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Authors: Amy Rebecca Sibley
Siobhan Strike
Sarah Catherine Moudy
Neale Anthony Tillin

Author Conflict: No competing interests declared

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The Effects of Long-Term Muscle Disuse on Neuromuscular Function in Unilateral Transtibial Amputees

Amy R. Sibley,[1,2] Siobhán Strike,[1] Sarah C. Moudy,[1,3] Neale A. Tillin[1]

[1]Department of Life Sciences, University of Roehampton, London, UK

[2]Department of Allied Health Sciences, London South Bank University, London, UK

[3]Department of Family Medicine, University of North Texas Health Science Center, Fort Worth, Texas, USA

Correspondence Address:

Amy R. Sibley

Department of Allied Health Sciences

103 Borough Road

London, UK SE1 0AA

Email: amy.sibley@lsbu.ac.uk

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Abstract

The purpose of this study was to determine: (1) whether individuals with unilateral transtibial amputations (ITTAs), who habitually disuse the quadriceps muscles of their amputated limb, provide an effective model for assessing the effects of long-term muscle disuse; and (2) the effects of such disuse on quadriceps muscle strength and neuromuscular function in this population. Nine ITTAs and nine controls performed isometric voluntary knee extensions in both limbs to assess maximal voluntary torque (MVT) and rate of torque development (RTD). The interpolated twitch technique and EMG normalised to maximal M-wave assessed neural activation, involuntary (twitch and octet) contractions assessed intrinsic contractile properties, and ultrasound images of the vastus lateralis assessed muscle architecture. Clinical gait analysis was used to measure knee kinetic data during walking at an habitual speed. ITTAs displayed 54-60% lower peak knee extensor moments during walking in the amputated than intact/control limbs, but the intact and control limbs were comparable for loading during walking and muscle strength variables, suggesting the intact limb provides a suitable internal control for comparison to the disused amputated limb. MVT and RTD were ~60% and ~75% lower, respectively, in the amputated than intact/control limbs. The differences in MVT appeared associated with ~40% and ~43% lower muscle thickness and neural activation, respectively, whilst the differences in RTD appeared associated with the decline in MVT coupled with slowing of the intrinsic contractile properties. These results indicate considerable changes in strength and neuromuscular function with long-term disuse, that could not be predicted from short-term disuse studies.

Introduction

Prolonged disuse of skeletal muscle poses a considerable threat to neuromuscular functional capacity and health (Narici & de Boer 2011). Just nine days of disuse causes considerable reductions in muscle strength, typically measured as maximum voluntary torque (MVT; Rozier et al. 1979) or rate of torque development during contractions performed from rest (RTD; Bamman et al. 1998). The knee extensor (quadriceps) muscles are particularly susceptible to degenerative changes resulting from disuse owing to their large contributions to locomotion, and so are frequently investigated in typical study models of disuse including spaceflight, unilateral lower-limb suspension (ULLS), limb immobilisation, bed rest, and immobilisation during intensive care following surgery (Narici & de Boer 2011). Studies show reductions in quadriceps MVT of approximately 2% per day for the first ten days (Berg & Tesch 1996, Puthucheary et al. 2017, Rozier et al. 1979), slowing to ~1% per week for up to 30 days, with an eventual plateau resulting in average strength losses of around 23% after 120 days of disuse (Dirks et al. 2016, Horstman et al. 2012, Narici & de Boer 2011). The effects of disuse on RTD have not been widely studied, yet RTD may be a more functionally relevant than MVT during rapid human movements such as recovering from a trip or loss of balance (Pijnappels et al. 2008, Behan, Pain & Folland 2018). Long-term muscle disuse is a default position for many clinical populations (Brown et al. 2004) and the sedentary, yet it is unclear how both MVT and RTD change with long-term, habitual disuse, as typical disuse study models last <90-120 days for logistical and ethical reasons.

Individuals with unilateral transtibial amputations (ITTAs, below-knee amputation on one limb) may provide a useful model for studying the effects of long-term, habitual disuse. ITTAs adopt an asymmetrical loading pattern characterised by considerably lower vertical ground reaction forces (vGRF) and knee extensor moments on the amputated compared to the intact limb, during movements such as walking (Fey & Neptune 2012), jumping (Schoeman, Diss & Strike 2012), and stair ascent/descent (Schmalz, Blumentritt & Marx 2007). This suggests the quadriceps of the amputated limb in ITTAs are chronically disused, which would explain observations of considerably lower (~50%) quadriceps MVT (Isakov et al. 1996, Lloyd et al. 2010, Pedrinelli et al. 2002) and size (Moirenfeld et al. 2000) in the amputated, compared to the intact and control limbs. Comparison of quadriceps neuromuscular function in the amputated vs. intact limb of ITTAs, coupled with comparison of limb loading during gait as an estimation of typical use, may therefore offer new insight into the long-term effects of habitual disuse. However, currently it is unclear whether the intact limb provides an internal control that is unaffected by the amputation and comparable to the limb of an able-bodied control, which would support the efficacy of ITTAs as a study model of long-term disuse. Despite similar peak vGRF and knee moments during walking gait (Lloyd et al. 2010, Nolan et al. 2003, Sanderson & Martin 1997), previous studies in ITTAs have shown lower MVT in the intact limb compared to able-bodied participant limbs (Isakov et al. 1996, Lloyd et al. 2010, Pedrinelli et al. 2002, Powers et al. 1996). However, the latter studies did not control for other factors known to independently affect muscle strength between the groups such as ageing, health, and sedentary lifestyle (Narici & de Boer 2011, Sacchetti et al. 2013).

Six studies (Isakov et al. 1996, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Powers et al. 1996, Renstrom et al. 1983) have previously measured quadriceps MVT in ITTAs, and none have assessed the changes in RTD in this population. Furthermore, the neuromuscular mechanisms of the considerable strength loss in the amputated limb of ITTAs have not been investigated. Neural activation, assessed via electromyography (EMG) amplitude or the interpolated twitch technique, is considered an important determinant of both MVT and RTD (Balshaw et al. 2016, Folland, Buckthorpe & Hannah 2014, Tillin, Pain & Folland 2011). However, evidence for changes in neural drive with short-term (≤89 days) disuse are equivocal with some studies reporting a decrease (Narici & de Boer 2011, Lambertz et al. 2001) and others no change (de Boer et al. 2007, Campbell et al. 2013). RTD also appears to be determined by the intrinsic contractile-speed properties of the muscle, such as RTD relative to peak torque recorded during electrically evoked-involuntary contractions (e.g. twitch or octets; Folland et al., 2014), and short-term disuse causes a shift towards faster contractile properties (Lambertz et al. 2001). Finally, the maximum force generating potential of a muscle is dependent upon its architecture (Blazevich et al. 2009), and just 21-30 days of disuse have elicited changes such as declines in muscle size (≤10%), pennation angle (≤13%), and fascicle length (≤9%; de Boer et al. 2007, Campbell et al. 2013). Determining the degree of change in these neuromuscular determinants of muscle strength with long-term habitual disuse may allow better targeting of preventative and rehabilitative interventions for populations subject to muscle disuse.

