid healing process and it should be customizable to the patient's native ACL morphometry (Dhammi *et al*., 2015; Hulet *et al*., 2019).

The commonly used grafts are quadriceps tendon (QT), the patellar tendon (PT) and semitendinosus tendon (ST) (Frank *et al*., 2017). The QT is one of the strongest tendons in the human body. It is situated on the anterior compartment of the thigh where it serves as a conjoint tendon for the quadriceps femoris muscles (rectus femoris, vastus intermedius, vastus medialis and vastus lateralis) at the patella. The muscles with its tendon flex the hip, extend the knee joint and keep the knee from buckling when standing (Drake *et al*., 2015: Slone *et al*., 2015). Thus the actions of the quadriceps femoris muscle enable walking. The conjoint tendon of the QT attaches the quadricep femoris muscles to the supero-anterior aspect of the patella (Ilan *et al*., 2003), passing anterior to it (by blending with the periosteum of patella) and finally inserting into the tibia tuberosity as the PT (Ilan *et al*., 2003; Slone *et al*., 2015; Drake *et al*., 2015). Through this connection, the PT and the patella improve the way the quadriceps femoris muscles pull on the tibia (Drake *et al*., 2015). The patella in this case is acting as a fulcrum (Olateju *et al*., 2013; Drake *et al*., 2015).

The ST is a cylindrical–shaped tendon of the semitendinosus muscle that is located on the postero-medial aspect of the thigh and knee. Semitendinosus originates from the supero-medial aspect of the ischial tuberosity via a common tendon of the hamstring muscles (Drake *et al*., 2015; Vadgaonkar *et al*., 2018) and then inserts via the ST on the anteromedial surface of proximal end of tibia. The tendons of gracilis and semimembranosus also insert on the anteromedial surface of proximal end of tibia via a conjoint tendon called *pes anserinus* (Drake *et al*., 2015). Similar to the hamstring muscles, the semitendinosus with its tendon flexes and medially rotates the knee (Drake *et al*., 2015).

The use of hamstring tendons in ACL reconstruction was first reported by Lipscomb *et al*. (1982) and it immediately gained popularity in Australia and Europe. The hamstring tendon as a graft has many advantages such as ease of harvest, suitable morphology, suitable plasticity that enables graft flexibility, reduced donor site morbidity, improved rehabilitation process and possibility of harvest tissue re–growth (Chiang *et al*., 2012; Stevanovic *et al*., 2013; Runer *et al*., 2018). However, the disadvantages of hamstring tendon graft are its slow graft incorporation (i.e. bone to ligament healing) and weakening of ACL antagonists e.g. hamstrings (Alkjær *et al*., 2012) and gastrocnemius (Fleming *et al*., 2001) which is accompanied by a reduced knee flexion strength (Runer *et al*., 2018). In addition, the semitendinosus supports the ACL by resisting external rotation and anterior displacement of tibia which are the presentations of an injured ACL (Messer, 2017). Thus, in harvested hamstring tendon, there may be an increased incidence of ACL rupture (Messer, 2017) and weakening of the medial stabilization of the knee in the limb from which the graft was taken (Barie *et al*., 2020).

Despite the shortfalls of ST graft, an interesting fact is that the whole of the ST can regenerate after being harvested (Eriksson et al., 2001; Stevanovic et al., 2013; Suydam et al., 2017; Dziedzic et al., 2020). Stevanovic et al. (2013) reported that a post-harvest haematoma may act as a scaffold for the mesenchymal stem cells that could invade the harvested area and then initiates healing in the harvest site by increasing tenocyte proliferation and collagen production (Stevanovic et al., 2013). Harvesting the ST in a majority of cases resulted in the regeneration of the ST tendon to its full length and which is structurally similar to the non-harvested tendon (Eriksson et al., 2001). However, ST regeneration is not seen in all patients (Nakamae *et al*., 2012). This could be attributed to added strain on the hamstring muscle as patients that experienced unsuccessful remodelling reported experiencing a sudden sharp/stabbing pain in the posterior aspect of the thighs (Nakamae *et al*., 2012). The pain was suggested to be caused by a strain on the muscle due to the added loads (Nakamae *et al*., 2012). In this instance, the muscle belly of semitendinosus is retracted proximally (Nakamae *et al*., 2012). It is thus recommended that hamstring strengthening exercises in the first month post-surgery should be completely avoided in order to increase the chances of tissue regeneration and remodelling (Nakamae *et al*., 2012).

The PT autograft is considered the gold standard for ACL reconstruction (Hadjicostas *et al*., 2007; Shani *et al*., 2016; Heffron *et al*., 2019). This is due to its high tensile strength and possibility of re-vascularization by the inferior lateral or inferior medial genicular artery as well as being able to provide a good bone integration where bone parts are harvested with the tendon (Cerulli *et al*., 2013; Hijazi *et al*., 2015). Despite the popularity of a bone–patellar tendon–bone graft in the late 70's and early 80's (Heffron *et al*., 2019) a survey conducted in Japan by Lee *et al*. (2015) to determine and understand surgeons' choice of graft and surgical technique for ACL reconstruction showed that the hamstring autograft was the more popular choice compared to the bone–patellar tendon–bone graft (Lee *et al*., 2015). In the same study, Lee *et* *al*. (2015) reported that surgeons preferred the hamstring single-bundle ACL reconstruction with meniscus preservation despite the advantages of a bone–patellar tendon–bone graft (Lee *et al*., 2015). This further shows that the choice of a graft often depends on the surgeon's preference.

In the 80's, the QT was used the least due to its poor biomechanical strength and the unsatisfactory clinical outcomes (Marshall *et al*. 1979; Noyes *et al*., 1984; Anderson *et al*., 2001; Cerulli *et al*., 2013) and it was the least studied compared to the PT, ST or gracilis tendon (Xerogeanes *et al*., 2013; Slone *et al*., 2015). Later, the QT gained popularity for use as a graft source because of its favourable biomechanics, low donor-site morbidity, large harvestable area, predictability of healing outcome and ease of harvest. Based on these, the use of the QT for ACL reconstruction has increased (Slone *et al*., 2015; Heffron *et al*., 2019). To increase the effectiveness of QT graft, it is important to harvest the portion of the tendon that would yield the best post-surgery outcomes and that would mimic the strength of the once healthy ACL (Harris *et al*., 1997). Following the studies conducted by Harris *et al*. (1997) and Staubli *et al*. (1999), it was recommended that the central quadriceps tendon–bone construct is adequate providing low donor site morbidity, adequate size and high tensile strength (Fulkerson and Langeland 1995; Harris *et al*., 1997; DeAngelis and Fulkerson, 2007).

Lund *et al*. (2014) reported on the outcome of QT over the PT autografts by documenting knee stability, kneeling pain and harvest site sensitivity loss in patients. The study found that kneeling pain and loss of sensitivity were reduced in the QT bone graft compared to the bone–patellar tendon –bone (Lund *et al*., 2014). Lee *et al*. (2016) also showed that the knee stability post-surgery was similar in both the QT and hamstring tendon autografts, but a more satisfactory recovery of strength of muscle flexion was observed in the QT. Thus the QT is biomechanically effective for ACL reconstruction that is considered safe, reproducible and versatile (Staubli *et al*., 1996; Garofalo *et al*., 2006; Slone *et al*., 2015; Shani *et al*., 2016). In addition, the QT is considerably thick and wide in diameter and provides abundant harvestable tissue (Fulkerson and Langeland, 1995). However, there is no report on the extent of the harvestable tissue area and its variability in individuals which may further empower its usability or adequacy in different individuals.

Despite many studies that have reported the use of PT and ST as autografts for ACL reconstruction, and to a lesser extent the use of the QT, there is still no consensus on the most suitable graft even though ST and PT are the most popular (Fulkerson and Langeland, 1995; DeAngelis and Fulkerson, 2007; Hadjicostas *et al*., 2007; Geib *et al*., 2009; Slone *et al*., 2015; Shani *et al*., 2016; Lee *et al*., 2016; Frank *et al*., 2017). It is quite challenging to reach a consensus on the best graft because factors such as length, harvestable tendon area, thickness, tensile strength, ease of graft harvesting and bone–to–bone healing after reconstruction are important factors to consider before deciding on a graft choice but unfortunately all of these factors are not obtainable in a single tendon (Hijazi *et al*., 2015). Other factors of importance are rapid healing, low donor site morbidity, immediate rigid fixation (i.e. should ligamentize once implanted), and similar structural properties that mimic the mechanical properties of ACL (Goldblatt *et al*., 2005; Hijazi *et al*., 2015). In all, it is imperative that the knee function of the graft site is not in any way compromised (Hijazi *et al*., 2015).

Due to the lack of consensus on the most adequate autograft, the present study investigated the morphometries of the commonly used autografts (e.g. QT, PT and ST) for ACL reconstruction. One factor this study focussed on was providing data on the harvestable area and the morphometry of graft using a simple morphometric approach on a cadaveric collection of South Africans of European ancestry. Another factor that was investigated was comparing the microstructure of the commonly used grafts. Understanding these will contribute to knowledge and will inform surgeons on the choice of autografts because tendons are measured preoperatively to determine if they are adequate for ACL reconstruction (Hamada *et al*., 1998). Therefore, this study also explored correlations between different paired parameters in order to determine whether a measured parameter can be used to predict the measure of another parameter. It is envisaged that the outcomes of this study will shed more light into the usability and suitability of each autograft and which will inform the choice of graft when performing an ACL reconstruction.

1.1. Aim:

This study aims to provide data on the morphometric profiles of the QT, PT and ST in both limbs of the male and female cadavers of South Africans of European Ancestry as well as to compare their microstructural profiles.

1.2. Specific objectives:

(A) To present the morphometric profile (i.e. the surface area, length and width) of the harvestable area of the QT using a simple tracing approach on cadavers of South Africans of European Ancestry.

(B) To present the morphometric profile (i.e. the surface area, length and width) of the harvestable area of the PT using a simple tracing approach on cadavers of South Africans of European Ancestry.

(C) To present the morphometric profile (i.e. the diameter, length and the derived cross-sectional area) of the ST of cadavers of South Africans of European Ancestry.

(D) To quantify and compare the tenocyte distribution and collagen deposition in each graft (i.e. QT, PT and ST) of cadavers of South Africans of European Ancestry using a light microscopy and imageJ software.

CHAPTER TWO

Materials and methods

2.1. Tendon morphometry

2.1.1. Demography of samples

Lower limbs of 79 (F = 40; M = 39) adult formalin–fixed cadavers of South African of European ancestry were used in the study. The cadavers were housed in the School of Anatomical Sciences at the University of the Witwatersrand, Johannesburg, South Africa for teaching purposes. Ethics Waiver was granted (Ethics Number: W-CJ-140604-1) by the Human Research Ethics Committee (Medical) of the same University (Appendix I). The mean age of the male cadavers was 76.2 years (range: 53 – 96 years) while that of the female cadavers was 75.3 years (range: 46 – 96 years). The QT, PT and ST were assessed in each cadaver. A cadaver limb with any obvious physical scar or deformity was excluded from the study. Thus, a total of 79 female and 77 male limbs were assessed (Table 1).

Table 1: Demography of cadavers used for QT, PT and ST morphometries

		Age		Side	
Sex	Number of cadavers assessed	Mean (mm)	SD	Number of left limbs assessed	Number of right limbs assessed
Female	40	75.3	13.6	40	39
Male	39	76.2	10.2	39	38

2.1.2. Dissections

To expose the QT and PT, the cadaver was placed in a supine position with the lower limbs in full extension. A longitudinal incision on the anterior surface of the lower limb was then made extending from midway of the thigh to midway of the leg and passing through the centre of the patella. Then, transverse incisions of about 10 cm each were made at the proximal and distal ends as well as at the level of the patella (perpendicular to the longitudinal incision) in order to expose the region of interest and to allow for adequate space for observation and morphometry. Subsequently, the skin was carefully reflected to reveal the underlying structures. The subcutaneous fascia, fat, fascia lata, and crural fascia were also carefully removed without altering the morphologies or positions of the structures of interest. These structures were carefully examined for any variations or abnormalities.

To expose the ST, the cadaver was placed in a prone position. The transverse incision lines on the anterior surface were extended to the posterior surface of the lower limb to expose the medial aspect of the lower limb, the popliteal fossa and proximal leg. Like the QT and PT, the skin, subcutaneous fascia, fat, fascia lata, and crural fascia were removed to expose the semitendinosus muscle and its tendon in the posterior compartments of the lower limb. For measurement accuracy, tissue (i.e. fascia or fat) surrounding the ST from its transition zone as a tendon to its insertion into the supero-medial aspect of the tibia (i.e. at the *pes anserinus* formation) was carefully removed. Examination of the regions of interest was also conducted to identify any variations or abnormalities.

2.1.3 Morphometry of quadriceps, patellar and semitendinosus tendons

For the morphometries of the QT or PT, both knees of a cadaver were flexed at an angle of about 45°. The QT or PT were carefully wiped with a dry absorbent cloth. Thereafter, a non-elastic transparent wax paper (dimension: 5 cm x 5 cm x 19 µm; Superior wax paper (cat no:

6003929000018) donated by Superhaze Trading Company, Tongaat, South Africa) was carefully placed in such a manner that it assumed the curvatures of the underlying QT (Figure 1) or PT (Figure 2) on both lower limbs. The wax paper on the curvatures of the tendons were firmly secured with pins. Thereafter, a permanent marker was then used to trace out the margins of the tendons onto the wax paper. To further enhance visibility and accuracy of the tracings, the margins of the tendons were pre-marked with a permanent marker before placing the wax paper onto the individual tendon.

After the tracing, the wax paper was removed and then a known scale bar was drawn on the wax paper. Then the wax paper with its inscribed scale bar was then scanned at 300 dpi using an Epson workforce DS-50000 scanner. From the digitized image, the surface area, straight distal width, curved distal width and length of tendon for the QT were measured using an ImageJ 1.47v software (NIH, USA). Similarly, the surface area, straight proximal width, curved proximal width, straight distal width, curved distal width and length of tendon for the PT was also measured using an ImageJ 1.47v software (NIH, USA). The measurement parameters for the QT and PT are described in Table 2.

Unlike the QT or PT, the measurements on the ST were conducted *in situ*. Due to its curvature, a non-elastic thread was used to measure the length of the ST from its musculotendinous junction (MTJ) to its insertion point (Figure 3) similar to Vadgaonkar *et al*. (2018). The thread was placed and pinned to the ST in such a way that it followed its curvature. In addition, the circumferences of ST were measured at the MTJ and at 3 cm away from the MTJ using a thin non-elastic thread and a metre rule. The measured parameters for the ST are also described in Table 2. The decision to measure at 3 cm away from the MTJ was arbitrary. To estimate the cross-sectional area (CSA) of the ST at the MTJ or at 3 cm away from the MTJ, the obtained circumferences at these points (i.e. at MTJ or 3 cm away from the MTJ) were used in the calculations with the assumption that the ST has a perfect cylindrical shape.

Estimated CSA = πr^2

Where r (i.e. ST radius at MTJ or at 3 cm away from MTJ) = C_{ST} (circumference at MTJ or 3 cm away from MTJ) divided by 2.

In order to normalize the data, the length of both lower limbs at full extension from the anterior superior iliac spine to the medial malleolus was measured with a measuring tape in order to give an indication of the 'stature' of each cadaver (Sabharwal and Kumar, 2008; Neelly *et al.*, 2013; Gupta *et al.*, 2017). The raw data was normalized by dividing the obtained values by the length of the corresponding lower limb (LLL).

