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MUSCLE-TENDON MORPHOLOGY AND FUNCTION FOLLOWING LONG-TERM EXPOSURE TO REPEATED AND STRENUOUS MECHANICAL LOADING

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Running Title: Chronic strain and muscle-tendon function

ABSTRACT

We mapped structural and functional characteristics of muscle-tendon units in a population exposed to very long-term routine overloading. Twenty-eight military academy cadets (age: 21.00 ± 1.1 yrs; height: 176.1 ± 4.8 cm; mass: 73.8 ± 7.0 kg) exposed for over 24 months to repetitive overloading were profiled via ultrasonography with a senior subgroup of them (n = 11; age = 21.4 ± 1.0 yrs; height = 176.5 ± 4.8 cm; mass = 71.4 ± 6.6 kg) also tested while walking and marching on a treadmill. A group of eleven ethnicity- and aged-matched civilians (age = 21.6 ± 0.7 yrs; height = 176.8 ± 4.3 cm; mass = 74.6 ± 5.6 kg) was also profiled and tested. Cadets and civilians exhibited similar morphology (muscle and tendon thickness and cross-sectional area, pennation angle, fascicle length) in 26 out of 29 sites including the Achilles tendon. However, patellar tendon thickness along the entire tendon was greater ($p<0.05$) by a mean of 16% for the senior cadets compared with civilians. Dynamically, cadets showed significantly smaller ranges of fascicle length change and lower shortening velocity in medial gastrocnemius during walking (44.0% and 47.6%, $p<0.05$ - 0.01) and marching (27.5% and 34.3%, $p<0.05$ - 0.01) than civilians. Furthermore, cadets showed lower normalised soleus electrical activity during walking (22.7%, $p<0.05$) and marching (27.0%, $p<0.05$). Therefore, 24-36 months of continuous overloading, primarily occurring under aerobic conditions, leads to more efficient neural and mechanical behaviour in the triceps surae complex, without any major macroscopic alterations in key anatomical structures.

Keywords (3-8): military; overloading; marching; triceps surae; medial gastrocnemius; patellar tendon; ultrasound; fascicle mechanics

1. INTRODUCTION

Although the morphological, cellular, and neural mechanisms of muscular adaptations have been described and explained in detail,[1,2] the literature regarding muscle-tendon architectural adaptations has developed considerably more recently with advancements in medical imaging technology. Adaptations in human muscle-tendon architecture, material properties and mechanical behaviour due to mechanical overloading have been observed and linked to a range of training regimens and habitual overloading, with patellar and Achilles tendons (PT, AT) and their attached muscle groups receiving most of the research attention.[2-8] Although a primary scientific consensus regarding short to medium exposure (<20 weeks) to external overloading and its effects on knee and ankle musculotendinous structures has been gradually formulated,[2,5,6,9] the evidence on long-term exposure is more variable. The studies examining adaptations to prolonged overloading (>6 months) have mainly targeted specific populations (e.g. long-distance runners) who had undergone some years of habitual overloading due to their routine physical activity.[7,10-18] This is understandable as long-term monitoring designs with pre-post measurements pose considerable challenges to the researcher. However, because of the large methodological heterogeneity surrounding these longer-term studies (population types, age differences and prospective vs. retrospective designs), additional investigation is needed to enrich our understanding.

A similar picture exists in the literature regarding the function of lower body muscle-tendon units (MTUs) with most of the studies focusing on acute or short- to mid-term adaptations with respect to the function of the triceps surae complex.[9,19-23] The triceps surae MTU has been studied extensively via ultrasonography during walking and running with the scientific consensus pointing to an efficient pattern of interaction between muscular and tendinous tissues that facilitates gait at low energetic cost.[24-26] However, research concerning the effects of long-term training and/or habitual overloading on the behaviour of tendinous and contractile structures supporting the gastrocnemius and soleus (SOL) muscles is scarce. The few studies in this area have specifically examined such long-term effects on MTU gait efficiency in trained runners under walking, running and isometric conditions.[27,28,29] However, this is a very limited sample to answer a research question so pertinent to several every day and competitive settings, which rely on economical MTU function to transport the body over long distances.

Obviously, apart from scientific interest, the mapping of the chronic structural and functional adaptations of MTUs, in particular those of the lower body, has an applied facet, especially for population groups negotiating high volumes of overloading on a daily basis for years. A good example of such a population is military personnel undergoing strenuous physical activity as part of their occupation in harsh and varying environments. Professional armed forces undergo a multidimensional and demanding physical activity programme during the early years of their career consisting of a large range of long duration activities on tough terrains with most of them requiring the carriage of substantial external load.[30,31] Furthermore, army units employ military-style marching as the most common form of gait to cover large distances in a short time but also as an essential part of training for exhibition drills and parades. Marching places substantial mechanical loads on the lower limbs, similar to or greater than those experienced during moderate running.[32] The effects of such loading are exacerbated with fatigue due to the deterioration of local muscular control.[33] It is therefore not surprising that marching has been identified as one of the principal activities associated with injuries in army populations.[34] Although research in military exercise and medicine is well developed, most studies are restricted to the effects of basic military training and short missions on physical fitness and injury incidence or the long-term effects of military life on physical and body composition characteristics.[30,35,36] As for marching, the key studies have focused on the acute effects of load carriage on energetic cost[31,37] and joint kinematics.[38] Attempts have also been made to capture advanced neuromuscular and gait data after heavy load carriage missions[30] but still with the focus on the acute effects of such extreme conditions.

Taking these considerations into account, an investigation into the chronic structural and functional properties of asymptomatic muscle-tendon structures in military personnel will strengthen the relevant military literature by providing a unique insight into the morphology and mechanics of key MTUs, especially those supporting lower-limb function. However, through the quantification of the above properties the present study aspires as an ultimate aim to offer original information to the wider body of literature with respect to adaptive responses of the human MTU to prolonged and repeated overloading. To this end we explored potential chronic adaptations in the structure and function of major limb muscles and tendons in a group of professional Army Cadets by comparing them with ethnicity- and age-matched civilians. Our hypothesis was that adaptations in structural and/or mechanical properties of the cadets' MTUs were responsible for more efficient function by the triceps surae complex during gait.

