UNIVERSITY OF CALIFORNIA, SAN DIEGO

Collagen Quantification Across Human Skeletal Muscles

A Thesis submitted in partial satisfaction of the requirements for the degree
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in

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by

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The Thesis of Evie Ya Hui Lin is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

To Christopher L. Berninger, who inspires and supports me to achieve my dreams, he is lovingly dedicated. Chris, I am grateful to be with you for the rest of my life.

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LIST OF ABBREVIATIONS

APL Abductor pollicis longus

BR Brachioradialis

ECM Extracellular matrix

ECRB Extensor carpi radialis brevis

ECRL Extensor carpi radialis longus

ECU Extensor carpi ulnaris

EDC Extensor digiti comunis

EDL Extensor digitorum longus

EDQ Extensor digiti quinti

EHL Extensor hallucis longus

EIP Extensor indicis proprius

EO External oblique

EPB Extensor pollicis brevis

EPL Extensor pollicis longus

FB Fibularis brevis

FCU Flexor carpi ulnaris

FDL Flexor digitorum longus

FDP Flexor digitorum profundus

FDS Flexor digitorum superficialis

FHL Flexor hallucis longus

FL Fibularis longus

FPL Flexor pollicis longus

GC Gracillis

Gmax Gluteus maximus

IM-ECM Intramuscular extracellular matrix

IO Internal oblique

LG Lateral gastrocnemius

MG Medial gastrocnemius

MMP Matrix metalloproteinase

PCSA Physiological cross sectional area

PL Palmaris longus

PLT Plantaris

PQ Pronator quadratus

PS Psoas

PT Pronator teres

QL Quadratus lumborum

RA Rectus abdominus

SOL Soleus

SR Sartorius

SU Supinator

TA Tibialis anterior

TIMP Tissue inhibitors of metalloproteinase

TP Tibialis posterior

TrA Transverse abdominus

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ABSTRACT OF THE THESIS

Collagen Quantification Across Human Skeletal Muscles

by

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Intramuscular connective tissue provides structural stability and facilitates force transmission in skeletal muscle. Additionally, it contains extracellular matrix that is crucial for muscle development and regeneration[1]. Alterations of collagen content within intramuscular connective tissue have been associated with aging or diseased muscle [2,3]. Data of baseline collagen content among different muscles, to provide deeper understanding of normal muscular functions, does not exist. Hence the aim of the current study is to quantify collagen content across one hundred skeletal muscles in order to elucidate intrinsic specialized functional roles of skeletal muscles. Muscle biopsies were obtained from 6 fresh cadavers (n=599). Hydroxyproline assay was performed to determine collagen content[4] in muscle samples measured in µg of collagen per mg of muscle. Differences in mean collagen content were analyzed within muscle regions and by muscle functions. Additionally, previous muscle architectural data was used to

examine the correlation between collagen content and muscle architectural properties [5-10]. Results of the current study demonstrated significant differences in mean collagen content of muscles in different regions, but no correlation between collagen content and muscle architectural properties. Average collagen content was high in the body trunk and in the upper and lower extremities. Regions close to the trunk had low collagen content, and regions in between the trunk and extremities exhibited intermediate collagen content.

Introduction

Skeletal muscles are dynamic and adaptive tissues that exhibit viscoelastic properties. That is, they exhibit diverse viscous behaviors depending on the rate of applied stretch, and elastic behaviors depending on the load of applied stretch. Passive stiffness is a term describing the physiological response of a specific muscle and can be defined as the slope of the stress-strain curve of a skeletal muscle during passive stretch. Stress is related to the strength of a material, which can be defined as the load applied on a material (i.e. force per unit of cross-sectional area). Strain describes the deformation of a material relative to the stress that is applied. Thus a stiff material will have a steep slope, which implies high passive stiffness, since it can withstand higher stress and is not easily deformed. Conversely, a compliant material will have a lower slope value (i.e. decreased passive stiffness), and thus it is easily deformed. The passive stiffness of a specific muscle is influenced by several factors when stretched passively under resting condition. One of the factors that influences passive stiffness of a particular muscle is the elasticity of inter- and intramuscular connective tissues within and surrounding the muscle belly [11].

Intramuscular connective tissue serves important physiological functions and offers dynamic adaptations to various mechanical stresses and biological demands in the musculoskeletal system. Intramuscular extracellular matrix (IM-ECM) consists mostly of collagen proteins and provides many crucial functions to maintain normal muscle function. In the events of mechanical loading, intramuscular connective tissues contribute passive stiffness and tensile strength to skeletal muscle as well as lateral force

transmission across muscle fibers and fascicles [12]. Additionally, IM-ECM is an important site of mechanotransduction in which intracellular signaling is initiated due to mechanical stimulus acting upon a cell, allowing skeletal muscle to adapt to mechanical challenges [13]. The ECM of skeletal muscle is composed of three interconnected layers. The outermost epimysium layer surrounds each muscle and is connected to the tendons that join the muscle and bone [14]. The intermediate perimysium layer surrounds muscle fascicles and extends further to the myotendinous junction where it joins the tendon [15]. Endomysium is the inner connective tissue layer that surrounds muscle fibers within fascicles [16]. Collagen types I, III, IV, V, VI, VII, XI, XII, XIV, XV, and XVIII have been identified among the various isoforms of collagen in skeletal muscle [17,18]. Previous studies show relatively even distribution of type III collagen in endomysium and epimysium and type I collagen as the major component in perimysium [19,20]. Type I collagen affects viscoelastic properties of muscle by conferring tensile strength [21,22], while type III collagen confers compliance [23]. The perimysium may be continuous with the tendon since both structures contain mainly type I collagen and decorin as the primary proteoglycan, whereas the epimysium and endomysium consist of approximately the same amount of type I and type III collagen along with diverse populations of proteoglycans [18]. The perimysium exhibits a unique morphological arrangement compared to epi- and endomysium, due to the presence of crimp patterns in the criss-cross lattice orientation and larger diameter of collagen fibers. Muscle contraction or stretch causes the angles of lattice to change with respect to the direction of muscle fiber; these can range from approximately 80° to 20 °. The crimp formation can be straightened out at long or short sarcomere length during muscle activation but increases when muscle

relaxation occurs [24]. The distinctive structural properties of perimysium allow it to serve as a load-bearing structure that prevents muscle fiber bundles from becoming overstretched and increases passive stiffness of muscles [25]. Thus, although the three connective tissue layers surrounding the muscle belly have been shown to provide resistance when a muscle is passively stretched, studies have shown that the perimysium may contribute the greatest resistance due to the orderly patterns of crimps across muscle fascicles and presence of large amounts type I and III collagen in the ECM [11]. As a result, it is possible that differences in relative content of connective tissue are coupled to specific roles and functions of a particular muscle [2].

Loss of normal muscle function has been associated with alterations of collagen content in aging or diseased skeletal muscle [3,26]. A decrease in the rate of collagen remodeling in aging muscles alters muscle tensile properties, causing an increase in passive stiffness [27]. Previous studies demonstrated an increase in collagen content in dystrophic, denervated, and immobilized muscles [28]. Elevation of collagen mRNA expression and a subsequent rise in collagen deposition in intramuscular tissues occurs in dystrophic muscle as a reparative process [29], leading to increased passive stiffness and compromised contractility [30]. Alterations in intramuscular tissues in muscles immobilized at short length have been well defined. Such changes include an increase in the thickness of endomysium and perimysium, resulting in greater separation of muscle fibers and a decrease in the capillary density in immobilized muscles [31]. Muscles immobilized in shortened positions exhibit a greater amount of connective tissues, which may be the cause of increases in passive elastic stiffness [32]. A decrease in muscle cross-section area

and/or muscle fibers has been shown to correlate with the abnormal muscular functions described above [28,31] and to decrease the force that a specific muscle can generate [33].

Previous architectural studies of muscle have mainly focused on measurements of muscle tissue without the presence of connective tissue. For example, measurements of physiological cross-sectional area (PCSA), pennation angle, muscle fiber length, muscle length, and muscle mass were reported for muscles across different regions [6]. The importance of diverse collagen isoforms and their exact functional implications in human skeletal muscle have not been well elucidated [34,35]. Deposition or removal of collagen networks or cross-linking constituents that are intimately associated with skeletal muscle can greatly affect normal muscular functions [36]. Hence, determination of normal collagen content across human skeletal muscles is critical for examining normal versus diseased muscle functions. No data exist from normal collagen content in human skeletal muscles. Having this information would contribute to a better understanding of the functional role and architectural properties of skeletal muscle. Thus the aim of the current study is to quantify collagen content across one hundred different fresh human muscles, which will provide estimates of average collagen content in order to correlate unique architectural properties and specialized intrinsic functional roles of different muscles.

Materials and Methods

Muscle biopsies were obtained from dissections of three male and three female (n=6) fresh human cadavers (Supplemental table 1). The donors were an average age of 83 years old and were participants of the Anatomy Bequest Program at the University of Minnesota. Several exclusion criteria were used to select donors, and these included history of spinal cord injury, muscular dystrophy, stroke, polio, myasthenia gravis, or Guillain-Barre syndrome. Biopsies of approximately 5 x 5 x 20 mm in size were obtained by dissecting the midpoint between the proximal and distal muscle-tendon junctions in muscles with a linear shape, or the approximate geometric midpoint in muscles with complex shape (e.g. trapezius, latissimus). Biopsies were obtained from each head in muscles with multi-head (e.g. biceps brachii, gastrocnemius). For repeated muscles in the hands such as lumbricals, dorsal interossei, and palmar interossei, biopsies were obtained from multiple locations. For digital flexors and extensors, one biopsy was obtained as a representative of the whole muscle. A total of one hundred biopsies were collected from each donor with the exception of opponens digit minimi in one donor (n=599 total muscle biopsies).

The protocol of hydroxyproline determination in skeletal muscle was modified from Woessner's (1961) original protocol [37]. L-hydroxyproline was used to make the hydroxyproline stock solution at a concentration of 100µg/mL. On the day prior to experiment, 20-25 mg wet weight of each individual muscle was weighed out and cut into small sections in the cryostat (-20°C), then placed in a glass tissue culture tube. Each muscle sample was hydrolyzed using 1 mL of 6 N HCl and placed in an oven set at 110°C for approximately 18 hours. In addition, the chloramine T and p-

Dimethylaminobenzaldehyde (pDAB) solutions were made a day prior to the experiment. The choloramine T solution was made by mixing 0.3525g of Chloramine T (Sigma Aldrich), 20 mL of pH 6 buffer, and 2.5 mL of isopropanol. The pH 6 buffer contained 17 g of NaOH, 25 g of Citric acid monohydrate, 60 g of sodium acetate trihydrate, 6 mL of glacial acetic acid 150 mL of isopropanol. It was brought to a final volume of 1 liter with distilled water and the pH was adjusted to 6.0. The pDAB solution was prepared by combining 3.75 g of pDAB, 15 Ml of isopropanol, and 6.5 mL of 60% perchloric acid. On the day of experiment, the hydroxyproline stock solution was diluted to 10µg/mL working solution. The working solution was further diluted to generate a hydroxyproline standard with a hydroxyproline concentration ranging from 0-7 µg/mL. The muscle samples were removed from the oven and neutralized to pH between 6.98-7.04 with 2.5M NaOH, 0.5M HCl, and 0.05M NaOH. Each muscle sample was diluted to the final volume of 6mL after neutralization, and 1 mL of sample was transferred to a new tube for assay. Subsequently, 0.5 mL of chloramine T solution was added to the hydroxyproline standard and sample tubes and they remained at room temperature for 20 min. Next, the pDAB solution of 0.5 mL was added and each tube was vortex until the schlieren disappeared. Both the standard and muscle sample tubes were incubated at 60°C water bath for 30 min. 150 µl in triplicate was transferred from each tube to a 96 well plate to measure the absorbance at 550 nm. The amount of hydroxyproline determined from the assay was converted to µg of collagen per mg of muscle sample by multiplication by the factor of 7.46 according to Neuman and Logan [38].

Data were analyzed using one-way analysis of variance with Bonferroni *post hoc* test of muscle groups divided by region and by function. Student's t-test was used to

compare anti-gravity versus gravity muscles and single-joint crossing versus multi-joint crossing muscles. Multiple linear regression was performed to examine the correlation between age, gender, and collagen content. Data was considered statistically significant at $p < 0.05$. Data of PCSA, muscle fiber length, muscle mass, and pennation angle from previous architectural studies were used in conjunction with data from current study to conduct multiple linear regression analysis (p-value < 0.05) to determine the strength of associations [5-10].

Results

The mean collagen content of different muscles varied greatly with up to a six-fold difference between iliacus and spinalis thoracis (Figure 2; Supplemental table 2). The average collagen content of each muscle is listed on supplemental table 2. Result of the one-way ANOVA showed significance in collagen content variation between different muscle regions ($p < 0.001$). Specifically, hand and hip muscles exhibited greater significance compared with other muscle regions (Table 1). The hand muscles had the highest average μg collagen per mg of muscle (17.41 μg/mg), whereas the hip muscles contained the lowest average μg collagen per mg of muscle (9.51 μg/mg). In the upper body, the axial (14.32 μg/mg) and shoulder (13.93 μg/mg) muscles exhibited relatively high mean collagen content compared to the hand muscles. The arm muscles (11.94 μg/mg) had the lowest mean collagen content in the upper body. In the lower extremity, the leg muscles contained the highest average collagen content (13.24 μg collagen/mg muscle) compared to the thigh muscles (10.91 μg collagen/mg muscle) and the hip muscles (9.51 μg/mg) (Figure 1). The average collagen content also varied between different muscles functions ($p < 0.05$). In particular, the external rotators (10.31 μg/mg) was statistically significant to abductors (14.67 μg/mg), and extensors (13.63 μg/mg) (Table 2). The external rotator contained the lowest average collagen content compare to other functional muscle groups (Figure 3).

The difference between the average collagen content of anti-gravity and gravity muscles was not statistically significant ($p > 0.05$), though anti-gravity muscles (13.30 μg/mg) contained slightly higher mean average collagen content compared to the gravity

muscles (12.93 μg/mg) (Figure 4). The differences in average collagen content between single-joint and multi-joint crossing muscles observed were significant ($p < 0.05$) with the multi-joint crossing muscles containing slightly higher mean collagen content (13.78 μg/mg) compared to the single-joint crossing muscles (12.50 μg/mg) (Figure 5).