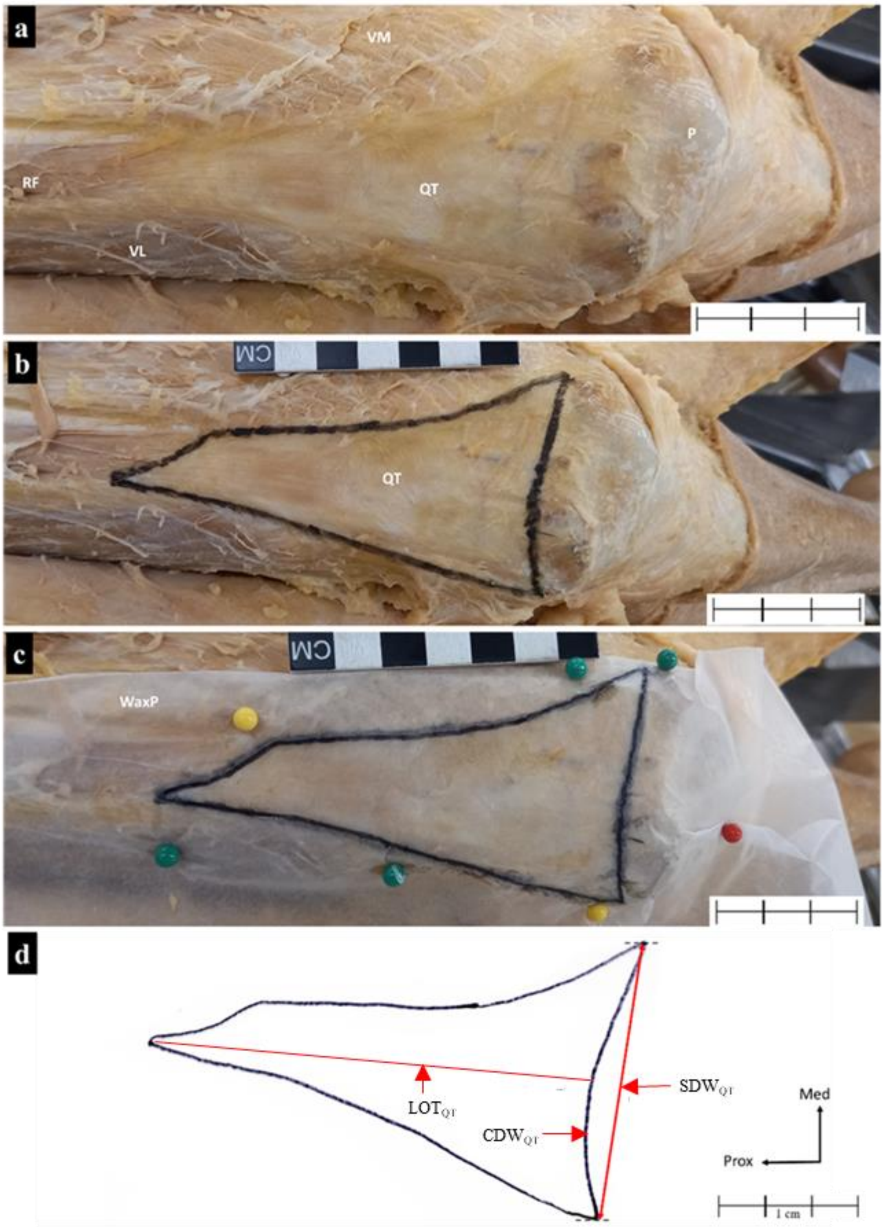


Figure 1. Illustrations of the QT tracings on the anterior compartment of the thigh showing (**a)** the exposed QT after removing the superficial structures, (**b)** the highlighted QT margins (i.e. its extent) with a permanent marker in order to improve visibility and tracing accuracy, **(c)** the QT tracing on a superimposed wax paper secured by coloured pins to assume the curvature of QT and **(d)** the digitized image of the tendon tracing from which parameters were measured. VM – Vastus medialis, VL – Vastus lateralis, RF – Rectus femoris, P – patella, QT – Quadriceps tendon, WaxP – Wax paper, LOT$_{QT}$ – length of QT tendon, CDW$_{QT}$ – curved distal width of QT, SDW$_{QT}$ – straight distal width of QT, Prox – proximal; Med – medial.

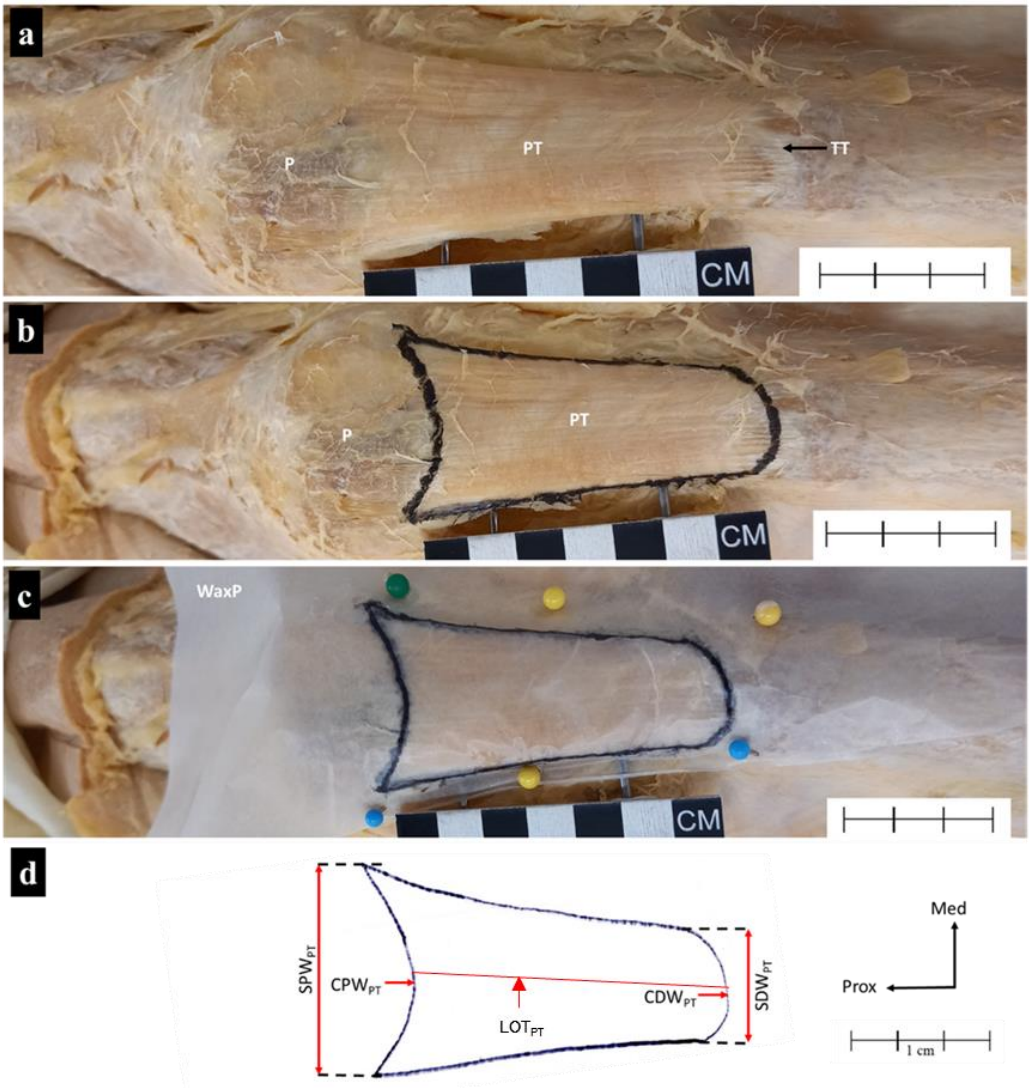


Figure 2. Illustrations of the PT tracings on the anterior compartment of the thigh showing (**a)** the exposed PT after removing the superficial structures, (**b)** the highlighted PT margins (i.e. its extent) with a permanent marker in order to improve visibility and tracing accuracy, **(c)** the PT tracing on a superimposed wax paper secured by coloured pins to assume the curvature of PT and **(d)** the digitized image of the traced tendon from which parameters were measured. PT – Patellar tendon, P – patella, TT – Tibial tuberosity, WaxP – Wax paper, LOT$_{PT}$ – length of PT tendon, SPW$_{PT}$ – straight proximal width, CPW$_{PT}$ – curved proximal width, CDW$_{PT}$ – curved distal width and SDW$_{PT}$ – straight distal width, Prox – proximal; Med – medial.

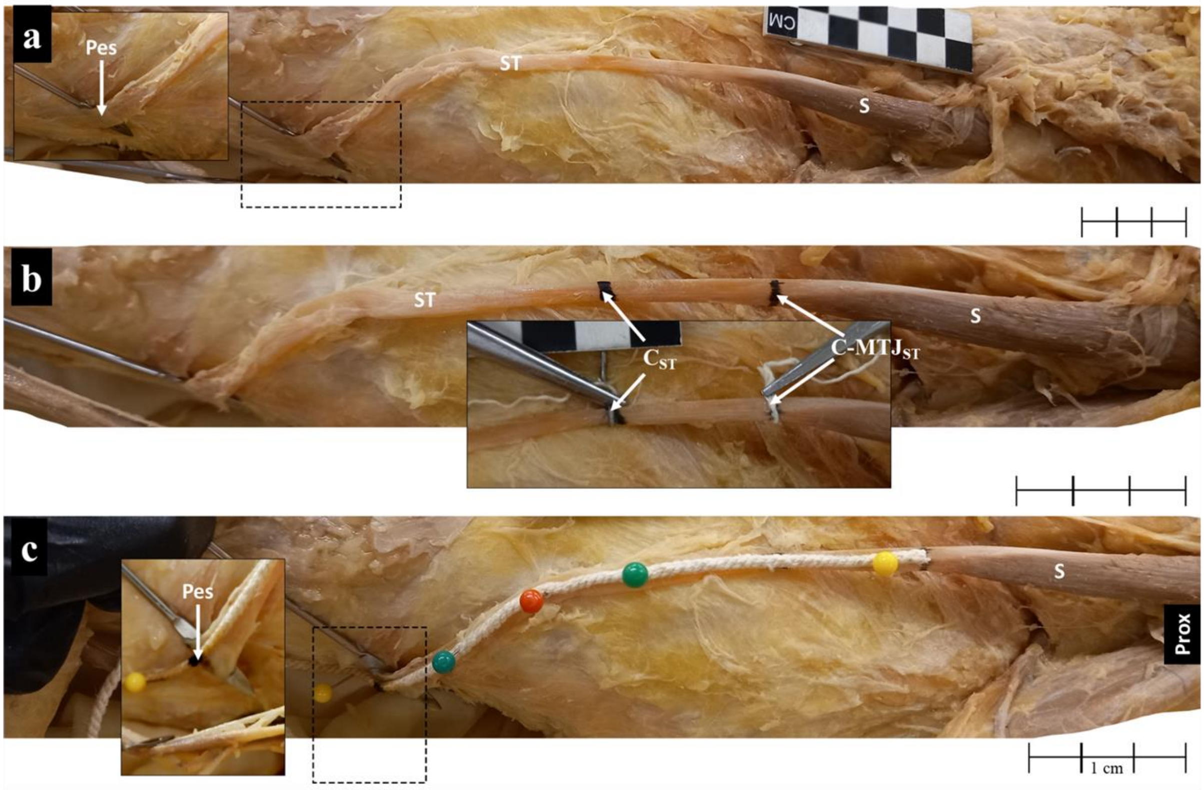


Figure 3. Photographs showing the ST on the postero-medial aspect of the thigh showing **(a)** the exposed ST after removing the superficial structures, **(b)** the MTJ and 3 cm away from the MTJ from where the circumferences were taken, **(c)** the extent of the length of tendon from the MTJ to Pes and how the length was measured using a non-elastic thread secured with coloured pins. S – semitendinosus muscle, ST – semitendinosus tendon, Pes – pes anserinus, C-MTJ$_{ST}$ – circumference at MTJ, C$_{ST}$ – circumference at 3cm away from MTJ, Prox – proximal.

Table 2: Measured parameters for the quadriceps, patellar and semitendinosus tendons.

Parameter	**Acronym**	**Description**
Quadriceps tendon		
Surface area	SA_{QT}	Area of tendon which is defined by the margins of the tendon i.e. infero-medial border – vastus medius; infero-lateral border – vastus lateralis; inferior border – tendon attachment on the base (superior border) of patella using the medial and lateral borders of the patella as landmarks.
Straight distal width	SDW_{QT}	Measurement taken as 'a crow flies' at the inferior border of QT using the medial and lateral borders of the patella as landmarks.
Curved distal width	CDW_{QT}	Measurement taken along the curvature of the inferior border of QT using the medial and lateral borders of the patella as landmarks.
Length of QT tendon	LOT_{QT}	Maximum height from the highest peak at the MTJ of QT to the halfway of the CDW at the inferior border of the tendon.
Patellar tendon		
Surface area	SA_{PT}	Defined by the borders of the tendon i.e. superior border – extent of PT attachment on the apex of patella; inferomedial and inferolateral borders of PT tendon; inferior border – at its insertion i.e. tibial tuberosity.
Straight proximal width	SPW_{PT}	Measurement taken as 'a crow flies' at the superior border.
Curved proximal width	CPW_{PT}	Measurement taken along the curvature of the superior border.
Straight distal width	SDW_{PT}	Measurement taken as 'a crow flies' at the inferior border.

Curved distal width	CDW_{PT}	Measurement taken along the curvature of the inferior border.
Length of PT tendon	LOT_{PT}	Maximum height extending from the halfway of the CPW_{PT} (i.e. proximal) to the halfway of the CDW_{PT} (i.e. distal)
Semitendinosus tendon		
Circumference at musculotendinous junction (MTJ)	$C\text{-}MTJ_{ST}$	Measurement of circumference taken at the MTJ.
Circumference at 3cm away from musculotendinous junction (MTJ)	C_{ST}	Measurement of circumference taken at 3cm away from the MTJ.
Estimated cross sectional area at musculotendinous junction (MTJ)	$CSA\text{-}MTJ_{ST}$	Calculated using the circumference taken at the MTJ. (Formula used: $CSA\text{-}MTJ_{ST} = (C\text{-}MTJ_{ST})^2 / 4\pi$).
Estimated cross sectional area at 3cm away from musculotendinous junction (MTJ)	CSA_{ST}	Calculated using the circumference taken at 3cm away from the MTJ. (Formula used: $CSA_{ST} = (CST)^2 / 4\pi$).
Length of ST tendon	LOT_{ST}	Length from the MTJ to its insertion at the *pes anserinus*

2.1.4 Statistical analysis:

To test for reliability of all measurements, an intra–observer reliability test was conducted for all the measurements. A Lin's Concordance test (which measures the degree of agreement between test and re-tests) was performed to determine the level of precision and accuracy between the measurements (Landis and Koch 1977). Normality test was conducted for each measured parameter using Shapiro-Wilk test. For parametric data, a Student's *t*-test was used to perform side and sex differences for each measured parameter. For non-parametric data, a Mann-Whitney U test was used. To determine a correlation between any two paired parameters, a Pearson's Correlation was used for normally distributed data while a Spearman's correlation was used for data that was not normally distributed. All statistical analyses were performed using SPSS software (version 22.0; IBM, US). Statistical difference of 5% was regarded as significant for all the statistical analyses.

2.2 Tendon microstructure

2.2.1 Demography of samples

Tissue samples of the QT, PT and ST were only collected from specific cadavers (i.e. from the scattered remains according to the Body Donation Policy of the School of Anatomical Sciences, University of the Witwatersrand). This aspect of the study is also covered by the Human Research Ethics Committee (Medical) approval (Ethics Number: W-CJ-140604-1). Tissue samples of the QT, PT and ST of the right leg were collected from female (F = 9; age range = 72 – 93 years) and male (M = 7; age range = 59 – 85 years) cadavers of South Africans of European Ancestry in order to reveal their microstructure using a light microscopy.

With the cadaver in a supine position and the knee flexed at an angle of about 45°, tissue sample of the QT or PT (1 cm x 0.5 cm) was removed from the central half of the tendon. For the ST, tissue sample of about 1 cm long was cut half-way along the tendon with the cadaver in a prone position and the lower limb in full extension. All tissue samples were immediately fixed in a 10% buffered formalin and stored at 4 °C until further histological processing.

2.2.2 Histological processing

Each fixed tissue was placed in a properly labelled plastic cassettes and then processed using an Automatic tissue processor (Thermo scientific - Citadel 2000) at the following schedules: 4 hr in 10% buffered formalin, 1 hr in 70% Alcohol, 2 hr in 95% Alcohol, 2 x 30 min in 95% Alcohol, 3 x 1 hr in 100% Alcohol. It was subsequently immersed in chloroform for 3 hr followed by immersion in paraffin wax for 4 hr. The tissue was then embedded in molten paraffin wax using embedding moulds and allowed to dry on an iced-cold surface. The tissue was sectioned in a sagittal plane at 9 μm thickness using a sliding microtome (Leica). In all, four sections per tissue for each stain were collected and then mounted on to 0.5% gelatine-coated slides. The slides containing the sections were kept at room temperature to dry overnight before staining with either Hematoxylin and Eosin (H & E) or Masson's Trichrome (MT).

2.2.3 Histological staining

For the H & E stain (to reveal the general microstructure and to quantify the distribution of tenocyte), the sections were initially incubated in the oven for 30 min and then de-paraffinized twice in xylene for 20 min each before hydrating the sections by dipping 10 times each in a decreasing series of alcohol concentrations (i.e. 100%, 95% and 70%). Thereafter, the sections were placed immediately in a running water for 1 min. Sections were immersed in Meyer's hematoxylin (for staining the cell nucleus) for 16 min before being placed in a running water for 5 min. The sections were then immediately placed in a Scott's tap water for 5 min and cleared in a running water for 5 min. Thereafter, sections were immersed in Eosin (to stain the cytoplasm) for 2 min and then quickly rinsed in water. The sections were subsequently dehydrated by dipping 10 times each in an increasing series of alcohol concentrations (i.e. 70%, 95% and 100%) and then cleared twice in xylene for 10 min each. Slides were cover-slipped using Entellan.