2. MATERIALS AND METHODS

2.1. Participants

A total of 39 men participated in the study. Three subgroups were randomly selected from wider populations as follows: 14 military academy 3rd year (Y3) officer cadets (age: 20.9 ± 1.4 yrs; height: 175.9 ± 5.1 cm; mass: 74.7 ± 5.0 kg); 14 military academy 4th year (Y4) officer cadets (age: 21.1 ± 0.6 yrs; height: 176.4 ± 4.5 cm; mass: 72.8 ± 8.7 kg); 11 healthy male civilians (age: 21.6 ± 0.7 yrs; height: 176.8 ± 4.3 cm; mass: 74.6 ± 5.6 kg). All cadets were attending a 4-year full-time education programme (44 weeks per year) consisting of theoretical and practical sessions at the Hellenic Army Academy whereas the civilians were 4th year university Physical Education students at the Aristotle University of Thessaloniki.

The typical daily physical training programme for the cadets involved running (twice a day), strength training-callisthenics, marching and military activities with and without additional load (e.g. weaponry) and on many occasions by wearing military footwear. Sprint-agility exercises and non-tactical hikes were also performed once a week. Furthermore, there were periods (in total 6 weeks) during the year where the cadets engaged in special training (winter, land and urban warfare, and parachute operations) under extreme conditions (e.g. freezing temperatures) and on tough terrains (e.g. rocky landscapes). To quantify the daily volume of physical activity (PA) and energy expenditure (EE) for all 28 cadets, each cadet wore a SenseWear Pro Armband (BodyMedia, Inc., Pittsburgh, PA, USA) for two periods of three days each (one per semester). The mean group value for total daily EE was 4094 ± 686 kcal, with 5.9 ± 1.01 hrs of >3 METs PA and 26893 ± 3884 steps. Therefore, each cadet on average underwent a daily volume of 21.5 km of walking/marching/running/training, equating to a mean annual volume of over 6,600 km.

The civilians were all highly active and members of the university's first football team who trained daily, as they were also playing semi-professionally for regional football clubs. As all participants were of Greek descent and entered the academy or university straight after high school studies (the year 4 cadets were from the same school cohort as the civilians), they offered a highly analogous sample for comparisons. The study received ethical approval from the Carnegie Faculty Research Ethics Committee of Leeds Beckett University. Following oral and written communication about the purpose and procedures of the study, all participants provided written informed consent.

2.2. Morphological Measurements

A Siemens Acuson P300 (Siemens Healthineers AG, Erlangen, Germany) B-mode ultrasound system with a 50 mm linear array probe (5 to 12 mHz) was used to take transverse scans from the following sites: anterior upper arm [at 60% length from acromion], posterior upper arm [at 60% length from acromion], lateral forearm [at 30% length from head of radius], anterior thigh [at 50% length from greater trochanter], posterior thigh [at 50% length from greater trochanter], lateral lower leg [at 30% length from tibial lateral condyle], anterior lower leg [at 30% length from tibial lateral condyle], posterior lower leg [at 30% length from tibial lateral condyle], and longitudinal scans from vastus lateralis (VL) [at 50% length from greater trochanter], medial gastrocnemius (MG) [at 30% length from tibial lateral condyle], lateral gastrocnemius (LG) [at 30% length from tibial lateral condyle], tibialis anterior (TA) [at 30% length from tibial lateral condyle], and SOL [at 50% length from tibial lateral condyle]. Longitudinal scans were also taken from the PT (12 mHz) and AT (40 mm linear array probe, 18 mHz) as follows: PT images were taken proximal to the patella at 25%, 50% and 75% of tendon length while AT images were taken 30, 40 and 50 mm proximal to the calcaneal insertion as well as at the calcaneal notch. Transverse images of the AT were taken 40 mm proximal to the calcaneal insertion to allow cross-sectional area (CSA) measurements. Finally, AT resting length was measured from the most distal point of the MG muscle-tendon junction to the tendon's insertion into the calcaneus. Three images per site were taken from the right side with all participants (n = 39) in a standing position apart from the tendon measurements where a seated (knee) and a prone (ankle) position was assumed with the joints in a neutral position. All scans were performed by an experienced operator who positioned the probe on the skin via acoustic gel and by applying minimal pressure to the underlying tissues. Ultrasound images were analysed using ImageJ (ImageJ, 1.55p, 64-bit, National Institutes of Health) with mean values per site used for statistical analysis. Muscle thickness was measured from the deep to superficial aponeuroses with tendon thickness measured from the superficial to deep edges of the tendon. The angle between the deep aponeuroses and a fascicle that met the deep aponeuroses was defined as the pennation angle. Fascicle length was established through trigonometry using the pennation angle and muscle thickness. Tendon CSA was measured by using a freehand drawing tool to outline the tendon area. Within-day test-retest repeatability expressed as Intraclass Correlation Coefficient (ICC 3,1) for all scanned sites ranged from 0.907 (VL pennation angle) to 0.999 (posterior lower leg thickness), whilst 48-hrs test-retest repeatability of identical scans ranged from 0.927 (VL pennation angle) to 100% absolute agreement for several sites (e.g., anterior upper arm thickness).

2.3. Functional Measurements

A subgroup of the Y4 military cadets (n = 11; age = 21.4 ± 1.0 yrs; height = 176.5 ± 4.8 cm; mass = 71.4 ± 6.6 kg) and the 11 civilians underwent the dynamic measurements. Participants were all habitual treadmill users and wore their normal training clothing and footwear. To become familiar with the specific testing conditions, participants initially walked on a motorised treadmill at a speed of 4.5 km/h and 0% gradient for at least 5 min before moving to a marching speed of 6 km/h for a further 5 min habituation bout. The 6 km/h speed was selected to match the upper end of the marching pace regularly required by the military rules.[37]

To allow knee and ankle joint angles to be calculated during gait trials, reflective markers were placed over the greater trochanter, lateral femoral condyle, lateral malleolus, with two additional markers placed between the trochanter and the femoral condyle, as the trochanter marker was occasionally blocked by a sidebar on the treadmill. Two-dimensional marker trajectories were recorded using a Fastec camera (TS3; Fastec Imaging, California, USA) positioned perpendicularly to the participants' right side and sampling at 100 Hz. The resolution of the camera was 1280 x 1024 pixels, and extra illumination was provided by two spotlights of 1000 W each. Bipolar electromyography (EMG) electrodes (Trigno wireless; Delsys, Massachusetts, USA) were positioned over the MG, SOL and TA muscles with an inter-electrode distance of 10 mm. Data were sampled wirelessly at 2 kHz via EMGworks software (Delsys) and stored in a computer for subsequent processing. Before electrode placement, the skin was shaved and cleaned with alcohol to decrease impedance from the outermost skin layers. An ultrasound device (Siemens Healthineers AG, Erlangen, Germany) was used to examine muscle fascicle lengths at a sampling rate of 42 Hz. The probe (7.5 MHz, 50 mm) was positioned over MG so that SOL fascicles were also visible and attached firmly with an elastic bandage. All data were synchronized using a common 5 V trigger pulse. Step cycle events were determined visually based on video data.