Results of linear regression showed correlation between gender and collagen content ($p < 0.001$), but no correlation between age and collagen content ($p > 0.5$) (Table 3). Additionally, the result of multiple regression analysis of average collagen content as criterion and PCSA, muscle fiber length, pennation angle, and muscle mass was not statistically significant ($p < 0.05$) (Table 4, Figure 6-9).

Discussion

The purpose of this study was to determine the collagen content of one hundred human skeletal muscles, in order to provide baseline data to further understand the functional roles of skeletal muscles. One limitation of the current study is that the collagen content measured in the muscles of elderly subjects may not be representative of normal collagen content in muscles of younger adults, but it provides an approximation of normal collagen content across human muscles. Previous studies showed an increase in advanced glycation end products (AGEs) in aging or diseased (e.g. diabetes) connective tissue reduces the rate of collagen turnover, and thus greatly increases the stiffness of tendon and IM-ECM structure. Thus accumulation of AGEs can greatly hinder the adaption to altered loading in tendons and IM-ECM [39,40]. The current study showed a significant variation of collagen content in different muscle regions. Particularly, mean collagen content was highest in the hand, shoulder, and axial regions followed by forearm and leg regions, and subsequently the arm and thigh regions. The hip muscles had the lowest collagen content. Interestingly, no correlation was found between the mean ECM content and various skeletal muscle architectural components (i.e. PCSA, muscle fiber length, pennation angle, and muscle mass) from previous architectural studies. Hence, the mean collagen content differences in various muscle regions may be due to the unique functional roles of specific muscles in each region [36]. The IM-ECM provides structural stability and serves as an important biological mechanotransducer, and is able to adapt to mechanical loadings depending on the functional demands and mechanical environment of a tissue [36,41]. Previous studies of passive elasticity measurements revealed that the intramuscular connective tissue could

prevent the overstretching of non-active muscle by the antagonist muscles or external forces [42]. Specifically the tensile properties of the intramuscular tissues accommodate shear strains due to changes in muscle shape during contractions [43], and thus provide structural stability and sufficient passive stiffness during active contractions or passive movements of muscle. Depending on the functional role, skeletal muscles undertake different levels of mechanical load during daily activity. Kadi and colleagues demonstrated that the satellite cells, the myogenic stem cells in skeletal muscle, are activated in skeletal muscle injury, certain pathological conditions, and muscle hypertrophy development in specific physiological conditions [44]. The activation of satellite cells in skeletal muscle is associated with an increase in collagen synthesis in the intramuscular connective tissue [45]. An increase in mechanical loading due to endurance and acute exercise had been shown to elevate collagen expression, synthesis, and accumulation in IM-ECM of skeletal muscle [46] as part of the repairing process. However, an increase in collagen synthesis may happen without muscle damage. Thus collagen content may be affected by the level of mechanical loading of muscles in different regions depending on their functional role [29].

The hand muscles contained the highest collagen content compared to other muscle regions. The high collagen content of hand muscles may be related to the functional biomechanical properties of hand muscles. The hands are capable of engendering fine motions and complex prehension patterns such as precision thumb-finger pinch grip (i.e. key pinch), passive palm pinch grip, and power grip [47]. Previous architectural studies of hand muscles demonstrated that hand muscles possess the most unique architectural properties in comparison to other muscle regions. Generally the

intrinsic hand muscles are designed to generate a wide range of excursion with high velocity production. Thus higher stiffness may provide stability during high excursions with great velocity. Additionally, higher stiffness also provides greater structural stability in hand muscles, which may promote precise and fine movements of hand. Previous studies done by Passerieux and colleagues demonstrated that the perimysium may serve as the mechanical linkage between the tendons and muscle fibers and the specialized "perimysial junction plate" may enhance the role of perimysium, serving as the mechanical linkage and enhancing lateral force transmission [14,15]. Hence elevated collagen content in the hand muscles may reflect the presence of a large amount of perimysium, which serves as an intimate mechanical linkage between the tendons and muscles in order to facilitate fine and precise hand movements.

The average collagen content of forearm muscles and leg muscles is almost identical. This result is unexpected since the primary function of lower limb muscles is to provide locomotion and balance whereas the primary function of wrist muscles is much more complex. The major functional role of forearm muscles is to provide movement of digits and wrist. Additionally, the forearm muscles bear the load from hand muscles during daily tasks. Thus higher stiffness prevents shear strains in forearm muscles due to their high excursion potential and provides stability in movements of digits and wrist [5]. The current study showed that the digit extensors extensor digiti quinti (EDQ), extensor digitorum comunis (EDC), extensor indicis proprius (EIP), extensor pollicis brevis (EPB), and extensor pollicis longus (EPL) have higher collagen content compared to the digital flexors flexor digitorum profundus (FDP), flexor digitorum superficialis (FDS), and flexor pollicis longus (FPL). A previous architectural study demonstrated that the

digital extensors have higher excursion potential, higher collagen content increases the stiffness and may provide stability and prevent shear stresses arising from high excursions to induce muscle strain in the digital extensors[6]. Higher stiffness was observed in the FDP and FDS tendons [48], and therefore greater passive compliance in the digital flexors may facilitate fine controls of finger positions and generation of a powerful grip. The collagen content of wrist flexors and extensors does not vary much with the exception of flexor carpi ulnaris (FCU) and extensor carpi ulnaris (ECU). A previous study showed that the FCU has a much more compliant tendon compared to FCR, and the tendon of ECU has greater compliance compared to extensor carpi radialis brevis (ECRB) and extensor carpi radialis longus (ECRL). Thus higher collagen content in the ECU and FCU may compensate for the greater compliance of their tendons and provide stability of wrist joint movement [49]. As a strong force generator at the thumb [6], the abductor pollicis longus (APL) has the highest mean collagen content among the forearm muscles. Higher stiffness in APL may provide stability during a power grip, as well as the precise control of the thumb. The brachioradialis (BR) contains higher collagen content compared to pronator teres (PT) and pronator quadratus (PQ). The BR serves as a weak flexor but enhances the excursion potential of forearm [6]. As a result, higher stiffness in the BR may increase the endurance of shear stress in BR during a wide range of excursion. The PT acts as a powerful flexor and pronator of forearm. Therefore greater passive compliance and lower collagen content in PT may better facilitate the movements of the forearm at the elbow joint. The PQ assists the pronation of the forearm by PT [6], and thus one would expect PQ to contain higher collagen content to provide mechanical linkage between the radius and ulna. Interestingly, the PQ has the lowest collagen content

among forearm muscles. Further study will need to be conducted in order to provide better understanding of PQ and its interaction with ECM. The anconeus has the highest collagen content in the arm muscle regions. It is a small muscle that is located between the lateral epicondyle of humerus and superior part of posterior ulna surface [50]. The exact functional role of anconeus remain unclear but it is generally regarded as a stabilizer of the elbow joint, and it assists the triceps in extending the forearm and abduction of ulna during pronation [50,51]. Consequently, higher stiffness may better accommodate the functional role of anconeus serving as the stabilizer for movements of the elbow joint. The palmaris longus (PL) has similar collagen content as the digital flexors. It is a weak wrist flexor but can assist in the cupping of the palm since its fibers extend into the palmar fascia [52]. As a result, greater passive compliance in PL may facilitate the tensioning of the palmar aponeurosis. The supinator (SU) muscle has the highest collagen content compared to other internal rotators PQ and PT in the forearm. The SU supinates the forearm as its name implies and also rotates the radius anteriorly or superiorly when the elbow is flexed [50]. Repetitive movements of forearm rotations, wrist flexion with pronation, or wrist extension with supination have been demonstrated to cause an increase or pathological thickening of SU muscle, leading to the development of radial tunnel syndrome [53,54]. Hence higher collagen content in SU may provide stability during supination, or may be caused by the thickening of SU due to repeated supination of forearm.

The arm muscles have the lowest average collagen content compared to other muscle regions in the upper body. The primary function of arm muscles is to flex or extend the arm at the elbow. The arm muscles usually do not experience strong

mechanical loading except in instances such as heavy weight training, which may lead to muscle tear and upregulate collagen remodeling [29]. The biceps brachii and brachilalis are powerful flexors of the arm. The coracobrachialis serves a synergistic muscle to assist in flexion and adduction of the arm and stabilization of the glenohumeral joint [50]. Conversely, the triceps brachii is the chief forearm extensor. Based on their functional roles, lower collagen content in the arm muscles may be an advantageous characteristic since higher stiffness may hinder movements such as flexion-extension and pronation-supination at the elbow joint.

The mean collagen content of shoulder muscles is relatively high compared to the forearm and arm muscles, and is close to the average collagen content of axial muscles. Many of the shoulder muscles provide glenohumeral stability, which is similar to many axial muscles that provide stabilization for the upper body. The deltoid and teres major have the highest collagen content among scapulohumeral muscles. The deltoid serves as a stabilizer during abduction [55], and is a powerful abductor of the arm. Similarly, the teres major is an important stabilizer during movement but acts as a strong adductor and rotator of the arm [56]. Additionally, protractions of the pendulum swinging motion of arms during normal walking are initiated by the synchronized coordination of the deltoid, upper latissimus dorsi, teres major, supraspinatus, and upper trapezius [57]. Thereby higher collagen content in the deltoid and teres major may provide sufficient passive stiffness in order to fulfill their functions as stabilizer in the movement of the arm. The scapulohumeral muscles include the deltoid, teres major and minor, supraspinatus, infraspinatus, and subscapularis. They are a group of short muscles that act on the glenohumeral joint [50]. Four of the scapulohumeral muscles are collectively known as the

rotator cuff muscles (i.e. supraspinatus, infraspinatus, teres minor, and subscapularis) since they form a musculotendinous rotator cuff around the glenohumeral joint to protect the joint and provide stability. The rotator cuff muscles contain relatively lower collagen compared to other shoulder muscles. Previous architectural studies of rotator cuff muscles demonstrated that their unique architectural properties are suitable for force production. The subscapularis is a powerful force generator among the rotator cuff muscles and contributes maximum passive tension at the maximum abduction and lateral rotation, indicating its stabilizing role in apprehension position [50]. The infraspinatus and supraspinatus are important stabilizers for the glenohumeral joint at rest since they are constantly under relatively high passive tension at rest[9]. An increase in collagen synthesis and content in the rotator cuff muscles is observed alongside rotator cuff tendinitis and rotator cuff tear as part of the pathogenesis [58], resulting in much higher passive stiffness in the shoulder. This evidence suggests high stiffness in the rotator cuff muscles may be associated with abnormalities in the rotator cuff muscles and may not be an adaptive characteristic for their functional role. Results of the current study showed higher collagen content in the extrinsic shoulder muscles (Trapezius, latissimus dorsi, and rhomboid major and minor) compared to the intrinsic shoulder muscles. In particular, the latissimus dorsi contained the highest collagen content, which may be related to its complex multifunctional mechanical role. It serves as a powerful extensor and adductor of the shoulder and extensor and lateral flexor of the back [59]. Additionally, the latissimus dorsi has a wide range of attachment from the trunk to the humerus and linkage to diverse aponeurosis and myotendinous junctions [50]. Higher collagen content in the latissimus dorsi may provide greater stability and intramuscular force transmission to better

accommodate its diverse mechanical functions [14]. A previous study showed higher excursion potential in the trapezius and rhomboid major and minor muscles and thus higher collagen content may prevent muscle strain due to shearing stress resulting from greater excursion potential. The anterior axioappendicular muscles form close association with the scapula and thoracic wall and assists in the movements of scapula. In particular, the seratus anterior connects the scapula close to the thoracic wall, and this attachment enables other muscles to attach to the scapula as a fix bone for movements of the humerus [50]. The pectoralis major participates in the adduction and medial rotation of humerus and assists in the movements of scapula anteriorly and inferiorly. The pectoralis minor stabilizes the scapula by drawing it inferiorly and anteriorly against the thoracic wall and extending when the arms elevate to allow external and upward rotation of scapula [60]. Results of the current study show pectoralis major and serratus anterior have higher collagen content than pectoralis minor. Based on the mechanical function, higher collagen content in pectoralis major muscle may be advantageous in order to prevent rupture of muscle since it is responsible for the movements of the scapula. Higher stiffness in serratus anterior may be suitable for its role in linking the scapula close to the thoracic wall. The pectoralis minor serves a supportive role by assisting in the stabilization of scapula and arm movements, and tightness in pectoralis minor is often associated with deviation of normal posture as seen in shoulder impingement [61]. Therefore lower stiffness in the pectoralis minor may be a suitable characteristic in order to fulfill its functional role.

The axial muscle region contained the second highest mean collagen content in the upper body. This result correlates with the mechanical functions of axial muscles,

since they support the upper body by transmitting shearing and compressive forces to the lower extremities during everyday activities [62]. The major lumbar extensors are longissimus and iliocostalis lumborum, with unique oblique orientations to support the anterior shearing stress resulting from forward flexion of the torso [63]. The current study showed that the longissimus and iliocostalis lumborum contained the highest collagen content compared to other paraspinal muscles. This higher collagen content may be a favorable characteristic in order to promote stabilization and to prevent shear strain from occurring in longissimus and iliocostalis lumborum. Interestingly, the spinalis thoracic, which resides nearest to the spine, contained the highest collagen content compared to other erector spinae muscles. A possible explanation would be that higher passive stiffness in spinalis thoracis may provide greater stability in accommodating spinal curvature and movements of vertebral column during rotation, flexion, and extension. The multifidus acts as a dynamic lumbar spine stabilizer and as an extensor of vertebral column along with the erector spinae muscles. A previous study examined the passive mechanical properties of multifidus and showed a much higher elastic modulus of multifidus at the fiber bundle level, which provided evidence that the higher elastic modulus of multifidus may arise from its extracellular matrix properties [64]. However, collagen content of multifidus proved lower than longissimus and iliocosalis lumborum in this study, suggesting other factors may contribute to the greater mean collagen content in multifidus. In the abdominal wall region, the transverse abdominus (TrA) showed the highest collagen content compared to external oblique (EO), internal oblique (IO), quadratus lumborum (QL), and rectus abdominus (RA). The TrA primarily supports and compresses the abdominal viscera [65]. Thus higher collagen content in TrA may be a

desirable property in its role in supporting the abdominal viscera. The QL has the second highest collagen content. The QL resides in close proximity to iliopsoas and aids in the lateral flexion of vertebral column and stabilizes the 12th rib during inspiration [50]. Hence QL serves a similar role in stabilizing the abdominal viscera, as the TrA and higher collagen content may be a suitable characteristic for its functional role. The EO and IO have complementing functional roles to flex and rotate the trunk. Specifically, the EO possesses greater excursion potential, whereas the IO has greater force production potential [65]. Hence greater stiffness in EO and IO may be favorable to stabilize the torso during flexion and/or rotation of a trunk. The RA contains similar collagen content as the EO and IO. It stabilizes and controls the tilt of the pelvis, and serves similar functional roles as other abdominal muscles such as flexion of the trunk and compression of abdominal viscera. Hence higher stiffness may facilitate the multifunctional role of RA.

