The effects of long-term muscle disuse on neuromuscular function in unilateral transtibial amputees

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Abstract
The purpose of this study was to determine: (i) 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 (ii) the effects of such disuse on quadriceps muscle strength and neuromuscular function in this population. Nine ITTAs and nine control subjects performed isometric voluntary knee extensions of both limbs to assess maximal voluntary torque (MVT) and the rate of torque development (RTD). The interpolated twitch technique and EMG normalized to maximal M-wave were used to assess neural activation, involuntary (twitch and octet) contractions to assess intrinsic contractile properties, and ultrasound images of the vastus lateralis to assess muscle architecture. Clinical gait analysis was used to measure knee kinetic data during walking at an habitual speed. The ITTAs displayed 54–60% lower peak knee-extensor moments during walking in the amputated compared with intact/control limbs, but the intact and control limbs were comparable for loading during walking and muscle strength variables, suggesting that the intact limb provides a suitable internal control for comparison with the disused amputated limb. The MVT and RTD were ∼60 and ∼75% lower, respectively, in the amputated than intact/control limbs. The differences in MVT appeared to be associated with ∼40 and ∼43% lower muscle thickness and neural activation, respectively, and the differences in RTD appeared to be 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.

KEYWORDS
muscle atrophy, muscle strength, neural activation

1 | INTRODUCTION

Prolonged disuse of skeletal muscle poses a considerable threat to neuromuscular functional capacity and health (Narici & de Boer 2011). A mere 9 days of disuse causes considerable reductions in muscle strength, typically measured as maximal voluntary torque (MVT; Rozier, Elder, & Brown, 1979) or the 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 therefore are frequently investigated in typical study models of disuse, including spaceflight, unilateral lower-limb suspension, limb immobilization, bed rest and immobilization during intensive care after surgery (Narici & de Boer 2011). Studies show reductions in quadriceps MVT of ∼2% day−1 for the first 10 days (Berg & Tesch 1996; Puthucheary et al., 2017; Rozier et al., 1979), slowing to ∼1% week−1 for ≤30 days, with an eventual plateau resulting in average strength losses of ∼23% after 120 days of disuse (Dirks et al., 2016; Horstman, Ruiter, Duijnhoven, Hopman, & Haan, 2012; Narici & de Boer 2011). The effects of disuse on RTD have not been studied widely, but RTD might be more functionally relevant than MVT during rapid human movements, such as recovering from a trip or loss of balance (Behan, Pain, & Folland, 2018; Pijnappels, Reeves, Maganaris, & Van Dieen, 2008). Long-term muscle disuse is
a default position for many clinical populations (Brown, Friedkin, & Inouye, 2004) and the sedentary, but it is unclear how both MVT and RTD change with long-term, habitual disuse, because 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) might provide a useful model for studying the effects of long-term, habitual disuse. These individuals adopt an asymmetrical loading pattern characterized by considerably lower vertical ground reaction forces and knee-extensor moments on the amputated compared with 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 that the quadriceps of the amputated limb in ITTAs are chronically disused, which would explain observations of considerably lower (~50%) quadriceps size (Moirenfeld, Ayalon, Ben-Sira, & Isakov, 2000) in the amputated compared with the intact limb. Further, studies comparing strength in ITTAs and healthy controls have found significantly reduced amputated limb (~50%) MVT (Isakov, Burger, Gregoric, & Marinecek, 1996; Lloyd, Stanhope, Davis, & Royer, 2010; Pedrinelli, Saito, Coelho, Fontes, & Guarniero, 2002) compared to the intact and control limbs. Comparison of quadriceps neuromuscular function in the amputated versus intact limb of ITTAs, coupled with comparison of limb loading during gait as an estimate of typical use, might therefore offer new insight into the long-term effects of habitual disuse. However, it is currently 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 subject, which would support the efficacy of ITTAs as a study model of long-term disuse. Despite similar peak vertical ground reaction forces 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 with the limbs of able-bodied participants (Isakov et al., 1996; Lloyd et al., 2010; Pedrinelli et al., 2002; Powers, Boyd, Fontaine, & Perry, 1996). However, the latter studies did not control between the groups for other factors known to have independent effects on muscle strength, 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; Renström, Grimby, & Larsson, 1983) have previously measured quadriceps MVT in ITTAs, and none has 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 EMG amplitude or the interpolated twitch technique, is considered an important determinant of both MVT and RTD (Balshaw, Massey, Maden-Wilkinson, Tillin, & Folland, 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 (Lambertz, Pérot, Kaspranski, & Goubel, 2001; Narici & de Boer, 2011) and others no change (Campbell et al., 2013; de Boer, Maganaris, Seynnes, Rennie, & Narici, 2007). The RTD also appears to be determined by the intrinsic contractile speed properties of the muscle, such as the RTD relative to peak torque recorded during electrically evoked involuntary contractions (e.g. twitch or octets; Folland, Buckthorpe & Hannah, 2014), and short-term disuse causes a shift towards faster contractile properties (Lambertz et al., 2001). Finally, the maximal force-generating potential of a muscle is dependent upon its architecture (Blazevich, Cannavan, Horne, Coleman, & Aagaard, 2009), and 21–30 days of disuse have elicited changes such as declines in muscle size (~10%), pennation angle (~13%) and fascicle length (~9%; Campbell et al., 2013; de Boer et al., 2007). Determining the degree of change in these neuromuscular determinants of muscle strength with long-term habitual disuse might allow better targeting of preventative and rehabilitative interventions for populations subject to muscle disuse.

