



Calf muscle behavior during walking and running under the gravity conditions of Earth, Space, Moon, and Mars:

a blessing for rehabilitative gait training and a curse for spaceflight exercise countermeasures

Doctoral thesis by **Charlotte Richter**



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Doctoral thesis accepted for the degree

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Date of disputation: August 30, 2021

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Hereby I declare:

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I further declare that I complied with the actual "guidelines of qualified scientific work" of the German Sport University Cologne.



GENERAL COMMENTS

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LIST OF ACRONYMS

AlterG Anti-Gravity Treadmill™

ARED Advanced Resistive Exercise Device

BW Body weight

BWS Body weight support

ESA European Space Agency

g Earth's gravitational acceleration

GM Gastrocnemius medialis

ISS International Space Station

LEO Low Earth Orbit

MTU Muscle-tendon unit

NASA National Aeronautics and Space Administration

PTS Preferred walk-to run transition speed

SEE Series elastic element

T2 Treadmill used on board the International Space Station

VTF Vertical Treadmill Facility

μg Microgravity

SUMMARY

Exposure to simulated hypogravity is the commonality between rehabilitative gait training and exercise countermeasures during human spaceflight. Patients with orthopedic or neurological disorders benefit from gait training with up to 30% body weight support (equivalent to simulated 0.7g), since less forces are acting on their lower extremities whilst gait kinematics are largely preserved. To restore walking function for those patients, in addition to preserved gait pattern, it is also important to maintain the contractile behavior of the plantar flexor muscles such as the gastrocnemius medialis (GM). However, in vivo measurements to determine whether walking with 30% body weight support (BWS) modulates GM fascicle and series elastic element (SEE) behavior have not been performed until this doctoral study.

In contrast to the intentionally applied BWS during rehabilitative gait training on Earth, astronauts exposed to micro- and hypogravity have to actively counteract the reduced loading to avoid musculoskeletal deconditioning. Therefore, International Space Station (ISS) crewmembers perform daily exercise countermeasures, including treadmill running with artificial force loading. Their maximum force loading happens to be at a similar level as the abovementioned recommendation for BWS gait training on Earth (i.e., simulated 0.7g). However, as with rehabilitative gait training, ultrasonic visualization of GM behavior during simulated running on ISS has not been performed before this doctoral study. These data now provide an insight into whether it is possible to replicate Earth-like contractile conditions in space, and thus apply similar stimuli exerted on the muscle. Moreover, for future mission scenarios it is crucial to know whether, and how, GM behavior would be modulated when reducing the hypogravity level to simulated Martian (0.38g) and Lunar gravity (0.16g).

Thus, the aim of the present doctoral study was to investigate in vivo the immediate effects of walking and running under different conditions of simulated hypogravity on GM fascicle and SEE behavior. Hypogravity was simulated on two different devices: on the Anti-Gravity Treadmill AlterG, to replicate rehabilitative gait training, and on the vertical treadmill facility (VTF) to replicate running on board ISS, Mars and Moon. Plantar forces of participants (n = 8, 32 ± 5 years, 178 ± 6 cm heights, 94 ± 6 cm leg lengths, 74 ± 7 kg body masses) were measured via force insoles to determine their stance phases and achieved loading levels. GM fascicle lengths and pennation angles were quantified using ultrasonography. Ankle and knee joint angles were recorded via electrogoniometers and analyzed to determine muscle–tendon unit (MTU) lengths, consisting of the muscle's contractile and series elastic elements. The lengths of the latter were calculated via an MTU model.

The results of this doctoral study are presented in three articles following the main fields of application: rehabilitation, ISS exercise countermeasures and future planetary exploration.

The main finding of the first article is that, in addition to gait kinematics, GM fascicle and SEE behavior is preserved during <u>walking</u> on the AlterG with 30% BWS. This is essential to recover "natural" locomotor patterns of patients and reinforces the recommendation of up to 30% BWS for rehabilitative gait training. In contrast, the results of the second and third article reveal significant differences in GM fascicle and SEE behavior, as well as gait kinematics between <u>running</u> on a conventional treadmill at 1g and running on the VTF at simulated 0.7g (Article 2), 0.38g and 0.16g (both Article 3). Modulation of GM behavior was found to increase with decreasing hypogravity levels. For instance, when decreasing the simulated gravity, decrements in values for SEE lengths, MTU lengths, pennation angles and shortening velocities are observed, whereas fascicle lengths increase.

These observations suggest that running on board ISS at simulated 0.7g does not provide an exact replication of Earth-like contractile behavior. Whether this functional adaptation to running under hypogravity conditions precipitates muscular deconditioning warrants further study. Nevertheless, it cannot be excluded that the observed alterations in contractile behavior, when not being compensated for elsewhere, affect the muscle's work capacity when being re-exposed to gravitational loading. This may not only require specific attention during astronauts' postmission rehabilitation phase back on Earth, but also when completing mission-specific tasks after landing on planetary bodies such as Moon and Mars. Moreover, the results indicate that fascicle and SEE behavior is sensitive to small absolute changes in hypogravity levels, which questions the 1:1 transferability of Lunar to Martian surface operations. It is thus concluded that, to maintain GM muscle mass and function, exercise countermeasures such as running should be optimized, to induce an Earth-like contractile behavior, be it on ISS, Moon, or Mars.

1 INTRODUCTION

1.1 Motivation

In the human body, there are more than 600 different muscles [1,2]. Most of them have distinct functions, some of which are vital for survival. For example, in this very moment, the cardiac muscle of you, the reader of this dissertation, keeps your heart beating. At the same time, your smooth muscles aid in transporting food throughout your digestive tract, and your extraocular muscles direct your eye movement over the words written here. The majority of your muscles are skeletal muscles, which account for approximately half of your body mass [3]. They play a fundamental role in maintaining your body posture and balance, but are also very important for all kinds of daily activities that involve movement.

Complex tasks that are frequently performed, such as locomotion (e.g., walking and running) require the coordination of a significant number of different muscles acting on various joints. The plantar flexor muscles are of particular importance in this respect. When walking, they provide nearly all the vertical body support and forward propulsion during the late stance phase of each step [4-6].

In more detail, the plantar flexor muscles consist of a multitude of individual muscles that differ in their individual contribution and energetic function [5]. The primary plantar flexor muscles are 1) the superficial two-headed gastrocnemius muscle (also referred to as calf muscle) consisting of a medial and lateral head, and 2) the soleus muscle. Both muscles merge via their aponeuroses into the Achilles tendon (Figure 1).



Figure 1. Anatomical sketch of the calf muscles

Anatomical model depicting the two major plantar flexor muscles, gastrocnemius and soleus, which merge into the Achilles tendon. For the gastrocnemius muscle only the medial head can be seen here. Artwork: Charlotte Richter

The soleus muscle mainly delivers energy to the trunk during locomotion, and is thus predominately responsible for body support and forward propulsion. Additionally, the gastrocnemius muscle delivers energy to the leg, and thus contributes to body support and swing initiation [5,7]. This is the reason why both, the soleus and two-headed gastrocnemius muscle appear to be particularly susceptible to changes in body weight (BW) loading [7]. This susceptibility is relevant for conditions of simulated or actual hypogravity, where gravitational force stimuli along the body's longitudinal axis are significantly reduced, e.g., by a change in body posture or where the residual gravitational force is below that of Earth [8]. Conditions where this is the case span from immobilization after injuries via bed rest situations to spaceflight, where astronauts are exposed to full microgravity (µg), i.e., the most extreme form of hypogravity, where residual gravitational forces are reduced to near zero.

Systematic reviews of the effects of actual or simulated µg on human muscle mass and function have shown that the plantar flexor muscles are particularly prone to atrophy [9,10]. After 7-14 days of simulated µg, moderate effects can already be observed for several muscle parameters, such as volume, cross sectional area, torques and strengths, contractile work capacity or thickness [9]. Without physical exercise to counteract these bed rest or µg-induced detrimental effects, muscle deteriorations can become a serious medical concern for bedridden patients and astronauts [11].

Therefore, in a wide range of situations, such as exercise countermeasures in space or patient rehabilitation on Earth, it has shown to be advantageous for the subjects' health to embed them in an artificial gravity level that is different from the one that is intrinsic to the given environment. Hypogravity can be simulated from two possible starting conditions: 1) starting from Earth's gravity (1g), by various means of unloading subjects with BW support mechanisms, as implemented during some form of rehabilitative gait training, and 2) starting from a µg situation, by providing artificial force loading such as implemented on exercise devices on the International Space Station (ISS; Figure 2).

To counteract the various detrimental physiological effects that are associated with µg exposure [12], astronauts on ISS perform daily exercise countermeasures including treadmill running (Figure 3), cycling, and resistance training [13,14] during their typical six-month-missions on board their outpost in Low Earth Orbit (LEO). The implementation of these countermeasures require an immense technical effort (e.g., upload mass, power supply and set-up).



Figure 2. The International Space Station (ISS)

A human outpost with an internal pressurized volume similar to that of a large passenger aircraft, orbiting Earth 15 to 16 times per day at an altitude of \sim 400 km. Credit: NASA / Roscosmos



Figure 3. Crewmember Alexander Gerst on the T2 treadmill during ISS Expedition 41

This system consists of two bungee assemblies that are clipped in series with carabiner clips that are attached to a harness at the left and right side of the hip. The applied amount of external force can be regulated by adding or removing carabiner. Credit: A. Gerst / ESA; with friendly permission.

To run in space, crewmembers strap themselves onto a dedicated treadmill by using a special harness-based subject loading system [15]. During running as countermeasure exercises in space, crewmembers benefit from the forces that generate both skeletal and muscular loading. This in turn provides important mechanical stimuli for their musculoskeletal system [16]. However, due to discomfort of the harness system, with only four major points of force application (hips and shoulders), ISS crewmembers typically limit the applied artificial force loading to about 70% equivalent BW [13], which, in this specific context, can also be referred to as simulated 0.7g. Hence, ISS crewmembers are not exposed to the same mechanical stimuli as on Earth [17-19], to the detriment of their plantar flexor muscles [20].

In fact, before the Advanced Resistive Exercise Device (ARED) was installed on ISS, plantar flexor muscle volume and peak power of returned crewmembers decreased by 13% and 32%, respectively after six months in µg [21]. Nowadays, this can largely be compensated for by the intensive use of ARED [22], which provides loads up to 2700 N and allows to perform a multitude of exercises including typical leg exercises such as squats or heel raises [13,23,24].

Due to these improvements of in-flight exercise hardware and exercise prescriptions, musculoskeletal deconditioning has been reduced during long-duration spaceflight to ISS [22,23,25], with some crewmembers even returning with a net increase in their overall muscle mass [personal communication A. Gerst, see online supplement of Article 2]. This, however, does not exclude losses in individual muscle groups, such as plantar flexors. Variable inter-individual physiological responses to the exercise induced stimuli still persist [20,26,27] and thus need to be taken into account. In fact, a recent study (Sarcolab) has demonstrated the effects of different countermeasure regimes on the plantar flexor muscles of two ISS crewmembers [20]. The conclusion is that even high intensity exercise countermeasures still appear to be insufficient to entirely prevent muscle deterioration [20].

The good news is that this detrimental effect can be influenced by the crewmembers themselves, as the effect appears to correlate with overall intensity of the exercise regime. I.e., the crewmember who trained more vigorously, as shown, for example, in more treadmill sessions at higher artificial force loadings and faster running speeds, was indeed able to largely attenuate decrements in muscle volume, lower limb strength and deteriorations in muscle architecture [20]. This demonstrates that exercise intensity (e.g., running velocity or resistance load [19]) is a critical component to elicit optimal physiological responses [23]. Following this logic, it has been suggested that with an appropriate harness and subject loading system, even replication of 1g conditions during µg running could be feasible, to serve as an effective exercise to counteract musculoskeletal deconditioning [28]. However, studies based on reports by crewmembers [13; see also online supplement of Article 2] suggest that the feasibility of such an increase in artificial

force loading to a simulated 1g is doubtful due to the discomfort caused by the limited options for force application points on the human body.

To strengthen the understanding of muscular responses to hypogravity conditions, the use of noninvasive real-time ultrasonic visualization of muscle architecture (fascicle length and pennation angle) provides useful information on whether the applied exercise intensity is sufficient to generate Earth-like contractile conditions [29]. It can be hypothesized that preservation of Earth-like loading conditions on the muscle—tendon units (MTU) of spaceflight crews would support the preservation of musculoskeletal health by providing Earth-like stimuli on their musculoskeletal system. The MTU, as the name already reveals, consists of the muscle's contractile elements and series elastic elements (SEE). The MTU for the gastrocnemius medialis (GM) muscle is depicted in Figure 1 in red (contractile elements) and beige (SEE). Presently, ultrasonic visualization of the plantar flexors' contractile behavior, as is presented in this doctoral study, has not yet been performed during running at simulated hypogravity, and certainly not during running at actual hypogravity.

The National Aeronautics and Space Administration (NASA) and European Space Agency (ESA), together with their international partners, are currently shifting their focus from LEO missions to human missions on the Moon and eventually on Mars. Plans include the construction of a Lunar Orbital Platform called Gateway, and eventually to set up a permanent human habitat on the Lunar surface. This will allow the development and testing of hardware and procedures towards the long-term goal of a human mission to Mars [30]. However, whilst the Apollo missions showed that humans can effectively operate in Lunar gravity [31] with a total stay time on the Moon (both inside and outside the landing module) of up to 75 hours [32], the physiological responses to chronic hypogravity exposure on Moon (0.16g) or Mars (0.38g) have yet to be evaluated [33]. Additionally, for Mars missions the consequences of prolonged exposure to μg during the interplanetary transit, abruptly followed by planetary surface activities in hypogravity are unknown [34,35].

It is most likely that exercise countermeasures for exploration missions exceeding 14 days [9] will still be required to compensate for the anticipated lack of mechanical and metabolic stimuli, and thus to maintain astronauts' health, safety, work performance and mission success [23,33,35]. However, the replication of current exercise countermeasure regimes as performed on ISS, including the use of relatively large, bulky and heavy exercise devices will be challenging, if not impossible, for Lunar and Martian missions. This is mainly due to differences in future mission timelines, manned space craft/habitat sizes, power supply and gravity level [26,36]. Therefore, to ensure mission success, future scientific research to attain optimum exercise equipment and

prescriptions, particularly with respect to the preservation of neuromuscular function is without alternative.

As described above, astronauts in space and bed-ridden patients suffer detrimental effects from hypogravity, in the shape of reduced BW loading compared to that to normal Earth-like conditions. In contrast, such an analogue hypogravity condition can become beneficial for patients with orthopedic or neurological impairments [37,38]. This may sound counterintuitive at first, however, early postoperative rehabilitation regimes often involve these patients performing gait training under simulated, or analogue, hypogravity conditions in an attempt to retrain "natural" walking gait function [39]. Simulation models include, but are not limited to, overhead suspension systems, therapist-assisted waist belts, robotic-assisted gait-training devices, vertical treadmill systems or lower body positive pressure treadmills [40-42]. During the implementation of rehabilitative gait training in simulated hypogravity, it is important that gait patterns remain similar and are thus transferable to subsequent unsupported walking while muscle and joint forces are reduced [38,41,43]. This is not only to decrease pain associated with ambulation, but also to protect the healing biological tissue or prostheses. At the same time, such exercises during early postoperative rehabilitation might help to prevent muscle deteriorations caused by bed rest or disuse [40].

For rehabilitative gait training, walking at simulated 0.7g is typically recommended [39,41,43]. It is equivalent to walking with 70% of the natural BW, and is sometimes also referred to as "30% body weight support (BWS)". Spatio-temporal and joint-kinematic characteristics were found to be largely preserved above this hypogravity level [39,41]. In addition, walking with up to 30% BWS was also found to largely preserve the knee joint moment curvature patterns, despite significant reductions in ground reaction forces and in knee joint moment [43]. However, as with hypogravity conditions in space, detailed knowledge of the plantar flexor muscle behavior during locomotion in simulated hypogravity on Earth is currently still lacking. This is unfortunate, because demonstrating a potential preservation of a "natural" Earth-like contractile behavior under simulated hypogravity conditions would support the validity of running exercises on ISS, and that of rehabilitative gait training¹ on Earth.

To fill the abovementioned knowledge gap, a biomedical study entitled "Mechanics and loading forces associated with Movement in simulated Low gravity – The MoLo Study" was launched. It was conducted through a cooperation between the German Sport University, the University of Applied Sciences Aachen, the European Space Agency, the German Aerospace Centre, King's College London and the London South Bank University. The MoLo study seeks to understand

¹ Please note, that in the context of this dissertation, rehabilitative gait training refers only to the gait of walking.

how locomotion biomechanics, and GM behavior, adapt to different external environmental conditions such as various levels of simulated hypogravity. The author of this cumulative dissertation contributed to the MoLo study by leading all ultrasound measurements, with the main goal of describing the modulation of GM behavior with respect to fascicle and SEE behavior in response to hypogravity exposure. Attaining this knowledge is crucial for the development of optimized future exercise countermeasures in LEO and deep space, as well as for future innovations in rehabilitative gait training, on Earth.

1.2 Overview of this doctoral study

In preparation for the MoLo study, the author of this cumulative dissertation and a team from ESA's Space Medicine Office conducted a systematic review of human biomechanical and cardiopulmonary responses to hypogravity [33]. Based on this review, in vivo knowledge of morphological muscle and tendon parameters and measures of the mechanical strain in the musculoskeletal system is lacking for hypogravity conditions [33]. Therefore, this doctoral study, which is part of the larger MoLo study, seeks to directly address the abovementioned knowledge gap by investigating the following main research questions:

Does locomotion under conditions of simulated hypogravity affect GM behavior? If yes, which exact alterations can be observed in the muscle's contractile and SEE behavior? What are potential implications for rehabilitative gait training on Earth, and for exercise countermeasures on ISS, Mars or Moon?