The hip muscles contained the lowest collagen content compared to other groups of muscle. Muscles around the hip joint provide motion and stability for the hip and upright posture, as well as to prevent excess bending stress acting on the femur [66]. The iliopsoas muscle group functions as the primary hip joint flexor in which the psoas acts inferiorly in conjunction with the iliacus to flex the thigh. The psoas (PS) also flexes the trunk inferiorly when sitting and acts superiorly to flex the vertebral column to balance the trunk [50]. Additionally, previous studies examining the architecture of PS demonstrated similar passive mechanical properties and average fiber length between PS and other paraspinal muscles such as longissimus and iliocostalis. This evidence suggests that PS may also serve as a lumbar stabilizer and provide support for upright posture [67]. The iliacus stabilizes the hip joint along with piriformis, obturator internus, and superior

and inferior gemelli [50]. The primary functions of gluteus maximus (Gmax) involve stabilization, extension, and lateral rotation of the thigh, and aids in rising from sitting position and the initial contact of stance phase during a gait cycle [50]. The Gmax is especially active during climbing, running, or activities that involve stabilization of the trunk against flexion [68]. Passive compliance of hip muscles plays an important functional role in normal gait movements since it contributes to the passive joint-moment arm and facilitates the power bursts arising from mid stance to initial swing [69]. Conversely, elevation in passive stiffness, which is often associated with an increase in intramuscular connective tissue, may be regarded as one factor that contributes to gait impairments. For example, in elderly populations, tightness and high stiffness in hip flexors may restrain step length and gait speed [70]. Hip contractures in progressive neuromuscular disease are also related to an increase in collagen deposition and fatty infiltration and a decrease in functional muscle fibers [71]. Hence many pathological gait movements have been attributed to an increase in passive stiffness in the hip muscles. This evidence suggest passive compliance is important in maintaining functional properties of hip muscles, and thus higher collagen content may not be a desirable characteristic since it increases the passive stiffness of the hip muscles.

The thigh muscles contained higher mean collagen content than that of the hip muscles. The thigh muscles have different specializations that contribute to high-speed activities [72] and/or normal gait movements. A previous study provided evidence that an increase in passive stiffness can greatly hinder the intrinsic functional properties of muscles in the thigh regions. For example, CP patients with hamstring contractures exhibit a significant increase in sarcomere length and ECM, leading to muscle weakness

and spasticity [73,74]. The quadriceps femoris muscle group includes rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius, and they collectively function as knee extensors. Additionally, RF assists the iliopsoas to flex the thigh while stabilizing the hip joint. The medial thigh muscles include adductor brevis, adductor longus, and adductor mangus that adduct the thigh to pull the legs together. The semitendinosus, semimembranosus, and biceps femoris in the posterior thigh region are known as the hamstrings and are involved in hip extension and knee flexion [50]. The hamstrings and quadriceps femoris are designed for engendering high-speed activities such as running, jumping, and cycling [75]. As a result, one would expect the quadriceps femoris and hamstrings to contain sufficient amount of collagen in order to withstand the tension arising from the hip and patellofemoral joint. The mean collagen content in the quadriceps femoris is slightly lower compared to the hamstrings, with the exception of rectus femoris. A previous study showed RF strain injuries frequently occur in sports that require sprinting or kicking motions, resulting in an increase in collagen content [76] and high stiffness. The collagen content in the hamstrings is the highest among the thigh muscles. Hamstring muscle strain also occurs frequently in high-speed sports activities such as running and jumping, and studies have shown that the hamstrings have the highest rate of re-injury [77,78]. Thus high collagen content observed in the hamstrings may be due to the upregulation of collagen synthesis as a result of a high frequency of hamstring strains. Higher collagen content was observed in gracillis (GC) and sartorius (SR). The GC and SR sartorius are long muscles that cross both the hip and knee joint and with great excursion potential.. Hence higher mean collagen content may provide protection against the shear strain of gracilis and sartorius during excursion. The

adductors in the thigh act to stabilize the hip and decelerate the lower limb during extraneous activities. Groin strains can occur often in the hip adductors, especially in the pectineus, adductor magnus, longus, and brevis. The collagen content of the hip adductors and hamstrings are similar. This result may be due to a high frequency of groin strains occurring at the hip adductors according to a previous study, and therefore the stiffness of hip adductors increases greatly [79].

The mean collagen content of leg muscles is highest in the lower extremity. The ankle plantarflexors showed higher collagen compared to the dorsiflexors. The soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) collectively form the triceps surae. The triceps surae is a strong plantarflexion force generator and acts in conjunction with the hip extensor and flexor to facilitate foot elevation during the stance phase of gait cycle [80]. The SOL is recruited during normal walking and running since it is a strong force generator and provides postural stability. The MG and LG are predominantly designed to generate higher excursion during plantarflexion[69]. The collagen content of MG and LG are larger than the SOL. The higher collagen content in MG and LG may be due to their functional role as the major plantarflexor in the posterior compartment of the leg. Another plausible reason for higher collagen content may be the higher risk of MG and LG tears (e.g. tennis leg) due to overstretching arising from dorsiflexion [81]. Consequently, an increase in collagen remodeling as part of the intrinsic regeneration process of injured muscle leads to the elevation of collagen content in the MG and LG muscles [82]. The primary function of plantaris (PLT) is to weakly assist gastrocnemius in ankle plantarflexion and is capable of generating large excursions. Hence, higher stiffness may prevent shearing strain from occurring in PLT due to its

large excursion potential [14]. The fibularis longus (FL) and fibularis brevis (FB) are weak ankle plantarflexor compared to the triceps surae. However, FL and FB are able to produce eversion of foot, and FL provides support for transverse arch of foot [50]. The FL and FB contain relatively high collagen contents compared to the MG and LG. One possible explanation may be that greater stiffness may enhance their supporting role in the foot, or prevent lateral ankle strain from occurring in FL[83]. The flexor hallucis longus (FHL), flexor digitorum longus (FDL), and tibialis posterior (TP) located in the deep posterior compartment of the leg contained lower collagen content compared to the other ankle plantarflexors described above. The FHL is a strong flexor of the joints of the great toe and is able to produce the final thrust of great toe flexion prior to the pre-swing of the gait cycle which follows the powerful delivery of plantarflexion thrust by triceps surae [84]. The FDL is responsible for the flexion of four digits of the foot. Interestingly, the FDL contained similar mean collagen content compared to the finger flexors FDP and FDS in the forearm. Both FHL and FDL provide longitudinal arch support of the foot [50]. The main function of TP is also to support longitudinal arch during weight bearing, and thus it contracts statically during the stance phase of gait [84]. The TP also serves as a synergistic muscle with the tibialis anterior to invert the foot when the foot is off the ground [50]. Higher passive stiffness in these muscles may promote their supportive functional role and withstand loading during the stance phase and flexion of the digits by FDL in the foot. The ankle dorsiflexors are designed to deliver powerful and fast excursions during running and walking [85]. In particular, the tibialis anterior (TA) is recruited during high speed activity and subjected to high mechanical load, and provides longitudinal support in the arch of the foot [50]. Besides acting as ankle dorsiflexors, the extensor hallucis

longus (EHL) extends the great toe, and the EDL extends the lateral four digits [8]. Interestingly, the collagen content of the TA, EHL, and EDL are similar in this study. Huijing and colleagues demonstrated that changes in the muscle fiber length of EDL and the active force generated by the TA and EHL complex can affect the amount of active force generated by proximal and distal EDL. They suggested that there may be interconnections of intra- and extramuscular connective tissue that allow force transmission between synergistic muscles. The continuation of the interconnection of connective tissues between the TA, EHL, and EDL may be one possible explanation of similar collagen content between them in order to facilitate optimal synergistic function in ankle dorsiflexion and during high speed activities [86].

In terms of comparison of collagen content by muscle function, the external rotator exhibited the lowest collagen content compared to other muscle groups. The post-hoc test also showed the mean collagen content of external rotator muscles to be significantly different from the others. Many of the external rotator muscles are located in the pelvic and gluteal region in this study. The pelvic floor is supported by the pelvic muscles and endo-pelvic fascia. When intra-abdominal pressure increases, contraction of pelvic muscles is important to maintain the support function of the pelvic floor. This contraction is also crucial in preventing involuntary loss of fecal and urine contents. The relaxation of pelvic muscles is also important in order to return the pelvic organs to their resting position and facilitate voluntary urination and defecation [87]. Many of the gluteal muscles are a powerful force generator that are recruited to flex or extend the thigh during the gait cycle, and primarily function in the locomotion and balance of lower extremity. Thus higher elasticity and compliance in the external rotator muscles may be

suitable for their intrinsic functional role. Additionally, the external rotator muscles of the hip are not subjected to high mechanical load during locomotion compared to the leg and thighs. Thus upregulation of collagen synthesis induced by high mechanical load may not occur in the external rotator muscles in the hip. In contrast, many of the internal rotator muscles located on the upper extremity are responsible for the pronation and supination of forearm and movements of the hand [8], or provide stabilization at the shoulder. Thus higher stiffness allows the internal rotator muscles to fulfill their stabilizing role and facilitate their movements. The average collagen content of flexor muscles is lower than the extensor muscles. One plausible explanation may be that many of the extensor muscles examined in this study provide stability in different muscle regions and balance for upright posture and locomotion. For example, many of the axial paraspinal muscles such as multifidus, iliocostalis lumborum, and longissimus are trunk extensors that have been shown to serve as a lumbar stabilizer [64]. Conversely, many flexors examined in the current study are designed to generate a large amount of force and excursion but are less suitable to act as a stabilizer. The abductor muscles have the highest collagen content compared to adductor muscles and muscles with different functions. Many abductor muscles are also anti-gravity muscles in this study. Although the differences in collagen content between the anti-gravity muscle and gravity muscle were not statistically significant, the anti-gravity muscles showed higher collagen content in the current study. Anti-gravity muscles must be able to generate force to support body weight and conduct locomotion against the gravitational load [88]. They also assist in the maintenance of upright posture during static positions. Thus higher stiffness may promote structural stability against gravitational load in abductors. Muscles that cross multiple

joints exhibited higher collagen content compared to muscles that cross a single joint. The multi-joint muscles usually have a large range of motion and higher excursion and velocity potential than the single-joint muscles, and thus they may experience greater shearing strain.

Results of linear regression showed correlation between collagen content and gender. This result may be due to the regulation of collagen remodeling mediated by estrogen and progesterone. During collagen remodeling, the MMPs regulate the degradation of collagen in the tissue, whereas the tissue inhibitors of metalloproteinases (TIMPs) oppose actions of MMPs. Studies have revealed mechanisms of regulation of various TIMPs and MMPs production by estrogen and progesterone [89,90]. Additionally, the level of estrogen secretion has been demonstrated to have a negative relationship with muscle stiffness in females [91]. Conversely, men experience less fluctuation in testosterone secretion and a low constant level of estrogen secretion, and thus the process of collagen remodeling may be more balanced in men compared to women [92].

Conclusion

The current study revealed that intramuscular collagen content varies greatly depending on the muscle region. The axial and hand regions had the highest mean collagen content, followed by the forearm and leg regions. The thigh and arm muscles contained intermediate mean collagen content, whereas the hip muscles had the lowest mean collagen content. Such variation may be due to the specific functional role of the muscles in each region.