During all trials, ultrasound, EMG and kinematic data were recorded synchronously. Each speed was maintained for at least 4 min before data collection to allow for adaptation. Because of the strict military rules, cadets performed the gait experiment separately at their base on a SportsArt T655L treadmill (SportsArt, Mukilteo, WA, USA) whereas PE students walked and marched on a h/p/Cosmos Pulsar treadmill (h/p/Cosmos, Nussdorf-Traunstein, Germany). Absolute agreement between treadmill belt speeds was ensured by calibrating treadmills via a marker placed on the belt and filmed at 100 Hz to obtain the true belt speed for each treadmill. Considering the low intensity of gait, we did not anticipate any effect of the belt's length and width on locomotion. Reproducibility of fascicle tracking between treadmill conditions was considered for a sequence of five steps of the same participant with the MG and SOL fascicle lengths showing ICC (3,1) and root mean square differences (RMSD) of 0.939 and 0.79 mm, and 0.918 and 0.56 mm respectively.

Reflective marker trajectories were tracked semi-automatically using SIMI software (Simi Reality Motion Systems GmbH, Unterschleissheim, Germany). EMG data were band-pass filtered online at 20-450 Hz and DC offset corrected. The cumulative muscle activity required to traverse a unit distance (CMAPD) was calculated for each muscle based on a modified version of the method of Carrier et al.[39] Individual strides were first identified, and those 20% above or below mean stride duration were excluded. Root mean square (RMS) EMG was then calculated for each stride. Finally, to calculate CMAPD, RMS values were normalized to a travel distance of 1 km by dividing the RMS value for a given stride by walking/running speed. MG and SOL fascicle lengths were determined using a semi-automated tracking algorithm validated previously in walking and running,[40,41] and fascicle velocities were obtained by differentiating length with respect to time. MG and SOL MTU lengths were determined by combining knee/ankle joint kinematic data with the equations of Hawkins and Hull.[42] Kinematic, EMG and fascicle data were averaged from 6-12 steps per condition and participant. Figure 1 shows a typical example of joint kinematics and fascicle behaviour during walking and marching.

FIGURE 1 HERE

2.4. Statistics

The following parametric statistical techniques were selected as the data met the assumptions of normality, independence, and homogeneity of variance and covariance. Anthropometric and static ultrasound variables were compared between the three groups using a one-way analysis of variance (ANOVA) followed by Tukey HSD tests where appropriate. For the AT and PT data a two-way mixed model ANOVA (site x group) was adopted. Dynamic EMG and fascicle data were compared between groups and conditions using a two-way mixed model ANOVA (condition x group) to establish whether there were any significant differences between the two gait speeds, the two groups and any interaction effects for each variable. All statistical tests were carried out using IBM SPSS statistics (version 24; Chicago, USA) and the significance level for all tests was set at $p < 0.05$.

3. RESULTS

3.1. Morphological Properties

The comparison amongst the three groups showed a homogeneous profile for all general characteristics. A similar picture of homogeneity amongst the groups was observed for most of the architectural characteristics derived from muscular sites of the upper and lower body (Table 1). Significant differences ($p<0.05$) were noted only for anterior leg thickness between the two cadet groups and the pennation angle for LG between Y3 cadets and civilians.

TABLE 1 HERE

Table 2 presents tendon measured characteristics for the three groups. All tendons in all three groups were asymptomatic. There were no differences between groups for the AT, with all groups showing the same thickness trend. There was a significant main effect ($p<0.01$) for the "site" factor, with thickness at the calcaneal notch being lower compared with the proximal sites by a cross-group mean of 15%. On the other hand, significant group differences ($p<0.05$) were noted for the PT, with the Y4 cadets exhibiting increased thickness across sites compared with civilians by a mean of 16%.

TABLE 2 HERE

3.2. Functional Properties

The 4.5 km/h walking condition displayed a greater knee joint range of motion during the stance phase when compared with the 6.0 km/h marching condition (*cadets*: walk: 53.2 ± 4.7°, march: 48.6 ± 6.0°; *civilians*: walk: 56.4 ± 7.7°, march: 48.6 ± 8.3°), irrespective of group ($p<0.01$). There was no difference between civilians and cadets in either condition. Additionally, ankle joint range of motion did not display any differences between the two conditions or between groups.

FIGURE 2 HERE

Figure 2 displays the time-series MTU and fascicle length changes during walking and marching for both groups, observably showing a net lengthening in the whole MTU, but a net shortening of the muscle fascicles during the stance phase of walking and marching in both muscles for both groups. A significant effect of condition was found for the MTU range of motion for MG (*cadets*: walk: 5.13 ± 0.59 cm, march: 5.36 ± 0.47 cm; *civilians*: walk: 4.96 ± 0.41 cm, march: 5.05 ± 0.52 cm) with no significant effect of condition or group for SOL (*cadets*: walk: 4.87 ± 0.59 cm, march: 4.83 ± 0.50 cm; *civilians*: walk: 4.75 ± 0.47, march: 4.63 ± 0.54 cm). However, the cadet group had a lower fascicle range in MG in both the walking (44.0% difference; Figure 3; $p<0.01$) and marching (27.5% difference; Figure 3; $p<0.01$)

conditions. No significant differences between groups were observed for SOL fascicle range for both gait conditions.

FIGURE 3 HERE

In addition to the lower fascicle ranges, the cadets also had a lower mean fascicle velocity during the stance phase for the MG in both the walking condition (47.6% difference; Figure 3; $p<0.05$) and marching condition (34.3% difference; Figure 3; $p<0.05$). Mean fascicle velocity in SOL was not significantly different between groups with a tendency for marching ($p = 0.058$) to produce higher fascicle velocities than walking (Figure 3).

Normalised EMG activity was higher in the MG during walking than marching, irrespective of group (Figure 4; $p<0.05$). However, the opposite trend was seen in the SOL, as marching required greater activity than walking, irrespective of group (Figure 4; $p<0.01$). There was no significant difference between groups for MG activity in either condition (Figure 4). However, the cadets had a lower SOL activity during walking (22.7% difference; Figure 4; $p<0.05$) and marching (27.0% difference; Figure 4; $p<0.05$). The TA activity showed no effect of group or condition, although a trend towards lower activity magnitudes in the cadets was observed (Figure 4).