To answer these questions, participants were asked to perform Earth-based walking and running trials while simulating different gravity conditions (1g, 0.7g, 0.38g and 0.16g)² by using two different support systems (Figure 4): 1) the vertical treadmill facility (VTF), which consists of a vertically mounted motorized treadmill, a supine suspension system and a subject loading system and 2) the Anti-Gravity Treadmill (AlterG), a conventionally (i.e., horizontally) orientated treadmill that generates BWS though a positive pressure differential below the participant's waist, thereby causing a vertical buoyancy force.

The VTF is considered by some as the most suitable ground based model for running in µg that is currently available. The system (also known as the Enhanced Zero-gravity Locomotion Simulator) was developed to mimic in-flight exercise and surface hypogravity exercise as close as possible, despite the unavoidable technical limitations and differences between any Earth-

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² Trials at 0.27g were also recorded but not used in this dissertation.

based system and a treadmill in actual µg [44]. Despite these differences, a study which compared running in actual µg (parabolic flight) and simulated µg (horizontal suspension) reported only marginal differences in joint kinematics and ground reaction forces between the two devices [45].

In contrast, the AlterG is frequently employed in gait training and considered a feasible and safe rehabilitation tool [40,46,47]. Its underlying concept of applying a pressure differential to the lower part of a human body was originally developed with space flight applications in mind [48].

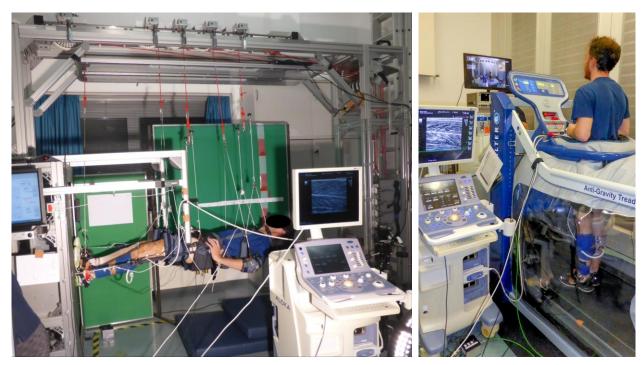


Figure 4. Hypogravity simulation systems used in the MoLo study

The vertical treadmill facility (VTF) on the left is a ground-based analog of the T2 treadmill used on board ISS. The Anti-Gravity Treadmill AlterG on the right is mainly used in medical facilities for gait rehabilitation. Photo credit: Charlotte Richter; participants provided written informed consent for their photo to be used for publication.

To compare locomotion at different levels of simulated hypogravity, one must account for decreasing gravity levels resulting in changes in the subjects' preferred walk-to-run transition speeds (PTS). At lower simulated gravity values, PTS occur at lower absolute speeds, yet at a similar Froude number [49-51]. Therefore, to ensure mechanically equivalent speeds at each gravity level (i.e., similar speeds relative to the PTS), participants in the MoLo study were required to walk at 75% and run at 125% of their PTS. While this method may limit the comparison to other hypogravity studies that use fixed speeds, it is a prerequisite to compare the same participants at different levels of simulated hypogravity [49]. It is therefore regarded as a suitable methodological approach with respect to the aim of this doctoral study, which investigates matched-pair comparisons between gravity levels.

Plantar forces, lower limb joint kinematics and GM muscle architecture were recorded non-invasively via force insoles, electrogoniometers, and B-mode ultrasonography, respectively (Figure 5).

Force insoles measure the normal component of ground reaction forces on the plantar surface of the foot. The used system demonstrates a high correlation (r > .96) and an adequate accuracy when being validated against a force platform [52]. Likewise, electrogoniometry has been demonstrated to produce reliable and reproducible lower limb joint kinematics [53-55]. It has already been used during running [56-59] and walking [37,47,59] at simulated hypogravity and is thus considered to be suitable for this study set-up.

Muscle ultrasound imaging has been frequently used in various dynamic 1g conditions [29] and is generally regarded as a reliable method to quantify muscle architecture. Consequently, fascicle length and pennation angle showed good reproducibility [60-62]. A prerequisite for this is a good location accuracy and the avoidance of relative motion of the transducer with respect to the muscle, which otherwise may lead to artifacts [29,60,63]. Therefore, in this study, the ultrasound transducer was secured with a custom-made cast on the participants' GM midbelly to ensure precise locations allowing for reproducibility and comparability of data.

Additionally, for all outcome parameters of this study, a state-of-the-art statistical data analysis was performed. Depending on the number of gravity levels that were compared, either Student's test or a one-way repeated analysis of variance was performed with α set to 0.05.

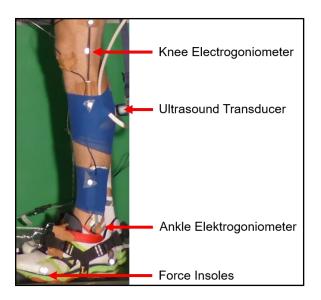


Figure 5. Participant set-up for the MoLo study

Joint angles were analyzed to determine GM muscletendon unit length. Ultrasonography was used to quantify GM fascicle length and pennation angle. On the basis of these parameters, series elastic element length were calculated. Plantar forces were measured to determine stance phase and loading level.

The results of this doctoral study are presented in three different publication articles that are connected by the overall theme of investigating GM behavior under simulated hypogravity conditions, but which focus on individual research questions within their different target communities.

Thus, for each article, a specific selection of the respective support system and simulated gravity level was made. Consequently, some terminologies were adapted to those of the respective target community. For example, in the area of rehabilitation the term "body weight support" is typically used instead of "simulated hypogravity". This affects mainly the first article, which focuses on walking on the AlterG treadmill at 1g versus simulated 0.7g (the latter is equivalent to "70% BW", or "30% BWS"), reaching out to exercise scientists and physical therapists working in the field of rehabilitative gait training.

The second article presents the data of <u>running</u> at 1g on a conventional treadmill (achieved by using the AlterG treadmill without its support mechanism) versus simulated 0.7g on the VTF. It addresses crewmembers and scientists involved in ISS medical operations, as well as exercise specialists working in the field of gravitational muscle physiology.

The third article compared <u>running</u> at 1g on a conventional treadmill with running at simulated 0.38g (Mars) and 0.16g (Moon) on the VTF, and will therefore be of interest to experts who deal with the preparation of sustainable future human space exploration missions but also gravitational physiology.

1.3 Research aims and hypotheses

Article 1

Walking with a 30% reduction of the full BW is typically recommended for rehabilitative gait training due to the preservation of kinematic and spatio-temporal gait parameters [39,41]. However, despite this recommendation, the influence of BWS on GM behavior was hitherto unknown.

Therefore, the aim of the first article was to determine, via ultrasonography, and compare GM fascicle and SEE behavior during walking at mechanically equivalent speeds (75% of the PTS) on the AlterG with full BW, and with 30% BWS (simulated 0.7g or 70% BW). The goal was to investigate whether the therapeutic dose of 30% BWS also preserves GM contractile behavior in addition to kinematic and spatio-temporal parameters, which would support the transferability of supported gait patterns to subsequent unsupported walking.

Forces that are acting on the SEE when walking with BWS are typically lower than when walking with full BW. Therefore, it was hypothesized that peak SEE lengths will be reduced during supported walking, and are compensated for by longer fascicles and/or smaller pennation angles, rather than by a shorter MTU (due to preserved joint angles).

Article 2

The intensity of spaceflight exercise countermeasures appears to be key for the extent of in-flight muscle wasting [20]. Therefore, understanding the impact of these countermeasures on muscular parameters is key to understanding their impact on muscle atrophy [20]. Despite this connection, the impact on fascicle and SEE behavior that is caused by running at simulated 0.7g, as is typically used as maximum running load on ISS, was unknown.

Therefore, the aim of the second article was to examine, via ultrasonography, the effects of running at mechanically equivalent speeds (125% PTS) on the VTF at simulated 0.7g vs. 1g on GM contractile behavior. The goal was to investigate whether crewmembers that perform running exercises in space are able to sufficiently replicate an Earth-like contractile behavior. It follows the assumption that stimuli exerted on the muscle similar to those required for musculoskeletal health on Earth are a prerequisite to maintain muscle mass and function in µg.

On the basis of the first article indicating a preserved GM fascicle–SEE behavior during <u>walking</u> at simulated 0.7g (i.e., 70% BW, achieved by 30% BWS), it was hypothesized that GM behavior is similarly preserved during <u>running</u> on ISS when artificially loaded to 0.7g or 70% BW.

Article 3

Before this doctoral study, it was unknown whether GM behavior is sensitive to similarly low hypogravity levels as are present on Mars or Moon. Furthermore, previous studies have indicated that alterations in running biomechanics do not necessarily scale to gravity level [64].

Therefore, the aim of the third article was to investigate, via ultrasonography, the effects on GM contractile behavior caused by running at mechanically equivalent speeds (125% PTS) at 1g (Earth), simulated 0.38g (Mars) and simulated 0.16g (Moon) on the VTF. The aim was to quantify the impact of running under the gravity conditions of Earth, Mars and Moon. It follows the assumption that a similar GM behavior is a prerequisite for the transferability of locomotion biomechanics, e.g., from a Lunar to a Martian gravity environment, and therefore astronauts' capabilities to perform planetary surface operations.

Based on the findings of simulated 0.7g running (Article 2), it was hypothesized that when running in even lower hypogravity conditions, ankle dorsiflexion and knee flexion at the time of peak SEE length are both smaller than at 1g. At the same time GM fascicles were hypothesized to operate at longer lengths, smaller pennation angles and faster velocities. These alterations are hypothesized to continue between simulated 0.38g and 0.16g. However, it remains to be determined by which extent GM behavior is altered, and whether absolute or relative differences in the gravity level dominate these alterations. The absolute differences in gravity between Moon and Mars surfaces are only 0.2g, which could be argued to be rather small, but the relative difference between them is large, i.e., Mars has more than twice as much surface gravity than the Moon.

2 PEER-REVIEWED ARTICLES

2.1 Article 1 - Gastrocnemius Medialis Contractile Behavior is Preserved during 30% Body Weight Supported Gait Training

Article published in Front. Sports Act. Living (2021) 2:614559. doi: 10.3389/fspor.2020.614559

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Article Information

Received: 06 October 2020

Accepted: 23 November 2020

Published: 18 January 2021

Impact: 3.9

Number of Figures: 3

Number of Tables: 2

Link to online article: https://doi.org/10.3389/fspor.2020.614559



2.1.1 Abstract

Rehabilitative body weight supported gait training aims at restoring walking function as a key element in activities of daily living. Studies demonstrated reductions in muscle and joint forces, while kinematic gait patterns appear to be preserved with up to 30% weight support. However, the influence of body weight support on muscle architecture, with respect to fascicle and series elastic element behavior is unknown, despite this having potential clinical implications for gait retraining. Eight males (31.9 ± 4.7 years) walked at 75% of the speed at which they typically transition to running, with 0% and 30% body weight support on a lower-body positive pressure treadmill. Gastrocnemius medialis fascicle lengths and pennation angles were measured via ultrasonography. Additionally, joint kinematics were analyzed to determine gastrocnemius medialis muscle-tendon unit lengths, consisting of the muscle's contractile and series elastic elements. Series elastic element length was assessed using a muscle-tendon unit model. Depending on whether data were normally distributed, a paired t-test or Wilcoxon signed rank test was performed to determine if body weight supported walking had any effects on joint kinematics and fascicle-series elastic element behavior. Walking with 30% body weight support had no statistically significant effect on joint kinematics and peak series elastic element length. Furthermore, at the time when peak series elastic element length was achieved, and on average across the entire stance phase, muscle-tendon unit length, fascicle length, pennation angle, and fascicle velocity were unchanged with respect to body weight support. In accordance with unchanged gait kinematics, preservation of fascicle-series elastic element behavior was observed during walking with 30% body weight support, which suggests transferability of gait patterns to subsequent unsupported walking.

Keywords: unloading, muscle fascicle behavior, series elastic element behavior, ultrasound imaging, walking, gait, rehabilitation, AlterG

2.1.2 Introduction

Orthopedic and neurological rehabilitation regimes often involve patients performing gait training with body weight support (BWS) in an attempt to retrain "natural" walking gait function. Whilst overhead suspension systems are largely employed to promote gait rehabilitation from neurologic disorders [41], lower-body positive pressure (LBPP) treadmills are frequently used following orthopedic injuries to re-expose patients to walking whilst bearing progressively greater proportions of their body weight [65,66]. In order to restore gait function, movement patterns should be as similar, and thus transferable to daily activities, as possible albeit with a reduction of lower limb muscle and joint forces [40]. Studies assessing LBPP have demonstrated that whilst ground reaction forces are reduced [37,40,67], gait kinematics are largely preserved [41].

During normal walking, mechanical energy is largely conserved due to the pendulum-like exchange between potential and kinetic energy [68]. Despite this, additional mechanical work by the muscle-tendon unit (MTU) is required to sustain the movement of the body's centre of mass. However, walking with BWS reduces the total mechanical energy of the centre of mass, and thus presumably requires less force and work from the MTU to vertically support and accelerate the body [68,69]. In fact, significant reductions in the metabolic cost of locomotion have been observed [69-71]. Furthermore, reductions of knee joint contact forces [47], ankle joint moments [39,72,73] ankle joint angular momentum [7] and ankle joint power [73] have been reported during unloading. Despite reduced kinetic and metabolic requirements for vertical body support and forward acceleration, LBPP (unless BWS is > 75%) has been reported to not induce significant differences in spatio-temporal gait parameters such as cadence, stride duration [47,67] and stride length [40,47,66], nor range of ankle [40,66] and knee [37,40] joint motion. In addition, whilst muscle activity patterns appear unchanged, lower limb muscle activity is reduced during LBPPtreadmill gait [56,66,74] with the plantar flexor muscles being particularly susceptible to manipulations of body weight [7]. This demonstrates their critical role in human locomotion by providing the majority of the force necessary for vertical body weight support and horizontal propulsion [4,5,7]. To gain a better understanding of the plantar flexors' response to different locomotor tasks, ultrasound imaging is a convenient technique to visualize architectural changes, which help to draw conclusions about muscle function.

Ultrasonic visualization of muscle fascicle behavior during locomotion without BWS has not only demonstrated the importance of the storage and release of elastic energy in the Achilles tendon for running and walking [75,76], but also that the plantar flexor muscles modulate their behavior depending on gait type, and speed [77]. In fact, increased walking speeds have been shown to increase gastrocnemius medialis (GM) shortening velocities [77], and to shorten soleus fascicles [78], thereby impairing plantar flexors force generation due to shifting the force–length–velocity relationship towards less favorable contractile conditions [79,80]. However, it is unknown whether

walking with BWS modulates fascicle and series elastic element (SEE) behavior to meet the reduced locomotor demands [33]. Knowledge of any changes in GM's muscle architecture (primarily fascicle length and pennation angle) in addition to fascicle shortening velocity, which affect the force-length-velocity relationships, would facilitate inference of the mechanisms determining mechanical power generation when BWS is applied. Whereas preservation of fascicle contraction behavior concurrent with preservation of gait kinematics would support the validity of rehabilitative gait training with BWS.

30% BWS is typically recommended for rehabilitative re-introduction to walking and running, due to the preservation of kinematic and spatio-temporal gait parameters [39,41] in addition to muscle activation patterns [58,81]. As during early postoperative rehabilitation patients usually start with recovering their walking function, the present study focuses on walking with BWS. Increasing BWS is known to result in walk-to-run transitions occurring at slower absolute walking speeds [51], but similar Froude number, a dimensionless number embedding gait speed, leg lengths and gravitational acceleration (in the present paper expressed as BWS) [50,51]. Thus, to obtain mechanically equivalent speeds (i.e., a similar walking speed relative to the preferred walk-to-run transition speed) at different BWS levels, walking speeds should be adjusted to the same Froude number [49,51,82], which requires a reduction in absolute walking speed.

Therefore, the aim of the present study was to determine via ultrasonography GM's fascicle-SEE behavior during walking at mechanically equivalent speeds, namely 75% of the preferred walk-to-run transition speed (PTS), on a LBPP treadmill, with, and without 30% BWS.

It was hypothesized that during walking with BWS (i.e., where forces acting on the SEE are reduced) peak SEE length decreases and is compensated for by longer fascicles and/or smaller pennation angles, rather than by a shorter MTU as ankle and knee joint kinematics are reported to be preserved.

2.1.3 Materials and Methods

2.1.3.1 Participants

Eight healthy male volunteers (mean \pm standard deviation: 31.9 \pm 4.7 years, 178.4 \pm 5.7 cm heights, 94.2 \pm 5.6 cm leg lengths and 73.5 \pm 7.3 kg body masses) with treadmill running experience provided informed written consent to participate in this observational study, which received approval from the "Ärztekammer Nordrhein" Ethical Committee of Düsseldorf, Germany. The study was conducted in the Physiology Laboratory of the Institute of Aerospace Medicine at the German Aerospace Center in Cologne, where all participants underwent a standard medical examination. Exclusion criteria included any cardiovascular, musculoskeletal or neurological

disorders within the previous two years in addition to any lower limb surgery that may affect MTU behavior.