Figures and Tables

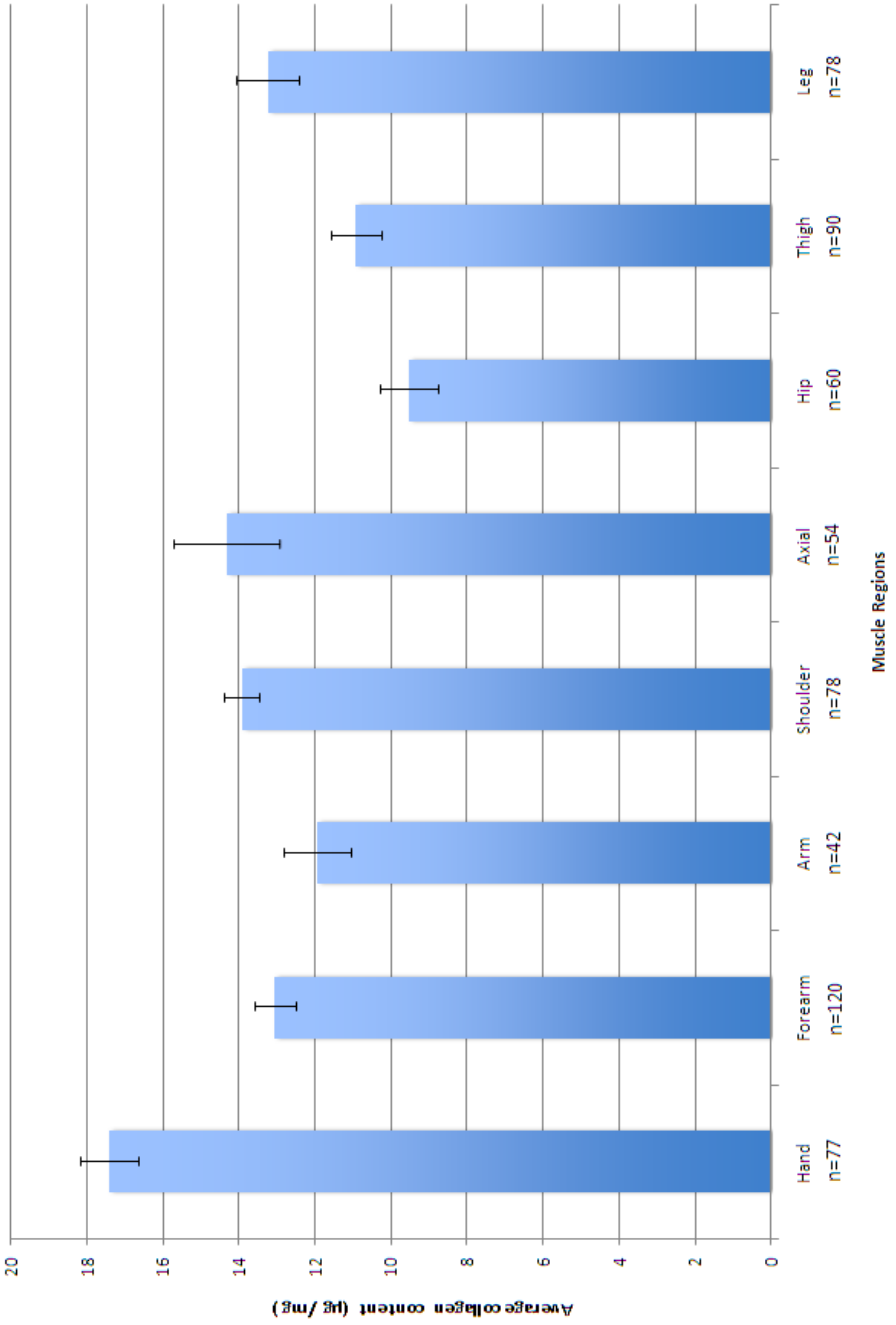

Figure 1. Mean collagen content of muscles by region. Average collagen contents from upper body to lower body are: hand region=17.41 µg/mg; forearm region=13.05 µg/mg; arm region=11.94 µg/mg; shoulder region= 13.93 µg/mg; axial region=14.32 µg/mg; hip region= 9.51 µg/mg; thigh region= 10.91 µg/mg; leg region=13.24 µg/mg.

Figure 2. Mean collagen content of 100 muscle samples (n=599) from upper extremity to lower extremity. The collagen content was measured in μg of collagen per mg of wet weight muscle. See supplemental figure 1 for the average collagen content of each musc

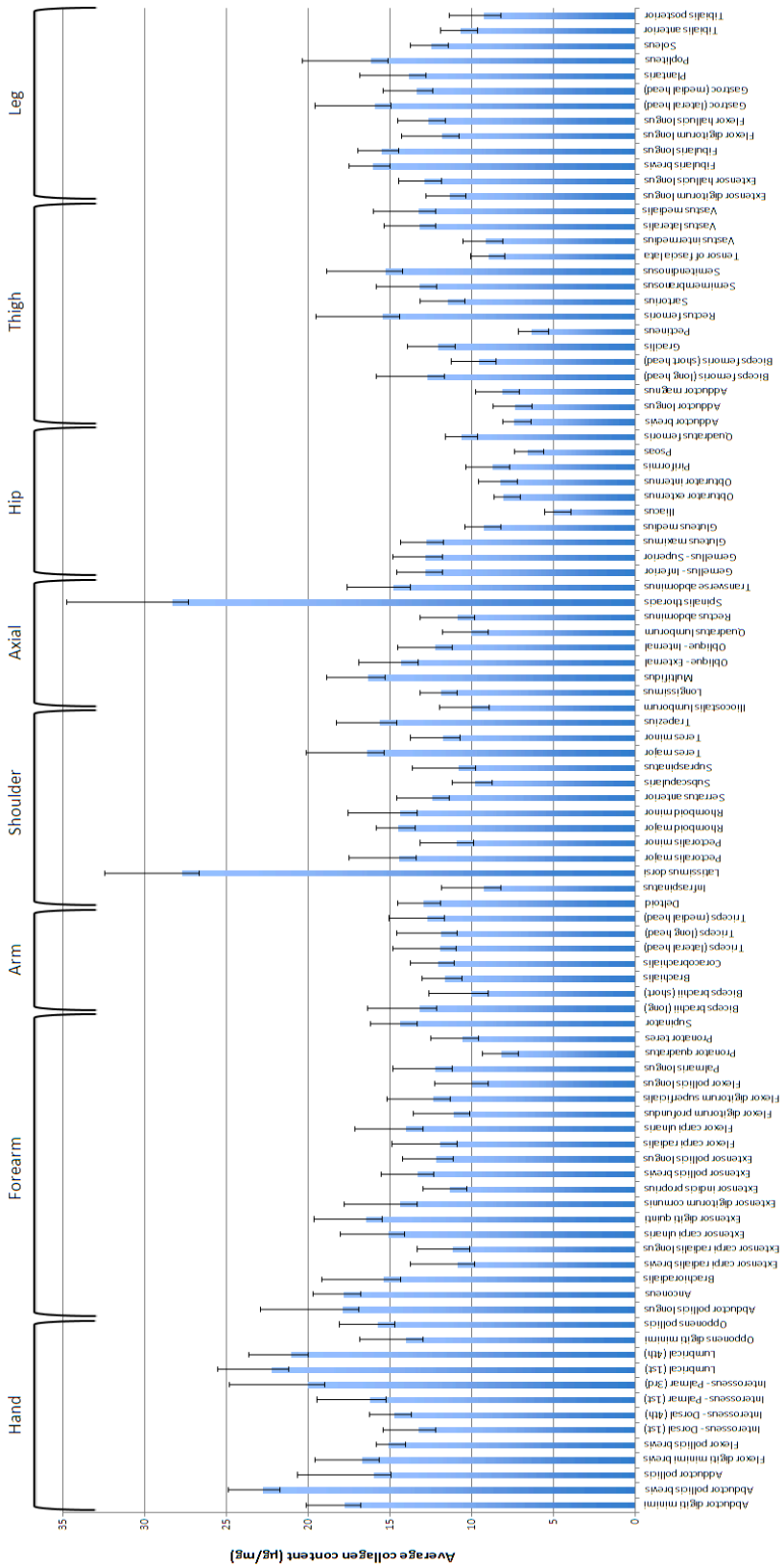
Average collagen content (µg/mg)
0
5
10
15
20
25
30
35
Hand
Forearm
Arm
Shoulder
Axial
Hip
Thigh
Leg
Abductor digiti minimi
Abductor pollicis brevis
Adductor pollicis
Flexor digiti minimi brevis
Flexor pollicis brevis
Interossseus - Dorsal (1st)
Interossseus - Dorsal (4th)
Interossseus - Palmar (1st)
Interossseus - Palmar (3rd)
Lumbrical (1st)
Lumbrical (4th)
Opponens digiti minimi
Opponens pollicis
Abductor pollicis longus
Anconeus
Brachioradialis
Extensor carpi radialis brevis
Extensor carpi radialis longus
Extensor carpi ulnaris
Extensor digiti quinti
Extensor digitorum communis
Extensor indicis proprius
Extensor pollicis brevis
Extensor pollicis longus
Flexor carpi radialis
Flexor carpi ulnaris
Flexor digitorum profundus
Flexor digitorum superficialis
Flexor pollicis longus
Palmaris longus
Pronator quadratus
Pronator teres
Supinator
Biceps brachii (long)
Biceps brachii (short)
Brachialis
Coracobrachialis
Triceps (lateral head)
Triceps (long head)
Triceps (medial head)
Deltoid
Infraspinatus
Latissimus dorsi
Pectoralis major
Pectoralis minor
Rhomboid major
Rhomboid minor
Serratus anterior
Subscapularis
Supraspinatus
Teres major
Teres minor
Trapezius
Iliocostalis lumborum
Longissimus
Multifidus
Oblique - External
Oblique - Internal
Quadratus lumborum
Rectus abdominus
Spinalis thoracis
Transverse abdominus
Gemellus - Inferior
Gemellus - Superior
Gluteus maximus
Gluteus medius
Iliacus
Obturator externus
Obturator internus
Piriformis
Psoas
Quadratus femoris
Adductor brevis
Adductor longus
Adductor magnus
Biceps femoris (long head)
Biceps femoris (short head)
Gracilis
Pectineus
Rectus femoris
Sartorius
Semimembranosus
Semitendinosus
Tensor of fascia lata
Vastus intermedius
Vastus lateralis
Vastus medialis
Extensor digitorum longus
Extensor hallucis longus
Fibularis brevis
Fibularis longus
Flexor digitorum longus
Flexor hallucis longus
Gastroc (lateral head)
Gastroc (medial head)
Plantaris
Popliteus
Soleus
Tibialis anterior
Tibialis posterior

Table 1. Results of Bonferroni *post hoc* test comparing group means of muscle regions. Blue boxes indicate $p<0.05$.

	Arm	Axial	Forearm	Hand	Leg	Hip	Shoulder	Thigh
Arm	–––	1	1	0.001	1	1	1	1
Axial	1	–––	1	0.254	1	0.003	1	0.083
Forearm	1	1	–––	< 0.001	1	0.022	1	0.589
Hand	0.001	0.254	< 0.001	–––	0.003	< 0.001	0.033	< 0.001
Leg	1	1	1	0.003	–––	0.032	1	0.664
Hip	1	0.003	0.022	< 0.001	0.032	–––	0.003	1
Shoulder	1	1	1	0.033	1	0.003	–––	0.097
Thigh	1	0.083	0.589	< 0.001	0.664	1	0.097	–––

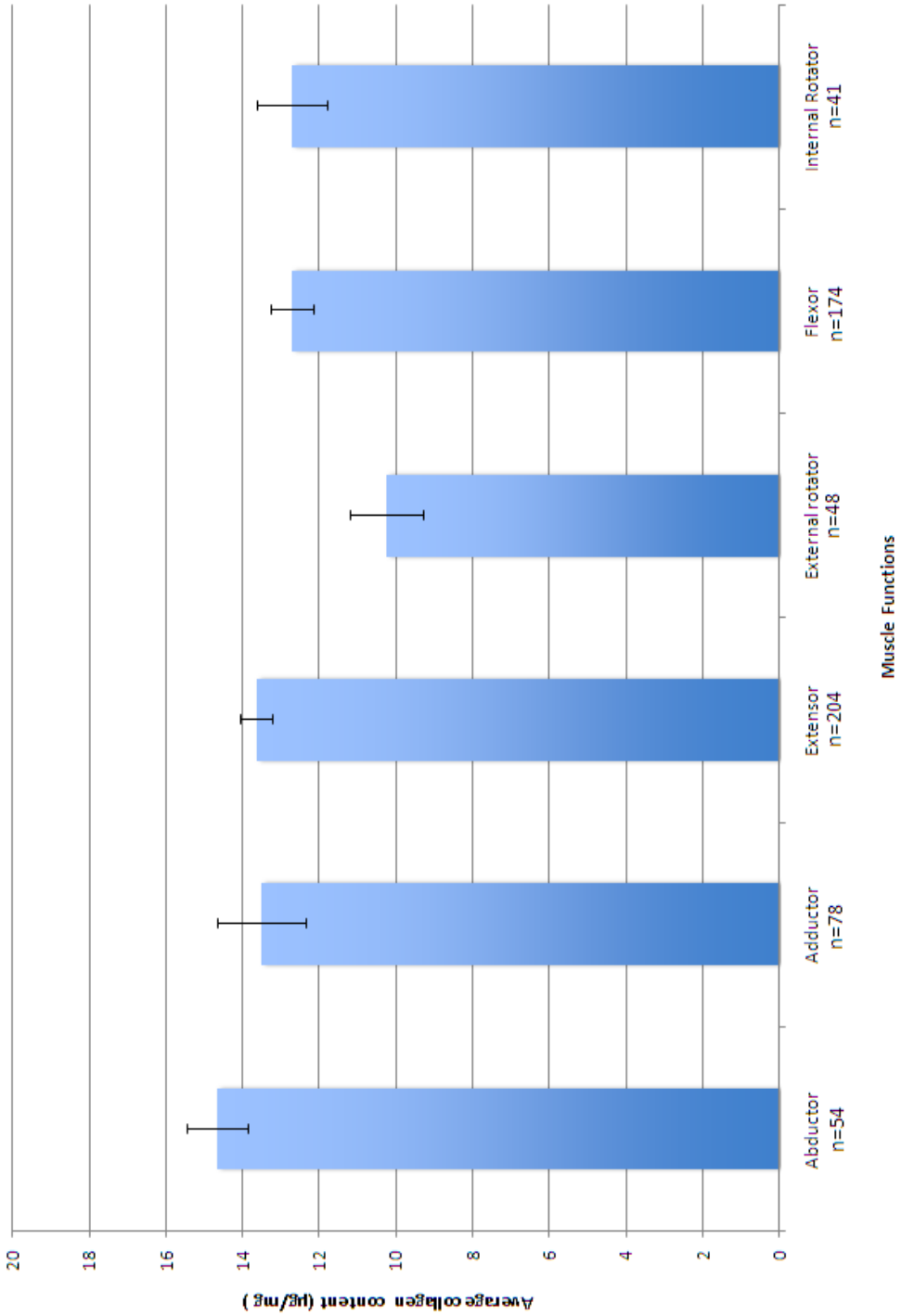

Figure 3. Mean collagen content of muscles by function. Average collagen contents of muscles grouped by function are: abductor=14.66 µg/mg; adductor=13.50 µg/mg; extensor= 13.63 µg/mg; external rotator=10.31 µg/mg; flexor=12.71 µg/mg; internal rotator=12.72 µg/mg.

Table 2. Results of Bonferroni *post hoc* test comparing group means of muscle functions. Blue boxes indicate $p<0.05$.