For the MT stain (to reveal collagen fibers and to quantify its distribution), sections were processed similar to the H & E staining above. Sections were stained with a celestine blue solution for 5 min and then washed under a running tap water for 2 min before staining with Alum hematoxylin for 9 min. The sections were immediately rinsed in a running water for 4 min and then stained with acid fuchsin for 3 min. Thereafter, sections were rinsed in distilled water before being treated with phosphomolybdic acid for 2 min and then treated with a light green stain for 10 min. The sections were allowed to dry before being treated with 1% Acetic acid in distilled water and 1% Acetic acid in absolute alcohol. Finally, sections were dehydrated and cleared before cover-slipping with Entellan.

2.2.4 Determination of tenocyte distribution

The H & E stain revealed the distribution of tenocytes in the microstructures of the QT, PT and ST. Photomicrographs were captured using a digital camera attached to a Zeiss Axioscope microscope at times 63 objective lens. For each section, photomicrographs were taken at randomly selected regions at every 2 mm interval along the length of the tissue section. An average of four images per section was taken for each tendon. All images were acquired under similar settings on the microscope. The photomicrograph was then fed into an ImageJ software where a 6–frame counting grid (220 μm^2) was superimposed on the image for ease of counting. The number of tenocytes in the six frames were then counted. The tenocyte distribution per image was calculated by dividing the total number of tenocytes (i.e. tenocyte) in the 6–frame grid by the area of the 6–frame grid.

Tenocyte distribution per image = tenocyte count / 220 μm^2 x 6

2.2.5 Determination of collagen distribution

The MT stain revealed collagen distribution in the microstructures of the QT, PT and ST tendons. Similar to the previous, photomicrographs were captured using a digital camera attached to a Zeiss Axioscope microscope at times 10 objective lens. For each section, photomicrographs were taken at every 3 mm interval along the length of the tissue section. An average of three images per section was taken for each tendon. All images were acquired under similar settings on the microscope. All acquired digitized images were stored in a jpeg file format with 24-bit RGB according to the colour deconvolution plugin of the imageJ software.

To quantify the collagen fibers, the method using the imageJ software as described by Chen *et al*. (2017) was used. With the scale bar set on the software, colours on the image were separated from overlapping regions using the colour deconvolution plugin of the software. This thus deconvolved the image into red, blue and green i.e. RGB components using the orthonormal transformation of the RGB information of the image (Chen *et al*., 2017). In this case, the green component represents the collagen fibers (Chen *et al*., 2017). A threshold was then set by manually adjusting the entire green–coloured component (i.e. the collagen fibers) until they were highlighted in red following which the highlighted area per image was then analysed. The collagen distribution (%) for each image was calculated by dividing the area covered by the collagen fibers by the area of the image (65450 μm^2).

Collagen distribution (%) = Area covered by collagen fibers / 65450 μm^2

2.2.6 Statistical analyses

The data obtained were not normally distributed (i.e. non–parametric) according to the Shapiro-Wilk test. A Kruskal-Wallis test was used to assess differences in the distribution of tenocyte or collagen distribution across the tendons (i.e. QT, PT and ST). In addition, sex differences in each tendon was conducted using a Mann-Whitney test. All statistical analyses were performed using the SPSS software (version 22.0; IBM, US). Statistical difference of 5% was regarded as significant for all the statistical analyses.

CHAPTER THREE

Results

3.1. Morphometry of the lower limb

To reiterate, all the measured parameters for the QT, PT and ST in each cadaver were normalized using the corresponding lower limb length (LLL). The test for repeatability using the Lin's concordance correlation of reproducibility (Pc) for the LLL was 0.997 which is an indication of a high degree of accuracy, reliability and reproducibility and the measurements are considered to have an error low enough to be acceptable. There was no statistically significant side difference ($p > 0.05$) in the LLL in both sexes (Table 3). In both limbs, the mean LLL of the male was significantly higher than the mean LLL of the female ($p < 0.05$).

Table 3: Comparison between the measurements of left and right lower limb lengths.

		Left			Right				
	N	Mean (mm)	SD	Median (mm)	N	Mean (mm)	SD	Median (mm)	p
Female									
LLL	40	860.550*	50.206	857.500	39	860.846**	48.393	865.000	0.979
Male									
LLL	39	926.692*	35.879	925.000	38	927.474**	38.055	920.500	0.926

* Significant difference p = 0.0000 (M > F)

**Significant difference p = 0.0000 (M > F)

LLL – length of lower limb; SD – standard deviation

3.2 Morphometry of the quadriceps tendon:

3.2.1 Test of reliability of measurements

The Pc values for the measured parameters of the QT are shown in Table 4. The Pc values ranged from 0.969 for SA_{QT} to 0.999 for LOT_{QT}. Based on these results, all the measured parameters of the QT are precise, accurate and reproducible. Therefore, the data analysed in this study can be considered free of measurement errors.

Table 4. Lin's concordance correlation of reproducibility for the measured parameters in the quadriceps tendon

Parameter	Pc
SA_{QT}	0.969
LOT_{QT}	0.999
SDW_{QT}	0.995
CDW_{QT}	0.989

SA_{QT} – surface area; LOT_{QT} – length of tendon; SDW_{QT} – straight distal width; CDW_{QT} –curved distal width; LLL – length of lower limb.

3.2.2 Comparison of morphometry in the quadriceps tendon

Morphologically, the QT often presents with either a dual or a single peak at its proximal end i.e. at the MTJ (Figure 1). In the cadavers assessed, a dual peak was observed in about 51% of left tendons and 48% of right tendons. Where a dual peak was absent, a single peak (diagram not shown) of the QT was observed. In a scenario of a dual peak, the maximum tendon length was taken from the more prominent peak.

For all the measured parameters in this study, both the raw data and the normalized data for all the tendons are presented. The descriptive analyses of the raw and the normalized data for each side and according to sex for the QT is shown in Table 5. For all the measured parameters in the female or the male (Table 5), there was no statistically significant difference in sides (i.e. left vs right). There was similarly no significant difference when the parameters in the female were compared with the male except for the SDW_{QT} of the male left limb which was significantly higher than the female left limb i.e. sexually dimorphic ($p = 0.033$). Despite the non-significant difference between the measures from both limbs, the SA_{QT} measure of one limb was different from the other limb in most individual cadavers assessed (indicated by red arrows in Figures 4). This is an indication that the surface area of the QT in both limbs are morphometrically different in some individuals compared to the similarity of the measures for the LLL (for illustrative purposes) in both limbs (Figure 4).

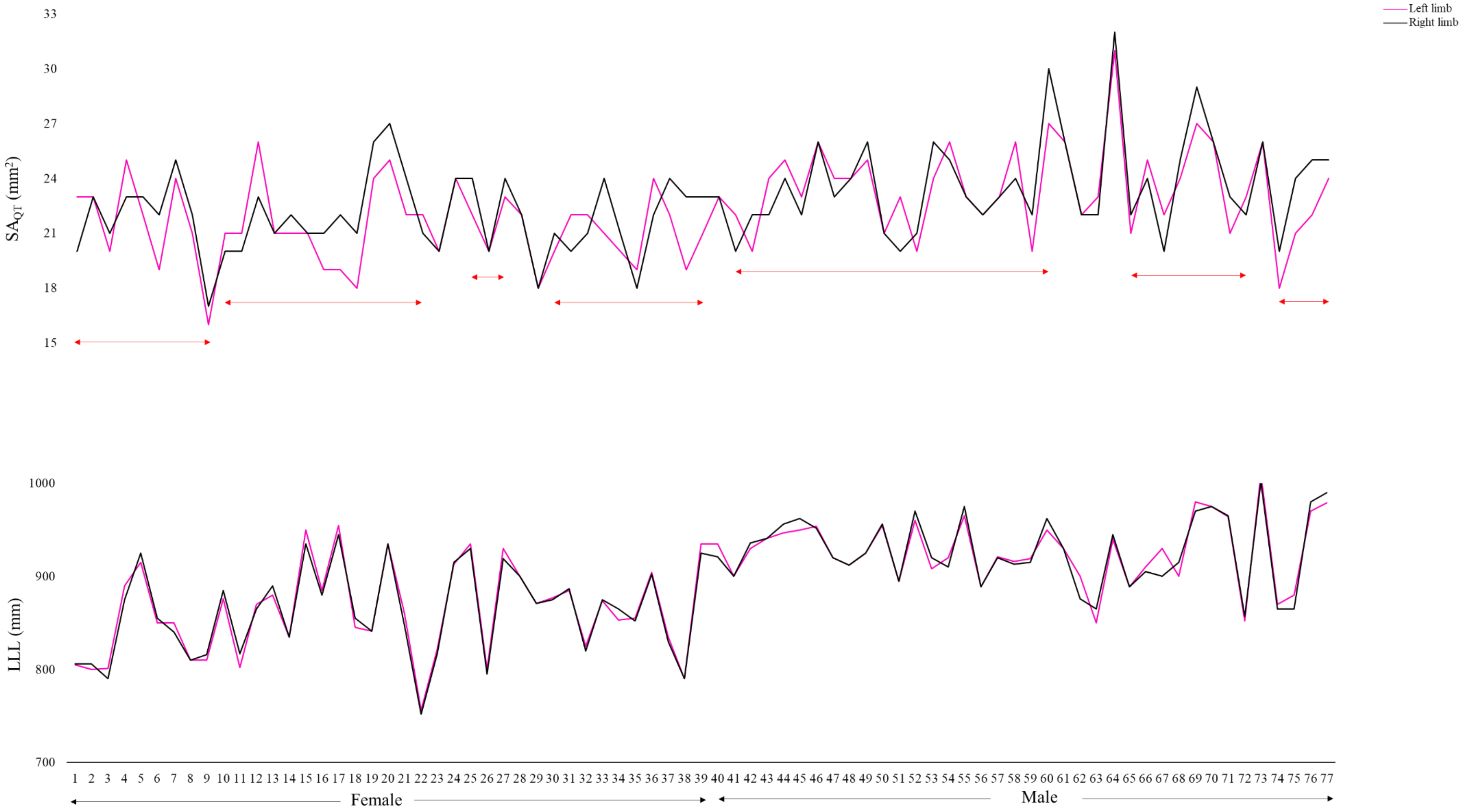
Table 5. Comparison of the measured parameters of the left and right quadriceps tendon

	Left					**Right**					
		Raw		Normalized			Raw		Normalized		
	Number of limbs assessed	Mean (mm)	Mean (mm^2)	Mean	SD	Number of limbs assessed	Mean (mm)	Mean (mm^2)	Mean	SD	p
Female											
SA_{QT}	40		21.23	0.03	0.003	39		21.90	0.03	0.002	0.440
LOT_{QT}	40	78.77		0.09	0.016	39	84.75		0.10	0.014	0.052
SDW_{QT}	40	49.96		0.06*	0.007	39	51.58		0.06	0.008	0.735
CDW_{QT}	40	53.18		0.06	0.008	39	54.75		0.06	0.008	0.344
Male											
SA_{QT}	39		23.44	0.03	0.003	38		23.74	0.03	0.003	0.421
LOT_{QT}	39	86.51		0.09	0.013	38	91.23		0.10	0.013	0.104
SDW_{QT}	39	57.24		0.06*	0.006	38	56.15		0.06	0.006	0.544
CDW_{QT}	39	60.74		0.07	0.006	38	60.63		0.07	0.007	0.442

*Significant difference $p = 0.033$ (M > F)

SA_{QT} – surface area; LOT_{QT} – length of tendon; SDW_{QT} – straight distal width; CDW_{QT} – curved distal width; SD – standard deviation

Figure 4. Superimposed line graphs showing the pattern of variations between the SA_{QT} or the LLL of both limbs for each individual cadaver. Red arrows indicate where patterns of the measures in both limbs of an individual are not visibly identical compared to the similarity of measures of the LLL for both limbs of same individual



3.2.3 Correlation analyses on the quadriceps tendon

The correlations between any paired QT dimensions showing a clear detail about the strength of their relationship are shown in Table 6. In this study, only paired parameters in all the tendons that revealed moderate or strong correlations are presented. Using the Pearson's correlation (R), strong correlations were observed between the paired measurements of SDW_{QT} and CDW_{QT} ($R \geq 0.7$) in both limbs of the female and the male (Table 6). A strong correlation was also observed between SA_{QT} and LOT_{QT} ($R \geq 0.7$) except for the left lower limb in the female that showed a moderate correlation ($R \geq 0.6$). In addition, the LLL of male or female were tested to determine whether they could be used to predict any of the measured parameters, only moderate correlations were observed in the paired parameters of LLL vs SA_{QT} or LLL vs CDW_{QT} for the male right limb. This is an indication that LLL may not be a good predictor for any of the measured parameters of the QT in either sex.

Table 6. Correlation coefficient between measurements of the left and right quadriceps tendon

	Female				**Male**			
	SA_{QT}	LOT_{QT}	SDW_{QT}	CDW_{QT}	SA_{QT}	LOT_{QT}	SDW_{QT}	CDW_{QT}
Left								
SA_{QT}		0.6				0.7	0.4	
LOT_{QT}								
SDW_{QT}				1.0				
CDW_{QT}							0.9	
LLL								
Right								
SA_{QT}		0.7		0.4		0.8		0.4
LOT_{QT}								
SDW_{QT}	0.5			1.0				
CDW_{QT}							0.8	
LLL					0.5			0.4

SA_{QT} – surface area; LOT_{QT} – length of tendon; SDW_{QT} – straight distal width; CDW_{QT} – curved distal width; LLL – length of lower limb

3.3 Morphometry of the patellar tendon:

3.3.1 Test of reliability of measurements

The Pc ranged from 0.931 for CDW_{PT} to 0.997 for LOT_{PT} (Table 7). Similar to the QT, this indicates a high degree of reproducibility and the data analysed in this study can be considered to be free of measurement errors.

Table 7. Lin's concordance correlation of reproducibility for the measured parameters in the patellar tendon

Parameter	Pc
SA_{PT}	0.953
LOT_{PT}	0.997
SPW_{PT}	0.983
CPW_{PT}	0.985
SDW_{PT}	0.981
CDW_{PT}	0.931

SA_{PT} – surface area; LOT_{PT} – length of tendon; SPW_{PT} – straight proximal width; CPW_{PT} – curved proximal width; SDW_{PT} – straight distal width; CDW_{PT} – curved distal width.

