Gastrocnemius medialis contractile behavior during running differs between simulated Lunar and Martian gravities

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Running Title: Contractile behavior during running on simulated Mars and Moon

Abstract

The international partnership of space agencies has agreed to proceed forward to the Moon sustainably. Activities on the Lunar surface (0.16g) will allow crewmembers to advance the exploration skills needed when expanding human presence to Mars (0.38g). Whilst data from actual hypogravity activities are limited to the Apollo missions, simulation studies have indicated that ground reaction forces, mechanical work, muscle activation and joint angles decrease with declining gravity level. However, these alterations in locomotion biomechanics do not necessarily scale to gravity level, the reduction in gastrocnemius medialis activation even appears to level off around 0.2g, whilst muscle activation pattern remains similar. Thus, it is difficult to predict whether gastrocnemius medialis contractile behavior during running on Moon will basically be the same as on Mars. Therefore, this study investigated lower limb joint kinematics and gastrocnemius medialis behavior during running at 1g, simulated Martian gravity and Lunar gravity on the vertical treadmill facility. The results reveal that hypogravity-induced alterations in joint kinematics and contractile behavior still persist between simulated running on Moon and Mars. This contrasts the idea of a ceiling effect and should be carefully considered when evaluating exercise prescriptions and the transferability of locomotion practiced in Lunar gravity to Martian gravity.

Keywords: hypogravity, Lunar gravity, Martian gravity, muscle fascicle behavior, series elastic element behavior, ultrasound imaging, running

Abbreviations

g	Gravitational acceleration
GM	Gastrocnemius medialis
ISS	International Space Station
MTU	Muscle–tendon unit
PTS	Preferred walk-to-run transition speed
SEE	Series elastic element
VTF	Vertical treadmill facility

Introduction

Human space exploration has fascinated humanity since the start of the space age in the 1950’s. Approximately 50 years after humans first set foot on the Moon, space agencies taking part in the international collaborative Artemis program have agreed to proceed forward to the Moon sustainably. Plans include to build the Lunar Orbital Platform−Gateway including a Human Lunar Lander and to set up a permanent surface habitat that may be a springboard for future human missions to Mars [1].

Although, Apollo missions showed that humans can effectively operate in Lunar gravity [2], with surface stay times up to 75 hrs [3], data collected during locomotion that provide useful information about biomechanical alterations required to enable surface activities and for the development of evidence-based exercise countermeasures are lacking. Leg muscles such as the gastrocnemius medialis (GM), that are largely involved in body support and forward acceleration [4], were observed to be particularly susceptible to atrophy and architectural changes induced by reduced loading [5,6]. Thus, on Earth but also on the International Space Station (ISS), running serves as a countermeasure as the forces that generate both skeletal and muscular loading provide important mechanical stimuli for the musculoskeletal system [7]. However, alterations in gravitational acceleration (g) appear to modify running gait. Thus, ground-based analogues have been developed to study locomotion in simulated hypogravity [8]. However, most hypogravity biomechanical studies have focused on identifying differences with Earth’s gravitational acceleration (1g) [9,10].

Studies investigating running at 1g and at simulated hypogravity levels broadly equivalent to Lunar and Martian gravity (0.16–0.40g) have indicated that the magnitudes of most gait parameters such as ground reaction forces [11,12], mechanical work [13], estimated joint forces [12] and muscle activation [12,14] are reduced with decreasing g-level. Similarly running kinematics such as ground contact times, cadence [11,12,15] and lower limb joint angles [15,16] tend to reduce with simulated g-level.

However, despite the fact that ankle dorsiflexion angles are smaller during running in simulated hypogravity, the ankle is reported to follow a similar joint movement profile [17]. Furthermore, the lower limb muscle activation patterns [12,14] and leg stiffness (considered as a linear spring) [11] are largely preserved.

Moreover, biomechanical parameters may not necessarily be proportional to the hypogravity level [18]. Indeed, GM is sensitive to changes in force loading evidenced by a reduction in muscle activation, although it appears that there might be a ceiling effect around 0.2g [14]. When running at simulated 0.7g, GM contractile behavior modulation has been observed. For instance, at peak series elastic element (SEE) length, where the force acting on the SEE is at its greatest, GM fascicles operated at longer lengths, with smaller pennation angles, but faster shortening velocities [19]. However, whether this pattern occurs in GM' muscle–tendon unit (MTU) at simulated Martian (0.38g) and Lunar gravity (0.16g) is unknown [9]. Thus, whether fascicle–SEE behavior is sensitive to low hypogravity levels, e.g. when running on the Lunar and Martian surfaces, remains to be determined. Such knowledge is important to assess the transferability of Lunar to Martian surface operations.

However, to compare conditions one must account for the fact that a decreasing g-level results in the walk-to-run transition occurring at slower absolute speeds, but with similar Froude numbers [20-22]. Thus, to investigate running at 'dynamically similar' speeds in simulated hypogravity (i.e., at a similar speed relative to the preferred walk-to-run transition speed, PTS) it is required to run at the same Froude number and hence at a slower speed [22,23].

Therefore, in order to determine whether hypogravity-induced modulation of GM fascicle–SEE interaction is sensitive to running at low hypogravity levels, we have required participants to run at 125% of the PTS at 1g in addition to simulated Martian gravity and Lunar gravity on the vertical treadmill facility (VTF).

Based on the findings of 0.7g running [19], it was hypothesized that at the time of peak SEE length, ankle dorsiflexion and knee flexion are both smaller, whilst GM fascicles are longer, less pennated and faster in shortening when running in simulated hypogravity vs. 1g. These alterations in joint kinematics and fascicle–SEE interaction are expected to persist between simulated Martian and Lunar gravity, although the question is by which extent, and whether absolute or relative differences in gravity between Moon and Mars surfaces dominate these alterations.

Results

Kinetic and spatio-temporal parameters

Participants running at predefined simulated hypogravity levels of 0.38g and 0.16g generated lower mean hypogravity levels, actually corresponding to 32.6% ± 10.3% and 14.8% ± 3.5% of the g-levels determined during running at 1g on a conventional treadmill. Running speeds corresponding to 125% of the participants' PTS, resulted in average running speeds of 2.62 $m \cdot s^{-1}$ ± 0.08 $m \cdot s^{-1}$ at 1g, 1.80 $m \cdot s^{-1}$ ± 0.05 $m \cdot s^{-1}$ at simulated Martian gravity and 1.50 $m \cdot s^{-1}$ ± 0.04 $m \cdot s^{-1}$ at simulated Lunar gravity.

There was a significant effect of g-level on peak plantar force, ground contact time, gait cycle duration, cadence and stride length (Table 1). Peak plantar forces were significantly reduced at both simulated Martian and Lunar gravity compared to 1g. At simulated Lunar gravity, peak plantar forces were significantly lower than during running at simulated Martian gravity (Table

2, Fig. 1a). Furthermore, ground-contact times and gait cycle durations were significantly longer at both simulated Martian and Lunar gravity vs. 1g. At simulated Lunar gravity, ground-contact times and gait cycle durations were significantly longer than at simulated Martian gravity (Table 2). Gait cadence was significantly reduced at both simulated Martian and Lunar gravity compared to 1g. At simulated Lunar gravity, participants ran at significantly lower cadence than at simulated Martian gravity (Table 2). In contrast, despite a significant effect of g-level, no significant post-hoc differences in stride length were observed between 1g and simulated Martian and Lunar gravity, as well as between Mars and Moon (Table 2).

