Effects of maturation and training on the development of the morphological and mechanical properties of the muscle-tendon unit

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Zusammenfassung

Bei jugendlichen Athleten wirken sowohl die körperliche Reifung als auch die erhöhte mechanische Belastung auf die Entwicklung des Muskel- und Sehnengewebes. Bislang gibt es jedoch keine Informationen hinsichtlich der Interaktion dieser beiden adaptationswirksamen Stimuli und es besteht Grund zu der Annahme, dass während der Adoleszenz, insbesondere bei Sportlern aus Sprungdisziplinen, Dysbalancen des Kraftpotenzials der Muskulatur und der Widerstandsfähigkeit (i.e. Steifigkeit) der Sehne auftreten und sich somit potentiell das Verletzungsrisiko der Sehne erhöhen könnte. Vor diesem Hintergrund untersucht die vorliegende Arbeit mittels Magnetresonanztomographie, Ultraschall und Dynamometrie die Entwicklung der morphologischen und mechanischen Eigenschaften der Knieextensoren und der Patellarsehne bei jugendlichen Kader-Volleyballathleten.

Zunächst zeigte ein Vergleich zu langjährig sportspezifisch trainierenden Athleten, dass sich die jugendliche Athleten hinsichtlich des Kraftpotenzials und der Morphologie der Muskulatur bereits in der mittleren Adoleszenz auf dem Niveau von Erwachsenen befanden, der Querschnitt der Sehne jedoch noch unterentwickelt war und somit erhöhte Belastungs- und Beanspruchungsparameter detektiert werden konnten. Die Annahme, dass sich das morphologische Anpassungspotential der Sehne vermutlich erst zu einem späteren Zeitpunkt in der Adoleszenz entfaltet, konnte durch eine zweijährige Längsschnittstudie bestätigt werden. Abschließend wurde der Entwicklungsverlauf von Muskelkraft und Widerstandsfähigkeit der Sehne in der mittleren Adoleszenz mit fünf Messzeitpunkten über ein Jahr näher untersucht und durch Einschluss einer jugendlichen Kontrollgruppe der Effekt der Reifung und des sportlichen Trainings ausdifferenziert. Dabei zeigten sich bei den Athleten signifikant größere Fluktuationen der Muskelkraft und eine Dysbalance der Muskel- und Sehnenadaptation. Als Konsequenz zeigte sich bei den jugendlichen Volleyballathleten eine sowohl chronisch als auch insbesondere episodisch erhöhte Beanspruchung der Sehne während maximaler Muskelkontraktionen, die für die Ausprägung von Überlastungsbeschwerden in der hier untersuchten Risikogruppe für Tendinopathien prädisponieren könnte.

Im Rahmen der Forschungsbemühungen wurde darüber hinaus ein Verfahren zur Vorhersage des Volumens von Oberschenkelmuskeln auf der Basis einfach zu erhebender Parameter entwickelt. Durch das Verfahren ließe sich zukünftig der Zeit- und Kostenaufwand muskelmorphologischer Diagnostik deutlich reduzieren.

Abstract

In adolescent athletes, the development of muscle and tendon tissue is influenced both by maturation and mechanical loading. However, no information is currently available on the interaction of these two driving stimuli although there is reason to believe that during adolescence imbalances between the muscle strength capacity and the tendon stiffness might develop, especially in athletes from sports that feature a high frequency of jumps. A consequence such an imbalance could be an increased mechanical demand placed upon the tendon by the working muscle, which might increase the risk of tendon injury. Considering the lack of information on the effects of maturation and superimposed mechanical loading on the muscle-tendon unit during adolescence, the present work examines the development of the morphological and mechanical properties of the knee extensors and patellar tendon in adolescent elite volleyball athletes by means of magnetic resonance imaging, ultrasound and dynamometry.

An initial comparison of mid-adolescent to middle-aged athletes, which were subjected to many years of sport-specific loading, demonstrated that mid-adolescent athletes feature similar muscle strength capacity and morphology, yet they show deficits with regard to the radial growth of tendons (i.e. tendon cross-sectional area) and, as a consequence, increased levels of external and internal loading parameters (i.e. tendon stress and strain). The assumption that the morphological plasticity of the tendon unfolds at later stages during development was supported by the results of a subsequent two-year longitudinal study. Finally, the time course of muscle and tendon development during mid-adolescence was investigated in five measurement sessions over one year in more detail and the effects of maturation and mechanical loading were differentiated by including a similar-aged control group. It was found that the development of muscle strength in athletes was characterised by significantly greater fluctuations and a non-uniformity of muscle and tendon adaptation. Consequently, tendon strain during maximum contraction, as a measure of internal loading, was increased episodically and chronically, which could contribute to the predisposition for the development of tendon overuse injury in this group, which is known to be at high risk of developing tendinopathy.

Moreover, in the context of the described research endeavour, a method for the volume prediction of thigh muscles on the basis of easily measurable parameters has been developed, which could greatly reduce the time and costs involved in the diagnostics of muscle morphology in the future.

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1 Introduction and literature review

This thesis investigates the development of the knee extensor muscle-tendon unit during adolescence and its adaptation to athletic volleyball training in light of the high incidence of tendon overuse in the population of volleyball athletes. The following introduction gives an overview of the morphological, mechanical and functional properties of muscles and tendons as well as their *in vivo* assessment. It is followed by a review of the current knowledge on muscle and tendon adaptation in response to mechanical loading and the development of the muscle-tendon unit from childhood to adulthood. In the final section of this chapter, the hypothesis of a non-uniform adaptation of muscles and tendons in response to increased mechanical loading during adolescence is explored and the potential implications with regard to tendon overuse are discussed. The focus of this thesis and literature review is on the morphological, mechanical and functional development and adaptation of muscles and tendons. Other aspects of the neuromuscular development and adaptation (e.g. neural, structural or molecular factors) are discussed briefly where necessary.

1.1 Muscle and tendon properties and *in vivo* assessment

In order to produce movement, muscles and tendons work as a unit, in which the muscle is the contractile element responsible for generating forces, while tendons, being the series elastic elements in muscle-tendon units, transmit the forces to the skeleton (Józsa and Kannus, 1997; Nigg and Herzog, 2007). The viscoelastic properties of tendons influence the dynamics of muscle fibres during the movement, facilitating the force-generating capacity of the fibres by optimising their operating range with regard to the force-velocity and force-length relationship, and allow for the storage and release of strain energy (Hof et al., 1983; Ettema et al., 1990; Roberts, 1997; Kawakami and Fukunaga, 2006). Consequently, the functional properties of muscles and the mechanical properties tendons both greatly influence movement performance (Zajac, 1989 for review; Sleivert et al., 1995; e.g.: Wisløff et al., 2004; Bojsen-Møller et al., 2005; Arampatzis et al., 2006; Stafilidis and Arampatzis, 2007; Karamanidis et al., 2008; Kubo et al., 2011b; Waugh et al., 2013) and, therefore, their assessment is of crucial importance for diagnostics in many scientific and medical contexts as well as in sports. The following section introduces the major musculotendinous determinants of force and power production, focussing on parameters that can be assessed non-invasively *in vivo* and providing a short

overview of the associated methodological approaches that have been established to date. With regard to the topic investigated in the present thesis, the diagnostics of the knee extensor muscle-tendon unit is emphasised.

1.1.1 Muscle morphology and architecture

The force generating capacity of a muscle is determined by the number of sarcomeres in parallel (Haxton, 1944; Goldspink, 1985) and intrinsic contractile properties (Bottinelli et al., 1996; i.e. specific tension; Fukunaga et al., 1996; Maganaris et al., 2001). It has been demonstrated *in vivo* that muscle strength is associated with the anatomical cross-sectional area of the muscle (ACSA; Ikai and Fukunaga, 1968; Moss et al., 1997). Though thigh muscle ACSA can be estimated on the basis of on anthropometric measurements (Knapik et al., 1996), a more accurate assessment and discrimination of single muscles relies on imaging techniques. The use of ultrasound for the visualisation of distinct muscles and determination of their ACSAs provides high reliability (Rankin and Stokes, 1998; Reeves et al., 2004), but the fitting of multiple images that is necessary when studying large muscles as the quadriceps vastii is challenging and time-consuming. Extended field-of-view ultrasonography incorporates automatised image-fitting algorithms (Weng et al., 1997) and produces promising results (Noorkoiv et al., 2010; Lixandrão et al., 2014). For this reason, it could be an alternative to the established gold standards for *in vivo* muscle ACSA assessment (i.e. magnetic resonance imaging [MRI] and computed tomography; Mitsiopoulos et al., 1998).

Theoretically, in a pennated muscle, the physiological cross-sectional area (PCSA) of a muscle provides a better estimate of the number of sarcomeres in parallel compared to the ACSA. Accordingly, the PCSA has been shown to be more closely associated to muscle strength in comparison to the ACSA (Fukunaga et al., 1996; 2001) and, furthermore, can be used to approximate muscle specific tension *in vivo* (Fukunaga et al., 1996; Maganaris et al., 2001; Erskine et al., 2011). However, it is currently not possible to directly measure muscle PCSA in humans. It is assessed indirectly, by calculating the quotient of muscle volume and fascicle length (Powell et al., 1984; Lieber and Fridén, 2000).

Fascicle length as well as muscle thickness and pennation angle can be assessed with good accuracy and reliability by means of ultrasound imaging (English et al., 2012; Kwah et al., 2013), especially in large muscles in inactive state (Kwah et al., 2013 for review). Fascicle length is related to the number of sarcomeres in series within muscle fibres and, therefore, is a determinant of maximum fibre shortening velocity (Bodine et al., 1982; Goldspink, 1985). Muscle thickness and pennation angle on the other hand are often used to explain observations on muscle strength, since

the thickness of a muscle can be an indicator of its morphological properties (e.g. volume or ACSA; Esformes et al., 2002; Miyatani et al., 2004; Giles et al., 2015) and the pennation angle is associated with the muscle PCSA (Kawakami et al., 1995; Aagaard et al., 2001), respectively.

Since the volume of a muscle is the best morphological descriptor of overall sarcomere number *in vivo* and includes both radial and longitudinal (and, thus, muscle force and shortening velocity) characteristics, it is a major determinant of the maximum mechanical muscle power (Sleivert et al., 1995; O'Brien et al., 2009a). Moreover, muscle volume is a necessary parameter for the calculation of muscle PCSA *in vivo*. The segmentation of transverse plane MRI images over the full length of a muscle is considered the gold standard for in vivo muscle volume assessment (Mitsiopoulos et al., 1998); however, it is costly and time-consuming. Three-dimensional freehand ultrasound scanning (MacGillivray et al., 2009) and predictions of muscle volume based on muscle thickness (Miyatani et al., 2004; Giles et al., 2015) or multiple ACSAs measured by means of ultrasound (Esformes et al., 2002) as well as using single plane MRI scans (Morse et al., 2007) are either time-consuming (Esformes et al., 2002) or strongly compromise accuracy (Miyatani et al., 2004; Morse et al., 2007; MacGillivray et al., 2009; Giles et al., 2015). Albracht and colleagues (2008) proposed a promising method for predicting the volume of the triceps surae muscles on the basis of their length, maximum ACSA and a muscle-specific scaling factor that is dependent on the muscle shape (and was, therefore, referred to as *shape factor*). The method and the underlying assumption that for a given muscle of the triceps surae the shape factor is quite constant across populations and independent of muscle dimensions has been validated only recently (Mersmann et al., 2014). However, the applicability of the method to the knee extensors still needs to be established.

1.1.2 Morphological, mechanical and material properties of tendons

The main parameters commonly used to assess the mechanical, morphological and material properties of tendons *in vivo* are stiffness, rest length, cross-sectional area (CSA) and Young's modulus. Tendon stiffness refers to the relationship between the applied tendon force and the resultant tendon elongation and has been demonstrated to affect the rate of muscle force development (Waugh et al., 2013), jump and sprint performance (Bojsen-Møller et al., 2005; Stafilidis and Arampatzis, 2007) as well as running economy (Arampatzis et al., 2006; Fletcher et al., 2010; Albracht and Arampatzis, 2013). Due to major advances in imaging technology over the past decades, tendon stiffness can be established *in vivo* using a combination of dynamometry and ultrasound (Fukashiro et al., 1995; Kubo et al., 1999; Maganaris

and Paul, 2002). Tendon force is calculated by dividing the respective joint moments generated by the muscles attached to the tendon, typically recorded during isometric contractions with gradually increasing effort from rest to maximum voluntary activation, by the tendon lever arm. However, it needs to be considered that the joint moment that is measured with a dynamometer is not only generated by the muscles that apply force to the tendon of interest but also synergistic and antagonistic muscles as well as gravitational forces. Moreover, the non-rigidity of the human-dynamometer system causes deviations of the joint axis with respect to the dynamometer during isometric contractions (Arampatzis et al., 2004; 2005). To account for most of these factors, respective correction procedures that are applicable to the knee joint have been established (Kellis and Baltzopoulos, 1997; Arampatzis et al., 2004; Mademli et al., 2004). The lever arm of the patellar tendon is frequently estimated from anthropometric data (Visser et al., 1990; Krevolin et al., 2004). However, considerable doubts have been raised about the validity of this approach (Tsaopoulos et al., 2007b; O'Brien et al., 2009b) and, therefore, the use of imaging techniques is recommended, especially for between-group comparisons where the inaccuracies of lever arm estimation would affect the comparability of all dependent parameters. Both, the tendon excursion method (An et al., 1984) and the geometric method (Reuleaux, 1875) can account for individual differences in joint anatomy and/or kinematics *in vivo.* The former involves the measurement of the tendon excursion in relation to joint rotation (Buford et al., 1997); however, the applicability of the method to the patellar tendon has been questioned (Sheehan, 2007). The geometric method is based on the measurement of the perpendicular distance from the line of action of the tendon to a reference point that approximates the joint centre of rotation (Tsaopoulos et al., 2006 for review). In the flexed knee positions that are relevant for the assessment of tendon properties, the variability of moment arm estimations using different reference points (e.g. instant centre of rotation, tibiofemoral contact point and geometrical centre of the posterior femoral condyles) is low (Tsaopoulos et al., 2009). However, it should be considered that the patellar tendon lever arm changes during contractions due to angular rotation and tendon loading (Smidt, 1973; Herzog and Read, 1993; Baltzopoulos, 1995; Tsaopoulos et al., 2007a).

Reeves and colleagues (2003), were the first to attempt an ultrasound-based assessment of patellar tendon elongation during isometric contractions *in vivo* by measuring the displacement of the patella in relation to a reference marker placed on the skin. It was demonstrated later by Hansen et al. (2006) that this procedure underestimates tendon elongation due to additional relative movement of the tibia. The use of longer transducers enabled researchers to visualise both the patellar apex

and either the tibial plateau (Kongsgaard et al., 2007; Carroll et al., 2008; Seynnes et al., 2011; Kösters et al., 2014) or even the actual insertion site at the tibial tuberosity (O'Brien et al., 2010b; Schulze et al., 2012; Kubo et al., 2014b) during contractions in a single recording (Figure 1.1). It seems reasonable to assume that the latter should be used, if possible, to reduce the probability of measurement error due to tibial tilt during contractions (Seynnes et al., 2015 for review). However, the precise visualisation and digitalisation of the anatomical reference points for elongation measurement is still challenging, and the tracking and averaging of multiple trials is thus strongly recommended to increase reliability (Schulze et al., 2012). When tendon force and elongation are established, tendon stiffness is calculated (either as a quotient or slope of a linear regression) in the elastic region of the force-elongation relationship, usually between 50% and 100% of the maximum tendon force (Seynnes et al., 2015 for review).

Tendon stiffness depends on (a) material properties (i.e. Young's modulus), (b) tendon CSA and (c) tendon rest length (Butler et al., 1978). The assessment of patellar tendon rest length is in line with the ultrasound-based elongation measurement. The CSA, on the other hand, should preferably be segmented in MRI images (Couppé et al., 2013b) since two systematic methodological studies recently

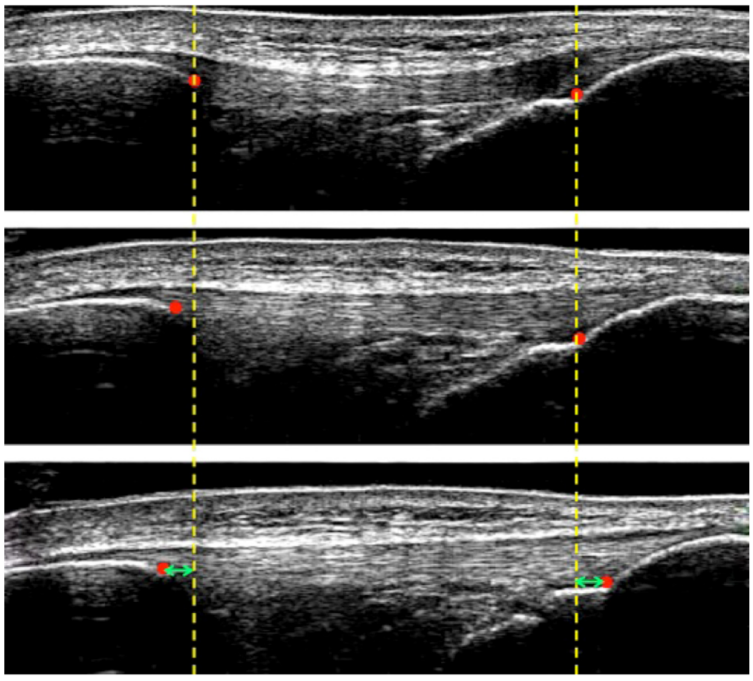

Figure 1.1 Ultrasound images of a patellar tendon during a maximal isometric knee extension contraction (top to bottom: at rest, ~50% and 100% of the maximal voluntary isometric contraction). The patella is seen on the left side and tibial tuberosity on the right side. The red marks (enlarged for illustration purposes) tag the anatomical reference points that were tracked for the elongation measurement. The yellow dashed lines illustrate the position of the marks at rest. (Schulze et al., 2012, Gait Posture 35, 334-338, p. 336. Reproduced with permission from Elsevier.)

reported an insufficient reliability and objectivity of ultrasound imaging for the assessment of CSA in the patellar tendon (Ekizos et al., 2013) as well as insufficient reliability, objectivity and validity in the Achilles tendon (Bohm et al., 2016). Due to potential regional tendon hypertrophy (Kongsgaard et al., 2007) or localised between-group differences (Couppé et al., 2013a), it is commendable to segment and analyse the CSA over the whole length of the tendon. By normalising the force-elongation relationship of the tendon on its morphological properties (i.e. force to CSA to obtain stress and elongation to rest length to obtain strain) the stress-strain relationship can be derived, which is solely dependent on the tendon material properties. Similarly to tendon stiffness, the slope of the stress-strain relationship between 50% and 100% of maximum stress is determined and referred to as Young's modulus (or elastic modulus). It is mainly influenced by the type and degree of intra- and interfibrillar collagen cross-linking (Thompson and Czernuszka, 1995; Depalle et al., 2015; Lin and Gu, 2015), glycosaminoglycan content (contributing to interfibrillar connections and force transfer; Cribb and Scott, 1995) and collagen area fraction (Robinson et al., 2004), which all strongly affect the micromechanical load-response behaviour of tendinous tissue.

1.2 Muscle-tendon unit plasticity

The following section provides a short overview of the scientific observations of loading-induced adaptation of muscle and tendon tissue in adults. The limited information that is available on adaptability of the muscle-tendon unit in children and adolescents is discussed with regard to maturation and development in Chapter 1.3.1. The synopsis focuses on adaptations that are relevant for strength and power production elicited by means of high-intensity mechanical loading, which predominantly is provided experimentally using heavy resistance or plyometric loading regimen. The respective sections on muscle and tendon adaptation are concluded with a summary of the current knowledge on the underlying mechanobiological mechanisms.

1.2.1 Mechanisms of muscle adaptation

When muscle tissue is chronically overloaded (e.g. due to resistance training), it demonstrates changes from the subcellular to the whole-muscle level that can be ultimately categorised into (a) radial adaptation, (b) longitudinal adaptation and (c) adaptation of specific tension (Goldspink, 1985; Bottinelli, 2001). A frequently reported response to strength training is the increase of muscle ACSA (e.g. Narici et

al., 1989; e.g. Johnson and Klueber, 1991; Kraemer et al., 2004; Moore, 2005; Blazevich et al., 2007; Farup et al., 2012), and this response seems to be irrespective of contraction type applied during training at a given loading volume (Wernbom et al., 2007; Moore et al., 2011; Farup et al., 2014; Rahbek et al., 2014).

In pennated muscle, the increase in ACSA can be mediated by both radial and longitudinal changes of muscle fibres. The PCSA of a muscle provides the best estimate of the number of sarcomeres in parallel and is therefore a more appropriate measure of radial adaptation (Haxton, 1944). Kawakami and colleagues (1995) were among the first to report an increase of muscle PCSA in response to strength training *in vivo* for the elbow flexors, which was later shown for the knee extensors as well (Seynnes et al., 2009; Erskine et al., 2010a). The changes were accompanied by an increase of fascicle pennation angle (Kawakami et al., 1995; Erskine et al., 2010a), which has been observed by several authors after applying interventions that facilitate muscle strength (Aagaard et al., 2001; Blazevich et al., 2007; Seynnes et al., 2007; Farup et al., 2012). Therefore, the remodelling of muscle architecture is considered to be a modulating factor of the PCSA (Alexander and Vernon, 1975) that enables fibre hypertrophy and hence radial muscle growth to exceed the changes of the whole muscle ACSA (Häkkinen et al., 1998; Aagaard et al., 2001). The increase in single muscle fibre CSA that governs the radial muscle adaptation (Johnson and Klueber, 1991; Aagaard et al., 2001; Shoepe et al., 2003; Farup et al., 2012) is in turn attributed to increased myofibrillar growth (McDougall et al., 1980) and proliferation (Goldspink, 1970).

The longitudinal adaptation of muscle refers to the modulation of the number of sarcomeres in series, which positively associates with the maximum shortening velocity and mechanical power of muscle fibres (Goldspink, 1985). Direct evidence for this adaptive mechanism comes predominantly from animal studies. Besides immobilisation (Williams and Goldspink, 1971; Pontén and Fridén, 2008) and surgical limb lengthening (Simpson et al., 1995; Lindsey et al., 2002), eccentric loading seems to effectively trigger an increase in serial sarcomere number, while concentric loading has been associated with their decrease (Lynn and Morgan, 1994; Butterfield et al., 2005). There are also a few reports of serial sarcomere modulation in humans (Yu et al., 2004; Boakes et al., 2007). However, in the majority of experiments with humans, changes of fascicle length were determined and used as an indirect indication of a modulation of sarcomere number in series. Though the findings on the effect of eccentric loading are in good agreement with the observations in animals (Blazevich et al., 2007; Duclay et al., 2009; Potier et al., 2009; Reeves et al., 2009; Sharifnezhad et al., 2014), a reduction of fascicle length

(and supposedly sarcomere number) in response to concentric loading finds no indication in human exercise studies (Blazevich et al., 2007).

Specific tension (or force) describes the intrinsic strength generating capacity of the muscle tissue (i.e. active force normalised to CSA) and can be assessed in single muscle fibres (Bottinelli, 2001 for review) or estimated on the whole muscle level (Narici et al., 1992; Maganaris et al., 2001; O'Brien et al., 2010a; Erskine et al., 2010a). Evidence of a loading-induced increase of the specific tension of single muscle fibres in adults is rare and inconsistent (Widrick et al., 2002; D'Antona et al., 2006; Pansarasa et al., 2009). Nevertheless, it is considered to be a potentially contributing factor to increases of the *in vivo* specific tension of muscles in response to resistance exercise (Erskine et al., 2010a; 2010b; 2011), along with an enhanced lateral force transmission between muscle fibres and intracellular matrix due to an increase of intramuscular connective tissue (Jones et al., 1989; Erskine et al., 2011). A shift in muscle fibre type from slow to fast twitch fibres could theoretically contribute to increased whole-muscle specific tension as well, under the premise that type II fibres possess higher specific tension compared to type I fibres (Bottinelli et al., 1996; Widrick et al., 2002; D'Antona et al., 2006; Pansarasa et al., 2009). This, however, is still under debate (Erskine et al., 2011), as is marked exercise-induced fibre type transformation in general (Folland and Williams, 2007 for review). Yet, it should be noted that a shift in the ratio of relative fibre CSA towards a higher percentage of type II fibres (due to selective hypertrophy; Aagaard et al., 2001; D'Antona et al., 2006; Martel et al., 2006) would greatly affect the power production of muscles due to the considerably greater shortening velocity of type II fibres (Bottinelli, 2001).

Despite extensive research, the mechanobiological machinery that regulates radial and longitudinal muscle adaptation is not completely understood and even unknown with regard to the potential modulation of fibre or muscle specific tension. It has been acknowledged that both mechanical and metabolic stress are unique but interacting stimuli that trigger muscle adaptation and growth (Goldberg et al., 1975; Vandenburgh and Kaufman, 1979; Rooney et al., 1994; Schott et al., 1995; Smith and Rutherford, 1995). Mechanical stress stimulates stretch-activated calcium channels (Kameyama and Etlinger, 1979), mechanosensitive intracellular enzymes and second messengers (Hornberger et al., 2006) as well as insulin-like growth factor I (IGF-I) release from the muscle cells (Perrone et al., 1995). These events induce an increase of protein synthesis via autocrine and direct intracellular signalling pathways (Tidball, 2005; Toigo and Boutellier, 2006 for review; Gonzalez et al., 2016). Increased nitric oxide efflux of stressed myofibres activates satellite cells (Anderson, 2000), which proliferate under the regulatory influence of IGF-I (Barton-Davis et al., 1999) and fuse with existing myofibres to provide new myonuclei to the parent fibre

(Allen et al., 1999). Moreover, muscle damage and corresponding inflammatory responses are considered to contribute to the growth-mediating processes (Schoenfeld, 2012). The role of metabolic stress, which refers to the exercise-related accumulation of metabolites (specifically lactate and hydrogen ions), is thought to originate, for instance, from the associated systemic growth-related hormone and local myokine up-regulation and/or the increased fibre recruitment with muscle fatigue (Schoenfeld, 2013; Ozaki et al., 2016 for reviews). Muscle hypertrophy was consequently suggested to be driven by the interaction of mechanical and metabolic stress, with the degree of contribution depending on the exercise modality (i.e. greater mechanical stress at high intensities and greater metabolic stress at moderate intensities; Ozaki et al., 2016). *Ergo*, given a sufficient overall training volume, muscle hypertrophy can be achieved with a wide range of exercise intensities (Campos et al., 2002; Tanimoto and Ishii, 2006; Mitchell et al., 2012; Schoenfeld et al., 2015; 2016).