FIGURE 4 HERE

4. DISCUSSION

4.1. Morphological Adaptations

Comparisons of muscle architecture between civilians and army cadets revealed no clear differences at most upper and lower limb measurement sites. Furthermore, the two cadet groups displayed very similar morphology for all sites apart from a difference in the anterior leg where Y4 cadets exhibited greater thickness. Therefore, the conclusion from this study about muscular architectural changes is that the occupational-specific overloading experienced by the cadets did not influence their muscular morphology. Some support for this finding can be extracted from past studies comparing muscle architecture between long distance runners with controls. Kubo et al[11] and Karamanidis and Arampatzis[15] suggested that chronic endurance-running exercise (volume of 30-100 km per week for 10 years for Karamanidis and Arampatzis[15] study) did not instigate any morphological changes (muscle thickness, pennation angle, fascicle length) for VL, vastus intermedius, rectus femoris and MG muscles.

Regarding tendon morphology, the thickness of the AT at the calcaneal notch site was lower than those at the sites proximal to the notch (30, 40 and 50 mm), a thickness pattern observed previously in heathy adults.[43] This was noted for all three groups with no between-group differences for any of the sites or tendon CSA. Moreover, Y4 cadets had greater thickness compared with civilians for all sites along the PT length with no differences noted between the two cadet groups. It has been shown previously that either short-term overloading,[3,44] long-term training or habitual overloading[7,16] of the PT results in increases in the tendon size. Such increases could be regionally sensitive with regions near the osteo-tendinous junctions, but also at the middle of the tendon’s longitudinal axis, showing greater hypertrophy.[3] Although the present study used a different (yet valid and reliable) method[45] from magnetic resonance imaging, the current findings agree in their entirety with these previous observations as the increased thickness for the Y4 cadets was observed at comparable tendon lengths. Considering the morphological similarities between cadets and civilians in all other 26 tested sites, including AT, these differences (15%) in PT thickness constitute a clear morphological distinction between the two populations. Since no dynamic measurements around the knee joint took place, it would be problematic to speculate about differences in behaviour of the quadriceps MTUs between cadets and civilians nor relate the changes in thickness to changes in the tendon stiffness. However, it would be safe to hypothesise that these thickness profiles are indicative of chronic adaptations due to the loading environment experienced by the cadets, as regular chronic physical activity of an endurance nature can affect collagenous tissues.[46] As mentioned in the methods, the cadets undergo a very strenuous physical programme on a daily basis, often in military gear, for 44 weeks per year, with the daily volume of activity exceeding 20 km. Unfortunately, no data are available for Y1-2 cadets but the progression in thickness values from Y3 to Y4, with the latter displaying significance against the civilians, indicate that these adaptations in tendon size follow a slow progressive rate. Tendons respond to overloading by increases in their size and/or mechanical properties.[15,46,47] This has been shown in studies employing short-term (≤14 weeks),[3,29,44] and long-term (12 months) resistance training[7] but it has also been supported by retrospective studies on populations exposed to habitual overloading.[12,16,17]

Although tendon hypertrophy has been linked with increased stiffness[3,29], there is also evidence suggesting that changes in material properties alone are responsible for changes in stiffness.[4,48,49] This means that it would be arbitrary to link the current changes in PT thickness with a parallel increase in stiffness, however, this increased thickness has to be considered as a positive adaptation, possibly contributing to muscle-tendon function and probably acting as a prophylactic mechanism to tendon damage due to a larger area exposed to stress.[3,6,12,44]

The fact that no differences between civilians and cadets were found for the AT agrees with a series of past findings pertaining to tendon-specific structural and functional adaptations resulting from short-term resistance training and long-term habitual overloading. Combined evidence from a number of studies in athletic populations with controls suggest that the mechanical loading threshold for adaptations in the AT is somewhat higher than that of the PT in the same populations.[4,10,11,13,14,29,45,50] We can therefore deduce that the load exerted on the AT in the group of cadets was not sufficient to produce any changes in tendon thickness and CSA. This assumption does not rule out other reasons for the differential response by the two tendons, for instance if certain characteristics of the repetitive external load (e.g. line of action) had a more optimal effect on the PT, however we have no data to substantiate this.

4.2. Functional Adaptations

During both walking and marching, MG fascicles underwent a larger range of length changes in civilians, with greater net shortening at a higher velocity. On the contrary, there were no clear differences in SOL fascicle behaviour between groups. In general, civilians exhibited higher normalised muscle activity, and this was especially true in SOL. The MG dynamic fascicle behaviour is consistent with more economical function in this MTU for cadets, since they limited the changes in muscle length and the rate at which this happens.[24,25] It has been shown that by reducing length changes in the contractile components during locomotion, the muscle fascicles can contract at very low speeds,[24,26] which can reduce the metabolic cost of contraction and increase force generation because of the force-velocity relationship. Moreover, minimising fascicle length changes can make effective use of the elasticity of tendinous structures, which undergo considerable lengthening and then recoil whereas the muscle fascicles maintain a nearly isometric behaviour.[24,26] The current MTU range data for the biarticular GM supports the above, as the lack of differences in MTU lengths between the groups alongside the shorter range and lower shortening velocities of the cadets suggest a greater role of tendon-aponeurosis complex elasticity, whereas civilians might rely more on the contractile elements for performing the equivalent work.

The fact that significant differences were not found between the groups for SOL muscle-tendon mechanics is not an unexpected observation. These two muscles differ considerably in their architecture, composition,[51] and joint articulation roles. Thus, they can function differently by retaining a degree of mechanical and neural independence, as evidenced previously under static and dynamic conditions.[19,20,23,25,52,53] In addition to morphological and neural factors, this mechanical independence is also explained by the fact that whilst GM and SOL attach to the same outer tendon, the stiffnesses of their respective tendinous tissues might differ because of the differential responses of each muscle's aponeuroses to loading, with the aponeuroses possibly exhibiting a range of variable compliance.[8,19,54,55] The above knowledge together with the fact that our protocol was of rather short duration suggests that the exercise did not require SOL to become the major work generator, and therefore both groups maintained similar SOL mechanical behaviour.