[Please insert Fig. 1 here]

Joint kinematics

Average participant knee (Fig. 1b) and ankle (Fig. 1c) joint movement profiles (plotted as a function of stance phase) are suppressed when running at both simulated Lunar gravity, and Martian gravity vs. 1g.

There was a significant effect of g-level on ankle joint angle and knee joint angle at the time of peak SEE length (Table 1). Ankle dorsiflexion (Fig. 2a) and knee flexion (Fig. 2b) angles at peak SEE length were both significantly smaller during running at simulated Martian and Lunar gravity compared to 1g. At simulated Lunar gravity, the ankle joint was also significantly less dorsiflexed and the knee joint significantly less flexed than at simulated Martian gravity (Table 2).

[Please insert Fig. 2 here]

GM muscle and SEE parameters

GM muscle−SEE parameters such as MTU length (Fig. 1d), SEE length (Fig. 1e), fascicle length (Fig. 1f), pennation angle (Fig. 1g) and fascicle velocity (Fig. 1h) (plotted as a function of stance phase) were modulated when running at 1g vs. simulated Martian gravity and Lunar gravity.

There was a significant effect of g-level on GM fascicle length, pennation angle and fascicle velocity at the time of peak SEE length (Table 1). At the time of peak SEE length, fascicles operated at a significantly longer length (Fig. 2e) but smaller pennation angle (Fig. 2g) at both simulated Martian and Lunar gravity compared to 1g. However, no significant differences between simulated Martian gravity and Lunar gravity were observed (Table 2). In contrast, whilst fascicles shortened significantly faster (at the time of peak SEE length) at simulated Martian gravity compared to 1g, no significant differences were observed at simulated Lunar gravity vs. 1g. Fascicle velocity was significantly slower when running at simulated Lunar gravity vs. Martian gravity (Table 2).

Furthermore, there was a significant effect of g-level on SEE length, MTU length at the time of peak SEE length, as well as MTU elongation (Table 1). The time point at which peak SEE length was reached (51.5% ± 7.5%, 54.3% ± 4.0%, 52.8% ± 5.2% of stance at 1g, Martian gravity, Lunar gravity) did not differ between g-levels (Table 1). Peak SEE length (Fig. 2h) and MTU length at the time of peak SEE length (Fig. 2c) were significantly shorter during running at simulated Martian and Lunar gravity compared to 1g. During running at simulated Lunar gravity, both peak SEE length and MTU length at the time of peak SEE length were significantly shorter than during running at simulated Martian gravity (Table 2). MTU elongation (Fig. 2d) was significantly lower in both simulated Martian and Lunar gravity vs. 1g. However, no differences were observed between simulated Mars and Moon (Table 2).

There was also a significant effect of g-level on fascicle shortening, the delta in pennation angle and average fascicle velocity during SEE elongation (from touch down to peak SEE length) (Table 1). Whilst no significant differences in fascicle shortening (Fig. 2f) were observed between 1g and simulated Martian gravity, significant reductions were induced when running at simulated Lunar gravity compared to 1g and simulated Martian gravity (Table 2). Delta pennation angle and average fascicle velocity between touchdown and peak SEE length were both significantly reduced at simulated Martian and Lunar gravity vs. 1g (Table 2). During running at simulated Lunar gravity, delta pennation angle and average fascicle velocity were also significantly reduced compared to simulated Martian gravity (Table 2).

[Please insert Table 1 and 2 here]

Discussion

The plantar force data of the present study suggest that participants actually ran at slightly lower hypogravity levels than originally intended in the experimental set-up (0.32g vs. 0.38g and 0.15g vs. 0.16g). According to the systematic review by Richter, et al. [9] the observed hypogravity levels are still in the range that has been defined for simulated Martian gravity (0.3g–0.4g) and Lunar gravity (0.1g–0.2g). Therefore, and in light of the fact that this is a pilot study, we do not expect this deviation from the actual values for Lunar and Martian gravities to strongly affect the overall interpretation of our results.

The main findings were that spatio-temporal, joint kinematic and most muscle–SEE outcomes during running at 125% PTS are affected by g-level. Decreasing g-level from 1g to simulated Martian and Lunar gravity resulted in prolonged ground contact times, decreased cadence, smaller ankle dorsiflexion and knee flexion angles at the time of peak SEE length, shorter peak SEE length as well as lower delta in pennation angle and average fascicle velocity during SEE elongation. Fascicle shortening during SEE elongation did not differ between 1g vs. Martian gravity, but was significantly reduced in Lunar gravity vs. Martian gravity and 1g. These outcomes appear to be sensitive to low hypogravity levels and thus indicate that there may be a Martian vs. Lunar effect. In addition, albeit not statistically significant, at the time of peak SEE length, fascicles operated at longer lengths and smaller pennation angles in simulated Lunar gravity vs. Martian gravity.

Running in simulated Martian and Lunar gravity resulted in prolonged ground contact times and decreased cadence at constant stride length, whereas previous studies investigating running at approximately 3.00 $m \cdot s^{-1}$ at simulated hypogravity reveal shorter ground contact times [11,12,15,24,25] and increased stride lengths [24,25] compared to 1g. This contradicts the present results. However, it should be noted that in the present study, participants ran at almost half of these speeds (1.8 $m \cdot s^{-1}$ and 1.5 $m \cdot s^{-1}$ at simulated Martian and Lunar gravity), because running speeds were intentionally decreased with decreasing g-level by adjusting running speeds to the same Froude number. This was done to ensure that subjects run at similar speeds relative to the PTS, which are considered to be mechanically equivalent independent of the gravity level. The Froude number approach can thus be regarded as a prerequisite to compare the same participants at different levels of simulated hypogravity [22]. Moreover, running at the same Froude number usually produces equal relative stride length [26]. Thus, maintenance of stride length could be attributed to the present methodological approach of running at a mechanically equivalent speed at each g-level.

However, ankle and knee joint kinematics were modulated by hypogravity running, demonstrating modification in participants running pattern compared to 1g. We did indeed expect that ankle dorsiflexion and knee flexion at peak SEE length become smaller with lower simulated hypogravity levels, as similar findings have already been observed in previous hypogravity studies [15,17,24]. However, we did not expect that the small absolute difference in hypogravity level between simulated Martian and Lunar gravity would produce reductions in ankle dorsiflexion and knee flexion angles, which are almost as large as the reductions in these joint angles between 1g and Martian gravity. Nevertheless, when looking at the relative difference between the two hypogravity levels, the distinct changes in joint kinematic characteristics between simulated running on Mars and Moon are less surprising, given that Martian gravity is more than twice as much as Lunar gravity.