The first aim of this study was to assess the efficacy of unilateral ITTAs as a model to study long-term habitual disuse, by comparing knee-extensor strength (MVT and RTD) and loading (knee extensor moments and impulse) during walking gait of the intact limb with a control able-bodied population, where both groups are healthy, young, and active. The second aim was to assess MVT and RTD, and the neuromuscular determinants of these (neural drive; intrinsic contractile properties; and vastus lateralis muscle architecture) in the disused quadriceps muscles of ITTAs, in comparison to both the intact and an able-bodied control limb.

Methods

Ethical Approval

Participants provided written informed consent prior to their involvement in the study, which complied with the standards set by the 2013 Declaration of Helsinki (except for registration in a database) and was approved by the University of Roehampton Ethics Committee (LSC 16/176) and the NHS Health Research Authority (17/NW/0566).

Participants

Nine male ITTAs and nine male controls took part in this study. Prior to data analysis, groups were matched to ensure similar group means and variability in age, height, body mass, and physical activity. Physical activity was assessed using the International Physical Activity Questionnaire (Short Format, http://ipaq.ki.se/downloads.htm). ITTAs were included if they had a unilateral transtibial amputation performed >6 months prior to involvement in the study, to ensure established ambulation and long-term disuse in the residual limb. ITTAs were excluded if they experienced any discomfort in the residual limb whilst using their prosthesis, and/ or if their amputation occurred due to congenital disorders, or complications arising from metabolic or vascular conditions (e.g. diabetes). Exclusion criteria for both groups included cardiovascular disease risk factors or neuro-musculoskeletal injuries (other than a transtibial amputation in the ITTAs).

Overview

Participants visited the laboratory on three separate occasions, with each visit 3-7 days apart, to complete a familiarisation (visit 1; identical to visit 2), neuromuscular function assessment of the quadriceps muscles of both limbs (visit 2), and a gait assessment (visit 3). Limb order for neuromuscular assessment was randomised. All three sessions commenced at a consistent time (±2 hours) of day for each participant, following at least 36 hours without strenuous exercise, and 24 hours without alcohol.

Experimental Setup and Measurements

Knee Extension Torque

Isometric strength data were collected using an isokinetic dynamometer (Humac Norm, Computer Sports Medicine Inc., Massachusetts, USA). Participants were seated with a hip angle of 100° (full extension = 180°) and with adjustable straps across the pelvis and shoulders tightened to ensure no extraneous movement. The knee joint angle was set so that the angle during active extension was 110°. Some basic modifications were made to minimise knee joint angle changes during isometric contractions, including the use of a dense foam padding on the seat and limb attachment, and a custom-made lower limb attachment which could be tightly clamped to the crank arm to remove unnecessary rotation around the crank arm. In all participants, the limb attachment was placed as distal on the tibia as anatomy and participant comfort permitted. For the amputated limb, the crank arm was flipped by 180° to account for the shorter residual tibia.

The analogue torque signal was sampled at 2000 Hz using an external A/D converter (16-bit signal recording resolution; Micro 1401, CED, Cambridge, UK) and interfaced with a PC using Spike 2 software (version 8; CED). Off-line, torque was filtered using a fourth-order low pass Butterworth filter with a cut-off frequency of 10 Hz and corrected for the passive weight of the limb.

Electromyography (EMG)

Electromyography signals were recorded from the superficial knee extensors (rectus femoris [RF], vastus medialis [VM], vastus lateralis [VL]) using a Noraxon TeleMyo Desktop DTS System (Noraxon, Arizona, USA). The skin was prepared by shaving, abrading and cleansing with 70% alcohol. Bipolar Ag/Ag/Cl surface electrodes (2 cm inter-electrode distance, Noraxon) were attached over the belly of each muscle at SENIAM recommended recording sites (Stegeman & Hermans, 2007), parallel to the presumed orientation of the muscle fibres. The raw EMG signals were wirelessly transmitted (Wireless Research EMG Probes, Part 542, Noraxon) to a receiver (Desktop DTS, Part 586, Noraxon), amplified x500, sampled at 2000 Hz in synch with torque via the same A/D converter and PC software, and band-pass filtered off-line between 6 and 500 Hz using a fourth-order zero-lag Butterworth filter.

Muscle Architecture

A static ultrasound image (Hitachi Noblus, Hitachi Medical Systems, UK) of the VL was taken using a linear array probe with a 94 mm scan width (HI VISION L53L, Hitachi Medical Systems, UK).The image was taken prior to any other measurements whilst the participant was seated in the dynamometer at rest, and with a joint angle of 100° (where 180° is full knee extension). The probe was placed perpendicular to the skin surface, over the thickest part of the belly of the VL, at 50% of the line between the greater trochanter and the knee joint centre, and aligned so that the muscle fascicles of the VL and their insertion into the deep aponeurosis were clearly visible.

Muscle thickness, pennation angle and fascicle length (Figure 1) were determined from the still images offline using Tracker software (an open source Video Analysis Tool, available from http://physlets.org/tracker/). Muscle thickness was defined as the mean distance between the deep and superficial aponeuroses at three points: at the middle and either end of the image. Pennation angle was defined as the mean of the angle between three separate muscle fascicles and their insertion on the deep aponeurosis. Fascicle length was extrapolated from the pennation angle and muscle thickness using trigonometry (de Brito Fontana, Roesler & Herzog 2014, Franchi et al. 2014, Tillin, Pain & Folland 2012), as the entire length of the fascicle was not visible in the image. Between-session reliability of muscle architecture measures was assessed in a pilot study of eight able-bodied controls using the same methods as described above. Coefficient of Variation (CV) was 4.4%, 10.9% and 9.3% for muscle thickness, fascicle length, and pennation angle, respectively.