Muscle Functions	Abductor	Adductor	Extensor	External rotator	Flexor	Internal rotator
Abductor	–––	1	1	0.023	1	1
Adductor	1	–––	1	0.181	1	1
Extensor	1	1	–––	0.042	1	1
External rotator	0.23	0.181	0.042	–––	0.505	1
Flexor	1	1	1	0.505	–––	1
Internal rotator	1	1	1	1	1	–––

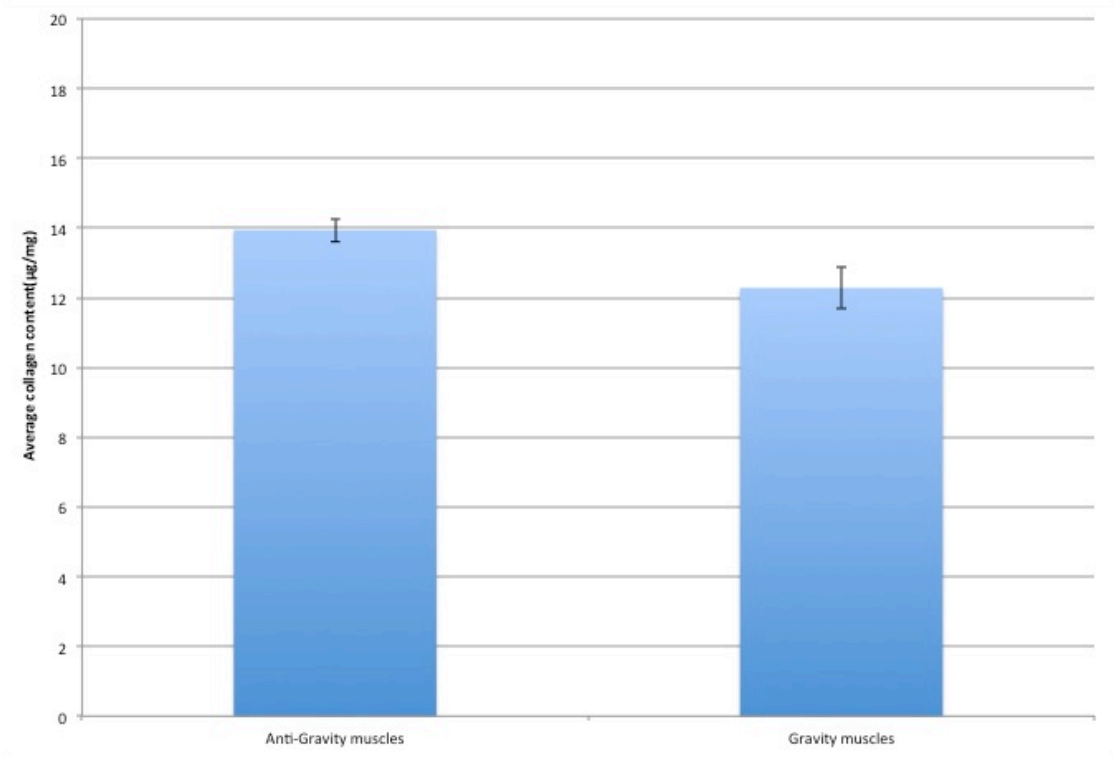

Figure 4. Mean collagen content of anti-gravity (n=300) versus gravity (n=299) muscles ($p<0.05$). Average collagen contents of anti-gravity and gravity muscles are 13.30 µg/mg and 12.93 µg/mg, respectively.

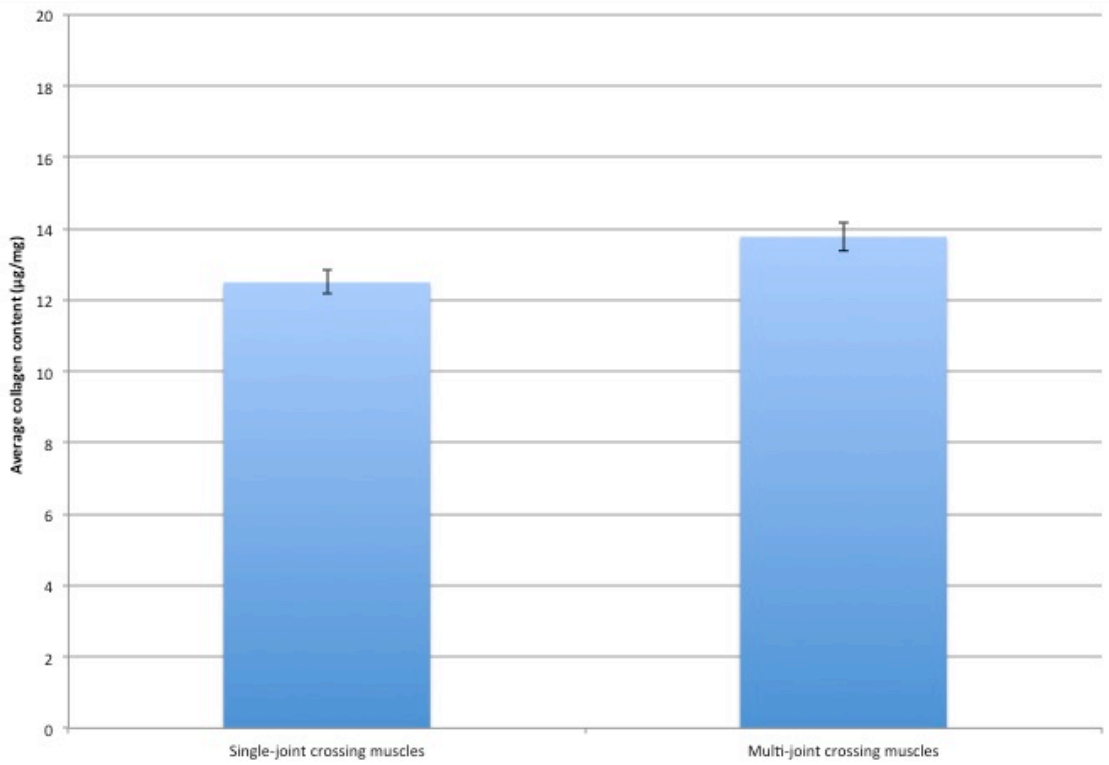

Figure 5. Mean collagen content of single-joint (n=311) versus multi-joint crossing muscles (n=288) ($p<0.05$). Average collagen contents of single and multi-joint crossing muscles are 12.50 µg/mg and 13.78 µg/mg, respectively.

Table 3. Result of multiple linear regression with average collagen content as the dependent variable (R^2=0.0247 and adjusted R^2=0.024). The p-value of gender was less than 0.05 but the R^2 value showed no correlation between the average collagen content and gender.

	Unstandardized coefficient		**Standardized coefficient**		**p-value**
	B	Std. Error	Beta	t	Sig.
Constant	11.959	3.669		3.259	0.001
Age	-0.028	0.044	-0.026	-0.629	0.53
Gender	2.297	0.564	0.165	4.073	0

Table 4: Result of the stepwise multiple linear regression with average collagen content as the dependent variable. The p-value of muscle fiber length was less than 0.05, but the R^2 value showed no correlation between the muscle fiber length and average collagen content. Data of muscle fiber length, PCSA, muscle mass, and pennation angle were obtained from previous architectural study [5-10]. PCSA=physiological cross sectional area.

	R^2	**Adjusted R^2**	**p-value**
Muscle fiber length (cm)	0.066	0.052	0.032*
PCSA (cm^2)	0.051	0.037	0.069
Muscle mass (g)	0.065	0.051	0.432
Pennation angle (deg)	0.005	-0.01	0.125

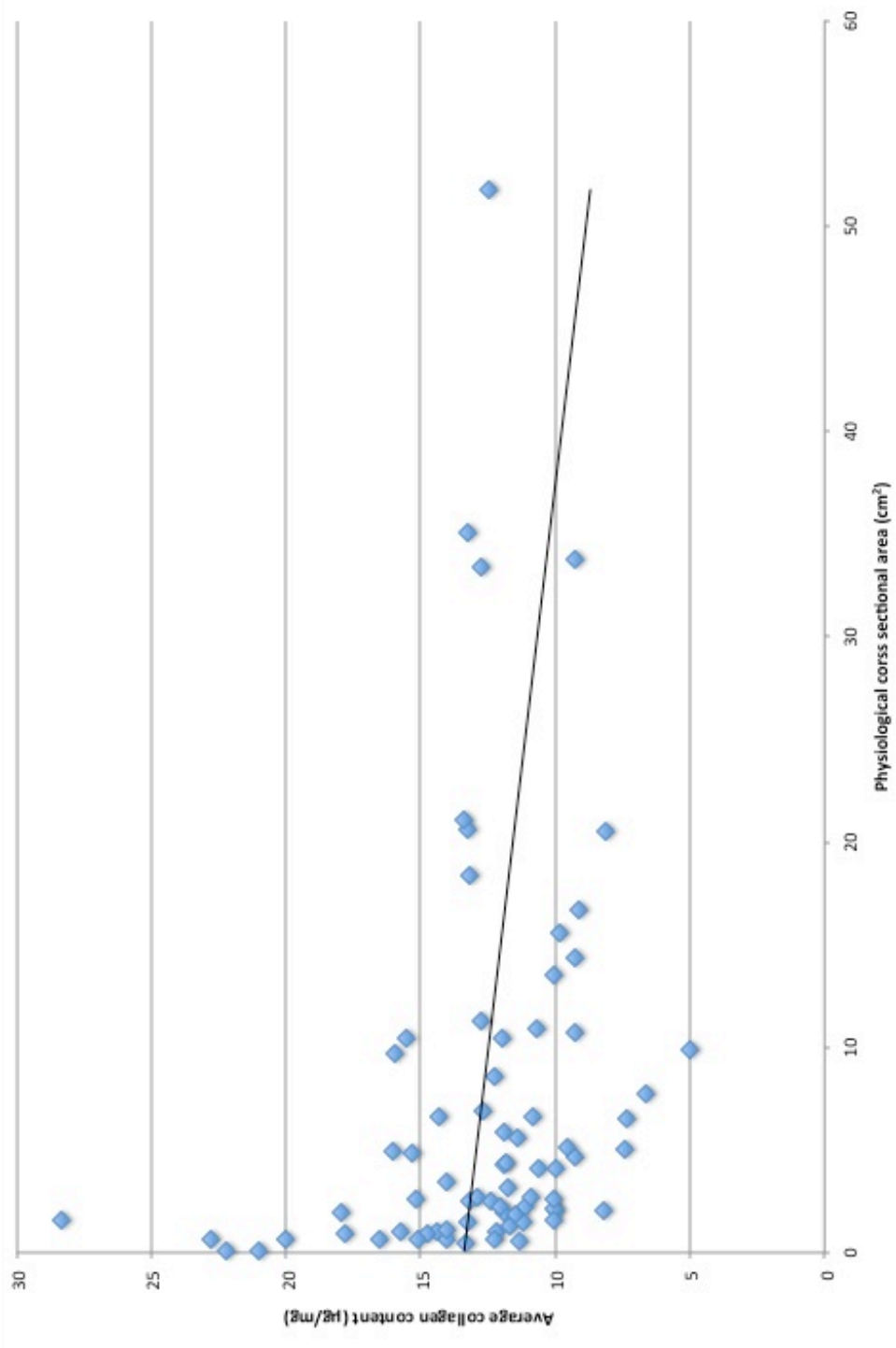

Figure 6. Scatter plot of average collagen content (μg/mg) versus physiological cross sectional area (cm^2) (R^2=0.051; $p<0.05$).

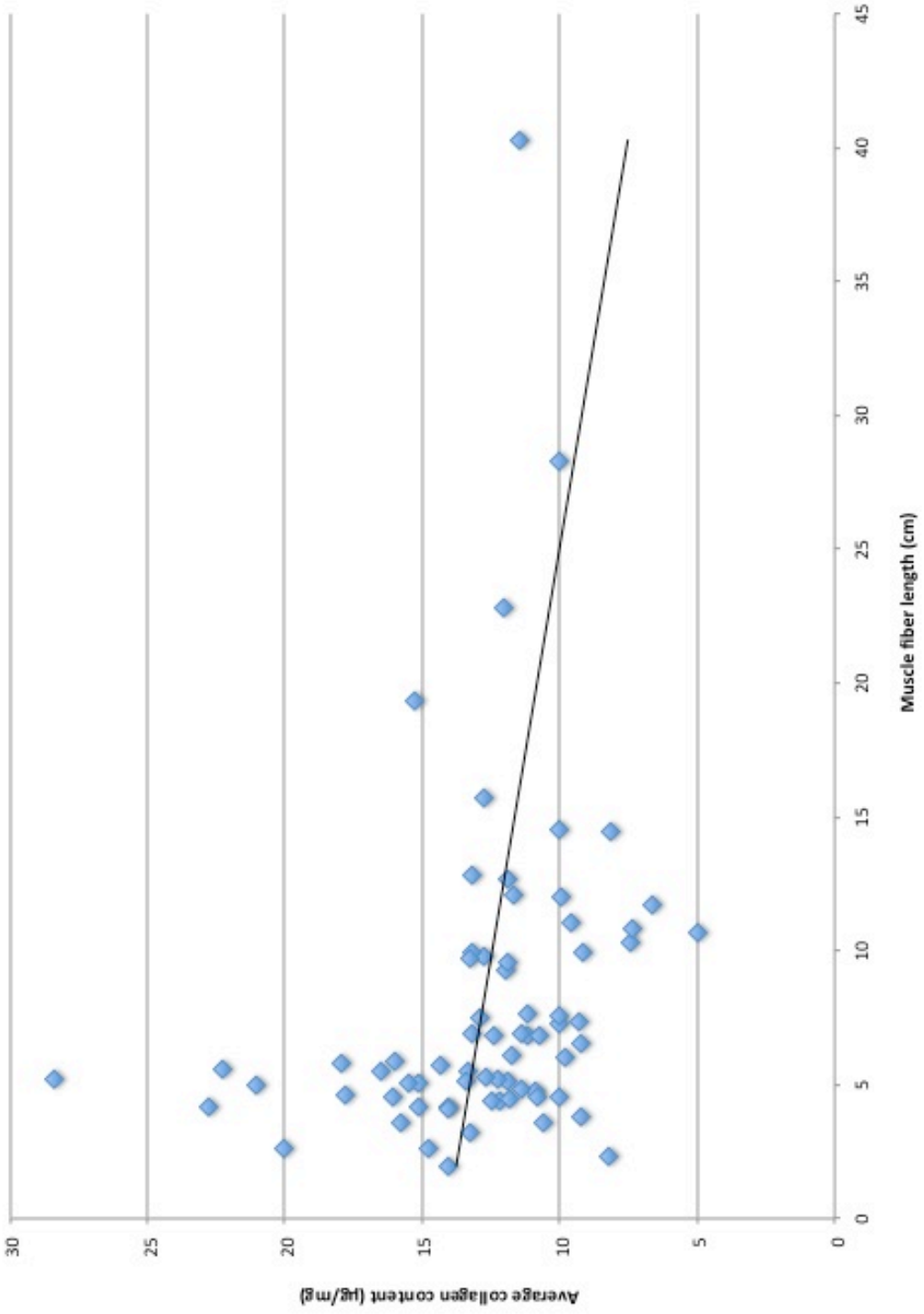

Figure 7. Scatter plot of average collagen content (µg/mg) versus muscle fiber length (cm) (R^2=0.066; p>0.05).