In the present study, participants' knee joint was less flexed the lower the hypogravity level, which supports the idea that participants adopt their running pattern due to the much lower energy absorption required with decreasing hypogravity levels [15]. In addition, the significantly smaller knee flexion angles at peak SEE length could also be the result of the reduced external work necessary to lift and forward-accelerate the body's centre of mass during simulated hypogravity running [13]. This effect could be even more pronounced by the fact that the present participants were not vertically but horizontally suspended on the VTF. Thus, participants presumably counteract their less flexed knee joints (that are likely the result of both, reduced g-level and unusual body position), by placing their ankle joints in a position of a smaller dorsiflexion. In fact, in the present study, despite a similar ankle joint angle at initial contact when running at simulated Lunar gravity vs. 1g, in the further course of the stance phase, ankle dorsiflexion angles were much smaller. This is also in accordance with previous hypogravity studies [15,24], which suggest that participants shift to a forefoot striking pattern [15].

Thus, from a joint kinematic point of view, running at simulated Lunar and Martian gravity is unequal to running at 1g, and running at simulated Lunar gravity differs from running at simulated Martian gravity, which in turn does not concur with the idea of a ceiling effect. This is further supported by the large effect sizes that were determined for lower limb joint angles.

As MTU lengths were calculated on the basis of ankle and knee joint angles it is not surprising that significant g-level effects were also observed for MTU lengths determined at the time of peak SEE length. The fact that MTU lengths become shorter during running at simulated hypogravity suggests that smaller ankle dorsiflexion compensates for the less-flexed knee joint, as it was already observed during simulated 0.7g running [19]. In addition to that, lower external forces acting on the SEE during hypogravity running presumably generate shorter peak lengths and thus confirm anticipated results that peak SEE length significantly decreases with hypogravity level. Shorter peak SEE lengths as a function of g-level point towards a reduced storage of elastic strain energy [27]. The smaller elastic stretch may thus also be a functional adaptation to the lower mechanical energy storage requirements during simulated running on Moon vs. on Mars [13].

Gastrocnemius medialis contractile behavior during running in simulated hypogravity appears more variable than joint kinematics or SEE length modulation. However, as expected, the present study showed that fascicles operated at longer lengths and smaller pennation angles in simulated Martian and Lunar gravity compared to 1g, similar to running in simulated 0.7g using the VTF [19]. Corresponding effect sizes for the comparisons to 1g were large.

Yet, contrary to the present hypothesis that significant alterations persist between Mars and Moon, fascicle length and pennation angle at the time of peak SEE length did not significantly differ between simulated Martian and Lunar running. This in turn suggests that for fascicle's operating length there might be a ceiling effect similar to that which was originally introduced by Mercer, et al. [14] for the reduction in muscle activation, which was limited around 0.2g. Albeit not statistically significant, at the time of peak SEE length, fascicles operated at 3 mm ± 3 mm longer lengths and 2° ± 2° smaller pennation angles in simulated Lunar gravity vs.

Martian gravity, still representing effect sizes of d = 0.5 and –0.4, respectively. Thus, further research using ultrasonography combined with measures of muscle activation and ideally including a larger sample size is warranted.

In terms of fascicle behavior, it should also be highlighted that during the SEE elongation (where muscular forces are naturally required to stretch the SEE and thus to store elastic energy), fascicle shortening, average shortening velocity and the delta in pennation angle were significantly reduced in hypogravity compared to 1g, but more importantly also between simulated Lunar vs. Martian gravity, as additionally indicated by the overall large effect sizes. Such alterations in GM contractile behavior in turn point to functional adaptations to hypogravity running.

For instance, a lower average shortening velocity, which may be associated with the longer ground contact times, suggests an enhanced force generation ability of the GM [28]. In 1g, GM contractile behavior adapts when switching from a walking to a running gait [29], however, no change in fascicle velocity is observed when running speeds are further increased [29,30]. The observation that the GM works on a similar part of the force-velocity relationship across various steady-state running speeds [29,30], however, appears to not account for conditions of simulated hypogravity where running speeds are intentionally decreased to run at the same Froude number. Thus, to state whether the observed decrease in fascicle velocity is solely attributed to the decrease in g-level or also by the decrease in running speed requires further studies.

As discussed above, shorter peak SEE lengths during hypogravity running might be part of the functional adaptation to the lower mechanical work output [13] (the muscle’s work or energy output is roughly proportional to cumulative SEE force multiplied by the change in muscle length). However, this is not the only adaptation that might influence the mechanical work output of the muscle. Reduced GM fascicle shortening alongside reduced delta in GM pennation angle is observed during the SEE elongation phase when reducing from simulated Martian to Lunar gravity. This means that the muscle shortening (the combined effect of fascicle length and pennation angle) also tends to be reduced at lower g-levels, which might be another way for the muscle to reduce its overall mechanical work output (by reducing not only the force, as described above, but also its change in length during every stance phase). Interestingly, when reducing simulated g-levels from Earth to Mars to Moon, peak SEE length (and thus implied SEE force) appears to reduce first, while fascicle shortening mainly reduces at lower g-levels (e.g. between Martian and Lunar gravity). This might be interpreted such that when reducing load, the muscle tends to reduce its mechanical work output first via reducing forces (and with it elastic energy stored in the SEE) before reducing its amount of shortening.

In fact, it appears that running in simulated hypogravity in-part impairs the MTU's stretch-shortening cycle. Plyometric-type exercises appear to be very effective to maintain stretch shortening cycle efficacy [31,32] by inducing relatively high vertical ground reaction forces and thus higher magnitudes of tissue strain [33]. For instance, peak vertical ground reaction forces have been revealed to be negatively related to simulated hypogravity level, but positively to hopping height. Moreover, submaximal hopping (> 15 cm height of flight) in simulated Lunar and Martian gravity is associated with forces similar to standing and running on Earth, respectively [32]. This may be why skipping and plyometric training, has been suggested to be the preferred gait on the Moon [13] and a promising countermeasure to prevent musculoskeletal deconditioning [32,33], respectively. One innovative gravity-independent countermeasure is spring-loaded horizontal jumping, but its applicability in space still needs to be evaluated [31].

In addition, it can be argued that reaching a terrestrial like fascicle–SEE behavior, and thus similar stimuli exerted on the GM muscle, is also a valid goal for effective running countermeasure exercises. In order to achieve this, the lower the hypogravity level, the more external loading needs to be applied as a compensation. In full microgravity, like on ISS, crewmembers strap themselves to a treadmill via a harness-based subject loading system [34]. In order to achieve terrestrial loading in such a setting, the crewmembers' full equivalent body weight force would have to be applied on their harness. However, due to harness discomfort, crewmembers typically limit their applied external loading to about 70% equivalent body weight [35], even if the bungee system would allow applying higher loads.