Electrical Stimulation

Square wave (0.2 ms duration) electrical impulses were delivered percutaneously to the femoral nerve, via a constant current variable voltage stimulator (Model DS7AH, Digitimer, Ltd, Welwyn Garden City, UK), to evoke supramaximal twitch, doublet and octet contractions of the knee extensors. The cathode stimulation probe (1 cm diameter, protruding 2 cm from a plastic base, Electro Medical Supplies, Wantage UK) was firmly pressed into the femoral triangle in the position that evoked the greatest twitch response for a submaximal (30–60 mA) electrical current. The anode (10 x 7 cm carbon rubber electrode) was taped in place over the greater trochanter. Single impulses were delivered with step-wise increments in the current, separated by 15 s, until a plateau in the amplitude of twitch torque and compound muscle action potentials (M-waves) were reached. The stimulus intensity was then increased by 20% to ensure supramaximal stimulation, and three supramaximal twitch contractions, separated by 20 s, were delivered. The current was reduced prior to commencing the octet contractions (eight pulses at 300 Hz), and stepwise increments in the current were delivered 15 s apart until the supramaximal current used for twitch contractions was attained. Subsequently, three supramaximal octet contractions were evoked.

The mean M-wave peak-to-peak amplitude of the three supramaximal twitch contractions was defined as the maximal M-wave (M_{max}) for each muscle. Torque measurements from the evoked contractions were twitch and octet peak torque (PT) and peak RTD (calculated using a 15 ms moving time window) presented as absolute and relative to PT. These variables were averaged across the three supra-maximal twitch and octet contractions recorded.

Knee Extension MVCs

Participants performed a series of ~20 warm-up contractions of 3-s duration at progressively higher intensities before completing six maximum voluntary contractions (MVCs). Each MVC lasted 3-5 s and was followed by 30-60 s rest. Participants were instructed to push ‘as hard as possible’ and strong verbal encouragement was given throughout the contractions. Real-time biofeedback of torque output was provided on a computer monitor in front of participants. MVT was defined as the greatest instantaneous peak voluntary torque (not due to superimposed stimulation) recorded during any of the MVCs or explosive contractions.

Explosive Voluntary Contractions

Participants completed 10-15 explosive isometric contractions, each separated by 20 s rest, utilising the method described by Folland, Buckthorpe & Hannah (2014). Three explosive voluntary contractions were chosen for analysis and all dependent variables assessed were averaged across these three explosive contractions. The three contractions were chosen as those with the highest peak RTD, peak torque >80% MVT, and no visible countermovement or pre-tension (quantified as change of baseline torque <0.5 Nm during the 100 ms prior to visible torque onset) were used for analysis. Peak RTD was extracted and expressed as both an absolute and relative to MVT.

To assess neural drive during the explosive contractions, the RMS amplitude of the EMG signal for each quadriceps muscle was calculated for the time period 0-100 ms from EMG onset ($EMG_{0\text{-}100}$), and normalised to M_{max} at the same muscle before averaging across the three quadriceps muscles. EMG onsets, defined as the onset of the first muscle to be activated, were identified with a standardised systematic protocol of visual identification (Tillin et al. 2010).

Voluntary Activation

The 2nd, 4th, and 6th MVCs had a single doublet superimposed at the plateau of the torque-time curve, and two further doublets evoked at rest immediately after the MVC. The difference between superimposed and resting potentiated doublet torque was used to VA (a measure of neural drive at MVT), using the equation:

$$VA(\%) = 100 \times (1 - D_s/D_c) \quad (1)$$

where D_s and D_c are the superimposed and control doublets, respectively.

The root mean square (RMS) of the EMG signal for each quadriceps muscle was calculated over the 500 ms window centred on or nearest to MVT, which was not influenced by stimulation artefact (EMG_{MVT}). EMG_{MVT} was normalised to M_{max} of the same muscle and averaged across the three quadriceps muscles.

Walking Gait

Kinematic data were collected using twelve Vicon Vantage V5 (Vicon Motion Systems Ltd.; Oxford, UK) motion capture cameras sampling at 200 Hz synched with three in-series Kistler force plates (Type 9281c; Kistler Instruments Ltd., Hampshire, UK) in the middle of a 15 m walkway sampling force data at 1000 Hz. Two sets of Brower TC timing gates (Brower Timing, Utah, USA) placed 2 m either side of the force plates were used to capture average walking pace. Retroreflective markers (14 mm diameter) were placed on the skin according to the Plug-In-Gait lower-body marker set. Markers for the shank, ankle and foot were placed in positions on the prosthetic corresponding as closely as possible to those on the intact limb.

Data collection involved participants walking along the 15 m walkway at a self-selected, habitual pace. Average walking pace was determined in preliminary trials by allowing participants to walk up and down the walkway until speed stabilised. Three 'good' trials, defined as a single pass with a successful force plate strike, walking speed within ±5% of average, and no gaps in marker data greater than 40 frames, were selected for analysis for each limb. Data were processed in Vicon Nexus 2.7.1. Raw marker trajectories and analogue force data were filtered using a low-pass zero-lag fourth-order Butterworth filter, at cut-off frequencies of 8 and 200 Hz respectively. Standard inverse dynamics techniques were used to calculate net internal joint moments, normalised by body mass (Winter and Sienko 1988).

Internal peak knee extension moment and total impulse (calculated as the integral of internal knee extension moment with respect to time) for the entire stance phase were extracted for each limb and averaged across the trials selected for analysis.

Statistical Analysis

Paired t-tests revealed no differences in either MVT or peak RTD between dominant vs. non-dominant (MVT: $p = 0.775$, g = 0.07; RTD: $p = 0.237$, g = 0.43) limbs in the control group, where the dominant limb was defined as the one in which the participant would favour to kick a ball. Thus, each dependent variable was averaged between the dominant and non-dominant limbs in the control group, and comparisons are made between the mean of the control limbs (CON) vs the amputated limb of ITTAs (AMP) vs the intact limb (INT).

Levene's test was used to check for equality of variances prior to running all analyses. A one-way mixed design ANOVA was used to analyse the influence of limb (AMP vs. INT vs. CON) on each dependent variable. In the instance of a main effect for any of the ANOVAs, post-hoc Bonferroni corrected t-tests (paired t-tests for AMP vs. INT, and independent t-tests for AMP or INT vs. CON) were used for paired comparisons. Effect size, Hedges *g*, was calculated for paired comparisons, and interpreted as small (0.2-0.5), medium (0.5-0.8) and large effects (>0.8). Statistical analysis was completed using SPSS version 24, and the significance level was set at $p < 0.05$. Data are reported as mean ± standard deviation (SD), with absolute percentage difference in values between each condition.