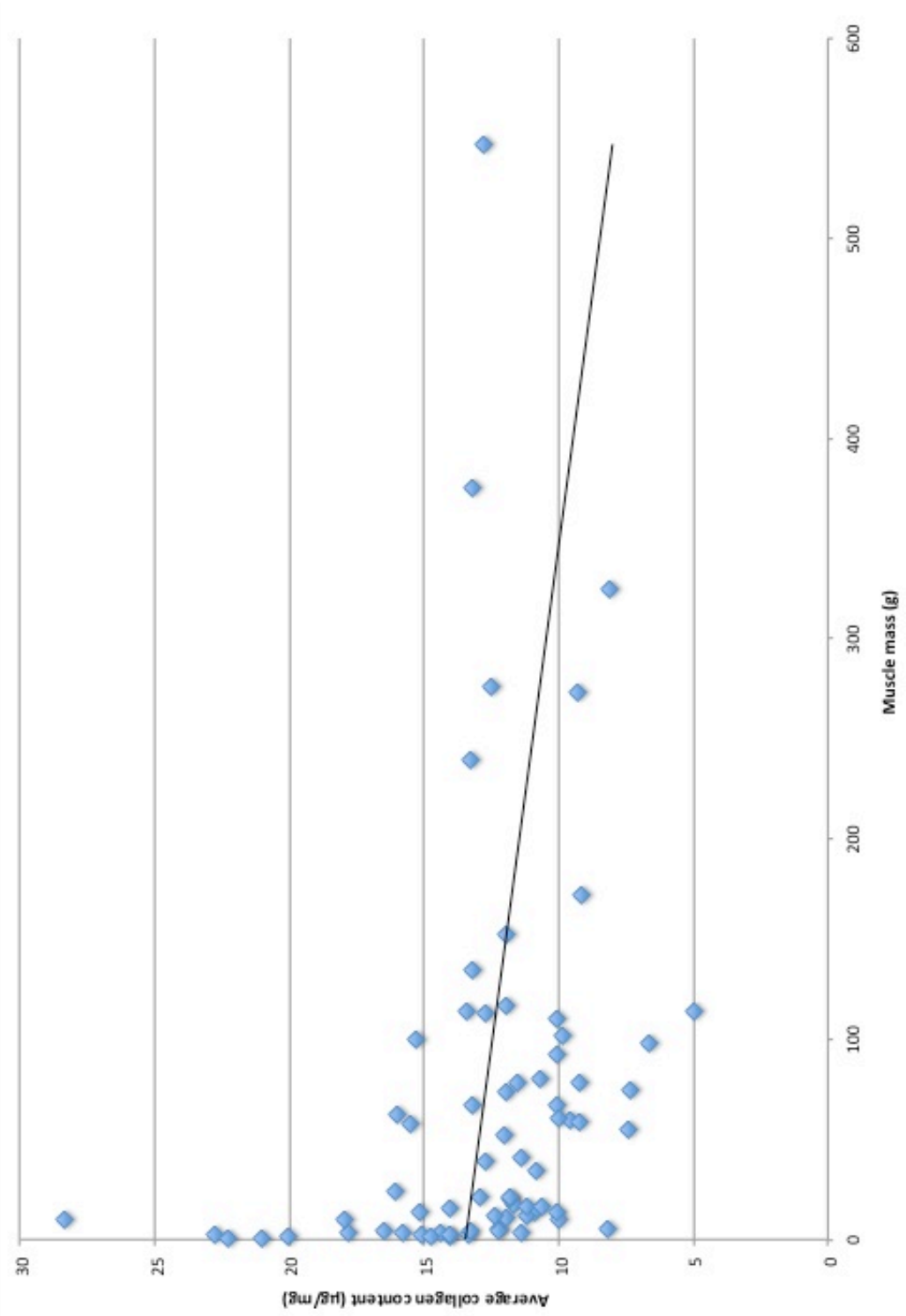

Figure 8. Scatter plot of average collagen content (µg/mg) versus muscle mass (g) (R^2=0.051; p>0.05).

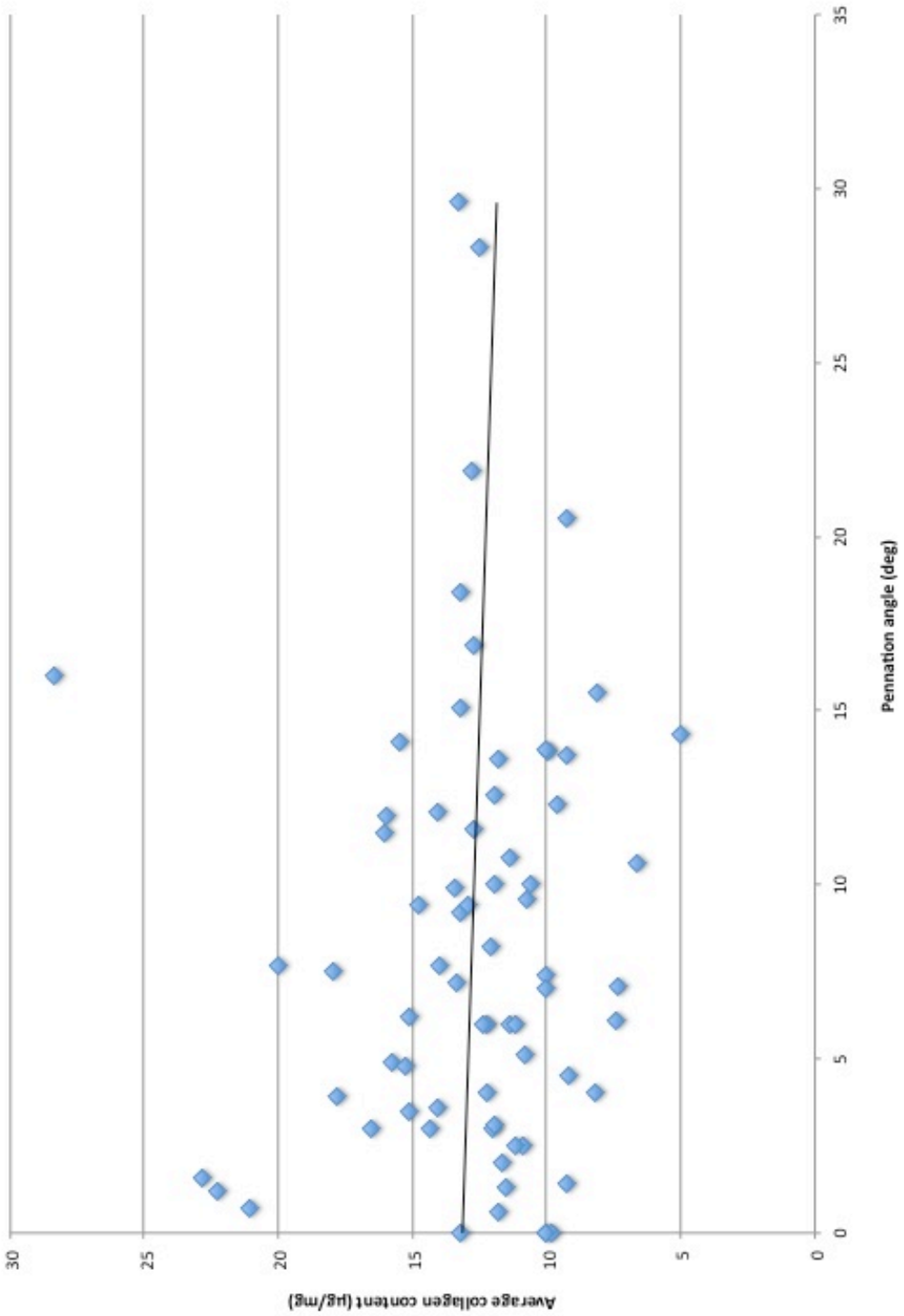

Figure 9. Scatter plot of average collagen content (µg/mg) versus pennation angle (deg) (R^2=0.005; p>0.05).

Supplemental table 1: One hundred different skeletal muscles were biopsied from each donor listed below.

Subjects	Age	Sex	Height	Weight
Donor A	84	F	5'5"	136
Donor B	73	M	6'1"	136
Donor C	91	F	5'0"	100
Donor D	86	M	5'8"	160
Donor E	76	F	5'4"	119
Donor F	88	M	5'7"	158

Supplemental table 2: Mean collagen content of 100 skeletal muscles by region (n=6 for each muscle). Opponens digiti minimi was not obtained from subject B.

Muscle	Function	A	B	C	D	E	F	Mean	S.D.	SEM
Abductor digiti minimi	Abductor	14.20	24.53	19.07	15.11	23.88	10.10	17.82	5.71	2.33
Abductor pollicis brevis	Abductor	21.88	17.90	32.43	18.54	22.84	23.21	22.80	5.22	2.13
Adductor pollicis	Adductor	9.50	38.50	15.73	8.86	7.56	15.71	15.98	11.58	4.73
Flexor digiti minimi brevis	Flexor	8.86	28.20	21.73	11.73	17.36	12.28	16.69	7.26	2.97
Flexor pollicis brevis	Flexor	14.04	14.80	17.18	17.35	12.40	14.87	15.10	1.89	0.77
Interosseus - Dorsal (1st)	Abductor	13.81	23.46	13.53	8.29	11.65	8.74	13.25	5.51	2.25
Interosseus - Dorsal (4th)	Abductor	17.60	20.29	10.32	15.45	11.24	13.60	14.75	3.81	1.56
Interosseus - Palmar (1st)	Adductor	17.29	18.46	27.97	8.96	19.21	5.73	16.27	7.95	3.25
Interosseus - Palmar (3rd)	Adductor	18.43	31.46	37.37	8.88	14.68	9.41	20.04	11.83	4.83
Lumbrical (1st)	Flexor	30.23	14.37	33.89	15.40	22.71	16.88	22.25	8.21	3.35
Lumbrical (4th)	Flexor	23.07	23.52	29.19	10.80	23.23	16.46	21.04	6.44	2.63
Opponens digiti minimi	Internal Rotator	24.61		11.84	10.76	7.93	15.00	14.03	6.44	2.88
Opponens pollicis	Internal Rotator	19.89	19.76	22.92	8.44	13.02	10.56	15.76	5.87	2.40
Abductor pollicis longus	Abductor	11.20	41.94	18.04	13.40	15.25	7.77	17.93	12.27	5.01
Anconeus	Extensor	15.54	26.57	17.56	14.94	14.02	18.59	17.87	4.59	1.87
Brachioradialis	Flexor	13.48	24.52	29.09	7.86	12.01	5.50	15.41	9.39	3.83
Extensor carpi radialis brevis	Extensor	6.81	19.68	20.23	5.83	8.45	4.25	10.88	7.17	2.93
Extensor carpi radialis longus	Extensor	8.10	12.10	19.74	13.04	10.41	3.62	11.17	5.38	2.20
Extensor carpi ulnaris	Extensor	14.27	22.89	25.23	8.50	9.33	10.64	15.14	7.22	2.95
Extensor digiti quinti	Extensor	14.09	18.54	31.17	13.32	12.79	9.12	16.50	7.79	3.18
Extensor digitorum comunis	Extensor	12.26	17.13	30.28	7.10	8.69	10.79	14.37	8.52	3.48
Extensor indicis proprius	Extensor	12.12	12.80	18.45	7.36	8.81	8.62	11.36	4.07	1.66
Extensor pollicis brevis	Extensor	10.51	19.56	19.70	7.72	14.47	8.13	13.35	5.43	2.22
Extensor pollicis longus	Extensor	14.35	18.48	15.53	5.00	7.74	12.10	12.20	5.04	2.06
Flexor carpi radialis	Flexor	12.66	8.78	25.32	9.53	12.08	3.20	11.93	7.37	3.01
Flexor carpi ulnaris	Flexor	11.05	21.43	25.75	7.94	11.12	6.93	14.04	7.71	3.15
Flexor digitorum profundus	Flexor	15.35	11.13	21.08	6.68	6.22	6.45	11.15	6.04	2.47
Flexor digitorum superficialis	Flexor	9.06	23.60	17.21	11.78	9.01	3.57	12.37	7.07	2.88
Flexor pollicis longus	Flexor	10.62	12.19	19.40	4.43	8.84	4.59	10.01	5.57	2.27
Palmaris longus	Flexor	16.08	13.41	22.73	8.81	6.12	6.33	12.25	6.49	2.65
Pronator quadratus	Internal Rotator	8.19	12.78	9.45	4.96	4.99	8.82	8.20	2.96	1.21
Pronator teres	Internal Rotator	13.23	9.14	19.10	7.34	8.69	6.19	10.61	4.80	1.96
Supinator	Internal Rotator	10.06	20.88	19.53	12.13	11.45	12.26	14.39	4.60	1.88
Biceps brachii (long)	Flexor	7.99	21.24	25.27	7.98	6.83	9.89	13.20	7.95	3.25