The first aim of the present 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 limb and an able-bodied control limb.

2 METHODS

2.1 Ethical approval

Participants provided written informed consent before 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
2.2 | Participants

Nine male ITTAs and nine male control subjects took part. Before data analysis, groups were matched to ensure similar group means and variability in age, height, body mass (BM) and physical activity. Physical activity was assessed using the International Physical Activity Questionnaire (Short Format; http://ipaq.ki.se/downloads.htm). The ITTAs were included if they had a unilateral transtibial amputation performed >6 months before involvement in the study, to ensure established amputation and long-term disuse in the residual limb. The ITTAs were excluded if they experienced any discomfort in the residual limb whilst using their prosthesis and/or if their amputation had occurred owing 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 neuromusculoskeletal injuries (other than a transtibial amputation in the ITTAs).

2.3 | Overview

Participants visited the laboratory on three separate occasions, with each visit 3–7 days apart, to complete a familiarization (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 randomized. All three sessions commenced at a consistent time (±2 h) of day for each participant, after ≥36 h without strenuous exercise and 24 h without alcohol.

2.4 | Experimental set-up and measurements

2.4.1 | Knee-extension torque

Isometric strength data were collected using an isokinetic dynamometer (Humac Norm; Computer Sports Medicine Inc., Stoughton, MA, USA). Participants were seated, with a hip angle of 100 deg (180 deg is full extension) 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 deg. Some basic modifications were made to minimize changes in knee joint angle 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 clamped tightly 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 deg to account for the shorter residual tibia.

The analog torque signal was sampled at 2000 Hz using an external A/D converter [16-bit signal recording resolution; Micro 1401; Cambridge Electronic Design (CED), Cambridge, UK] and interfaced with a PC using Spike2 software (v8; CED). Offline, 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.