Results

Due to an injury to one ITTA occurring between visits 2 and 3 (neuromuscular and gait assessment) data are for 9 and 8 ITTAs, respectively. One control withdrew from octet and doublet stimulation, so control data for VA and octet variables are presented for 8 controls, but all other variables are for 9 controls. The groups had similar age, height, body mass and physical activity scores ($p \geq 0.354$; g = 0.10-0.64; Table 1). There was a large effect size for the controls to walk faster (g = 1.21), although this difference was not statistically significant ($p = 0.616$; Table 1).

Muscle Architecture

There was no main effect ($p = 0.226$) of limb on pennation angle (Table 2). However, muscle thickness in AMP was lower than both INT (-41%, $p = 0.030$, g = 1.78) and CON (-38%, $p = 0.002$, g = 1.58; Figure 1), but similar between INT and CON ($p = 1.000$, g = 0.23; Table 2).

Fascicle length was shorter in AMP than INT (-36%, $p < 0.001$, g = 0.95), but similar between AMP and CON ($p = 0.187$; g = 0.50), and INT and CON ($p = 1.000$; g = 0.49).

<u>Contractile Properties</u>

PT and absolute RTD in both the twitch and octet (Table 2) were lower in AMP than both INT and CON (-72% to -50%, p = 0.001-0.004, g = 1.97-2.84), but similar between INT and CON ($p \geq 0.284$, g = 0.40-0.68).

When expressed relative to PT, twitch RTD was 18% lower ($p = 0.006$, g = 1.35), and octet RTD 25% lower ($p < 0.001$, g = 2.60) in AMP when compared to INT (Table 2). Relative twitch and octet RTD were also both 14% lower in AMP compared to CON (twitch RTD: $p = 0.036$, g = 1.59; octet RTD: $p = 0.037$, g = 1.63). Despite being statistically similar, there was a large effect for relative octet RTD to be greater in INT than CON ($p = 0.120$, g = 1.03; Table 2), whilst relative twitch RTD was similar between INT and CON ($p = 1.000$, g = 0.18).

<u>Maximal and Voluntary Explosive Torque</u>

MVT (both absolute and relative to body mass) was significantly lower in AMP compared to both INT (~-60%, $p < 0.002$, g = 1.74-1.97) and CON (~-64%, $p < 0.001$, g = 2.05-2.33). There were no differences between INT and CON in absolute ($p = 1.000$, g = 0.35) or relative MVT ($p = 1.000$, g = 0.28; Figure 2A and C).

Absolute peak voluntary RTD (Figure 2B) was ~75% lower in AMP than INT ($p = 0.001$, g = 2.22), ~76% lower in AMP than CON ($p < 0.001$, g = 2.36), but similar between INT and CON ($p = 1.000$, g = 0.14). Relative to MVT, peak RTD was significantly smaller in AMP than INT (-43%, $p = 0.027$, g = 1.37) and CON (-39%, $p = 0.031$, g = 1.09), while INT and CON were similar ($p = 1.000$, g = 0.23; Figure 2D).

<u>Neural Drive</u>

Both VA and RMS EMG_{MVT} (Table 2) were lower in AMP than INT (-44% for VA, $p < 0.001$, g = 3.63; and -43% for EMG_{MVT}, $p < 0.001$, g = 1.97) and CON (-43% for VA, $p < 0.001$, g = 3.54; -32% for EMG_{MVT}, $p = 0.021$, g = 1.23), but similar between INT and CON ($p \geq 0.271$, g = 0.14-0.70).

During the voluntary explosive contractions, there was no main effect of limb ($p = 0.304$) on the amplitude of explosive RMS $EMG_{0\text{-}100}$ (Table 2). However, there was a moderate effect for RMS $EMG_{0\text{-}100}$ to be greater in INT than AMP (g = 0.75), but only small to moderate effects for other comparisons (AMP vs. CON, g = 0.30; INT vs. CON, g=0.45).

<u>Knee Kinetics in Gait</u>

Knee moments throughout stance are presented in Figure 3. Both absolute and relative peak knee extensor moment during the stance phase of gait was significantly lower in the AMP compared to INT (absolute -59%, $p = 0.011$, g = 1.77; BM -60%, $p = 0.005$, g = 1.78) and CON (absolute -54%, $p = 0.005$, g = 1.61; BM -59%, $p = 0.006$, g = 1.72) limbs, but similar between INT and CON ($p = 1.000$; g = 0.05-0.14; Table 2). While there was no main effect of limb on absolute or relative knee extensor moment impulse during stance ($p > 0.069$), there were medium to large effects for it to be lower in AMP than INT (-36%; absolute g = 0.99, BM g = 1.15) and CON (-27%; absolute g = 0.56, BM g = 0.90), respectively (Table 2).

Discussion

In this study, we compared quadriceps strength and neuromuscular function in the amputated limb of ITTAs with their intact limb and a control group limb, providing a novel model for studying the long-term (>1.5 years) effects of chronic disuse. Long-term disuse of the amputated limb in ITTAs was evidenced from the ~60% lower peak knee extensor moments during walking compared to the intact and control limbs. This disuse was accompanied by ~60% lower MVT and ~75% lower RTD in the amputated limb, which are much greater differences than may be predicted from short-term disuse studies. Declines in MVT appeared to be largely due to reduced muscle size (evidenced by lower muscle thickness in AMP) and neural drive (evidenced by lower VA and EMG_{MVT} in AMP). Declines in RTD appeared to be due primarily to declines in MVT and a shift towards slower intrinsic contractile properties, with neural drive in explosive contractions being unaffected in AMP.

TTAs as a model for long-term disuse

In the current study, there were large effects for knee extensor kinetics during gait to be lower in amputated than intact or control limbs which, coupled with the considerable reductions in knee extensor strength in the amputated limb, suggests the amputated limb undergoes substantially less habitual loading during ambulation. The reduced knee extensor moments in gait may also be partly due to increased co-contraction at the knee on the amputated limb during gait (Culham et al., 1986; Isakov et al., 2001). Future research should therefore aim to quantify internal loading of the knee extensors for a more direct estimation of disuse and its association with strength changes in the amputated limb. Consistent with our results, previous studies have reported decreased knee moments (Powers, Rao & Perry, 1998, Winter & Sienko, 1988); powers (Powers, Rao & Perry, 1998, Winter & Sienko, 1988); and work (Silverman & Neptune, 2012) on the amputated limb in walking. In contrast to these previous studies however, the ITTAs of the current study were young, healthy, and moderate-highly active. As a result, the effects of the evident disuse on strength and neuromuscular function could be isolated from factors such as ageing, disease, and sedentary behaviour, which are known to independently affect muscle strength and function (Narici & de Boer 2011, Sacchetti et al. 2013).