Longitudinal muscle adaptation is commonly explained by (active) stretch induced muscle damage and titin-based signalling. The *popping sarcomere* hypothesis (Morgan, 1990) suggests that when muscle fibres are actively stretched on the descending limb of the force-length relationship, the sarcomere length becomes increasingly non-uniform in its distribution, which leads to a reduction of sarcomere stability, uncontrolled rapid lengthening (*popping*) of sarcomeres and structural disruption (Morgan and Allen, 1999; Proske and Morgan, 2001). However, it is unlikely that muscle damage and inflammatory responses are the sole drivers of longitudinal adaptation. The kinase domain of titin has been identified to act as mechanosensor in the sarcomeres (Lange et al., 2005; Puchner et al., 2008). The activation of titin kinase by a mechanical strain-induced conformational change initiates a signalling cascade that regulates muscle gene expression (Tskhovrebova and Trinick, 2008) and is thought to play a key role in the adaptational response to eccentric exercise (Butterfield, 2010; Sharifnezhad et al., 2014).

1.2.2 Mechanisms of tendon plasticity

The early work of Ingelmark (1945; 1948) already suggested that tendons adapt to changing functional demands, and the extensive research of the last two decades broadened our understanding of the underlying mechanisms and basic dose-response relationships of tendon plasticity *in vivo*. When the muscle-tendon unit is repeatedly subjected to increased mechanical loading, for instance in the course of resistance exercise, the associated increases of muscle strength are commonly accompanied by an increase of tendon stiffness (Kubo et al., 2001a; Kongsgaard et al., 2007; Arampatzis et al., 2007a). The adaptation of stiffness may serve as a protective mechanism since the ultimate strain of tendons is considered to be relatively constant

(Abrahams, 1967; Loitz et al., 1989; LaCroix et al., 2013; Shepherd and Screen, 2013) and greater strength-generating capacity would otherwise increase tendon strain during maximum effort muscle contractions and challenge the integrity of the tendinous tissue. Two candidate mechanisms can account for exercise-induced increases of tendon stiffness: (a) changes of the material properties (i.e. Young's modulus) and (b) radial tendon growth. The longitudinal studies that reported a facilitation of tendon stiffness following an exercise intervention and explored the adaptive mechanisms behind this response almost exclusively (except for Kongsgaard et al., 2007) also documented an increase of Young's modulus by 17-77% (Kubo et al., 2001a; Arampatzis et al., 2007a; Seynnes et al., 2009; Arampatzis et al., 2010; Carroll et al., 2011; Malliaras et al., 2013; Bohm et al., 2014). In comparison, increases of tendon CSA are reported less consistently, with some evidence of significant changes in response to strength training, yet of moderate magnitude (4-10%) (Kongsgaard et al., 2007; Arampatzis et al., 2007a; Seynnes et al., 2009; Bohm et al., 2014), and several findings of increased tendon stiffness without concomitant tendon hypertrophy (Kubo et al., 2001a; 2002; 2007; e.g. Arampatzis et al., 2010; Kubo et al., 2010a; Carroll et al., 2011; Malliaras et al., 2013). However, cross-sectional studies with athletes from sports that are associated with increased tendon loading suggest that tendon hypertrophy of 20-35% is well possible (Rosager et al., 2002; Magnusson and Kjaer, 2003; Kongsgaard et al., 2005; Seynnes et al., 2013). Moreover, Couppé and colleagues (2008) reported ~20% difference in tendon CSA between the dominant and non-dominant leg in badminton players. The emerging picture and current consensus is that the modulation of tendon material properties is a short-term response to increased mechanical loading, while tendon hypertrophy contributes to the adaptation of stiffness in the long-term (Kjaer et al., 2009; Bohm et al., 2015; Wiesinger et al., 2015).

The plasticity of tendons is based on the mechanotransduction of the external load into biological signals that initiate metabolic responses. Fibroblasts lie embedded in the extracellular matrix and are physically connected with it via specific transmembrane proteins. These connections allow to transmit external strain, when it is applied to the extracellular matrix, to the cytoskeleton of the fibroblasts (Wang, 2006; Heinemeier, 2011). It has been suggested that the conformational changes of the transmembrane proteins that occur during load application and the activation of stretch-sensitive ion channels in the cell membrane trigger intracellular signalling cascades, which stimulate gene and growth factor expression for the up-regulation of collagen and matrix protein synthesis (Sackin, 1995; Chiquet, 1999; Wang, 2006). Accordingly, an increase of both interstitial growth factor and binding protein concentration (Hansson et al., 1988; Heinemeier et al., 2003; Olesen et al., 2006;

Dideriksen et al., 2013; Jones et al., 2013) as well as collagen synthesis (Langberg et al., 1999; 2001; Miller et al., 2005) has been observed in mechanically loaded tendon tissue. Moreover, increased levels of enzymes mediating collagen cross-linking were reported following following mechanical loading of tendon in a rat model (Heinemeier et al., 2007a), which is thought to modulate the collagen cross-link profile in humans in response to resistance exercise as well (Kongsgaard et al., 2009). More recently, loading induced proliferation and collagen production of tendon stem cells have been discovered, which extends the range of potential mechanisms that mediate the anabolic response of tendons to mechanical loading (Bi et al., 2007; Zhang et al., 2010).

It has been proposed that fibroblast cell deformation and fluid flow-induced shear stress are important regulators of the adaptive response of tendons (Lavagnino et al., 2008). The cyclic application of high-level magnitude strains to tendons *in vitro* has been associated with greater cell deformation (Arnoczky et al., 2002), fibre recruitment (Kastelic et al., 1980; Hansen et al., 2002), inhibition of catabolic activity (Lavagnino et al., 2003; Arnoczky et al., 2004) and adaptive response (Yamamoto et al., 2003) in comparison to lower levels of strain. Accordingly, Arampatzis and colleagues (2007a; 2010) found significant changes of the mechanical and morphological properties of the human Achilles tendon *in vivo* only in response to a high-intensity loading regimen (i.e 90% isometric maximum voluntary contraction [MVC] corresponding to 4.6% of tendon strain), while no adaptive changes were evidenced after moderate-intensity training (i.e. 55% MVC corresponding to 2.9% of tendon strain) with equal overall training volume. Experimental studies on the patellar tendon (Kongsgaard et al., 2007; Malliaras et al., 2013) and two recent meta-analyses (Bohm et al., 2015; Wiesinger et al., 2015) confirmed the importance of high-intensity tendon loading for *in vivo* tendon adaptation, while, against earlier assumptions, the contraction type (i.e. isometric, concentric, eccentric) does not seem to influence the adaptive response (Bohm et al., 2015). Interestingly, although high strain rates are thought to increase fluid flow-related shear stress (Haut and Haut, 1997; Lavagnino et al., 2008) and therefore to stimulate tendon metabolism (Archambault et al., 2002), the main body of experimental *in vivo* evidence suggests that the induction of high strain rates via plyometric exercise fails to elicit significant adaptive changes of human tendons (Kubo et al., 2007; Fouré et al., 2009; 2010; Houghton et al., 2013; Bohm et al., 2014). It has been proposed that the short strain durations of loading regimen featuring high-frequency load-relaxation cycles might compromise the effective transmission of the external load into cellular signals (Arampatzis et al., 2010; Bohm et al., 2014). It seems that increasing the strain duration of single loading cycles by up to 3 s facilitates tendon adaptation

(Arampatzis et al., 2010; Bohm et al., 2014), while additional increases of strain duration do not further promote the effect and might even be less effective if the strain duration is increased at the expense of the number of loading cycles (Bohm et al., 2014). Collectively, evidence suggests that tendon adaptation can be most effectively triggered if the loading regimen incorporates cyclic high-intensity muscle contractions (≥ 85 MVC, irrespective of contraction type) and contraction durations of ~3 s (Bohm et al., 2014). High strain rate and frequency modes of loading, for instance, plyometric exercise, do not provide an optimal stimulus for tendon adaptation.

1.3 Maturation, growth and development

Irrespective of environmental mechanical loading, maturation affects the development of the muscle-tendon unit during adolescence. Following a short introduction on the general somatic and hormonal changes during the development from childhood to adulthood, this section reviews the current knowledge on associated changes of the mechanical, morphological and structural properties of muscle and tendon. With regard to the terminology, *maturation* is henceforth used to refer to processes initiated by the progression of biological age *per se*, while *development* is used as a broader concept for biological changes, integrating additional stimuli, such as the increased mechanical loading due to somatic growth.

1.3.1 Somatic growth

Human body height and mass increase in an almost linear manner from childhood until the onset of adolescence (Tanner et al., 1966). However, while the annual gains of body height demonstrate slow reduction, the opposite is true for the development of body mass, indicating that the accumulation of tissue mass exceeds the degree that can be expected from of longitudinal growth. Puberty marks the onset of adolescence (at approximately 10-11 years of age in girls and 11-12 in boys Kail and Cavanaugh, 2015) and is associated with the activation of the hypothalamo-pituitary-gonadal axis, which results in a dramatic increase in the sex steroid hormone secretion (mainly testosterone in boys and oestrogen in girls), growth hormone and IGF-I (Murray and Clayton, 2013). The elevated levels of these (and other) circulating hormones and growth factors stimulate the progression of both sexual and somatic maturity (for the specific effects on muscles and tendons see Chapter 1.3.2, 1.3.3 and 1.4.1). The adolescent growth spurt between early and mid-adolescence is characterized by rapid increases in body height and mass, with peak height velocity preceding peak weight

velocity and both occurring on average about two years earlier in girls in comparison to boys (Malina et al., 2004). At around 15 years of age in girls and 17 in boys, the longitudinal body growth comes to an end, while body mass increases towards adulthood (Tanner, 1990).

1.3.2 Maturation and development of skeletal muscle

Similarly to the overall body dimensions, skeletal muscle mass increases progressively from childhood to adulthood, demonstrating a pronounced surge during the adolescence, especially in boys (Malina et al., 2004; McCarthy et al., 2014; Kim et al., 2016). Muscle mass relative to body mass increases as well, with marked changes in boys yet modest ones in girls (McCarthy et al., 2014; Kim et al., 2016). Consequently, studies that investigated the development on the individual muscle level reported an increase in muscle length, ACSA and volume (Kanehisa et al., 1995a; 1995b; Kubo et al., 2001b; Neu et al., 2002; Tonson et al., 2008; e.g. O'Brien et al., 2010c). Recent studies employing ultrasound-based assessments of muscle architecture provided additional indications that the gain of muscle volume is governed by both an increase in PCSA and fascicle length, yet the changes of PCSA seem to exceed those of fascicle length in pennated muscles, suggesting a remodelling of muscle architecture in favour of force production (Morse et al., 2008; O'Brien et al., 2010c; Bénard et al., 2011). The results on the development of pennation angle suggest muscle specificity. While no differences in pennation angle between children and adults were evidenced in the quadriceps femoris muscles (O'Brien et al., 2010c) and the lateral gastrocnemius (Morse et al., 2008), it seems that in the more pennated gastrocnemius medialis the fascicle angle remains unchanged during prepubertal growth (Legerlotz et al., 2010; Bénard et al., 2011) but increases during adolescence (Weide et al., 2015). Thus, adolescence might not only promote the development of the morphological determinants of force production in general, but also the specific functional differentiation between muscles (Huijing, 1985; Kawakami et al., 1998).

The increase in muscle PCSA from childhood to adulthood is most likely mediated by an increase of fibre CSA and not hyperplasia (Bowden and Goyer, 1960; Aherne et al., 1971; Oertel, 1988; Lexell et al., 1992). It has been demonstrated that the growth hormone-IGF-I axis, which is of major importance for the overall body growth and experiences a marked activation during adolescence, stimulates fibre hypertrophy and protein content, but not myotube formation (Grohmann et al., 2005). For instance, myoblast proliferation and fusion with myotubes, which is a prerequisite for radial and longitudinal fibre growth, depends on growth hormone and IGF-I secretion (Cheek et al., 1971; Allen et al., 1999; Grohmann et al., 2005). Therefore, it is not surprising that there is also an association between single fibre

CSA and body height (Aherne et al., 1971). The hormonal changes during adolescence, specifically the increasing systemic levels of sex steroid hormones, further initiate the emergence of marked differences in both muscle strength and fibre CSA between boys and girls (Oertel, 1988; Round et al., 1999).

The differentiation of muscle fibre type during child to adulthood development is generally unclear and the findings are inconsistent. The work of Vogler and Bove (1985) as well as Bell and colleagues (1980) suggests that at about six to eight years of age no major changes of the fibre type profile would occur. However, some findings indicate that the percentage of type II fibres might as well increase in the course of childhood and adolescence, at least in boys (Glenmark et al., 1994; Jansson, 1996). Some authors related the potential sex-related differences of fibre type differentiation to the influence of testosterone (Jansson, 1996; Van Praagh and Doré, 2002). Indeed, evidence from animal models suggests that testosterone (Gutmann et al., 1970; Holmäng et al., 1990; Holmes et al., 2007) as well as oestrogen and thyroid hormone might elicit some fibre type-specific effects (Haizlip et al., 2015). However, biopsy results from human adults suggest that it is more likely that the most important difference in muscle structure between males and females is the greater fibre CSA of males, which is, though evident in all fibre types, specifically pronounced in type IIA fibres (Staron et al., 2000). Sexual dimorphism in human gene expression of myosin isoforms and proteins regulating the IFG-1 and myostatin signalling pathways might contribute to the development of these differences (Welle et al., 2008), which is likely to be triggered during the adolescent endocrine development, specifically via the interaction of growth hormone with IGF-I (Hulthen, 2001; Grohmann et al., 2005) and androgens with myostatin (Mendler et al., 2007).

Little information is available on the development of muscle specific tension. To date, only two studies have investigated the specific tension of muscles in children and adults *in vivo*. Morse and colleagues (2008) estimated the intrinsic force generating capacity of the gastrocnemius lateralis in prepubescent boys and adult men. However, a number of assumptions were made in that study (e.g. regarding the relative contribution of gastrocnemius lateralis PCSA to total plantar flexor PCSA, moment arm lengths during muscle contraction, length-tension relations) that might have accounted for the unexpected findings of a greater specific tension of the gastrocnemius in boys compared to men. From a methodological perspective, the study of O'Brien et al. (2010a) on the quadriceps femoris is considered the most valid approach for the estimation of muscle specific tension *in vivo* (Bouchant et al., 2011; Herzog et al., 2011). The conclusion that there seems to be no significant difference in specific force between children and adults is also in accordance with results obtained in a rodent model (Brooks and Faulkner, 1988) and the crude estimates of "muscle

quality", determined as ratio of knee extensor muscle volume to joint torque, reported for prepubescent children and adolescents (Pitcher et al., 2012; Fukunaga et al., 2014).

It is now well established that increased mechanical loading, superimposed on the increase due to body growth (e.g. by means of resistance exercise), facilitates the development of the strength generating capacity during childhood (Falk and Eliakim, 2003; Matos and Winsley, 2007) and especially in adolescence (Moran et al., 2016). While it is generally accepted that neuronal adaptation contributes to the observed strength gains (Ramsay et al., 1990; Ozmun et al., 1994), there is disagreement on contribution of muscle morphological changes (Matos and Winsley, 2007). Though there is some evidence, that exercise-induced muscle hypertrophy can be elicited already at prepubescent age (Fukunaga et al., 1992; Daly et al., 2004), longitudinal assessments often fail to support this assumption (Ramsay et al., 1990; Granacher et al., 2011). For this reason, the morphological plasticity of muscles in response to strength training is considered to be lower in children compared to adults. However, in adolescence, differences of quadriceps femoris ACSA between athletic and non-athletic boys can reach up to 28% (Kanehisa et al., 1995a; 2003) and the findings of Fukunaga et al. (1992) and Virjens (1978) support the assumption that the contribution of radial muscle growth to the exercise-induced development of strength increases with maturation. No information, on the other hand, is available with regard to changes of fibre CSA, fibre type distribution (of number and area) and their intrinsic contractile capacity in response to superimposed mechanical loading. Similarly, it is unclear whether muscle architecture is modulated in response to training during childhood or adolescence. However, reports of physical activity-dependent increases of vastus lateralis and gastrocnemius medialis pennation angle in children with cerebral palsy (Moreau et al., 2010; Lee et al., 2015) suggests that, similar to adults (Aagaard et al., 2001), the modification of muscle architecture is a possible response to sport and exercise loading that warrants further investigation in healthy children and adolescents.

In conclusion, human skeletal muscle generally grows proportionally to the overall body dimensions. However, the increase of muscle PCSA is more pronounced in comparison to the longitudinal changes and, thus, contributes greatly (along with increased moment arms (O'Brien et al., 2009b; Waugh et al., 2011) and muscle activation (Dotan et al., 2012) to the disproportionate increases in muscle strength (O'Brien et al., 2010a). The radial muscle growth is mediated by the increase of fiber CSA, which might be more pronounced in type II fibres. A significant modulation of fibre type distribution or specific force on the other hand seems unlikely. Adolescence

and the associated hormonal changes facilitate these processes and seem to increase the muscle responsiveness to mechanical loading.

1.3.3 Maturation and development of tendons

The following section gives an overview of the current knowledge on the effects of maturation on tendinous tissue. With only a few studies providing insight into the development of the morphological and mechanical properties of human tendons *in vivo,* supplementary information derived predominantly from animal models is used to review the structural and endocrine mechanisms that potentially contribute to the mechanical and morphological changes observed during development.

Kubo and colleagues (2001b) were the first to provide information about the developmental changes of the mechanical properties of human tendinous tissue *in vivo* by comparing the compliance of the vastus lateralis tendon and aponeurosis of children, adolescents and adults. It was shown that tendon-aponeurosis compliance was significantly greater in young boys in comparison to adolescent boys and was the lowest in adults. Thus, these results indicate an increase of tendinous stiffness (i.e. the inverse of compliance) from childhood to adulthood despite the longitudinal growth of the muscle-tendon unit. Given similar material properties and CSA, an increase in length of the series elastic elements would reduce stiffness (Butler et al., 1978). However, the increase of stiffness as suggested by Kubo et al. (2001b) was later supported by data from O'Brien et al. (2010b), who reported a greater stiffness of the patellar tendon in adults compared to pre-pubertal children.

Furthermore, O'Brien and colleagues were able to provide insight into the mechanisms underlying the apparent increase in stiffness with maturation by measuring the patellar tendon length and CSA, calculating Young's modulus and discriminating between sexes (O'Brien et al., 2010b). Significantly greater tendon lengths, CSAs and Young's moduli were found in adults than in children. Yet, as the tendon length-to-CSA ratios of boys and men were similar, the authors concluded that in males tendon stiffness mainly increases due to the modulation of the material properties, while in females tendon hypertrophy not only compensates the longitudinal tendon growth but together with an increase of Young's modulus contributes to the increase of patellar tendon stiffness.

In vitro studies on juvenile mice tendons have shed some light on the potential structural changes of the tissue governing the development of its mechanical properties during growth. Newly synthesised collagen molecules are assembled longitudinally (end-to-end) and subsequently associate laterally to adjacent fibril intermediates to form mechanically functional fibrils; a process that is regulated by minor collagens (e.g. collagen type III, IV, XI and XIV) and proteoglycans (Zhang et

al., 2005; Connizzo et al., 2013). The formation of *intra*fibrillar cross-links between collagen molecules and the maturation of these cross-links (by conversion of divalent to trivalent bonds) as well as the development of further *inter*fibrillar cross-link interactions are thought to be two key mechanisms in tendon development that promote its mechanical strength (Davison, 1978; Bailey et al., 1998; Eyre and Wu, 2005; Depalle et al., 2015). The expression of specific proteoglycans (e.g. decorin and fibromodulin) that are associated with the regulation of radial fibril growth (Ezura et al., 2000; Zhang et al., 2006) and cross-linking (Seidler et al., 2005; Kalamajski and Oldberg, 2010; Kalamajski et al., 2014) peaks during distinct phases of development and facilitates the structural properties of the fibrils (Ezura et al., 2000; Zhang et al., 2006). Accordingly, greater average CSAs of both collagen fibrils and tendon (Figure 1.2) as well as tendon Young's modulus were found in older juvenile in comparison to younger juvenile mice (Ansorge et al., 2011; Miller et al., 2012), which resulted in altered ultra-structural load responses and increased tendon stiffness. Moreover, it seems that the tendon material properties are modulated predominantly during early development, while the tendon CSA increases throughout maturation (Ansorge et al., 2011; Miller et al., 2012).

The limited information available to estimate the time course of human tendon development *in vivo* point to a similar trend. The results of a study by Kubo et al. (2014b) comparing elementary and high school boys to adult men suggest that the major developmental increase of patellar tendon material properties from childhood to adulthood takes place until the early-adolescence, with only minor increments of

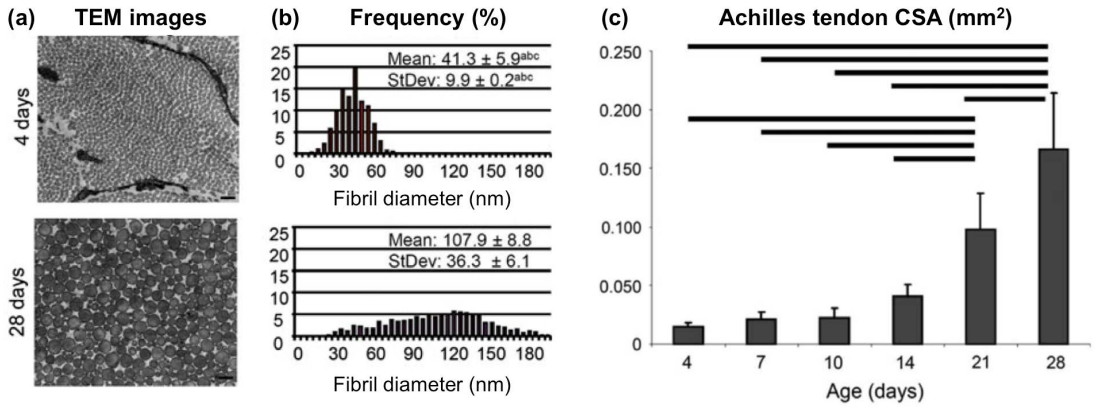

Figure 1.2 (a) Representative transmission electron microscopy (TEM) images of mouse collagen fibrils throughout post-natal mouse development. Scale bar = 200 µm. (b) Histogram of fibril distribution demonstrating increased fibril diameter mean and spread throughout post-natal mouse Achilles tendon development. [a]: significantly different from 28 days; data for other age-groups are not shown here. (c) Mean values and standard deviation (error bars) of mouse Achilles tendon cross-sectional area throughout post-natal development. Horizontal bars indicate significant differences between age groups ($p < 0.05$). Note that mice reach maturity at about 25-40 days of age. (Ansorge et al., 2011, Ann Biomed Eng 39, 1904-13, p. 1908 & 1911. Adapted with permission from Kluwer Academic Publishers.)

Young's modulus occurring thereafter. An analogous behaviour is indicated for the Achilles tendon by the study of Waugh et al. (2011), reporting differences of similar order in Achilles tendon Young's modulus between younger (5-7 years) and older pre-pubertal children (aged 8-10 years) compared to the differences between the latter group and the notably older adults (~26 years). Thus, the material properties of tendons might develop to a great extent early in youth (i.e. before the growth spurt at the onset of adolescence).

In contrast to the material properties, patellar tendon hypertrophy is indicated to considerably progress further throughout adolescence (Kubo et al., 2014b). Interestingly, no significant differences of CSA between boys in early adolescence and adult men were found for the Achilles tendon (Kubo et al., 2014a). Furthermore, the only longitudinal study to date on *in vivo* tendon development in humans, conducted by Neugebauer and Hawkins (2012), even demonstrated a reduction of Achilles tendon CSA in boys and girls in early adolescence over a period of six month, which resulted in increased tendon stress. Though an association of the decrease of tendon CSA to overall body growth rate could not be established (potentially because the overall body growth does not necessarily predict lower leg or tendon growth; Roche and Malina, 1983; Martorell et al., 1988; Kubo et al., 2014b), the authors speculated that this behaviour could be associated with the significant increases of Achilles tendon length and might be of transient nature. Though in contrast to the findings of Kubo and colleagues (2014a), further Achilles tendon hypertrophy towards adulthood seems in fact to be quite likely. Firstly, the CSA values for children in early adolescence reported by Neugebauer and Hawkins (2012) are markedly lower when compared to those which were obtained from adults using methodologically similar approaches (~39 mm^2 and 52-67 mm^2, respectively; Kongsgaard et al., 2011; Waugh et al., 2011; Stenroth et al., 2012; Waugh et al., 2013; Bohm et al., 2016), except that Neugebauer and Hawkins (2012) assessed the CSA during a moderate application of force (i.e. ~20% MVC). However, the different loading conditions between these studies and the radial force-deformation behaviour of tendons (Poission's ratio, i.e. the ratio of transverse to axial strain) cannot explain the magnitude of differences in CSA between cohorts (Abrahams, 1967; Vergari et al., 2011). Secondly, it is very well documented for adults (Bohm et al., 2015; Wiesinger et al., 2015 for reviews) and has also been recently shown in pre-pubertal children (Waugh et al., 2014) that tendons adapt to increased mechanical loading and tendon hypertrophy is considered to occur with habitually increased loading over time (Rosager et al., 2002; Magnusson and Kjaer, 2003). It is known that body mass increases not only until early-adolescence but until adulthood (Tanner et al., 1966), and it is reasonable to assume that this holds true for lower limb joint moments and tendon loading during daily activities as

well (Mullineaux et al., 2006; Spanjaard et al., 2008), which in turn would stimulate radial tendon growth.

The age-related development indicated above could therefore be attributed to a great extent to increased mechanical loading due to anthropometric changes and muscle strength development. Using the data of the Achilles tendon properties of 52 children (5 to 12 years of age) and 19 adults, Waugh et al. (2011) tried to identify factors influencing the development of tendon stiffness and Young's modulus in a stepwise multiple regression analysis. The results indicate that the increase of tendon stiffness and Young's modulus with age (Figure 1.3) can be explained to a great extend by the associated increase of body mass and maximum tendon force (with regard to stiffness) or stress (with regard to Young's modulus). A close association of tendon stiffness with tendon force has been demonstrated earlier in a population of either untrained, endurance or strength trained adults (Arampatzis et al., 2007b) and there are indications that such an association exists within a sedentary population as well (Muraoka et al., 2005). Interestingly, the inclusion of age as a factor in the regression model of Waugh and colleagues (2011) did not contribute to the prediction of tendon stiffness, while it was a significant additional predictor of Young's modulus (i.e. independent of body mass and tendon stress). Although statistical models do not clarify the causality, it seems very likely that maturation is a unique factor besides the effects of increased mechanical loading. Several hormones and growth factors that mediate general somatic development (see Murray and Clayton, 2013 for reviews) have been demonstrated to effect the metabolism of tendons as well and could influence the maturation of tendons aside from mechanical loading. For instance, growth hormone and IGF-I were shown to stimulate gene expression, collagen synthesis and cross-linking (Abrahamsson et al., 1991; Choy et al., 2005; Doessing et al., 2010; Nielsen et al., 2014), while low serum levels of these growth factors caused disruptions of tendon ultrastructure (Nielsen et al., 2014). Thyroid hormones are thought to be involved in the regulation of tenocyte growth and proliferation as well as collagen synthesis (Oliva et al., 2013; Berardi et al., 2014). Moreover, oestrogens have been reported to have inhibitory effects on collagen synthesis (Miller et al., 2007) and could reduce the adaptability of female tendons to exercise (Magnusson et al., 2007), whereas fluctuations of oestrogen due to the menstrual cycle seems not to be associated with changes of tendon mechanical properties in women (Bryant et al., 2008; Burgess et al., 2010).