On Mars, crewmembers will be exposed to a force of 0.38g, which corresponds to 38% equivalent body weight. Therefore, a harness loading of around 60-70% bodyweight, which is similarly tolerable than the typical loading used on ISS [35], should therefore be able to effectively compensate for reduced gravity level and should result in an external loading that is in the range of full body weight on Earth. In Lunar gravity, the force of 0.16g acting on the crewmembers body will most likely not be sufficient to reach full body weight at a similar harness loading, only adding up to 75%-85% body weight. For a Lunar habitat scenario, if this resulting loading is regarded as too low, one might think about complementing the harness-based subject loading system by wearing an additional weight vest. However, to add a missing 15% equivalent body weight loading in Lunar gravity, such a weight vest would have to be in the mass range of the crewmember's own body mass, which will likely add strong discomfort through its inertial behavior in response to the crewmember's running motion. Nevertheless, determination of the optimal body weight loading in hypogravity conditions should be subject to further research. Additionally, future studies should also investigate whether crewmembers exposed to 0.16g could carry equipment that is approximately six times as heavy as on Earth without any risks, once their GM behavior has functionally adapted in response to the lower musculoskeletal loading.

In conclusion, simulated hypogravity running (Martian and Lunar gravity) vs. 1g induced alterations in joint kinematics (e.g., smaller ankle dorsiflexion and knee flexion angles at peak SEE length) and GM contractile behavior (e.g. longer fascicles and smaller pennation angles at peak SEE length and slower average shortening velocities during SEE elongation). Moreover, joint kinematics and GM contractile behavior during running in simulated Lunar gravity are not equivalent to that on Mars as indicated by their sensitivity to the small absolute difference but, more importantly, large relative difference in gravity between Moon and Mars surfaces. This could impair the transferability of Lunar to Martian surface operations that involve locomotion. Finally, whilst crewmembers performing running countermeasures on Mars would be able to apply full body weight loading at a similar perceived harness discomfort as on ISS, crewmembers exposed to Lunar gravity would have to apply greater external loading to induce mechanical stimuli that are similar to those on Earth.

Methods

The methods of the present study are the same as in a previous publication [19], except for the hypogravity levels, some additional outcome parameters and the statistical analysis. Some parts that are identical to the methods in Richter, et al. [19] have thus been shortened.

Participants

Eight healthy male volunteers (31.9 years ± 4.7 years, 178.4 cm ± 5.7 cm height, 94 cm ± 6 cm leg lengths, 73.5 kg ± 7.3 kg body masses) were examined medically and provided informed written consent to participate in this study, which received approval from the 'Ärztekammer Nordrhein' Ethical Committee of Düsseldorf, Germany, in accordance with the ethical standards of the 1964 Helsinki declaration. Exclusion criteria included any cardiovascular, musculoskeletal or neurological disorders within the previous two years of the present study.

Study design and experimental protocol

Participants attended the laboratory on a single occasion and ran on the vertical treadmill facility (VTF; Arsalis, Glabais, Belgium, Fig. 3) at simulated Martian and Lunar gravity (randomized order) in addition to on a conventional treadmill at 1g. Before each running trial, participants familiarized themselves (~ 4 min) until they have acclimatized to the simulated gravity level and the predefined running speed. After another 2 min accommodation time [36], data were collected for 30 s. As this protocol was conducted within a larger study, 1g data of all eight participants have already been served as a control condition in a recent publication [19].

To obtain mechanically equivalent running speeds at all tested g-levels, running speeds were defined as 125% of the preferred walk-to-run transition speed (PTS) estimated by fitting an exponential regression equation ($PTS_{FR}\,(a) = 1.183e^{-5.952a} + 0.4745$) with a least-squares method ($r^2 = 0.99$) to the data provided by Kram, et al. [20] using the resulting acceleration (a) as the independent variable. By accounting for the participants' leg length (l), the individual $PTS(a) = \sqrt{PTS_{FR}(a)\ \cdot a \cdot l}$ was determined. To ensure a running gait, 25% were added to this PTS which resulted in participants running at predefined speeds of 2.62 $\text{m}\cdot\text{s}^{-1}$ ± 0.08 $\text{m}\cdot\text{s}^{-1}$ at 1g, 1.80 $\text{m}\cdot\text{s}^{-1}$ ± 0.05 $\text{m}\cdot\text{s}^{-1}$ at simulated Martian gravity and 1.50 $\text{m}\cdot\text{s}^{-1}$ ± 0.04 $\text{m}\cdot\text{s}^{-1}$ at simulated Lunar gravity.

[Please insert Fig. 3 here]

Data collection

To determine the stance phase (touchdown to toe-off), participant plantar force was acquired at 83 Hz via shoe insoles (novel GmbH, loadsol® version 1.4.60, Munich, Germany). The gait cycle events were automatically detected via a custom-made script (MATLAB R2018a, MathWorks, Inc., Natick, United States) using a 20 N force threshold for 0.1 s.

Knee and ankle joint angle data were sampled at 1500 Hz via the TeleMyo 2400 G2 Telemetry System (Noraxon USA., Inc., Scottsdale, USA) and the MyoResearch XP software (Master Edition 1.08.16) using a twin-axis (Penny and Giles Biometrics Ltd., Blackwood Gwent, UK) and a custom-made 2D-electrogoniometer, respectively. Electrogoniometer and loadsol signals were time-synchronized via recording of a rectangular TTL pulse generated by pressing on a custom-made pedal. Before each running trial, the electrogoniometers were zeroed when in the anatomical neutral position (standing).

B-mode ultrasonography (Prosound α7, ALOKA, Tokyo, Japan) was used to image the GM fascicles at a frame rate of 73 Hz. The T-shaped 6-cm linear array transducer (13 MHz), was positioned in a custom-made cast over the GM mid-belly, and secured with elastic Velcro. The ultrasound recordings and electrogoniometer signals were time-synchronized via a rectangular TTL pulse generated by a hand switch recorded on the electrocardiography channel of the ultrasound device and the MyoResearch XP software. GM fascicle lengths (distance between the insertions into the superficial and the deep aponeuroses) and pennation angles (angle between the fascicle and the deep aponeurosis) were quantified (Fig. 4) and where appropriate manually corrected using a semi-automatic tracking algorithm (UltraTrack Software, version 4.2) [37].

[Please insert Fig. 4 here]

To calculate SEE length (Achilles tendon, aponeuroses and proximal tendon; Figure 4), muscle fascicle lengths multiplied by the cosine of their pennation angles were subtracted from the MTU lengths [38]. Muscle−tendon unit length was calculated via a multiple linear regression equation [39] using the participant's shank length as well as knee and ankle joint angles.

Data processing

For each participant and each outcome measure at each g-level, eight consecutive left foot stance phases were analyzed via a custom-made script (MATLAB R2018a, MathWorks, Inc., Natick, United States). Prior to being resampled to 101 data points per stance phase, ultrasound data were smoothed with a five-point moving average, whereas electrogoniometer signals were smoothed with a fifth-order Butterworth low-pass filter at a 10-Hz cut-off frequency. Fascicle velocities were calculated as the time derivative of the respective length using the central difference method [40].