Supplemental table 2, continued

Biceps brachii (short)	Flexor	7.07	15.38	20.61	6.57	6.64	3.94	10.04	6.48	2.65
Brachialis	Flexor	10.65	15.40	12.57	15.70	8.03	7.67	11.67	3.50	1.43
Coracobrachialis	Flexor	9.08	19.06	15.25	10.33	8.69	10.22	12.10	4.14	1.69
Triceps (lateral head)	Extensor	8.94	22.12	19.46	5.78	9.78	5.81	11.98	7.06	2.88
Triceps (long head)	Extensor	6.81	14.91	23.68	11.43	8.24	6.42	11.92	6.59	2.69
Triceps (medial head)	Extensor	7.43	18.84	20.84	9.73	12.43	6.99	12.71	5.89	2.40
Deltoid	Abductor	14.31	14.39	19.28	8.82	9.13	11.76	12.95	3.93	1.60
Infraspinatus	External Rotator	5.63	12.02	21.28	6.02	4.48	6.08	9.25	6.46	2.64
Latissimus dorsi	Adductor	14.00	31.07	42.28	13.02	31.86	34.06	27.72	11.70	4.78
Pectoralis major	Adductor	8.54	17.08	28.23	12.88	12.88	6.96	14.43	7.65	3.12
Pectoralis minor	Adductor	8.81	21.38	13.13	7.48	7.95	6.96	10.95	5.57	2.27
Rhomboid major	Adductor	12.39	16.54	19.70	15.74	12.31	10.27	14.49	3.46	1.41
Rhomboid minor	Adductor	10.86	28.88	18.15	7.61	10.45	10.28	14.37	7.94	3.24
Serratus anterior	Abductor	10.52	12.60	22.76	8.54	7.40	12.69	12.42	5.49	2.24
Subscapularis	Internal Rotator	6.42	7.47	12.11	11.93	14.68	6.33	9.82	3.54	1.44
Supraspinatus	Abductor	8.30	13.13	24.23	6.95	6.15	6.18	10.82	7.07	2.89
Teres major	Internal Rotator	10.66	16.57	34.36	15.53	10.36	11.15	16.44	9.17	3.74
Teres minor	External Rotator	16.00	11.23	19.39	7.45	9.82	6.76	11.78	4.98	2.03
Trapezius	Extensor	13.00	24.15	24.02	11.75	10.44	10.63	15.67	6.58	2.69
Iliocostalis lumborum	Extensor	6.57	18.83	13.08	7.80	7.17	6.39	9.97	5.00	2.04
Longissimus	Extensor	10.00	15.78	11.59	7.22	15.12	11.84	11.92	3.19	1.30
Multifidus	Extensor	10.55	26.92	10.18	13.79	17.82	18.93	16.36	6.31	2.57
Oblique - External	Flexor	9.51	18.16	25.51	11.50	12.71	8.66	14.34	6.41	2.62
Oblique - Internal	Flexor	8.34	17.21	21.63	8.49	10.12	7.61	12.23	5.80	2.37
Quadratus lumborum	Extensor	6.39	18.49	9.36	6.90	10.37	8.69	10.03	4.40	1.80
Rectus abdominus	Flexor	5.53	11.13	21.43	5.80	10.76	10.50	10.86	5.76	2.35
Spinalis thoracis	Extensor	19.51	36.70	16.07	13.06	29.86	54.96	28.36	15.77	6.44
Transverse abdominus	Flexor	11.69	24.64	22.43	11.60	11.59	7.05	14.83	7.01	2.86
Gemellus - Inferior	External Rotator	13.33	10.05	12.43	13.04	20.48	7.87	12.87	4.27	1.74
Gemellus - Superior	External Rotator	12.26	13.19	8.62	9.28	11.68	22.14	12.86	4.88	1.99
Gluteus maximus	Extensor	9.84	11.83	19.34	11.17	15.65	8.85	12.78	3.97	1.62
Gluteus medius	Abductor	8.64	10.78	14.42	6.39	7.06	8.33	9.27	2.94	1.20
Iliacus	Flexor	6.44	5.82	5.36	2.56	5.62	4.12	4.99	1.41	0.58
Obturator externus	External Rotator	8.46	9.77	9.63	7.96	5.89	6.71	8.07	1.55	0.63
Obturator internus	External Rotator	5.34	13.56	9.02	5.93	10.60	5.22	8.28	3.39	1.38
Piriformis	External Rotator	10.61	6.91	14.98	3.88	10.45	5.57	8.73	4.06	1.66
Psoas	Flexor	6.63	7.67	5.90	5.00	10.00	4.52	6.62	2.00	0.82
Quadratus femoris	External Rotator	8.81	14.54	12.88	8.89	9.11	9.81	10.67	2.43	0.99
Adductor brevis	Adductor	7.42	9.48	9.39	6.90	6.36	4.89	7.41	1.78	0.73
Adductor longus	Adductor	6.46	9.61	12.94	7.02	3.71	4.43	7.36	3.44	1.40
Adductor magnus	Adductor	6.98	9.79	14.90	8.72	5.19	3.25	8.14	4.07	1.66
Biceps femoris (long	Extensor	5.77	17.98	25.75	8.77	11.14	6.94	12.72	7.71	3.15

Supplemental table 2, continued

head)										
Biceps femoris (short head)	Flexor	6.45	6.61	17.68	10.68	8.52	7.60	9.59	4.25	1.74
Gracilis	Adductor	8.07	7.78	19.43	15.64	12.98	8.39	12.05	4.81	1.96
Pectineus	Adductor	4.72	6.12	10.41	6.11	5.01	5.67	6.34	2.07	0.85
Rectus femoris	Flexor	7.30	18.59	34.22	14.11	10.14	8.51	15.48	10.07	4.11
Sartorius	Flexor	9.27	13.93	18.87	9.34	10.03	7.54	11.50	4.19	1.71
Semimembranosus	Extensor	9.00	8.69	24.95	9.96	17.54	8.95	13.18	6.68	2.73
Semitendinosus	Extensor	7.08	21.74	29.22	13.72	14.07	5.96	15.30	8.88	3.62
Tensor of fascia lata	Flexor	6.79	12.39	11.74	6.75	9.80	6.55	9.00	2.67	1.09
Vastus intermedius	Extensor	7.64	8.46	12.72	7.43	13.95	4.77	9.16	3.48	1.42
Vastus lateralis	Extensor	10.87	19.85	16.77	11.48	15.68	4.72	13.23	5.36	2.19
Vastus medialis	Extensor	9.68	9.08	26.10	7.52	14.95	12.26	13.26	6.81	2.78
Extensor digitorum longus	Extensor	8.95	12.78	13.81	8.15	16.76	7.89	11.39	3.62	1.48
Extensor hallucis longus	Extensor	9.03	15.04	18.23	14.66	7.87	12.55	12.90	3.91	1.60
Fibularis brevis	Extensor	15.56	16.09	23.10	15.07	13.19	13.21	16.04	3.66	1.50
Fibularis longus	Extensor	19.62	13.60	19.82	13.05	16.23	10.74	15.51	3.70	1.51
Flexor digitorum longus	Flexor	8.62	13.30	23.03	9.55	11.20	5.17	11.81	6.13	2.50
Flexor hallucis longus	Flexor	11.25	11.55	20.71	10.92	14.78	6.90	12.69	4.66	1.90
Gastroc (lateral head)	Extensor	27.27	13.66	26.81	11.58	10.75	5.79	15.98	8.95	3.65
Gastroc (medial head)	Extensor	10.38	21.26	16.30	6.63	11.69	14.20	13.41	5.08	2.07
Plantaris	Extensor	8.28	15.28	28.03	10.90	12.06	8.53	13.85	7.41	3.03
Popliteus	Flexor	13.15	9.03	33.68	7.76	23.63	9.71	16.16	10.34	4.22
Soleus	Extensor	7.92	14.68	13.28	14.95	15.11	8.95	12.48	3.22	1.31
Tibialis anterior	Extensor	9.23	14.93	13.88	10.06	7.70	8.50	10.72	2.98	1.22
Tibialis posterior	Flexor	12.63	8.14	18.09	4.51	8.41	3.72	9.25	5.38	2.19

Reference

1 Meyer, G. A. & Lieber, R. L. Elucidation of extracellular matrix mechanics from muscle fibers and fiber bundles. *Journal of Biomechanics* **44**, 771-773, doi:10.1016/j.jbiomech.2010.10.044 (2011).

2 Haus, J. M., Carrithers, J. A., Trappe, S. W. & Trappe, T. A. Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *Journal of Applied Physiology* **103**, 2068-2076, doi:10.1152/japplphysiol.00670.2007 (2007).

3 Booth, C. M., Cortina-Borja, M. J. F. & Theologis, T. N. Collagen accumulation in muscles of children with cerebral palsy and correlation with severity of spasticity. *Developmental Medicine & Child Neurology* **43**, 314-320, doi:doi:10.1017/S0012162201000597 (2001).

4 Woessner, J. F. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Archives of Biochemistry and Biophysics* **93**, 440-447, doi:10.1016/0003-9861(61)90291-0 (1961).

5 Lieber, R. L., Fazeli, B. M. & Botte, M. J. Architecture of selected wrist flexor and extensor muscles. *J Hand Surg Am* **15**, 244-250 (1990).

6 Lieber, R. L., Jacobson, M. D., Fazeli, B. M., Abrams, R. A. & Botte, M. J. Architecture of selected muscles of the arm and forearm: anatomy and implications for tendon transfer. *J Hand Surg Am* **17**, 787-798 (1992).

7 Delp, S. L., Suryanarayanan, S., Murray, W. M., Uhlir, J. & Triolo, R. J. Architecture of the rectus abdominis, quadratus lumborum, and erector spinae. *Journal of Biomechanics* **34**, 371-375 (2001).

8 Ward, S. R., Eng, C. M., Smallwood, L. H. & Lieber, R. L. Are current measurements of lower extremity muscle architecture accurate? *Clin Orthop Relat Res* **467**, 1074-1082, doi:10.1007/s11999-008-0594-8 (2009).

9 Ward, S. R. *et al.* Rotator cuff muscle architecture: implications for glenohumeral stability. *Clin Orthop Relat Res* **448**, 157-163, doi:10.1097/01.blo.0000194680.94882.d3 (2006).

10 Jacobson, M. D. *et al.* Architectural design of the human intrinsic hand muscles. *J Hand Surg Am* **17**, 804-809 (1992).

11 Gajdosik, R. L. Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clinical Biomechanics* **16**, 87-101, doi:10.1016/s0268-0033(00)00061-9 (2001).

12 Aumailley, M. & Gayraud, B. Structure and biological activity of the extracellular matrix. *Journal of Molecular Medicine* **76**, 253-265, doi:10.1007/s001090050215 (1998).

13 Hamill, O. P. & Martinac, B. Molecular Basis of Mechanotransduction in Living Cells. *Physiological Reviews* **81**, 685-740 (2001).

14 Purslow, P. P. Muscle fascia and force transmission. *Journal of Bodywork and Movement Therapies* **14**, 411-417, doi:10.1016/j.jbmt.2010.01.005 (2010).

15 Passerieux, E., Rossignol, R., Letellier, T. & Delage, J. P. Physical continuity of the perimysium from myofibers to tendons: involvement in lateral force transmission in skeletal muscle. *Journal of Structural Biology* **159**, 19-28, doi:10.1016/j.jsb.2007.01.022 (2007).

16 Trotter, J. A. Interfiber tension transmission in series-fibered muscles of the cat hindlimb. *Journal of Morphology* **206**, 351-361, doi:10.1002/jmor.1052060312 (1990).

17 Gosselin, L. E. in *Sarcopenia – Age-Related Muscle Wasting and Weakness* (ed Gordon S. Lynch) 159-172 (Springer Netherlands, 2011).

18 Gillies, A. R. & Lieber, R. L. Structure and function of the skeletal muscle extracellular matrix. *Muscle & Nerve*, n/a-n/a, doi:10.1002/mus.22094 (2011).

19 Light, N. & Champion, A. E. Characterization of muscle epimysium, perimysium and endomysium collagens. *The Biochemical journal* **219**, 1017-1026 (1984).

20 Bailey, A. J., Restall, D. J., Sims, T. J. & Duance, V. C. Meat tenderness: Immunofluorescent localisation of the isomorphic forms of collagen in bovine muscles of varying texture. *Journal of the Science of Food and Agriculture* **30**, 203-210, doi:10.1002/jsfa.2740300216 (1979).

21 Mays, P. K., Bishop, J. E. & Laurent, G. J. Age-related changes in the proportion of types I and III collagen. *Mechanisms of Ageing and Development* **45**, 203-212, doi:10.1016/0047-6374(88)90002-4 (1988).

22 Marijianowski, M. M. H., Teeling, P., Mann, J. & Becker, A. E. Dilated cardiomyopathy is associated with an increase in the type I/type III collagen ratio: A quantitative assessment. *Journal of the American College of Cardiology* **25**, 1263-1272, doi:10.1016/0735-1097(94)00557-7 (1995).

23 Horwitz, A. L., Hance, A. J. & Crystal, R. G. Granulocyte Collagenase: Selective Digestion of Type I Relative to Type III Collagen. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 897-901 (1977).

24 Purslow, P. P. Strain-induced reorientation of an intramuscular connective tissue network: Implications for passive muscle elasticity. *Journal of Biomechanics* **22**, 21-31, doi:10.1016/0021-9290(89)90181-4 (1989).

25 Schleip, R. *et al.* Passive muscle stiffness may be influenced by active contractility of intramuscular connective tissue. *Medical Hypotheses* **66**, 66-71, doi:10.1016/j.mehy.2005.08.025 (2006).

26 Gajdosik, R. *et al.* Viscoelastic properties of short calf muscle-tendon units of older women: effects of slow and fast passive dorsiflexion stretches in vivo. *European Journal of Applied Physiology* **95**, 131-139, doi:10.1007/s00421-005-1394-4 (2005).

27 Gosselin, L. E., Adams, C., Cotter, T. A., McCormick, R. J. & Thomas, D. P. Effect of exercise training on passive stiffness in locomotor skeletal muscle: role of extracellular matrix. *Journal of Applied Physiology* **85**, 1011-1016 (1998).