Although basically all knowledge on the effect of hormones and growth factors on tendon metabolism has been derived from studies involving human adults or animal models (see Oliva et al., 2016 for review), and the dynamic changes of, for example, growth factor receptor sensitivity and binding protein activity with

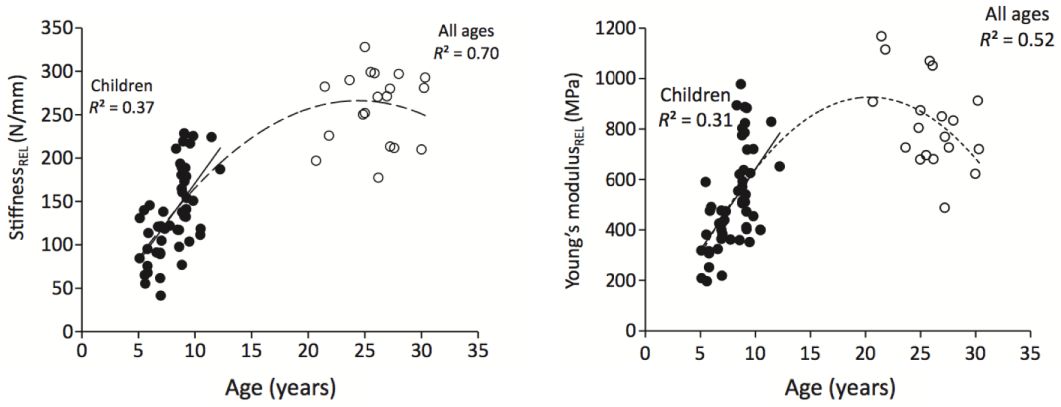

Figure 1.3 Relationship between age and tendon stiffness (left) and age and Young's modulus (right) in children (filled circles) and adults (open circles). Lines of best fit are shown for children (solid line) and all ages combined (dashed line). (Waugh et al., 2011, J Anat 220, 144-155, p. 150. Adapted with permission from John Wiley and Sons Inc.)

maturation (Malina et al., 2004) complicate the transferability of these results on tendon development during childhood and adolescence, it is evident that tendinous tissue is a target for several growth related factors of the endocrine system. Therefore, it seems to be very likely that biological maturation might be a separate stimulus driving the development of the mechanical properties of tendons. However, this interplay is widely unknown to date. Moreover, effects triggered by the interaction between maturation, growth and superimposed mechanical loading (i.e. increased loading is not confined to developmental gains in body mass due to growth, e.g. due to athletic training during childhood and adolescence), are also unknown. To date, only a single longitudinal study has investigated the effect of a strength training intervention on the properties of the Achilles tendon in children and reported an exercise-induced increase of tendon stiffness (Waugh et al., 2014). However, due to the short intervention period (ten weeks), the data does not offer an insight into long-term effects of athletic activity during maturation (e.g. months or years of training). A cross-sectional comparison between 500 adolescent athletes from different sports provided an indication of a morphological adaptation of the Achilles and patellar tendon in response to increased loading, yet only the thickness of tendons was assessed (Cassel et al., 2016).

Collectively, the currently available information about the development of human patellar and Achilles tendon from child to adulthood *in vivo* strongly suggests that tendon stiffness increases with age due to radial tendon hypertrophy and a modulation of the material properties. The maturation and increased mechanical loading due to gains in body mass and muscle strength both seem to be unique, but interacting factors contributing to the development of the mechanical properties of tendons, however, the dissociation of these two factors is challenging. The time course of tendon adaptation during development is widely unknown was well. The

increase of Young's modulus might be pronounced in early phases of childhood, while the results on changes of tendon CSA are inconclusive. It is well possible that radial tendon hypertrophy might also contribute markedly to the increase of stiffness during the later stages of development, but that the size of the tendon CSA is characterized by fluctuations during growth. However, it must be clearly stated that these assumptions need to be made with caution, due to the cross-sectional design of most of the studies (Kubo et al., 2001b; O'Brien et al., 2010b; Waugh et al., 2011; 2013; Kubo et al., 2014b) and the measurement inaccuracies (see chapter) associated with the use of ultrasonic imaging for the assessment of tendon CSA (O'Brien et al., 2010b; Waugh et al., 2011; Neugebauer and Hawkins, 2012; Kubo et al., 2014a; 2014b).

1.4 Non-uniformity of muscle and tendon adaptation

The final section of this introduction reviews current evidence on factors and conditions that can lead to a non-uniform adaptation of muscles and tendons. Furthermore, it provides a discussion how maturation could be an influential factor and adolescence a critical phase for the development of an imbalance of muscle and tendon properties. Finally, the potential implications of a non-uniform adaptation are discussed and related to the increasing risk of tendon overuse injuries during adolescence.

1.4.1 Non-uniform adaptation and influential factors

It is widely acknowledged that tendons are characterised by a significantly lower degree of vascularization (Smith, 1965) and a less active metabolism (Laitinen, 1967) in comparison to muscle tissue. Half-life estimates of collagen in tendinous tissue are almost tenfold higher when compared to muscle proteins actin and myosin (Lundholm et al., 1981; Thorpe et al., 2010). Thus, it seems possible that morphological and structural changes in response to mechanical loading occur at a lower rate in tendon tissue. Accordingly, changes of muscle morphology and architecture were detected as early as three to four weeks following heavy resistance training (Seynnes et al., 2007; DeFreitas et al., 2011), while no reports of such rapid changes in morphological or mechanical properties exist considering tendon adaptation. Moreover, it is known that muscle strength increases can even precede major muscle morphological changes due to neuronal adaptation (Folland and Williams, 2007). All mechanisms facilitating tendon stiffness on the other hand rely on the modulation of tissue metabolism and subsequent structural and morphological

modifications, which supports the idea that the time-course of muscle and tendon adaptation during a training process can differ substantially. Kubo and colleagues addressed this issue in two separate 3-month exercise intervention studies on the patellar (2010b) and the Achilles tendon (2011a), respectively. In both studies, a marked increase of muscle strength preceded significant changes of tendon stiffness by one to two month and morphological changes were solely detectable at muscle but not tendon level during the intervention period. Urlando and Hawkins (2007) monitored Achilles tendon force and strain during maximum voluntary contractions at six time-points over an eight week strength training intervention. The mean values of tendon strain did not differ significantly throughout the intervention, despite an increase in tendon force, which could be interpreted as uniform muscle and tendon adaptation. However, it is at least notable that the individual strain values seemed to fluctuate greatly between measurement sessions. For instance, the highest values of tendon strain measured in each session varied between 8.6% and 13.5%. Therefore, it seems possible that imbalances in the development of muscle strength and tendon stiffness remained undetected due to the analysis of group means only.

In addition to the potentially different time course of adaptive changes, there is now substantial evidence that the mechanical stimuli that effectively elicit adaptation differ between muscles and tendons as well. It is widely accepted that muscle growth and strength gains can be achieved using moderate loads during training (Moss et al., 1997; Wernbom et al., 2007; Mitchell et al., 2012; Schoenfeld et al., 2016), even in well trained individuals (Schoenfeld et al., 2015), as metabolic stress is a potent trigger for muscle hypertrophy and might compensate the reduced levels of mechanical stress compared to higher loading intensities (see also Chapter 1.2.1) (Schoenfeld, 2013; Ozaki et al., 2016). However, evidence strongly suggests that moderate loads do not provide a sufficient stimulus for tendon adaptation. In two studies, Arampatzis and colleagues (2007a; 2010) compared isometric plantar flexion protocols with similar overall loading volume using either high (i.e., 90% MVC) or moderate (i.e., 55% MVC) loading intensity (merely the applied strain frequency differed between protocols of the two studies). While plantar flexor strength increased significantly following all exercise protocols applied, only the high magnitude loading protocols induced significant Achilles tendon adaptation. The consequence of the unchanged tendon stiffness following the moderate magnitude loading protocol was a higher Achilles tendon strain during maximal isometric contractions, which indicates an increase of the mechanical demand for the tendon (Arampatzis et al., 2007a; 2010; Figure 1.4). Kongsgaard et al. (2007) confirmed for the patellar tendon that stiffness seems to increase only in response to heavy and not light load (work-matched) resistance training and a recent meta-analysis on human tendon adaptation sub-

grouping studies using training intensities of either higher or lower than 70% MVC (or one-repetition maximum, respectively) reported a significant effect size only for the high intensity sub-group (Bohm et al., 2015). Furthermore, it is well documented that plyometric training is effective in facilitating muscle strength, also in trained athletes (Sáez-Sáez de Villarreal et al., 2010). However, plyometric loading does not seem to significantly change tendon stiffness (Kubo et al., 2007; Houghton et al., 2013). Even at high loading magnitudes, plyometric training failed to elicit significant Achilles tendon adaptation in contrast to equivolume isometric loading (Bohm et al., 2014). The dissimilar responsiveness of muscles and tendons to plyometric loading seems particularly interesting with regard to the dramatic prevalence of tendon overuse injuries in sports with a plyometric loading profile like volleyball or basketball (Lian et al., 2005).

Differently graded responses of muscle and tendon to specific types of loading have also been described on the transcriptional level of growth factors. Heinemeier and colleagues (2007a; 2007b) subjected rats to either isometric, concentric or eccentric strength training and found contraction type-specific expression of growth factors in muscle but not tendon tissue. More recently, the same group investigated musculotendinous gene and growth factor expression following a fatiguing one-leg kicking exercise model in humans (Heinemeier et al., 2011). While at tendon level no major changes of genes relevant for an adaptive response were documented, the expression of muscle tissue-related growth factors increased significantly. The authors concluded that an imbalanced adaptation of muscle and tendon might develop under specific loading conditions (Heinemeier et al., 2007a; 2011).

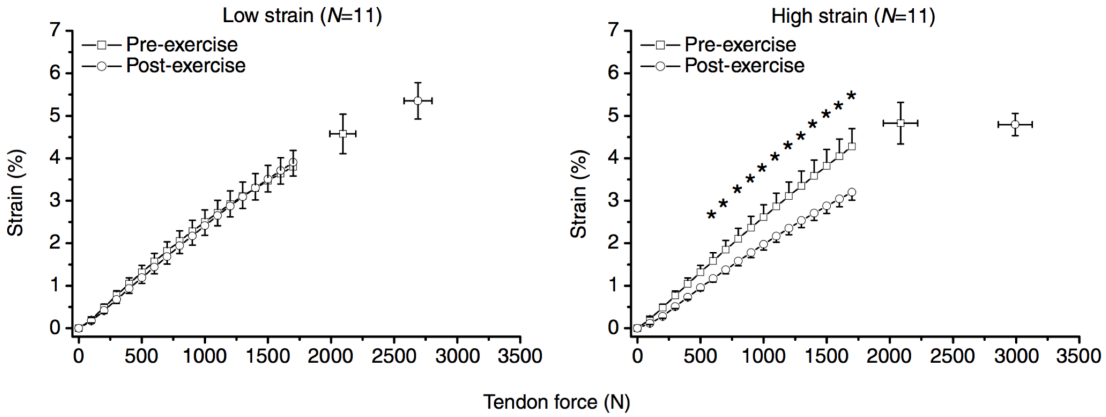

Figure 1.4 Tendon force-strain relationship following two isometric exercise protocols applying either moderate (i.e. 55% maximum voluntary contraction [MVC]; low strain) or high loading (90% MVC; high strain). Both protocols induced an increase of tendon force. However, following moderate loading (i.e. low strain protocol) tendon stiffness did not change significantly and, thus, there was a significant increase of tendon strain during maximum muscle contractions. (Arampatzis et al., 2007, J Exp Biol 210, 2743-2753, p. 2749. Adapted with permission from The Company of Biologists Limited.)

Maturation and the related hormonal and somatic changes might additionally influence the risk of a divergent development of muscle and tendon properties. Neugebauer and Hawkins (2012) provided evidence that the longitudinal growth of the muscle-tendon unit during adolescence can be associated with a temporary reduction of tendon CSA and, in turn, increased tendon stress. At given material properties, increased tendon stress results in higher tendon strain and, thus, a greater challenge for the integrity of the tendinous tissue. However, both Young's modulus and maximum tendon strain increased only in tendency in this study (Neugebauer and Hawkins, 2012) and, therefore, the implications of the observed morphological development still need to be elucidated. Similarly, no conclusive information is available on the effects of the rapid increase of circulating sex hormones (i.e. testosterone in boys and oestrogens in girls) during puberty on the uniformity of muscle and tendon development, but a potential influence is indicated. While it is well established that testosterone is one of the most potent hormones promoting muscle hypertrophy and thus increasing muscle strength (Vingren et al., 2010 for reviews), its role in the development of tendon mechanical properties is basically unknown to date (Hansen and Kjaer, 2014). The available information on the effects of anabolic-androgenic steroid supplementation on tendon properties, which are thought to stimulate collagen synthesis (Pärssinen et al., 2000) and increase tendon stiffness (Inhofe et al., 1995; Marqueti et al., 2011; Seynnes et al., 2013), but impair tissue remodeling (Marqueti et al., 2006) as well as ultimate stress and strain (Inhofe et al., 1995; Marqueti et al., 2011; Tsitsilonis et al., 2014), is not likely to be representative of the physiological mechanisms of testosterone action. Nevertheless, it can at least be concluded that the anabolic and strength-promoting effects of testosterone are more clearly established for muscle tissue than tendon tissue. On the other hand, the effects of oestrogens on muscle and tendon metabolism have been explored quite extensively and were reviewed recently by Hansen and Kjaer (2014). The authors concluded that scientific evidence renders oestrogen as anabolic with regard to muscle by decreasing protein turnover and increasing the sensitivity to mechanical loading. Conversely, oestrogens seem to reduce tendon collagen synthesis and the plasticity of tendon mechanical properties in response to exercise. Though it should be noted that the current understanding of the effects of sex hormones is based primarily on studies administering exogenous hormones to adults (Hansen and Kjaer, 2014), it seems well possible that the change of endogenous sex hormone levels could contribute to an imbalanced adaptation of muscle and tendon during adolescence.

The collective evidence reviewed above strongly supports the idea that a non-uniform development of muscles and tendons can occur during a training process.

Maturation, which acts as an additional stimulus on the muscle-tendon unit of young athletes, could potentially further contribute to such a development and adolescence, which is characterized by marked increase of sex hormones, could be a critical phase in that context. Recent meta-analyses confirmed the widely held opinion that the trainability of muscle strength increases significantly during adolescence (Behringer et al., 2010; Moran et al., 2016). However, information on the development of tendon plasticity as a function of age is currently not available, and the balance in the adaptation of muscles and tendons during adolescence has not received scientific consideration thus far.

1.4.2 Potential implications

A non-uniform adaptation of muscles and tendons - when gains of muscle strength are not accompanied by an adequate change of tendon properties - increases the mechanical demand placed upon the tendon by the working muscle. The mechanical strain theory is currently considered the most probable injury mechanism in the etiology of tendinopathy (, attributing the histological, molecular and functional changes of the affected tissue to mechanical overload (Archambault et al., 1995; Fredberg and Stengaard-Pedersen, 2008; Magnusson et al., 2010; Legerlotz, 2013). In fact, evidence suggests that repetitive loading of the tendon at high strain magnitudes (Butler et al., 1978; Lavagnino et al., 2006; Legerlotz et al., 2014) leads to cumulative damage in the extracellular matrix by successive collagen denaturation and fibril tears (Woo, 1982; Veres et al., 2013). It is though that consequently the load needs to be redistributed among intact fibrils, which probably increases the risk of damage upon subsequent loading cycles (Neviaser et al., 2012) and might explain the reported decrease of stiffness and ultimate stress (Schechtman and Bader, 2002; Fung et al., 2009; 2010; Legerlotz et al., 2013). While some authors ascribe the manifestation of tendinopathy to the progression of cumulative mictrotrauma into higher structural levels and successive matrix breakdown, others relate the degenerative cascade to the discontinued mechanotransduction of ruptured and, in turn, relaxed fibrils (Knörzer et al., 1986), which leads to the understimulation of fibroblasts (i.e. stress-shielding) and associated catabolic responses (Arnoczky et al., 2007). However, both scenarios are based on initial strain-induced damage, and though it should be acknowledged that there is a multitude of other potentially contributing factors (Fredberg and Stengaard-Pedersen, 2008 for an overview), it seems reasonable to assume that a non-uniform adaptation of muscle strength and tendon loading capacity could increase the risk of overload-induced tendinopathy. Wren and colleagues (2003) investigated the fatigue of human Achilles tendon in response to static and cyclic loading and found that initial high strain during both

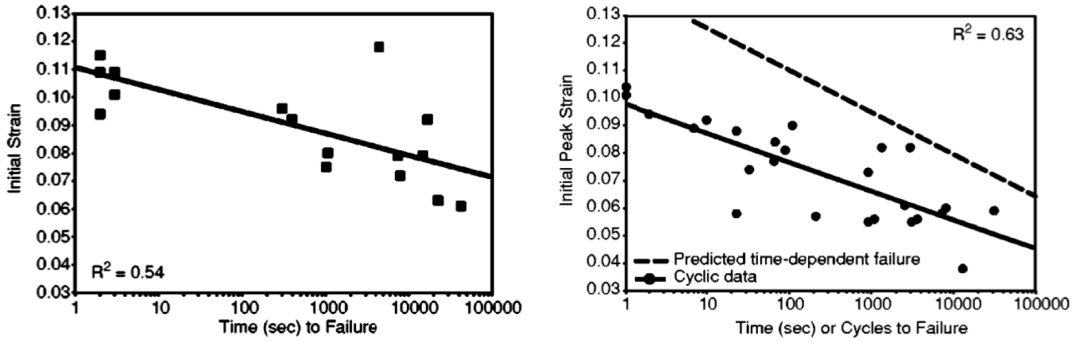

Figure 1.5 Static (left) and cyclic loading (right) tendon lifetime results as a function of initial (peak) tendon strain during loading and associated coefficients of determination (R^2). These data demonstrate that tendon strain magnitude determines the challenge for the tissue integrity. (Wren et al., 2003, Ann Biomed Eng 31, 710-717, pp. 713-714. Adapted with permission from Springer.)

static and cyclic loading was inversely correlated with time or loading cycles until failure (Figure 1.5). This suggests that increased tendon strain during repetitive maximum effort muscle contractions due to an imbalance of muscle strength and tendon stiffness could induce significant sub-rupture fatigue and trigger pathological processes. Accordingly, *in vivo* studies with patients and athletes suffering from tendinopathy report increased tendon strain during maximum voluntary contractions (Arya and Kulig, 2010; Child et al., 2010). Other studies did not identify increased levels of strain, yet showed an increase of tendon stress (Couppé et al., 2013a) or a decrease of tendon stiffness (Helland et al., 2013). Although cause and effect still remain unclear, collectively, the *in vivo* data supports the notion that tendinopathy is related to a mechanical weakening of the tendon.

Several epidemiological observations lend support to the idea that an imbalanced adaptation of the muscle-tendon unit could contribute to the development of overuse injuries and that adolescence might be a critical phase for tissue plasticity. First, it is interesting to note that there is a significant increase in the probability of non-contact soft-tissue injury when training loads are increased rapidly (Gabbett, 2016), which suggests that the differing time course of muscle and tendon adaption (characterised by a delayed tendon adaptation in comparison to a rapid increase of the strength-generating capacity of an associated muscle) might be of clinical relevance. Furthermore, the prevalence of tendinopathy in both elite and recreational athletes is highest in sports that feature a plyometric loading profile (Lian et al., 2005; Zwerver et al., 2011). Moreover, in adolescent volleyball players it was found that jumping ability, the weekly hours of volleyball training (and not strength training) increase the risk of tendinopathy, and it was suggested that the frequency of jumps during training and competition might be of critical importance in this regard (Visnes et al., 2013; Bahr and Bahr, 2014). These findings support the idea that the different responsiveness of muscle and tendon, especially to plyometric

loading, might play a role in the pathogenesis of tendon overuse injury. Lastly, a recent meta-analysis of the current epidemiological evidence on tendinopathy in sportive children and adolescents indicated an increasing risk with age (Simpson et al., 2016). This observation is in line with the earlier reported higher prevalence of general soft-tissue overuse injuries in adolescents compared to children reported earlier (Stracciolini et al., 2014) and underlines the necessity to deepen our understanding of the adaptation of muscles and tendons during adolescence and the influence of mechanical loading.

In summary, the evidence on both the pathogenesis and epidemiology of tendinopathy provides indirect support for the idea that a non-uniform adaptation of muscle and tendon might have consequences for the risk of tendon injury as (a) the resultant increased mechanical demand on the tendon is a well-explored mechanism to induce overload, (b) the prevalence of soft-tissue overuse injuries is high at time-points in the training process (i.e. sudden increase of loading) and in sport disciplines that favour the development of a muscle-tendon imbalance and (c) maturation seems to be a potential risk factor for both tendinopathy and the non-uniform development of muscle and tendon. Though it is premature to infer associations between non-uniform muscle and tendon adaptation and overuse, the development of the muscle-tendon unit, especially during maturation, and the interaction with mechanical loading appears to be a potentially relevant issue in this context and calls for further research. It seems well possible that muscle and tendon develop in a non-uniform manner in sportive populations that are at an increased risk to develop tendinopathy, however, this has not been shown experimentally thus far.

2 Purpose of the thesis

Although considerable advances have been made in the last decades with regard to the muscle and tendon adaptation in adults, there is still a clear lack a clear lack of knowledge on mechanisms governing the plasticity of muscle-tendon unit during child to adulthood development. Our current understanding is predominantly derived from cross-sectional comparisons of children, adolescents and adults and there is very little longitudinal data on both normal development (i.e. maturation and loading due to body growth) and the effects of superimposed mechanical loading (e.g. sports participation or systematic strength training). Moreover, no information is available on the uniformity of muscle and tendon adaptation during development, though there is convincing evidence on adults that the balance of muscle strength and tendon loading capacity can be disturbed during a training process (see Chapter 1.4.1). Adolescence might be a particularly crucial phase of plasticity in this context, as there is reason to assume that the associated surge of sex steroid hormone secretion, the increased responsiveness of the neuromuscular system to mechanical loading and the accelerated longitudinal body growth might predestine adolescents, specifically those engaging in sportive activities featuring a plyometric loading profile, for a non-uniform development within the muscle-tendon unit (see Chapters 1.3.2 and 1.4.2). Since the tendon core formation during adolescence is thought to reduce the regenerative capacity of the tendon (Heinemeier et al., 2013), the increased mechanical demand placed upon the tendinous tissue and the associated risk of fatigue damage as a potential consequence of an imbalanced adaptation of muscle and tendon could be a contributing factor to the observed increase of tendinopathy in athletic adolescents.

The present thesis aims to provide an insight into the development of the morphological, mechanical and functional properties of muscle and tendon during adolescence and the effects of superimposed mechanical loading. For the investigation of the uniformity of muscle and tendon development, Volleyball athletes are an intriguing population. Firstly, the plyometric loading profile has been evidenced to be a potent stimulus for muscle but not tendon adaptation (Kubo et al., 2007; Sáez-Sáez de Villarreal et al., 2010; Bohm et al., 2014) and, secondly, the prevalence of tendon overuse injury is the highest reported in the literature (Lian et al., 2005) and affects adolescents as well (Gisslèn et al., 2005). The time course in the short-term development of the knee extensor muscle and patellar tendon properties and the long-term adaptation in adolescent volleyball athletes are both addressed with the research design of this thesis. Moreover, this thesis aims to develop and incorporate a

method for the prediction of muscle volume of the quadriceps femoris vastii on the basis of the work by Albracht and colleagues (2008) that could simplify the assessment of volume within this research endeavour as well as in future studies. The issues stated above were addressed in four working steps:

(1) Development of a method for predicting the muscle volume of the quadriceps vastii for the potential application in the diagnostics of muscle morphological development
(2) Investigation of the developmental state of the knee extensor muscle and tendon properties of mid-adolescent volleyball athletes in comparison to adult counterparts subjected to many years of sport-specific loading
(3) Examination of the changes of the mechanical and morphological properties of muscle and tendon from mid- to late adolescence in a long-term longitudinal design
(4) Detailed analysis of the time course of muscle and tendon adaptation over the course of one year in adolescent volleyball athletes and similar-aged controls

It was hypothesised that (1) the prediction of quadriceps vastii muscle volume on the basis of the maximum ACSA, muscle length and a muscle-specific scaling factor would be possible with reasonable precision, (2) mid-adolescent volleyball athletes feature adult-like muscle morphological and functional properties, yet they demonstrate a lag of tendon development with regard to muscle, resulting in increased tendon stress and strain, (3) deficits of tendon loading-capacity in young volleyball athletes that are present at mid-adolescence would be compensated in long-term and (4) adolescent volleyball athletes demonstrate greater fluctuations in the time course of muscle and tendon development and a lower uniformity of changes of muscle strength and tendon stiffness compared to similar-aged recreationally active controls, which increases the mechanical demand for the tendon.

The topics were addressed in four separate studies that are presented in the following chapters as submitted to the respective journal (including the reference list, yet with the exception of figure and table numbering). Note that the citation style has been preserved in accordance to the journal the respective study was published in.

3 First study | Muscle-shape consistency and muscle volume prediction of thigh muscles

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3.1 Abstract

The present study investigated the applicability of a muscle volume prediction method using only the muscle length (L_M), the maximum anatomical cross-sectional area ($ACSA_{max}$) and a muscle-specific shape factor (p) on the quadriceps vastii and the effect of sex on muscle shape. L_M, $ACSA_{max}$, muscle volume and p were obtained from magnetic resonance images of the vastus intermedius (VI), lateralis (VL) and medialis (VM) of female (n=20) and male (n=17) volleyball athletes. The average p were used to predict muscle volumes (Vp) using the equation $Vp = p*ACSA_{max}*L_M$. Though there were significant differences in the muscle dimensions between male and female athletes, p was similar and on average 0.582, 0.658, 0.543 for the VI, VL and VM, respectively. The position of $ACSA_{max}$ showed low variability and was at 57, 60 and 81% of the thigh length for VI, VL and VM. Further, there were no significant differences between measured and predicted muscle volumes with root mean square differences of 5% to 6%. These results suggest that the muscle shape of the quadriceps vastii is independent of muscle dimensions or sex and that the prediction method could be sensitive enough to detect changes in muscle volume related to degeneration, atrophy or hypertrophy.