2.1.3.2 Study design and experimental protocol

Participants attended the laboratory on a single occasion and walked on an Anti-Gravity Treadmill (AlterG; AlterG®, M320, Fremont, USA; Figure 6), an LBPP treadmill, with 0% BWS and thereafter with ~30% BWS [recommended load for rehabilitative gait training; 39,83]. Before each trial, participants familiarized them-selves until they have acclimatized to the BWS level and the predefined walking speed (~4 min). After another 2 min accommodation time given to produce reproducible gait kinematics [84] and hence a total warm-up time of ~6 min, which is further required for the Achilles tendon to achieve a relatively stable steadystate behavior [85], data were collected for 30 s. Blinding of participants was not applicable due to the nature of the experimental set-up. Walking speeds were defined as 75% of the prefer-red walk-to-run transition speed (PTS) expressed as a Froude number (PTS_{FR}).



Figure 6. AlterG experimental set-up

Participant walking on the lower-body positive pressure treadmill (the AlterG) with an ultrasound transducer attached to the midbelly of the gastrocnemius medialis muscle and electrogoniometers (added in green to accentuate placement) to measure ankle and knee joint angles.

PTS_{FR} was estimated by fitting an exponential regression equation (PTS_{FR} (a) = 1.183 $e^{-5.952a}$ + 0.4745) with a least-squares method (r^2 = 0.99) to the experimental data of Kram and co-workers [51] using the resulting acceleration (a) as the independent variable. Hence, for a = 0.7 g (g = 9.81 m·s⁻²), a PTS_{FR} value of 0.49 was obtained. By accounting for the participants' leg lengths (I), measured from the greater trochanter to the ground, the individual $PTS(a) = \sqrt{PTS_{FR}(a) \cdot a \cdot l}$ expressed in meters per second was determined resulting in walking speeds of 1.58 ± 0.05 m·s⁻¹ at 0% BWS, and 1.34 ± 0.04 m·s⁻¹ at 30% BWS.

The AlterG was enclosed within a sealed height-adjustable chamber, which allowed air pressure to increase inside the chamber and generated an additional vertical buoyant force to produce

controlled and stable BWS levels. A seal between the participant and the chamber was created through a neoprene kayak-type skirt that could be zipped into the aperture of the chamber.

2.1.3.3 Joint kinematics

Knee and ankle joint angles were recorded using a twin-axis (Penny and Giles Biometrics Ltd., Blackwood Gwent, UK) and a custom-made potentiometer based electrogoniometer, respectively. The end blocks of the knee electrogoniometer were placed along the leg from the greater trochanter to the lateral femur epicondyle and along the leg from the lateral epicondyle of the femur to the lateral malleolus. The end blocks of the ankle electrogoniometer were placed along the leg from the lateral femur epicondyle to the lateral malleolus and from the lateral malleolus to the most distal end of the fifth metatarsal. Before each walking trial, a reference measurement was taken in the anatomical neutral position to define the 0° joint angles. Data were sampled at a frequency of 1500 Hz via the TeleMyo 2400 G2 Telemetry System (Noraxon USA., Inc., Scottsdale, USA) and MyoResearch XP software (Master Edition 1.08.16).

2.1.3.4 Spatio-temporal parameters

To determine gait cycle events and thereby define stance phases of the left leg, participant plantar pressure was measured (83 Hz) via insoles (Novel GmbH, loadsol® version 1.4.60, Munich, Germany). Touchdown and toe-off were automatically detected using a 20 N threshold for 0.1 s via a custom-made script (MATLAB R2018a, MathWorks, Inc., Natick, United States). Insole and electrogoniometer signals were time-synchronized via recording of a rectangular pulse generated by pressing on a custom-made pedal.

2.1.3.5 GM muscle fascicle length and pennation angle

Real-time B-mode ultrasound (Prosound α7, ALOKA, Tokyo, Japan) captured at 73 Hz using a T-shaped 6-cm linear array transducer (13 MHz) was performed over the midbelly of the left GM muscle. Transducer position was standardized by determining the intersection of the mediolateral and proximodistal midline of the GM and aligning the transducer longitudinally to the fascicles, while transducer movement was minimized by using a custom-made cast, which was secured with elastic Velcro. Ultrasound recordings and electrogoniometer signals were time-synchronized via a rectangular pulse generated by a hand switch, which was recorded synchronously through the electrocardiography channel of the ultrasound and the MyoResearch software. A semi-automatic tracking algorithm [UltraTrack Software, version 4.2; 86] was used to quantify muscle fascicle length (distance between the insertion of the fascicles into the superficial and the deep aponeuroses) and pennation angles (angle between the fascicle and the deep aponeurosis) during the stance phase. Manual correction of the digitized fascicle and the deep aponeurosis, defined as a second fascicle, were performed where appropriate. If the field of view of the transducer was not sufficiently wide to capture the entire fascicle, the missing portion was

estimated via manual extrapolation based on the assumption that the fascicle and the aponeurosis extend linearly. Ultrasonography has been frequently used in dynamic conditions [29] and has been demonstrated to provide reliable measures of GM fascicle lengths and pennation angles [60,62].

2.1.3.6 SEE and MTU lengths

Series elastic element length was estimated using an MTU model by subtracting muscle fascicle lengths multiplied by the cosine of their pennation angles from the MTU lengths [75]. Muscle-tendon unit length was calculated via a linear regression equation [87], using participant's shank length data (the distance from the lateral malleolus to the lateral femur epicondyle) in addition to recorded knee and ankle joint angles.

2.1.3.7 Data processing

For each participant, and each outcome, eight consecutive stance phases (touchdown to toe-off) of the left foot per condition were analyzed and averaged using custom-made scripts (MATLAB R2018a, MathWorks, Inc., Natick, United States). Prior to being resampled to 101 data points per stance phase (to represent data as a percentage), fascicle lengths and pennation angles were smoothed with a five-point moving average. Electrogoniometer signals were smoothed with a fifth-order Butterworth low-pass filter, and a 10-Hz cut-off frequency. Fascicle velocity was calculated as the time derivative of its length using the central difference method [88].

Based on the ultrasound and joint-angle recordings SEE length, MTU length, fascicle length, pennation angle and fascicle velocity were determined at the time when peak SEE length was achieved, and thus force acting on the SEE is presumably at its greatest. Furthermore, average values across the stance phase were determined. Overall fascicle shortening was calculated by subtracting the minimum from maximum fascicle length. Knee and ankle joint range of motion were defined as the delta between their respective minimum and maximum joint angles. Additionally, the difference in knee and ankle joint angles between touchdown to the time of first local maximum and maximum dorsiflexion, were defined as knee flexion and ankle dorsiflexion, respectively. Knee and Ankle joint angles at touchdown and toe-off as well as ground-contact times were determined. To estimate the level of BWS achieved by applying LBPP, average plantar forces over the stance phase were determined and expressed as percentage of the average plantar forces when walking without BWS.

2.1.3.8 Statistical analysis

Distribution normality was assessed using the Shapiro–Wilk normality test. If normally distributed, a two-tailed paired t-test was performed, whereas if not, a non-parametric Wilcoxon (matched-pairs) signed rank test was used to compare conditions (30% vs. 0% BWS).

All tests were performed in GraphPad Prism (v 7.04) with a significance level of α = 0.05. Effect Sizes (dz) were calculated using the G*Power software version 3.1.9.4 [89]. Thresholds of 0.2, 0.5 and 0.8 were defined as small, moderate and large effects between the two comparison groups [90].

2.1.4 Results

Participants walking with 30% BWS generated significantly lower average plantar forces (-194 ± 32 N, P < 0.001, $d_z = -6.07$) corresponding to $68 \pm 4\%$ of the average plantar forces when walking without BWS, which did not differ significantly from the target of 70% (P = 0.223). Ground-contact times were 0.03 ± 0.03 s longer when walking with 30% BWS, however, the effect was statistically not significant (P = 0.078, $d_z = 0.80$) (Table 1).

Figure 7 presents the averages and standard errors of joint angles and muscle-SEE outcomes time normalized to a single stance phase for participants walking with 0% and 30% BWS. No statistically significant differences in knee and ankle joint angles at touchdown (P = 0.164, $d_z = -0.55$; P = 0.635, $d_z = -0.18$), at toe-off (P = 0.848, $d_z = -0.07$; P = 0.641, $d_z < 0.01$) and at the time of the peak SEE length (P = 0.461, $d_z = 0.42$; P =0.742, $d_z = 0.04$) were observed (Table 1). Furthermore, knee and ankle joint range of motion (P = 0.860, $d_z = 0.06$; P = 0.844, $d_z =$ -0.04), knee flexion (P = 0.347, $d_z = -0.36$) and ankle dorsiflexion (P = 0.204, $d_z = 0.50$) were unaffected by walking with 30% BWS (Table 1).

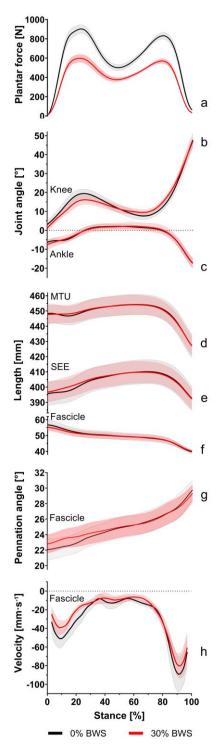


Figure 7. Kinetic, kinematic and GM fascicle-SEE parameters during the stance phase of walking without and with 30% BWS

Sample average and standard error of plantar forces (a), knee joint angle (b), ankle joint angle (c), muscletendon unit length (d), series elastic element length (e), fascicle length (f), pennation angle (g), and fascicle velocity (h) for participants walking at 75% of their preferred walk-to-run transition speed with 0% body weight support (black line) and 30% body weight support (red line) during the entire stance phase. The solid lines represent the sample average, and the corresponding shaded areas represent the standard error of measurement.

Table 1. Kinematic outcome measures while participants walked at 75% of their PTS with 0% and 30% body weight support

	0% BWS	30% BWS	Differences	95% CI	P	Effect Size
Ground contact time [s]	0.59 ± 0.04	0.62 ± 0.04	0.03 ± 0.03	-0.01 to 0.05	0.078 ^w	0.80
Ankle joint angle at touch-down [°]	-6.35 ± 3.26	−7.61 ± 7.74	−1.26 ± 7.17	-7.25 to 4.73	0.635 ^t	-0.18
Knee joint angle at touch-down [°]	3.07 ± 5.92	1.24 ± 5.21	−1.84 ± 3.34	-4.63 to 0.95	0.164 ^t	-0.55
Ankle joint angle at toe-off [°]	−17.47 ± 7.42	-17.45 ± 7.21	0.02 ± 10.69	-8.92 to 8.95	0.641 ^w	< 0.01
Knee joint angle at toe-off [°]	47.67 ± 11.38	46.94 ± 7.91	-0.73 ± 10.38	-9.41 to 7.94	0.848 ^t	-0.07
Ankle joint range of motion [°]	21.04 ± 5.47	20.88 ± 4.93	−0.16 ± 3.55	-3.12 to 2.81	0.844 ^w	-0.04
Knee joint range of motion [°]	45.21± 9.09	45.72 ± 3.77	0.51 ± 7.82	-6.03 to 7.04	0.860 ^t	0.06
Ankle dorsiflexion [°]	10.26 ± 3.04	11.82 ± 3.77	1.56 ± 3.15	-1.07 to 4.19	0.204 ^t	0.50
Knee flexion [°]	17.82 ± 4.42	15.81 ± 6.49	-2.01 ± 5.65	-6.73 to 2.71	0.347 ^t	-0.36
Ankle joint angle at peak SEE length [°]	2.41 ± 3.18	2.72 ± 6.06	0.31 ± 7.91	-6.30 to 6.92	0.742 ^w	0.04
Knee joint angle at peak SEE length [°]	9.19 ± 6.83	11.04 ± 4.75	1.85 ± 4.43	-1.86 to 5.55	0.461 ^w	0.42

Data are presented as mean \pm standard deviation. PTS: preferred walk-to-run transition speed; BWS: body weight support; CI: confidence interval; P: result of the paired t test ($^{\text{t}}$) or Wilcoxon matched-pairs signed rank test ($^{\text{w}}$) indicating a statistically significant effect of body weight support (α = 0.05). n = 8

Walking with 30% BWS had no effect on peak SEE length (P = 0.976, $d_z = -0.01$) (Figure 8a). Furthermore, at the time when peak SEE length was reached, no statistically significant differences from 0% BWS were observed for MTU length (P = 0.641, $d_z = -0.04$), fascicle length (P = 0.890, $d_z = -0.05$), pennation angle (P = 0.945, $d_z = -0.03$) and fascicle velocity (P = 0.576, $d_z = -0.21$) (Figure 8a-c).

No statistically significant differences of the average values across the entire stance phase were also observed for SEE length (P = 0.945, $d_z = 0.05$), MTU length (P = 0.641, $d_z = 0.01$), fascicle length (P = 0.790, $d_z = -0.10$), pennation angle (P = 0.641, $d_z = 0.16$) and fascicle velocity (P = 0.148, $d_z = 0.51$) between 0% and 30% BWS walking (Table 2).

Furthermore, overall fascicle shortening did not differ between conditions (P = 0.313, $d_z = -0.43$) (Table 2).

Table 2. Gastrocnemius medialis muscle and SEE outcome measures while participants walked at 75% of their PTS with 0% and 30% body weight support

	0% BWS	30% BWS	Differences	95% CI	P	Effect Size
Average SEE length [mm]	404.19 ± 23.40	404.62 ± 20.74	0.42 ± 7.70	-6.02 to 6.86	0.945 ^w	0.05
Average MTU length [mm]	449.11 ± 19.80	449.18 ± 18.27	0.07 ± 6.24	-5.15 to 5.28	0.641 ^w	0.01
Average fascicle length [mm]	49.44 ± 6.48	49.18 ± 5.28	−0.25 ± 2.58	-2.41 to 1.90	0.790 ^t	-0.10
Average pennation angle [°]	24.92 ± 3.93	25.16 ± 3.84	0.24 ± 1.46	-0.98 to 1.46	0.641 ^w	0.16
Average fascicle velocity [mm·s ⁻¹]	-31.03 ± 16.18	-26.01 ± 8.72	5.02 ± 9.91	-3.27 to 13.31	0.148 ^w	0.51
Overall fascicle shortening [mm]	17.23 ± 7.54	15.12 ± 4.64	−2.10 ± 4.91	-6.20 to 2.00	0.313 ^w	-0.43

Data are presented as mean \pm standard deviation. PTS: preferred walk-to-run transition speed; BWS: body weight support; CI: confidence interval; *P* result of the paired t test (*) or Wilcoxon matched-pairs signed rank test (*) indicating a statistically significant effect of body weight support ($\alpha = 0.05$). n = 8

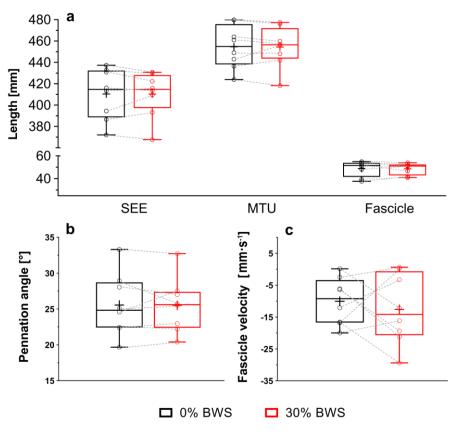


Figure 8. GM fascicle—SEE behavior at the time of peak SEE length when walking without and with 30% BWS

Series elastic element length (a), muscle–tendon unit length (a), fascicle length (a), pennation angle (b) and fascicle velocity (c) at the time of the peak series elastic element length as presented as boxplots for participants walking without body weight support (black box) and 30% body weight support (red box). The lower and upper parts of the box represent the first and third quartile, respectively. The length of the whisker represent the minimum and maximum values. The horizontal line in the box represents the statistical median of the sample; + the mean of the sample; \circ individual data points

2.1.5 Discussion

2.1.5.1 Effects of walking with 30% BWS on contractile and series elastic element behavior

During the walking trials, participants were successfully unloaded by 30% of their body weight, as the average plantar forces actually achieved by inducing LBPP did not differ significantly from the target average plantar forces. The main findings were that walking with 30% BWS did not significantly affect joint kinematics. Furthermore, in contrast to the hypotheses, walking with 30% BWS induced no statistically significant differences from 0% BWS in peak SEE length as well as MTU length, fascicle length and pennation angle neither at the time of the peak SEE length, nor on average across the stance phase. Also, in contrast with the hypotheses, no statistically significant effect of 30% BWS was found on fascicle shortening velocity at the time of the peak SEE length, nor on average across stance, despite a reduction in absolute walking speed (albeit same Froude number). These findings are further supported by the overall small effect sizes.

Previous studies and simulation models have shown that the GM force-length-velocity behavior shifts with gait type and speed to meet the varying locomotor demands [77,79]. However, in the present study, fascicle length and pennation angle were unchanged when walking with 30% BWS, which implies that the GM remains operating on a similar part of the force-length relationship, thereby preserving its force generation ability [79]. Moreover, GM fascicle velocity has been reported to decrease with decreasing walking speed, thereby increasing GM's force generation ability [77,79]. In fact, in the present study average fascicle velocity was $5.0 \pm 10 \text{ mm} \cdot \text{s}^{-1}$ slower when walking at a slightly slower speed at 30% vs. 0% BWS ($-0.24 \text{ m} \cdot \text{s}^{-1}$) reaching a moderate effect ($d_z = 0.51$), however, high variability may have contributed to it failing to reach statistical significance.

It has been reported that the ankle plantarflexion moment decreases with increasing BWS [39,72,73]. In fact, the present results suggests a reduction in average plantar force by almost 200 N whilst ankle joint kinematics were largely preserved when walking with 30% BWS suggesting that ankle joint moment was reduced. Interestingly, this did not affect peak SEE length, which incorporates the length of the free tendon and aponeurosis. As aponeurosis stiffness varies upon contractile conditions (e.g., reduced muscle activity results in lower orthogonal muscle expansion linked to lower transverse strain) [91], SEE length can remain similar despite a reduction in ankle joint moment.