2.4.2 | Electromyography

Electromyography signals were recorded from the superficial knee extensors [rectus femoris (RF), vastus medialis (VM) and vastus lateralis (VL)] using a Noraxon TeleMyo Desktop DTS System (Noraxon, Scottsdale, AZ, USA). The skin was prepared by shaving, abrading and cleansing with 70% alcohol. Bipolar Ag/AgCl surface electrodes (2 cm inter-electrode distance; Noraxon) were attached over the belly of each muscle at surface EMG for non-invasive assessment of muscles (SENIAM) recommended recording sites (Hermens et al., 1999), parallel to the presumed orientation of the muscle fibres. The raw EMG signals were transmitted wirelessly (Wireless Research EMG Probes, Part 542; Noraxon) to a receiver (Desktop DTS, Part 586; Noraxon), amplified x500, sampled at 2000 Hz in synchrony with torque via the same A/D converter and PC software, and bandpass filtered offline between 6 and 500 Hz using a fourth-order zero-lag Butterworth filter.

2.4.3 | Muscle architecture

A static ultrasound image (Hitachi Noblus; Hitachi Medical Systems, Wellingborough, UK) of the VL was taken using a linear array probe with a 94 mm scan width (HI VISION L53L; Hitachi Medical Systems). The image was obtained before any other measurements whilst the participant was seated in the dynamometer at rest, and with a joint angle of 100 deg (180 deg 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), because 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 control subjects using the same methods as described above. The coefficient of variation was 4.4, 10.9 and 9.3% for muscle thickness, fascicle length and pennation angle, respectively.

2.4.4 | 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
Explosive voluntary contractions

Knee-extension MVCs

Voluntary activation

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 individual with unilateral transtibial amputation and one control limb (CON). Architectural measures taken included the pennation angle (ϴ) 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, as indicated by numbered circles in the middle image). A significant reduction in the amputated limb VL muscle thickness is evident, and similarities in pennation angle in all three limbs, and in muscle thickness between INT and CON, can be seen clearly.

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 cm × 7 cm carbon rubber electrode) was taped in place over the greater trochanter. Single impulses were delivered with stepwise increments in the current, separated by 15 s, until a plateau in the amplitude of twitch torque and compound muscle action potentials (M-waves) was 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 before 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 value and relative to MVT. Torque onset). Peak RTD was extracted and expressed as both an absolute value 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_{0–100}) and normalized to M_max of the same muscle before averaging across the three quadriceps muscles. The EMG onsets, defined as the onset of the first muscle to be activated, were identified with a standardized systematic protocol of visual identification (Tillin, Jimenez-Reyes, Pain, & Folland, 2010).

2.4.5 Knee-extension MVCs

Participants performed a series of ~20 warm-up contractions of 3 s duration at progressively higher intensities before completing six maximal 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. The MVT was defined as the greatest instantaneous peak voluntary torque (not attributable to superimposed stimulation) recorded during any of the MVCs or explosive contractions.

2.4.6 Explosive voluntary contractions

Participants completed 10–15 explosive isometric contractions, each separated by 20 s rest, using the method described by Folland et al. (2014). Three explosive voluntary contractions were chosen for analysis, and all dependent variables assessed were averaged across these three explosive contractions. The three contractions chosen for analysis were 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 N m during the 100 ms before visible torque onset). Peak RTD was extracted and expressed as both an absolute value 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_{0–100}) and normalized to M_max of the same muscle before averaging across the three quadriceps muscles. The EMG onsets, defined as the onset of the first muscle to be activated, were identified with a standardized systematic protocol of visual identification (Tillin, Jimenez-Reyes, Pain, & Folland, 2010).

2.4.7 Voluntary activation

The second, fourth and sixth 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 determine the voluntary activation (VA; a measure of neural drive at MVT), using eqn (1):

\[ VA(\%) = 100 \times \left(1 - \frac{D_s}{D_c} \right) \]  

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 the stimulation artefact (EMG_{MVT}). The EMG_{MVT} was normalized to M_max of the same muscle and averaged across the three quadriceps muscles.
2.4.8 | Walking gait

Kinematic data were collected using 12 Vicon Vantage V5 (Vicon Motion Systems Ltd, Oxford, UK) motion capture cameras sampling at 200 Hz synchronized with three in-series Kistler force plates (type 9281c; Kistler Instruments Ltd, Hook, UK) in the middle of a 15 m walkway, sampling force data at 1000 Hz. Two sets of Brower TC timing gates (Brower Timing Systems, Draper, UT, USA) placed 2 m either side of the force plates were used to capture average walking pace. Retroreflective markers (14 mm in 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 stabilized. 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 >40 frames, were selected for analysis for each limb. Data were processed in Vicon Nexus v.2.7.1 (Vicon Motion Systems LTD, Yarnton, UK). Raw marker trajectories and analog 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, normalized by body mass (Winter & 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.