Key words: MRI, leg muscles, muscle segmentation, volume distribution, gender

3.2 Introduction

Information on muscle volume differences between populations and changes of volume due to environmental or pathological conditions are necessary in a wide range of research fields. Changes of muscle volume are associated with maturation (O'Brien et al. 2010), ageing (Morse et al. 2005), several pathologies (Zoabli et al. 2008; Hiba et al. 2011; Ji et al. 2013), mechanical loading (Folland and Williams 2007), unloading (Adams et al. 2003) or immobilization (Oates et al. 2010). The volume of a muscle has major implications for its mechanical power (Sleivert et al. 1995; O'Brien et al. 2009). Therefore, the measurement of muscle volume is of special importance in research designs with the objective to explain differences in physical performance between groups or to evaluate interventions with regard to changes in muscle morphology (Thom et al. 2005; O'Brien et al. 2009). Another major determinant of physical performance is the maximum force generating capacity of a muscle (i.e. muscle strength) (Delecluse et al. 1995), which is predominantly determined by the physiological cross-sectional area (PCSA) (Haxton 1944; Aagaard et al. 2001). To date, it is not possible to measure the PCSA of pennate muscle *in vivo*. However, the indirect calculation of the PCSA by dividing the muscle volume by fascicle length as proposed by Powell et al. (1984) as well as Lieber and Frieden (2000) is well accepted. Furthermore, the relation of muscle force to PCSA is an accepted approach to estimate the specific tension of a muscle *in vivo* (Erskine et al. 2011). Therefore, it is evident that muscle volume measurement is necessary not only for the assessment of the mechanical muscular capacity, but also to differentiate peripheral mechanisms leading to increased muscle power or strength.

The measurement of muscle volume is, however, a time-consuming procedure, since it involves the segmentation of the muscle from magnetic resonance imaging (MRI) recordings (Mitsiopoulos et al. 1998). A promising approach to reduce the time and effort of muscle volume assessment was presented by Albracht et al. (2008) for the triceps surae. Following a derivation of the volume calculation of solids, the muscle volume is the product of the mean anatomical cross-sectional area (ACSA) and the muscle length. Expressing the mean ACSA as a fraction of the maximum ACSA describes the shape of a given muscle and there is evidence that this fraction (or shape factor) is constant for each triceps surae muscle (Albracht et al., 2008). Consequently, the muscle volume can be assessed using only the muscle length, the maximum ACSA and the shape factor (Albracht et al. 2008). However, to date no study provided evidence that the muscle shape of other muscles than the triceps surae feature a similar consistency and provide the opportunity for the application of the reported timesaving assessment method. Further, the consistency of muscle shape

has only been examined on the basis of data obtained from male individuals (Albracht et al. 2008). However, since the anthropometry and whole-body muscle volume distribution of males and females differs significantly (Abe et al. 2003) and there are some reports of sex differences in muscle architecture (Abe et al. 1998; Chow et al. 2000), it is well conceivable that there might be differences in the muscle shape between men and women as well. Moreover, for the proposed method to be applicable to populations with great differences in body anthropometry, the robustness of the muscle shape and its predictive validity need to be verified within a cohort with a great range of muscle lengths and cross-sectional areas.

The contribution of the quadriceps femoris muscles (QF) during locomotion is essential, because it is one of the strongest muscles within the lower extremities. The force and power potential of the QF significantly affects athletic performance in sports involving sprinting and jumping (Sleivert et al. 1995; Chelly et al. 2010) as well as important locomotor functions in the elderly (Karamanidis et al. 2008) and the muscle volume assessment of the QF muscles is, thus, a crucial diagnostic and experimental procedure.

Therefore the purpose of the current study was to extend the examination of muscle shape consistency and the applicability of the shape factor based assessment method on the vastii muscles (i.e. lateralis, medialis and intermedius). We hypothesized that neither differences in body anthropometry between males and females, nor in muscle dimensions would significantly affect the muscle shape and that the muscle volume prediction using only the maximum ACSA, muscle length and the muscle specific shape factors of the vastii muscles, would provide acceptable results compared to whole-muscle segmentation.

3.3 Methods

3.3.1 Participants

Twenty female and 17 male volleyball athletes participated in the present study. The competition-level of the athletes ranged from regional to top-national league and the number of training-sessions from 2 to 7 sessions per week. The anthropometric data of all groups are shown in Table 3.1. All participants signed informed consent to the experimental procedure, which had been approved by the local university ethics committee.

Table 3.1 *Mean values ± standard deviations of age, body height and mass of the female and male volleyball athletes*

Parameter	Female (n = 20)	Male (n = 17)
Age	31 ± 17	32 ± 16
Body height [cm]	179 ± 6	193 ± 5
Body mass [kg]	70 ± 7	91 ± 13

3.3.2 Data acquisition

Transversal plane MRI images were obtained from the dominant leg of every participant (i.e. leading leg in the spike jump) between the greater trochanter and the femur condyles (T1 weighed, slice thickness 4.0 mm, 0.8 mm inter-slice spacing, echo time 11 ms, repetition time 641 ms, field of view 230 × 420 mm^2) lying supine with the knee fully extended in a 1.5 Tesla Magnetom Avanto scanner (Siemens, Erlangen, Germany). For the muscle volume measurement of the three uniarticular quadriceps femoris muscles (i.e. vastus lateralis, VL; vastus medialis, VM; vastus intermedius, VI), the boundaries of the muscles were tracked manually in every transversal plane image (Figure 3.1) using Osirix (Version 4.0, 64 bit, Pixmeo SARL, Bernex, CH). Hence, the muscle volume V was calculated as the integral of the cross-sectional areas of the obtained muscle contours along the muscle length L_M, which in turn was measured on the longitudinal axis of the coordinate system (along which the transversal images were obtained) as the distance between the two marginal transversal slices contributing to the whole-muscle segmentation. Further, as an external reference, the femur length was measured as the distance between the most proximal part of the femoral head to the most distal part of the medial femoral condyle on longitudinal axis of the common MRI coordinate system. Thus, it was possible to relate the position of the muscle cross-sectional areas to the length of the femur.

3.3.3 Assessment of muscle specific shape

The assessment of a parameter describing the muscle specific shape can be derived from the following theoretical consideration (Albracht et al. 2008): Locating the origin of a Cartesian coordinate system to the myotendinous junction of a muscle with the z-axis aligned with the longitudinal axis of the muscle, the anatomical cross-sectional area (ACSA) at a certain location (z) of the muscle is

$$ACSA(z) = \iint_{A(z)} f(x,y)\ dx\ dy. \quad (1)$$

Accordingly, the volume V of a muscle is calculated as

$$V = \int_0^{L_M} ACSA(z)\ dz, \tag{2}$$

which equals the product of the mean ACSA and the muscle length (L_M). Describing the mean ACSA as the fraction p of the maximum ACSA ($ACSA_{max}$), equation (2) can be rewritten as

$$V = p \cdot ACSA_{max} \cdot L_M. \tag{3}$$

The size of the fraction p depends on the shape of the respective muscle (Albracht et al. 2008) and is henceforth referred to as shape factor.

In the present study, the VL, VM and VI shape factors (p) of the female and male volleyball athletes were obtained from the whole-muscle segmentations by dividing the measured volume by the product of the maximum ACSA and the muscle length for each muscle:

$$p = \frac{V}{ACSA_{max} \cdot L_M} \tag{4}$$

For the evaluation of the applicability of the muscle volume assessment based on the muscle shape, we compared the values measured by whole-muscle segmentation to those predicted based on equation 3, using only the measured maximum ACSA, muscle length and average shape factor obtained from the whole group of participants (pooled data of female and male athletes). Finally, we investigated the muscle dimensions and the obtained shape factors between the female and male athletes for the VL, VM and VI, respectively.

3.3.4 Statistics

A two-way analysis of variances (ANOVA) with the fixed factors sex and investigated muscle (i.e. vastus intermedius, lateralis and medialis) was performed to examine the specificity of muscle shape. A Bonferroni post-hoc test was applied to identify differences between the three uniarticular quadriceps femoris muscles regarding the shape factor of the muscle, muscle volume, maximum ACSA, the position of the maximum ACSA relative to femur length and muscle length.

A repeated measures ANOVA was used to compare the muscle volumes measured by means of whole-muscle segmentation with those assessed with the simplified method applying the obtained shape factors of VI, VL and VM, respectively. For accuracy evaluation, the root mean squares (RMS) of the differences between the volumes determined by means of the two methods as well as the coefficients of determination (R^2) were calculated.

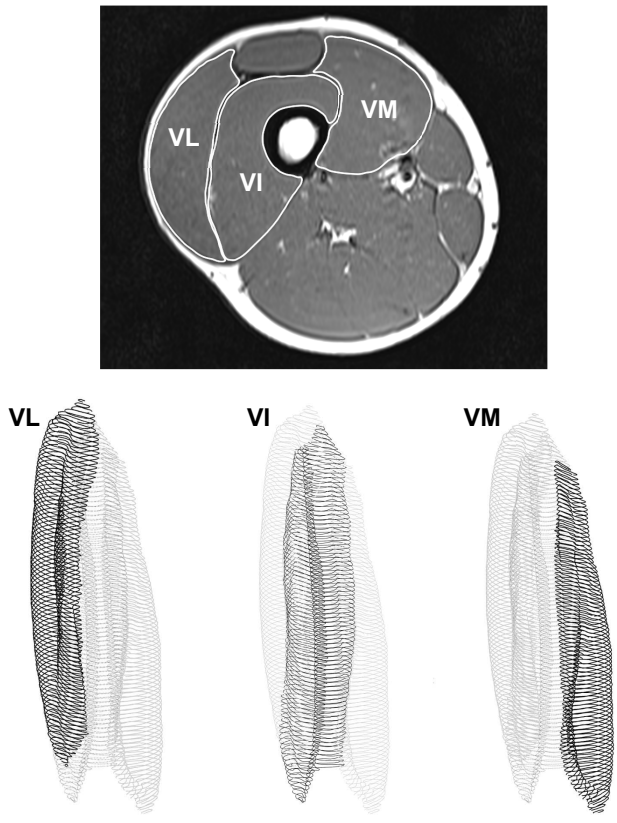

Figure 3.1 Manually tracked contours of the vastus lateralis (VL), vastus intermedius (VI) and vastus medialis (VM) in a MRI slice of one male participant at ~50% of the femur length (top) and the respective whole-muscle segmentation (bottom).

All statistical procedures were performed in SPSS (IBM Corp., Version 19.0, NY, USA) and the level of significance was set to $\alpha = 0.05$.

3.4 Results

There was a significant effect of sex ($p < 0.05$) on the muscle volume, the muscle length and the maximum ACSA with significantly greater values for the male compared to the female athletes (Table 3.2). However, there was no effect of sex on the muscle shape factors or the position of the maximum ACSA and there was no sex-by-muscle interaction on any of the investigated parameters (Table 3.2). There was a significant effect of investigated muscle on muscle volume, muscle length, the position of the maximum ACSA and the muscle shape factors ($p < 0.05$; Table 3.2). Post-hoc comparisons showed that muscle length was significantly ($p < 0.05$) greater in VI compared to VL and VM, with no significant differences between the latter. The maximum ACSA position was significantly ($p < 0.05$) more distal (i.e. greater values of relative femur length position) in VM compared to VI and VL, yet similar

between the latter. Figure 3.2 shows the cross-sectional areas and the position of the maximum ACSA as a function of relative femur length. The muscle volumes of VI and VL were similar and significantly ($p < 0.05$) greater compared to VM. The muscle shape factors were significantly ($p < 0.05$) different between all three uniarticular quadriceps femoris muscles (Table 3.2).

There was no significant difference between the muscle volumes obtained from whole-muscle segmentation (means ± standard deviation of VI: 639 ± 153 cm^3, VL: 692 ± 183 cm^3, VM: 514 ± 121 cm^3) and the volumes predicted using the measured maximum ACSA, muscle length and the shape factors obtained from the pooled data of both female and male participants (VI: 637 ± 140 cm^3, VL: 694 ± 191 cm^3, VM: 515 ± 123 cm^3; Figure 3.3). The pooled data could be used due to the absence of an effect of sex on the shape factors (Table 3.2). The RMS differences were ~5% (Figure 3.3) and the coefficients of determination (R^2) were 0.955, 0.972 and 0.943 for VI, VL and VM, respectively.

Table 3.2 *Mean values ± standard deviations of muscle length, maximum anatomical cross-sectional area (ACSAmax), position of ACSAmax relative to femur length (Pos. ACSAmax; from proximal to distal), muscle volume (measured by whole-muscle segmentation) and shape factor of the vastus intermedius (VI), vastus lateralis (VL) and vastus medialis muscle (VM) of female and male athletes*

	Main Effects	Muscle	Female athletes (n = 20)	Male athletes (n = 17)	Total (n = 37)
Muscle length [cm]	* #	VI	38.3 ± 2.3	40.8 ± 1.2	39.4 ± 2.3
		VL†	36.3 ± 2.3	38.9 ± 2.0	37.5 ± 2.5
		VM†	35.4 ± 2.9	37.6 ± 1.2	36.4 ± 2.5
$ACSA_{max}$ [cm^2|]	*	VI	24.6 ± 4.3	31.1 ± 3.4	27.6 ± 5.0
		VL	23.6 ± 4.2	32.9 ± 4.6	27.9 ± 6.4
		VM	22.7 ± 4.3	29.7 ± 3.3	25.9 ± 5.2
Pos. $ACSA_{max}$ [%]	#	VI	56.9 ± 5.8	58.3 ± 4.3	57.5 ± 5.2
		VL	60.3 ± 7.0	60.0 ± 6.0	60.2 ± 6.5
		VM†‡	81.3 ± 3.3	82.3 ± 2.4	81.8 ± 2.9
Muscle volume [cm^3]	* #	VI	544.9 ± 126.0	749.7 ± 97.8	639.0 ± 152.8
		VL	570.2 ± 128.1	834.9 ± 125.9	691.8 ± 183.3
		VM†‡	431.0 ± 85.1	611.3 ± 74.0	513.8 ± 120.7
Shape factor	#	VI	0.574 ± 0.029	0.590 ± 0.032	0.582 ± 0.031
		VL†	0.662 ± 0.028	0.652 ± 0.026	0.658 ± 0.027
		VM†‡	0.539 ± 0.028	0.548 ± 0.028	0.543 ± 0.028

Main effects: * significant effect of sex, # significant effect of muscle, $p < 0.05$

Post-hoc comparison: † significant difference to VI, ‡ significant difference to VL, $p < 0.05$

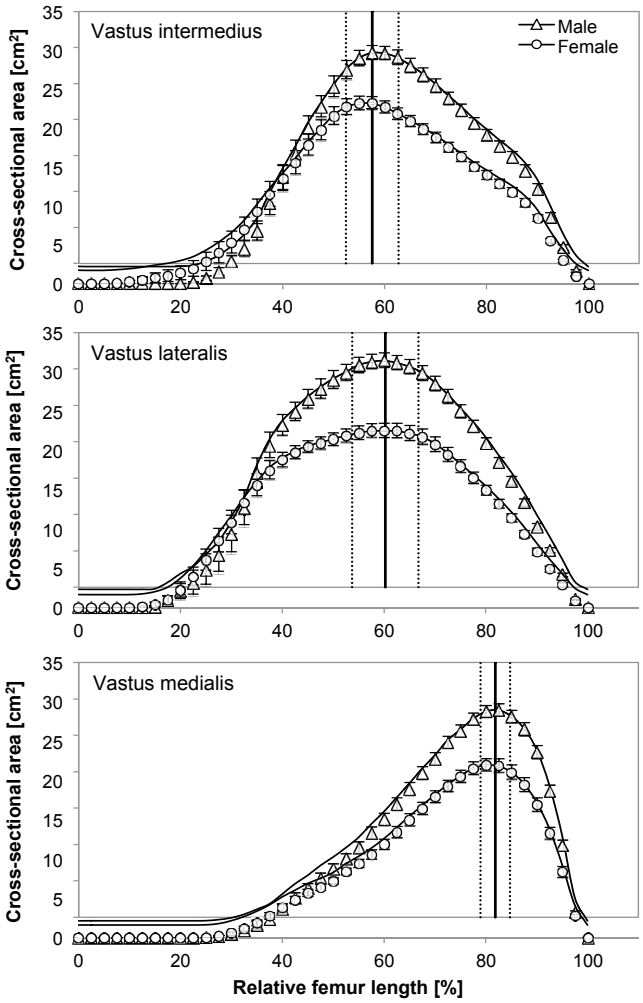

Figure 3.2 Mean cross-sectional area (CSA) and standard error of mean (error bars) of the vastus intermedius, vastus lateralis and vastus medialis muscle of female (n = 20) and male (n = 17) athletes as a function of relative femur length (from proximal [0%] to distal [100%]). The vertical lines indicate the mean position ± standard deviation (dotted vertical lines) of the maximum CSA of the pooled data.

3.5 Discussion

The purpose of the present study was to investigate the muscle shape consistency and the applicability of a shape factor based assessment method (Albracht et al. 2008) on the quadriceps femoris vastii muscles (i.e. lateralis, medialis and intermedius) across a population featuring a) both sexes and b) a wide range of anthropometric characteristics (body height ranged from 166 to 201 cm). Though the muscle dimensions (i.e. muscle volume, length and maximum ACSA) were significantly different, it was found that the muscle shape as well as the location (relative to femur length) of the maximum ACSA was similar in female and male athletes. Further the

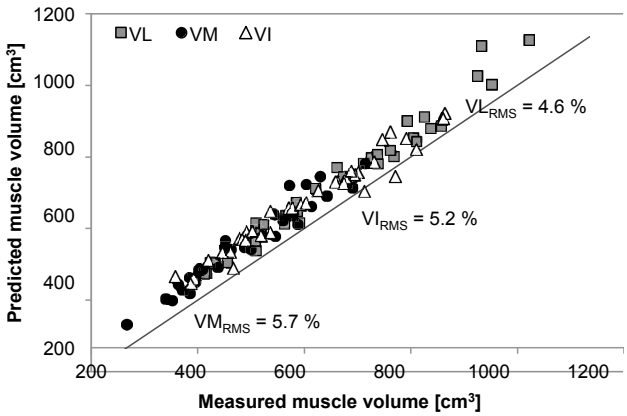

Figure 3.3 Muscle volumes of vastus intermedius (VI), vastus lateralis (VL) and vastus medialis (VM) (n = 37) measured from whole-muscle segmentation (abscissa) or predicted using the muscle specific shape factor, the maximum anatomical cross-sectional area and muscle length (ordinate). The solid diagonal line represents the identity line. The relative root mean square differences (RMS) between the two assessment methods are included in the figure.

shape factors, locations of the maximum ACSA and most dimensional parameters were different between the three vastii muscles. Taken together, this suggests that muscle shape of volleyball athletes is muscle- but not sex-dependent. The muscle volume predicted using the calculated average shape factors of each muscle, the muscle length and the maximum ACSA was not significantly different to whole-muscle segmentation. Therefore, our hypotheses were confirmed.

It is self-evident that muscle volume is an important measure in a wide range of research fields. Assessment approaches predicting muscle volume on the basis of easily measurable parameters may, therefore, provide a promising alternative to the time-consuming procedure of whole-muscle segmentation. In the present study, the concept proposed by Albracht et al. (2008) for the triceps surae was applied to the vastii muscles of the quadriceps femoris. The knee extensor muscle is a major contributor to athletic performance during sprint start (Sleivert and Taingahue 2004), sprinting (Stafilidis and Arampatzis 2007), jumping (Pääsuke et al. 2001; Kubo et al. 2005) as well as to physical performance during everyday functional tasks as chair rise, stair ascend/descend (Ploutz-Snyder et al. 2002; Karamanidis and Arampatzis 2009; 2011) or gait (Ploutz-Snyder et al. 2002) and balance recovery (Karamanidis et al. 2008) in the elderly. The findings of the present study provide evidence that the prediction of the vastii muscle volumes using only the shape factors, the muscle length and the maximum ACSA shows good accuracy ($R^2 > 0.94$). Furthermore, the RMS differences to the volume measured from whole-muscle segmentation were about 4-5%. Thus, the muscle-shape based assessment method could be sensitive enough to detect for example increases in muscle volume of 10-12% that were reported for the quadriceps muscles in response to strength training in young and elderly adults (Tracy et al. 1999; Aagaard et al. 2001) or decreases of quadriceps vastii volume

during 29 and 89 days of bed rest of 10% and 19%, respectively (Alkner and Tesch 2004). Suetta et al. (2009) found a decrease of quadriceps muscle volume following immobilization of 9% in young male adults and baseline differences to elderly men of 11%. Further, pathologic increases of thigh muscle volume can reach differences to healthy individuals of 22% (Ji et al. 2013). The generalizability of the obtained QF vastii to those populations needs further research, yet a recent study by Mersmann et al. (2014) investigating the triceps surae provided promising evidence of muscle shape consistency between populations with different habitual loading conditions (i.e. untrained individuals, endurance and strength athletes) and of a high predictive validity of the proposed method when using the obtained shape factors of one sample for volume prediction of an independent one.

The position of the maximum ACSA was at 58%, 60% and 82% of the thigh length (from proximal to distal) for VI, VL and VM, respectively, and showed low inter-individual variability. For example, in 95% of the cases an image acquisition of 20%, 26% and 11% of the thigh length around the reported position would be sufficient to include the maximum ACSA of the VI, VL and VM, respectively. Given the unimodal distribution of the muscle ACSA the maximum ACSA can easily be identified with a few segmentations around the reported positions and the subsequent calculation of muscle volume using the proposed method greatly reduces the required time compared to full muscle segmentation.

Sex-differences in muscle size were expected and are in accordance with previous studies (Tracy et al. 1999; O'Brien et al. 2010; Maden-Wilkinson et al. 2014). The present study further provides evidence that despite pronounced differences in muscle length, maximum ACSA and volume, the muscle shape factor is similar between sexes in volleyball athletes. This suggests that sex-related differences in whole-body muscle mass distribution (Abe et al. 2003), muscle tissue structure (Toft et al. 2003; Galbán et al. 2005) and potential differences in muscle architecture that were reported in some (Abe et al. 1998; Chow et al. 2000) but not all studies (Abe et al. 2001; Blazevich et al. 2006), do not significantly influence the muscle shape of the quadriceps femoris vastii in athletes. On the basis of the data of the present study, a generalizability of this finding to an untrained population can only be assumed. However, the data of Mersmann et al. (2014), providing evidence that training induced muscle hypertrophy does not effect the muscle shape factors of the triceps surae, as well as the consistency of muscle shape despite considerable differences of muscle dimensions within and between sexes in the present study give reason to believe that the reported shape factors should be widely applicable for muscle volume prediction in both sexes.

In conclusion, the results of the present study indicate that the muscle volume prediction method proposed by Albracht et al. (2008) for the triceps surae is also applicable to other leg muscles (i.e. quadriceps vastii), as the shape factors of the investigated muscles showed relative consistency across a population featuring a wide range of anthropometric measures and muscle dimensions. We further provide evidence that there seems to be no effect of sex on the muscle shape of the quadriceps vastii. The prediction of quadriceps vastii muscle volume using the averaged shape factors and the easily measurable parameters muscle length and maximum CSA, should be sensitive enough to detect atrophic responses due to unloading or aging as well as muscle hypertrophy following training interventions.

3.6 Perspectives

The presented prediction method using the shape factors and respective locations of the maximum ACSA reported in the present study features the potential to greatly reduce a) the length of acquired MRI sequences and b) the time for muscle segmentation needed to obtain a reliable measure of quadriceps vastii muscle volume. Further research may extend the investigation of the applicability of the proposed method to other muscles of the human body.

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4 Second study | Evidence of imbalanced adaptation between muscle and tendon in adolescent athletes

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4.1 Abstract

Adolescence may be regarded as a critical phase of tissue plasticity in young growing athletes, as the adaptation process of muscle-tendon unit is affected by both environmental mechanical stimuli and maturation. The present study investigated potential imbalances of knee extensor muscle strength and patellar tendon properties in adolescent compared to middle-aged athletes with long-term musculotendinous adaptations. Twenty adolescent elite volleyball athletes ([A], 15.9±0.6 years) and 18 middle-aged competitively active former elite volleyball athletes ([MA], 46.9±0.6 years) participated in magnetic resonance imaging and ultrasound-dynamometry sessions to determine quadriceps femoris muscle strength, vastus lateralis morphology and patellar tendon mechanical and morphological properties. There was no significant effect of age on the physiological cross-sectional area of the vastus lateralis and maximum knee extension moment ($p > 0.05$) during voluntary isometric contractions. However, the patellar tendon cross-sectional area was significantly smaller (A: 107.4±27.5 mm^2; MA: 121.7±39.8 mm^2) and the tendon stress during the maximal contractions was significantly higher in adolescent compared to the middle-aged athletes (A: 50.0±10.1 MPa; MA: 40.0±9.5 MPa). These findings provide evidence of an imbalanced development of muscle strength and tendon mechanical and morphological properties in adolescent athletes, which may have implications for the risk of tendon overuse injuries.

Key words: tendon morphology, muscle strength, knee joint, muscle morphology

4.2 Introduction

The mechanical and morphological properties of muscles and tendons have been shown to influence athletic performance (Arampatzis et al., 2006; Stafilidis & Arampatzis, 2007) and relate to the risk of injury (Arya & Kulig, 2010; Couppé et al., 2013; Hansen et al., 2013). Since the maximum strain of tendon tissue cannot be significantly altered (LaCroix et al., 2013), the increase of force as a result of physical exercise (Kongsgaard et al., 2007; Arampatzis et al., 2007a) has to be accompanied by an increase of tendon stiffness to maintain physiological ranges of strain during maximum muscle contractions. This increase of stiffness may be a result of an increase of Young's Modulus (i.e. changes of material properties; Arampatzis et al., 2007a, 2010) and/or an increase of the tendon's cross-sectional area (Kongsgaard et al., 2007; Arampatzis et al., 2007a; Couppé et al., 2008; Seynnes et al., 2009; O'Brien et al., 2010b), whereupon the former may be considered an early mechanism leading to an increased stiffness and the latter a rather long-term effect of mechanical loading (Heinemeier, 2011). However, there is evidence that on the transcriptional level of growth factors in response to loading tendons feature delayed responses compared to muscle (Heinemeier et al., 2011). Further, the mechanical stimuli eliciting adaptation may be different for tendon than for muscle tissue (Arampatzis et al., 2010). Thus, it is reasonable to argue that the development of the muscle-tendon unit within the course of athletic training may be characterized by imbalances of muscle capacity and tendon properties, resulting in episodes of high tendon strain and stress. Besides environmental mechanical stimuli maturation affects the properties of muscle and tendon (O'Brien et al. 2010a, 2010b). Therefore, it may be argued that young athletes are in a critical phase of muscle and tendon plasticity, as they are subjected to high mechanical loads as well as the physical growth that accompanies adolescence. The above argumentation can be supported by clinical evidence that early manifestations of overload injuries like patellar tendinopathy concern adolescent athletes (Le Gall et al., 2006). While the long-term effects of athletic training with different loading histories on muscle and tendon properties in adults have been investigated previously (Rosager et al., 2002; Kongsgaard et al., 2005; Arampatzis et al., 2007b; Kubo et al., 2011), there is little information about the morphological and mechanical properties of muscle and tendon in adolescent athletes. Imbalances between muscle strength and tendon loading capacity in adolescent athletes might increase the risk of tendon injury. However, possible differences of the training induced long-term adaptation within the muscle-tendon unit in young compared to adult athletes have to date not been investigated. The identification of differences between the early and later phases of the athletic career would deepen the

understanding of adaptational processes that are associated with long-term athletic engagement and, more importantly, shed light on the composition of muscle-tendon unit properties that are crucial for health and performance in a dynamic phase of tissue development (i.e. adolescence).