The knee extensors of the intact limb in the ITTAs did not differ from those of an able-bodied control population for kinetics during walking, MVT, RTD, or any of the neuromuscular determinants of strength. This suggests that, for these parameters, the intact limb of the

ITTAs provides an ideal internal control for comparison to the amputated limb, from which to draw conclusions about the effects of chronic disuse.

Changes in Strength

The declines in MVT found in the amputated limb compared to the intact limb (-59%) are comparable to, albeit at the high end of, differences observed in previous amputee studies (-33 to -57%; Isakov et al. 1996, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002), but considerably greater than the reduction in strength typically observed after a period of short-term disuse of up to 120 days (~23%; Narici & de Boer, 2011). Short-term intervention studies suggest that MVT decreases exponentially over time following unloading, plateauing out after ~90 days; however, the results of this study suggest the strength declines with longer-term disuse are considerably more than could be predicted from short-term intervention studies.

To the authors' knowledge, only two previous studies have investigated the effect of disuse on voluntary RTD of the knee extensors, reporting 54% (Bamman et al. 1998) and 42% (de Boer et al. 2007) decreases in RTD after 16 days of bed rest, and 23 days of ULLS, respectively. The considerable reductions in peak RTD (-75%) in the amputated vs. intact limb are important, as RTD is considered more functionally relevant than MVT, in many sports-specific and daily tasks, such as sprinting, jumping, and balance recovery (Behan, Pain & Folland 2018, Pijnappels et al. 2008, Tillin, Pain & Folland 2013).

When expressed relative to MVT, peak RTD was significantly reduced in the amputated compared to the non-amputated limbs, although limb differences were considerably smaller for relative than absolute peak RTD. Thus, the reduction in MVT appears to be a large contributing factor to reduced absolute RTD in the amputated limb; however, this only partially contributed to the reduction in peak RTD, which was likely also influenced by the slowing of the contractile properties (discussed in more detail below).

Mechanisms of Strength Differences

Neural Drive

A broad suppression in neuromuscular activity at maximal force production – indicated by reduced amputated limb VA (~44%) and EMG_{MVT} (~38%) compared to non-amputated limbs – likely contributes to the reduction in amputated limb MVT. Whilst previous studies have reported reduced quadriceps EMG amplitude (-16 to -35%; Alkner & Tesch 2004, Deschenes et al. 2002) and VA (-7%; Kawakami et al. 2001), others have not observed changes in these measurements (de Boer et al. 2007, Campbell et al. 2013, Horstman et al. 2012), following periods of disuse of up to 89 days. Thus, the large limb effects on VA and EMG responses observed in the present study suggest that reductions in neural drive with disuse become more pronounced and observable over time. Of note is the specificity of the neural deficits in the ITTAs to the amputated limb. Evidence from unilateral injury and training studies suggest a cross-over effect of neural function, in that neural drive adaptations occur at the contralateral, as well as the injured/trained limb (Bogdanis et al. 2019, Tillin et al. 2011). In this study however, there was no evidence that reduced neural drive on the amputated side had affected neural drive on the intact side, which was similar to the control limb. Perhaps this is because ITTAs rely more on the intact limb for most activities of daily living and exercise (e.g. Winter & Sienko 1988), which may negate any cross-over effects of reduced neural drive from the amputated to the intact limb.

Despite the substantial differences between the amputated and non-amputated limbs evident in neural drive during maximum force production, no such differences were observed in this study in explosive-phase EMG amplitude (Table 2). This suggests that altered neural drive does not explain the lower peak RTD in the amputated limb, which is interesting given that neural drive is a key determinant of RTD (del Vecchio et al. 2019, Folland, Buckthorpe & Hannah 2014). The large variability in EMG, even after normalisation to M_{max} (Buckthorpe et al. 2012), greater variability in RTD compared to MVT (Folland, Buckthorpe & Hannah 2014, Tillin, Pain & Folland 2013), and small sample sizes (n = 9 per limb) may have reduced the chances of observing a significant effect. Alternatively, the amputated limb's role in ambulation may explain the lack of differences in neural drive during the explosive contractions. Specifically, whilst the knee extensors of the amputated limb experience reduced load compared to the intact during ambulation, the amputated side does contribute to stability and postural correction, for which RTD appears to be important (Behan, Pain & Folland 2018). Thus, typical physical activity in the amputees may provide sufficient stimulus to maintain the neural drive during short, rapid contractions, which typically underpins RTD.

Muscle Architecture

The VL muscle was 41% thinner in the amputated limb compared to the intact, which is a larger difference than the declines in MRI and CT scanner measurements of muscle size (-3 to -18%) observed in short-term disuse studies (Alkner & Tesch 2004, Campbell et al. 2013, de Boer et al. 2007, Dirks et al. 2016). Thus, similar to the changes observed for strength and neural drive, reductions in muscle size with long-term disuse are much greater than could be predicted from short-term disuse studies. Muscle size is considered an important determinant of MVT (Blazevich et al. 2009), and thus the reduction in muscle thickness is likely to contribute to the declines in both MVT, and by association RTD, in the amputated limb.

Fascicle length was reduced by 36% in the amputated limb compared to the intact. Again, this difference is considerably greater than the decline in knee extensor fascicle length (6-9%) typically observed with short-term unloading (Campbell et al. 2013, de Boer et al. 2007). ITTAs walk with a comparatively stiff knee joint on the amputated limb (Powers, Rao & Perry 1998, Winter & Sienko 1988), which would theoretically isolate loading to shorter fascicle lengths, and limit the stimulus likely required to maintain longer fascicle lengths. Decreases in fascicle length may reduce maximum shortening velocities and power (Blazevich & Sharp 2005), and shift the torque-angle relationship towards more extended knee positions

(Blazevich et al. 2009). Given our strength measurements were made at a typical plateau region of the torque-angle relationship (Chow et al. 1999), a shift away from this region in the amputated limb may have partly contributed to the observed differences in MVT and RTD.