However, as in the present observational study MTU interaction was only modelled for the GM, which accounts for a modest fraction (~ 17%) of the physiologic cross-sectional area of the plantar flexor muscles [92], changes that influence the ankle joint moment might not be fully reflected. Furthermore, joint moments were not determined and SEE length was not measured directly but

estimated using an MTU model. Thus, if tuning of the mechanical properties of the SEE actually causes preserved SEE and fascicle kinematics warrants further study.

2.1.5.2 Implications for rehabilitative body weight supported gait training

Maintenance of joint kinematics and GM behavior may facilitate rehabilitative gait training by preserving "natural" movement patterns, despite joint loads and related pain being reduced [37,40]. Preserved fascicle's operating range suggests that the stimuli exerted on the muscle remain the same and thus help to maintain optimum fascicle length for force production, which is key for locomotor recovery. Furthermore, the maintenance of SEE strain, as possibly achieved by an increased aponeurosis strain, might help to prevent degeneration and maintain function of the aponeurosis despite external unloading. Patients who may benefit from LBPP gait training during their early postoperative rehabilitation include not only those with tendon, ligament and meniscus repairs but also joint replacements or fractures [37]. However, the increased aponeurosis strain, which is required to compensate for the decreased free tendon strain (and thus to maintain SEE strain), could pose a potential risk to patients after Achilles tendon rupture if the rupture does not exclusively affect the free tendon. Therefore, BWS rehabilitation should be individualized to the specific pathological characteristics of patients, depending on the impaired biological tissues that require unloading, e.g., rehabilitation after total knee arthroplasty vs. ankle or Achilles tendon injury. Based on the current findings, further studies including different patient groups are required.

The present data, are not only in agreement with a recent systematic review, which concluded that spatio-temporal and kinematic gait parameters can be preserved with up to 30% BWS [41], but extends this view to preserved muscle-SEE mechanics. In fact, healthy individuals appear able to retain normal walking kinematics even when unloaded by up 50% BWS [93,94]. The absence of any effects when BWS was increased from 0% to 30% suggests that the modulation of fascicle-SEE behavior does not develop linearly with increasing BWS but is determined by a certain threshold, however if this threshold is below or above 50% BWS remains to be determined. Additionally, if non-LBPP BWS systems, such as overhead suspension harnesses, therapistassisted waist belts or robotic-assisted gait-training devices, are also able to preserve GM behavior warrants further study. Nevertheless, the present observational study supports the recommendation [39] for LBPP-induced 30% BWS in rehabilitative gait training. Finally, it should be noted that walking speed was intentionally reduced with increasing BWS via the adjustment to the same Froude number to obtain mechanically equivalent walking speeds [82]. Thus, the observation that the neural system appears to largely preserve GM overall contraction behavior in addition to joint kinematics suggests that the approach of producing comparable gait patterns across the different walking conditions was successful and should be considered for future gait rehabilitation.

2.1.6 Conclusions

This is the first study to examine *in vivo* GM fascicle–SEE behavior during walking at 30% BWS, frequently employed in gait rehabilitation, at 75% PTS on an LBPP treadmill. The present findings reveal that during walking with 30% BWS fascicle–SEE behavior was largely preserved, in contrast to the hypothesis. Thus, the present study not only supports the contention made in previous studies that walking with the recommended therapeutic dose of 30% BWS largely retains spatio-temporal and joint kinematic characteristics but extends this to GM fascicle and SEE mechanics. This may be advantageous during rehabilitative gait training with BWS as it indicates transferability of gait patterns to subsequent unsupported walking.

2.1.7 Additional Information

2.1.7.1 Data Availability Statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

2.1.7.2 Ethics Statement

The study involving human participants were reviewed and approved by Ärztekammer Nordrhein Ethical Committee of Düsseldorf, Germany. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for publication of any potentially identifiable images or data included in this article.

2.1.7.3 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.

2.1.7.4 Funding

This study was funded by the ESA's Space Medicine Team (HRE-OM) of the European Astronaut Center (EAC) in Cologne, Germany and the University of Applied Sciences Aachen obtained funding by the Federal Ministry for Economic Affairs and Energy (50WB1728). Spaceship EAC provided the lower body positive pressure treadmill (AlterG) used in this study.

2.1.7.5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.1.8 References

Please refer to the common reference list at the end of this dissertation.

2.2 Article 2 - Contractile Behavior of the Gastrocnemius Medialis Muscle during Running in Simulated Hypogravity

Article published in npj Microgravity (2021) 7:32. doi: 10.1038/s41526-021-00155-7

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Article Info

Received: 03 November 2020

Accepted: 11 June 2021

Published: 09 August 2021

Impact: 4.3

Number of Figures: 4

Number of Tables: 1

Link to online article: https://doi.org/10.1038/s41526-021-00155-7

2.2.1 Abstract

Vigorous exercise countermeasures in microgravity can largely attenuate muscular degeneration, albeit the extent of applied loading is key for the extent of muscle wasting. Running on the International Space Station is usually performed with maximum loads of 70% body weight (0.7g). However, it has not been investigated how the reduced musculoskeletal loading affects muscle and series elastic element dynamics, and thereby force and power generation. Therefore, this study examined the effects of running on the vertical treadmill facility, a ground-based analogue, at simulated 0.7g on gastrocnemius medialis contractile behavior. The results reveal that fascicle-series elastic element behavior differs between simulated hypogravity and 1g running. Whilst shorter peak series elastic element lengths at simulated 0.7g appear to be the result of lower muscular and gravitational forces acting on it, increased fascicle lengths and decreased velocities could not be anticipated, but may inform the development of optimized running training in hypogravity. However, whether the alterations in contractile behavior precipitate musculoskeletal degeneration warrants further study.

Keywords: unloading, muscle fascicle behavior, series elastic element behavior, ultrasound imaging, running

2.2.2 Introduction

Astronauts exposed to a micro-g-force environment, often referred to as microgravity (µg), experience many physiological adaptations, including musculoskeletal deconditioning, with the plantar flexor muscles appearing particularly susceptible to atrophy [10,95]. To prevent these detrimental effects, the crewmembers of the International Space Station (ISS) perform daily exercise countermeasures, including treadmill running, cycling, and resistance training [13]. Due to the implementation of new exercise hardware and improvements of the in-flight exercise hardware and exercise prescriptions, µg-induced physiological deconditioning has been reduced, although variable inter-individual physiological responses to the exercise induced stimuli persist [26,27].

For instance, a recent study investigating the plantar flexor muscles of two ISS crewmembers suggests that vigorous treadmill and resistive training reduces the decrements in muscle volume and lower limb strength and the deteriorations in muscle architecture [20]. Moreover, the muscle wasting seems to affect the organism's systemic inflammatory/anti-inflammatory balance [96], which highlights the requirement to safeguard musculoskeletal health in space. The extent of muscle wasting is likely related to the training volume (sets, repetition, and duration) and training intensity in particular with regard to the maximum external loading that can comfortably be applied during countermeasure exercises [20].

ISS crewmembers that are part of U.S. Orbital Segment currently perform locomotion countermeasures on the T2 treadmill. On this treadmill, subject loading is currently provided via a harness system connected to a bungee assembly that is clipped in series with several carabiner clips. The applied harness load is usually an individual crew choice, mainly limited by increasing discomfort of the harness system at higher loads (A. Gerst, Personal Communication 2021, see Supplementary Reference). Running sessions are thus usually performed with ~70% of the equivalent body weight (BW) at 1g ($g = 9.81 \text{ m·s}^{-2}$) [13], resulting in lower peak ground reaction forces (~1.3 BW when running at 2.2 m·s⁻¹) compared to terrestrial running [19].

A ground-based analogue to simulate hypogravity running on the ISS is the vertical treadmill facility (VTF), where subjects are suspended horizontally with graded "pull-down" forces toward a vertically mounted treadmill provided via a harness-based subject loading system [28,97,98]. Despite marginal differences in joint kinematics and ground reaction forces between running in actual µg (parabolic flight) vs. running in simulated µg (VTF), the latter is still regarded as a valid analogue, even though it does not provide a 1:1 representation of running in actual µg since on the VTF the (vertically suspended) body and in particular the suspended limbs are still exposed to gravity [45].

Running on the ISS or on ground-based hypogravity simulation systems is not only associated with reduced ground reaction forces but also with lower plantar load [17,99,100]. In addition, metabolic cost was found to be reduced during running in simulated hypogravity [70,100,101]. Furthermore, estimated ankle joint forces [102] and peak ankle dorsiflexion and knee flexion as well as range of motion [58] were reported to be reduced when running at different velocities (2.2–3.5 m·s⁻¹) on a lower body positive pressure treadmill to simulate hypogravity.

In contrast, running with additional mass (120% of BW, equivalent to 1.2g), was found to require more mechanical work at the ankle and knee joints [103]. Despite these changes in kinetic gait parameters, the overall gastrocnemius medialis (GM) muscle fascicle behavior and peak series elastic element (SEE) length were found to be largely preserved. Interestingly, essentially preserved fascicle–SEE behavior was also observed when walking with only 70% BW achieved by lower body positive pressure [104]. In 1g, whilst changes in walking speed between 0.75 and 2.00 m·s⁻¹ have been shown to affect fascicle velocity (at the time of peak force), no effects were observed when changing running speed between 2.00 and 3.25 m·s⁻¹ [77]. Taken together, these findings make it difficult to predict if and how the neuromuscular system modulates fascicle–SEE dynamics when running in simulated hypogravity.

When reducing the loading level, gait transitions occur at a slower preferred walk-to-run transition speed (PTS) but at a similar Froude number, a dimensionless number embedding gait speed, leg lengths, and gravitational acceleration [49-51]. Thus, to run at "dynamically similar" speeds (i.e., at a similar running speed relative to the PTS) in hypogravity, one must run at the same Froude number, which means a reduction in absolute running speed. However, the influence of hypogravity running at a dynamically similar speed on the interaction between the contractile and series elastic elements within GM's muscle—tendon unit (MTU) has not been investigated.

Therefore, the aim of this study was to investigate, via ultrasonography, GM fascicle-SEE behavior in addition to joint kinematics during running with 125% of the PTS at a simulated hypogravity level of 0.7g (on the VTF) versus 1g. We hypothesized that fascicle-SEE behavior will be preserved when running in simulated hypogravity at 125% of the PTS.

2.2.3 Results

2.2.3.1 Kinetic and spatio-temporal parameters

Running speeds in this study were selected to correspond to 125% of the participants' PTS, resulting in average running speeds of $2.62 \pm 0.08 \text{ m} \cdot \text{s}^{-1}$ at 1g and $2.23 \pm 0.07 \text{ m} \cdot \text{s}^{-1}$ at simulated 0.7g. Participants running on the VTF at simulated hypogravity of 0.7g were subjected to lower (t(7) = 11.465, P <0.001, $d_z = -4.1$) mean loading levels than at 1g, corresponding to 63.4 ± 4.8% (mean ± standard deviation) of the loading levels determined during running on a conventional treadmill. Peak plantar forces (t(7) = 9.070, P < 0.001, $d_z = -3.2$) were reduced by 633.3 ± 197.5 N (95% confidence interval (CI), -798.4 to -468.2) at simulated 0.7g compared to 1g (Figure 9a). In contrast, groundcontact times (t(7) = 5.597, P < 0.001, $d_z =$ 2.0) were increased by $0.05 \pm 0.02 \text{ s}$ (95% CI, 0.03 to 0.07) when running at simulated 0.7g (Table 3). Accordingly, cadence (t(7) =5.442, P = 0.001, $d_z = -1.9$) was decreased by $10.1 \pm 5.2 \text{ steps} \cdot \text{min}^{-1}$ (95% CI, -14.4 to-5.7) at simulated 0.7g compared to 1g.

2.2.3.2 Joint kinematics

The participant's knee and ankle joint movement patterns during running at simulated 0.7g vs. 1g are displayed in Figure 9b and Figure 9c, respectively. Knee joint range of motion (t(7) = 3.057, P = 0.018, $d_z = -1.1$) was lower by $4.3 \pm 4.0^{\circ}$ (95% CI, -7.7 to -1.0) at simulated 0.7g compared to 1g, whereas ankle joint range of motion

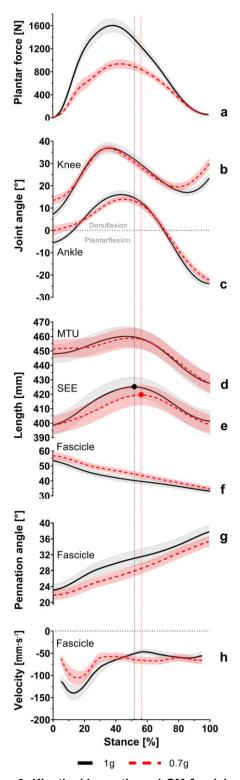


Figure 9. Kinetic, kinematic and GM fascicle–SEE parameters during the stance phase of running at 1g and simulated 0.7g

Participants' average (mean ± standard error of the mean) patterns of plantar forces (a), knee (b) and ankle (c) joint angles, and MTU (d) and SEE (e) lengths as well as muscle fascicle length (f), pennation angle (g), and velocity (h) change during the stance phase of running at 1g (black line) and simulated 0.7g (red dashed line). The vertical lines mark the peak SEE length achieved at 1g (black) and simulated 0.7g (red). n = 8 participants

(t(7) = 1.595, P = 0.155, d_z = -0.6) was not affected by unloading, with a mean difference of 3.7 \pm 6.6° (95% CI, -9.3 to 1.8) (Table 3). Furthermore, ankle dorsiflexion (t(7) = 6.629, P < 0.001, d_z = -2.3) and knee flexion (t(7) = 4.503, P = 0.003, d_z = -1.6) during the first half of the stance phase were both lower at simulated 0.7g, by 6.8 \pm 2.9° (95% CI, -9.2 to -4.4) and 5.9 \pm 3.7° (95% CI, -9.0 to -2.8), respectively (Table 3). At the time of peak SEE length, both ankle (t(7) = 3.144, P = 0.016, d_z = -1.1) and knee (t(7) = 2.706, P = 0.030, d_z = -1.0) joint angles were lower during running at simulated 0.7g, by 3.0 \pm 2.7° (95% CI, -5.3 to -0.7) and 3.3 \pm 3.4° (95% CI, -6.2 to -0.4), respectively (Table 3).

Table 3. Spatio-temporal and kinematic parameters while participants ran at 125% of their PTS at 1g and simulated 0.7g

Parameters	1g	0.7g	P
Ground contact time [s]	0.30 ± 0.04	0.35 ± 0.04*	< 0.001
Cadence [steps⋅min ⁻¹]	83.25 ± 5.90	73.19 ± 4.21*	0.001
Ankle joint range of motion [°]	40.18 ± 7.72	36.44 ± 5.99	0.155
Knee joint range of motion [°]	30.03 ± 5.15	25.69 ± 3.65*	0.018
Ankle dorsiflexion [°]	21.91 ± 3.92	15.11 ± 5.02*	< 0.001
Knee flexion [°]	29.98 ± 5.20	24.06 ± 4.99*	0.003
Ankle joint angle at peak SEE length [°]	15.19 ± 5.09	12.81 ± 4.06*	0.016
Knee joint angle at peak SEE length [°]	31.92 ± 6.25	28.63 ± 4.75*	0.030

Data are presented as mean \pm standard deviation. PTS: preferred walk-to-run transition speed. *significantly different (two-tailed paired t-test) from 1g ($P \le 0.05$). Peak SEE length at simulated 0.7g and 1g occurred at 57 \pm 4% and 52 \pm 7% of stance, respectively. n = 8 participants

2.2.3.3 GM muscle and SEE parameters

Temporal differences in muscle-SEE parameters within the single stance phase between running at simulated 0.7g and 1g are depicted in Figure 9 d-h.

Loading level had no effect upon overall fascicle shortening (t(7) = 1.646, P = 0.144, d_z = 0.6), with a mean difference of 1.6 ± 2.7 mm (95% CI, -0.7 to 3.9). However, at the time of peak SEE length, muscle fascicles operated at a longer length (Δ = 3.3 ± 1.9 mm, 95% CI 1.7 to 4.9, t(7) = 4.922, P = 0.002, d_z = 1.7, Figure 10a), smaller pennation angle (Δ = -2.7 ± 2.0°, 95% CI -4.3 to -1.0, t(7) = 3.789, P = 0.007, d_z = -1.3, Figure 10b), and faster shortening velocity (Δ = 19.0 ± 16.6 mm·s⁻¹, 95% CI -32.9 to -5.1, t(7) = 3.230, P = 0.014, d_z = -1.1, Figure 10c) at simulated 0.7g compared to 1g.

The peak SEE length (t(7) = 4.315, P = 0.004, $d_z = -1.5$) and the MTU length at the time of peak SEE length (t(7) = 2.547, P = 0.038, $d_z = -0.9$) were shorter during running at simulated 0.7g

compared to 1g, by 5.6 ± 3.7 mm (95% CI, -8.7 to -2.5) and 1.8 ± 2.0 mm (95% CI, -3.5 to -0.1), respectively (Figure 10a).

The time at which peak SEE length was achieved (t(7) = 1.860, P = 0.105, d_z = 0.7) did not differ between loading levels although peak SEE length was attained slightly later (56.9 ± 4.1% vs. 51.5 ± 7.5% of stance phase, 95% CI, -1.5 to 12.2) at simulated 0.7g compared to 1g.