3.3.2 Comparison of morphometry of the patellar tendon

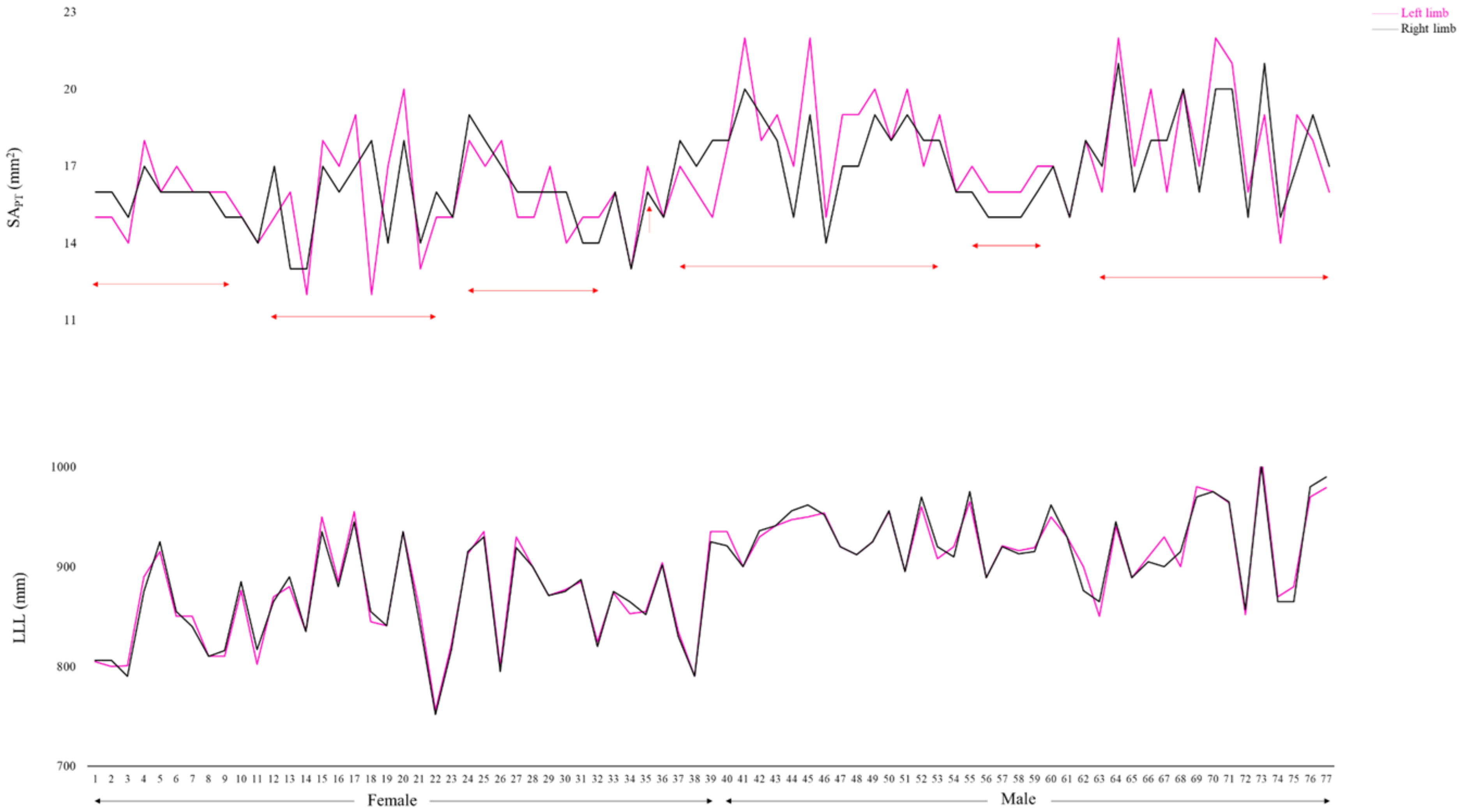
The descriptive statistics of the raw data and the normalized data for each side and according to sex is shown in Table 8. For all the measured parameters in the male or female (Table 8), there was no statistically significant difference in sides (i.e. left vs right). There was similarly no significant difference when the measured parameters in both sexes were compared. Thus, none of the measures in the PT exhibited a side dominance nor sexual dimorphism. Despite the non-significant difference between measures from both limbs, like in the QT the SA_{PT} measure of one limb was different from the other limb in most individual cadavers assessed (indicated by red arrows in Figures 5). This is also an indication that the surface area of the PT in both limbs are morphometrically different in some individuals compared to the similarity of the LLL measures (for illustrative purposes) in both limbs (Figure 5).

Table 8. Comparison of the measured parameters of the left and right patellar tendon

	Left					Right					
		Raw		Normalized			Raw		Normalized		
	Number of limbs assessed	Mean (mm)	Mean (mm^2)	Mean	SD	Number of limbs assessed	Mean (mm)	Mean (mm^2)	Mean	SD	p
Female											
SA_{PT}	40		15.68	0.02	0.002	39		15.87	0.02	0.002	0.584
LOT_{PT}	40	51.01		0.06	0.011	39	51.19		0.06	0.009	0.706
SPW_{PT}	40	34.09		0.04	0.005	39	34.92		0.04	0.005	0.571
CPW_{PT}	40	37.14		0.04	0.007	39	38.04		0.04	0.006	0.137
SDW_{PT}	40	24.83		0.03	0.004	39	25.50		0.03	0.004	0.639
CDW_{PT}	40	25.95		0.03	0.004	39	26.90		0.03	0.004	0.279
Male											
SA_{PT}	39		17.90	0.02	0.002	38		17.42	0.02	0.002	0.324
LOT_{PT}	39	57.39		0.06	0.012	38	56.20		0.06	0.011	0.937
SPW_{PT}	39	37.34		0.04	0.005	38	37.81		0.04	0.005	0.835
CPW_{PT}	39	41.35		0.05	0.006	38	41.81		0.05	0.005	0.590
SDW_{PT}	39	27.63		0.03	0.004	38	28.20		0.03	0.004	0.784
CDW_{PT}	39	29.61		0.03	0.004	38	29.79		0.03	0.005	0.909

SA_{PT} – surface area; LOT_{PT} – length of tendon; SPW_{PT} – straight proximal width; CPW_{PT} – curved proximal width; SDW_{PT} – straight distal width; CDW_{PT} – curved distal width; LLL – length of lower limb

Figure 5. Superimposed line graphs showing the pattern of variations between the SA$_{PT}$ or the LLL of both limbs for each individual cadaver. Red arrows indicate where patterns of the measures in both limbs of an individual are not visibly identical compared to the similarity of measures of the LLL for both limbs of same individual



3.3.3 Correlation analyses

Like the QT, the correlations between any paired PT dimensions showing a clear detail about the strength of their relationships are shown in Table 9. A strong correlation ($R \geq 0.7$) was observed between the paired parameters of SA_{PT} vs LOT_{PT}, SPW_{PT} vs CPW_{PT} and SDW_{PT} vs CDW_{PT} in both limbs of the male and female. Other paired parameters showed a positive moderate correlation while others revealed a negative moderate correlation as shown in Table 9. In addition, only a moderate correlation ($R = 0.4$) was observed in the female between LLL vs SA_{PT} or LLL vs LOT_{PT} of the left limb and between LLL vs LOT_{PT} of the right limb. This is also an indication that LLL may not be a good predictor for any of the measured parameters of the PT in either sex.

Table 9. Correlation coefficient between measurements of the left and right patellar tendon

	Female						**Male**					
	SA$_{PT}$	LOT$_{PT}$	SPW$_{PT}$	CPW$_{PT}$	SDW$_{PT}$	CDW$_{PT}$	SA$_{PT}$	LOT$_{PT}$	SPW$_{PT}$	CPW$_{PT}$	SDW$_{PT}$	CDW$_{PT}$
Left												
SA$_{PT}$		0.7						0.9	0.5			
LOT$_{PT}$					-0.6	-0.6					-0.4	-0.4
SPW$_{PT}$	0.5									0.8		
CPW$_{PT}$	0.6		1.0				0.6					
SDW$_{PT}$			0.4	0.4								
CDW$_{PT}$					0.9						0.9	
LLL	0.4	0.4										
Right												
SA$_{PT}$		0.7		0.5				0.8	0.5			
LOT$_{PT}$												
SPW$_{PT}$	0.4									0.8	0.4	
CPW$_{PT}$			0.9		0.4	0.4	0.5					0.5
SDW$_{PT}$										0.4		
CDW$_{PT}$					0.9				0.4		0.9	
LLL		0.4										

SA$_{PT}$ – surface area; LOT$_{PT}$ – length of tendon; SPW$_{PT}$ – straight proximal width; CPW$_{PT}$ – curved proximal width; SDW$_{PT}$ – straight distal width; CDW$_{PT}$ – curved distal width; LLL – length of lower limb

3.4 Morphometry of semitendinosus tendon

3.4.1 Test of reliability of measurements

The Pc ranged from 0.956 for C-MTJ$_{ST}$ to 0.977 for LOT$_{ST}$ (Table 10). Similar to the QT and PT, this indicates a high degree of reproducibility and the data analysed in this study can be considered to be free of measurement errors.

Table 10. Lin's concordance correlation of reproducibility for the measured parameters in the semitendinosus tendon

Parameter	Pc
C–MTJ$_{ST}$	0.956
C$_{ST}$	0.971
LOT$_{ST}$	0.977

C–MTJ$_{ST}$ – circumference at muscular tendinous junction; C$_{ST}$ – circumference at 3cm away from muscular tendinous junction; LOT$_{ST}$ – length of tendon.

3.4.2 Comparison of morphometry of the semitendinosus tendon

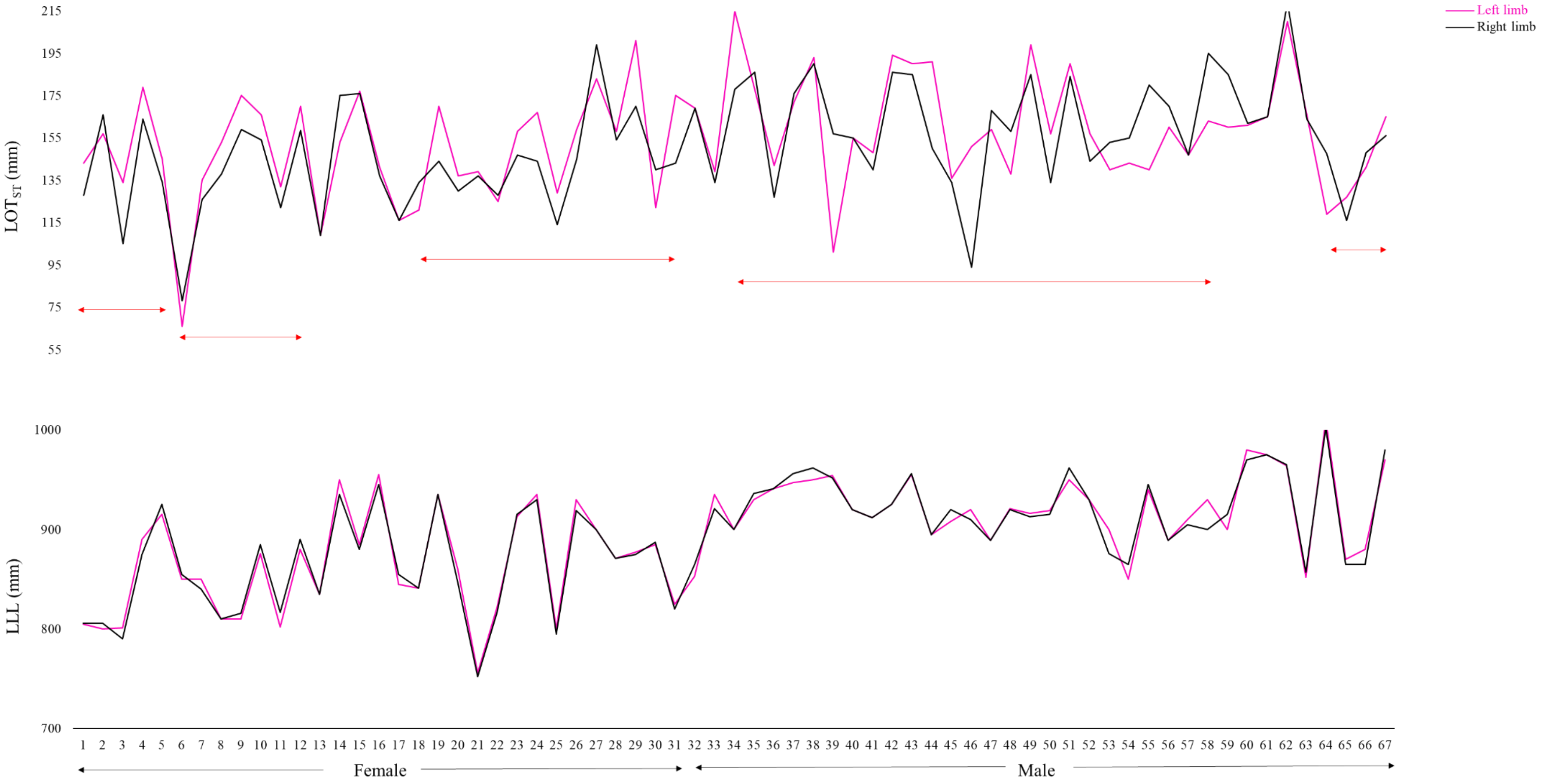
The descriptive statistics of the data for each side and according to sex is shown in Table 11. For all the measured parameters in the male or female, there was no statistically significant difference in sides (i.e. left vs right limb). There was also no significant difference when the measured parameters in both sexes were compared. This also indicates that the ST measures did not exhibit a side dominance nor were they sexually dimorphic. Like the QT and PT, the LOT_{ST} measure of one limb was different from the other limb in most of the individual cadavers assessed (indicated by red arrows in Figures 6). This is also an indication that the length of ST in both limbs are morphometrically different in some individuals compared to the similarity of the measures for the LLL (for illustrative purposes) in both limbs (Figure 6).

Table 11. Comparison of the measured parameters of the left and right semitendinosus tendon

	Left					**Right**					
		Raw		Normalized			Raw		Normalized		
	Number of limbs assessed	Mean (mm)	Mean (mm^2)	Mean	SD	Number of limbs assessed	Mean (mm)	Mean (mm^2)	Mean	SD	p
Female											
C-MTJ_{ST}	36	16.57		0.02	0.004	33	15.86		0.02	0.003	0.650
CSA-MTJ_{ST}	36		22.72	0.03	0.012	33		20.52	0.02	0.008	0.805
C_{ST}	36	13.81		0.02	0.002	33	13.70		0.02	0.002	0.240
CSA_{ST}	36		15.45	0.02	0.005	33		15.11	0.02	0.003	0.484
LOT_{ST}	36	150.03		0.17	0.029	33	141.92		0.17	0.026	0.141
Male											
C-MTJ_{ST}	36	16.64		0.02	0.002	38	16.59		0.02	0.003	0.725
CSA-MTJ_{ST}	36		22.35	0.02	0.006	38		22.64	0.03	0.010	0.279
C_{ST}	36	14.43		0.02	0.002	38	14.59		0.02	0.002	0.174
CSA_{ST}	36		16.71	0.02	0.003	38		17.27	0.02	0.005	0.937
LOT_{ST}	36	160.72		0.17	0.028	38	160.62		0.17	0.026	0.772

C–MTJ_{ST} – circumference at muscular tendinous junction; CSA–MTJ_{ST} – cross sectional area at muscular tendinous junction; C_{ST} – circumference at 3cm away from muscular tendinous junction; CSA_{ST} – cross sectional area at 3cm away from muscular tendinous junction; LOT_{ST} – length of tendon; SD – standard deviation

Figure 6. Superimposed line graphs showing the pattern of variations between the LOT$_{ST}$ or the LLL of both limbs for each individual cadaver. Red arrows indicate where patterns of the measures in both limbs of an individual are not visibly identical compared to the similarity of measures of the LLL for both limbs of same individual



3.4.3 Correlation analyses

Like the QT and PT, the correlations between any paired ST dimensions showing a clear detail about the strength of their relationships are shown in table 12. The correlation between some paired parameters in the limbs of male or female were identical (Table 12). They revealed a strong (R = 0.7) to a moderate (R = 0.4 – 0.6) correlation. In addition, a moderate correlation (R = 0.4 – 0.6) was observed in some paired parameters only in the female (for examples: LLL vs C_{ST}, LLL vs CSA_{ST} or LLL vs LOT_{ST}) (Table 12) however these relationships produced a weak correlation in the male. This is also an indication that the LLL may not be a good predictor for any of the parameters in the ST in both sexes.

Table 12. Correlation coefficient between measurements of the left and right semitendinosus tendon

	Female					**Male**				
	C-MTJ$_{ST}$	CSA-MTJ$_{ST}$	C$_{ST}$	CSA$_{ST}$	LOT$_{ST}$	C-MTJ$_{ST}$	CSA-MTJ$_{ST}$	C$_{ST}$	CSA$_{ST}$	LOT$_{ST}$
Left										
C-MTJ$_{ST}$										
CSA-MTJ$_{ST}$			0.7	0.7	0.4			0.6	0.6	0.5
C$_{ST}$	0.7					0.6				
CSA$_{ST}$	0.7					0.6				
LOT$_{ST}$	0.4					0.5				
LLL			0.5	0.5	0.4					
Right										
C-MTJ$_{ST}$										
CSA-MTJ$_{ST}$			0.6	0.7	0.6			0.7	0.7	0.4
C$_{ST}$	0.7					0.7				
CSA$_{ST}$	0.7					0.7				
LOT$_{ST}$	0.6		0.5	0.5						
LLL	0.4		0.4	0.5	0.5					

C–MTJ$_{ST}$ – circumference at muscular tendinous junction; CSA–MTJ$_{ST}$ – cross sectional area at muscular tendinous junction; C$_{ST}$ – circumference at 3cm away from muscular tendinous junction; CSA$_{ST}$ – cross sectional area at 3cm away from muscular tendinous junction; LOT$_{ST}$ – length of tendon; LLL – length of lower limb

3.5. General morphologies of tissue architecture of the quadriceps, patellar and semitendinosus tendons

At the microscopic level, the three tendons (QT, PT and ST) have similar micro-architectural arrangements (Figure 7). Fascicles made up of aggregates of collagen molecules were organised side–by–side and end–to–end along the tendon as seen in the H & E and MT stains (Figure 7). Several fascicles aggregate to then form the tendon fibres. The tenocytes or tenocytes appeared blue-stained in the H & E (Figure 7a, 7b and 7c) and are responsible for the production of collagen in the tendon. Numerous collagen deposits appeared green-stained in the MT staining (Figure 7d, 7e, and 7f) and they constitute the major component of the tendons as well as contributing to the strength and structure of the tendons. The tenocytes were mostly spindle or flat in shape and they were sparsely distributed along the fascicles in the form of longitudinal arrays where they contribute to the tensile load of the tendons. Similarly, the collagen distribution was conspicuous and widely distributed in all the tendons assessed.