In contrast to the results of previous research which demonstrated decreases in pennation angle during short periods of ULLS (de Boer et al. 2007, Campbell et al. 2013), our results appear to suggest that pennation angle does not change with long-term disuse. In healthy populations, angles of pennation of the VL muscle have been reported to be 6-27° (Blazevich et al. 2006, Rutherford & Jones 1992); the pennation angle of all three limbs in this study (~12-14°) falls within this range. This suggests that the structural re-modelling that seems to take place in the early phases of disuse are not representative of long-term adaptations. It is possible muscle thickness declines at a faster rate than fascicle length with short term disuse, causing a decline in pennation angle; whilst over longer periods of disuse, fascicle length reductions "catch-up" with muscle thickness loss, causing a return to baseline pennation angle, but this hypothesis cannot be tested with our data.

Intrinsic contractile properties

The significant reductions in evoked (twitch and octet) contractile peak torque in the amputated compared to the intact and control limbs (Table 2) are reflective of the reduced capacity of the amputated limb knee extensors for torque production. These changes were accompanied by reductions in RTD, both absolute and relative to peak torque, reflecting a shift towards slower contractile properties in the intact limb. This is in contrast to the results of short-term disuse studies in both healthy controls and pathological populations, which have reported a shift towards faster contractile properties owing to a greater expression of fast-contracting myosin-heavy-chain isoforms (MHC; Bamman et al. 1998, Kapchisky et al. 2018, Trappe et al. 2004). The results of the current study therefore provide novel evidence that changes in intrinsic contractile properties with long-term disuse are more characteristic of ageing muscle, which also displays a slowing of the contractile properties (Roos et al. 1999). This slowing may be due to preferential atrophy of type II muscle fibres, and potentially also to an increased dominance of type I MHC in fibres co-expressing MHCs commonly seen with old age (Lexell et al. 1988). The slower contractile properties in the amputated limb likely contributed to the lower voluntary peak RTD also observed in the amputated limb, as twitch and octet RTD are important determinants of voluntary RTD (Folland, Buckthorpe & Hannah 2014).

Conclusion

This study was the first to utilise ITTAs as a novel study model to investigate the effects of long-term muscle disuse on strength and neuromuscular function, in young, healthy, active adults. Strength, neuromuscular function and loading during gait, of the intact limb of ITTAs were comparable to a control able-bodied limb, suggesting the intact limb provides a suitable internal control for comparison to the amputated limb for these parameters. The quadriceps muscles of the amputated limb displayed considerably less habitual loading during gait, than the intact side. This disuse of the amputated limb was accompanied by larger reductions in MVT and RTD than could be predicted from short-term disuse studies. The reductions in MVT were likely due to the declines in muscle size and neural drive, whilst the reductions in RTD appeared due to the decline in MVT coupled with a slowing of the contractile properties.

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Tables

Table 1. Participant information. Data are mean ± SD, and presented for n = 9 for both groups, except walking speed (n = 8 for ITTAs and n = 9 for controls). Cause of amputation was trauma for all ITTAs.

	ITTAs		Controls	
	Mean ± SD	Range	Mean ± SD	Range
Age (years)	40.3 ± 8.5	24 – 48	38.6 ± 6.3	27 – 46
Height (cm)	179 ± 8.2	165 – 186	177 ± 4.1	171 – 184
Body Mass (kg)	84.7 ± 16.7	54.6 – 114	80.0 ± 10.5	58.3 – 97.5
Activity Level (MET-min.week^{-1})	7890 ± 6122	480 – 15918	5686 ± 3256	2577 – 11817
Walking Speed (m.s^{-1})	1.34 ± 0.16	1.05 – 1.61	1.51 ± 0.10	1.34 – 1.71
Years since Amputation	12.2 ± 11.5	1.5 – 29.0	-	-

Table 2. Knee extensor kinetics and neuromuscular determinants of strength in the amputated (AMP) and intact (INT) limbs of unilateral transtibial amputees, and in an able-bodied control limb (CON). Data are presented as mean ± SD for n = 9 (AMP and INT) and n = 9 (CON). Data in italics correspond to those variables where n = 8 due to participant withdrawal. Differences compared to AMP are denoted by * ($p < 0.05$) or ** ($p < 0.001$).

	AMP	INT	CON
Knee Extensor Kinetics			
Moment (Nm)	*26.1 ± 13.3*	*65.4 ± 38.1* *	57.0 ± 13.7 *
Moment_{BM} (Nm.kg^{-1})	*0.30 ± 0.14*	*0.75 ± 0.31* *	0.71 ± 0.24 *
Impulse (Nm·s)	*1.14 ± 0.84*	*2.23 ± 1.21*	1.75 ± 0.43
Impulse_{BM} (Nm·s.kg^{-1})	*0.013 ± 0.009*	*0.025 ± 0.011*	0.022 ± 0.008
Neural Drive			
Voluntary Activation (%)	*50.6 ± 12.7*	89.2 ± 5.75 **	*90.4 ± 4.07* **
RMS EMG_{MVT} (% M_{max})	5.19 ± 1.20	9.10 ± 2.39 **	7.64 ± 1.47 *
Explosive RMS $\text{EMG}_{0\text{-}100}$ (% M_{max})	5.38 ± 3.12	7.92 ± 3.66	7.00 ± 1.75
Evoked Twitch			
PT (Nm)	11.6 ± 6.00	30.8 ± 11.6 **	39.0 ± 11.9 **
Absolute RTD (Nm.s^{-1})	223 ± 171	650 ± 247 **	808 ± 243 **
Relative RTD (PT.s^{-1})	16.7 ± 3.23	20.4 ± 1.79 *	21.1 ± 4.60 *
Evoked Octet			
PT (Nm)	47.1 ± 31.2	94.5 ± 32.3 **	*116 ± 28.0* **
Absolute RTD (Nm.s^{-1})	609 ± 387	1647 ± 541 **	*1840 ± 365* **
Relative RTD (PT.s^{-1})	13.3 ± 1.62	17.7 ± 1.66 **	*16.0 ± 1.43* *
Muscle Architecture			
Muscle Thickness (mm)	15.4 ± 5.19	26.3 ± 6.38 *	25.0 ± 3.34 *
Pennation Angle (°)	12.0 ± 1.66	13.9 ± 3.79	13.7 ± 1.46
Fascicle Length (mm)	73.8 ± 23.2	117 ± 50.8 **	96.5 ± 14.8