28 Alnaqeeb, M. A., Al Zaid, N. S. & Goldspink, G. Connective tissue changes and physical properties of developing and ageing skeletal muscle. *J Anat* **139 (Pt 4)**, 677-689 (1984).

29 Porter, J. D. *et al.* A chronic inflammatory response dominates the skeletal muscle molecular signature in dystrophin-deficient mdx mice. *Human Molecular Genetics* **11**, 263-272, doi:10.1093/hmg/11.3.263 (2002).

30 Watchko, J. F., O'Day, T. L. & Hoffman, E. P. Functional characteristics of dystrophic skeletal muscle: insights from animal models. *Journal of Applied Physiology* **93**, 407-417, doi:10.1152/japplphysiol.01242.2001 (2002).

31 Järvinen, T. A. H., Józsa, L., Kannus, P., Järvinen, T. L. N. & Järvinen, M. Organization and distribution of intramuscular connective tissue in normal and immobilized skeletal muscles. *Journal of Muscle Research and Cell Motility* **23**, 245-254, doi:10.1023/a:1020904518336 (2002).

32 Williams, P. E. & Goldspink, G. Connective tissue changes in immobilised muscle. *J Anat* **138 (Pt 2)**, 343-350 (1984).

33 Maughan, R. J., Watson, J. S. & Weir, J. Strength and cross-sectional area of human skeletal muscle. *The Journal of Physiology* **338**, 37-49 (1983).

34 Kovanen, V., Suominen, H., Risteli, J. & Risteli, L. Type IV collagen and laminin in slow and fast skeletal muscle in rats--effects of age and life-time endurance training. *Coll Relat Res* **8**, 145-153 (1988).

35 Goldspink, G., Fernandes, K., Williams, P. E. & Wells, D. J. Age-related changes in collagen gene expression in the muscles of mdx dystrophic and normal mice. *Neuromuscul Disord* **4**, 183-191 (1994).

36 Kjaer, M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiological Reviews* **84**, 649-698, doi:10.1152/physrev.00031.2003 (2004).

37 Woessner, J. F., Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Archives of Biochemistry and Biophysics* **93**, 440-447 (1961).

38 Neuman, R. E. & Logan, M. A. The determination of collagen and elastin in tissues. *J Biol Chem* **186**, 549-556 (1950).

39 Sell, D. R. & Monnier, V. M. End-stage renal disease and diabetes catalyze the formation of a pentose-derived crosslink from aging human collagen. *J Clin Invest* **85**, 380-384, doi:10.1172/JCI114449 (1990).

40 James, V. J., Delbridge, L., McLennan, S. V. & Yue, D. K. Use of X-ray diffraction in study of human diabetic and aging collagen. *Diabetes* **40**, 391-394 (1991).

41 Silver, F. H., Freeman, J. W., Horvath, I. & Landis, W. J. Molecular basis for elastic energy storage in mineralized tendon. *Biomacromolecules* **2**, 750-756 (2001).

42 Purslow, P. P. The structure and functional significance of variations in the connective tissue within muscle. *Comp Biochem Physiol A Mol Integr Physiol* **133**, 947-966 (2002).

43 Trotter, J. A. & Purslow, P. P. Functional morphology of the endomysium in series fibered muscles. *J Morphol* **212**, 109-122, doi:10.1002/jmor.1052120203 (1992).

44 Kadi, F. *et al.* The behaviour of satellite cells in response to exercise: what have we learned from human studies? *Pflugers Arch* **451**, 319-327, doi:10.1007/s00424-005-1406-6 (2005).

45 Crameri, R. M. *et al.* Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. *The Journal of Physiology* **558**, 333-340, doi:10.1113/jphysiol.2004.061846 (2004).

46 Myllyla, R., Salminen, A., Peltonen, L., Takala, T. E. & Vihko, V. Collagen metabolism of mouse skeletal muscle during the repair of exercise injuries. *Pflugers Arch* **407**, 64-70 (1986).

47 Carmeli, E., Patish, H. & Coleman, R. The aging hand. *J Gerontol A Biol Sci Med Sci* **58**, 146-152 (2003).

48 Ward, S. R., Loren, G. J., Lundberg, S. & Lieber, R. L. High stiffness of human digital flexor tendons is suited for precise finger positional control. *J Neurophysiol* **96**, 2815-2818, doi:10.1152/jn.00284.2006 (2006).

49 Loren, G. J. & Lieber, R. L. Tendon biomechanical properties enhance human wrist muscle specialization. *Journal of Biomechanics* **28**, 791-799 (1995).

50 Moore, K. L., Agur, A. M. R. & Dalley, A. F. *Essential clinical anatomy*. 4th edn, (Lippincott Williams & Wilkins, 2011).

51 King, G. J. W., Morrey, B. F. & An, K.-N. Stabilizers of the elbow. *Journal of Shoulder and Elbow Surgery* **2**, 165-174, doi:10.1016/s1058-2746(09)80053-0.

52 Sebastin, S. J., Lim, A. Y., Bee, W. H., Wong, T. C. & Methil, B. V. Does the absence of the palmaris longus affect grip and pinch strength? *J Hand Surg Br* **30**, 406-408, doi:10.1016/j.jhsb.2005.03.011 (2005).

53 Chatterjee, D. S. Repetition strain injury--a recent review. *J Soc Occup Med* **37**, 100-105 (1987).

54 Erak, S., Day, R. & Wang, A. The role of supinator in the pathogenesis of chronic lateral elbow pain: a biomechanical study. *J Hand Surg Br* **29**, 461-464, doi:10.1016/j.jhsb.2004.06.001 (2004).

55 Billuart, F., Devun, L., Skalli, W., Mitton, D. & Gagey, O. Role of deltoid and passives elements in stabilization during abduction motion (0 degrees-40 degrees): an ex vivo study. *Surg Radiol Anat* **30**, 563-568, doi:10.1007/s00276-008-0374-x (2008).

56 Johnson, G. R., Spalding, D., Nowitzke, A. & Bogduk, N. Modelling the muscles of the scapula morphometric and coordinate data and functional implications. *Journal of Biomechanics* **29**, 1039-1051 (1996).

57 Webb, D., Tuttle, R. H. & Baksh, M. Pendular activity of human upper limbs during slow and normal walking. *Am J Phys Anthropol* **93**, 477-489, doi:10.1002/ajpa.1330930407 (1994).

58 Riley, G. P. *et al.* Tendon degeneration and chronic shoulder pain: changes in the collagen composition of the human rotator cuff tendons in rotator cuff tendinitis. *Ann Rheum Dis* **53**, 359-366 (1994).

59 Bogduk, N., Johnson, G. & Spalding, D. The morphology and biomechanics of latissimus dorsi. *Clinical Biomechanics* **13**, 377-385 (1998).

60 Borstad, J. D. & Ludewig, P. M. Comparison of three stretches for the pectoralis minor muscle. *J Shoulder Elbow Surg* **15**, 324-330, doi:10.1016/j.jse.2005.08.011 (2006).

61 Herzberg, G., Urien, J. P. & Dimnet, J. Potential excursion and relative tension of muscles in the shoulder girdle: relevance to tendon transfers. *J Shoulder Elbow Surg* **8**, 430-437 (1999).

62 Cholewicki, J. & McGill, S. M. Mechanical stability of the in vivo lumbar spine: implications for injury and chronic low back pain. *Clinical Biomechanics* **11**, 1-15 (1996).

63 McGill, S. M., Hughson, R. L. & Parks, K. Changes in lumbar lordosis modify the role of the extensor muscles. *Clinical Biomechanics* **15**, 777-780 (2000).

64 Ward, S. R. *et al.* Passive mechanical properties of the lumbar multifidus muscle support its role as a stabilizer. *Journal of Biomechanics* **42**, 1384-1389, doi:10.1016/j.jbiomech.2008.09.042 (2009).

65 Brown, S. H., Ward, S. R., Cook, M. S. & Lieber, R. L. Architectural analysis of human abdominal wall muscles: implications for mechanical function. *Spine (Phila Pa 1976)* **36**, 355-362, doi:DOI: 10.1097/BRS.0b013e3181d12ed7 (2011).

66 Hughes, P. E., Hsu, J. C. & Matava, M. J. Hip Anatomy and Biomechanics in the Athlete. *Sports Medicine and Arthroscopy Review* **10**, 103-114 (2002).

67 Regev, G. J. *et al.* Psoas Muscle Architectural Design, In Vivo Sarcomere Length Range, and Passive Tensile Properties Support its Role as a Lumbar Spine Stabilizer. *Spine (Phila Pa 1976)*, doi:10.1097/BRS.0b013e31821847b3 (2011).

68 Lieberman, D. E., Raichlen, D. A., Pontzer, H., Bramble, D. M. & Cutright-Smith, E. The human gluteus maximus and its role in running. *J Exp Biol* **209**, 2143-2155, doi:10.1242/jeb.02255 (2006).

69 Whittington, B., Silder, A., Heiderscheit, B. & Thelen, D. G. The contribution of passive-elastic mechanisms to lower extremity joint kinetics during human walking. *Gait Posture* **27**, 628-634, doi:10.1016/j.gaitpost.2007.08.005 (2008).

70 Kerrigan, D. C., Todd, M. K., Della Croce, U., Lipsitz, L. A. & Collins, J. J. Biomechanical gait alterations independent of speed in the healthy elderly: evidence for specific limiting impairments. *Arch Phys Med Rehabil* **79**, 317-322 (1998).

71 Farmer, S. E. & James, M. Contractures in orthopaedic and neurological conditions: a review of causes and treatment. *Disabil Rehabil* **23**, 549-558 (2001).

72 Kumagai, K. *et al.* Sprint performance is related to muscle fascicle length in male 100-m sprinters. *Journal of Applied Physiology* **88**, 811-816 (2000).

73 Smith, L. R., Lee, K. S., Ward, S. R., Chambers, H. G. & Lieber, R. L. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased in vivo sarcomere length. *The Journal of Physiology* **589**, 2625-2639, doi:10.1113/jphysiol.2010.203364 (2011).

74 Zhao, C. G., He, X. J., Lu, B., Li, H. P. & Kang, A. J. Increased expression of collagens, transforming growth factor-beta1, and -beta3 in gluteal muscle contracture. *BMC Musculoskelet Disord* **11**, 15, doi:10.1186/1471-2474-11-15 (2010).

75 Austin, N., Nilwik, R. & Herzog, W. In vivo operational fascicle lengths of vastus lateralis during sub-maximal and maximal cycling. *Journal of Biomechanics* **43**, 2394-2399, doi:10.1016/j.jbiomech.2010.04.016 (2010).

76 Hasselman, C. T., Best, T. M., Hughes, C. t., Martinez, S. & Garrett, W. E., Jr. An explanation for various rectus femoris strain injuries using previously undescribed muscle architecture. *Am J Sports Med* **23**, 493-499 (1995).

77 Lempainen, L., Sarimo, J., Heikkila, J., Mattila, K. & Orava, S. Surgical treatment of partial tears of the proximal origin of the hamstring muscles. *Br J Sports Med* **40**, 688-691, doi:10.1136/bjsm.2006.028191 (2006).

78 Petersen, J. & Holmich, P. Evidence based prevention of hamstring injuries in sport. *Br J Sports Med* **39**, 319-323, doi:10.1136/bjsm.2005.018549 (2005).

79 Tyler, T. F., Nicholas, S. J., Campbell, R. J. & McHugh, M. P. The association of hip strength and flexibility with the incidence of adductor muscle strains in professional ice hockey players. *Am J Sports Med* **29**, 124-128 (2001).

80 Walmsley, B. & Proske, U. Comparison of stiffness of soleus and medial gastrocnemius muscles in cats. *J Neurophysiol* **46**, 250-259 (1981).

81 Jamadar, D. A. *et al.* Sonography of the painful calf: differential considerations. *AJR Am J Roentgenol* **179**, 709-716 (2002).

82 Kaariainen, M., Jarvinen, T., Jarvinen, M., Rantanen, J. & Kalimo, H. Relation between myofibers and connective tissue during muscle injury repair. *Scand J Med Sci Sports* **10**, 332-337 (2000).

83 Grindstaff, T. L. *et al.* Immediate effects of a tibiofibular joint manipulation on lower extremity H-reflex measurements in individuals with chronic ankle instability. *J Electromyogr Kinesiol* **21**, 652-658, doi:10.1016/j.jelekin.2011.03.011 (2011).

84 Jacob, H. A. Forces acting in the forefoot during normal gait--an estimate. *Clinical Biomechanics* **16**, 783-792 (2001).

85 Reber, L., Perry, J. & Pink, M. Muscular control of the ankle in running. *Am J Sports Med* **21**, 805-810; discussion 810 (1993).

86 Maas, H., Baan, G. C. & Huijing, P. A. Intermuscular interaction via myofascial force transmission: effects of tibialis anterior and extensor hallucis longus length on force transmission from rat extensor digitorum longus muscle. *Journal of Biomechanics* **34**, 927-940 (2001).

87 Messelink, B. *et al.* Standardization of terminology of pelvic floor muscle function and dysfunction: report from the pelvic floor clinical assessment group of the International Continence Society. *Neurourol Urodyn* **24**, 374-380, doi:10.1002/nau.20144 (2005).

88 Eng, C. M. *et al.* Scaling of muscle architecture and fiber types in the rat hindlimb. *J Exp Biol* **211**, 2336-2345, doi:10.1242/jeb.017640 (2008).

89 Yan, C. & Boyd, D. D. Regulation of matrix metalloproteinase gene expression. *J Cell Physiol* **211**, 19-26, doi:10.1002/jcp.20948 (2007).

90 Salamonsen, L. A. & Woolley, D. E. Matrix metalloproteinases in normal menstruation. *Hum Reprod* **11 Suppl 2**, 124-133 (1996).

91 Bell, D. R. *et al.* Estrogen and muscle stiffness have a negative relationship in females. *Knee Surg Sports Traumatol Arthrosc*, doi:10.1007/s00167-011-1577-y (2011).

92 Slauterbeck, J., Clevenger, C., Lundberg, W. & Burchfield, D. M. Estrogen level alters the failure load of the rabbit anterior cruciate ligament. *J Orthop Res* **17**, 405-408, doi:10.1002/jor.1100170316 (1999).