However, it is important to highlight that the civilians exhibited greater normalised SOL EMG activity than the cadets during both walking and marching, implying a greater neural cost in this muscle. This is an important finding as it provides a further indication of possible positive adaptations in the cadet group resulting from their long-term exposure to occupational overloading. On the other hand, this pattern was not observed in GM at either speed. A secondary observation, evident in both groups, confirming the different roles of the two muscles during gait was the higher normalised EMG for MG for walking compared with marching with SOL displaying the reverse trend. Notable differences in activation costs during walking between the two muscles have been observed previously,[56] offering another important insight into the functional flexibility of different synergistic muscles.

In terms of the exact mechanism behind the differences in fascicle behaviour and EMG activity between the groups, we hypothesise that this is related to the chronic repetitive overloading experienced by the cadets.[12,18,57] We are unable to detect the location of these physiological adaptations, but it is reasonable to assume that changes in the elastic elements of the MTU (e.g. altered tendon and aponeuroses stiffness) might have facilitated the more economical function of the contractile elements of the cadets' triceps surae muscles. A further interesting finding of this study was that the cadets exhibited signs of more efficient neural and mechanical behaviour than the civilians during marching at a speed (6.0 km/h) that was more familiar because of their occupational demands, but also at the intermediate walking speed of 4.5 km/h where the energetic cost for heathy adults is expected to be lowest.[20,56] It therefore seems that possible adaptations in the cadets' neuromechanical function carry over to different walking speeds. This could mean that the well-known U-shaped relationship between walking speed and energetic cost may be altered after chronic military training, and this is worthy of further study.

4.3. Ecological Validity and Limitations

Although the current findings were obtained from gait on a motorised treadmill, we are confident that the observed differences between groups would have also been present whilst moving on hard ground surfaces and in different footwear, as previous research has demonstrated muscle contractile behaviour to be similar between treadmill and overground gait and under different shod conditions.[22] The second consideration about the ecological validity of our findings relates to the length of our protocol, which for logistical reasons was quite short. However, based on a series of previous studies examining the effect of prolonged gait on fascicle and neural behaviour of the triceps surae muscle group, it seems that fascicle behaviour remains nearly isometric throughout long durations (>30 min), with the tendinous tissues showing an increased compliance over time, whilst overall EMG activity remains similar through inter-muscle neural adjustments to compensate for muscle-specific compliance changes.[19,20] Therefore, we believe that both groups would have shown very similar neuromuscular behaviour if a longer protocol had been adopted, with the cadets retaining their advantageous neuromechanical efficiency. In fact, the energetic advantage could have even increased as SOL, because of properties discussed earlier, would have been expected to play the primary role of work generation to maintain the necessary ankle torque over the long period of time.[20,23,54]

The study undoubtedly contained limitations with the following being the most important: the study is retrospective, and all between-group comparisons are made at a single time without historical data on all three groups. Furthermore, we did not have the capacity to acquire daily physical activity data from the civilians, so we were unable to quantity their movement volume, which is expected to be lower than those of the cadets. However, the comparisons of control sites (e.g. upper limb) between groups and trends between year 3 and 4 cadets, as well as the fact that all groups were drawn from the same ethnic group, support the hypothesis of a similar baseline state for all groups. Second, the absence of tendon stiffness and muscle strength measurements of the triceps surae muscle-tendon complex limits our understanding of the whole spectrum of morphological adaptations, as well as our capacity to link the MTU and fascicle data with intrinsic qualities of the AT and its connecting muscles. Moreover, data from other key MTUs (e.g. knee joint) would have allowed a fuller picture of lower limb adaptions and synergies/compensations. For instance, evidence from Arampatzis et al[27] and Bohm et al[58] shows that a combination of a compliant quadriceps tendon and aponeurosis with VL fascicles contracting almost isometrically during walking and running can promote more economical movement by reducing metabolic cost. Finally, the ultrasound and kinematic techniques leading to the MTU length calculations were two-dimensional and could thus have resulted in some errors.

5. PERSPECTIVE

Our findings reveal, apart from the PT, no adjustments in muscle-tendon morphological properties for structures subjected to a particular type of chronic mechanical overloading. This does not rule out changes in tendon stiffness and material properties. However, the same overloading conditions promoted increases in PT thickness, an adaptation that could offer at least an additional prophylactic mechanism. On the other hand, the exposure to such prolonged overloading resulted in neuromechanical modifications in the cadet population that enhance the ability to perform occupation-specific and routine locomotion tasks at a lower muscle mechanical and neural cost. This was evident in the crucial triceps surae muscle group with prominent changes in MG. Such adaptations in military populations have not been studied previously. Regarding the broader meaning of our findings, we have measured the effects of 24-36 months continuous overloading, primarily occurring under aerobic conditions and involving mainly upright postures, on lower limb MTUs. The evidence collected strongly suggests that modulations in the triceps surae complex can lead to more efficient behaviour, at least in this muscle group, without any major macroscopic alterations in key anatomical structures.

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Figure captions

Figure 1. Example of knee and ankle joint kinematics and fascicle behaviour of medial gastrocnemius and soleus during the stance phase of marching at 6.0 km/h and walking at 4.5 km/h. Data are from a single participant in the cadet group. MG = medial gastrocnemius; SOL = soleus.

Figure 2. Group mean curves for medial gastrocnemius and soleus muscle-tendon unit length change and fascicle length change during the stance phase of walking and marching for both cadets and civilians. Length changes are expressed relative to their respective lengths at the instant of ground contact. MG = medial gastrocnemius; SOL = soleus.

Figure 3. Mean fascicle range and mean fascicle velocity during the stance phase of walking and marching for cadets and civilians. For mean fascicle velocities, a negative value indicates a net fascicle shortening. Dashed horizontal lines indicate significant differences at $p<0.05$. Solid horizontal lines indicate a significant difference at $p<0.01$. MG = medial gastrocnemius; SOL = soleus.

Figure 4. Normalised EMG activity for medial gastrocnemius, soleus and tibialis anterior during the stance phase of walking and marching for cadets and civilians. Dashed horizontal lines indicate significant differences at $p<0.05$. The solid horizontal line indicates a significant difference at $p<0.01$. MG = medial gastrocnemius; SOL = soleus; TA = tibialis anterior.