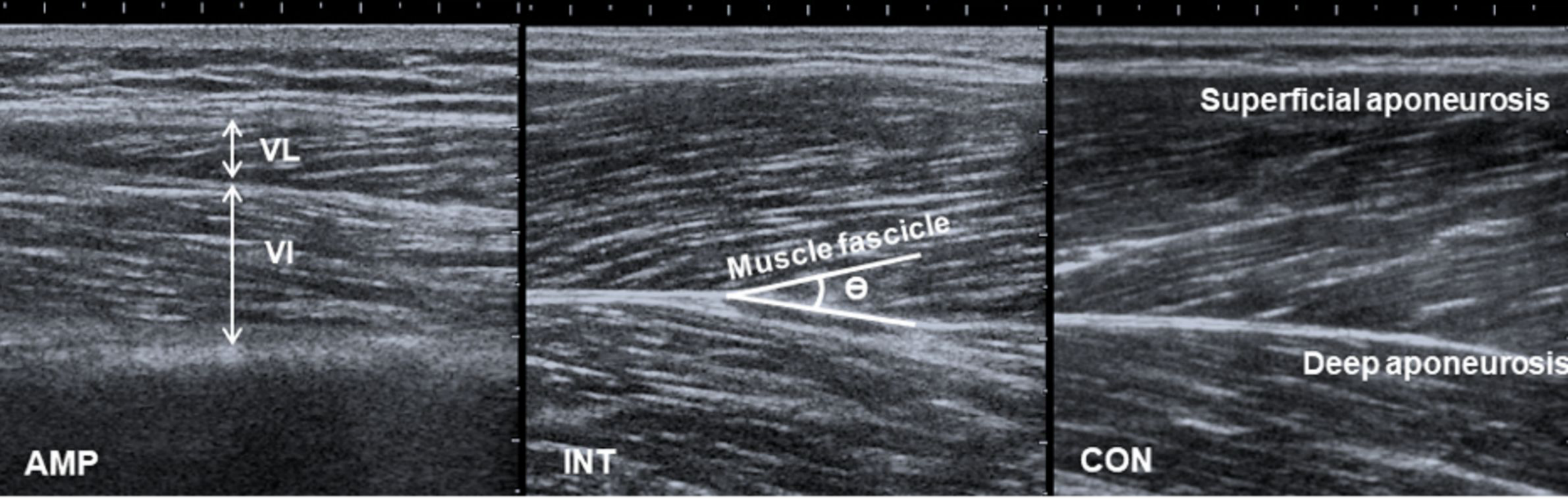
RTD, Rate of Torque Development; subscript BM, relative to body mass; subscript MVT, relative to Maximum Voluntary Torque; RMS EMG_{MVT}, root mean squared electromyography at MVT; RMS $\text{EMG}_{0\text{-}100}$, root mean squared electromyography from 0-100 ms of an explosive voluntary contraction; M_{max}, maximal M-wave; PT, peak torque.