To estimate the loading achieved on the VTF, average simulated g-levels over the stance phase were calculated via plantar force and impulse, and expressed as percentage of the average g-levels determined similarly during running on a conventional treadmill. Peak plantar force was defined as the maximum force value observed during stance. Ground-contact times and gait cycle durations were calculated as the time between left foot touchdown and toe-off and between left foot touchdown to the next ipsilateral touchdown, respectively. Cadence was defined as steps (gait cycle duration) per minute. Stride lengths were determined by multiplying gait cycle durations with running velocities. Ankle and knee joint angles as well as SEE-, fascicle-, and MTU lengths in addition to fascicle pennation angles and velocities were determined at the time of the peak SEE length, where the force acting on the SEE is at its greatest. MTU elongation was calculated as the difference between touchdown and peak length. Fascicle shortening and changes in pennation angle occurring during SEE elongation were calculated by subtracting the respective values at touchdown from the values measured at peak SEE length. Average fascicle velocity was determined for the phase of SEE elongation.

Statistical analysis

Data distribution for all outcome measures was assessed using the Shapiro−Wilk normality test. If normal distribution was confirmed, a one-way repeated analysis of variance (ANOVA) with the Geisser-Greenhouse correction in case of violation of sphericity was used to determine whether g-level (1g, Martian gravity and Lunar gravity) had any effects on joint kinematics and fascicle–SEE outcomes ($n = 8$). If a significant effect of g-level was observed, Tukey's post-hoc test to correct for multiple comparisons using statistical hypothesis testing (1g vs. Martian gravity, 1g vs. Lunar gravity, and Martian gravity vs. Lunar gravity) was used.

If the data were not normally distributed, as was the case for the time of peak SEE length, fascicle velocity at the time of peak SEE length and stride length, the non-parametric Friedman test and Dunn's post test was used (n = 8). The statistical analysis was performed in GraphPad Prism (v 7.04) with α set to 0.05. Data is reported as mean (± standard deviation). Furthermore, effect sizes f(U) for the ANOVA were calculated using the G*Power software version 3.1.9.4 [41]. Effect sizes for the respective post-hoc comparisons are presented as Cohen's d. Thresholds of d = 0.2, d = 0. 5 and d = 0.8 were defined as small, moderate and large effects [42]. Whilst data (mean ± standard deviation) acquired at 1g have already been presented in a previous publication [19], the differences to simulated Martian and Lunar gravity as well as between Mars and Moon have not been published elsewhere.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon request.

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Author Contributions

BB, TW and DG conceptualized research. BB, JA, TW, KM, JR, DG and KA designed research. CR, BB, BS, JA and AS acquired data. CR, BB, BS, AS and KA analyzed data. CR, BB, JR, DG and KA interpreted data. CR and DG drafted manuscript. BB, JA, TW, KM, JR, DG and KA revised manuscript. All authors approved manuscript and agreed to be personally accountable for the author's own contributions. Furthermore, all authors ensured that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and the resolution documented in the literature.

Additional Information

Competing Interests Statement

DG and TW are employed by KBR GmbH on behalf of the European Space Agency. The funder KBR GmbH provided support in the form of salaries for the authors DG and TW but did not have any role in the study design, data collection, and analysis, decision to publish, or preparation of the manuscript. All authors declare that the research was conducted in the absence of any commercial, financial or non-financial relationships that could be construed as a potential conflict of interest.

Materials and Correspondence

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Figure Legends

Fig. 1 Kinetic, kinematic, gastrocnemius medialis fascicle and series elastic element parameters during the stance phase of running at 1g, simulated Martian gravity and Lunar gravity

Participants' average (mean ± standard error) patterns of plantar forces (a), knee (b) and ankle (c) joint angles, and muscle–tendon unit (d) and series elastic element (e) lengths as well as muscle fascicle lengths (f), pennation angles (g), and velocities (h) change during the stance phase of running at 1g (black line), simulated 0.32g (orange line) and 0.15g (blue line). The vertical dashed lines mark the time at which peak series elastic element length was achieved (in % of stance) at 1g (black), simulated 0.32g (orange) and 0.15g (blue). Please note that the observed hypogravity levels were slightly lower than the actual values for Martian and Lunar gravity. Means and standard error of the 1g condition have previously been published by Richter, et al. [19]. n = 8 participant

Fig. 2 Gastrocnemius medialis fascicle and series elastic element behavior at the time of peak series elastic element length when running at 1g, simulated Martian gravity and Lunar gravity

Ankle joint angle (a), knee joint angle (b), muscle–tendon unit length (c), fascicle length (e), pennation angle (g) and series elastic element length (h) at the time of the peak series elastic element length as well as muscle–tendon unit elongation (d) and fascicle shortening during series elastic element elongation (f) when running at 1g (black box), 0.32g (orange box) and 0.15g (blue box). Please note that the observed hypogravity levels were slightly lower than the actual values for Martian and Lunar gravity. The lower and upper parts of the box represent the first and third quartile, respectively. The length of the whisker represents the minimum and maximum values. The horizontal line in the box represents the statistical median of the sample; + the mean of the sample; ○ individual data points; * significantly different (Tukey post-hoc, $p \leq 0.05$). The boxplots of the 1g condition in c, e, g and h have previously been published by Richter, et al. [19]. n = 8 participants

Fig. 3 VTF Experimental set-up

Participant being suspended horizontally on the vertical treadmill facility (VTF) with an ultrasound transducer attached to the midbelly of the GM muscle and electrogoniometers placed over the knee and ankle joint to record joint angles. Photo credit: Charlotte Richter; informed consent was obtained to publish this photograph.

Fig. 4 Schematic and anatomical muscle-tendon unit model (a) in addition to an actual annotated ultrasound image of the gastrocnemius medialis (b)

The SEE consists of all tendon-like elements, i.e. free tendon and aponeuroses, as shown in in beige (a). The pennation angle (φ) of the muscle fascicles is defined with respect to the deep aponeurosis. Fascicle length is measured as the length following the pennation between the deep and the superficial aponeuroses (b).

Table 1. ANOVA results for kinetic, spatio-temporal, kinematic, gastrocnemius medialis fascicle and series elastic element parameters while participants ran at 125% PTS at 1g and simulated Martian gravity and Lunar gravity