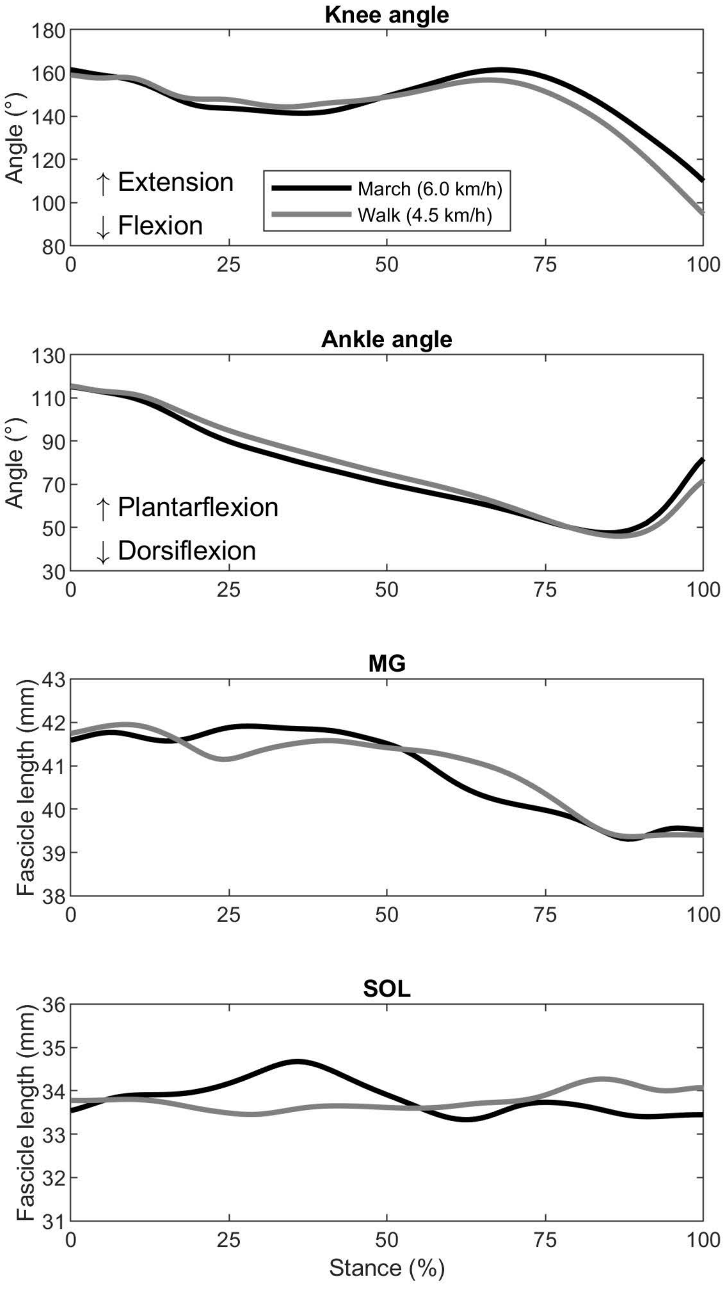

Knee angle
Angle (°)
180
160
140
120
100
80
↑ Extension
↓ Flexion
March (6.0 km/h)
Walk (4.5 km/h)
0
25
50
75
100
Ankle angle
Angle (°)
130
110
90
70
50
30
↑ Plantarflexion
↓ Dorsiflexion
0
25
50
75
100
MG
Fascicle length (mm)
43
42
41
40
39
38
0
25
50
75
100
SOL
Fascicle length (mm)
36
35
34
33
32
31
0
25
50
75
100
Stance (%)