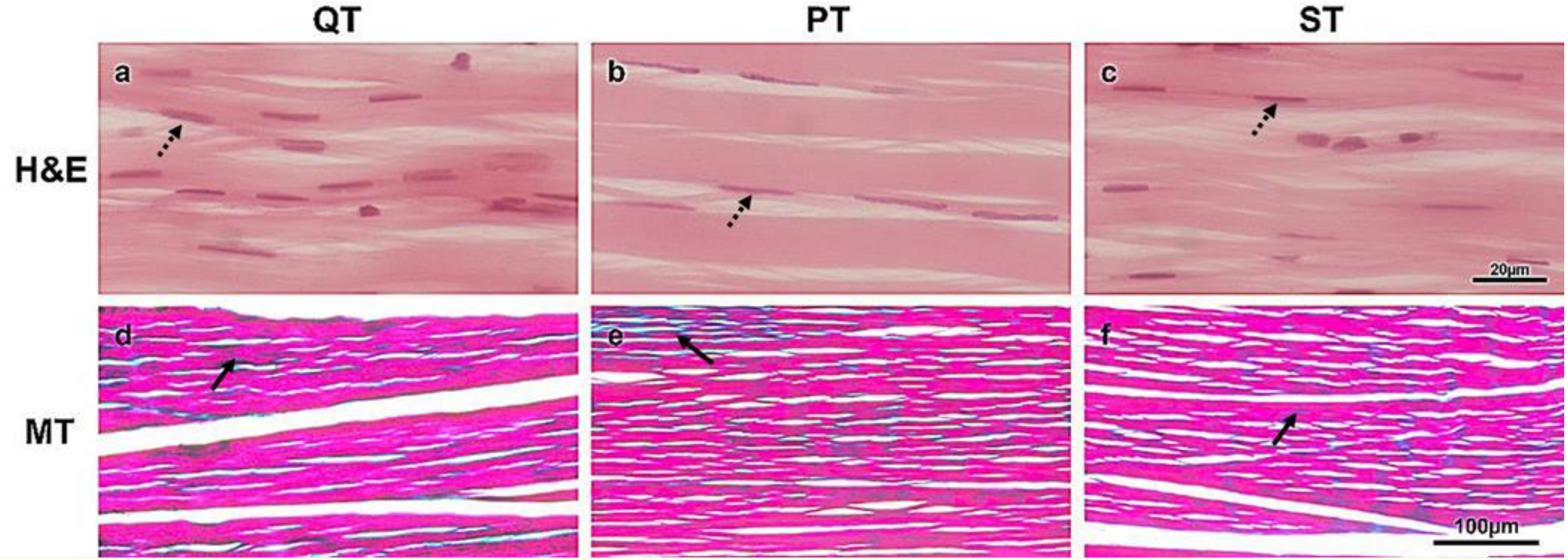


Figure 7. Photomicrographs showing representative longitudinal sections of the quadriceps tendon (a, d), the patellar tendon (b, e) and the semitendinosus tendon (c, f). The micro-architecture of the three tendons was closely identical with the fascicles arranged in parallel and end–to–end in the longitudinal plane along the length of the tendons. In the H&E staining (a, b, c), the blue–stained tenocytes (broken arrows) appeared spindle or flat in shape and were uniformly distributed in all the tendons. In the MT staining (d, e, f), collagen appeared green–stained (solid arrows) and appeared to be widely distributed in the fascicles.

3.6 Tenocyte distribution

The descriptive analyses of the tenocyte distribution per tendon for both sexes are shown in Table 13. Boxplots showing the characteristics of the data (i.e. the quartile ranges and median) for both sexes are shown in Figure 8. In the female, the tenocyte distribution was significantly different across the tendons ($p = 0.006$). PT had the lowest tenocyte distribution and post hoc analyses using a Dunn’s test showed that the tenocyte distribution in the PT was significantly lower than the QT ($p = 0.019$) or the ST ($p = 0.016$). However in the male, the tenocyte distribution was not significantly different across the tendons ($p = 0.872$). In addition, there was no sex difference in the tenocyte distributions when the distribution of tenocyte in each tendon were compared in both sexes ($p > 0.05$).

Table 13. Descriptive statistics of tenocyte distribution in the QT, PT and ST

	QT				PT				ST				
	Number of images assessed	Mean	SD	Median	Number of images assessed	Mean	SD	Median	Number of images assessed	Mean	SD	Median	p
Female	42	0.008	0.004	0.008	31	0.007	0.009	0.005	42	0.009	0.007	0.006	0.008*
Male	29	0.008	0.006	0.007	34	0.008	0.006	0.006	30	0.007	0.003	0.007	0.924

*Significant difference $p < 0.05$

QT — quadriceps tendon; PT — patellar tendon; ST — semitendinosus tendon; SD — standard deviation

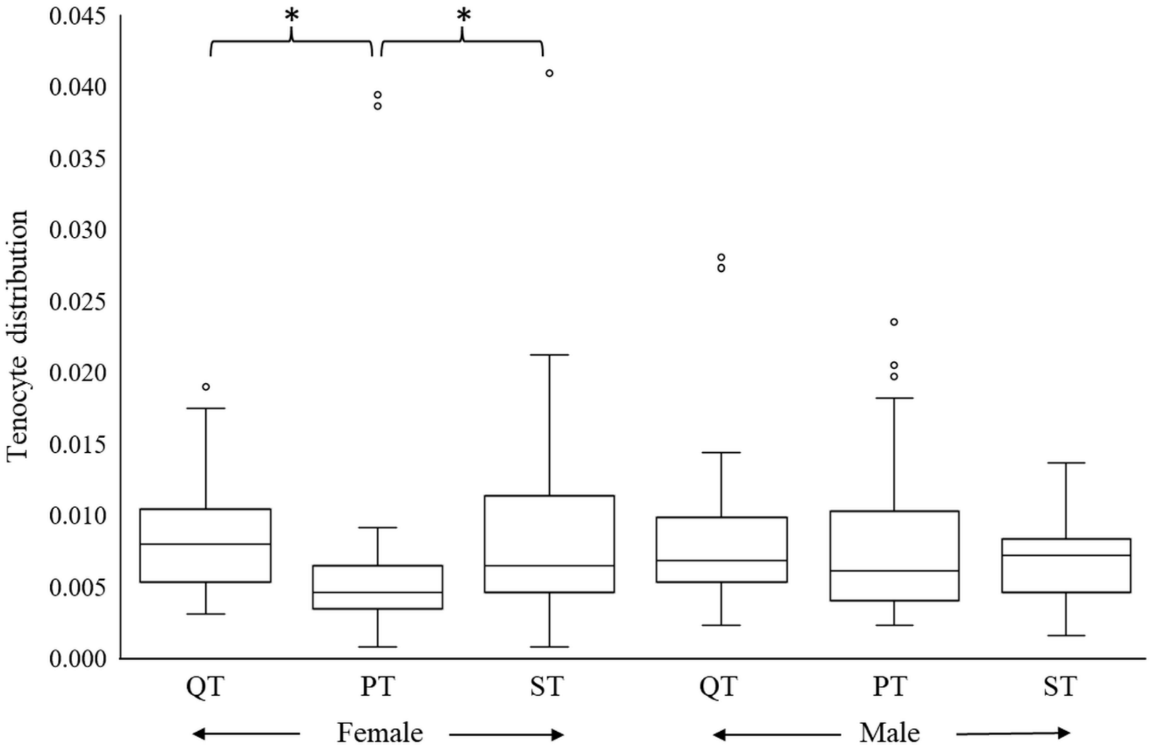


Figure 8. A box plot showing the comparison of the tenocyte distribution across the tendons (quadriceps tendon, QT; patellar tendon, PT and semitendinosus tendon, ST) in the female and the male cadavers. Tenocyte distribution is significantly lower in the PT than in the QT or in the ST in the female cadavers. *$p < 0.05$

3.7 Collagen distribution

The average percentage of collagen distribution per tendon in the female and the male cadavers is shown in Table 14. A boxplot showing the characteristics of the data for the female and the male population is shown in Figure 9. Despite the ST having the lowest percentage collagen distribution in both sexes, a Kruskal–Wallis one-way analysis of variance showed that the percentage collagen distribution was not significantly different across the tendons in the female ($p = 0.383$) or in the male ($p = 0.567$). In addition, a Mann Whitney test showed that there were no sex differences in the collagen distribution in each tendon when the male and female were compared ($p > 0.05$).

Table 14. Descriptive statistics of collagen distribution in the QT, PT and ST

	QT				PT				ST				
	Number of images assessed	Mean	SD	Median	Number of images assessed	Mean	SD	Median	Number of images assessed	Mean	SD	Median	p
Female	33	25.779	21.488	16.773	20	26.870	17.241	24.403	21	17.917	15.981	11.717	0.383
Male	17	29.514	18.995	29.869	17	33.501	22.986	36.692	18	25.931	21.641	19.717	0.567

QT — quadriceps tendon; PT — patellar tendon; ST — semitendinosus tendon SD — standard deviation

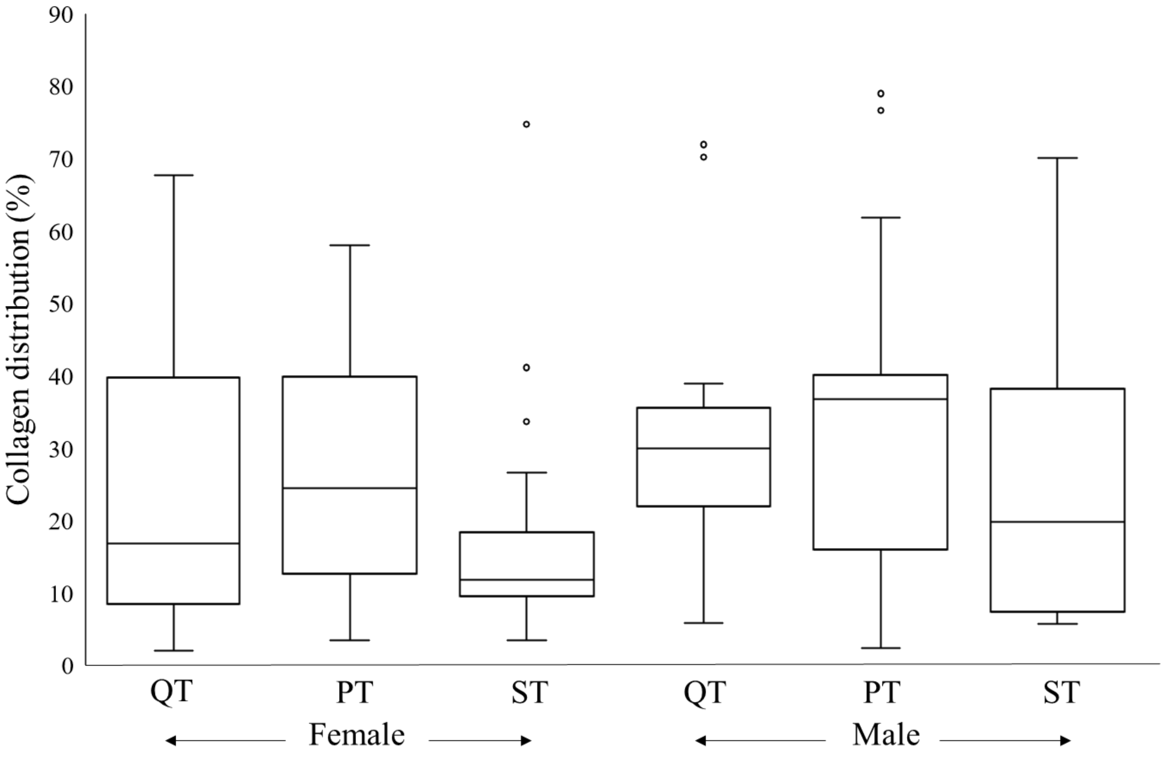


Figure 9. A box plot showing the comparison of the percentage distribution of collagen across the tendons (quadriceps tendon, QT; patellar tendon, PT and semitendinosus tendon, ST) in the female and the male cadavers. Percentage collagen distribution across the tendons are similar in both sexes.

CHAPTER FOUR

Discussion

In the event of an ACL injury, repair of the ligament is necessary in order to restore the anatomical and functional biomechanics of the damaged ligament (Macaulay *et al*., 2012). Poor management or treatment plan will lead to unbearable pain, knee function impairment, deterioration of the surrounding cartilages as well as increasing the chances of developing osteoarthritis (Oiestad *et al*., 2010; Hijazi *et al*., 2015; Shultz, 2015; Sayampanathan *et al*., 2017). Surgical repair using an autograft sourced or harvested from a patient is the commonly used approach for ACL reconstruction. Various tendons could be used as an autograft however, preference for an autograft often depends on the experience of the surgeon. The presented morphometric data and details of the microstructure of the commonly used autografts (QT, PT and ST) for ACL reconstruction will contribute to knowledge and will be beneficial for preoperative surgical planning.

4.1 Lower limb morphometry

Generally, human height is a useful parameter for stature in anthropometry and morphometry studies (Xerogeanes *et al*., 2013; Gupta *et al*., 2017; Krebs *et al*., 2019). However, where the height of the subject cannot be accurately measured as in the case of cadavers due to post-mortem variation in body sizes (Cardoso *et al*., 2016; Ferorelli *et al*., 2017), the lower limb is often used as an indication of stature. Several studies have used the LLL (i.e. distance from the ASIS to the medial malleolus of each limb) as an indication of stature (e.g. Treme *et al*., 2008; Chiang *et al*., 2012; Sundararajan *et al*., 2016; Gupta *et al*., 2017; Mohd Asihin *et al*., 2018; Sakti *et al*., 2019; Dziedzic *et al*., 2020). Even though the LLL parameter used in the present study is consistent with the previous studies, the focus of this study was not to determine stature in this population group. Instead, LLL was used to normalize the measures and to determine whether LLL could be a useful and a dependable factor to predict the morphometries of the commonly used autografts preoperatively.

It is also a known fact that stature is different in different races and ethnicities, and this is the case in the length of limb. Gupta *et al*. (2017) reported a longer (but not significant) LLL in the male than in the female of an Indian population while Treme *et al*. (2008) found the LLL in an American population to be significantly shorter in the female than in the male. Similar observations were reported in a South Sulawesi (Sakti *et al*., 2019) and a Chinese (Chiang *et al*., 2012) population groups, which are consistent with the findings of the present study where the LLL was significantly higher in the male than in the female (i.e. sexually dimorphic).

In morphometric studies of structures (e.g. tendons or ligaments in the lower limb), some measured parameters may indicate a leg dominance because a measure on a structure on one limb may be significantly different from the other limb. Leg dominance has been reported in semitendinosus tendon (Pichler *et al*., 2008; Bundi *et al*., 2016) and patellar ligament (Olateju *et al*., 2013). The factors that may contribute to leg dominance are age, sex, lifestyle and activity level of an individual as well as tendon pathology arising from injuries (Latiff *et al*., 2021). In the study by Gupta *et al*. (2017), the lengths of both limbs were not significantly different, which is also consistent with the findings of the present study. This means that leggedness/leg dominance may not be associated with limb length but may be directly linked to the tendon or ligament morphometries of the limbs as is the case in Pichler *et al*. (2008), Olateju *et al*. (2013) and Bundi *et al*. (2016).

4.2 Quadriceps tendon morphometry

The morphometric profile of the QT provides useful information that are beneficial to ACL reconstruction surgery (Lippe *et al*., 2012). The large area of harvestable tissue of the QT is an important feature that makes it a commonly used autograft. Other advantages of the QT are that it can be reliably harvested (Sheean *et al*., 2018) and it has a similar microstructure to other graft sources e.g. PT and hamstrings (Macaulay *et al*., 2012). The present study, for the first time, presents data on the surface area (SA) of the QT. The SA of the QT was higher in the male than in the female but this difference was not significant. Similarly, SA was higher in the right limb than in the left limb but was also not significant in the population group assessed. Unfortunately, there is no report in the literature to compare the findings of the present study thus this is not discussed further. The SA of the QT may be of benefit to the recovery of the left-over tissue at the harvest site and the functionality of the tendon post-harvest since there is often an abundant tissue left. It is thus proposed that this parameter should be considered in preoperative planning where the QT is being considered as a graft in an individual.