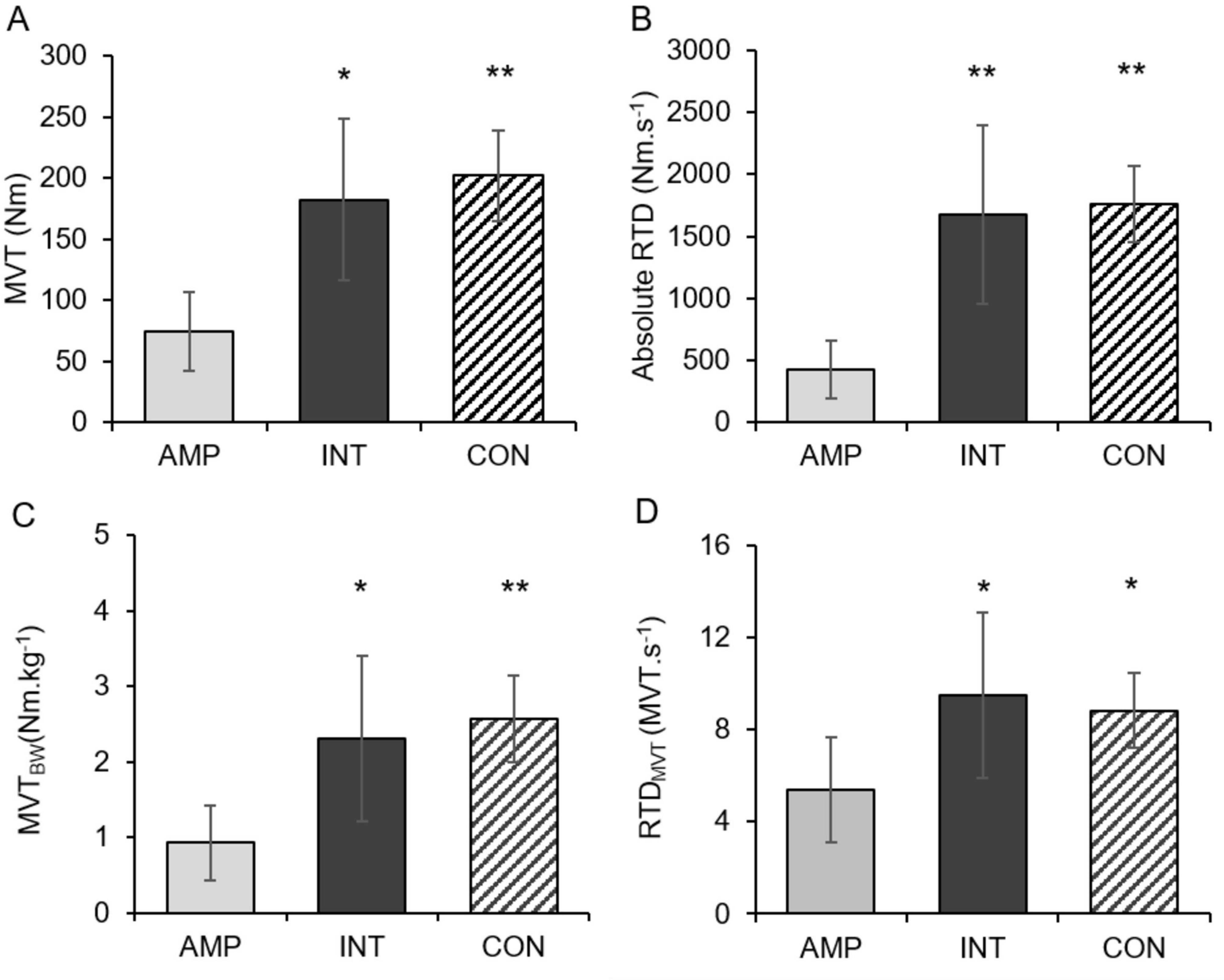
Figure Headings

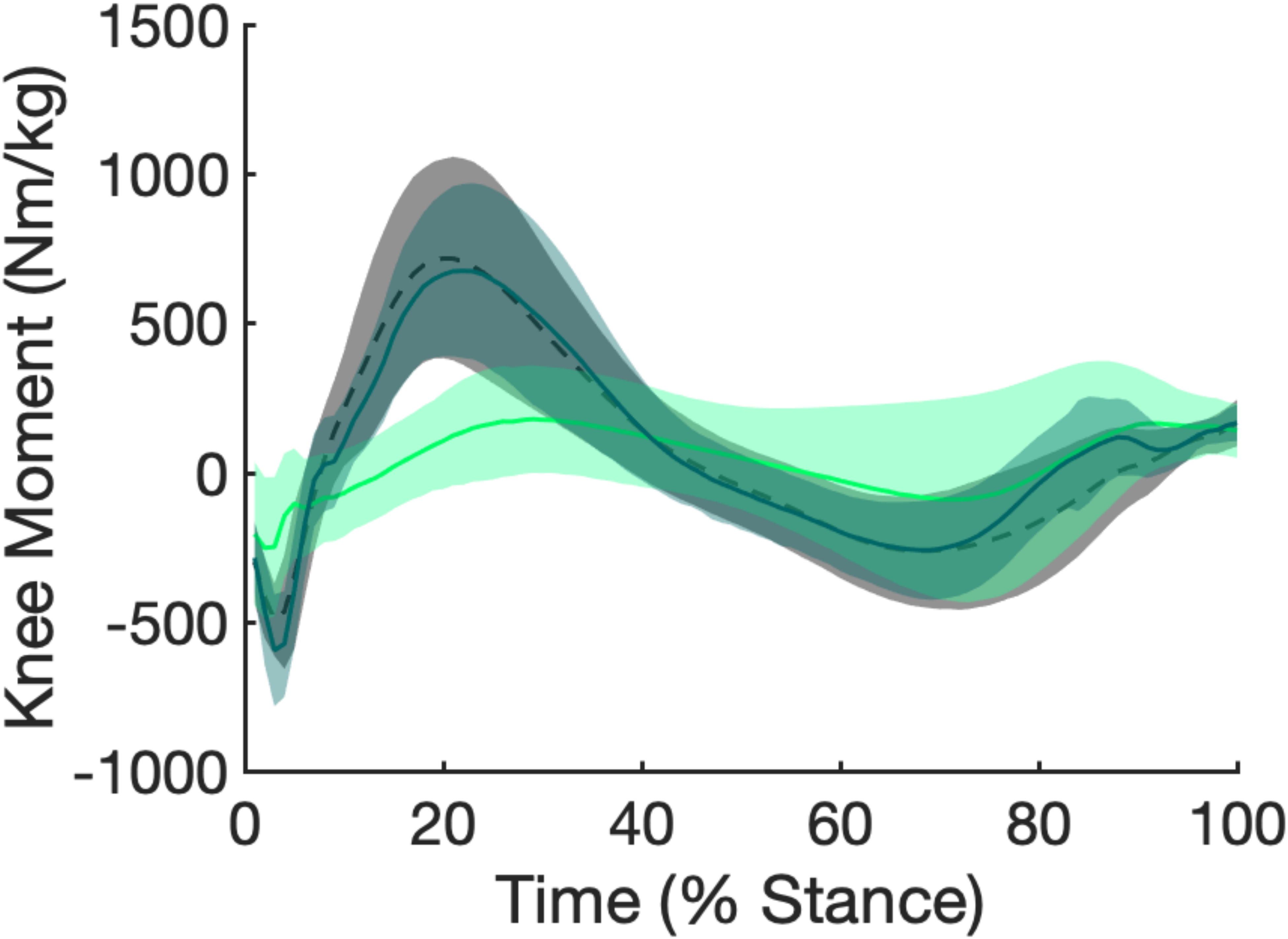
Figure 1. Static B-mode ultrasound image of the Vastus Lateralis (VL) and Vastus Intermedius (VI) muscles at rest for the amputated (AMP) and intact (INT) limb of one ITTA, and one control limb (CON). Architectural measures taken included pennation angle (Ɵ) relative to the deep aponeurosis and extrapolated fascicle length, which were each determined from three fascicles; and muscle thickness, measured between the superficial and deep aponeuroses at three separate points (the centre and either end of each image – indicated by numbered circles in the middle image). Significant reduction in amputated limb VL muscle thickness is evident, while similarities in pennation angle in all three limbs, and muscle thickness between INT and CON, can clearly be seen.

Figure 2. Maximal voluntary torque (MVT) and absolute peak rate of torque development (RTD) recorded during respective maximal and explosive voluntary isometric knee extensions, in both the amputated (AMP, light grey) and intact (INT, dark grey) limbs of unilateral transtibial amputees ($n = 9$), and an able-bodied control group (CON, striped; $n = 9$). Data are presented as mean ± SD absolute values (A,B) and relative to body mass (C) or MVT (D). Differences compared to AMP are indicated by * ($p < 0.05$) or ** ($p < 0.001$).

Figure 3. Sagittal plane knee moments during the stance phase of walking for the amputated (AMP, light grey line) and intact (INT, dark grey line) limbs of unilateral transtibial amputees, and of an able-bodied control limb (CON, dashed line). INT and CON display substantial overlap. Joint moment is expressed as internal moment. Positive and negative values indicate knee extension and flexion moments, respectively. Data are presented as mean ± SD for $n = 8$ (AMP and INT) and $n = 9$ (CON).


VL
VI
AMP
Muscle fascicle
θ
INT
Superficial aponeurosis
Deep aponeurosis
CON


A
B
C
D
MVT (Nm)
Absolute RTD (Nm.s-1)
MVTBW(Nm.kg-1)
RTDMVT (MVT.s-1)
AMP
INT
CON
*
**


Knee Moment (Nm/kg)
1500
1000
500
0
-500
-1000
0
20
40
60
80
100
Time (% Stance)