Outcomes	**1g**		**0.32g**		**0.15g**		**Test Statistic**	***P***	**f(U)**
	M	**SD**	**M**	**SD**	**M**	**SD**			
Peak plantar force [N]	1612.3	348.3	616.0	159.7	315.7	154.1	F(1.1, 7.8) = 199.6	< .0001	5.3
Ground contact time [s]	0.30	0.04	0.38	0.06	0.41	0.08	F(1.3, 8.8) = 39.7	< .0001	2.4
Gait cycle duration [s]	0.72	0.05	0.97	0.08	1.18	0.18	F(1.3, 9.3) = 48.3	< .0001	2.6
Cadence [steps·min^{-1}]	83.3	5.9	62.3	4.9	52.0	7.4	F(1.8, 12.8) = 117.8	< .0001	4.1
Stride length [m]	1.9 (0.1)		1.7 (0.2)		1.7 (0.3)		$\chi^2(2) = 6.8$	.0375	0.7
Ankle joint angle at peak SEE length [°]	15.2	5.1	7.3	4.9	1.5	3.8	F(1.6, 10.9) = 47.5	< .0001	2.6
Knee joint angle at peak SEE length [°]	31.9	6.3	24.6	5.5	18.1	3.7	F(1.5, 10.2) = 23.2	.0003	1.8
Fascicle length at peak SEE length [mm]	40.3	5.8	45.7	5.8	48.5	5.8	F(1.2, 8.2) = 32.7	.0003	2.2
Pennation angle at peak SEE length [°]	31.2	5.8	27.5	3.6	26.0	3.4	F(1.4, 9.8) = 20.8	.0006	1.7
Fascicle velocity at peak SEE length [mm·s^{-1}]	-49.0 (18.2)		-72.8 (33.8)		-52.6 (24.4)		$\chi^2(2) = 12.0$	.0011	
Peak SEE length [mm]	425.5	20.8	414.3	20.5	407.8	21.3	F(1.4, 9.8) = 47.0	<.0001	2.6
Time of peak SEE length [% Stance]	52.0 (11.8)		53.5 (7.8)		54.5 (5.3)		$\chi^2(2) = 0.8$	.7147	
MTU length at peak SEE length [mm]	460.1	20.5	454.9	20.2	451.4	20.0	F(1.6, 11.2) = 32.7	<.0001	2.2
MTU elongation [mm]	13.0	2.8	7.0	3.3	5.3	2.6	F(1.5, 10.2) = 39.6	<.0001	2.4
Fascicle shortening (during SEE elongation) [mm]	13.3	3.3	12.0	2.9	8.9	3.4	F(2.0, 13.9) = 17.5	.0002	1.6
Delta pennation angle (during SEE elongation) [°]	8.1	3.2	5.8	1.6	4.2	1.4	F(1.4, 9.7) = 16.7	.0014	1.5
Average fascicle velocity (during SEE elongation) [mm·s^{-1}]	-97.0	20.8	-64.6	13.5	-44.7	12.0	F(1.7, 12.0) = 75.9	<.0001	3.3

PTS = preferred walk-to-run transition speed; M = mean; SD = standard deviation; *P* = result of the ANOVA (F-statistic) or Friedman test (χ^2) indicating a significant effect of g-level (α set to 0.05); f(U) = effect size ANOVA; Results of the Friedman test are presented as median (interquartile range). Peak SEE length at 1g, simulated 0.32g (Mars) and 0.15g (Moon) occurred at 52% ± 8%, 54% ± 4% and 53% ± 5% of stance, respectively. Mean and standard deviation for ground contact time, cadence, and joint angles for the 1g condition have previously been published by Richter, et al. [19]. n = 8

Table 2. Post-hoc results for kinetic, spatio-temporal, kinematic, gastrocnemius medialis fascicle and series elastic element parameters while participants ran at 125% PTS at 1g and simulated Martian gravity and Lunar gravity

Outcomes	Difference 1g vs. 0.32g					Difference 1g vs. 0.15g					Difference 0.32g vs. 0.15g				
	M	SD	95% CI	*P*	d	M	SD	95% CI	*P*	d	M	SD	95% CI	*P*	d
Peak plantar force [N]	-996.4	221.9	-1227.4; -765.3	< 0001	-3.7	-1296.6	239.5	-1546.0; -1047.3	<.0001	-4.8	-300.3	64.8	-367.8; -232.8	<.0001	-1.9
Ground contact time [s]	0.08	0.03	0.05; 0.11	.0006	1.7	0.11	0.05	0.06; 0.16	.0008	1.9	0.03	0.03	0.01; 0.06	.0168	0.5
Gait cycle duration [s]	0.25	0.07	0.17; 0.32	< 0001	3.9	0.45	0.16	0.29; 0.62	.0002	3.4	0.21	0.14	0.06; 0.36	.0116	1.5
Cadence [steps·min^{-1}]	-21.0	5.6	-26.9; -15.1	< 0001	-3.9	-31.2	6.7	-38.2; -24.3	<.0001	-4.7	-10.3	5.2	-15.7; -4.9	.0020	-1.6
Stride length [m]	-0.2 (0.2)			.0733	-1.4	-0.1 (0.3)			.0733	-0.8	-0.01 (0.2)			>.9999	0.1
Ankle joint angle at peak SEE length [°]	-7.9	3.3	-11.3; -4.5	0006	-1.6	-13.7	4.9	-18.9; -8.6	.0003	-3.0	-5.8	3.6	-9.5; -2.1	.0063	-1.3
Knee joint angle at peak SEE length [°]	-7.3	4.9	-12.4; -2.2	.0096	-1.2	-13.9	7.3	-21.4; -6.3	.0026	-2.7	-6.5	4.7	-11.4; -1.6	.0138	-1.4
Fascicle length at peak SEE length [mm]	5.4	1.5	3.9; 7.0	<.0001	0.9	8.1	3.8	4.2; 12.1	.0013	1.4	2.7	2.9	-0.3; 5.8	.0758	0.5
Pennation angle at peak SEE length [°]	-3.7	2.3	-6.1; -1.2	.0073	-0.8	-5.2	2.9	-8.2; -2.1	.0039	-1.1	-1.5	1.5	-3.1; 0.1	.0630	-0.4
Fascicle velocity at peak SEE length [mm·s^{-1}]	-25.2 (25.6)			.0081	1.4	-3.3 (17.6)			>.9999	0.3	13.8 (24.9)			.0081	-0.9
Peak SEE length [mm]	-11.2	3.8	-15.1; -7.3	.0002	-0.5	-17.7	6.7	-24.6; -10.7	.0003	-0.8	-6.5	4.8	-11.5; -1.4	.0165	-0.3
Time of peak SEE length [% Stance]	-0.5 (11.3)				0.5	2.5 (7.0)				0.2	1.0 (7.0)				-0.3
MTU length at peak SEE length [mm]	-5.2	2.5	-7.8; -2.6	.0016	-0.3	-8.6	3.7	-12.5; -4.8	.0008	-0.4	-3.5	2.8	-6.4; -0.6	.0226	-0.2
MTU elongation [mm]	-6.0	2.3	-8.3; -3.6	.0004	-1.9	-7.7	3.2	-11.0; -4.3	.0007	-2.8	-1.7	2.0	-3.8; 0.4	.1051	-0.6
Fascicle shortening (during SEE elongation) [mm]	-1.3	2.1	-3.5; 0.8	.2305	-0.4	-4.4	2.2	-6.7; -2.1	.0022	-1.3	-3.1	2.2	-5.3; -0.8	.0124	-1.0
Delta pennation angle (during SEE elongation) [°]	-2.3	2.0	-4.5; -0.2	.0342	-0.9	-4.0	2.4	-6.5; -1.5	.0057	-1.6	-1.6	1.2	-2.9; -0.4	.0156	-1.1
Average fascicle velocity (during SEE elongation) [mm·s^{-1}]	32.3	13.8	18.0; 46.7	.0008	-1.8	52.3	12.7	39.1; 65.5	< 0001	-3.1	20.0	9.4	10.2; 29.8	.0014	-1.6