Volleyball players have been found to have greater quadriceps femoris muscle strength and thigh circumference compared to non-active controls and middle-distance runners (Sleivert et al., 1995). On the other hand volleyball athletes are predisposed to developing patellar tendinopathy (Lian et al., 2005) and, thus, an intriguing subject cohort for the examination of the knee extensor muscle-tendon unit properties and their development. As it has been shown that athletic training has the potential to maintain muscle mass in master athletes (Wroblewski et al., 2011), middle-aged sportsmen seem to be a relevant group to investigate long-term effects of sport-specific training. We further believe that former elite athletes might be more suitable to provide information about a muscle-tendon unit steady-state that develops in response to long-term sport specific mechanical loading than a younger active elite athlete group, as the latter might be more susceptible to specific plastic changes due to a high rate of adaptation and inhomogeneous training intensity during the season in elite sports.

Our purpose in the present investigation was to provide information about m. quadriceps femoris muscle strength and morphology as well as patellar tendon mechanical and morphological properties of adolescent and middle-aged elite volleyball athletes. We hypothesized that adolescent athletes feature equivalent muscle capacities due to the rapid development of muscle function, yet lower tendon stiffness and cross-sectional areas (i.e. long-term adaptation), resulting in higher tendon stress and strain. As there is evidence of degraded collagen synthesis in response to mechanical loading in women (Miller et al., 2007), we further hypothesized that these imbalances would be more pronounced in female athletes.

4.3 Methods

4.3.1 Experimental design

Thirty-seven volleyball athletes participated in the present study. The adolescent group consisted of 10 male and 9 female athletes of the extended pool of the junior national team. The middle-aged group was composed of 8 male and 10 female former elite athletes who still engaged actively in volleyball at least twice a week. Anthropometric data of the participants are shown in Table 4.1. The study has been approved by the university ethics committee and all participants signed informed

consent to the experimental procedure, which included (a) the measurement of the maximum knee joint moment during isometric contractions by dynamometry, (b) the assessment of the patellar tendon and m. vastus lateralis morphology using ultrasonography and magnetic resonance imaging and (c) the measurement of the patellar tendon elongation during maximum knee extension contractions by ultrasonography. All measurements were conducted on the jumping leg (i.e. leading leg in the spike jump).

Table 4.1 *Mean values ± standard deviations of age, height and mass of the female and male adolescent and middle-aged athletes respectively*

	Adolescent		Middle-aged	
	Female (n = 9)	Male (n = 10)	Female (n = 10)	Male (n = 8)
Age [years]	15.7 ± 0.5	16.1 ± 0.7	46.9 ± 6.4	46.9 ± 3.3
Height [cm]	182.3 ± 4.2	194.8 ± 5.6	176.2 ± 4.9	191.6 ± 5.6
Mass [kg]	69.7 ± 8.4	86.2 ± 7.4	70.2 ± 5.8	97.4 ± 14.9

4.3.2 Measurement of maximum knee joint moment

The participants were seated on a dynamometer (Biodex Medical, Inc., Shirley, NY) and fixed with a pelvic strap with a trunk flexion of 85° (supine = 0°). Appropriate correction techniques were used to account for the effect of gravitational forces, the misalignment of the joint axis and the axis of the dynamometer during the maximal isometric contractions (Arampatzis et al., 2004) and the antagonistic coactivation (Mademli et al., 2004) on all measured knee extension moments. For this purpose, kinematic, kinetic and electromyographic (EMG) data were recorded during the contractions. Six reflective markers were fixed to the following anatomical landmarks on the leg: anterior iliac spine, greater trochanter, lateral and medial femoral epicondyles and malleoli. Furthermore, two bipolar surface electrodes (2 cm interelectrode distance, 0.8 cm^2 pickup surface, Myon m320RX, Myon AG, Baar, CH) were fixed over the lateral head of the biceps femoris in the direction of the muscle fibres after shaving and cleaning the skin with alcohol to reduce skin impedance (Mademli et al., 2004). Kinematic data was recorded using a Vicon motion capture system (Version 1.7.1., Vicon Motion Systems, Oxford, UK) integrating eight cameras (6x Vicon F20, 2x Vicon T20) operating at 250 Hz. The analog data of the dynamometer as well as the EMG-signals were sampled at a rate of 1000 Hz and transmitted to the Vicon system via a 16-channel A-D converter.

Following a standardized warm-up including five minutes of ergometer cycling and ten deep squats, ten submaximal isometric knee extension contractions were used as preconditioning of the tendon and as accustoming for the participants. Three to five trials of isometric maximum voluntary knee extension contractions (MVC) were performed starting from a range of resting knee joint angles of 60° to 80° (0° = full knee extension) to measure the maximal knee joint moment (note that due to the soft tissue deformation and dynamometer compliance the knee joint angles between rest and moment maximum are different (Arampatzis et al., 2004)). Two trials with submaximal knee flexion contractions were used to establish an activation-flexion moment relationship and, thus, to account for the knee flexion moment generated during maximum effort knee extension due to antagonistic coactivation (for more information on the procedure see Mademli et al., 2004).

4.3.3 Morphology of the patellar tendon and vastus lateralis muscle

The measurement of the m. vastus lateralis fascicle length was obtained during a passive knee extension (i.e. inactive muscle) throughout the full range of motion driven by the isokinetic device at a speed of 5 °/s using a 7.5 MHz ultrasound probe of 10 cm width (My Lab60, Esaote Canada, Georgetown, Canada). The ultrasound probe was positioned over the medial part of the muscle belly and fixed with Velcro straps. The recordings were analyzed by means of a semi-automatic feature-tracking algorithm implemented in a custom written MATLAB interface. In short, following the manual tracking of the upper and deeper aponeuroses throughout the whole recording a key-frame in the mid-portion of the recording was selected and the visible features of multiple fascicles were digitalized manually. Subsequently, these features were tracked automatically throughout the recording by processing the shift of the brightness profiles within discrete horizontal lines between the aponeuroses using a non-linear least squares fitting. A representative reference fascicle was calculated on the basis of the behavior of the tracked fascicle portions (Figure 4.1). Hence, fascicle length (FL) was reported as a function of knee angle and was then normalised to femur length (rFL; Mohagheghi et al., 2008). In the following we present the data measured at 50°, which was the average knee joint angle obtained from the kinematic data where the highest knee joint moment was accomplished during the MVC assessment. The measurement and semi-automatic tracking procedure was tested for reproducibility in a pilot-study on the vastus lateralis of six independent male participants (Age: 30.5 ± 3.3 years; body height: 181.8 ± 16.5 cm, body mass: 83.8 ± 21.9 kg) showing an intra-class correlation for absolute agreement between the fascicle lengths at 50° knee joint angle measured on two consecutive days of 0.8 (Day 1: 10.04 ± 1.93 cm; Day 2: 9.97 ± 1.73 cm).

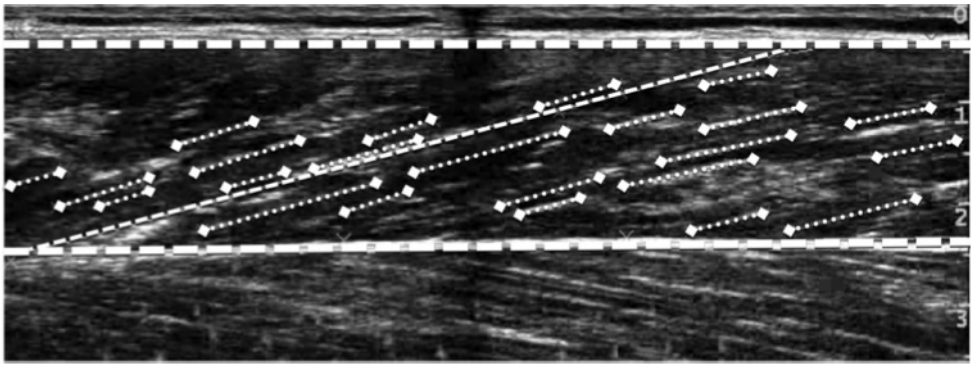

Figure 4.1 Measurement of fascicle length by a semi-automatic feature-tracking approach, which involved the manual tracking of the upper (upper thick dashed line) and deeper (lower thick dashed line) aponeuroses throughout the whole and the manual digitalization of visible features of multiple fascicles (pointed lines). Subsequently, these features were tracked automatically throughout the recording by processing the shift of the brightness profiles. A representative reference fascicle (thin dashed line) was then calculated on the basis of the behavior of the tracked fascicle portions.

In an MRI-session sagittal and transversal plane images were obtained from the jumping leg of every participant between the pelvic spine and the tibial tuberosity (sagittal: 2D-MESE, slice thickness 3 mm, inter-slice spacing 3.51 mm, tansversal: 3D-MEDIC, slice thickness 1.2 mm, inter-slice spacing 0 mm,) lying supine with the knee fully extended in a 1.5 Tesla Magnetom Avanto scanner (Siemens, Erlangen, Germany). To assess vastus lateralis volume (VL_{vol}) the boundaries of the muscle were tracked manually in the transversal plane images between the origin at the line aspera and the insertion at the patella using Osirix (Version 4.0, 64 bit, Pixmeo SARL, Bernex, CH). Muscle volume was calculated as the sum of the products of the respective cross-sectional areas multiplied by slicke thickness. The vastus lateralis physiological cross-sectional area (VL_{PCSA}) was then calculated as the quotient of VL_{vol} to FL at a knee joint angle position of 50° (Lieber & Fridén, 2000).

The patellar tendons cross-sectional areas (PT_{CSA}) were also digitalized manually in the transversal plane MRI between the caudal pole of the patellar and the insertion at the tibial tuberosity. Unfortunately, not all the transversal plane recordings included the most distal part of the tendon. Thus, the reported PT_{CSA} values relate to mean cross-sectional area of the longest common relative length, which was 0-70% of the patellar tendon length. The digitized PT_{CSA} were transformed perpendicular to the line of action of the patellar tendon, which in turn was defined as the line of best fit through the geometrical centers of the respective cross-sectional areas. The patellar tendon moment arm at full knee extension (PT_{MA}) was measured in a three-dimensional coordinate system as the perpendicular distance of the tendon's line of action to the rotation axis of the knee. The rotation axis of the knee joint was determined by outlining the lateral and medial femoral epicondyles in the sagittal MRI scans and connecting the centers of the respective best fitting circles

according to Churchill et al. (1998). Subsequently, the tendon moment arm as a function of knee joint angle was calculated by processing moment arm changes in relation to joint angle on the basis of the data reported by Herzog and Read (1993).

4.3.4 Determination of the mechanical properties of the patellar tendon

The measurement of the patellar tendon elongation during isometric contractions was performed in the same experimental setup as described earlier (in the section *measurement of maximum knee joint moment*) following the MVC assessment and using the same considerations for joint moment calculation. The ultrasound probe was fixed in a modified knee brace covering the tendon in the sagittal plane. The knee joint angle for the subsequent contractions was adjusted according to the resting knee joint angle where the highest individual joint moments were accomplished during the MVC assessment. Based on the findings from Schulze et al. (2012) regarding the reliability of patellar tendon elongation measurements, five trials of isometric ramp contractions were performed by every participant increasing the force steadily from rest to maximum effort in about five seconds. The shank was fixed to the lever arm of the dynamometer with a Velcro strap to allow full relaxation and accurate tendon resting length assessment.

Tendon force (TF) was then calculated by dividing the knee joint moment by the angle specific tendon moment arm. The patellar tendon elongation was digitalized by manually tracking the deep insertion of the tendon at the caudal pole of the patella and the tibial tuberosity frame by frame using a custom written MATLAB user interface (version R2011b, The Mathworks, Natick, USA) to navigate through the video images and mark the respective insertion points. Subsequently, the mean force-elongation relationship for each participant was calculated using the highest force value that was reached in all of the five ramp contractions (and the respective elongations) as peak force. The resultant function was fitted by a second-order polynomial and tendon stiffness as well as Young's modulus was calculated between 50% and 100% of the peak tendon force. For the calculation of the respective stress values we used the mean patellar tendon cross-sectional area. The data obtained in the ultrasound sessions of four out of the 37 participants had to be excluded due to artefacts in the ultrasound recordings.

4.3.5 Statistics

Statistical analysis was performed in SPSS (SPSS Inc., Version 19.0, Chicago, USA). After testing for normal distribution of the data using the Kolmogorov-Smirnov test,

the parameters were analyzed by means of a two-way ANOVA with the fixed factors sex and age. The level of significance was set to $\alpha = 0.05$.

4.4 Results

4.4.1 Muscle morphology and strength

There was a significant effect of sex and age ($p < 0.05$) on the VL volume (Table 4.2). The male athletes had a greater muscle volume compared to the female, and the adolescent compared to the middle aged counterparts respectively. Male athletes showed a greater ($p < 0.05$) PCSA of the vastus lateralis muscle and a higher maximal resultant knee-extension moment during the MVC compared to the female athletes (Table 4.2). However, we did not find any effect of age ($p > 0.05$) on the PCSA and maximal knee joint moment. Further, there was no effect of sex and age on the relative fascicle length of the vastus lateralis muscle (Table 4.2).

4.4.2 Tendon morphological and mechanical properties

Males showed a greater ($p < 0.05$) moment arm and resting length of the patellar tendon compared to the female athletes (Table 4.3). There was no significant effect of sex or age on patellar tendon stiffness and Youngs Modulus but a tendency ($p = 0.07$) towards higher maximum tendon strain in adolescent compared to middle-aged athletes (Table 4.3). There was a significant effect of sex and age ($p < 0.05$) on the P_{CSA} and the maximum tendon stress. We found smaller cross-sectional areas, yet higher tendon stress in the female compared to male and the adolescent compared to middle-aged athletes (Figure 4.2).

Table 4.2 *Mean values ± standard deviations of vastus lateralis muscle volume, physiological cross-sectional area (PCSA), fascicle length relative to femur length (rFL) and maximum knee joint moment (MVC) of female and male adolescent and middle-aged athletes respectively*

	Adolescent		Middle-Aged		Effects
	Female (n = 9)	Male (n = 10)	Female (n = 10)	Male (n = 8)	
Volume [cm^3]	620 ± 155	862 ± 129	476 ± 68	776 ± 78	#,*
PCSA [cm^2]	45.5 ± 8.0	53.4 ± 13.1	36.0 ± 10.0	55.0 ± 10.8	#
rFL	0.287 ± 0.047	0.355 ± 0.094	0.309 ± 0.091	0.305 ± 7.1	
MVC [Nm]	248 ± 49	351 ± 39	214 ± 28	340 ± 58	#

#: significant effect of sex ($p < 0.05$), *: significant effect of age ($p < 0.05$)

Table 4.3 *Mean values ± standard deviations of the patellar tendon moment arm (PT_{MA}), maximum tendon force (TF_{max}), maximum strain, stiffness, Youngs Modulus and rest length of female and male adolescent and middle-aged athletes respectively*

	Adolescent				Middle-Aged				Effects
	Female (n = 8)		Male (n = 10)		female (n = 7)		male (n = 8)		
PT_{MA} [mm]	55.4	± 2.9	62.9	± 2.4	57.4	± 3.1	63.6	± 3.8	#
TF_{max}	4688	± 529	5622	± 639	3994	± 748	5383	± 1031	
Strain [%]	8.20	± 2.27	8.40	± 2.36	6.16	± 2.03	7.49	± 2.26	
Stiffness [N/mm]	1031	± 285	1258	± 463	1370	± 540	1345	± 480	
Youngs Modulus [GPa]	0.64	± 0.18	0.59	± 0.25	0.69	± 0.23	0.54	± 0.24	
Rest length [mm]	51.2	± 3.1	56.8	± 3.0	48.5	± 3.4	55.5	± 4.4	#

#: significant effect of sex ($p < 0.05$)

4.5 Discussion

The present study investigated the muscle strength of the knee extensors and the morphological and mechanical properties of the m. vastus lateralis and patellar tendon in adolescent and middle-aged athletes. While muscle strength of the knee extensors and PCSA of the vastus lateralis muscle were similar between the two groups, the adolescent athletes featured a lower tendon cross-sectional area and, thus,

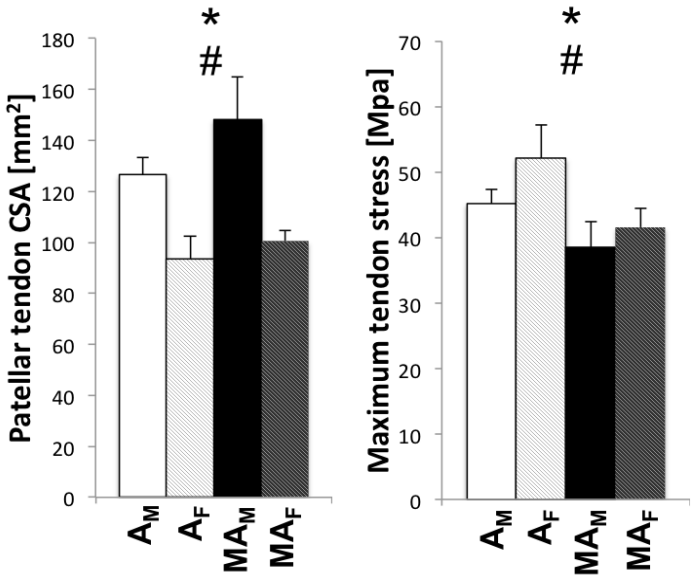

*Figure 4.2 Mean values and standard error of mean (error bars) of the patellar tendon cross-sectional area (left) and maximum tendon stress (right) in female ($_F$) and male ($_M$) adolescent (A) and middle-aged (MA) athletes. * significant effect of age ($p < 0.05$), # significant effect of sex ($p < 0.05$)*

higher tendon stress. Further, tendon stress was greater in female compared to male athletes. Therefore, our hypotheses were partly confirmed.

It is well known that muscle strength increases during maturation (O'Brien et al., 2010b). Though still under debate, it is likely that this increase is mainly due to increases in muscle size (especially the PCSA), moment arm length and activation level and not changes in muscle specific contractile capacity (Bouchant et al., 2011). In the present study, the maximal knee extensor moments did not differ between the adolescent and middle-aged athletes. Further, there were no differences in patellar tendon moment arm and vastus lateralis PCSA. These findings may be interpreted in terms of the adolescents featuring middle-age adult-like muscular capacities in volleyball athletes. As mechanical loading and strength training have been shown to increase muscle strength in adolescents and even pre-pubertal children (Matos & Winsley, 2007) and hormonal changes during puberty facilitate muscle growth (Hulthen, 2001) the developmental status of the muscular capacities of the adolescent athletes in the present study is well explicable. However, the present study provides evidence of considerable differences between adolescent and middle-aged athletes regarding the properties of the patellar tendon, which has the crucial role to transmit the force of the muscle to the bone. A strong muscle acting on a tendon characterized by a low cross-sectional area induces high levels of tendon stress. In the present study, the maximum patellar tendon stress during isometric knee extension contractions was significantly increased (~27%) in adolescent compared to middle-aged athletes and can be explained by an unfavorable relation of muscle strength to tendon cross-sectional area, indicating an imbalance within the muscle-tendon unit of the quadriceps muscle. While muscle strength did not differ significantly between groups, the average cross-sectional area of the patellar tendon was smaller in the adolescents. It has been shown recently that patellar tendinopathy in athletes is associated with tendon stress (Couppé et al., 2013). Thus, the high levels of tendon stress may increase the injury risk of the patellar tendon in the adolescent athletes. Patellar tendinopathy is a well known phenomenon in sports with a high frequency in jumping and sprinting (Lian et al., 2005; Le Gall et al., 2006) and considerably affects the sporting development in adolescent and young athletes. As there is evidence that the time course of plastic changes of muscle and tendon in response to loading and unloading is different (de Boer et al., 2007; Heinemeier et al., 2011) and that the effective mechanical stimuli eliciting adaptational responses in muscle and tendon might differ as well (Arampatzis et al., 2007a, 2010), it is well possible that adolescent athletes develop imbalances between muscle and tendon. It has been shown that even though maturation is accompanied by a general increase of tendon cross-sectional area (O'Brien et al., 2010b), the lengthening of the muscle-tendon unit

during growth can be accompanied by episodes of cross-sectional area reductions and increases of tendon stress (Neugebauer & Hawkins, 2012). Furthermore we found a tendency ($p = 0.07$) for higher tendon strain in the adolescent compared to middle-aged athletes, which might be interpreted as a mechanical weakening of the patellar tendon (Hansen et al., 2013) and can be explained by a slightly lower average tendon stiffness, due to the lower tendon cross-sectional area, in the adolescent group.

Specific differences between sexes in the capacity and morphology of the muscle tendon unit are well documented (Neder et al., 1999; Westh & Kongsgaard, 2008; O'Brien et al., 2010a) and replicated in the present study (i.e. greater muscle strength, muscle volume, muscle PCSA and tendon CSA for male compared to female athletes). However, a novel finding of the present study is that the female featured higher maximum tendon stress compared to the male athletes. In previous studies tendon stress has been found to be similar between sexes (Westh & Kongsgaard, 2008) in runners or higher in males (Onambele et al., 2007) in moderately active individuals. However, the mechanical loading profile of volleyball is characterized by a high frequency of maximum effort muscle contractions and, thus, may be considered an effective stimulus for increasing muscle strength. Regarding the potentially degrading effect of oestrogen on the collagen response to mechanical loading (Miller et al., 2007), it may be possible that the higher tendon stress found in the female volleyball players might be due to a marked imbalance in the development of muscle strength and tendon cross-sectional area as a result of the sport-specific mechanical loading. There might be a sex-related disadvantage regarding the long-term adaptation of the tendon (i.e. tendon hypertrophy) to the developing levels of muscle force. This assumption can be supported by reports of reduced hypertrophic tendon responses to loading and greater risks to develop soft tissue injuries in women compared to men (Magnusson et al., 2007).

The rationale of choosing a group of middle-aged former elite athletes as comparison to the adolescents entails some limitations of the present study. Though there is strong evidence that continuous mechanical loading has the potential to preserve muscle strength (Wroblewski et al., 2011), an effect of age-related degeneration in the middle-aged participants cannot be ruled out completely. However, the isometric torque production of the middle-aged male athletes (340 Nm) in the present study were similar to those reported from varsity athletes (~350 Nm; Sleivert et al., 1995). This is a strong indication that the muscle capacity of the athletes of the present study has been preserved on a very high level and gives reason to believe that this would hold true for the properties of the tendon as well. However, due to a lack of untrained adolescent and middle-aged controls, the effect of

maturation and long-term mechanical loading on the properties of the muscle-tendon unit cannot be separated with the current study-design.

In conclusion, the present study provides evidence of an imbalanced development of muscle strength and tendon mechanical and morphological properties in adolescent athletes. While muscle strength was similar to that of middle-aged athletes, tendon hypertrophy, as a potential consequence of long-term mechanical loading, was not similarly developed and resulted in greater tendon stress in the adolescent athletes. This imbalance is even more pronounced in female athletes and may, among other factors (Lian et al., 2005), be associated with the development of overuse injuries.

4.6 Perspectives

Taking into account recent reports of Couppé et al. (2013) and Hansen et al. (2013), who were able to associate overload injuries to the mechanical properties of tendons, the present findings of high tendon stress in adolescent athletes demonstrate the need for exercise interventions increasing the mechanical strength of tendons to meet the demands placed upon the tissue by the muscle. Facilitating tendon hypertrophy seems to be of particular importance, since it has the potential to reduce tendon stress and has been identified in the present study as a main deficit within the muscle-tendon unit of adolescent compared to long-term adapted middle-aged athletes.

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5 Third study | Muscle and tendon adaptation in adolescent athletes: A longitudinal study

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5.1 Abstract

There is evidence that a non-uniform adaptation of muscle and tendon in young athletes results in increased tendon stress during mid-adolescence. The present longitudinal study investigated the development of the morphological and mechanical properties of muscle and tendon of volleyball athletes in a time period of two-years from mid- to late adolescence. Eighteen elite volleyball athletes participated in magnetic resonance imaging and ultrasound-dynamometry sessions to determine quadriceps femoris muscle strength, vastus lateralis, medialis and intermedius morphology and patellar tendon mechanical and morphological properties in mid- (16 $\pm$1 years) and late adolescence (18$\pm$1 years). Muscle strength, anatomical cross-sectional area (CSA) and volume showed significant ($p < 0.05$) but moderate increases of 13%, 6% and 6% respectively. The increase of patellar tendon CSA ($p < 0.05$) was substantially greater (27%) and went in line with increased stiffness ($p < 0.05$; 25%) and reduced stress ($p < 0.05$; 9%). During late adolescence, a pronounced hypertrophy of the patellar tendon led to a mechanical strengthening of the tendon in relation to the functional and morphological development of the muscle. These adaptive processes may compensate the unfavourable relation of muscle strength and tendon loading capacity in mid-adolescence and might have implications on athletic performance and tendon injury risk.

Key words: Muscle size, Growth, Maturation, Imbalance, Knee joint, Tendinopathy, Jumper's knee

5.2 Introduction

Within the musculoskeletal system, tendons have the crucial role to transmit the forces generated by the muscles to the bones. Since the ultimate strain of tendons cannot be significantly altered (LaCroix et al. 2013), it is commonly observed that muscle strength in healthy individuals develops in line with tendon stiffness (Kubo et al. 2001). Hence, tendon strain during maximum muscle contractions remains on physiological levels. While changes of the material properties of a tendon (i.e. Young's Modulus) have been proposed to characterize the initial phase of a loading-induced tendon adaptation, an increase of the cross-sectional area (CSA) of a tendon is considered the major mechanism to increase stiffness in the later phase of long-term mechanical loading (Bohm et al. 2015 for reviews). For example, Couppé et al. (2008) demonstrated that unilaterally increased habitual loading results in a marked increase of tendon CSA on the dominant leg of badminton and fencing athletes.

However, the loading-induced adaptation of muscle and tendon can show differences in its time course (Kubo et al. 2010; 2011). It seems well possible that due to the lower metabolic rate (Laitinen 1967) of tendon compared to muscle tissue and its reduced responsiveness to loading on the transcriptional level of growth factors (Heinemeier et al. 2011) there might develop an imbalance of muscle strength and tendon material and morphological properties during a training period. Moreover, there is evidence of differences between muscle and tendon regarding the characteristics of mechanical loading (e.g. magnitude, frequency, duration) that effectively facilitate adaptive processes (Arampatzis et al. 2007; Kubo et al. 2007; Arampatzis et al. 2010), which possibly leads to a non-uniform adaptation of muscle and tendon properties under certain conditions.