Table 1. Muscular architectural characteristics for all three groups

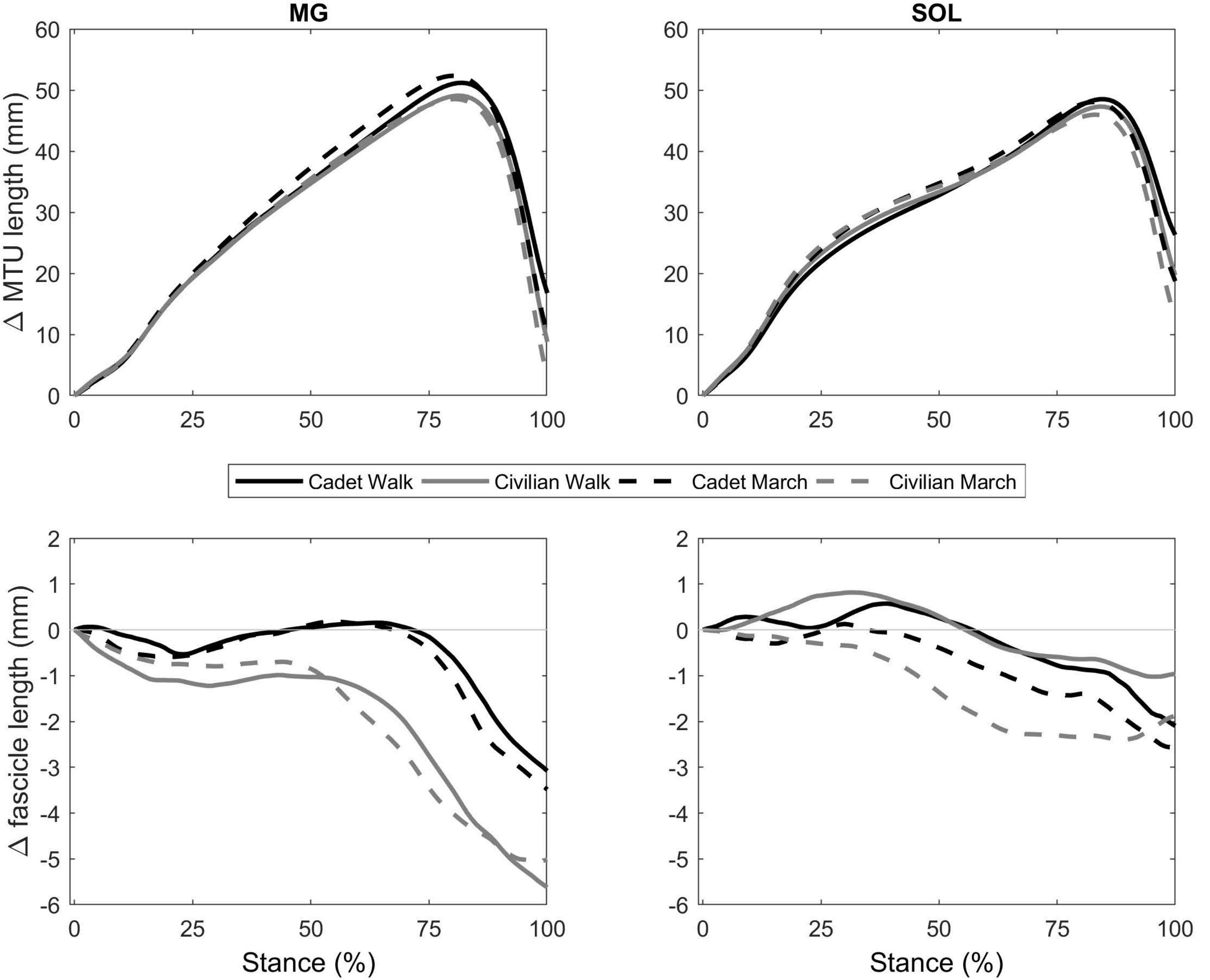
	Muscular Architectural Characteristics			
	Civilians (n=11)	**Cadets Year 3** (n=14)	**Cadets Year 4** (n=14)	***Group Differences***
Anterior Upper Arm TN† (mm)	34.22 ± 4.27	34.16 ± 3.96	35.83 ± 3.91	*ns*
Posterior Upper Arm TN (mm)	32.96 ± 3.74	33.56 ± 3.62	32.80 ± 6.16	*ns*
Lateral Forearm TN (mm)	20.76 ± 2.02	21.59 ± 2.47	22.77 ± 2.73	*ns*
Anterior Upper Thigh TN (mm)	57.81 ± 5.44	55.24 ± 5.50	57.54 ± 6.71	*ns*
Posterior Upper Thigh TN (mm)	65.45 ± 7.17	65.84 ± 5.13	66.84 ± 5.99	*ns*
Anterior Leg TN (mm)	30.58 ± 1.90	28.58 ± 3.65*	31.74 ± 2.70*	*Cadets 4 > 3*
Posterior Leg TN (mm)	67.31 ± 6.86	66.42 ± 10.13	72.95 ± 4.34	*ns*
Lateral Leg TN (mm)	24.21 ± 1.98	23.03 ± 2.45	23.74 ± 1.80	*ns*
Vastus Lateralis TN (mm)	26.05 ± 2.66	25.81 ± 2.88	25.52 ± 3.39	*ns*
Lateral Gastrocnemius TN (mm)	14.84 ± 2.96	16.21 ± 3.42	15.65 ± 2.98	*ns*
Medial Gastrocnemius TN (mm)	21.80 ± 2.69	23.21 ± 2.32	21.57 ± 2.70	*ns*
Tibialis Anterior TN (mm)	17.28 ± 2.51	17.51 ± 4.35	18.04 ± 3.94	*ns*
Soleus TN (mm)	15.83 ± 2.65	14.51 ± 2.49	14.59 ± 2.39	*ns*
Vastus Lateralis FL‡ (mm)	85.66 ± 10.28	85.10 ± 11.30	86.91 ± 12.23	*ns*
Lateral Gastrocnemius FL (mm)	65.18 ± 14.80	60.14 ± 15.04	64.19 ± 8.50	*ns*
Medial Gastrocnemius FL (mm)	63.20 ± 11.90	60.35 ± 12.27	57.11 ± 6.84	*ns*
Tibialis Anterior FL (mm)	87.14 ± 15.45	78.37 ± 25.16	92.43 ± 23.88	*ns*
Vastus Lateralis PA§ (°)	17.88 ± 1.59	17.70 ± 2.03	17.23 ± 2.24	*ns*
Lateral Gastrocnemius PA (°)	13.49 ± 1.65*	16.08 ± 3.02*	14.17 ± 2.05	*Cadets 3 > Civil*
Medial Gastrocnemius PA (°)	20.84 ± 4.46	23.02 ± 2.92	22.22 ± 1.56	*ns*
Tibialis Anterior PA (°)	11.64 ± 1.81	13.54 ± 3.15	11.40 ± 1.24	*ns*

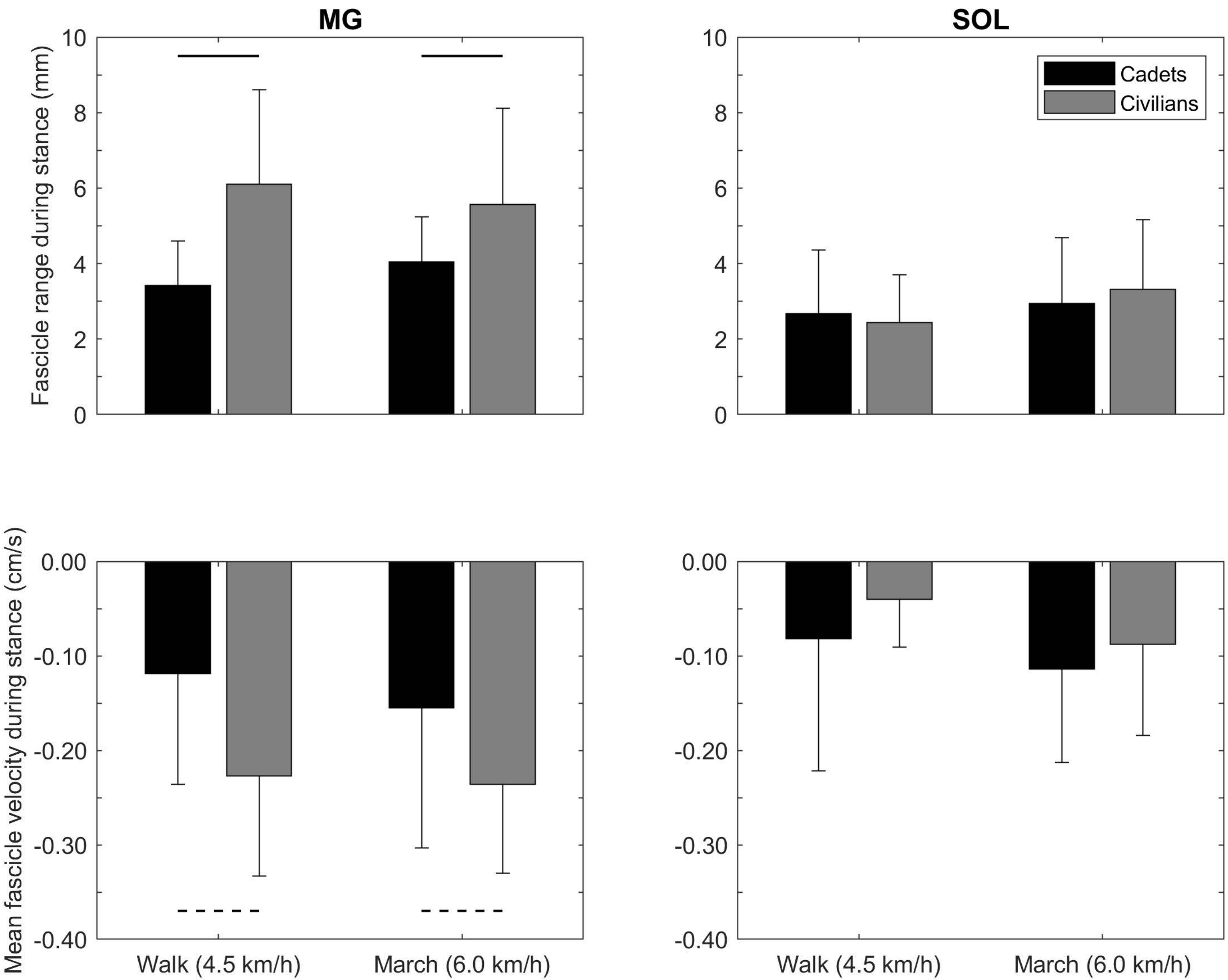
Values are means ± SD. †TN: Thickness; ‡FL: Fascicle Length; §PA: Pennation Angle. *p<0.05, ns: not significant.