PTS = preferred walk-to-run transition speed; M = mean; SD = standard deviation; CI = Confidence Interval; *P* = result of the post-hoc test indicating a significant effect between conditions (α set to 0.05); d = effect size (Cohen's d) for the post-hoc test. Results of the Friedman test are presented as median (interquartile range). Peak SEE length at 1g and simulated 0.32g (Mars) and 0.15g (Moon) occurred at 52% ± 8%, 54% ± 4% and 53% ± 5% of stance, respectively. n = 8

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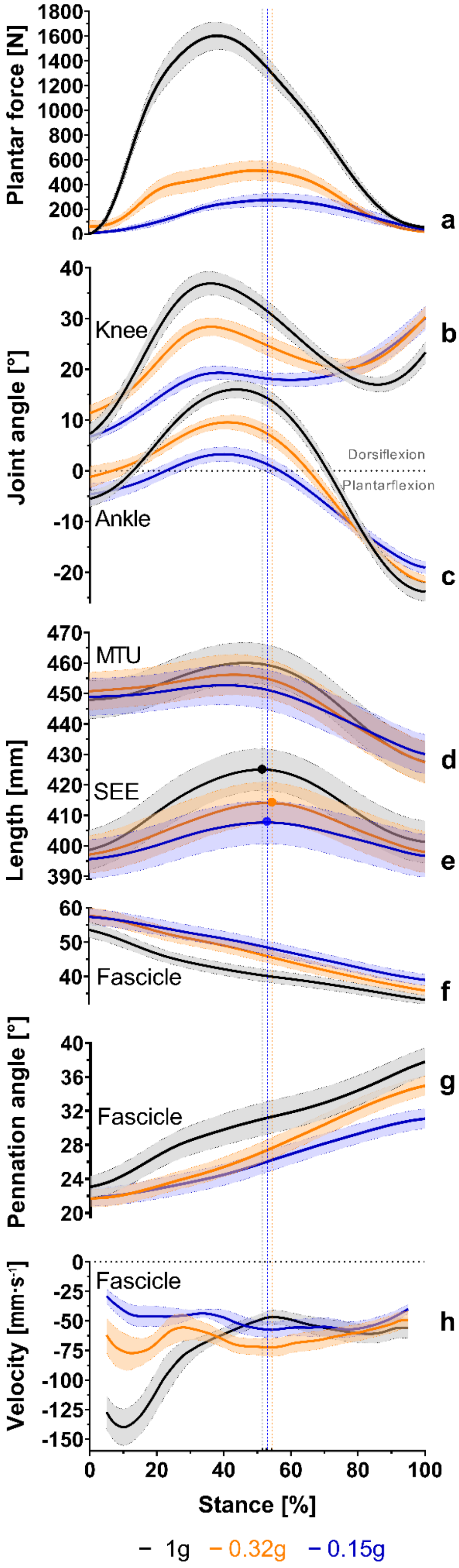
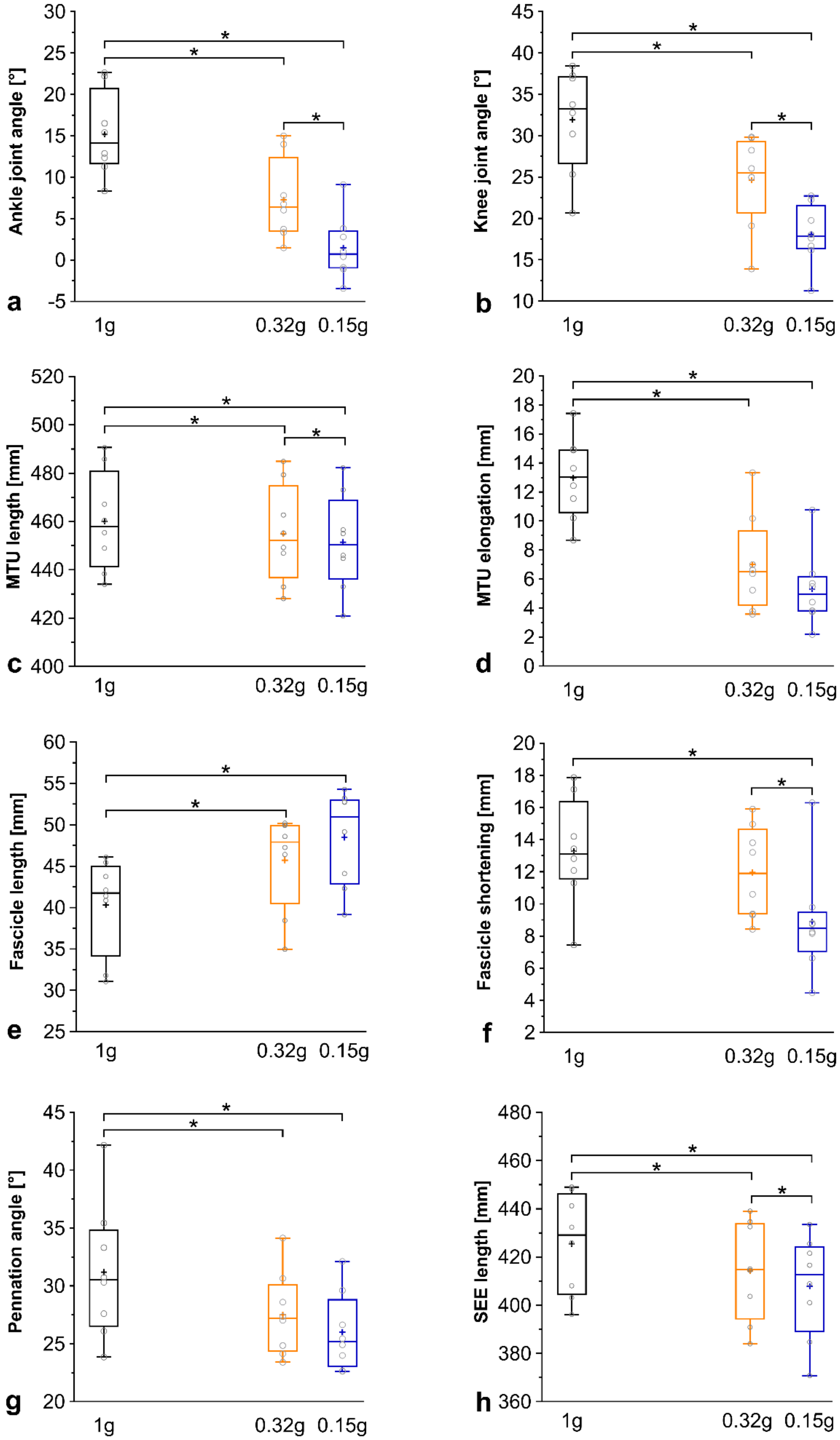