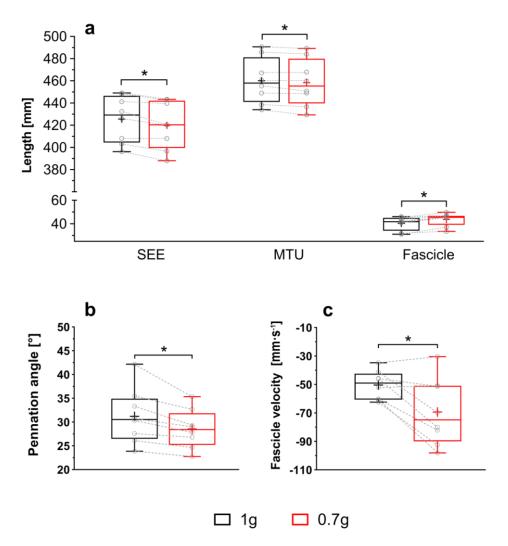


Figure 10. GM fascicle—SEE behavior at the time of peak SEE length when running at 1g and simulated 0.7g

SEE length (a, left), MTU length (a, middle), fascicle length (a, right), pennation angle (b), and fascicle velocity (c) at the time of the peak SEE length differ between running at 1g (black box) and simulated 0.7g (red box). 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; \circ individual data points; * significantly different (two-tailed paired t-test) from 1g (P \leq 0.05). n = 8 participants

2.2.4 Discussion

Running on the VTF with 125% of the PTS at simulated 0.7g vs. 1g induced peak plantar forces corresponding to ~1.3 BW, which are similar to those observed when running with bungee loading on board the ISS [19]. The main findings of the present study were that simulated 0.7g running increased ground contact time, reduced cadence, and lowered ankle dorsiflexion and knee flexion at the time of peak SEE length. Concurrently, GM fascicles operated at longer lengths, smaller pennation angles, and faster shortening velocities, whilst MTU and SEE lengths were shorter.

Hypogravity (0.7g) running induced a significant reduction in peak plantar forces (-39.3%), whilst ground-contact times were slightly increased, resulting in a greater time available for the neuromuscular system to adopt GM's contractile behavior. In addition, ankle and knee joint angles at the time of the peak SEE length were significantly reduced, consistent with a previous lower body positive pressure study reporting that participants adapted their running pattern when the loading level was < 0.8g [58]. Smaller ankle joint flexions were also observed in studies investigating running on a treadmill equipped with a vertical body weight support system [59] or a subject loading system as used during parabolic flights [98]. Changes in GM's MTU behavior may thus result from an altered movement pattern induced by prolonged stance phase durations and altered joint kinematics. For instance, lower ankle dorsiflexion at peak SEE length may compensate for the less-flexed knee joint, significantly shortening MTUs.

Furthermore, the present study indicates that at the time of peak SEE length, where the force acting on the SEE is at its greatest, GM fascicles are less contracted compared to 1g running. Simultaneously, the pennation angle was found to be significantly smaller, potentially facilitating fascicles to operate at longer lengths. However, this contrasts with preliminary findings indicating that overall fascicle behavior is relatively stable against a reduction or increase in loading by 30% BW [104] or 20% BW [103], respectively.

During 1g running, fascicles barely reach the plateau region of the force-length relationship [76,105], thereby limiting their ability to generate force. Thus, the increase in fascicle length observed during simulated (0.7g) hypogravity running may enable the GM to operate closer to its optimum length, thereby enhancing force-generation ability. By using a normalized active force-length relationship represented by a Gaussian function [106] and an optimum GM fascicle length of 51.0 ± 9.8 mm (99% confidence interval, 45.0 - 58.0 mm), as determined by a cadaveric study [92], we estimate an increase in GM's force-generation ability by as much as 6% when running at simulated 0.7g. Moreover, a shift in the fascicles' operating range toward longer lengths may result in an increased strain on the z-discs, potentially preserving or increasing the number of sarcomeres in-series, which in turn may be beneficial for muscle mass preservation [107].

On the other hand, fascicle-shortening velocity at peak SEE length was found to be significantly increased during running at simulated 0.7g. This may result in less-favorable contractile conditions as fascicles are less able to generate force with increasing speed of contraction [108]. In fact, it has been reported that fascicle-shortening velocity is a major determinant of the preferred walk-to-run transition by improving fascicles' contractile conditions after switching gait to counteract impaired muscle force production [77,80]. Indeed, a change in fascicle velocity is noted to have a greater impact on muscular performance than a change in fascicle length, especially at high running speeds [109].

Interestingly, at 1g, fascicles operated at a sub-optimal length but at a slower and more optimal shortening velocity for generating force. Assuming that fascicle neuro-motor control is optimally adapted to 1g, an increase in fascicle velocity and thus induction of less-favorable contractile conditions in simulated 0.7g may outweigh the benefits from an increase in fascicle length. In fact, the significantly shorter peak SEE length observed during simulated 0.7g running may be the direct result of lower muscular forces acting on the SEE. In addition, according to the MTU's stretch-shortening cycle, the smaller SEE strain should result in less stored and thus released elastic energy. However, as an exact replication of running in actual microgravity is not possible using the VTF, the present results strongly suggest but do not prove that running on the ISS induces a significant change in muscle—tendon dynamics in response to lower musculoskeletal loading.

Although, given a largely preserved fascicle behavior when running with increased loading [103], one might speculate that when the musculoskeletal loading is increased, it is more important to preserve the well-adapted contractile conditions to favor economical force production.

It has been proposed that the provision of (non-standardized) external force loading while exercising in µg may underlie the observation of variable muscular degeneration during long-term spaceflight [20]. The present (in vivo) study supports this notion as significant alterations in GM fascicle–SEE outcome parameters were observed between running at simulated 0.7g on a ground-based analogue and 1g. Such alterations point to functional adaptations in response to a reduced locomotor demand during hypogravity running, involving not only lower gravitational but also muscular forces that may precipitate musculoskeletal degeneration [16]. Thus, it appears that the consequences of hypogravity running are not limited to a mere reduction in mechanical loading but also to an altered contractile behavior, which could affect the muscle's work capacity upon the return to daily activities in a 1g environment. The longer fascicles may be beneficial to preserve muscle mass but may also result in long-term adaptations in optimal fascicle length that are no longer functional for the requirements on Earth and may require specific attention during the rehabilitation phase upon return to Earth's 1g environment [107,110].

To increase the mechanical loading on the MTU during hypogravity running, and hence to induce muscle fascicle-SEE behavior that is similar to that in 1g conditions (to ensure the stimuli exerted on the muscle remain the same), it would be required that the harness applies a higher external force to the body than what is typically chosen by crewmembers. This force is mainly limited by considerable discomfort of the harness at higher loads, especially during gait cycle phases with maximum stretch of the bungee assembly [13,15]. Moreover, whether the provision of full BW loading is actually optimal, is subject to further research. An alternative approach may be to increase running speed [19,100,111], which has been shown to augment maximum plantar force [99]. Interestingly, many ISS crewmembers appear to intuitively increase their running speed to achieve a perceived workout intensity that is similar to what they are used to on Earth despite the reduced external loading (A. Gerst, Personal Communication 2021, see Supplementary Reference). This sensation may relate to the fact that increasing running speed reduces the ground-contact time but requires a higher force production, evident by greater plantar flexor muscle activity [103]. However, further research is needed to investigate the interaction between loading level and running speeds on fascicle-SEE behavior in vivo. Another possibility to mitigate µg-induced muscle wasting would be to increase the volume of in-flight treadmill running. However, one goal of optimizing exercise countermeasures in space is the reduction of crew time spent on exercise whilst maintaining or improving the effectiveness of currently prescribed exercise countermeasures [112].

To conclude, simulated 0.7g running significantly alters fascicle—SEE interaction. For instance, a shorter peak SEE length seems to be the result of lower muscular forces acting on it. However, to answer the question as to whether there is a loading and running speed combination above which muscular deconditioning is prevented, additional measurements of torque and neuromuscular activation are required to estimate the effects of (various-level) hypogravity running on GM strain and resultant contractility/excitability. Such knowledge is crucial to inform the development of optimized running training in hypogravity but may also inform the mechanisms of contractile behavior regulation on Earth.

2.2.5 Methods

2.2.5.1 Participants

Eight healthy male volunteers (31.9 ± 4.7 years, 178.4 ± 5.7 cm heights, 94 ± 6 cm leg lengths, 73.5 ± 7.3 kg body masses) 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. All participants were examined medically. Exclusion criteria included cardiovascular, musculoskeletal, or neurological diseases and/or surgery within two years prior to participation.

2.2.5.2 Study design and experimental protocol

Participants attended the laboratory on a single occasion and familiarized themselves with running on the vertical treadmill facility (VTF; Arsalis, Glabais, Belgium, Figure 11) at their predefined running speed (125% PTS). After achieving a stable gait, 30 s were recorded while they ran on the VTF at simulated 0.7g in addition to on a conventional treadmill at 1g.

The VTF comprises a customized, motorized treadmill (Woodway, Waukesha, WI, USA) mounted vertically onto a chassis with an overhead suspension system, allowing supine suspension of the participant using a customized cradle and harness. Fabric cuffs attached to cords support the participants' torso and pelvis and each foot, thigh, and arm. An adjustable piston-based loading system generates a constant controllable force pulling the participant toward the treadmill belt via fixation to the harness at the pelvis (Figure 11). Written informed consent was obtained for publication of this photograph (Figure 11).

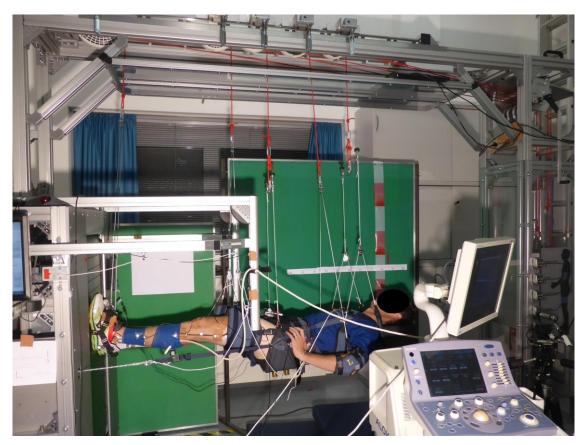


Figure 11. VTF experimental set-up

Participant being suspended horizontally on the vertical treadmill facility (VTF) with an ultrasound transducer attached to the mid-belly of the GM muscle and electrogoniometers to record knee and ankle joint angles. Photo credit: Charlotte Richter; participant provided written informed consent to publish this photo

At both loading levels, running speeds were defined as 125% of the PTS to obtain mechanically equivalent running speeds. PTS, expressed as a Froude number (PTS_{FR}), was 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. ⁵¹ using the resulting acceleration (a) as the independent variable. Hence, for a = 0.7 g, a PTS_{FR} value of 0.49 was obtained. By accounting for the participants' leg length (I), the individual $PTS(a) = \sqrt{PTS_{FR}(a) \cdot a \cdot l}$ was determined for each participant; moreover, adding 25% to this PTS resulted in running speeds of 2.62 \pm 0.08 m·s⁻¹ at 1g and 2.23 \pm 0.07 m·s⁻¹ at simulated 0.7g.

2.2.5.3 Data collection

Joint kinematics

Knee and ankle joint angles were recorded using a twin-axis (Penny and Giles Biometrics Ltd., Blackwood Gwent, UK) and a custom-made 2D-electrogoniometer, respectively. The end blocks of the knee electrogoniometer were positioned along the line from the greater trochanter to the lateral femur epicondyle and from the lateral femur epicondyle to the lateral malleolus. The end blocks of the ankle electrogoniometer were placed along the line from the lateral femur epicondyle to the lateral malleolus and from the lateral malleolus to the most distal end of the fifth metatarsal bone. Before each running trial, the goniometers were zeroed when in the anatomical neutral position (standing). Electrogoniometry data were sampled at 1500 Hz via the TeleMyo 2400 G2 Telemetry System (Noraxon USA., Inc., Scottsdale, USA) using the MyoResearch XP software (Master Edition 1.08.16). Electrogoniometry has been revealed to produce reliable and reproducible knee and ankle joint kinematics [53-55] and has already been used during running with reduced loading [56-59].

Spatio-temporal parameters

Shoe insoles (novel GmbH, loadsol® version 1.4.60, Munich, Germany) were used to measure plantar forces during running and hence to determine the stance phase. Touchdown and toe-off were automatically detected from the signal acquired with a sampling rate of 83 Hz via a custom-made script (MATLAB R2018a, MathWorks, Inc., Natick, United States) using a 20 N force threshold for 0.1 s. Insole and electrogoniometer signals were time-synchronized via recording of a rectangular TTL pulse generated by pressing on a custom-made pedal before each running trial.

GM muscle fascicle length and pennation angle

Real-time 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),

placed inside a custom-made cast to prevent shifting, was positioned at the intersection of the mediolateral and proximodistal midline 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. Ultrasonography has been frequently used in dynamic conditions [29] and is regarded as a reliable method to quantify fascicle architecture. Fascicle length and pennation angle show good reproducibility not only within sessions but also between sessions [60,61].

A semi-automatic tracking algorithm (UltraTrack Software, version 4.2) [86] was used to quantify GM fascicle lengths and pennation angles. Manual correction of the digitized fascicle and the deep aponeurosis, defined as a second fascicle, was performed where appropriate. Fascicle length was defined as the distance between the insertions to the superficial and deep aponeurosis parallel to the lines of collagenous tissue (Figure 12). If the transducer's field of view was too small to display the entire fascicle, the missing portion was extrapolated, assuming that the fascicle and the aponeuroses extended linearly. The pennation angle (ϕ) was defined as the angle between the fascicle and the deep aponeurosis (Figure 12).

SEE and MTU length

To calculate SEE length (Achilles tendon, aponeuroses, and proximal tendon) on the basis of an MTU model [75], muscle fascicle lengths multiplied by the cosine of their pennation angles were subtracted from the MTU lengths. MTU length was calculated via a multiple linear regression equation [87] using the participant's shank length as well as their knee and ankle joint angles.

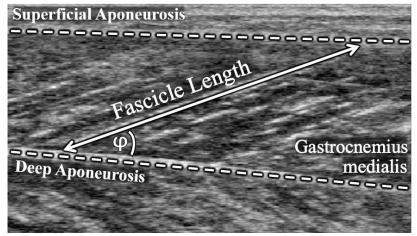


Figure 12. Ultrasound image of the gastrocnemius medialis with schematic representation of the extracted fascicle parameters

The pennation angle (ϕ) of the muscle fascicles (double-headed arrow) is defined with respect to the deep aponeurosis (lower dashed line). Fascicle length is measured as the length following the pennation between the deep and the superficial (upper dashed line) aponeuroses.

2.2.5.4 Data processing

For each participant and each outcome measure at each loading level, the first eight consecutive left foot stance phases (from the 30 s of data recording) were analyzed using a custom-made script (MATLAB R2018a, MathWorks, Inc., Natick, United States). Fascicle length and pennation angle 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. Muscle fascicle velocities were calculated as the time derivative of the respective length using the central difference method [88]. Data were time-normalized by being resampled to 101 data points per stance phase.

To estimate the loading achieved on the VTF, average simulated gravity levels over the stance phase were calculated via plantar force and impulse and expressed as percentage of the average gravity 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 were calculated as the time between left foot touchdown and toe-off. Cadence was defined as steps (duration from touchdown to the next ipsilateral touchdown) per minute. Ankle and knee joint angles as well as SEE-, fascicle-, and MTU lengths in addition to fascicle pennation angle and velocity were determined at the time of the peak SEE length, where the force acting on the SEE is at its greatest. Overall fascicle shortening was calculated by subtracting the minimum from the maximum fascicle length. Ankle and knee joint ranges of motion were defined as the differences between their respective minimum and maximum joint angles. The differences in knee and ankle joint angles between touchdown to the time of first local maximum and maximum dorsiflexion were defined as knee flexion and ankle dorsiflexion, respectively.

2.2.5.5 Statistical analysis

Data distribution for all outcome measures was assessed using the Shapiro–Wilk normality test. As normal distribution was confirmed for all outcome measures, a two-tailed paired t-test (n = 8 participants) was performed to test for significant differences in joint kinematics and fascicle–SEE outcomes between loading levels (1g vs. simulated 0.7g). All statistical analysis was performed in GraphPad Prism (v 7.04) with α set to 0.05. Data is reported as mean (\pm standard deviation). Effect sizes (d_z) were calculated using the G*Power software version 3.1.9.4 [89]. Thresholds of 0.2, 0.5, and 0.8 were defined as small, moderate, and large effects between the two comparison groups [90].

2.2.6 Additional Information

2.2.6.1 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

2.2.6.2 Code Availability Statement

A custom-made MATLAB code (MathWorks, Inc., Natick, United States) used for data analysis is available from the corresponding author upon reasonable request.

2.2.6.3 Acknowledgments

This study was supported with funding from the ESA Space Medicine Team (HRE-OM) of the European Astronaut Centre in Cologne, Germany, and the University of Applied Science Aachen obtained funding from the Federal Ministry for Economic Affairs and Energy (50WB1728). ESA provided the VTF used in this study.

2.2.6.4 Competing Interests Statement

Authors Dr. David A. Green and Dr. Tobias Weber are employed by KBR on behalf of the European Space Agency. All authors declare that the research was conducted in the absence of any commercial and financial relationships or non-financial interests that could be construed as a potential conflict of interest.

2.2.6.5 Author Contributions

BB, TW, and DG conceptualized research. BB, JA, TW, KM, JR, DG, and KA designed the research. CR, BB, BS, JA, and AS acquired the data; CR, BB, BS, AS, and KA analyzed the data; and CR, BB, JR, DG, and KA interpreted the data. CR and DG drafted the manuscript; BB, BS, JA, TW, KM, JR, DG, and KA revised the manuscript. All authors approved the manuscript and account 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.

2.2.6.6 Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1038/s41526-021-00155-7.