2.5 | Statistical analysis

Student’s paired t tests revealed no differences in either MVT or peak RTD between dominant versus non-dominant (MVT, $P = 0.775, g = 0.07$; and RTD, $P = 0.237, g = 0.43$) limbs in the control group, where the dominant limb was defined as the one that 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) versus the amputated limb of ITTAs (AMP) versus the intact limb (INT).

Levene’s test was used to check for equality of variances before running all analyses. A one-way ANOVA was used to analyse the influence of limb for independent comparisons (AMP versus CON and INT versus CON) on each dependent variable. In the instance of a main effect, post-hoc Student’s t-tests were performed. Paired Student’s t-tests compared dependent variables between the intact and amputated limb. 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 v.24 (IBM, Armonk, NY, USA), and the significance level was set at $P < 0.05$. Data are reported as the mean ± SD, with absolute percentage difference in values between each condition.

3 | RESULTS

Owing to an injury to one ITTA occurring between visits 2 and 3 (neuromuscular and gait assessment), data are for nine and eight ITTAs, respectively. One control subject withdrew from octet and doublet stimulation; therefore, control data for VA and octet variables are presented for eight control subjects, but all other variables are for nine control subjects. 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 control subjects to walk faster ($g = 1.21$), although this difference was not statistically significant ($P = 0.616$; Table 1).

3.1 | 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 in 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 in INT (−36%, $P < 0.001, g = 0.95$), but similar between AMP and CON ($P = 0.187; g = 0.50$) and between INT and CON ($P = 1.000; g = 0.49$).

3.2 | Contractile properties

The PT and absolute RTD in both the twitch and the octet (Table 2) were lower in AMP than in both INT and CON (−72 to −50%,

### TABLE 1 Participant information

| Parameter | ITTAs | Control subjects |
|-----------|-------|------------------|
| Age (years) | 40.3 ± 8.5 | 38.6 ± 6.3 |
| Height (cm) | 179 ± 8.2 | 177 ± 4.1 |
| Body mass (kg) | 84.7 ± 16.7 | 80.0 ± 10.5 |
| Activity level (MET-min week$^{-1}$) | 7890 ± 6122 | 5686 ± 3256 |
| Walking speed (m s$^{-1}$) | 1.34 ± 0.16 | 1.51 ± 0.10 |
| Time since amputation (years) | 12.2 ± 11.5 | – |

Data are mean values ± SD and are presented for $n = 9$ subjects for both groups, except for walking speed [$n = 8$ for individuals with unilateral transtibial amputations (ITTAs) and $n = 9$ for control subjects]. The cause of amputation was trauma for all ITTAs.
Knee kinetics in gait

Maximal and voluntary explosive torque

| Parameter | AMP | INT | CON |
|-----------|-----|-----|-----|
| Moment (N m) | 26.1 ± 13.3 | 65.4 ± 38.1* | 57.0 ± 13.7* |
| Moment<sub>BM</sub> (N m kg<sup>-1</sup>) | 0.30 ± 0.14 | 0.75 ± 0.31* | 0.71 ± 0.24* |
| Impulse (N m s) | 1.14 ± 0.84 | 2.23 ± 1.21 | 1.75 ± 0.43 |
| Impulse<sub>BM</sub> (N m kg<sup>-1</sup>) | 0.013 ± 0.009 | 0.025 ± 0.011 | 0.022 ± 0.008 |