Besides mechanical loading, maturation affects the mechano-morphological properties of the muscle-tendon unit (O'Brien et al. 2010). With the associated hormonal and molecular mechanisms being beyond the scope of this study, it is intuitive considering the dynamic changes of the morphology and structure of the musculoskeletal system (Malina et al. 2004) that until the end of adolescence the twofold stimulus of loading and maturation might promote the development of imbalances within muscle-tendon units of young athletes. A recent study has provided evidence for this assumption. Mersmann et al. (2013) found adult-like knee extensor muscle morphology and strength, yet significantly smaller patellar tendon CSAs of mid-adolescent volleyball athletes (i.e. ~16 years of age) compared to competitive adult athletes that were subjected to years of sport-specific loading. The resultant high levels of tendon stress might predispose for the development of tendinopathy (Couppé et al. 2013), which is the major overuse injury in adolescent

athletes (Le Gall et al. 2006). Therefore, it is crucial to gain more insight into the time course of muscle and tendon adaptation during adolescence to deepen our understanding of a critical phase of tissue plasticity with regard to overuse injury.

The present longitudinal study investigates the development of the morphological and mechanical properties of the quadriceps femoris vastii muscles as well as the patellar tendon of volleyball athletes in a time period of two-years from mid- (i.e. ~16 years) to late adolescence (i.e. ~18 years). With regard to the low patellar tendon CSA and the resultant high stress values identified in mid-adolescent athletes in a preceding study by Mersmann et al. (2013), we hypothesized that an increased morphological change of the tendon compared to the muscles compensates the initial deficits.

5.3 Methods

5.3.1 Experimental design

Nine female (f) and nine male (m) adolescent volleyball athletes of the extended pool of the German junior national team participated in the present study. All measurements were conducted on the jumping leg (i.e. leading leg in the spike jump) with approximately two years distance at the age of 16 ± 1 and 18 ± 1 years, respectively. The athletes, which were already trained at the beginning of the investigation period, trained on average 16 hours a week, including ~3 hours of strength training, ~4 hours of athletic training (i.e. jump and sprint drills, core stability training, etc.) and ~9 hours of ball practice. All participants took part in the magnetic resonance imaging (MRI) sessions, where we assessed muscle and tendon morphology. Muscle strength and tendon mechanical properties were measured in a separate dynamometry/ultrasound session. However, only 12 participants (6 f, 6 m) were able to attend the second session due to scheduling difficulties. The study has been approved by the university ethics committee and all participants signed informed consent to the experimental procedures.

5.3.2 Morphology of the quadriceps vastii muscles and patellar tendon

In the MRI-session, sagittal and transversal plane images were obtained between the pelvic spine and the tibial tuberosity while the participants lying supine with the knee fully extended in a 1.5 Tesla Magnetom Avanto scanner (Siemens, Erlangen, Germany). To assess the muscle volume of the vastus lateralis, intermedius and medialis, the boundaries of the muscles were tracked manually in transversal plane

images (T1 weighted, slice thickness 4.0 mm, 0.8 mm inter-slice spacing) between the respective origins and insertions using Osirix (Version 4.0, 64 bit, Pixmeo SARL, Bernex, CH). Muscle volume was calculated as the sum of the products of the respective CSAs multiplied by the sum of slice thickness and inter-slice spacing. The muscle volumes of two participants were predicted using the approach suggested by Mersmann et al. (Mersmann et al. 2015), as the muscles were not covered in full length during the recording in the second session (i.e. late adolescence). In this approach, muscle volume is calculated as the product of a muscle-specific shape factor, which was obtained from the individual muscle reconstructions of the first session and has been shown to be independent from muscle dimensions (Mersmann et al. 2015), and the maximum anatomical CSA ($ACSA_{max}$) and muscle length, which could be identified in transversal images and localizer traces of the second session, respectively.

The patellar tendon CSAs were also digitalized manually in transversal plane MRI images (3D-MEDIC, slice thickness 1.2 mm, inter-slice spacing 0 mm) between the caudal pole of the patellar and the deep insertion at the tibial tuberosity. However, the axis of the scanner did not coincide with the longitudinal axis of the tendon, which would lead to an overestimation of the CSAs in simple transversal plane segmentations. Therefore, the digitized patellar tendon CSAs were transformed orthogonal to the line of action of the patellar tendon, which in turn was defined as the line of best fit through the geometrical centers of the respective cross-sectional areas. Since not all the transversal plane recordings included the most distal part of the tendon, the CSAs are reported in 10% intervals from 0-70% of the total patellar tendon length (identified in sagittal plane images: 2D-MESE, slice thickness 3 mm, inter-slice spacing 3.51 mm), which was the longest common relative length within the participants. The patellar tendon moment arm at full knee extension was measured in a three-dimensional coordinate system as the perpendicular distance of the tendon's line of action to the rotation axis of the knee, which was determined by outlining the lateral and medial femoral epicondyles in the sagittal MRI scans and connecting the centers of the respective best fitting circles according to Churchill et al. (1998).

5.3.3 Measurement of maximum knee joint moment

The maximum strength capacity of the knee extensor muscles was assessed by dynamometry. The participants were seated on a dynamometer (Biodex Medical, Inc., Shirley, NY) with a trunk flexion of 85° (supine = 0°) and fixed with a pelvic strap around the waist. To account for the effects of gravitational forces, the misalignment of the joint axis and the axis of the dynamometer as well as the antagonistic

coactivation on the measured knee extension moments, we applied established correction techniques (Arampatzis et al. 2004; Mademli et al. 2004) on the basis of kinematic and electromyographic (EMG) recordings. For this purpose, six reflective markers were fixed to the following anatomical landmarks: anterior iliac spine, greater trochanter, lateral and medial femoral epicondyles and malleoli. Furthermore, two bipolar surface electrodes (2 cm interelectrode distance, 0.8 cm^2 pickup surface, Myon m320RX, Myon AG, Baar, CH) were fixed over the lateral head of the biceps femoris in the direction of the muscle fibres after proper skin preparation (Mademli et al. 2004). Kinematic data was recorded using a Vicon motion capture system (Version 1.7.1., Vicon Motion Systems, Oxford, UK) integrating eight cameras (6x Vicon F20, 2x Vicon T20) operating at 250 Hz. The analog data of the dynamometer as well as the EMG-signals were sampled at a rate of 1000 Hz and transmitted to the Vicon system via a 16-channel A-D converter.

The experimental protocol included a standardized warm-up with five minutes of cycling on an ergometer, ten deep squats and ten submaximal isometric knee extension contractions as preconditioning of the tendon and as accustoming for the participants. Subsequently, three trials of isometric maximum voluntary knee extension contractions (MVC) were performed in resting knee joint angles of 60° to 80° (0° = full knee extension) to measure the maximal knee joint moment (note that due to the soft tissue deformation and dynamometer compliance the knee joint angles between rest and moment maximum are different (Arampatzis et al. 2004). Two trials of submaximal knee flexion contractions were used to establish an activation-flexion moment relationship that was used to account for the knee flexion moment generated during the maximum effort knee extensions due to antagonistic coactivation (for more information on the procedure see Mademli et al. 2004).

5.3.4 Determination of the mechanical properties of the patellar tendon

The measurement of the patellar tendon elongation during isometric contractions was conducted in the same experimental setup as described earlier (in the section *measurement of maximum knee joint moment*) following the MVC assessment, using the same considerations for joint moment calculation. A 7.5 MHz ultrasound probe of 10 cm width (My Lab60, Esaote Canada, Georgetown, Canada) was fixed overlying the patellar tendon in the sagittal plane using a modified knee brace. The knee joint angle for the subsequent contractions was adjusted with respect to the angle where the highest individual joint moments were accomplished during the MVC assessment. The shank was fixed to the lever arm of the dynamometer with a Velcro strap to allow full relaxation and accurate tendon resting length assessment. Considering the reliability of patellar tendon elongation measurements (Schulze et al. 2012), five trials

of isometric contractions were performed by every participant increasing the force steadily from rest to maximum effort in about five seconds (ramp contractions). The ultrasound images were synchronized offline with the data captured in the Vicon system by a voltage peak, which was generated by pressing a trigger button and could be identified in both the ultrasound images and the analog data stream of the Vicon system.

Tendon force was calculated by dividing the knee joint moment by the tendon moment arm, which was adjusted for knee joint angle position on the basis of data from Herzog and Read (1993) to account for the differences in knee joint angle during MRI recording (0°) and dynamometry (~50°). The patellar tendon elongation was assessed by tracking the deep insertion of the tendon at the caudal pole of the patella and the tibial tuberosity using a custom written MATLAB interface (version R2011b, The Mathworks, Natick, USA). Subsequently, the mean force-elongation relationship for each participant was calculated using the highest common force value of the five ramp contractions (and the respective elongations) as peak force. The resultant function was fitted by a second-order polynomial and tendon stiffness as well as Young's modulus was calculated between 50% and 100% of the peak tendon force and stress, respectively. Maximum tendon tress was calculated using the mean patellar tendon CSA (0-70% of tendon length) and the maximum tendon force during the MVCs.

5.3.5 Statistics

The statistical analysis was performed in SPSS (IBM Corp., Version 20.0, Armonk, USA). Normal distribution of the data was tested using the Kolmogorov-Smirnov test. The changes of the morphology of the vastus intermedius (VI), lateralis (VL) and medialis (VM) muscle as well as the patellar tendon was analyzed by means of a repeated measures analysis of variances (RM ANOVA) using the within-subjects factors time (mid-, late adolescence) and muscle (VI, VL, VM) or tendon site (0-70% tendon length in 10% intervals) respectively. The relative changes of muscle and tendon morphology (calculated as: (late adolescence value – mid-adolescence value) * 100 / mid-adolescence value) were analyzed in a RM ANOVA with parameter (muscle volume, ACSAmax, mean tendon CSA) as within-subject factor. Pairwise comparisons with Bonferroni adjustment were used as *post-hoc* test where appropriate. All other parameters were compared using a paired samples t-test. The alpha level for all statistical tests was set to 0.05. In case of significant effects or differences respectively, effect sizes (d) were calculated using G*Power (Version 3.1.6, HHU Düsseldorf, Germany).

5.4 Results

From mid- (~16 years of age) to late adolescence (~18 years), there was a significant increase ($p < 0.05$) of body height (mid- 188±7 cm, late 190 ± 8 cm; $d = 1.01$) and mass (mid- 77 ± 10 kg, late 80 ± 13 kg; $d = 0.85$).

There were significant main effects of time and muscle ($p < 0.05$) on muscle volume (time $d = 0.85$; muscle $d = 2.11$) and $ACSA_{max}$ (time $d = 0.91$; muscle $d = 0.81$) with greater values in late adolescence and for VI and VL compared with VM (Table 5.1). A time by muscle interaction ($p < 0.05$; $d = 0.45$) on the $ACSA_{max}$ followed by *post-hoc* t-tests indicated a greater increase of $ACSA_{max}$ for VM compared to VI and VL (table 5.1). There was no effect of time on muscle length ($p > 0.05$), however, the effect of muscle was significant ($p < 0.05$; $d = 1.58$) with the muscle length of VI being greater than both VL and VM, and VL being greater than VM (Table 5.1). Further, muscle strength increased significantly over time ($p < 0.05$; $d = 0.80$; Table 5.1)

Table 5.1 *Mean values ± standard deviation of patellar tendon morphological and mechanical properties of volleyball athletes (n = 12) in mid- and late adolescence*

		Mid-adolescence (n = 18)	Late adolescence (n = 18)	Main effects (RM ANOVA)
Muscle volume [cm^3]	VL	742 ± 181	782 ± 188	* ‡
	VI	680 ± 160	735 ± 187	
	VM°	515 ± 107	549 ± 142	
$ACSA_{max}$ [cm^2]	VL	29.6 ± 6.5	30.8 ± 6.3	* ‡ ×
	VI	28.3 ± 5.8	29.7 ± 6.6	
	VM°§	25.7 ± 5.2	28.0 ± 6.3	
Muscle length [cm]	VL	38.7 ± 2.5	39.2 ± 3.3	‡
	VI⁺	40.9 ± 1.5	41.6 ± 2.7	
	VM°	37.1 ± 2.3	36.5 ± 2.2	
		n = 12	n = 12	t-test
MVC [Nm]		290 ± 53	326 ± 68	*

Top: Significant main effect of * time and ‡ muscle ($p < 0.05$), × significant muscle by time interaction ($p < 0.05$); *Post-hoc* comparisons: ° Significantly different to VI and VL ($p < 0.05$), ⁺ significantly different to VL ($p < 0.05$), § significantly greater effect of time compared to VL and VI ($p < 0.05$). *RM ANOVA* Repeated measures analysis of variances.
Bottom: * Significant difference between mid- and late adolescence ($p < 0.05$).

Table 5.2 *Mean values ± standard deviation of patellar tendon morphological and mechanical properties of volleyball athletes (n = 12) in mid- and late adolescence*

	Mid-adolescence	Late adolescence
Rest length [mm]	53.7 ± 3.7	52.0 ± 4.4
Moment arm [mm]	59.2 ± 4.8	60.1 ± 4.7
Stiffness [N/mm]	1154 ± 428	1445 ± 369 *
Young's modulus [GPa]	0.64 ± 0.24	0.64 ± 0.16
Maximum strain [%]	8.12 ± 2.2	7.71 ± 1.64

* Significant difference between mid- and late adolescence ($p < 0.05$).

There was a main effect of time and tendon site on the patellar tendon CSA ($p < 0.05$; time $d = 1.99$; site $d = 1.06$; Figure 5.1A), yet no time by tendon site interaction ($p > 0.5$), indicating substantial tendon hypertrophy along the whole investigated length of the tendon. Maximum tendon force ($d = 0.99$) and stiffness ($d = 0.97$) increased significantly over time ($p < 0.05$; Table 5.2; Figure 5.1B,C), while no significant changes were observed for the tendon resting length, moment arm, maximum strain or Young's modulus ($p > 0.05$; Table 5.2) and a significant decrease of maximum tendon stress ($p < 0.05$; $d = 0.91$; Figure 5.1C).

Figure 5.2 shows the relative changes from mid- to late adolescence of the total volume and $ACSA_{max}$ of the quadriceps vastii (sum of VI, VL and VM) and average tendon CSA. There was a significant main effect of parameter on the relative changes ($p < 0.05$; $d = 1.22$), demonstrating greater morphological changes of the tendon compared to both measures of muscle morphology.

To allow for an association of the morphological changes of muscle and tendon ($n = 18$) to the changes of strength and mechanical properties respectively ($n = 12$), we also statistically analyzed the morphological parameters excluding the six participants that did not attend the ultrasound/dynamometry session. The exclusion did not change the presence or absence of any main effects of time, muscle, tendon position or parameter in the statistical analysis of the morphological properties and the comparison of the relative changes. The relative increases of the quadriceps vastii volume, $ACSA_{max}$ and tendon CSA were 6.6%, 6.4% and 24.2% and, thus, similar to the results from the full data set.

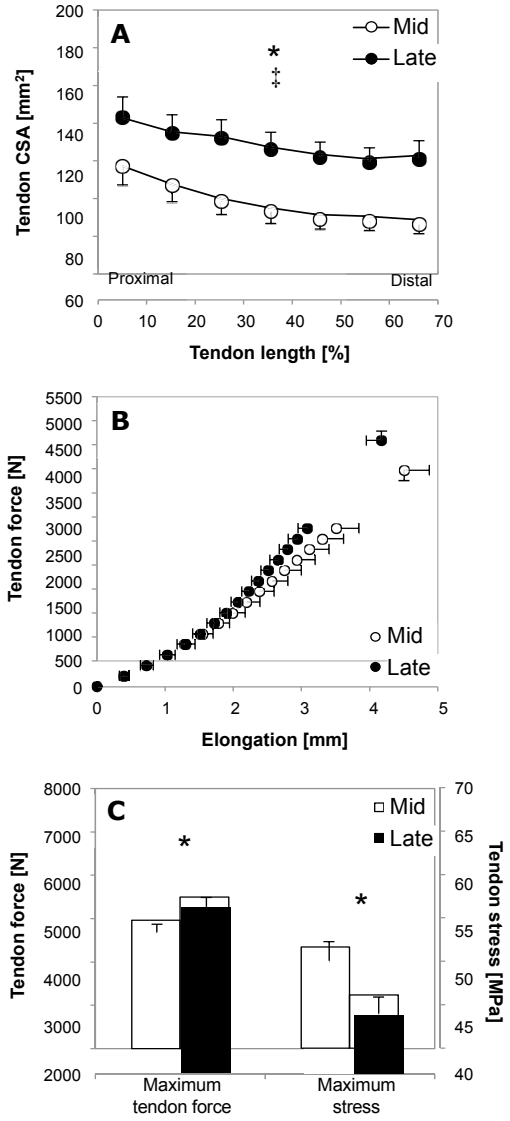

Figure 5.1 Mean values ± standard error (bars) of A) patellar tendon cross-sectional area (CSA) as a function of tendon length (in 10% intervals from proximal to distal; n = 18), B) tendon force-elongation relationship (obtained from ramp contractions, see methods section; n = 12), and C) maximum tendon force and stress (calculated for MVCs; n = 12) of volleyball athletes in mid- (white) and late adolescence (black). A: * Significant main effect of time and ‡ tendon site ($p < 0.05$); The post-hoc comparison revealed significant differences between the most proximal interval (i.e. 0-10% of tendon length) to all distal sites and the intervals 10-20% and 20-30% to the intervals from 30-70%. C: * Significant difference between mid- and late adolescence ($p < 0.05$)

5.5 Discussion

The present longitudinal study investigated the development of the morphological and mechanical properties of muscle and tendon of trained adolescent volleyball athletes from mid- to late adolescence in a time period of two-years. We hypothesized a non-uniform development of muscle and tendon, with greater morphological changes in the tendon, compensating the unfavourable relation of muscle strength to

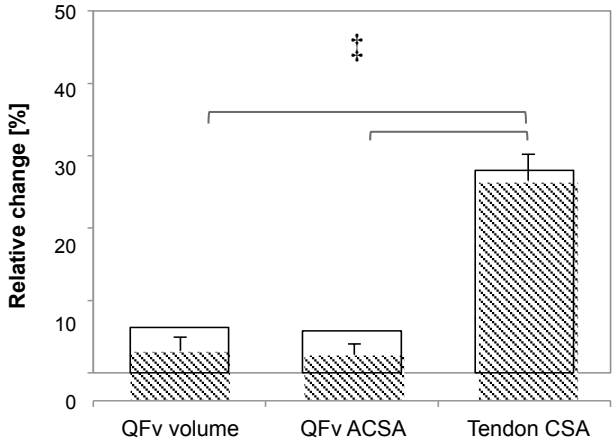

Figure 5.2 Mean values ± standard error (bars) of the relative changes of muscle volume and maximal anatomical cross-sectional area (ACSA) of the quadriceps vastii (QFv), and the patellar tendon cross-sectional area (CSA) of the from mid- to late adolescence in volleyball athletes ($n = 18$). ‡ Significant main effect of parameter ($p < 0.05$); significant post-hoc differences ($p < 0.05$) are indicated by brackets.

tendon loading capacity that was identified in an earlier study comparing mid-adolescent to middle-aged athletes (Mersmann et al. 2013). In fact, the hypertrophy of the tendon (i.e. ~27% increase in mean CSA; $d = 1.99$) was substantially greater than muscle growth (i.e. 5-7% increase of volume and $ACSA_{max}$; $d = 0.85$ and 0.91, respectively). Therefore, our hypothesis was confirmed. The marked enlargement of tendon CSA resulted in significantly greater tendon stiffness. Thus, the increases of tendon force (~11%, due to increased muscle strength) were compensated and levels of tendon strain maintained, while tendon stress was even reduced by around 10%.

A recent study that was conducted on volleyball players demonstrated that mid-adolescent elite athletes feature an adult-like morphology and contractile capacity of the quadriceps muscle, yet a relative deficit of the patellar tendon morphology, which resulted in high levels of stress during maximum voluntary contractions compared to middle-aged athletes (i.e. 50MPa vs. 40 MPa, respectively) (Mersmann et al. 2013). Considering that the ultimate stress of human patellar tendon tissue was reported to range from 65 to 78 MPa in cadaver studies (Johnson et al. 1994; Flahiff et al. 1995), the tendon stress values of ~52 MPa in the mid-adolescence athletes of the present study seem to be quite high (i.e. 67-80% of the reported ultimate stress). The association of tendon stress to patellar tendinopathy, suggested by the findings of Couppé et al. (2013), support the assumption that the high levels of tendon stress we found in athletes during mid-adolescence might have implications on the risk of tendon injury. In fact, there is epidemiological evidence that (2006) tendinopathies are the major overuse injury in athletes of that age. Though the present study design does not allow for a direct association of the found imbalanced adaptation of muscle and tendon to overuse injuries, it appears that in

young elite athletes the morphological adaptation of the tendon that reduces the tendon stress at a given tendon force unfolds at a later stage in adolescence compared to muscular adaptation (Mersmann et al. 2013). The exact role of this non-uniform development within the multifactorial etiology of tendinopathy could be an important matter to be addressed in future studies.

The increase of patellar tendon stiffness as a result of tendon hypertrophy further has the potential to facilitate athletic performance. It has been shown that rate of torque development and vertical jump performance are associated with the stiffness of the force-transmitting structures of the knee-extensor muscle-tendon unit (Bojsen-Møller et al. 2005). Moreover, it can be argued that when muscle strength and, thus, force production during a movement task (e.g. jumping) increases, an increase of stiffness of the series elastic elements is necessary to maintain fascicle kinetics within an optimal operating range (Lichtwark and Wilson 2007).

The non-uniform adaptation of muscle and tendon discussed above might be explained by differences between these two tissues regarding the temporal dynamics of adaptation and the mechanical loading parameters that effectively elicit the associated processes, as well as processes related to maturation. It is generally accepted that tendons show a low metabolic rate (Laitinen 1967) and poor blood supply compared to muscle (Smith 1965). The assumption that these differences might result in discrepancies between muscle and tendon in the time course of adaptation in response to loading is supported by two separate three-month intervention studies by Kubo et al. on the patellar (2010) and the Achilles tendon (2011). In the latter study for example, muscle functional and morphological changes were detected after one and two month, respectively. Significant changes of Achilles tendon stiffness on the other hand were found only after three month, while morphological changes of the tendon did not occur at all in this time period (Kubo et al. 2011). Moreover, there is substantial evidence that muscle and tendon tissue differ with regard to the mechanical stimuli that elicit adaptation in response to loading. Muscle strength gains can be achieved even using low loading magnitudes (Moss et al. 1997), however, to the best of our knowledge no such reports exists on human tendon adaptation. Arampatzis et al. (2007) compared two exercise interventions with equal overall loading volume using either high (i.e. 90% MVC) or moderate (i.e. 55% MVC) loading magnitudes. While muscle strength increased significantly following both protocols, only the high magnitude loading facilitated tendon adaptation. Furthermore, it is well documented that plyometric training, as a common form of loading in volleyball athletes, is effective in promoting muscle strength (Sáez-Sáez de Villarreal et al. 2010) but fails to significantly change tendon mechanical and morphological properties (Kubo et al. 2007), even at high loading magnitudes (Bohm

et al. 2014). A dissimilar responsiveness of muscle and tendon to loading has also been described on the transcriptional level of growth factors in humans (Heinemeier et al. 2011). Finally, in the development from child- to adulthood, the functional balance within the muscle-tendon unit may be additionally challenged by growth and maturation, as there are reports of episodes of tendon CSA reductions, which results in increased tendon stress (Neugebauer and Hawkins 2012). However, as the differentiation between the influences of loading- and maturation-induced changes within the muscle-tendon unit as well as the analysis of a potentially sex-specific development is not possible with the present experimental design, more research is needed to elucidate the contribution of these stimuli and to clarify the associated hormonal and mechanobiological processes.

The findings reviewed above strongly support the view that muscle and tendon in general, and during growth in particular, do not necessarily follow a coordinated adaptation. However, as the literature exclusively provides evidence of an earlier and/or more pronounced muscular adaptations, the results of the present study most likely indicate a compensatory long-term adaptation from mid- to late-adolescence following an initial imbalance within the muscle-tendon unit at mid-adolescence (Mersmann et al. 2013). The increases of muscle strength and size were quite moderate under the premise that greater changes can be expected following two years of training (Narici et al. 1996), which supports the idea of an already advanced developmental status of muscle morphology and function in trained mid-adolescents. Provided a sufficient training intensity, loading-induced muscle hypertrophy can be elicited already at prepubescent age (see Matos and Winsley 2007 for reviews) and differences of quadriceps femoris CSA between athletic and non-athletic adolescents can reach up to 28% (Kanehisa et al. 1995; 2003). However, muscle hypertrophy in response to loading is reaching a plateau eventually (Alway et al. 1992) and muscle mass changes due to maturation are reduced in late adolescence (Malina et al. 2004). Accordingly, Kanehisa et al. (Kanehisa et al. 2003) found only regional increases of CSA in the smaller distal portion of the quadriceps femoris, while the greater medial and proximal areas remained unchanged in elite mid-adolescent weightlifting athletes over 18 month of training. Therefore, the moderate increases of muscle volume (i.e. 5-7%) in the present study may indicate that the adolescent athletes approach a plateau of muscle hypertrophy in late adolescence. The tendon on the other hand seems to unfold its morphological adaptation potential at a later stage of the maturational progress. We found an increase of tendon CSA of 27%, while no changes of the material properties (i.e. Young's modulus) were observed. Long-term intervention studies typically report initial and predominant changes of material properties and, if present at all, a small to moderate tendon hypertrophy (Bohm et al.

2015 for reviews). As tendons adapt to mechanical loading by changing its material properties already at prepubescent age (Waugh et al. 2014) and mid-adolescent athletes already feature similar tendon material properties compared to adults that were subjected to years of sport-specific loading (Mersmann et al. 2013), it seems conclusive that the increase of tendon stiffness observed in the present study was attributed to the candidate long-term mechanism of tendon adaptation, being the enlargement of the tendon CSA. It is well possible that tendon hypertrophy during adolescence might be further facilitated by the formation of the tendon core that has been hypothesized to occur during adolescence (Heinemeier et al. 2013).

In conclusion, the present study provides evidence of a non-uniform adaptation of muscle and tendon in adolescent elite volleyball athletes. During late adolescence, a pronounced hypertrophy of the patellar tendon led to increased stiffness and reduced stress and, thus, to a mechanical strengthening of the tendon in relation to the functional and morphological development of the muscle. These adaptive processes may compensate the unfavourable relation of muscle strength and tendon loading capacity that was identified in mid-adolescence (Mersmann et al. 2013) and could be the result of different temporal dynamics of muscle and tendon adaptation.