2.2.7 References

Please refer to the common reference list at the end of this cumulative dissertation.

2.3 Article 3 – Gastrocnemius Medialis Contractile Behavior during Running differs between Simulated Lunar and Martian Gravities

Article published in Scientific Reports (2021) 11:22555. doi: 10.1038/s41598-021-00527-9

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Article Info

Received: 18 June 2021

Accepted: 13 October 2021

Published: 19 November 2021

Impact: 4.3

Number of Figures: 4

Number of Tables: 2

Link to online article: https://doi.org/10.1038/s41598-021-00527-9



2.3.1 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 the gravity level, the reduction in gastrocnemius medialis activation even appears to level off around 0.2g, while 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 simulated Lunar gravity on the vertical treadmill facility. The results indicate that hypogravity-induced alterations in joint kinematics and contractile behavior still persist between simulated running on the Moon and Mars. This contrasts with the concept 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

2.3.2 Introduction

Human space exploration has fascinated mankind since the start of the Space Age in the 1950s. 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 building the Lunar Orbital Platform–Gateway, including a Human Lunar Lander, and setting up a permanent surface habitat that may serve as a springboard for future human missions to Mars [30].

Although the Apollo missions showed that humans can effectively operate in Lunar gravity [31], with surface stay times of up to 75 hrs [113], the data collected during locomotion which would provide useful information about biomechanical alterations required to enable surface activities and 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 [7] were observed to be particularly susceptible to atrophy and to architectural changes induced by reduced loading [9,10]. Thus, on Earth as well as 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 [16]. However, alterations in gravitational acceleration (g) appear to modify running gait. Thus, ground-based analogues have been developed to study locomotion in simulated hypogravity [42]. However, most hypogravity biomechanical studies have focused on identifying differences with Earth's gravitational acceleration (1g) [33,114].

Previous studies investigating running at 1g and at simulated hypogravity levels broadly equivalent to Lunar and Martian gravity (0.16–0.40g) have indicated reductions in the magnitudes of most gait parameters, such as ground reaction forces [102,115], mechanical work [69], estimated joint forces [102], and muscle activation [57,102] with decreasing g-level. Similarly, running kinematics, such as ground contact times, cadence [58,102,115], and lower limb joint angles [58,116] also tend to reduce with simulated g-level.

However, despite the fact that the ankle dorsiflexion angles are smaller when running in simulated hypogravity, the ankle is reported to follow a similar joint movement profile [59]. Furthermore, the lower limb muscle activation patterns [57,102] and leg stiffness (considered as a linear spring) [115] are largely preserved.

Moreover, the biomechanical parameters may not necessarily be proportional to the hypogravity level [64]. Indeed, the GM is sensitive to changes in force loading, as evidenced by a reduction in muscle activation, even though it appears that there might be a ceiling effect around 0.2g [57]. Running at simulated 0.7g has shown to modulate GM contractile behavior. For instance, at peak

series elastic element (SEE) length, where the force acting on the SEE is at its greatest, the GM fascicles operated at longer lengths, with smaller pennation angles but faster shortening velocities [117]. However, whether this pattern occurs in the GM muscle–tendon unit (MTU) at simulated Martian (0.38g) and Lunar gravity (0.16g) is unknown [33]. 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 surface operations to Martian ones.

However, to compare conditions, one must consider the fact that a decrease in the g-level results in the walk-to-run transition occurring at slower absolute speeds but with similar Froude numbers [49-51]. Thus, to achieve 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 suggested to run at the same Froude number and, hence, at a slower speed [49,82].

Therefore, 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 [117], it was hypothesized that, at the time of peak SEE length, ankle dorsiflexion and knee flexion are both smaller, whereas 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 to what extent and whether the absolute or relative differences in gravity between Moon and Mars surfaces dominate these alterations.

2.3.3 Results

2.3.3.1 Kinetic and spatio-temporal parameters

Participants running at the predefined simulated hypogravity levels of 0.38g and 0.16g generated lower mean hypogravity levels, actually corresponding to 32.6% \pm 10.3% and 14.8% \pm 3.5%, respectively, 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·s⁻¹ \pm 0.08 m·s⁻¹ at 1g, 1.80 m·s⁻¹ \pm 0.05 m·s⁻¹ at simulated Martian gravity, and 1.50 m·s⁻¹ \pm 0.04 m·s⁻¹ at simulated Lunar gravity.

A significant effect of g-level was noted on peak plantar force, ground contact time, gait cycle duration, cadence, and stride length (Table 4). 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 than during running at simulated Martian gravity (Table 5, Figure 13a). Ground-contact times and gait cycle durations were significantly longer at both simulated Martian and Lunar gravity vs. 1g and were significantly longer at simulated Lunar gravity than at simulated Martian gravity (Table 5). 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 they did at simulated Martian gravity (Table 5). 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 or between Martian and Lunar gravity (Table 5).

2.3.3.2 Joint kinematics

The participants' average knee (Figure 13b) and ankle (Figure 13c) joint movement profiles (plotted as a function of stance phase) are suppressed when running occurred 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 when the peak SEE length was reached (Table 4). Ankle dorsiflexion (Figure 14a) and knee flexion (Figure 14b) angles 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 was significantly less flexed than at simulated Martian gravity (Table 5).

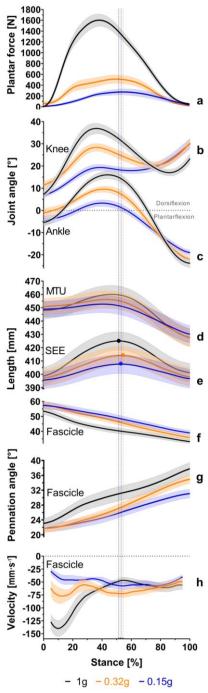


Figure 13. Kinetic, kinematic and GM fascicle-SEE parameters during the stance phase of running at 1g, simulated Martian gravity and Lunar gravity

Participants' average (mean \pm standard error) patterns of plantar forces (a), knee (b) and ankle (c) joint angles, and MTU (d) and SEE (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 point of time at which peak SEE 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 errors of the 1g condition have previously been published by Richter et al. (see Article 2). n = 8

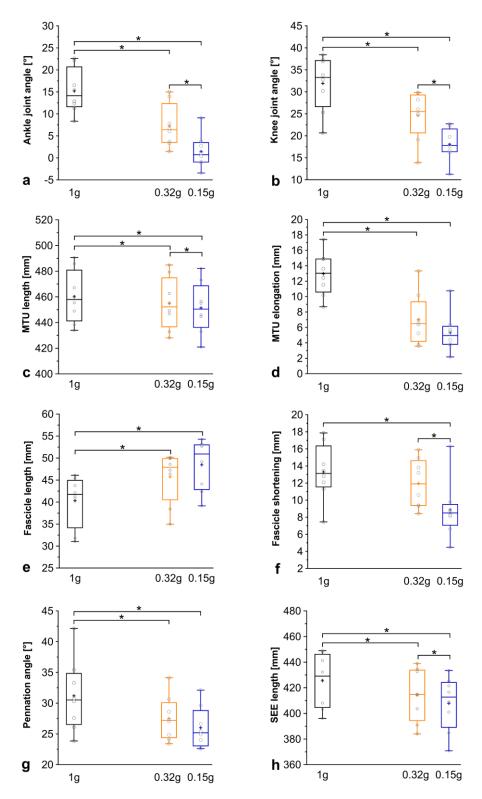


Figure 14. GM fascicle—SEE behavior at the time of peak SEE length when running at 1g, simulated Martian gravity and Lunar gravity

Ankle joint angle (a), knee joint angle (b), MTU length (c), fascicle length (e), pennation angle (g) and SEE length (h) at the time of the peak SEE length as well as MTU elongation (d) and fascicle shortening during SEE elongation (f) when running at 1g (black box), simulated 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; \circ individual data points; * significantly different (Tukey post-hoc, p \le 0.05). The boxplots of the 1g condition in c, e, g and h have previously been published by Richter et al. (see Article 2). n = 8 participants

2.3.3.3 GM muscle and SEE parameters

GM muscle-SEE parameters, such as MTU length (Figure 13d), SEE length (Figure 13e), fascicle length (Figure 13f), pennation angle (Figure 13g), and fascicle velocity (Figure 13h) (plotted as a function of stance phase), were modulated when running was performed at 1g vs. simulated Martian gravity and Lunar gravity.

A significant effect of g-level was observed on GM fascicle length, pennation angle, and fascicle velocity at peak SEE length (Table 4). At the time of peak SEE length, the fascicles operated at a significantly longer length (Figure 14e) but a smaller pennation angle (Figure 14g) at both simulated Martian and Lunar gravity compared to 1g. However, no significant differences were noted between simulated Martian gravity and Lunar gravity (Table 5). In contrast, while the 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. The fascicle velocity was significantly slower when running was performed at simulated Lunar vs. Martian gravity (Table 5).

Furthermore, there was a significant effect of g-level on SEE length and MTU length at the time of peak SEE length, as well as on MTU elongation (Table 4). The time point at which peak SEE length was reached ($51.5\% \pm 7.5\%$, $54.3\% \pm 4.0\%$, and $52.8\% \pm 5.2\%$ of stance at 1g, Martian gravity, and Lunar gravity, respectively) did not differ between g-levels (Table 4). Both, the peak SEE length (Figure 14h) and MTU length at the time of peak SEE length (Figure 14c) were significantly shorter when running at simulated Martian and Lunar gravity compared to 1g and when running was performed at simulated Lunar gravity compared to simulated Martian gravity (Table 5). MTU elongation (Figure 14d) was significantly lower in both simulated Martian and Lunar gravity vs. 1g. However, no differences were observed between the findings for simulated Mars and Moon (Table 5).

The g-level also had a significant effect on fascicle shortening, the delta in pennation angle, and average fascicle velocity during SEE elongation (from touch down to peak SEE length) (Table 4). Fascicle shortening (Figure 14f) showed no significant differences for running at 1g and simulated Martian gravity, but showed significant reductions when running at simulated Lunar gravity compared to 1g and simulated Martian gravity (Table 5). 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 5). Running at simulated Lunar gravity significantly reduced delta pennation angle and average fascicle velocity compared to simulated Martian gravity (Table 5).

Table 4. ANOVA results for kinetic, spatio-temporal, kinematic, fascicle and SEE parameters while participants ran at 125% PTS at 1g, simulated Martian and Lunar gravity

Comospi.C		19	0.3	0.32g	0.	0.15g	Toct Statistic	d	1179
	Σ	SD	Σ	SD	Σ	SD		L	<u>(</u>
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	90.0	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	1.9 (0.1)	1.7	1.7 (0.2)	1.7	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	9000.	1.7
Fascicle velocity at peak SEE length [mm·s-¹]	49.0	-49.0 (18.2)	-72.8	-72.8 (33.8)	-52.6	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	52.0 (11.8)	53.5	53.5 (7.8)	5.4.5	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	7.44.7	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 (x²) 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 medians (interquartile ranges). 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. ¹¹⁷. n = 8

Table 5. Post-hoc results for kinetic, spatio-temporal, kinematic, fascicle and SEE parameters while participants ran at 125% PTS at 1g, simulated Martian and Lunar gravity

Outcomes		19	g vs. 0.32g					1g vs. 0.15g				0:0	0.32g vs. 0.15g		
	Σ	SD	95% CI	٩	ъ	Σ	SD	95% CI	۵	ъ	Σ	SD	95% CI	٩	ъ
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	8.48	-367.8; -232.8	<.0001	-1.9
Ground contact time [s]	80.0	0.03	0.05; 0.11	9000	1.7	0.11	0.05	0.06; 0.16	8000	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	7.4	-10.3	5.2	-15.7; -4.9	.0020	-1.6
Stride length [m]	-0.2 (0.2)	0.2)		.0733	4:1-	-0.1 (0.3)	.3)		.0733	-0.8	-0.01	-0.01 (0.2)		>.9999	0.1
Ankle joint angle at peak SEE length [°]	-7.9	3.3	-11.3; -4.5	9000	-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	9600:	-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	6.0	8.1	3.8	4.2; 12.1	.0013	4.1	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)	25.6)		.0081	4.1	-3.3 (17.6)	(9.7		>.9999	0.3	13.8 (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	8.8	-11.5; -1.4	.0165	-0.3
Time of peak SEE length [% Stance]	-0.5 (11.3)	1.3)			0.5	2.5 (7.0)	(O:			0.2	1.0 (7.0)	(0.7			-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	8000	-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	9.0-
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	8000	-1.8	52.3	12.7	39.1; 65.5	< 0001	-3.1	20.0	9.4	10.2; 29.8	.0014	-1.6

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 medians (interquartile ranges). Peak SEE length at 1g and 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 simulated 0.32g (Mars) and 0.15g (Moon) occurred at $52\% \pm 8\%$, $54\% \pm 4\%$ and $53\% \pm 5\%$ of stance, respectively. n = 8

2.3.4 Discussion

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, and 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 peak SEE length, fascicles operated at longer lengths and smaller pennation angles in simulated Lunar gravity as compared to Martian gravity.

The plantar force data acquired in the present study suggest that the 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. ³³ 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.

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·s⁻¹ at simulated hypogravity reveal shorter ground contact times [58,102,115,118,119] and increased stride lengths [118,119] 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·s⁻¹ and 1.5 m·s⁻¹ at simulated Martian and Lunar gravity, respectively), 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. Moreover, running at the same Froude number usually produces equal relative stride length [120]. 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 modifications in the participants' running pattern in relation to 1g. We did indeed expect ankle dorsiflexion and knee flexion at peak SEE length to become smaller with lower simulated hypogravity levels, as similar findings have been reported in previous hypogravity studies

[58,59,118]. However, we did not expect that the small absolute difference in the 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 adapt their running pattern according to the much lower energy absorption required with decreasing hypogravity levels [58]. In addition, the significantly smaller knee flexion angles at peak SEE length could also be the result of the reduced external work required to lift and forward-accelerate the body's centre of mass when running in simulated hypogravity [69]. This effect could be even more pronounced by the fact that the present participants were not vertical but, instead, were horizontally suspended on the VTF. Thus, participants presumably counteracted their less flexed knee joints (which is likely caused by both, reduced g-levels and unusual body positions), by placing their ankle joints in a position involving 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 subsequent stages of the stance phase, ankle dorsiflexion angles were found to be much smaller. This is also in alignement with previous hypogravity studies [58,118], which suggest that participants shift to a forefoot striking pattern [58].

Thus, from a joint kinematic point of view, running at simulated Lunar and Martian gravity is not equivalent to running at 1g; further, 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 identified for lower limb joint angles.

As MTU lengths were calculated on the basis of ankle and knee joint angles, it is unsurprising 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 was already observed when running in simulated 0.7g [117]. In addition, 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, indicate a reduced storage of elastic strain energy [121]. Thus, the smaller elastic stretch may also be a functional adaptation to the lower

mechanical energy storage requirements of running on the simulated surfaces of the Moon as compared to those of Mars [69].

Gastrocnemius medialis contractile behavior during running in simulated hypogravity appears to be 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, which is similar to running in simulated 0.7g using the VTF [117]. 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 for the simulated Martian and Lunar running. This, in turn, suggests that for fascicle's operating length, there might exist a ceiling effect that is similar to the one originally introduced by Mercer, et al. 57 for the reduction in muscle activation, which was stabilized around 0.2g. Albeit not statistically significant, at the time of peak SEE length, fascicles operated at 3 mm \pm 3 mm longer lengths and $2^{\circ} \pm 2^{\circ}$ 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 is warranted using ultrasonography combined with measures of muscle activation and ideally including a larger sample size.

With regard to 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 as compared to 1g; more importantly, they were also reduced for simulated Lunar in relation to Martian gravity, as additionally indicated by the overall large effect sizes. Such alterations in GM contractile behavior, in turn, point to functional adaptations associated with 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 [108]. In 1g, GM contractile behavior adapts when switching from a walking to a running gait [77]. However, no change in fascicle velocity is observed when running speeds are further increased [77,103]. The observation that the GM works on a similar part of the force-velocity relationship across various steady-state running speeds [77,103], however, appears to not account for conditions of simulated hypogravity when running speeds are intentionally decreased to match the Froude number. Thus, to determine whether the observed decrease in fascicle velocity can be solely attributed to the decrease in g-level or in running speed requires further studies.

As discussed above, the shorter peak SEE lengths observed during running in hypogravity might be a part of the functional adaptations to the lower mechanical work output [69] (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 along with 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 the extent to which it is shortened.

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 for maintaining the stretch shortening cycle efficacy [122,123] as they induce relatively high vertical ground reaction forces and thus higher magnitudes of tissue strain [124]. 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 that are similar to standing and running on Earth, respectively [123]. This may be why skipping and plyometric training, have been suggested as the preferred gait on the Moon [69] and a promising countermeasure for preventing musculoskeletal deconditioning [123,124], respectively. One innovative gravity-independent countermeasure is spring-loaded horizontal jumping, but its applicability in space remains to be evaluated [122].

In addition, it can be argued that achieving a terrestrial-like fascicle—SEE behavior, and, thus, having similar stimuli exerted on the GM muscle, is also a valid goal for effective running countermeasure exercises. To achieve this, the lower the hypogravity level, the more external loading that needs to be applied as compensation. In full microgravity, like on ISS, crewmembers strap themselves to a treadmill via a harness-based subject loading system [15]. 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 [13] 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 as the typical loading used on ISS [13], should be able to effectively compensate for reduced gravity level and 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 be insufficient to reach their 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 consider complementing the harness-based subject loading system by wearing an additional weight vest. However, to add the missing 15% equivalent body weight loading in Lunar gravity, the weight vest would have to be in the mass range of the crewmember's personal body mass, which will likely create considerable 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 examined in future research. Additionally, 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 after their GM behavior has functionally adapted in response to the lower musculoskeletal loading.