Neural drive

| Parameter | AMP | INT | CON |
|-----------|-----|-----|-----|
| Voluntary activation (%) | 50.6 ± 12.7 | 89.2 ± 5.75** | 90.4 ± 4.07** |
| RMS EMG<sub>MVT</sub> (% M<sub>max</sub>) | 5.19 ± 1.20 | 9.10 ± 2.39** | 7.64 ± 1.47* |
| Explosive RMS EMG<sub>0–100</sub> (% M<sub>max</sub>) | 5.38 ± 3.12 | 7.92 ± 3.66 | 7.00 ± 1.75 |

Evoked twitch

| Parameter | AMP | INT | CON |
|-----------|-----|-----|-----|
| PT (N m) | 11.6 ± 6.00 | 30.8 ± 11.6** | 39.0 ± 11.9** |
| Absolute RTD (N m s<sup>-1</sup>) | 223 ± 171 | 650 ± 247** | 808 ± 243** |
| Relative RTD (PT s<sup>-1</sup>) | 16.7 ± 3.23 | 20.4 ± 1.79* | 21.1 ± 4.60* |

Evoked octet

| Parameter | AMP | INT | CON |
|-----------|-----|-----|-----|
| PT (N m) | 47.1 ± 31.2 | 94.5 ± 32.3** | 116 ± 28.0** |
| Absolute RTD (N m s<sup>-1</sup>) | 609 ± 387 | 1647 ± 541** | 1840 ± 365** |
| Relative RTD (PT s<sup>-1</sup>) | 13.3 ± 1.62 | 17.7 ± 1.66* | 16.0 ± 1.43* |

Muscle architecture

| Parameter | AMP | INT | CON |
|-----------|-----|-----|-----|
| Muscle thickness (mm) | 15.4 ± 5.19 | 26.3 ± 6.38* | 25.0 ± 3.34* |
| Pennation angle (deg) | 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 |

Data are presented as the mean value ± SD for n = 9 (AMP and INT) and n = 9 (CON). Data in italics correspond to those variables where n = 8 owing to participant withdrawal. Differences compared with AMP are denoted by * (P < 0.05), ** (P < 0.001). Abbreviations: subscript BM, relative to body mass; M<sub>max</sub>, maximal M-wave; MVT, relative to maximal voluntary torque; PT, peak torque; RMS EMG<sub>MVT</sub>, root mean squared electromyography at MVT; RMS EMG<sub>0–100</sub>, root mean squared electromyography from 0 to 100 ms of an explosive voluntary contraction; RTD, rate of torque development.

P = 0.001–0.004, g = 1.97–2.84), but similar between INT and CON (P ≥ 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 with INT (Table 2). Relative twitch and octet RTD were also both 14% lower in AMP compared with CON (twitch RTD, P = 0.036, g = 1.59; and 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 in 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).

### 3.3 Maximal and voluntary explosive torque

The MVT (both absolute and relative to body mass) was significantly lower in AMP compared with 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,c).

Absolute peak voluntary RTD (Figure 2b) was ~75% lower in AMP than in INT (P = 0.001, g = 2.22) and ~76% lower in AMP than in 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 in 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).

### 3.4 Neural drive

Both VA and RMS EMG<sub>MVT</sub> (Table 2) were lower in AMP than in INT (−44% for VA, P < 0.001, g = 3.63; and −43% for EMG<sub>MVT</sub>, P < 0.001, g = 1.97) and CON (−43% for VA, P < 0.001, g = 3.54; −32% for EMG<sub>MVT</sub>, P = 0.021, g = 1.23), but similar between INT and CON (P ≥ 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<sub>0–100</sub> (Table 2). However, there was a moderate effect for RMS EMG<sub>0–100</sub> to be greater in INT than in AMP (g = 0.75), but only small to moderate effects for other comparisons (AMP versus CON, g = 0.30; INT versus CON, g = 0.45).