663

664 **Fig. 1**


a
Ankle joint angle [°]
30
25
20
15
10
5
0
-5
1g
0.32g
0.15g
*
*
*
b
Knee joint angle [°]
45
40
35
30
25
20
15
10
1g
0.32g
0.15g
*
*
*
c
MTU length [mm]
520
500
480
460
440
420
400
1g
0.32g
0.15g
*
*
*
d
MTU elongation [mm]
20
18
16
14
12
10
8
6
4
2
0
1g
0.32g
0.15g
*
*
e
Fascicle length [mm]
60
55
50
45
40
35
30
25
1g
0.32g
0.15g
*
*
f
Fascicle shortening [mm]
20
18
16
14
12
10
8
6
4
2
1g
0.32g
0.15g
*
*
g
Pennation angle [°]
45
40
35
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1g
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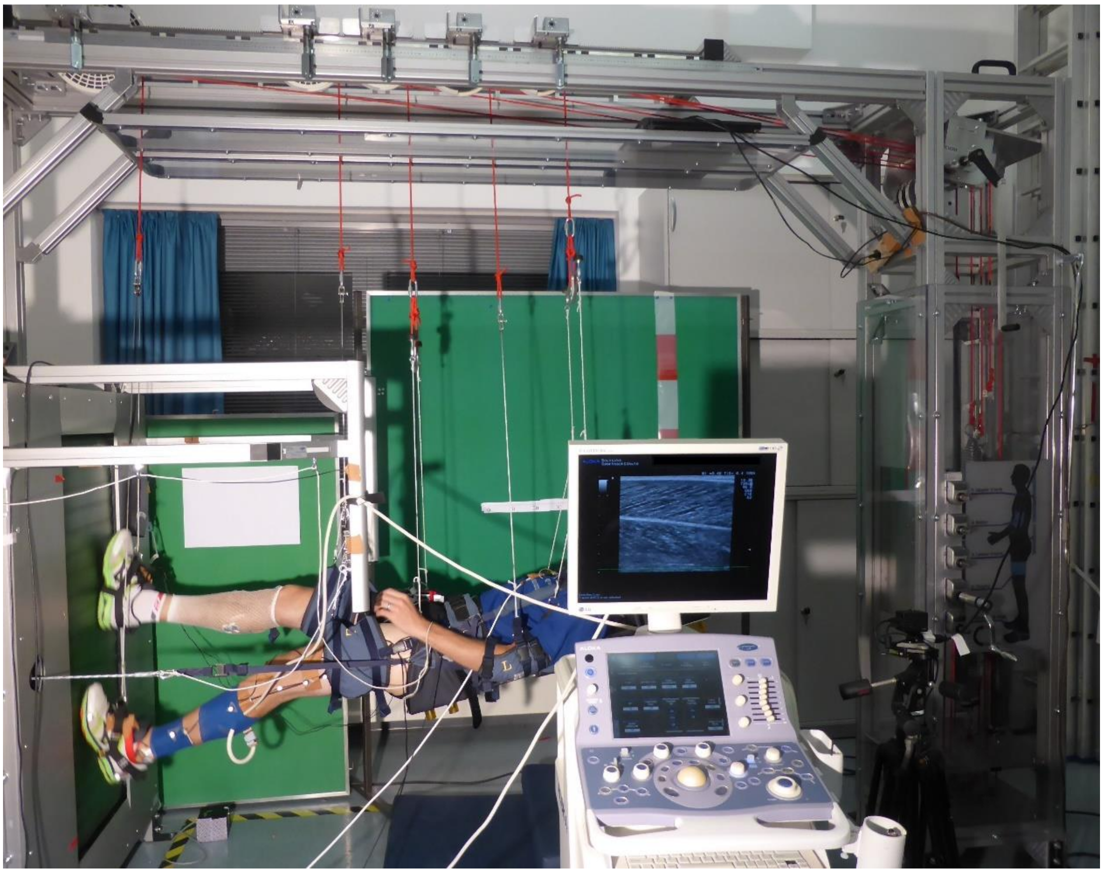
h
SEE length [mm]
480
460
440
420
400
380
360
1g
0.32g
0.15g
*
*
*

Fig. 2

669 

670 **Fig. 3**

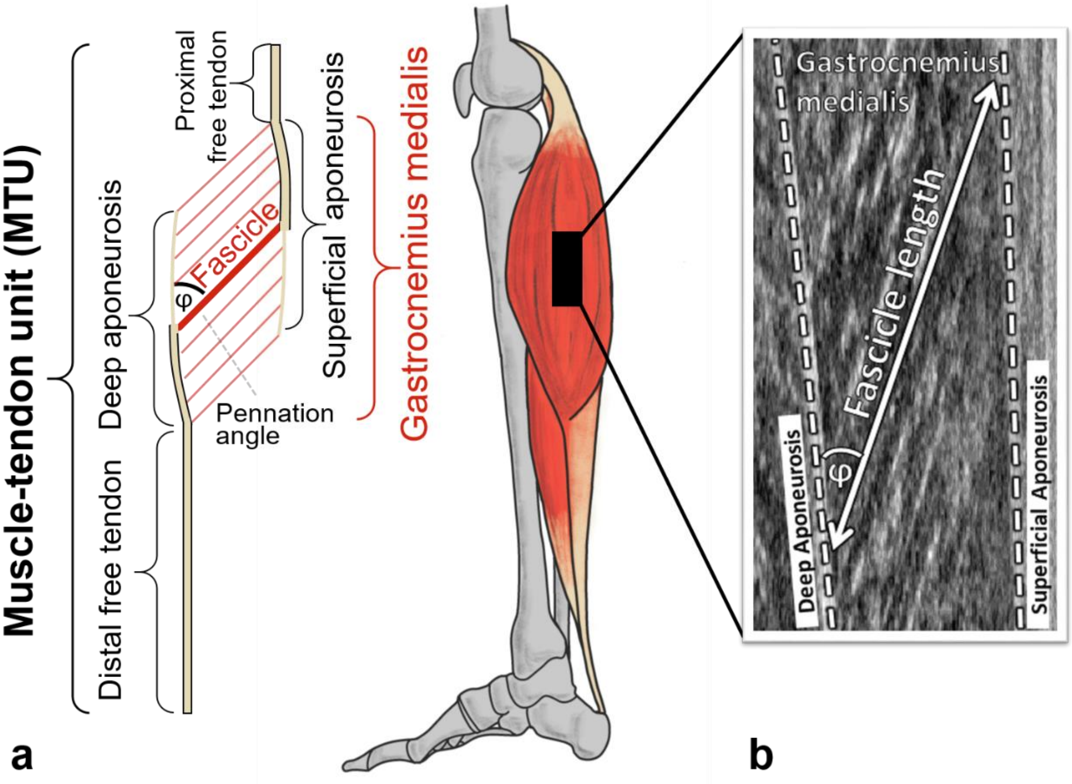

Muscle-tendon unit (MTU)
Distal free tendon
Deep aponeurosis
Fascicle
φ
Pennation angle
Proximal free tendon
Superficial aponeurosis
Gastrocnemius medialis
a
Gastrocnemius medialis
Fascicle length
φ
Deep Aponeurosis
Superficial Aponeurosis
b

Fig. 4