In conclusion, simulated hypogravity running (Martian and Lunar gravity) as compared to running at 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 those for 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, while crewmembers performing running countermeasures on Mars would be able to apply full body weight loading at a similar perceived harness discomfort as that on ISS, crewmembers exposed to Lunar gravity would have to apply greater external loading to induce mechanical stimuli that are similar to those experienced on Earth.

2.3.5 Methods

The methods used in the present study are the same as reported in a previous publication [117], 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. ¹¹⁷ have been shortened.

2.3.5.1 Participants

Eight healthy male volunteers (31.9 years \pm 4.7 years, 178.4 cm \pm 5.7 cm height, 94 cm \pm 6 cm leg lengths, and 73.5 kg \pm 7.3 kg body masses) were examined medically, and informed written consent to participate in this study was obtained from them. This study 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 the occurrence of any cardiovascular, musculoskeletal, or neurological disorders within two years of the study.

2.3.5.2 Study design and experimental protocol

The participants visited the laboratory on a single occasion and ran on the vertical treadmill facility (VTF; Arsalis, Glabais, Belgium, Figure 15) at simulated Martian and Lunar gravity (randomized order), in addition to running on a conventional treadmill at 1g. Before each running trial, the participants familiarized themselves (~ 4 min) until they had acclimatized to the simulated gravity level and the predefined running speed. After another two minutes of accommodation time [84], data were collected for 30 s. As this protocol was conducted as part of a larger study, the corresponding data of all eight participants for 1g have already been included as a control condition in a recent publication [117].

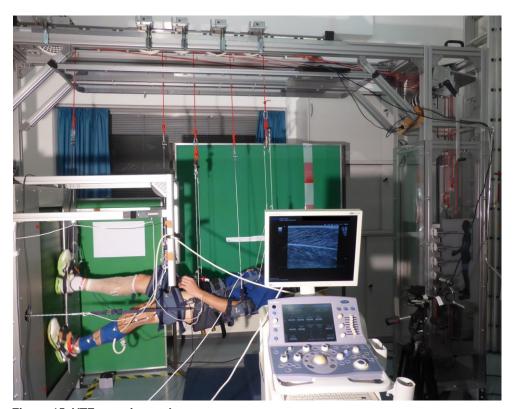


Figure 15. VTF experimental set-up

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 the respective joint angles. Photo credit: Charlotte Richter; informed consent was obtained to publish this photograph.

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). This was 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. ⁵¹ using the resulting acceleration (a) as the independent variable. By accounting for each participant's leg length (I), the individual $PTS(a)=\sqrt{PTS_{FR}(a)\cdot a\cdot l}$ was determined. A running gait was ensured by adding 25% to this PTS, and this resulted in participants running at predefined speeds of 2.62 m·s⁻¹ ± 0.08 m·s⁻¹ at 1g, 1.80 m·s⁻¹ ± 0.05 m·s⁻¹ at simulated Martian gravity, and 1.50 m·s⁻¹ ± 0.04 m·s⁻¹ at simulated Lunar gravity.

2.3.5.3 Data collection

To determine the stance phase (touchdown to toe-off), each participants' 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) that used 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 MyoResearch XP software (Master Edition 1.08.16) using a twin-axis electrogoniometer (Penny and Giles Biometrics Ltd., Blackwood Gwent, UK) for the knee and a custom-made 2D-electrogoniometer for the ankle joint. Electrogoniometer and loadsol signals were time-synchronized by recording a rectangular TTL pulse generated by pressing on a custom-made pedal. Before each running trial, the electrogoniometers were zeroed when the participant was in an anatomical neutral position (standing).

B-mode ultrasonography (Prosound α 7, 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 inside 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, which was 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 (Figure 16) and, where appropriate, were manually corrected using a semi-automatic tracking algorithm (UltraTrack Software, version 4.2) [86].

The SEE length (Achilles tendon, aponeuroses and proximal tendon; Figure 16), was calculated by multiplying the muscle fascicle lengths by the cosine of its pennation angle and then subtracting that value from the MTU length [75]. MTU length was calculated by a multiple linear regression equation [87] using the participant's shank length and their knee and ankle joint angles.

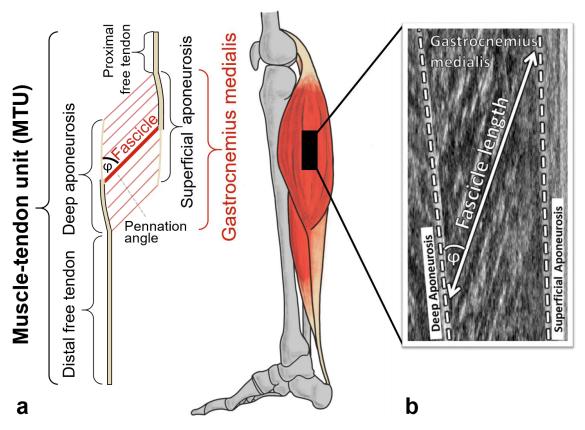


Figure 16. Schematic and anatomical muscle-tendon unit model (a) in addition to an actual annotated ultrasound image of the gastrocnemius medialis (b)

The series elastic element 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).

2.3.5.4 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 [88].

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 a percentage of the average g-levels that were 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, when 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 that occurred 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.

2.3.5.5 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 was used to correct for multiple comparisons using statistical hypothesis testing (1g vs. Martian gravity, 1g vs. Lunar gravity and Martian gravity vs. Lunar gravity). 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 were used (n = 8). The statistical analysis was performed in GraphPad Prism (v 7.04) with α set to 0.05. Data are 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 [89]. Effect sizes for the respective posthoc 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 [90]. While the data (mean ± standard deviation) acquired at 1g have already been presented in a previous publication [117], the differences to simulated Martian and Lunar gravity as well as between Mars and Moon have not been published elsewhere.

2.3.6 Additional Information

2.3.6.1 Data Availability Statement

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

2.3.6.2 Acknowledgments

This study was supported with funding from the European Space Agency's (ESA) Space Medicine Team (HRE-OM) of the European Astronaut Centre in Cologne, Germany, and the University of Applied Science Aachen obtained funding from the Federal Ministry for Economic Affairs and Energy (50WB1728). ESA provided the vertical treadmill facility used in this study. The corresponding author, Charlotte Richter, has designed and drawn Figure 16.

2.3.6.3 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.

2.3.6.4 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.

2.3.6.5 Materials and Correspondence

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2.3.7 References

Please refer to the common reference list at the end of this cumulative dissertation.

3 SUMMARIZED DISCUSSION

3.1 Hypogravity: a blessing and a curse

The three articles that are integrated in this cumulative dissertation discuss the effects of simulated hypogravity upon GM behavior during human locomotion, not only in the context of spaceflight but also in a rehabilitation setting.

During rehabilitative gait training, patients with orthopedic or neurological impairments receive reduced forces acting on their lower extremities' joints, bones, muscles, ligaments and tendons, because they are unable to bear their weight adequately, or they suffer from impaired motor function [40,41]. Another key aspect of rehabilitative gait training with BWS is the generation of locomotor pattern and sensory input that is essential to replicate "normal" walking. The level of applied BWS has thus to be chosen wisely, as natural gait characteristics including muscles' contractile behavior should be preserved. Too much support could adversely affect the outcome of the treatment [41].

In contrast, astronauts exposed to μg experience significant physiological space deconditioning, with the musculoskeletal system being particularly susceptible to reduced force loading [12]. Antigravity muscles such as the plantar flexors appear to be prone to atrophy [9,10], which likely relates to the fact that muscle loading forces on ISS are well below those on Earth [18]. Therefore, crewmembers seek to replicate Earth-like forces and muscular loading while exercising in μg , which is not always possible due to various physical and physiological constraints [15,17] that are limiting force application. Similar challenges will very likely also apply for Lunar and Martian gravity scenarios.

Knowledge about the impact of simulated hypogravity on gait kinetics (parameters related to forces), gait kinematics (spatio-temporal parameters and parameters related to orientation of body segments), and on neuromuscular activation (parameters related to electrical signals associated with muscle contraction) is available in the literature [9,33,41,64,125]. Furthermore, knowledge exists on GM fascicle—SEE behavior (assed via ultrasonography) in 1g [29] with respect to movement/gait type [75,126-133], gait speed [76,77,103,105], age [134-136], diabetics [137], usage of exoskeletons [138] or even added BW loading [103]. However, the combination of both, GM fascicle—SEE behavior and hypogravity exposure, was unknown [33].

The common goal of all three articles is thus to describe GM behavior, with respect to muscle's contractile behavior (fascicle length, pennation angle and shortening velocity) and SEE behavior, associated with locomotion under different hypogravity conditions.

From the data presented and analyzed here, it became apparent that the general relation between hypogravity level and the parameters of gait kinematics and GM behavior is more complex than initially expected. Interestingly, the most significant changes were observed during <u>running</u>, mainly between 1g and the various simulated hypogravity levels (0.7g, 0.38g and 0.16g). Yet, even for relatively small absolute differences in hypogravity level, such as between simulated Martian (0.38g) and Lunar (0.16g) gravity, significant changes were observed. These changes do not exclusively include GM behavior, but it was shown that even the overall locomotion pattern of running is strongly altered when changing gravity levels. A consequence of this is that the impact of locomotion at different levels of simulated hypogravity on gait kinematics and GM behavior is complex. Therefore, before the implementation of future exercise devices that use running as a countermeasure exercise in future space flight scenarios, assumptions of the precise impact of the respective hypogravity level on locomotion patterns and GM behavior should be validated.

In contrast to running, it appears that <u>walking</u> at simulated 0.7g is less prone to modulation of overall locomotion pattern and GM behavior. While the data presented in this doctoral study allow for a variety of clear conclusions on locomotion biomechanics and GM behavior, the relatively small data set also has clear limitations. The doctoral study should therefore be treated as an observational pilot study. Accordingly, effect sizes were presented for the parameters in all three articles, and individual data points are depicted in each box-plot figure.

The results of each article are discussed in more detail in the following sections. They will be analyzed with respect to their contribution to 1) filling of current knowledge gaps and 2) formulation of rehabilitative gait training requirements, running countermeasure exercises on ISS and future planetary surface operations. In this context it will be summarized in which situations simulated hypogravity is potentially beneficial, or detrimental, with respect to maintaining the subjects' GM contractile behavior.

3.1.1 Article 1 – Benefits for rehabilitative gait training on Earth

This article focused on GM's fascicle and SEE behavior during walking at 75% of the PTS, on the AlterG with full BW and subsequently with 30% BWS, as is frequently employed in gait rehabilitation [39,41,43]. The aim was to investigate whether walking with 30% BWS (simulated 0.7g) also preserves GM behavior in addition to preserved gait kinematics.

The findings of the first article contrast the initial hypothesis of an altered fascicle and SEE interaction. Remarkably, walking with 30% BWS had no effect on peak SEE length, MTU length, fascicle length, fascicle shortening velocity and pennation angle, neither at the time of the peak SEE length, nor on average. In addition, ankle and knee joint kinematics were largely preserved [104].

To exclude that the observed preservation of GM parameters (SEE and MTU length) is a masking effect caused by systematic measurement errors, an error propagation analysis of the MTU model (used to calculate SEE length) has been performed, including assumed systematical errors in fascicle length [139], pennation angle [62,140] and joint angles [53]. Even when using conservative error ranges, resulting systematic errors of SEE length were found in the range of \leq 10 mm compared to an overall SEE length of 400 mm. This means that systematic errors can only account for potentially masking a 2% hypothetical change in SEE length, therefore supporting the observation of preserved SEE parameters.

In summary, GM behavior was surprisingly stable with respect to reductions in simulated gravity level during walking.

For rehabilitative gait training to be effective, it is required to reduce forces acting on the lower extremities while maintaining gait pattern [39-41]. Previous studies on walking with BWS have already demonstrated significant changes in gait kinetics, e.g., reduced ground reaction forces [37,40,43,67], knee joint moment [43] or ankle joint moment and power [39,73] but limited changes in gait kinematics [41], such as ankle and knee joint range of motion [37,40]. In addition, kinetic curvature patterns of hip and knee joint trajectories were found to be largely preserved up to 30% BWS [43]. The results of the first article extend this view to a largely maintained GM contractile behavior and thus support the recommended therapeutic dose of 30% BWS for rehabilitative gait training.

Maintenance of GM fascicle and SEE behavior despite external unloading is beneficial for rehabilitative gait training for various reasons: 1) Preservation of gait pattern supports the transferability to subsequent unsupported walking. 2) Preservation of fascicles' operating range suggests that that the associated stimulus exerted on the muscle remains the same. 3) Preservation of SEE strain (presumably as a result of a lower free tendon strain but higher aponeurosis strain) might help to prevent degeneration and thus maintain function of the aponeurosis. Patients who may benefit from this potential redistribution of strain within the SEE are, for example, patients recovering from certain Achilles tendon injury. However, a differentiated assessment is necessary in this case. It could be argued that patients can benefit from this effect if the rupture solely affects their free tendon, but that they might be adversely affected if the rupture affects their aponeurosis.

To determine whether walking with 30% BWS is, in addition to maintaining GM behavior, also sufficient to maintain muscle mass, additional measurements of torque and neuromuscular activation are required to estimate the actual load on the GM. In this context it is remarkable that the abovementioned fascicle and SEE key parameters, including peak SSE length, apparently remain unaltered by 30% reduction in external loading. The possible mechanisms leading to this

observation range from redistribution of joint torques to an acute variation of the mechanical SEE properties (e.g., in aponeuroses stiffness). Alternatively, it could be an effect of the performed adjustment of walking speed to the same Froude number. The detailed determination of this underlying mechanism should therefore be subject to future studies.

To conclude, rehabilitative gait training with intentionally applied 30% BWS generally appears to be beneficial to the patient by preserving natural gait characteristics including the behavior of GM contractile and series elastic elements.

3.1.2 Article 2 - Concerns for running countermeasures in space

Crewmembers running on ISS in µg artificially load themselves with up to 70% BW equivalent, i.e., they effectively run in a simulated 0.7g environment. This happens to be a similar hypogravity level as is typically used for rehabilitative gait training (with 30% BWS), despite the different starting conditions (µg vs. 1g). Taking into account that walking at simulated 0.7g does not modulate GM behavior compared to 1g, as demonstrated in the first article, a logical follow-up question is whether this also applies for running at simulated 0.7g vs. 1g.

Therefore, it was investigated whether GM fascicle and SEE behavior is similarly preserved when running (at 125% PTS) on the VTF at simulated 0.7g. This was done to examine whether the provision of 0.7g artificial force loading during treadmill running in space sufficiently replicates an Earth-like contractile behavior.

As GM fascicle behavior was observed to be relatively stable with respect to alterations in gravity level, both when walking at simulated 0.7g (Article 1) and running at simulated 1.2g [103], similar findings of a preserved GM contractile behavior were initially expected for running at simulated 0.7g. However, in contrast to this expectation, during running at simulated 0.7g, GM fascicles were observed to be operating at longer lengths, smaller pennation angles, and faster shortening velocities than at 1g, whilst MTU and SEE lengths were shorter (all outcomes measured at peak SEE length). Additionally, significant changes in kinematics (reduced ankle and knee joint angles) were observed [117].

It thus appears that it makes a difference for GM behavior whether participants walk or run at a given hypogravity level. This underlines the complexity of GM behavior during locomotion in simulated and likely also in actual hypogravity. Whilst walking at simulated 0.7g largely preserves GM contractile behavior, running at simulated 0.7g apparently alters GM contractile behavior. These alterations between running vs. walking in hypogravity might be explained through changes in cost of transport, which is determined by the energetic cost of muscular force generation that is necessary to carry the subject over a unit of distance [70,101]. When lowering the loading for both running and walking, this cost of transport is unequally reduced, with a

stronger reduction taking place for running compared to walking at simulated hypogravity [70]. In other words, compared to 1g, runners save more energy in hypogravity than walkers do.

However, it should also be taken into account that in the MoLo study, walking and running were performed on two different support systems: The AlterG was used to replicate hypogravity for rehabilitative gait training (Article 1) and for the 1g baseline used in Article 2; The VTF was used to replicate running on board ISS (i.e., the simulated 0.7g dataset used in Article 2). Therefore, while the authors consider this unlikely, it cannot be fully excluded that the observed differences in GM contractile behavior between walking and running at simulated 0.7g are the result of undiscovered systematic differences between the two different methodologies that were used to simulate hypogravity.

Next to the observed differences between walking and running at simulated 0.7g, another significant observation here is the difference between running at 1g and running at simulated 0.7g.

Previous investigations of running under various hypogravity simulations (e.g., vertical BWS systems, supine suspension systems, lower body positive pressure treadmills or parabolic flights) have reported reductions in ground reaction forces and plantar load [17,99,100], metabolic cost [70,100,101], estimated ankle joint forces [102], muscle activation [56,81], peak ankle dorsiflexion, peak knee flexion and joint range of motion [58], i.e., significant changes in kinetics and kinematics compared to 1g. The second article of this cumulative dissertation extends that list to alterations in GM fascicle and SEE behavior, which could be the result of the abovementioned changes in kinetics and predominantly kinematics, which were both observed in this study and by others [64].

The acute alterations in GM behavior observed during steady-state hypogravity running at levels of simulated 0.7g or below are likely to cause long-term functional adaptations to the reduced locomotor demand, and are thus relevant for exercise regimes in space. The following potential mechanisms might play a role in this adaptation process: 1) The observation of shorter peak SEE lengths is the result of a reduction of external forces, which also causes lower internal muscular forces, which consequentially may precipitate musculoskeletal degeneration [16]. 2) The observed longer fascicles may result in an increased strain on the z-discs, potentially preserving or increasing the number of sarcomeres in-series, which could in turn preserve muscle mass despite the reduced loading [107]. 3) The longer fascicles represent functional adaptations of the muscle that are no longer optimal for the requirements on Earth, which results in the necessity of post-flight reconditioning [107,110].