### 3.5 Knee kinetics in gait

Knee moments throughout stance are presented in Figure 3. Both absolute and relative peak knee-extensor moment during the stance phase of gait were significantly lower in the AMP compared with INT.
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; grey bars) and intact (INT; black bars) limbs of unilateral transtibial amputees ($n = 9$) and an able-bodied control group (CON; hatched bars; $n = 9$). Data are presented as the mean ± SD absolute values (a,b) and relative to body mass (c) or MVT (d). Differences compared with 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 green line) and intact (INT; dark green line) limbs of unilateral transtibial amputees and of an able-bodied control limb (CON; dashed grey 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 the mean ± SD for $n = 8$ (AMP and INT) and $n = 9$ (CON)

(absolute $-59\%$, $P = 0.011$, $g = 1.77$; relative to BM $-60\%$, $P = 0.005$, $g = 1.78$) and CON (absolute $-54\%$, $P = 0.005$, $g = 1.61$; relative to 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). Although 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 in INT ($-36\%$; absolute $g = 0.99$, relative to BM $g = 1.15$) and CON ($-27\%$; absolute $g = 0.56$, relative to BM $g = 0.90$), respectively (Table 2).

4 | 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 new 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 $\sim 60\%$ lower peak knee-extensor moments during walking compared with the intact and control limbs. This disuse was accompanied by $\sim 60\%$ lower MVT and $\sim 75\%$ lower RTD in the amputated limb, which are much greater differences than might be predicted from short-term disuse studies. Declines in MVT appeared to be largely attributable to reduced muscle size (evidenced by lower muscle thickness in AMP) and neural drive (evidenced by lower VA and EMG\textsubscript{MVT} in AMP). Declines in RTD appeared to be attributable primarily to declines in MVT and a shift towards slower intrinsic contractile properties, with neural drive in explosive contractions being unaffected in AMP.

4.1 | Transtibial amputees as a model for long-term disuse

In the present 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 that the amputated limb undergoes substantially less habitual loading during ambulation. The reduced knee-extensor moments in gait may also be attributable, in part, to increased co-contraction at the knee of 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 changes in strength in the amputated limb. Consistent with our results, previous studies have reported decreased knee moments (Powers, Rao, & Perry, 1998; Winter & Sienko, 1988), power (Powers et al., 1998; Winter & Sienko, 1988) and work (Silverman & Neptune, 2012) in the amputated limb in walking. In contrast to these previous studies, however, the ITTAs of the present study were young, healthy and moderately to 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 independently to 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 with the amputated limb, from which to draw conclusions about the effects of chronic disuse.

### 4.2 Changes in strength

The declines in MVT found in the amputated limb compared with the intact limb (−59%) are comparable to, albeit at the high end of, differences observed in previous studies of amputees (−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 after unloading, plateauing after ~90 days; however, the results of the present study suggest that the reductions in strength with longer-term disuse are considerably more than could be predicted from short-term intervention studies.

To our 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 unilateral lower-limb suspension, respectively. The considerable reductions in peak RTD (−75%) in the amputated versus intact limb are important, because RTD is considered more functionally relevant than MVT in many sport-specific and daily tasks, such as sprinting, jumping and recovery of balance (Behan et al., 2018; Pijnappels et al., 2008; Tillin, Pain, & Folland, 2013).

When expressed relative to MVT, peak RTD was significantly reduced in the amputated compared with the non-amputated limbs, although the differences between limbs 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 contributed to the reduction in peak RTD, which was probably also influenced by the slowing of the contractile properties (discussed in more detail below, Section 4.3.3).