5.6 Perspectives

The non-uniform adaptation between muscle and tendon in adolescent athletes and the potential implications call for the development of exercise intervention facilitating tendon adaptation already at an early stage in the athletic career. As the sport-specific plyometric loading profile is not the most effective mechanical stimulus for tendon adaptation (Kubo et al. 2007; Bohm et al. 2014), an integration of more suitable mechanical stimuli in the training programmes might be beneficial for young athletes of sports that feature a high frequency of jumps (i.e. volleyball) and prevalence of tendon overuse injuries (Lian et al. 2005). The work of Arampatzis et al. (2010) and Bohm et al. (2014) provided evidence that loading regimen featuring high contraction intensities ($\geq 85\%$ MVC) and long contraction durations (≥ 3 s;) effectively augmented the morphological and material properties of tendons. Accordingly, heavy slow resistance training (i.e. high intensity, long duration of contractions) has been shown to improve tendon fibril morphology and the clinical outcome of patellar tendinopathy (Kongsgaard et al. 2010).

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6 Fourth study | Athletic training affects the uniformity of muscle and tendon adaptation during adolescence

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6.1 Abstract

With the double stimulus of mechanical loading and maturation acting on the muscle-tendon unit, adolescent athletes might be at increased risk to develop imbalances of muscle strength and tendon mechanical properties. This longitudinal study aims to provide detailed information on how athletic training affects the time-course of muscle-tendon adaptation during adolescence. In twelve adolescent elite athletes and eight similar-aged controls knee extensor muscle strength and patellar tendon mechanical properties were measured over one year in three-month intervals. A linear mixed effects model was used to analyze time-depended changes and the residuals of the model to quantify fluctuations over time. The cosine similarity (CS) served as a measure of uniformity of the relative changes of tendon force and stiffness. Muscle strength and tendon stiffness increased significantly in both groups ($p < 0.01$). However, the fluctuations of muscle strength were greater (A: 17±7 Nm, C: 6±2 Nm, $p < 0.05$) and the uniformity of changes of tendon force and stiffness was lower in athletes (CS A: -0.02±0.5, C:0.5±0.4, $p < 0.05$). Further, athletes demonstrated greater maximum tendon strain (A: 7.6±1.7%, C: 5.5±0.9%, $p < 0.05$) and strain fluctuations (A: 0.9±0.4, C: 0.3±0.1, $p < 0.05$). We conclude that athletic training in adolescence affects the uniformity of muscle and tendon adaptation, which increases the demand on the tendon with potential implications for tendon injury.

Key words: knee, tendinopathy, plasticity, adolescence, biomechanics, time-course

6.2 Introduction

Tendons have the crucial role to transmit the forces generated by the muscles to the skeleton. However, as the ultimate strain of tendons cannot be significantly altered (26), tendons need to adapt in their mechanical properties when the force generating potential of muscles increases (e.g., due to physical training), to maintain physiological levels of strain during maximum muscle contractions (24). Therefore, it is frequently observed in healthy adults that both muscle strength and tendon stiffness increase in response to chronic mechanical loading (and 7 for reviews, see 41). Yet, there seems to be only a poor association of strength gains and changes of tendons structural and mechanical properties (38) and the time course of loading-induced adaptation of muscle and tendon can differ as well (22, 23), potentially due to the lower responsiveness to loading on the transcriptional level of growth factors (15) or differences between muscle and tendon in the mechanical stimuli that effectively elicit adaptational processes (1, 3, 8, 25). In conclusion, during a training process imbalances might develop between the adaptation of muscle and tendon.

In adolescent athletes, the effects of mechanical loading interact with the stimulus of maturation, which also triggers changes in both muscles and tendons (34, 35). This twofold stimulus might expose young athletes to an increased risk to develop imbalances within the muscle-tendon unit. Recently, we provided evidence of an imbalance in the adaptation of muscle and tendon in junior volleyball athletes (31, 33), which resulted in high levels of tendon stress and tendon strain (31). While stress is a measure of external load (normalized to tendon cross-sectional area) and the ultimate stress of a tendon can be increased or decreased via a modulation of the tendon material properties, ultimate tendon strain is more or less constant (26). Therefore, tendon strain is an adequate indicator of the internal mechanical demand placed upon the tendon (i.e. how the integrity of the tissue is challenged) during muscle contractions and has been associated previously to tendon overuse (5, 10).

Tendinopathy has not only an exceptionally high prevalence in Volleyball athletes (29), but is also the most frequent overuse injury in adolescent elite athletes (28). This supports the assumption that an imbalance in the adaptation of muscle and tendon in adolescent athletes might be of clinical relevance and demonstrates the necessity to deepen our understanding of the development of muscle and tendon during adolescence and the effect of mechanical loading. To date, however, the time-course of muscle-tendon unit adaptation during adolescence and the effect of athletic training are largely unknown. Consequently, the present longitudinal study investigates for the first time the development of muscle and tendon properties in three-month intervals during one year in adolescent athletes and non-athletes *in vivo*.

Due to the seasonal variations in mechanical loading in athletes, we hypothesized that there will be greater fluctuations in the development of muscle and tendon functional, morphological and mechanical properties and indications of an increased mechanical demand for the tendon due to a lower uniformity of muscle and tendon adaptation (i.e., imbalance) in adolescent athletes compared to similar-aged controls.

6.3 Methods

6.3.1 Participants and experimental design

As an *a priori* power analysis to determine a target sample size was not possible due to a lack of appropriate longitudinal data on the development of muscle and tendon in adolescence, we tried to recruit as many participants as possible. Twenty-six adolescent volleyball athletes of the extended pool of the German junior national team and a group of 13 similar-aged habitually active controls agreed to participate in the present longitudinal study and measurements were scheduled every three months for one year. Nineteen participants dropped out of the study due to the time-consuming procedures and, thus, twelve adolescent athletes (5 female, 7 male; $\geq$ 16 h of sport-specific training per week; age: 16 $\pm$ 1 years) and eight controls (5 female, 3 male; $\leq$ 4h recreational training per week; age: 16 $\pm$ 1 years) completed the investigation period. All measurements were conducted on the dominant leg (i.e. leading leg in the spike jump or leg used for kicking a ball, respectively). The study has been approved by the university ethics committee and all participants (and respective legal guardians) signed informed consent to the experimental procedures.

6.3.2 Measurement of maximum knee joint moment

Knee extensor muscle strength was measured combining dynamometry, kinematic and electromyographic (EMG) recordings. Effects of gravitational forces and the misalignment of rotation axes of knee joint and dynamometer were accounted for on the basis of the inverse dynamics approach suggested by Arampatzis et al. (2). For this purpose, six reflective markers were fixed to the following anatomical landmarks: anterior superior iliac spine, greater trochanter, lateral and medial femoral epicondyles and malleoli. Kinematic data was recorded using a Vicon motion capture system (Version 1.7.1., Vicon Motion Systems, Oxford, UK) integrating eight cameras operating at 250 Hz. For estimating the contribution of the antagonistic muscles to the resultant joint moment (6, for details on the procedure see 30), the

electromyographic (EMG) activity of the lateral head of the biceps femoris was recorded (Myon m320RX, Myon AG, Baar, CH).

Following a standardized warm-up, the participants performed three trials of isometric maximum voluntary knee extension contractions on a dynamometer (Biodex Medical, Inc., Shirley, NY) at 85° trunk flexion (supine = 0°) and resting knee joint angles of 65° to 75° (0° = full extension) in 5° intervals to measure the maximum knee extension moment (MVC). Additionally, a passive knee extension trial (driven by the dynamometer at 5 °/s) and two trials of knee flexion contractions were recorded to account for moments of gravity (2) and to establish an activation-flexion moment relationship that was used to estimate the knee flexion moment generated during maximum effort knee extension due to antagonistic coactivation (for more information on the procedure see 25).

6.3.3 Measurement of tendon mechanical properties

For the assessment of the patellar tendon mechanical properties, the participants completed five trials of isometric ramp contractions (i.e., steadily increasing effort from rest to maximum effort in about five seconds) in the knee joint angle where the highest individual joint moments were accomplished during the MVC assessment. The tendon elongation during the contractions was captured at 25 Hz by a 10 cm ultrasound probe (7.5 MHz; My Lab60, Esaote Canada, Georgetown, Canada) overlying the patellar tendon in the sagittal plane fixed by a modified knee brace. The knee extension moments were calculated using the same considerations as for the MVC calculation (i.e. correction for axes misalignments, gravitational forces and antagonistic coactivation) and the ultrasound images were synchronized offline with the data captured in the Vicon system by an externally induced voltage peak, which could be identified in both the ultrasound images and the analog data stream.

Tendon force was calculated by dividing the knee extension moment (measured as in MVC assessment) by the tendon moment arm. In ten athletes the tendon moment arm at the first measurement session could be assessed by magnetic resonance imaging (MRI) using the procedure described earlier (31). For all other participants the moment arm (MA) was predicted using an equation derived from a regression analysis of 77 MRI-based data sets from earlier studies with stepwise inclusion of anthropometric data. The final model included mass in kg (m), sex (s; coded as 0 for male and 1 for female), and height in centimeters (h) as prediction variables, while age and knee width were excluded. The regression equation was

$$\mathrm{MA} = 25.88 + 0.078 \cdot \mathrm{m} - 2.242 \cdot \mathrm{s} + 0.128 \cdot \mathrm{h} \quad (1)$$

with an R^2 of 0.55 ($p < 0.001$) and a standard error of 2.25 mm. The tendon moment arm for the subsequent sessions was scaled considering the relative changes predicted by the regression equation. For each session the moment arm was adjusted to the knee joint angle position on the basis of the regression equation suggested by Herzog and Read (19).

The patellar tendon elongation was determined by a manual frame-by-frame tracing of its deep insertion at the patella apex and tibial tuberosity using a custom written MATLAB interface (version R2015a, The Mathworks, Natick, USA). The force-elongation relationship of the five trials of each participant was averaged to achieve an excellent reliability (37), using the highest common force value as peak force. The resultant function was fitted by a second-order polynomial and tendon stiffness was calculated between 50% and 100% of the peak tendon force.

6.3.4 Architecture of vastus lateralis muscle

Vastus lateralis (VL) architecture was assessed at a knee joint angle of 60° with the ultrasound probe positioned over the muscle belly at ~60% of thigh length (32). The upper and deeper aponeuroses were marked in custom written MATLAB by setting reference points along the aponeuroses that were approximated by a linear least-squares fitting. Subsequently, we digitized the visible features of multiple fascicles and calculated pennation angle and muscle thickness with respect to the average inclination of the fascicle portions and the distance of the aponeuroses, respectively (31).

6.3.5 Statistics

Normality and homoscedasticity of the data was tested in SPSS (IBM Corp., Version 20.0, Armonk, USA) using the Kolmogorov-Smirnov and Levene's test, respectively, using an adjusted (more conservative) significance level of $\alpha = 0.2$ considering the small sample sizes. Normality was present in all target parameters, homoscedasticity in most, but not all parameters.

A linear mixed-effects model (LMM) was formulated and processed in MATLAB to analyze the time- and group-dependent development. Further, we were able to use the residuals to the model as a measure of fluctuations of muscle and tendon properties, as, in contrast to general linear models, the inclusion of random effects accounts for individual differences in the development over time. The model equation was

$$y_{ii} = \beta_0 + \beta_1 g_i + \beta_2 t_{ij} + \beta_3 t_{ij} g_i + b_{i0} + b_{i1} t_{ij} + \varepsilon_{ij} \quad (2)$$

i: index for participant (1,...,20)

j: index for session (0,...,4)

g_i: athletes = 0; control = 1

t_ij: measurement session (0, ..., 4)

β_0: y-intercept constant for athletes

β_1: y-intercept constant for difference between controls and athletes

β_2: slope constant for athletes

β_3: slope constant for difference between controls and athletes

bi_0: subject-specific y-intercept (random effect)

bi_1: subject-specific slope (random effect)

ε_{ij}: residual

Hence, in athletes the terms including *g* equal zero and, thus, β_0 and β_2 represent the constants for intercept and slope, while in controls $\beta_0 + \beta_1$ and $\beta_2 + \beta_3$ represent these constants. This allows testing the significance of changes over time (slope constant β_2), baseline differences between groups (intercept constant β_1) and group-differences with respect to time-dependent changes (slope constant β_3). When the latter was significant, changes over time were tested for both groups separately. The LMM was applied to all target parameters, as those models have been shown to be robust even in presence of heteroscedasticity (40). Considering the unbalanced design with regard to sex, differences between groups were tested for selected parameters (i.e., MVC normalized to body mass, pennation angle, and maximum strain). The absolute residuals to the LMM fit ($|\varepsilon_{ij}|$, averaged over the five measurement sessions for the individual as a measure of fluctuations) of the contextually most relevant parameters (i.e., MVC, tendon stiffness and strain, and VL thickness and pennation angle) were compared between groups using Welch's *t*-test.

The association of muscle and tendon properties was modeled in the LMM as well (by exchanging the measurement session variable by the respective prediction variable), predicting muscle strength by muscle thickness or pennation angle and tendon stiffness by tendon force. The absolute residuals to the model fit of tendon stiffness predicted by tendon force were compared between groups to analyze respective prediction uncertainties.

The uniformity of the development of tendon force and stiffness was calculated as cosine similarity (CS) of the relative changes between the 5 measurement sessions in a 4-dimensional vector space:

$$\mathrm{CS} = \cos(\vartheta) = \frac{\mathrm{A}\cdot\mathrm{B}}{\|\mathrm{A}\cdot\mathrm{B}\|} = \frac{\sum_{i=1}^{4} \mathrm{A_i}\,\mathrm{B_i}}{\sqrt{\sum_{i=1}^{4} \mathrm{A_i^2}}\sqrt{\sum_{i=1}^{4} \mathrm{B_i^2}}} \tag{3}$$

A and B represent the vectors of the relative inter-session changes (i = 1,...,4). CS can take values from 1 (indicating equal orientation of the vectors, i.e., high similarity) to -1 (opposing orientation, i.e., low similarity).

The significance level for the LMM hypothesis testing and the Welch's t-test for the residuals and the CS was set to $\alpha = 0.05$.

6.4 Results

Anthropometric data are shown in Table 6.1. There was a marginal but statistically significant increase of body height and mass (~0.6%, $p = 0.001$; ~2.8%, $p = 0.003$), without group-difference of change over time ($p = 0.76$ and 0.33, respectively).

6.4.1 Muscle strength and tendon properties

Both absolute and normalized muscle strength increased significantly over time ($p < 0.001$ and $p = 0.003$, respectively; Figure 6.1, Table 6.2). The increase was independent of group, as there was no difference between groups with regard to the change over time ($p = 0.54$ and 0.58, respectively). There were greater fluctuations of muscle strength over time in athletes compared to controls (i.e., greater residuals, $p < 0.001$; Figure 6.2) and normalized muscle strength was significantly greater in athletes ($p = 0.016$; Table 6.2). Tendon stiffness increased significantly ($p = 0.003$; Figure 6.1), again independent of group ($p = 0.2$), however, without differences in the fluctuations between groups ($p = 0.33$, Figure 6.2). Maximum tendon strain did not

Table 6.1 *One-year development of anthropometric data of adolescent volleyball athletes and controls measured in three-month intervals*

		Session				
		Baseline	3 Month	6 Month	9 Month	12 Month
Body height [cm], #	Athletes	187.4 ± 6.8	187.8 ± 7.2	188.0 ± 7.3	188.2 ± 7.1	188.5 ± 7.6
	Controls	169.4 ± 8.5	169.8 ± 8.6	170.3 ± 9.0	170.4 ± 9.1	170.4 ± 9.1
Body mass [kg], #	Athletes	73.0 ± 9.5	73.3 ± 9.8	73.9 ± 10.4	74.3 ± 10.3	74.8 ± 10.6
	Controls	56.8 ± 8.8	57.4 ± 9.5	58.0 ± 9.8	59.1 ± 9.2	59.4 ± 9.6

Values are means ± standard deviations. # significant change over time (i.e. slope; $p < 0.05$). There were no group-differences of change over time (i.e. slope; $p > 0.05$). Note that differences between groups (i.e. intercept) were not tested.

change significantly over time ($p = 0.56$; Figure 6.1) in both groups ($p = 0.88$). However, maximum strain was greater in athletes ($p < 0.001$) and demonstrated greater fluctuations ($p = 0.001$, Figure 6.2). A *post hoc* stepwise regression analysis identified the fluctuations of MVC as significant predictor of the fluctuations of strain ($p = 0.006$, $R^2 = 0.346$), while the fluctuations of tendon stiffness did not contribute significantly ($p = 0.42$). Independent of group ($p = 0.63$), tendon force increased significantly over time ($p = 0.014$; Table 6.2) and was a significant predictor of tendon stiffness in the LMM ($R^2 = 0.82$; $p = 0.036$). The prediction uncertainties, however, were significantly greater in athletes ($p = 0.035$; Figure 6.3) and there was less uniformity (i.e., significantly lower CS) in the development of tendon force and stiffness compared to controls ($p = 0.045$; Figure 6.3).

Further, significant time-dependent increases were found for the tendon moment arm ($p < 0.001$; Table 6.2), irrespective of group ($p = 0.72$). No significant changes over time or differences in the time-dependent changes were observed in tendon rest length ($p = 0.45$ and 0.26, respectively; Table 6.2).

Table 6.2 *One-year development of normalized knee extensor muscle strength (MVC), vastus lateralis architecture and patellar tendon properties of adolescent volleyball athletes and controls measured in three-month intervals*

		Session				
		Baseline	3 Month	6 Month	9 Month	12 Month
Normalized MVC [Nm/kg], * #	Athletes	4.15 ± 0.53	4.19 ± 0.57	4.21 ± 0.53	4.31 ± 0.55	4.50 ± 0.73
	Controls	3.36 ± 0.81	3.33 ± 0.81	3.58 ± 0.84	3.59 ± 0.92	3.52 ± 0.88
Muscle thickness [mm], ‡ #[A]	Athletes	21.9 ± 4.1	24.6 ± 3.8	23.9 ± 4.7	26.1 ± 4.0	27.1 ± 3.9
	Controls	18.2 ± 1.6	19.5 ± 1.8	20.5 ± 2.0	19.7 ± 1.6	18.9 ± 2.2
Pennation angle [°], *	Athletes	10.1 ± 1.9	10.3 ± 2.1	9.9 ± 2.0	10.2 ± 1.9	10.9 ± 1.9
	Controls	7.6 ± 0.7	8.2 ± 0.4	9.2 ± 0.7	9.1 ± 0.9	9.1 ± 0.7
Tendon moment arm [mm], #	Athletes	55.2 ± 3.7	55.3 ± 3.7	55.4 ± 3.8	55.4 ± 3.8	55.5 ± 3.9
	Controls	50.6 ± 2.5	50.7 ± 2.5	50.8 ± 2.6	50.9 ± 2.6	50.9 ± 2.7
Rest length [mm]	Athletes	52.7 ± 4.3	52.2 ± 5.3	52.8 ± 4.3	52.6 ± 4.5	53.1 ± 6.1
	Controls	49.4 ± 4.3	50.6 ± 5.2	49.5 ± 4.4	48.9 ± 4.2	49.4 ± 4.2
Tendon force [N], #	Athletes	5117 ± 981	5120 ± 944	4971 ± 1184	5229 ± 1182	5690 ± 1292
	Controls	3351 ± 566	3672 ± 822	3739 ± 434	3669 ± 506	3788 ± 371

Values are means ± standard deviations. MVC was normalized to body mass. * significant difference between groups (i.e. intercept; $p < 0.05$), # significant change over time (i.e. slope; $p < 0.05$) and ‡ significant group-difference in change over time (i.e. slope; $p < 0.05$). [A] indicates a significant change over time was present only in athletes. Note that differences between groups were tested only for normalized MVC and pennation angle.

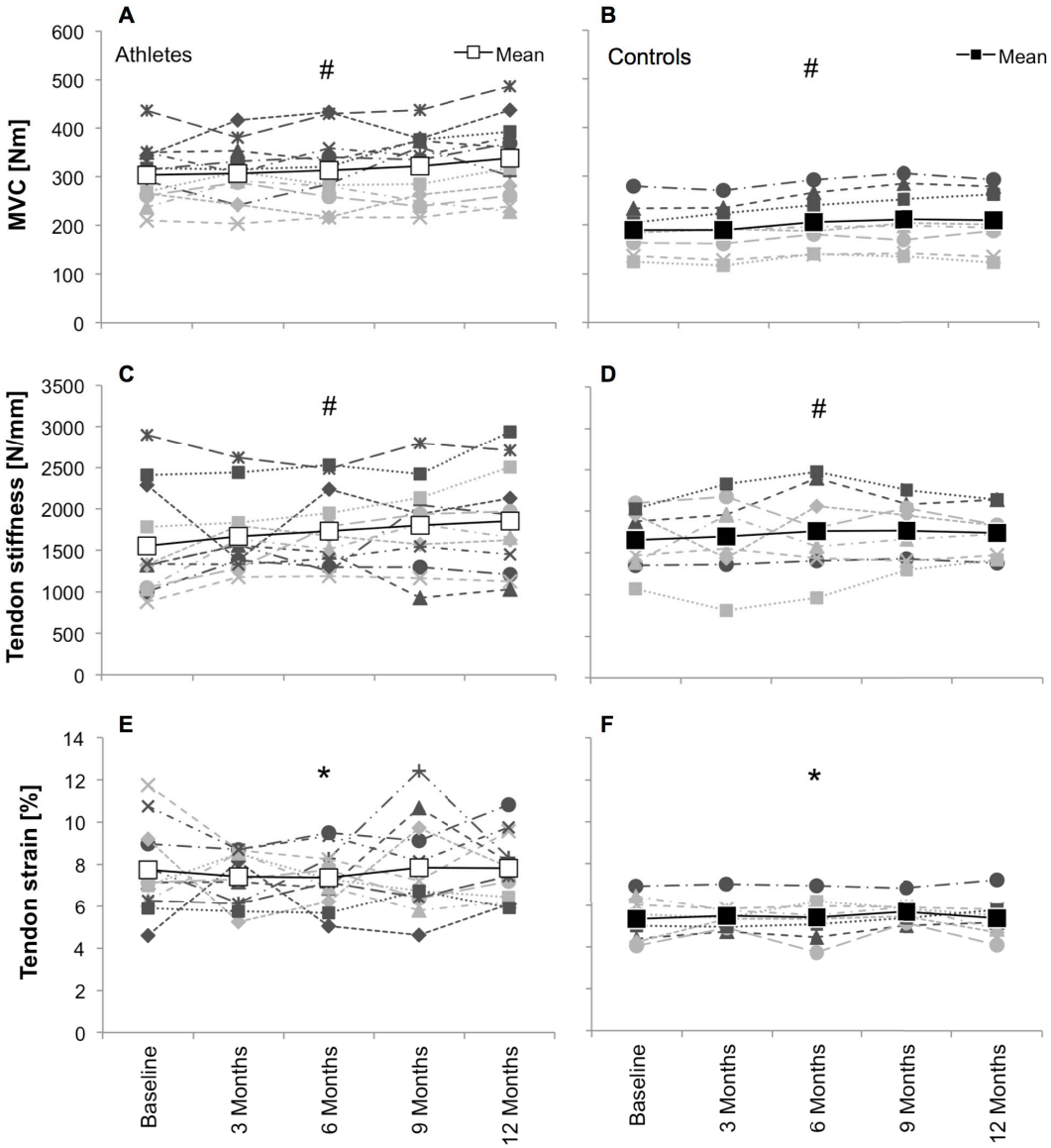

Figure 6.1 On-year development (in three-month intervals) of knee extensor muscle strength (MVC; A, B), patellar tendon stiffness (C, D) and maximum tendon strain (E, F) in adolescent volleyball athletes (n = 12; A, C, E; white symbols show mean values) and controls (n = 8; B, D F; black symbols show mean values), including individual data of female (light grey) and male participants (dark grey) in both groups. * significant difference between groups (i.e. intercept; $p < 0.05$); # significant change over time (i.e. slope; $p < 0.05$). Note that differences between groups were not tested for MVC and stiffness.

The mean values of the female sub-groups (n = 5 each) of the crucial fluctuation and uniformity measures (e.g., MVC residuals of 15 Nm and 5 Nm or CS of -0.04 and 0.38 for female athletes and controls, respectively) were representative of the reported total group means and, thus, we are confident that our findings were not biased by the unbalanced sample composition with regard to sex.

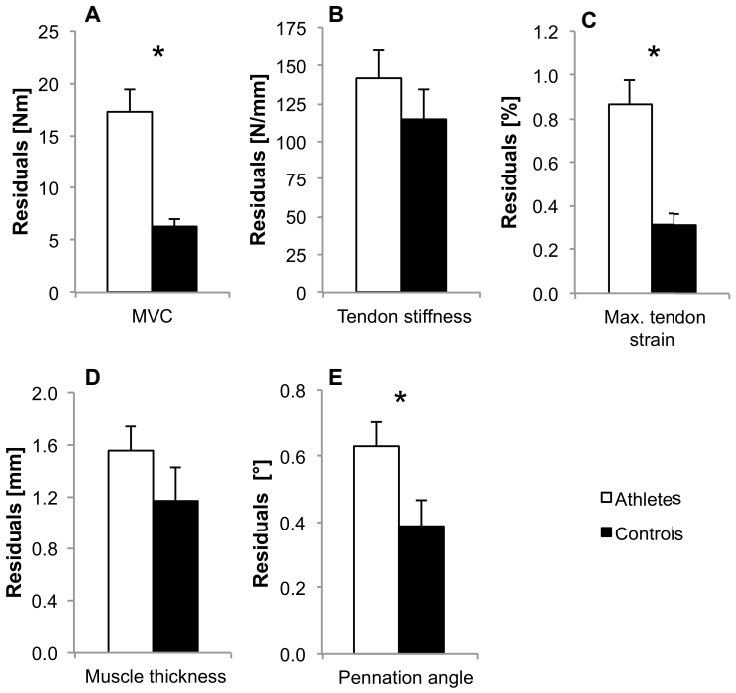

Figure 6.2 Measures of fluctuations (mean ± standard error of averaged absolute residuals to linear mixed model fit) of the development of muscle strength (MVC; A), patellar tendon stiffness (B), maximum tendon strain (C) and vastus lateralis thickness (D) and pennation angle (E) in adolescent volleyball athletes (white bars) and controls (black bars). * significant difference between groups ($p < 0.05$).

6.4.2 Vastus lateralis architecture

There was a significant increase in VL thickness in athletes ($p < 0.001$; Table 6.2), but not controls ($p = 0.59$). No significant group-differences were found in the fluctuations of VL thickness ($p = 0.22$). Pennation angle was greater in athletes compared to controls ($p = 0.002$) and did not show a systematic change over time ($p = 0.12$). The fluctuations of pennation angle were significantly greater in athletes as well ($p = 0.036$). Both VL thickness and pennation angle were significant predictors of muscle strength in the LMM ($R^2 = 0.94$, $p = 0.017$ and $R^2 = 0.96$, $p < 0.001$, respectively).