To attain a morphometric profile of the QT, a simple approach utilizing an *in situ* tracing of tendon was used. Similar anatomical landmarks as described in other studies (e.g. Lippe *et al*., 2012; Krebs *et al*., 2019; Tanpowpong *et al*., 2019; Yamasaki *et al*., 2020) were used. Morphologically, the QT of the population group assessed was similar to the previous studies. Some cadavers exhibited a dual peak while others had a single peak. The dual peaks are because the vastus lateralis and vastus medialis become tendons (i.e. the QT) before inserting at the base of the patella (Lippe *et al*., 2012). Understanding and acknowledging this characteristic feature of the QT is important during harvesting in order to avoid harvesting from the short peak (which is mostly positioned medially), instead of from the long peak that provides adequate graft tissue (i.e. the maximum tendon length) for harvesting (Lippe *et al*., 2012). In this study, the maximum tendon length was used similar to other studies (Lippe *et al*., 2012;

Tanpowpong *et al*., 2019). In addition, none of the measured parameters of the QT exhibited a leg dominance as there was no significant difference in the measured parameters of both limbs, which is also consistent with the cadaveric studies by Hijazi *et al*. (2015) and Tanpowpong *et al*. (2019).

In the present study, the average maximum tendon length for both limbs was 81.76 mm in the female and 88.87 mm in the male. Due to differences in morphometric approaches, other studies reported a lower maximum tendon length than in the present study. For example, Yamasaki *et al*. (2020) in a MRI study on a Japanese population reported a QT length of about 59.5 mm, while a QT length of 61 mm was reported in cadavers by Harris *et al*. (1997) and a length of 63 mm in a Thai population by Tanpowpong *et al*. (2019). However, other studies reported a QT length within the range observed in the present study. For example, Staubli *et al*. (1999) reported an average tendon length of about 86 mm for both limbs, Krebs *et al*. (2019) reported an average of about 83 mm in a cadaveric study while Thi and Ha (2021) and Lippe *et al*. (2012) reported averages of about 79 mm and 88 mm respectively. With the similarities in the mean QT length of cadavers (e.g. the present study, Staubli *et al*. (1999), Lippe *et al*. (2012), Krebs *et al*. (2019), Thi and Ha, 2021), it seems that the *in situ* tendon tracing and methodologies used in the present study are adequate. However, variations in the QT length across different studies and population groups may be attributed to differences in study approaches (e.g. MRI or cadaveric), race, age, stature or ethnicity (Gupta *et al*., 2017).

The QT length in the present study was not sexually dimorphic despite the observed higher tendon length in the male than in the female. This however contradicts the MRI study by Xerogeanes *et al*. (2013) that found a significant higher tendon length in the male (~ 81.1 mm) than in the females (~ 73.5 mm). A cadaveric study in a Saudi Arabian population also reported a significantly higher QT length in the male (Hijazi *et al*., 2015). The difference in significance may also be attributed to the normalized data that was used in the statistical tests in the present study unlike in the studies by Xerogeanes *et al*. (2013) and Hijazi *et al*. (2015). To reiterate, all measures (except where not stated) were normalised for the length of the corresponding limb to give a better reflection of the morphometry with respect to the ‘stature’ of each cadaver.

To further give a detailed morphometry that could be used preoperatively, the SDW_{QT} and CDW_{QT} were measured on the tracings. The tendon tracings provided an additional advantage in that the actual or curved widths can be easily measured unlike in radiological records e.g. MRI. One or both measurements may be adequate for preoperative investigation and planning for the use of the QT as an autograft. The study however did not find a significant difference between the straight and curved distal widths of the QT. Unfortunately, there is no similar study in the literature to compare these findings. It is the view of this study that both measurements may be important to better understand the morphometry of the QT and its curvature.

The SDW_{QT} or the CDW_{QT} did not reveal a side difference. However, SDW_{QT} (not CDW_{QT}) showed a sex dimorphism where the male QT width was wider than the female. This is consistent with the report by Hijazi *et al*. (2015) despite the narrower widths reported compared to the present study. The present study found SDW_{QT} to be about 50.8 mm in the female and about 56.7 mm in the male (left and right limbs combined) while the QT widths of about 26.7 mm in the female and about 28.5 mm in the male (left and right limbs combined) were reported by Hijazi *et al*. (2015). Thi and Ha (2021) also reported a narrower QT distal width (~ 36.0 mm) than the present study. On the other hand, Lippe *et al*. (2012) (~ 43.3 mm), Krebs *et al*. (2019) (~ 44.8 mm) and Tanpowpong *et al*. (2019) (~ 46.2 mm) reported QT distal widths in cadavers within the range observed in the present study.

Considering the usability of the QT as an autograft, the area of harvestable tissue is high and this is one advantage the QT has over the other commonly used autografts (e.g.

semitendinosus and patellar tendons which are discussed later in this chapter). Harvesting a standard 10–mm wide (Adams *et al*., 2006; Shelton and Fagan., 2011; Iriuchishima *et al*., 2013; Hijazi *et al*., 2015) and a 70–mm long (Van Eck *et al*., 2010) QT tissue for an ACL reconstruction means that there is a sufficient tissue that could be harvested, and at the same time leaving a sufficient tissue for tendon functionality and healing at the harvest site. This is also highlighted in other reports e.g. Yamasaki *et al*. (2020), Krebs *et al*. (2019) and Xerogeanes *et al*. (2013). Based on the observed morphometries of the tendon length (female: 52.1 – 116.7 mm and male: 67.3 – 121.8 mm) and width (female: 35.8 – 68.9 mm and male: 48 – 70.1 mm) of the QT in the present study, only about 8% (i.e. 6 limbs out of 77) of the male limbs and about 17% (i.e. 13 limbs out of 79) of the female limbs fall short of the required dimensions of a QT autograft and thus these individuals may not qualify for an ACL reconstruction using the QT autograft. Interestingly, these observations were found bilaterally in 3 cadavers (i.e. 1 male and 2 females). This completely nullifies these subjects as a QT autograft recipient if they were to undergo an ACL reconstruction. In comparison to Tanpowpong *et al*. (2019), about 61% of the Thai population group assessed were considered not a suitable candidate for a QT autograft due to their short QT length. In these individuals, a bone–patellar tendon–bone autograft may be an alternative (Yamasaki *et al*., 2020). Predicting the size of the autograft for use in surgery may be a difficult task (Krebs *et al*., 2019), but this is where morphometric studies have become useful (Helito *et al*., 2015; Zakko *et al*., 2017).

Similarly, it may be possible that the QT of one limb may be adequate but not the other limb. Depending on the strategies or plans of the surgeon, QT may be harvested from the limb with a damaged ACL or from the undamaged limb (Shelbourne and Urch, 2000; von Essen et al., 2021). Whatever the scenario, there is need for caution as there are some individuals with observable differences in the morphometry of the QT of both limbs. For these individuals and depending on which leg is injured, the surgeon must be cautious in choosing which graft to use by conducting a morphometry. Thus, the preference of a surgeon based on the surgical training may not be applicable in this case but rather by an informed decision provided by tendon morphometry must be applied. It is not advisable to harvest a graft from the injured limb as there is a high risk of compounding the problems in this limb. In the event of harvesting a graft from the contralateral limb as explained by Shelbourne and Urch (2000), the trauma of the surgery is being divided between the limbs and this allows the patient to focus on the rehabilitation process for each limb. For the recovery of both knees and the return of knee function, patients may not have to worry about a major loss of strength in the limb where the ACL was reconstructed as the graft was harvested from the non-injured limb. Even though a non-injured limb is being 'disturbed', this proves to be advantageous for recovery of both limbs (Shelbourne and Urch, 2000).

In the present study, correlation between paired parameters of the QT was tested to determine whether one parameter could be a good predictor of the other. Strong correlation was found between the distal width (i.e. straight and curved) in both limbs and for both sexes. This relationship further shows that one or both measurements is adequate for preoperative investigation of QT as a possible autograft because the two measurements of the distal width are not significantly different, and one parameter of the distal width is a good predictor of the other. A strong correlation was also found between the SA_{QT} and LOT_{QT} (except for the female left limb that had a moderate correlation). This relationship is another important factor to consider during preoperative investigation of the QT because the surface area of the QT can be used to predict the length of the tendon and vice versa. Furtherance to predictability of measurements, correlation was extended to determine whether the LLL could be a good predictor of any of the QT measures. It is important to note that the height of a subject and the length of limb in the same subject may not be directly related. Interestingly, none of the parameters paired with the LLL showed a strong correlation. Unlike in previous reports by

Xerogeanes *et al*. (2013) and Krebs *et al*. (2019) that found a strong correlation between the height of individual and the tendon length.

4.3 Patellar tendon morphometry

The harvesting of PT as an autograft is common and it is considered a gold standard for ACL reconstruction. The morphometry of the PT is beneficial to the planning of an ACL reconstruction (Milankov *et al*., 2015; Pudar *et al*., 2021). The present study also for the first time presented data on the surface area of the PT. The surface area of the harvestable tissue seems to be an important parameter to be considered in surgical planning when considering the recovery and functionality of the limb after the removal of the graft. In the present study, the dimension of the SA_{PT} in the population group was similar in both limbs despite the variations in surface area in both limbs of most of the individuals. Similarly, the surface area was also not significantly different between the sexes. Unfortunately, there is no report in the literature to compare these findings. An important step in the methodology of the present study is that the actual measurements were obtained *in situ* in order to obtain a more realistic measurement with no derivation of dimensions which requires the use of mathematical formulae e.g. calculating the area of shapes (cylinder or circle) such as in deriving the cross sessional area of a tendon from the obtained circumference (Toritsuka *et al*., 2003; Milankov *et al*., 2015). The *in situ* approach used in this study (even though it was on cadavers) mimics the living state. However the present study acknowledges the possible influences of tissue fixatives on morphometry, but the extent of tissue shrinkage has not yet been elucidated (Olateju *et al*., 2013).

Despite the non-significant difference in the dimension of the SA_{PT} in this study, the dimensions in both limbs of some individuals were not observably similar just like in the QT. Even though the reason for the variations between the limbs could not be ascertained, it is assumed that daily activities such as standing, sitting and/or postural stance when exercising may impact the morphology and morphometry of the muscle or its tendon as a result of a possible gradient in the muscle tone in both limbs (Aithal Padur *et al.*, 2021). Despite the advantages of a bone–patellar tendon–bone graft that this tendon provides, one implication of the present observation (like in the QT) is that one or both PT may not be suitable as an autograft in an individual (bone pathologies being additional reasons). However, the anatomical disparity between the limbs of an individual does not seem to be a major problem when considering the PT as an autograft because of the additional advantages of the PT being able to incorporate bone blocks at the two ends of the graft. Despite the average tendon length being lower than the required 70–mm graft length, but with the incorporation of the bone blocks it means that most individuals may still be considered suitable recipients of PT autograft. The benefit associated with using the bone–patellar tendon–bone graft is that it incorporates and heals at a much faster rate within the bone tunnels at the attachment sites of the ACL in the knee joint (Mehran *et al.*, 2013; Frank *et al.*, 2017). This has made the patellar tendon a gold standard for use as a graft in ACL reconstruction thus affirming its popularity. Apart from the bone–patellar tendon–bone benefits, graft site morbidity and return of tendon and knee functions are the other important factors to consider before deciding on harvesting tissues from the contralateral limb. To reiterate, harvesting grafts from the contralateral side is more advantageous than harvesting from the ipsilateral. Shelbourne and Urch (2000) reported a faster restoration of motion range and strength and return to full sporting capacity with no compromised stability when the contralateral PT graft was used.

In the present study, the length of PT was similar in both limbs and in both sexes. The recorded tendon length for the present population group was about 51.1 mm in the female and about 56.8 mm in the male compared to similar studies on the PT of South Africans of European ancestry e.g. Van Zyl *et al*. (2016) reported an average of 45.7 mm for a male population while Olateju *et al*. (2013) reported an average of about 65.9 mm for both sexes with a significantly higher tendon length in the male. Similarly, Xerogeanes *et al*. (2013) reported a significantly higher tendon length in a male population sample of Americans. The reported mean tendon length in a Swiss population (about 51.9 mm) (Staubli *et al*., 1996) and in the male samples of a South Indian population (about 47.8 mm) (Aithal Padur *et al*., 2021) are within the range that was observed in the present study. The differences in the mean length of tendon across the different studies may also be due to the differences in methodologies, age, race or ethnicity (Gupta *et al*., 2017).

For the widths of the PT, the present study reported no significant differences between the limbs and between sexes for the proximal or distal widths. A common morphology of the PT is that the proximal width is wider than the distal and this is often attributed to the convergence of fascicles towards the midline of the tendon (Yoo *et al*., 2007; Milankov *et al*., 2015; Aithal Padur *et al*., 2021). There was similarly no significant difference between the curved and straight measurements of the widths suggesting that one or both measurements may be adequate for preoperative planning when considering the PT as an autograft. In comparison to other studies, the patellar width of about 25.5 mm of an Arabian population (Hijazi *et al*., 2015) is within the range reported for the distal width in the present study. In a study using CT scans from cadavers of Japanese population, Oikawa *et al*. (2019) reported a mean proximal width of about 29.9 mm and a mean distal width of about 25.0 mm while Aithal Padur *et al*. (2021) reported no significant difference in the proximal width (~ 35.3 mm) or in the distal width (~ 25.5 mm) of both limbs of a male South Indian population.

Similar to the QT in the present study, association between the paired parameters of the PT was tested to determine whether one parameter could be a good predictor of the other. A strong correlation was found between the straight and the curved proximal or distal widths in both limbs and for both sexes. This relationship further shows that one or both measurements is adequate for preoperative investigation of the PT as a possible autograft because one parameter is a good predictor of the other. However, correlation between proximal and distal widths (e.g. CPW_{PT} vs CDW_{PT} or SPW_{PT} vs SDW_{PT}) for both limbs and sexes range from weak to moderate positive correlation. Similarly, a positive moderate correlation was reported in a male South Indian population between the distal and proximal widths (Aithal Padur *et al*., 2021). Interestingly, a strong correlation was found between the SA_{PT} and the LOT_{PT} in both limbs and for the two sexes (similar to the correlation between the similarly paired parameters of the QT in this study). This relationship also indicates that the surface area of the PT can be used to predict the length of the PT when considering the use of this tendon for ACL reconstruction. Furtherance to the predictability of measurements, none of the parameters paired with the LLL showed a strong correlation suggesting that the LLL is not a good predictor of any parameters of the PT.

4.4 Morphometry of the semitendinosus tendon

The ST is also a commonly used graft for ACL reconstruction and a preoperative planning is also important to determine whether the harvested graft is adequate (Hamada *et al*., 1998). Unlike the QT or PT, the entire ST is harvested and it has been demonstrated that the ST can fully regenerate (Eriksson *et al*., 2001; Ferretti *et al*., 2002; Stevanovic *et al*., 2013; Janssen *et al*., 2013; Dziedzic *et al*., 2020). To accurately determine the tendon morphometry, the circumferences at two different points – at the musculotendinous junction and at 3 cm away from its insertion were measured *in situ* to prevent impacting on its morphometry. From the measured circumferences, the diameter of the tendon was derived to calculate the cross-sectional area (CSA) of the tendon according to previous studies where diameter or circumference of a tendon was used to determine the tendon CSA (e.g. Cavaignac *et al*., 2014; Iriuchishima *et al*., 2014). The present study found no significant difference in the CSA in both limbs and in both sexes. CSA was about 21.6 mm^2 in the female and 22.5 mm^2 in the male at the musculotendinous junction while CSA was about 15.3 mm^2 in the female and 17.0 mm^2 in the male at 3 cm away from the insertion. Despite the CSA being higher at the musculotendinous junction than at 3 cm away from the insertion, this difference was not significant.

In some cadaveric studies, an average of 52.0 mm^2 was reported for a Japanese population (Iriuchishima *et al*., 2014) while an average of 38.9 mm^2 was reported for a French population (Cavaignac *et al*., 2014). In both studies, the ST was harvested and then prepared for ACL graft before the measurements were taken and then the CSA derived (Iriuchishima *et al*., 2014; Cavaignac *et al*., 2014). In another cadaveric study on an Australian population, the CSA was derived by multiplying the thickness and width of the tendon after compressing the tendon at the musculotendinous junction to a thickness of about 2 mm and then the width at this point was measured with a calliper, the reported CSA was about 11.45 mm^2 for both sexes (Pichler *et al*., 2008). A MRI study on an Indian population that utilized Osirix software to determine the CSA of a semitendinosus tendon reported an average of 12.0 mm^2 in male and 11.1 mm^2 in female (Raja *et al*., 2020) while a CSA of about 10.1 mm^2 was reported for a Japanese population (Hamada *et al*., 1998). An ultrasound examination in a Malaysian population also reported CSA of about 10.1 mm^2 (Mohd Asihin *et al*., 2018). With these marked differences in the reported dimensions of the CSA, it seems that measurements using MRI, ultrasound or the methodology by Pichler *et al*. (2008) produced a more consistent CSA dimension.