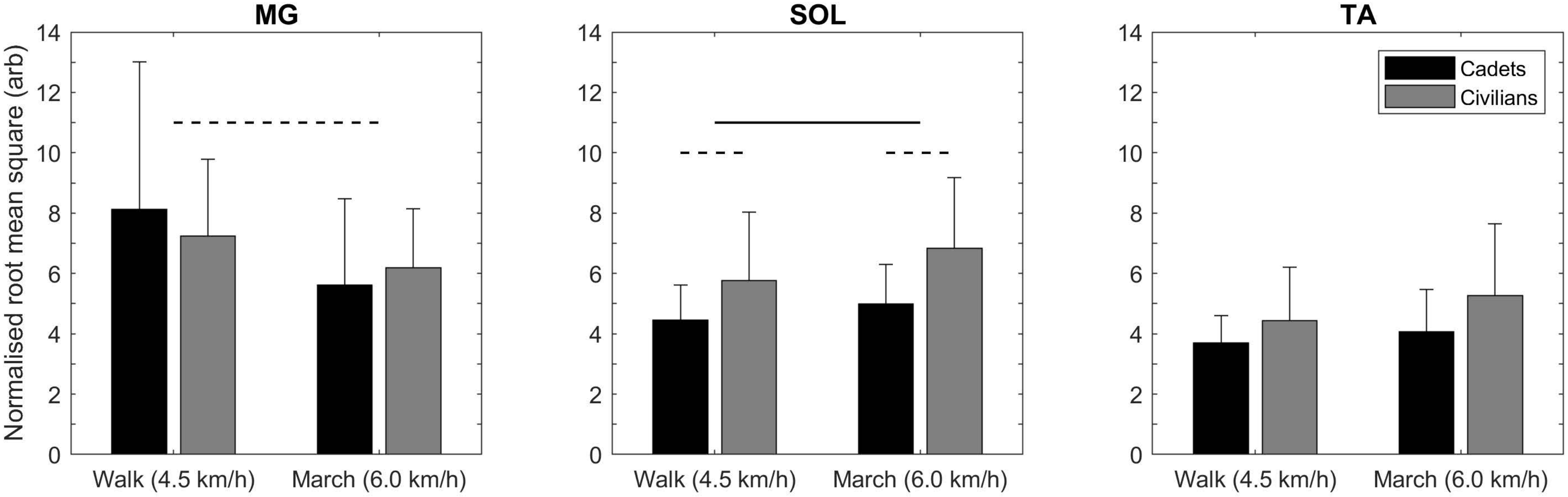
Table 2. Tendon architectural characteristics for all three groups

	Tendon Architectural Characteristics			
	Civilians (n=11)	**Cadets Year 3** (n=14)	**Cadets Year 4** (n=14)	***Group/Site Differences***
Achilles Tendon resting length (cm)	20.04 ± 2.31	20.35 ± 2.51	21.07 ± 0.62	*ns*
Achilles Tendon thickness - Calcaneal Notch (mm)	4.28 ± 0.83**	4.26 ± 0.76**	4.21 ± 0.79**	*Calc. Notch < 3,4,5cm*
3cm (mm)	4.94 ± 0.82	5.15 ± 0.60	4.91 ± 0.64	*ns*
4cm (mm)	4.98 ± 0.77	5.16 ± 0.68	4.96 ± 0.53	*ns*
5cm (mm)	4.91 ± 0.82	5.16 ± 0.75	4.90 ± 0.56	*ns*
Achilles Tendon cross-sectional area (mm^2)	50.74 ± 8.77	54.38 ± 4.43	57.47 ± 5.52	*ns*
Patellar Tendon thickness 25% (mm)	3.38 ± 0.30*	3.48 ± 0.58	3.84 ± 0.44*	*Cadets 4 > Civil*
50% (mm)	3.30 ± 0.30*	3.62 ± 0.70	3.93 ± 0.54*	*Cadets 4 > Civil*
75% (mm)	3.30 ± 0.24*	3.69 ± 0.73	3.82 ± 0.52*	*Cadets 4 > Civil*
Average[§] (mm)	3.33 ± 0.26*	3.60 ± 0.63	3.86 ± 0.46*	*Cadets 4 > Civil*

Values are means ± SD. For patellar tendon, 25-75% represent % of total length proximal to the patella;
[§] Average of three sites. *p<0.05, **p<0.01, ns: not significant.


MG
SOL
Δ MTU length (mm)
60
50
40
30
20
10
0
0
25
50
75
100
Cadet Walk
Civilian Walk
Cadet March
Civilian March
Δ fascicle length (mm)
2
1
0
-1
-2
-3
-4
-5
-6
Stance (%)
Stance (%)


MG
SOL
Fascicle range during stance (mm)
10
8
6
4
2
0
Cadets
Civilians
Mean fascicle velocity during stance (cm/s)
0.00
-0.10
-0.20
-0.30
-0.40
Walk (4.5 km/h)
March (6.0 km/h)
Walk (4.5 km/h)
March (6.0 km/h)


MG
SOL
TA
Normalised root mean square (arb)
0
2
4
6
8
10
12
14
Walk (4.5 km/h)
March (6.0 km/h)
Cadets
Civilians