6.5 Discussion

The present study investigated *in vivo* the development of knee extensor muscle and tendon properties in three-month intervals during one year in adolescent elite volleyball athletes and non-athletes. As main results we found a) increased fluctuations of muscle strength (and tendon force) and b) less uniformity in the

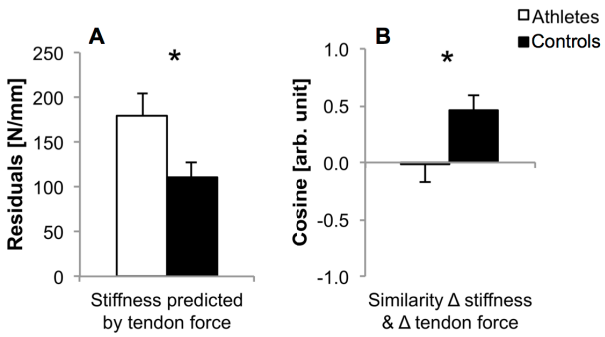

Figure 6.3 Prediction uncertainties of tendon stiffness by tendon force and similarity between tendon force and stiffness in adolescent volleyball athletes (white bars) and controls (black bars). The left chart (A) illustrates the absolute residuals (means ± standard error) to the linear mixed model fit of stiffness predicted by tendon force and the right chart (B) shows the cosine similarity (means ± standard error) of the relative changes (Δ) of tendon force and stiffness (see also methods section). * significant difference between groups ($p < 0.05$).

changes of tendon force and stiffness over time in athletes compared to non-athletes. In consequence, maximum tendon strain demonstrated considerable fluctuations over time with increased average levels and episodically markedly elevated individual levels of strain compared to controls. Therefore, our hypotheses were confirmed.

The current investigation demonstrated for the first time that athletic training might disrupt the uniformity of muscle and tendon adaptation during adolescence. We found evidence that the marked fluctuations of muscle strength in athletes were not accompanied by a matched adaptive response of the tendon, as indicated by the lower association and uniformity measures for tendon force and stiffness. In consequence, the tendon strain during maximum contractions demonstrated considerable fluctuations as well. In contrast to controls, the development over time in athletes was characterized by episodes of very high individual levels of strain in the range of 10-12.5%. With regard to the generally accepted strain-dependency of tendon failure (26), this implies an increased mechanical demand on the tendon and, thus, a greater challenge for the integrity of the tissue. Average maximum tendon strain over time was significantly greater in athletes compared to controls as well (~7.6% vs. 5.5%, respectively). The high prevalence of tendon overuse in adolescent athletes (28) and volleyball players (29) and the consistent reports of a mechanical weakening of the tendon concomitant with tendinopathy (5, 11, 18) give reason to believe that an imbalance in the adaptation of muscle and tendon might increase the risk of tendon injury.

Our regression analysis suggests, that the fluctuations of strain were due to the fluctuations of muscle strength rather than tendon stiffness. Considering the lower metabolic rate (27) and vascularization (39) of tendon compared to muscle, it seems possible that muscle adapts at a higher rate to altered mechanical loading. Moreover,

it is known that changes in muscle activation can lead to rapid gains of muscle strength (14), while changes of tendon properties require morphological and/or structural changes. In the present study, both VL thickness and pennation angle were associated with muscle strength, yet only pennation angle demonstrated significantly greater fluctuations over time in athletes compared to controls. Changes of pennation angle are thought to be indicative of a modulation of the physiological cross-sectional area of a muscle (20) and, thus, its strength capacity. Therefore, the architectural remodeling of the muscle might be associated with the observed fluctuations of strength in the adolescent athletes, which in turn might have been induced by variations in training volume and content, as it has been described previously in adult athletes (see 21 for reviews).

As maturation alone seems to be associated with a uniform adaptation of muscle and tendon (reflected in our data by the greater cosine similarity and lower prediction uncertainties of tendon stiffness by tendon force) and, thus, quite constant levels of maximum strain, the observed imbalanced adaptation in adolescent athletes can be explained by the different temporal dynamics of muscle and tendon in the adaptation to altered mechanical loading and the dissimilar responsiveness to specific mechanical stimuli. Discrepancies between muscle and tendon in the time course of adaptation in response to loading were reported by Kubo et al. for the patellar (23) and the Achilles tendon (22), respectively. The studies provided evidence that muscle functional and morphological changes can precede significant changes of tendon mechanical or morphological properties. Moreover, there is convincing evidence that the mechanical stimuli that effectively elicit tissue adaptation differ between muscle and tendon as well, as both high and moderate loading magnitudes can facilitate muscle strength, but only high magnitude loading facilitates tendon adaptation (1). It has also been observed on the transcriptional level of growth factors in rats (16, 17) and humans (15) that muscle and tendon tissue show differentially graded responses to specific loading regimen and it has been hypothesized by the authors that this might induce an imbalanced adaptation of muscle and tendon following certain training modes (16). Interestingly, plyometric loading, which is the predominant type of loading in volleyball and, thus, for the athletic group of the present study, has been demonstrated to be an effective stimulus for muscle but not tendon adaptation (25). It has been hypothesized that short strain durations (as in plyometric jumps) could be less effective to trigger the transmission of the external strain to the tendon cells via the viscoelastic extracellular matrix and thus induce a lower biological response compared to other loading regimen featuring greater strain durations (7). Therefore, preventive intervention strategies would need to incorporate mechanical stimuli that more effectively increase the tendon loading capacity (i.e., increasing

Young's modulus and/or cross-sectional area). A series of systematic controlled experimental studies (1, 3, 8) provided evidence that such an intervention should include repetitive loading using high intensity muscle contractions (≥ 85% MVC; irrespective of contraction type) and long contraction durations (~3 s).

Though the present study investigated a cohort at high risk for the development of tendinopathy and the mechanical strain theory is held as the most probable explanation of the underlying injury mechanism (4, 13), the association of the observed non-uniform muscle and tendon adaptation and the resultant high levels of strain with injury risk or tendinopathy remains an assumption and needs further investigation. Moreover, it was not possible to obtain MRI for all participants and measurement sessions, which is strongly suggested for the assessment of tendon cross-sectional area (12) and, thus, the time-course of the development tendon morphology, total and regional tendon stress, and the material properties of the tendon during adolescence with and without the influence of mechanical loading is still largely unknown. Additionally, the analysis of regional strain distribution might provide further insight into the complex loading pattern of tendons during contractions, though it should be acknowledged that increased total tendon strain, as found in the present study, is associated with increased regional strains as well (9, 36).

In conclusion, the present study provides evidence that athletic (volleyball) training during adolescence is associated with increased fluctuations of the knee extensor strength capacity. The fluctuations of strength were not compensated accordingly by changes of the patellar tendon mechanical properties and result in an increased demand on the tendon (i.e., maximum strain) in athletes compared to controls. Future studies might address the potential of training interventions facilitating tendon adaptation to restore the balance of muscle and tendon development in adolescent athletes.

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6.8 Disclosures

There are no conflicts of interest, financial or otherwise, to declare.

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7 Main findings and conclusions

The present thesis provides an insight into the development of the morphological, mechanical and functional properties of muscle and tendon during adolescence and the effects of superimposed mechanical loading. Furthermore, a volume prediction method for the vastii muscles (vastus lateralis, medialis and intermedius) has been established in the first study. Separated by topic, the following chapter provides an overview about the main findings and conclusions. Subsequently, practical implications and limitations of the work are discussed. The final section for the respective matter at hand outlines open and new questions for future lines of research.

7.1 Non-uniform adaptation of muscle and tendon in adolescent athletes

This thesis demonstrated for the first time that superimposed mechanical loading during adolescence can result in a non-uniform adaptation of muscle and tendon. In normally developing recreationally active adolescents, tendon strain during maximum muscle contractions (i.e. a marker for the mechanical demand placed upon the tendon by the working muscle) was quite consistent throughout one year of mid-adolescence (fourth study). This indicates that the increases of tendon force were paralleled by an adequate development of patellar tendon stiffness. However, a significantly lower association and uniformity in the development of tendon force and stiffness compared to the control group was evidenced in adolescent volleyball athletes. In addition, their muscle strength demonstrated increased fluctuations over time, probably mediated to a certain extent via a modulation of muscle pennation angle. This resulted in episodes of very high individual tendon strains during maximum effort muscle contractions (i.e. 10-12.5%). Moreover, average levels of tendon strain over the investigation period were elevated in comparison to controls as well. This indicates that the adaptation of the knee extensor muscles to the superimposed mechanical loading by means of athletic training might be more advanced when compared to tendon adaptation, an assumption that is supported by the results of the second and third study.

The comparison of mid-adolescent athletes with adult counterparts that were subjected to many years of sport-specific loading (i.e. middle-aged former elite athletes; second study) as well as the longitudinal screening of muscle and tendon adaptation from mid- to late adolescence (third study) both suggest that in well-

trained youth athletes the development of the morphological and functional properties of the muscle (under the influence of both maturational and mechanical stimuli) is already far progressed in mid-adolescence. First, there were no significant differences in muscle strength and PCSA between mid-adolescent volleyball athletes and their adult counterparts and, second, there were only moderate changes of muscle morphology and function from mid- to late adolescence despite two years of continuous training. This is in accordance with the results of the fourth study, which demonstrated greater normalised strength (normalised to body mass) and vastus lateralis pennation angle in mid-adolescent athletes compared to similar-aged controls (i.e. non-athletes) at baseline, but no difference between these groups regarding the moderate strength gains over time and no further increments of pennation angle were evident in response to continued athletic training. The patellar tendon, on the other hand, seems to unfold its morphological adaptation potential at a later stage of adolescence. While the material properties of the patellar tendon were not different between mid-adolescent and the adult athletes and no further changes were observed in the longitudinal study, a significant tendon hypertrophy was evidenced from mid- to late adolescence. As it is known that increased mechanical loading can modulate the material properties of tendons already at prepubescent age (Waugh et al., 2014), it seems possible that further increases of tendons stiffness need to be mediated predominantly by radial tendon growth. However, in mid-adolescent athletes, there might be a transient deficit of radial tendon adaptation that, considering the attained level of muscle strength, results in increased tendon stress and strain (second study).

The results collectively suggest that superimposed mechanical loading (in terms of athletic volleyball training) perturbs the development of muscle and tendon during adolescence. The increased mechanical loading early in adolescence seems to stimulate the morphological and functional development of muscle to a greater extent than the morphological and mechanical properties of tendons, which results in increased stress and strain. Moreover, increased fluctuations of muscle strength in volleyball athletes, probably due to seasonal variations in loading (Koutedakis, 1995; Ogasawara et al., 2013), and a marked non-uniformity in the adaptation of muscle and tendon induces episodes of individual peak strains more than twice as high as in controls. Potential implications of the observed imbalances within the muscle-tendon unit are discussed in the subsequent section.

7.1.1 Practical implications

The development of the morphological, mechanical and functional properties of muscle and tendon during adolescence and the non-uniform adaptation to superimposed mechanical loading evidenced in volleyball athletes might have some

major implications for movement performance and risk of injury. The increase of muscle strength observed during adolescence in controls and athletes (fourth study) is likely to be associated with improvements of general sports skills such as sprinting, jumping or cutting (Suchomel et al., 2016 for a recent review). Moreover, the morphological development of the knee extensors from mid- to late adolescence that was observed in athletes (third study) could be linked to an increase of maximum mechanical power output (O'Brien et al., 2009a). The increase of patellar tendon stiffness in both controls and volleyball athletes (third and fourth study) probably facilitates the rate of force development (Bojsen-Møller et al., 2005; Waugh et al., 2013) and might allow the muscle fascicles to still operate within an optimal range despite increased force production during a movement task (Lichtwark and Wilson, 2007). However, more importantly, the non-uniform development of muscle strength and tendon loading-capacity in adolescent volleyball athletes might predispose them for tendon overuse injury.

This thesis provides evidence that the patellar tendon of adolescent volleyball athletes is subjected to a chronically and episodically increased mechanical demand when the muscle is working at a given relative contraction intensity (second and fourth study). There is conclusive evidence that a higher magnitude of tendon strain during cyclic (or static) loading increases the risk of accumulative microtrauma and tendon failure (Butler et al., 1978; Wren et al., 2003; Lavagnino et al., 2006; Legerlotz et al., 2014). Thus, it seems reasonable to assume that in adolescent volleyball athletes the integrity of the tendon tissue is challenged by the non-uniform adaptation of muscle and tendon. It was suggested by Bahr and Bahr (2014) that within a population of adolescent elite volleyball athletes the frequency of jumps could be an important risk factor for tendinopathy. Since both the initial strain and the number of loading cycles determine tendon fatigue (Wren et al., 2003; Fung et al., 2009; Legerlotz et al., 2013), it seems possible that the chronically and episodically increased tendon strain during muscle activity (fourth study) in combination with the high frequency of jumps (or other maximum effort muscle contractions) might predispose the patellar tendon of adolescent volleyball athletes to sub-rupture fatigue damage and tendinopathy. Although studies on the mechanical properties of tendons in tendinopathy patients *in vivo* generally support the notion that tendon overuse injury is related to a mechanical weakening of the tendon (Arya and Kulig, 2010; Child et al., 2010; Helland et al., 2013; Couppé et al., 2013a), it must be clearly stated that association of the observed non-uniform adaptation of muscle and tendon and the resultant high levels of strain with injury risk or tendinopathy remains an assumption.

Though the exact causes for the observed non-uniform development of muscle and tendon in adolescent volleyball athletes still need to be elucidated, there is reason to believe that the sport-related loading profile might play a role, due to the dissimilar responsiveness of muscle and tendon to plyometric loading (Kubo et al., 2007; Sáez-Sáez de Villarreal et al., 2010; Bohm et al., 2014). It is quite likely that a similar behaviour of muscle-tendon development can be observed in adolescent athletes of other sports whose specific loading profile does not provide an effective stimulus for tendon adaptation as well (e.g. basketball or athletic jump disciplines). Therefore, it might be beneficial with regard to both sports performance and risk of injury to promote the tendon mechanical properties in adolescent athletes in these sports by means of training regimen that were optimised for eliciting tendon adaptation. A series of well-controlled experimental intervention studies have demonstrated the superior effects of high compared to low strain magnitudes, low compared to high strain rates and frequencies, and moderate (i.e. ~3 s) compared to short (≤ 1 s) or long strain durations (~12 s) with regard to the facilitation of tendon mechanical properties (Arampatzis et al., 2007a; 2010; Bohm et al., 2014). On the basis of this loading profile, several dynamic and isometric exercises could be designed and implemented into the athletic training in those types of sports in which a non-uniform development of muscle and tendon is indicated.

7.1.2 Limitations

The dissociation of biological and chronological age is an issue commonly discussed in paediatric science. In this thesis, biological age was not controlled in the respective studies, which might have biased the comparisons between adolescent groups of similar chronological age (second and fourth study). However, with regard to the chronological age of the adolescent participants (i.e. ≥ 15 years) and common chronological ages at peak height velocity (11 – 12 years for girls and 13 – 14 years for boys; Malina et al., 2004), around which the greatest inter-individual variations of biological age can be expected, it seems unlikely that maturational differences between groups confounded the results and conclusions of this work. The marginal and similar increases of body height in the adolescent athletes and controls (i.e. ~1 cm * year^{-1}) supports this assumption.

The two longitudinal studies conducted within the framework of this thesis (third and fourth study) did not investigate the effect of sex on the development of muscle and tendon properties. However, the similar values of male and female adolescent athletes in the measures of fluctuation of muscle strength and similarity of muscle-tendon adaptation suggest that the non-uniform development of muscle and tendon is likely an issue in both sexes. Nevertheless, this assumption needs

confirmation by studies that specifically address this issue using appropriate sample sizes for a sound sub-group analysis.

The *in vivo* assessment of muscle and tendon properties is based on several simplified assumptions. Tendon force was calculated from joint moments that were measured solely in the sagittal plane. Thus, moments generated by the quadriceps in the frontal and transverse plane and the respective components of the tendon force needed to be neglected. Yet, the resultant underestimation of tendon force is likely to be small. Furthermore, the tendon moment arm of the adolescent athletes was measured at rest and full knee extension, while the assessment of the mechanical properties of the tendon was performed at flexed knee positions using maximum voluntary isometric contractions. Though differences in knee joint angle were accounted for using the data of Herzog and Read (1993), no adjustment was performed with regard to the change of moment arm due to muscle contraction, which might well introduce an overestimation in the calculation of tendon force of ~10% (approximated from data for 20° and 90° knee joint angle of Tsaopoulos et al., 2007a). However, to date no data is available on the contraction-induced change of patellar tendon moment arm for the knee joint angles used in the studies of this thesis and the waiver of adjustment can be considered a systematic error. Yet, as the major contraction-induced changes of patellar tendon moment arm occur between rest and 50% of MVC, the error introduced in the calculation of tendon stiffness is low. As different assessment methods for the tendon moment arm were used for adolescent athletes and controls, no between-group comparisons were made for dependent parameters in the fourth study. Moreover, since it was not possible to obtain MRIs for all participants at all measurement sessions and the reliability of tendon CSA assessment by means of ultrasound was reported to be low (Ekizos et al., 2013), there is a lack of information on the underlying mechanisms accounting for the increase of tendon stiffness in both groups, though the results of the third study suggest that an increase of tendon CSA likely contributed to the facilitation of stiffness, at least in athletes. In the second and third study, where the tendon CSA could be determined based on MRI sequences obtained at rest, tendon stress was calculated without considering the transverse deformation of the tendon during force application. However, it is currently not possible to account for individual Poisson's ratios during *in vivo* tendon stress assessment and there is no reason to believe that a group-specific influence might have biased the results. Lastly, it is acknowledged that a measurement of total tendon strain using single plane ultrasound can hardly reflect the complex three-dimensional load-response distribution of tendons during loading (Lersch et al., 2012; Khodabakhshi et al., 2013). Though local strain variations could not be investigated using the present methodological approach, there is convincing

evidence that increased total tendon strain is associated with increased local strains, specifically in regions susceptible to tendon injury (Butler et al., 1990; Pearson et al., 2014). Therefore, it can be assumed that the differences and fluctuations of total strain evidenced in this work well represent the differences and fluctuations of the mechanical demand placed upon the tendon by the working muscle.

Similarly to tendon elongation measurements, the assessment of muscle architecture and PCSA using planimetric modelling based on ultrasound imaging simplifies the complex geometry of the muscle. However, to the best of my knowledge, no non-invasive techniques for the assessment of three-dimensional muscle architecture and PCSA have been established to date. Moreover, there is now convincing evidence that the assessment of muscle architecture (i.e. fascicle length, pennation angle and muscle thickness) using ultrasound as well as the calculation of muscle PCSA from muscle volume and fascicle length provide valid estimates (Powell et al., 1984; Kwah et al., 2013; Ando et al., 2014; Giles et al., 2015) and the variations of muscle architecture over the length of the muscle (Blazevich et al., 2006; O'Brien et al., 2010c) were accounted for by standardising the ultrasound probe position with respect to femur length.

7.1.3 New questions and future lines of research

The work presented in this thesis has demonstrated for the first that time superimposed mechanical loading by means of athletic volleyball training during adolescence can induce a non-uniform adaptation of muscle and tendon lead to an increased mechanical demand for the tendon. However, more research is clearly needed to gain more insight into the prevalence, the causes and consequences of the imbalanced muscle-tendon development. For instance, no information is available on the time course of muscle and tendon adaptation in younger age groups. In this regard, early adolescence might be an intriguing phase to be investigated due to the accelerated somatic development, though it will be challenging to control inter-individual differences in biological maturity. Research targeting the association of changes of endogenous hormone levels with muscle and tendon development and adaptation to superimposed mechanical loading in different age groups would improve our understanding of the interaction of these stimuli and the influence of sex. Moreover, it is yet unclear if the observed development is specific to volleyball athletes or the associated loading profile. Further studies could investigate cohorts from different sports or examine the effects of experimental modulation of distinct mechanical loading parameters on muscle and tendon adaptation during development. In the fourth study, it was not possible to schedule the individual measurement sessions of the athletes at similar time points during the competitive season. It still

needs to be elucidated in detail how seasonal variations of training volume or content contribute to the observed imbalanced adaptation of muscle and tendon. Similarly, more research is needed to clarify the consequences of the increased mechanical demand placed upon the tendon. It might be promising to investigate if a non-uniform adaptation of muscle and tendon could be associated with future injury or common ultrasound-based indications of tendon abnormalities that were related to tendinopathy (e.g. hypoechoic regions, neovascularisation or local swelling; Gisslèn et al., 2005; Gisslén et al., 2007; Hoksrud et al., 2008; Visnes et al., 2015). Establishing a clear relationship between total tendon strain *in vivo* and tendon overuse will be a further methodological challenge, as an accurate assessment of tendon mechanical properties incorporates maximum voluntary contractions and pain reduces the level of muscle activation (Hart et al., 2010; Palmieri-Smith et al., 2013). This particularly concerns the magnitude of strain measured during the assessment and might explain the inconsistent findings with regard to tendinopathy and maximum strain (Arya and Kulig, 2010; Child et al., 2010; Helland et al., 2013; Couppé et al., 2013a). Additionally, regional and three-dimensional strain field analyses might provide more detailed information on how a non-uniform adaptation of muscle and tendon might affect the internal load-response in tendinous tissue.

7.2 Muscle volume prediction

The present thesis has established a method for the prediction of thigh muscle volume based on the approach proposed by Albracht and colleagues (2008), using only the maximum ACSAs, muscle lengths and muscle-specific shape factors as input parameters. For this purpose, MRI images of the quadriceps femoris vastii of volleyball athletes, mostly those obtained for the second study, were segmented over the full length of the muscle to calculate the muscle volume, length, maximum ACSA and the respective shape factors for subsequent volume prediction. There were no significant differences between the muscle volumes of the quadriceps vastii obtained from whole-muscle segmentations and the predicted values. The RMS differences between the volume measurement (i.e. whole-muscle segmentation) and the prediction were in the order of 5-6%. As the longitudinal changes of muscle volume in adolescent athletes (third study) were very low (5-7%), the prediction method was used exclusively on two datasets, in which whole-muscle segmentation was not possible. However, the precision of the prediction method is high enough to detect changes of the knee extensor muscle volume that can be expected following resistance training in untrained young or elderly adults (Tracy et al., 1999; Aagaard et al.,

2001), and, under the premise of muscle shape consistency, the changes of muscle volume in response to unloading or immobilization (Alkner and Tesch, 2004; Suetta et al., 2009) as well as due to ageing (Suetta et al., 2009) and specific pathologies (Ji et al., 2013). Moreover, the precision of the prediction method using muscle-specific shape factors for the quadriceps vastii is higher than the precision reported for ultrasound-based approaches (Miyatani et al., 2004; Giles et al., 2015) or the regression-model incorporating a single MRI scan of the maximum ACSA proposed by Morse and colleagues (2007). Thus, the proposed prediction method using only the muscle length, maximum ACSA and the respective shape factor is a promising alternative to whole-muscle segmentation.

7.2.1 Practical implications

The method for predicting the muscle volume of the quadriceps vastii established in the first study has the potential for application in many fields of research as muscle volume changes occur in response to loading or unloading (Adams et al., 2003; Folland and Williams, 2007; Oates et al., 2010), maturation and ageing (Morse et al., 2005; O'Brien et al., 2010c), or pathology (Hiba et al., 2011; Ji et al., 2013). With regard to the relative consistency of the position of the maximum ACSA relative to femur length, the shape factor-based prediction method could greatly reduce the length of acquired MRI sequences. For instance, a scan of 11%, 20% and 26% of the thigh length would be sufficient to capture the maximum ACSA of the muscle of interest in 95% of the cases for the vastus medialis, vastus lateralis and vastus intermedius, respectively. Moreover, given the unimodal distribution of CSA of the muscle over its lengths that was found in all quadriceps vastii, the maximum ACSA can be easily identified around the reported positions with only a few segmentations, which is a time-saving of around 85% compared to a full muscle segmentation as performed in the first study of this thesis.

7.2.2 Limitations

The development of the method for the prediction of thigh muscle volume was conducted using data from volleyball athletes. Thus, the generalizability of the results of the first study to other (e.g. less athletic) populations remains an assumption. However, the validity of the prediction method for the triceps surae muscles was not affected by muscle hypertrophy (Mersmann et al., 2014) and no differences in muscle-specific shape factors were found between male and female participants of the first study, despite marked differences in muscle dimensions. Further, recent work validated the prediction method for the vastus lateralis in a cadaveric study

incorporating ultrasound for the assessment of muscle length and maximum ACSA (Infantolino and Challis, 2016). Finally, a sub-group cross-validation and application of the prediction method on longitudinal data (using the data of the first and third study) indicates that (a) the shape factors obtained in one group of athletes can be applied to an independent group of adolescent athletes and (b) moderate muscle hypertrophy does not induce a change in the muscle shape factors. The corresponding data was recently presented at the 21st Congress of the European Society of Biomechanics in Prague (Mersmann et al., 2015).

7.2.3 New questions and future lines of research

The method for muscle volume prediction initially proposed by Albracht and colleagues (2008) has been demonstrated to be applicable to the triceps surae of differentially trained adults (Mersmann et al., 2014) and there are strong indications that the method is applicable for adult thigh muscles of very different dimensions as well (Infantolino and Challis, 2016). Although changes in muscle dimension due to physiological hypertrophy does not seem to significantly the shape factors of the thigh muscles, it should be noted that this might not hold true, for example, for pathological hypertrophy or muscle atrophy (Miokovic et al., 2012). Future studies might systematically explore the underlying assumption of muscle shape consistency in children or the very elderly, in specific diseases or following unloading, as well as in other skeletal muscles. The sub-group cross-validation and application of the prediction method on longitudinal data (using the data of the first and third study) further indicated that the precision of the proposed method might be higher when, in contrast to the absolute muscle volume, intra-individual changes of muscle volume are predicted, since over- or underestimation of muscle volume is likely to be in a similar direction for an individual during a baseline and follow-up assessment (RMS differences between prediction and whole-muscle segmentation were 3-4%; Mersmann et al., 2015). However, this first indication might need further confirmation. Finally, recent advances in extended field-of-view ultrasound imaging for the assessment of muscle ACSA *in vivo* (Noorkoiv et al., 2010; Lixandrão et al., 2014) suggest that it might be possible to refrain from MRI imaging for the application of the proposed prediction method. This assumption was recently supported by the cadaver-study of Infantolino and Challis (2016), yet a validation *in vivo* using an appropriate sample size remains an outstanding issue.

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Eidesstattliche Erklärung | Statutory declaration

Ich erkläre, dass ich die vorliegende Dissertation selbständig und nur unter Verwendung der angegebenen Hilfsmittel angefertigt habe. Alle Zitate sowie sinngemäße wörtliche Wiedergaben, die anderen Werken entnommen wurden, sind unter Angabe der Quelle kenntlich gemacht. Die Abbildungen, Diagramme und Tabellen sind von mir erstellt, sofern diese nicht als Entlehnung gekennzeichnet sind. Weder diese noch eine andere Arbeit wurde von mir an einer anderen Universität oder Hochschule zum Zwecke der Einleitung eines Promotionsverfahrens vorgelegt.

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