For the length of the tendon, there was no side or sex differences in the measures. The tendon length was about 146.0 mm in the female and about 160.6 mm in the male in the present study. Comparison with previous studies on CSA is summarized in Table 15. The variation in dimensions across the different studies could also be attributed to differences in approaches

Table 15. The literature review of the reported semitendinosus tendon length in different population groups

Population studied	**Age range**	**Approach**	**Measuring tool**	**Definition of parameter**	**Length of ST (mm)**
Indian (Challa and Satyaprasad, 2013)	27.9	Intraoperative	Operative ruler	Length taken from the proximal to the distal end	243.9
Greek (Papastergiou *et al*., 2012)	27.02	Intraoperative	Operative ruler	Length taken from the proximal to the distal end	293.9
Australian (Pichler *et al*., 2008)	71.5	Cadaver	Sliding calliper		263.0
Kenyan (Bundi *et al*., 2016)		Cadaver	Tape rule		298.0
Nigerian (Ashaolu *et al*., 2016)		Cadaver	Vernier calliper	Measured from the distal end of muscle mass to the point where it conjoined to other tendons of the pes anserinus or its distal attachment	116.8
Chinese (Xie *et al*., 2012)	28.57	Intraoperative			279.0
Polish (Dziedzic *et al*., 2020)		Ultrasound on cadaver		Measured from the insertion site to the end of the tendinous fibres	322.3
South Indian (Vadgaonkar *et al*., 2018)		Cadaver	Cotton thread	From the transition zone as tendon to the pes anserinus formation	~154.8
Japanese (Tohyama *et al*., 1993)	62.5	Cadaver	Linear scale	Measured from the tibial insertion to the musculotendinous junction	~235.0
Brazilian (Pereira *et al*., 2016)	31.78	Intraoperative	Ruler	Tendon dissected out and measured from end to end	~287.5
Virginian (Treme *et al*., 2008)	31.45	Intraoperative	Ruler	Tendon dissected out and measured from end to end	~297.5

Thai (Limitlaohaphan *et al*., 2009)		Cadaver			~230.0
South Sulawesi (Sakti *et al*., 2019)	27.23	Intraoperative		Measured end to end after harvested	~249.3
South African (present study)	75.75	Cadaver	Cotton thread	From the MTJ to its insertion at the *pes anserinus*	~152.9

and measurement parameters. The measurement that is closest to the present study is by Vadgaonkar *et al*. (2018) on cadavers of South Indian population which utilized similar approach like in the present study. It is also important to state that although the ST provides an adequate and abundant tendon length that can be used as an autograft in most individuals, but the tendon length differs in both limbs in most cadavers assessed.

Due to the fact that the whole ST is harvested for ACL reconstruction and that there are other hamstring muscles (e.g. semimembranosus and biceps femoris) that can compensate for the lost function of the muscle, from anatomical point of view (although not tested in the present study) it is possible that knee function may still be (to some extent) negatively impacted in the limb from which the tendon is removed and may be worse if an ipsilateral–approach was utilized than a contralateral–approach. It is also worth noting that ST supports the ACL (Biscarini *et al*., 2013) and in the event of an ACL rupture the surgeon must consider the weakening of the medial stabilization of the knee and as such harvesting a ST graft from the contralateral limb will be ideal. This suggestion is backed by von Essen *et al*. (2021) that the strength of knee flexion was significantly weaker in the patients that had their ST harvested from the ipsilateral limb than from those that the tendons on the contralateral limbs were removed. It was also reported that the patients with ipsilateral approach did not recover from a loss of flexor strength within a 2–year assessment period. Using a ST graft from an undamaged–ACL limb facilitates early isokinetic and isometric strength recovery in both limbs without any adverse effects thus it is considered an effective approach to reduce the risk of a long term defect in the ACL–damaged limb (von Essen *et al*., 2021). Surprisingly, McRae *et al*. (2013) reported no measurable benefit or drawback in the quality of life of patients whose ST was harvested from the undamaged–ACL limb.

Similar to the QT and PT in the present study, a strong correlation was found between the paired parameters of the tendon circumferences versus the derived CSA for both sexes and both limbs. This relationship also shows that the circumference close to the tendon insertion can be used to predict the circumference at the musculotendinous junction which is an important relationship to consider during preoperative planning. Similar to the other tendons assessed in this study, the LLL is also not a good predictor of any parameters of the ST. The paired parameters showed a moderate to weak correlation. Limitlaohaphan *et al*. (2009) similarly observed a moderate correlation between the tendon length and length of limb of a Thai population. On the other hand, a strong correlation between the length of limb and tendon length was reported for an Indian population (Gupta *et al*., 2017) and a Chinese population (Chiang *et al*., 2012) while there was no correlation between similar parameters for a Malaysian population according to Mohd Asihin *et al*. (2018).

4.5 Tissue microstructure of the quadriceps, patellar and semitendinosus tendons

The microstructure and composition of harvestable tissues are important in order to determine their suitability as a graft. To further provide additional information that will be useful to surgeons on their choice of graft, the present study compared the tenocyte distribution pattern and the collagen deposition in the QT, PT and ST. The tenocytes and collagen contribute to the strength of tendons which are essential for the success of an ACL reconstruction. From the present observation, the microstructure of the QT, PT and ST were similar and were not different from the previous studies where they are generally composed of closely packed collagen fibres with tenocytes interspersed within the collagen bundles (Hadjicostas *et al*., 2007).

Tendon strength is attributed to collagen deposition and the QT has an advantage over the PT in that it has a more (approximately 20%) collagen deposition (Hadjicostas *et al*., 2007a) which enables the QT to endure a higher load to failure and strength than the PT (Shani *et al*., 2016; Krebs *et al*., 2019). Considering the surface areas of the QT and the PT, the implication of this is that the remaining collagen content after the harvesting of a graft should be sufficient for the QT to function more satisfactorily than the PT with a lesser collagen content due to its surface area. This is evident by reports that showed that the QT produces a better functional and clinical outcome with no serious donor site morbidity than the PT (Hadjicostas *et al*., 2007a; Cavaignac *et al*., 2017). To reiterate, the QT is also considered a biomechanically efficient alternative for ACL reconstruction that is considered safe and reproducible with an abundant harvestable tissue (Staubli *et al.*, 1996; Garofalo *et al*., 2006; DeAngelis & Fulkerson, 2007; Geib *et al*., 2009; Slone *et al.*, 2015; Shani *et al*., 2016). Unfortunately, the present study did not find a significant difference in the collagen distributions of the tendons despite the ST having the lowest collagen distribution in both sexes.

Tenocytes facilitate the healing of tendon and tendon regeneration after harvesting (Hadjicostas *et al*., 2007). Similar to collagen, it is assumed that the QT will fare better in healing compared to the other tendons due to its large surface area and the abundance of tenocytes to initiate a more rapid healing at the donor site and the production of more collagen to sustain the remaining tendon after harvest. The activities of the cells should promote a faster return of knee functions when a QT was harvested compared to the PT or ST. However, the present study found no significant difference in the tenocyte distribution across the tendons in the male population assessed. However, tenocyte distribution was significantly higher in the QT or in the ST than in the PT for the female population assessed. In a combined data by Hadjicostas *et al*. (2007a and b), the tenocyte distribution in the ST is significantly higher than in the PT (Hadjicostas *et al*., 2007b) and the tenocyte distribution in the QT is also significantly higher than in the PT (Hadjicostas *et al*., 2007a). The findings in the two studies by Hadjicostas *et al*. (2007a and b) are similar to the observations found in the female cadavers of the present study.

The disparities in the outcomes of tenocyte distributions in the tendons for both sexes observed in the present study highlight the possible impact of biological differences (e.g.

hormones) on the healing process and the return of knee functions. This thus necessitates further investigation as healing at the donor site and improved knee function has been reported to be better in the male than in the female (Kim and Park, 2015). Factors such as mechanical, hormonal and genetic factors are important factors for increased risk of ACL injuries in female compared to male (Vaudreuil *et al*., 2020) and which are also suggested to play an important role in the healing process and return of knee function at both the harvest site and ACL-reconstructed knee. As described in the literature, the graft used to reconstruct the ACL goes through stages of healing through the processes of remodelling and neo-ligamentization (i.e. cells making new connections at a new site) (Zhu et al., 2012). During these processes, blood supply is re-established and the cell number increases in the re-constructed knee (Zhu et al., 2012). The healing process at the harvest site also occurs in phases (Janssen and Scheffler, 2014). The early healing phase is characterized by a partial graft necrosis owing to the cessation of blood supply after the removal of graft from the harvest site. This process is immediately followed by the proliferation phase (influenced by growth factors) where new cells are formed to replace the dead cells. An increased number of myotenocytes appear and function to restore the *in situ* tension required for ligamentization at a later stage. The ligamentization phase involves establishing an ACL morphology and mechanical strength (Janssen and Scheffler, 2014). The healing process at the graft site is finalized by structural remodelling, cell maturation and collagen synthesis which are influenced by environmental forces e.g. exercise/therapy (Zhu et al., 2012). However, strain is required to trigger the last stage of healing but this has to be done cautiously in order to prevent failing at this early stage because of the reduced strength at the harvest site (Janssen and Scheffler, 2014).

The collagen distribution across the tendons were similar despite the large surface area of the QT. It would therefore be interesting to investigate changes in the collagen distribution at the harvest site of each tendon after an ACL reconstruction. This will shed more light into the healing process of the tendons. However, the present study understands the ethical implication of such study thus the reasons may remain unknown as cadaveric approach may not be suitable for such investigation.

4.6 Conclusion

The cadaveric approach used in this study is considered reliable and reproducible evident by the results of the test of reliability. It is true that a cadaveric approach has many limitations e.g. sample size, tissue shrinkage, population representation etc. but it provides a 3–Dimensional structure from which several measurements can be obtained *in situ* (Olateju *et al*., 2013) and which are of benefit to preoperative planning of knee surgeries. The morphometric profiles of the harvestable tissues of the QT, PT and ST presented in this study may be considered a representative of the morphometric profiles of these tendons in South Africans of European ancestry who are within 46 to 96 years old.

The QT provides an abundant harvestable tissue which is superior to either the PT or ST but surgeons must be cautious as the QT in some individuals may not be adequate as an autograft. When it comes to faster healing and integration of graft, the benefits of the bone–patellar tendon–bone cannot be matched by either the QT or ST. This makes the PT a commonly used graft and a preferred choice especially when a faster return to activity level is desired. However, where the PT cannot be used in an individual (e.g. osteoporosis or other osteological degenerative diseases), then the ST may become the most preferred option. From an anatomical point of view (and in our opinion), the ST is the easiest to harvest and the most convenient due to its position. It is also the least likely tendon to cause a major compromised knee/leg function from the limb the harvest was taken because its function is shared by the semimembranosus and partially by the gracilis and biceps femoris (Drake *et al*., 2015). Most importantly, the ST can fully regenerate to its native capacity. Another advantage of exploring the ST as a graft is that within the immediate vicinity of the tendon there is an option of using the gracilis should in case the initially desired ST in an individual becomes unusable or inadequate upon close examination during surgery (Cavaignac *et al*., 2014; Yeh *et al*., 2018; Goto *et al*., 2020).

Finally, the microstructure of the three tendons discussed in this study are similar thus they are adequate as graft for ACL reconstruction. However, the differences in tenocyte distribution across the tendons in the female is an interesting observation that necessitates further investigation. The findings of this study will contribute to knowledge and will assist orthopaedic surgeons in making an informed decision on the choice of graft.

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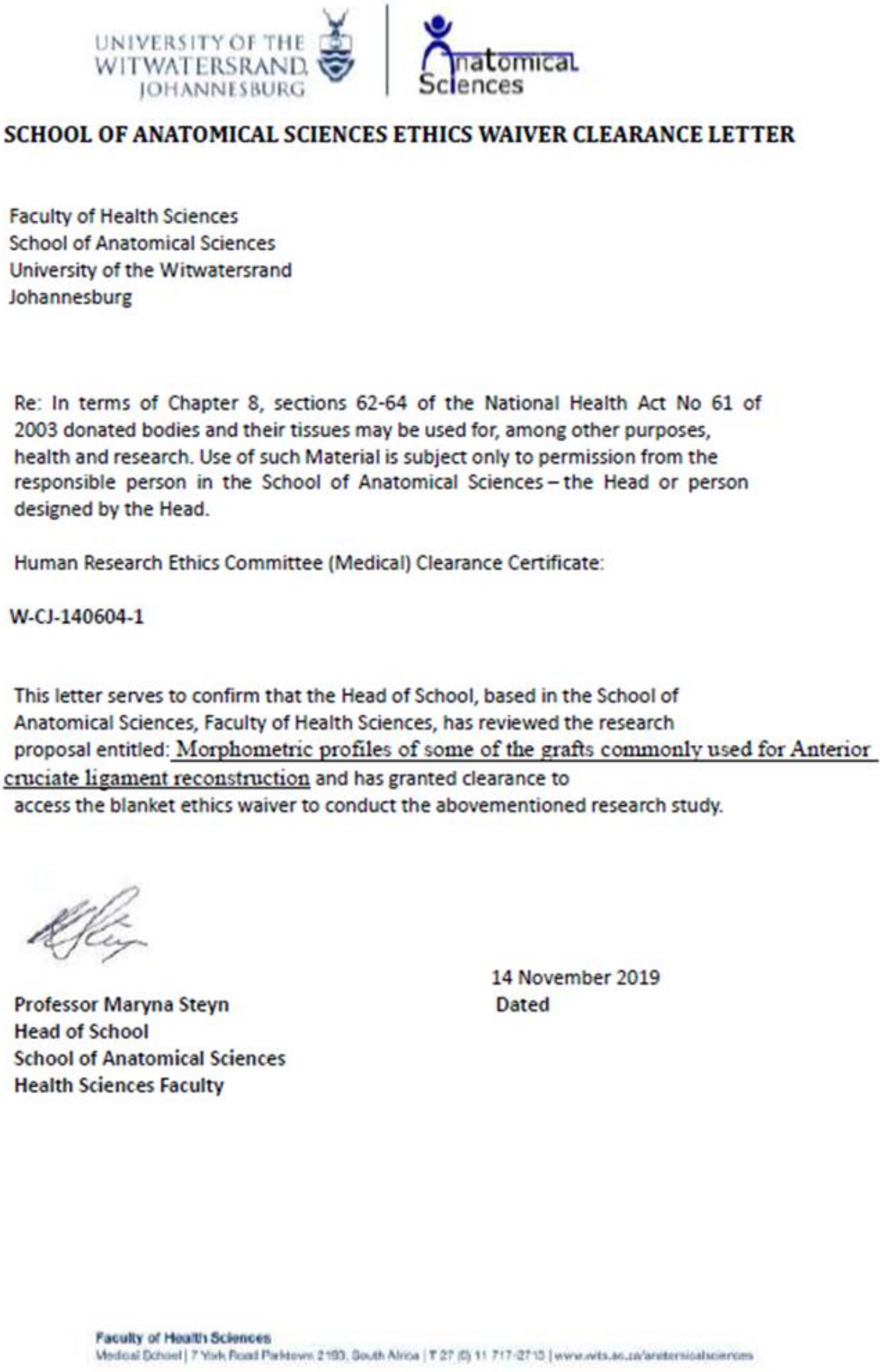
Appendix I – Ethics Waiver Clearance



UNIVERSITY OF THE
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Anatomical
Sciences

SCHOOL OF ANATOMICAL SCIENCES ETHICS WAIVER CLEARANCE LETTER

Faculty of Health Sciences
School of Anatomical Sciences
University of the Witwatersrand
Johannesburg