### 4.3 Mechanisms of strength differences

#### 4.3.1 Neural drive

A broad suppression in neuromuscular activity at maximal force production, indicated by a reduction in VA (−44%) and EMG\(_\text{MVT}\) (−38%) in the amputated compared with the non-amputated limb, is likely to contribute to the reduction in amputated limb MVT. 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), whereas others have not observed changes in these measurements (Campbell et al., 2013; de Boer et al., 2007; Horstman et al., 2012), after periods of disuse of ≤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 suggests a crossover effect of neural function, in that neural drive adaptations occur in the contralateral limb in addition to the injured/trained limb (Bogdanis et al., 2019; Tillin, Pain & Folland, 2011). In the present 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 might negate any crossover 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 maximal force production, no such differences were observed in the present 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 (Folland et al., 2014; Vecchio et al., 2019). The large variability in EMG, even after normalization to \(M_{\text{max}}\) (Buckthorpe, Hannah, Pain, & Folland, 2012), greater variability in RTD compared with MVT (Folland et al., 2014; Tillin et al., 2013) and small sample sizes \((n = 9\) per limb) might have reduced the chances of observing a significant effect. Alternatively, the role of the amputated limb in ambulation might explain the lack of differences in neural drive during the explosive contractions. Specifically, although the knee extensors of the amputated limb experience reduced load compared with the intact side during ambulation, the amputated side does contribute to stability and postural correction, for which RTD appears to be important (Behan et al., 2018). Thus, typical physical activity in the amputees might provide sufficient stimulus to maintain the neural drive during short, rapid contractions, which typically underpins RTD.
4.3.2 | Muscle architecture

The VL muscle was 41% thinner in the amputated limb compared with the intact limb. 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). Individuals with unilateral transtibial amputations walk with a comparatively stiff knee joint on the amputated limb (Powers et al., 1998; Winter & Siemko 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 might reduce maximal shortening velocities and power (Blazevich & Sharp 2005) and shift the torque–angle relationship towards more extended knee positions (Blazevich et al., 2009). Given that our strength measurements were made at a typical plateau region of the torque–angle relationship (Chow, Darling, & Ehrhardt, 1999), a shift away from this region in the amputated limb might have 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 unilateral lower-limb suspension (Campbell et al., 2013; de Boer et al., 2007), 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 deg (Blazevich et al., 2009; Rutherford & Jones 1992); the pennation angle of all three groups of limbs in the present study (~12–14 deg) falls within this range. This suggests that the structural remodelling that seems to take place in the early phases of disuse is not representative of long-term adaptations. It is possible that muscle thickness declines at a faster rate than fascicle length with short-term disuse, causing a decline in pennation angle, whereas over longer periods of disuse, reductions in fascicle length ‘catch up’ with muscle thickness loss, causing a return to baseline pennation angle, but this hypothesis cannot be tested with our data.

4.3.3 | Intrinsic contractile properties

The significant reductions in evoked (twitch and octet) contractile peak torque in the amputated compared with 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 control subjects and pathological populations, which have reported a shift towards faster contractile properties owing to a greater expression of fast-contracting myosin heavy-chain (MHC) isoforms (Bamman et al., 1998; Kapchinsky et al., 2018; Trappe et al., 2004). The results of the present study therefore provide new 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, Rice, Connelly, & Vandervoort, 1999). This slowing might be attributable 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 in old age (Lexell, Taylor, & Sjöström, 1988). The slower contractile properties in the amputated limb probably contributed to the lower voluntary peak RTD also observed in the amputated limb, because twitch and octet RTD are important determinants of voluntary RTD (Folland et al., 2014).

4.4 | Conclusion

This study was the first to use ITTAs as a 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 those of a control able-bodied limb, suggesting that the intact limb provides a suitable internal control for comparison with 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 probably attributable to the declines in muscle size and neural drive, whereas the reductions in RTD appeared to be attributable to the decline in MVT coupled with a slowing of the contractile properties.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

Data collection was performed in the biomechanics laboratory at the University of Roehampton, Whitelands College. All authors conceived and designed the research. A.R.S. and S.C.M. performed experiments. A.R.S. analysed the data. A.R.S. and N.A.T. interpreted results of experiments and drafted the manuscript. All authors edited and revised the manuscript, approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work...
are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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