Re: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

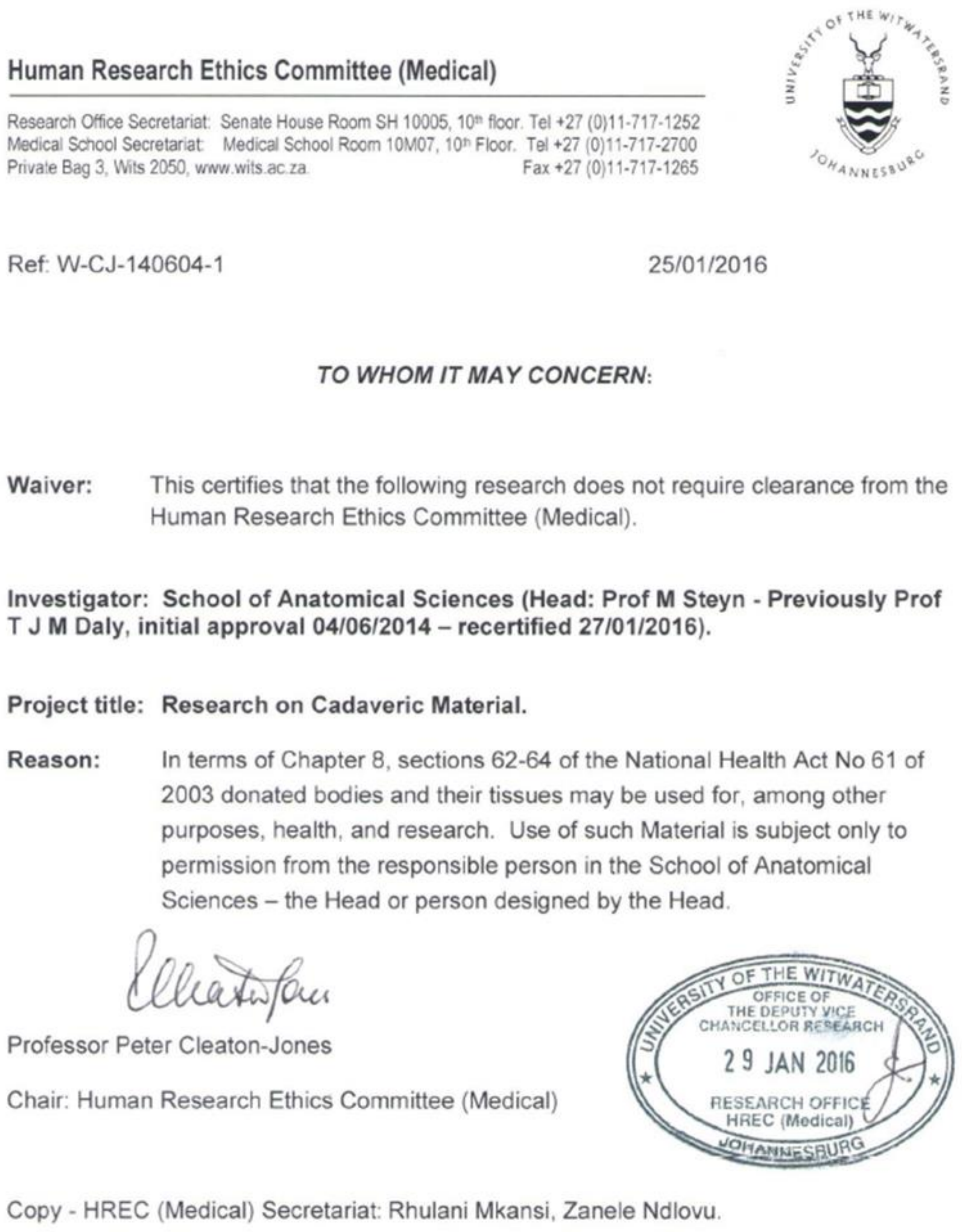
Human Research Ethics Committee (Medical) Clearance Certificate:

W-CJ-140604-1

This letter serves to confirm that the Head of School, based in the School of Anatomical Sciences, Faculty of Health Sciences, has reviewed the research proposal entitled: Morphometric profiles of some of the grafts commonly used for Anterior cruciate ligament reconstruction and has granted clearance to access the blanket ethics waiver to conduct the abovementioned research study.

Professor Maryna Steyn
Head of School
School of Anatomical Sciences
Health Sciences Faculty

14 November 2019
Dated



Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor. Tel +27 (0)11-717-2700
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Ref: W-CJ-140604-1 25/01/2016

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: School of Anatomical Sciences (Head: Prof M Steyn - Previously Prof T J M Daly, initial approval 04/06/2014 – recertified 27/01/2016).

Project title: Research on Cadaveric Material.

Reason: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health, and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

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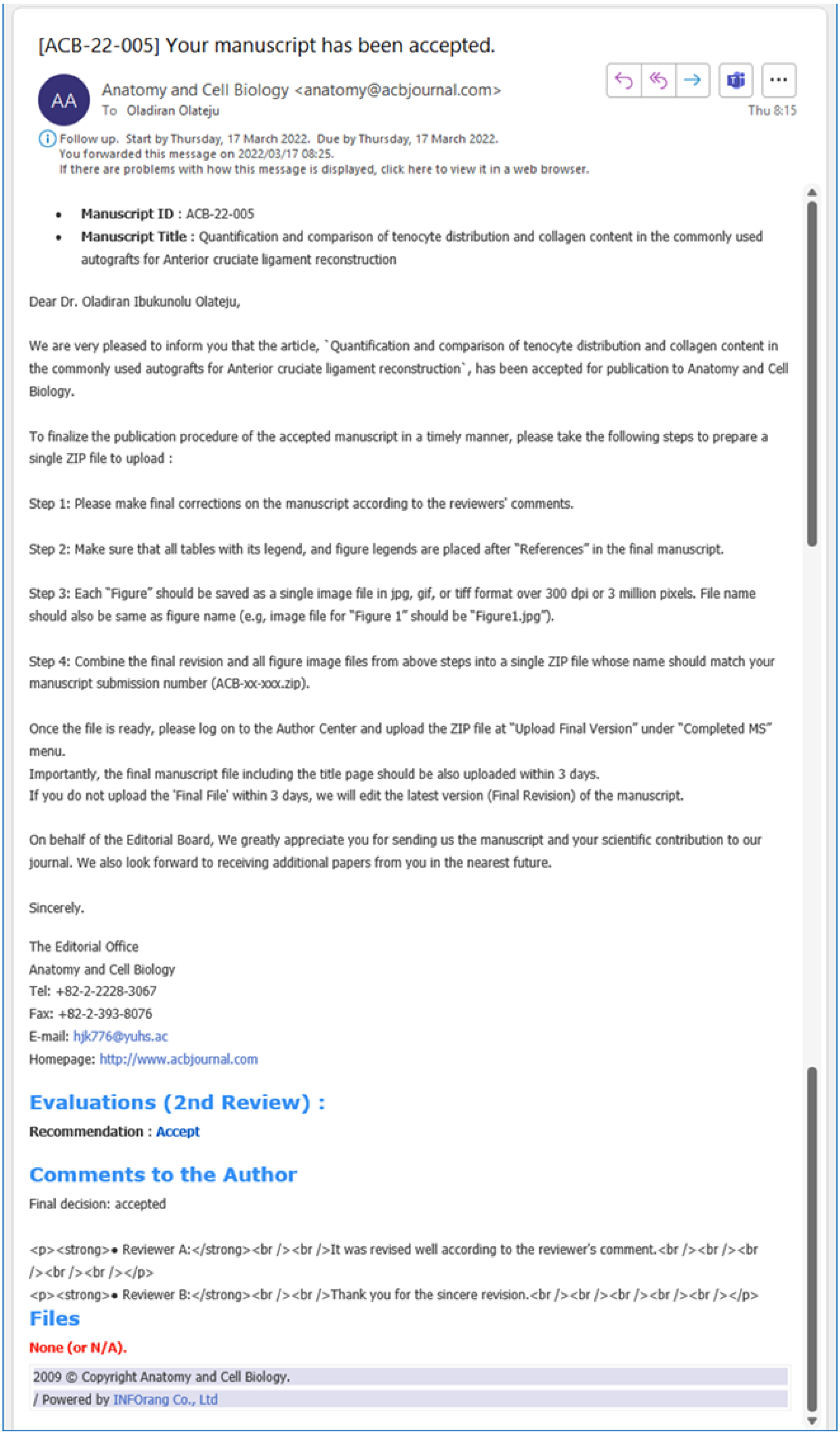
Appendix II – Proofs of manuscripts submitted to journals



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AA Anatomy and Cell Biology <anatomy@acbjournal.com>
To Oladiran Olateju
Thu 8:15

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- **Manuscript ID** : ACB-22-005
- **Manuscript Title :** Quantification and comparison of tenocyte distribution and collagen content in the commonly used autografts for Anterior cruciate ligament reconstruction

Dear Dr. Oladiran Ibukunolu Olateju,

We are very pleased to inform you that the article, `Quantification and comparison of tenocyte distribution and collagen content in the commonly used autografts for Anterior cruciate ligament reconstruction`, has been accepted for publication to Anatomy and Cell Biology.

To finalize the publication procedure of the accepted manuscript in a timely manner, please take the following steps to prepare a single ZIP file to upload :

Step 1: Please make final corrections on the manuscript according to the reviewers' comments.

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Homepage: http://www.acbjournal.com

Evaluations (2nd Review) :

Recommendation : Accept

Comments to the Author

Final decision: accepted

<p>• Reviewer A:

It was revised well according to the reviewer's comment.

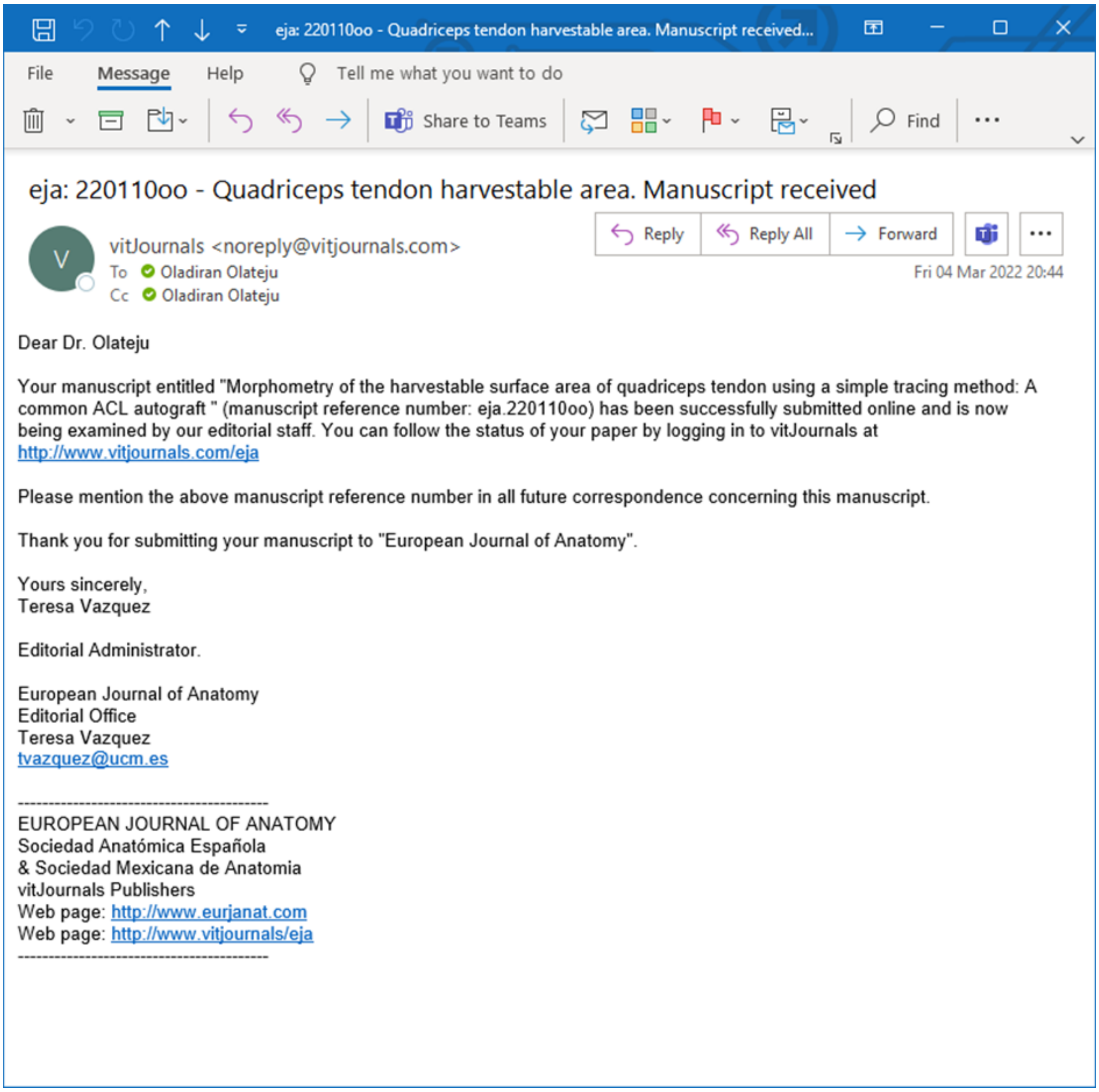
</p>
<p>• Reviewer B:

Thank you for the sincere revision.

</p>

Files

None (or N/A).



eja: 220110oo - Quadriceps tendon harvestable area. Manuscript received

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Fri 04 Mar 2022 20:44

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Yours sincerely,
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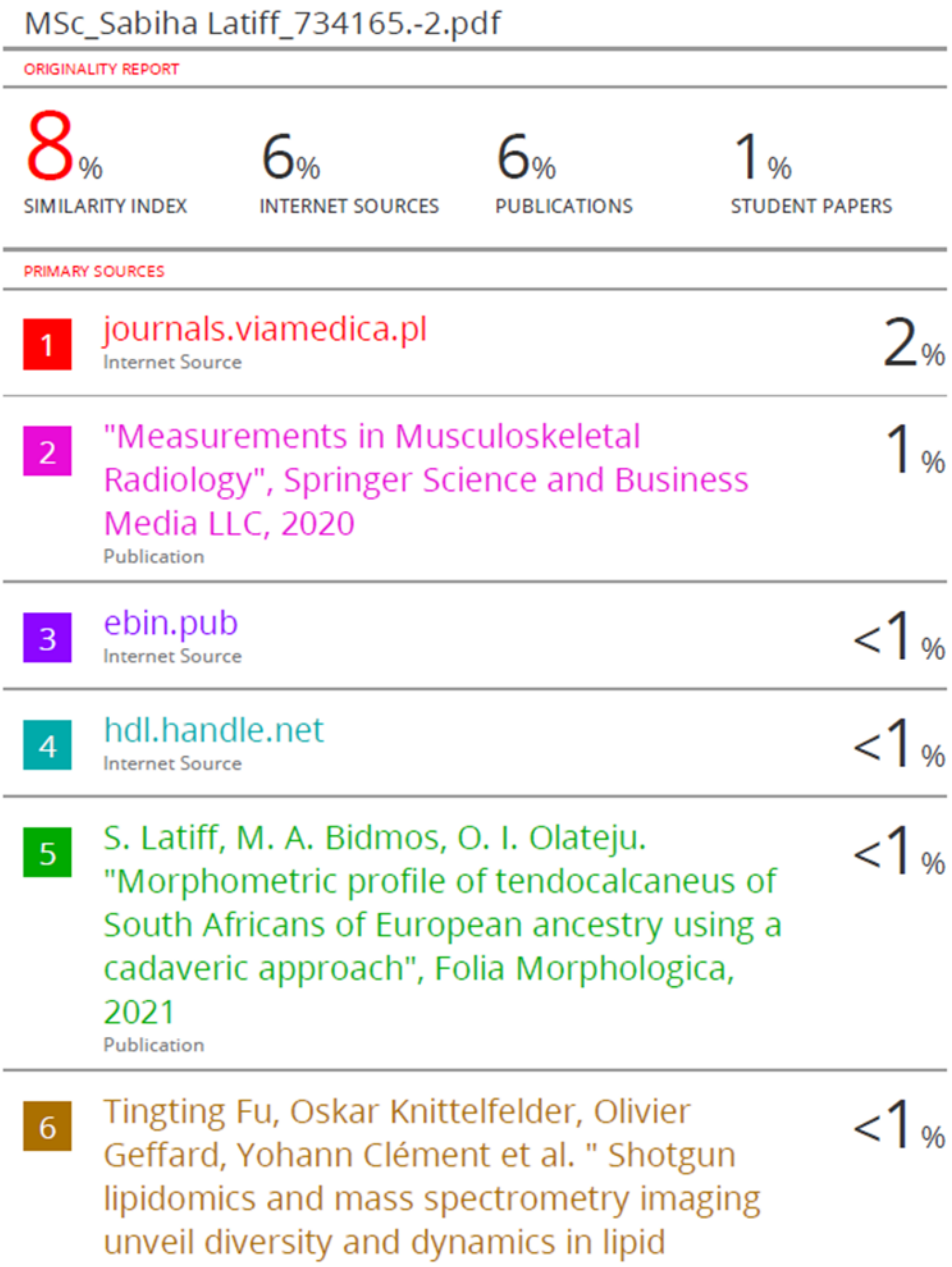
Appendix III – Turnitin Report



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