While the observed muscle degeneration of many returned long-duration crewmembers suggests that the detrimental mechanisms (1 & 3) dominate the adaptation process, it requires further study to determine whether crewmembers could potentially benefit from mechanism two.

In any case, to avoid harmful effects both in space and upon return, it can be argued that maintaining an Earth-like contractile behavior, and thus similar stimuli exerted on the GM, is a valid goal for effective running countermeasure exercises. The necessary adaptations in the exercise regimes to increase the mechanical stimuli (e.g., greater artificial force loading, faster running speeds, increased training volume) to preserve fascicle—SEE interaction, require further research. Interestingly, previous findings suggest that running mechanics are largely preserved at simulated hypogravity levels at or above 0.8g [58]. Therefore, it might be possible that crewmembers running at simulated 0.7g are only a remarkably small step (0.1g) apart from reaching the minimum required mechanical stimuli to induce Earth-like contractile conditions during running. Generally speaking, determination of "optimal" artificial force loading during treadmill running in space requires further study, in combination with running duration and volume. Even by assuming that on-board running could be optimized to represent Earth-like conditions, the minimum daily or weekly running duration that leads to full prevention of muscular deconditioning needs to be determined, considering the general lack of significant muscle loading between exercise sessions.

It can be argued that maximizing on-board exercise countermeasure efficiency not only maximizes crew health, but also frees up available crew time for mission tasks by minimizing the time spent exercising. Both of these effects can be positive factors for mission success, and it is tempting to see the minimization of exercise time as a valid goal for future in-flight exercise regimes. However, it should not be neglected that many crewmembers enjoy their time spent with exercises as "quality time" [personal communication A. Gerst, see online supplement of Article 2]. This contributes to the crewmembers' mental health, which is as important as their physical health for mission success [141]. Thus, the design of future exercise regimes should take all these effects into account.

To conclude, despite the advantage of performing mission specific tasks in µg without significant physical exertion, vigorous exercise countermeasures are required on board space vehicles to prevent musculoskeletal deconditioning. The findings of the second article support that steady-state running in space with maximum loads of about 0.7g does not provide a sufficient replication of an Earth-like GM contractile behavior, which, however, is likely to be an important prerequisite to preserve muscle mass and function. Whether the modulation in GM behavior actually precipitates musculoskeletal degeneration requires further study.

The above findings have, in addition to their value for space flight applications, interesting consequences for the area of rehabilitative gait training of patients on Earth, as was discussed in Article 1. As a reminder, the first article has shown that <u>walking</u> at simulated 0.7g vs. 1g did not cause changes in kinematics, and consequently also no significant changes in GM behavior. In contrast, Article 2 shows that such changes exist for <u>running</u> at simulated 0.7g vs. 1g. This suggests that running at simulated 0.7g might be less suitable for rehabilitative gait training than walking at 0.7g. At the same time, there is evidence that alterations in running mechanics are limited when running at levels of 0.8g or above [58], suggesting that there is a hypogravity threshold above which running kinematics and GM behavior is preserved, albeit at a different hypogravity level than for walking. Thus, for rehabilitative gait training, there should potentially be different BWS recommendations for walking vs. running.

3.1.3 Article 3 – Implications for surface operations on Moon and Mars

Taken into account that running at simulated 0.7g already modulates GM behavior compared to 1g, it appears likely that the replication of terrestrial locomotion is even more challenging on Moon and Mars.

The aim of the third article was therefore to investigate whether alterations in GM contractile and SEE behavior, which were observed during simulated 0.7g running, are also apparent during running at simulated Martian (0.38g) and Lunar gravity (0.16g), on the VTF. The underlying question was whether fascicle behavior is sensitive to small absolute changes in hypogravity levels (such as between 0.38g and 0.16g), and whether this could impact the transferability of Lunar to Martian surface operations.

As hypothesized, in addition to the change of gravitational loading, running on simulated Mars and Moon is not the same as on Earth. This is indicated by modulations in spatio temporal parameters (e.g., prolonged ground-contact times and decreased cadence), joint kinematics (e.g., smaller ankle dorsiflexion and knee flexion at peak SEE length) and contractile behavior (e.g., longer fascicles and smaller pennation angles at peak SEE length, as well as slower average shortening velocities during SEE elongation) [142].

When comparing simulated running on Moon to running on Mars, these alterations of spatio-temporal parameters, lower limb joint angles and muscle—SEE parameters are statistically significant, except for fascicle length and pennation angle at peak SEE length. These latter two parameters nevertheless show a trend to be longer and smaller at simulated Lunar gravity than at simulated Martian gravity, respectively. In summary, in terms of locomotion biomechanics and GM behavior it appears that running on Moon is significantly different than running on Mars [142].

To the author's knowledge, very few studies have compared locomotion in simulated Lunar and Martian gravity [58,69], despite this having potential implications for the transferability of Lunar to Martian surface operations. Therefore, the third article contributes to fill this knowledge gap by evaluating running in simulated 0.38g vs. 0.16g.

The difference in hypogravity level between 0.38g and 0.16g might appear rather small in absolute terms, potentially leading to the false conclusion that this difference is insignificant for astronaut operations on the surface of the Moon compared to the surface of Mars. The above findings however, suggest that instead of the absolute difference between the two hypogravity levels, the relative difference is the more relevant parameter. I.e., 0.38g is 238% more than 0.16g. This large relative difference may well explain the differences observed in running kinematics and GM behavior between simulated Lunar and Martian gravity.

The fact that even a seemingly small difference of ~0.2g has a notably effect on joint kinematics and GM contractile behavior should therefore be carefully considered in the following respects: 1) During astronaut training, preparing for future missions to the Moon and eventually Mars, simulation of the exact hypogravity level should be mandatory. 2) Awareness of distinct differences in joint kinematics and GM contractile behavior should be integrated into mission capability and mission risk assessments. For example, being exposed to 1/6th of the Earth's gravitational acceleration does not necessarily mean that astronauts could also carry weights with a six-fold mass once muscles have adapted their contractile behavior to the Lunar gravity environment. This is potentially very important for Lunar long-duration missions but requires further study. 3) In terms of exercise countermeasures it is very likely that running at 0.16g and 0.38g, without applying artificial force loading, is insufficient the attenuate musculoskeletal deconditioning. However, especially on Mars, crewmembers could benefit from the force of 0.38g acting on their body. A harness loading of ~70% BW, which is similarly tolerable than the typical loading used on board the ISS [13], will result in an artificial force loading that is in the range of 100% BW, i.e., Earth-like conditions. On Moon, a similar harness loading only adds up to 85% BW and thus might require additional strategies to increase forces, such as increasing running speeds [19,100,111]. Moreover, to safeguard musculoskeletal health in (deep) space, plyometrictype exercises, i.e., exercises that include jumping, might be advantageous above running by inducing higher impact forces and thus higher magnitudes of tissue strain [124], which in turn helps to maintain stretch shortening cycle efficacy [122,123].

Finally, it may be summarized that effects on GM contractile behavior, which were already observed at simulated 0.7g running, are even more pronounced when reducing simulated hypogravity to Martian and Lunar levels. This reinforces that running on Moon and Mars is likely insufficient to elicit optimal mechanical stimuli if not addressed by additional artificial force loading.

4 CONCLUSION AND PERSPECTIVES

4.1 Concluding remarks

This cumulative dissertation discusses simulated hypogravity as the common link between Earth-based rehabilitative gait training and spaceflight missions, since in both cases hypogravity is intentionally applied despite the fundamentally different starting conditions. It expands the current understanding of the modulation of GM behavior during locomotion under different conditions of gravitational loading:

- 1) Walking on the AlterG at 1g and simulated 0.7g (rehabilitative gait training).
- 2) Running at 1g and running on the VTF at simulated 0.7g (running on board ISS).
- 3) Running at 1g and running on the VTF at simulated 0.38g (Mars) as well as 0.16g (Moon).

The main results of the doctoral study can be summarized as follows, by answering the three key research questions stated in the introduction (Chapter 1.2).

1) Does locomotion under conditions of simulated hypogravity affect GM behavior?

For the first time, this study demonstrated the preservation of GM behavior during <u>walking</u> with 30% BWS (simulated 0.7g vs. 1g) as frequently employed during rehabilitative gait training. In contrast, the results of simulated <u>running</u> on ISS with maximum loads of about 70% BW (simulated 0.7g), reveal that GM contractile and SEE behavior differs from what is observed during "normal" running on Earth (1g). Similarly, modulation of GM behavior is also observed in an even more pronounced form during simulated running on Mars (0.38g) and Moon (0.16g).

2) If GM behavior is modulated in response to locomotion under simulated hypogravity, which exact alterations can be observed in the muscle's contractile and SEE behavior?

The main alterations in GM contractile and SEE behavior during simulated hypogravity <u>running</u> can be summarized as follows: With decreasing gravity level, SEE lengths and MTU lengths become shorter, while fascicle lengths become longer, pennation angles become smaller and shortening velocities become slower. In addition, ankle and knee joint angles indicate decreasing ankle dorsiflexion and knee flexion angles, respectively (all parameters measured at the time of peak SEE length).

3) What are potential implications for rehabilitative gait training on Earth and exercise countermeasures on ISS, Mars or Moon?

The findings of the first article support the recommendation of <u>walking</u> at 30% BWS for rehabilitative gait training for patients suffering from neurological and orthopedic impairments. But

more importantly, it extends this rehabilitation regime, which until now was only valid for the preservation of the gait kinematics, to also preserve GM behavior. This combined preservation of both GM behavior and gait kinematics is beneficial because it supports the transferability of the practiced gait pattern at hypogravity to subsequent unsupported activities of daily life.

In contrast, and important for spaceflight exercise countermeasures, the observed alterations in GM behavior when <u>running</u> at simulated 0.7g, 0.38g and 0.16g point to functional adaptations caused by the reduced external forces and lower musculoskeletal loading. When not being combined with resistive exercise countermeasures, these adaptations might affect the GM muscle's work capacity, which in turn could precipitate muscular degeneration and become a hazard when being re-exposed to gravitational loading after extended periods in space. Thus, running countermeasure exercises during long-duration space missions in µg, and when expanding human presence to the Moon and Mars, should be optimized to maintain sufficient muscular function. Crewmembers will require this capability to successfully complete nominal and off-nominal mission tasks, especially those immediately after landing, be it on Earth, Moon or Mars.

Consistent with the goals to generate synergies with mission-related activities at ESA, the findings of Articles 2 & 3 support the goals of ESA's Space Medicine Team in promoting astronaut health and well-being by advancing Europe's scientific knowledge on the impact of hypogravity exposure on human physiology. They are expected to have a direct impact on the achievement of the "Humans beyond LEO" corner stone within ESA's European Exploration Envelope Programme "Terrae Novae". Consistent with ESA's "Agenda 2025" vision, the results of this doctoral study will help to prepare European contributions to future human exploration activities.

4.2 Future outlook

This doctoral study did not investigate the effect of variations in the applied running and walking protocols (i.e., locomotion speed) on GM behavior. Therefore, to state whether, and how, exercise countermeasure protocols should be modified in the future is outside the scope of this doctoral study. To answer this intriguing question, the next step will be to investigate if, and how, alterations in GM contractile behavior actually precipitate musculoskeletal degeneration. In this context, it might be possible to establish a potential gravity threshold, below which significant alterations in GM contractile behavior take place during walking and running. Thereupon it might be investigated whether there is, for example, a loading and locomotion speed combination, above which muscular deconditioning is fully prevented.

To create an evidence base for answering this question, the logical next step is to systematically review and analyze all available anonymized running data that was collected during ISS exercise countermeasures, in terms of loading level, speed and duration. Following this, a ground-based analogue should be developed, to mimic µg-running with a simple subject loading system that has the advantage of reducing the difficulties associated with setting up complex support systems such as the VTF. This could be achieved by using a bungee-based vertical support system, where subjects are fully suspended by the main suspension system (to simulate µg) while at the same time being back-strapped to the treadmill using exactly the same subject loading system as used on ISS (to simulate hypogravity loading starting from µg). The subjects should then be asked to replicate frequently used ISS running protocols, and trials should be repeated at different running speeds. Kinematic and ultrasound data are required to be complemented with additional measurements such as torque and neuromuscular activation parameters, to allow for estimating the effects of running at various levels of simulated hypogravity and speed on GM strain, contractility and excitability. Future research should additionally include a larger sample size than was available for this doctoral study. Taking all these aspects into account has the potential to finally answer the important question of how much artificial force loading and speed is needed to induce a similar level of stimulus than during terrestrial running. Such a study design will also be applicable to investigate Lunar and Martian gravity scenarios.

Another important point for future investigations is that so far, many studies that are investigating hypogravity responses ignore individual differences between subjects, and show average responses instead [27]. However, there is evidence that some astronauts experience greater musculoskeletal deconditioning effects than others [26,27]. Future studies should therefore also consider the inter-individual variability when performing different gaits at various levels of simulated hypogravity. In addition to that, since the VTF, due to technical imitations, does not provide a 1:1 replication of treadmill running on board the ISS, it would be beneficial if ultrasonic visualization of the GM could also be realized during in-flight running in actual µg. This technique additionally has a large potential to contribute to a deeper understanding of the individual differences in deconditioning between various astronauts. Furthermore, a better understanding of underlying factors from a biomechanical perspective might also help to develop personalized healthcare, and improved on-board exercise routines, as well as to reveal the deeper mechanisms of contractile behavior regulation on Earth.

Finally, the results and conclusions of this study highlight the great importance of continued investigations of locomotion under hypogravity conditions. It is an inspiring outlook that such future studies will enable and prepare the next step forward in human deep-space exploration. After all, when the first human will set their foot on Mars, one of the first muscles that he or she will have to rely on, is their gastrocnemius medialis.

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DEUTSCHE ZUSAMMENFASSUNG

Die Entlastung des Körpergewichtes ist eine Gemeinsamkeit von rehabilitativem Gangtraining und bemannter Raumfahrt. Patienten mit orthopädischen oder neurologischen Erkrankungen profitieren von einem Gangtraining mit bis zu 30 % Entlastung (entsprechend 0.7g), da weniger Kräfte auf ihre unteren Extremitäten wirken, während jedoch die Gangkinematik weitestgehend erhalten bleibt. Zur Wiederherstellung einer natürlichen Gehfunktion ist neben dem Erhalt des Gangbildes auch der Erhalt der kontraktilen Eigenschaften der Plantarflexoren, wie z. B. des Muskels gastrocnemius medialis (GM), wichtig. In vivo Messungen zur Klärung der Frage, ob das Gehen mit 30 % Entlastung das Muskelfaser- und Sehnenverhalten moduliert, wurden bis zum Zeitpunkt dieser Doktorarbeit noch nicht durchgeführt.

Im Gegensatz zur applizierten Entlastung während des rehabilitativen Gangtrainings auf der Erde müssen Astronauten, die der Mikro- und Hypogravitation ausgesetzt sind, aktiv der Entlastung ihres Körpers entgegenwirken, um eine Degeneration ihres muskuloskelettalen Systems zu vermeiden. Daher führen die Besatzungsmitglieder der Internationalen Raumstation (ISS) tägliche Trainingsmaßnahmen, einschließlich dem Laufen mit künstlich applizierter Belastung durch. Die auf der ISS verwendete maximale Belastungsniveaus liegen zufällig auf dem gleichen Niveau, wie sie für das körpergewichtsunterstützte Gangtraining auf der Erde empfohlen sind (entsprechend 0,7g). Jedoch wurde bis zum Zeitpunkt dieser Doktorarbeit noch keine entsprechende Visualisierung des kontraktilen Verhaltens des GM Muskels mittels Ultraschall durchgeführt, weder für das rehabilitative Gangtraining, noch für das simulierte Laufen auf der ISS. Eine derartige Visualisierung kann aber Aufschluss darüber geben, ob es im Weltraum oder auf planetaren Oberflächen möglich ist, erdähnliche Kontraktionsbedingungen und damit ähnliche auf den Muskel ausgeübte Reize zu replizieren. Darüber hinaus blieb im Hinblick auf zukünftige Missionsszenarien bisher unerforscht, ob und wie das kontraktile Verhalten moduliert wird, wenn das Gravitationslevel auf simulierte Marsgravitation (0,38g) und Mondgravitation (0,16g) verringert wird. Daher war das Ziel der Doktorarbeit, die unmittelbaren Auswirkungen des Gehens und Laufens unter verschiedenen Entlastungsbedingungen auf das muskuläre Verhalten des GM zu untersuchen.

Die Entlastungsbedingungen wurden in dieser Studie durch den Einsatz zweier verschiedener Laufbandsysteme simuliert: Zum einen das AlterG Laufband, um ein rehabilitatives Gangtraining zu simulieren, und zum anderen das vertikale Laufbandsystem "VTF", um das Laufen auf der ISS, dem Mars und dem Mond zu replizieren. Bei den Studienteilnehmern (n = 8, 32 \pm 5 Jahre, 178 \pm 6 cm Körpergröße, 94 \pm 6 cm Beinlänge, 74 \pm 7 kg Körpermasse) wurden die plantaren Kräfte über spezielle Kraftmesssohlen gemessen, um die Standphasen und das erreichte Entlastungsniveau zu bestimmen. Die Faserbündellängen und Fiederungswinkel des GM wurden

mittels Ultraschall quantifiziert. Sprung- und Kniegelenks-winkel wurden über Elektrogoniometer aufgezeichnet und zur Bestimmung der Länge der Muskelsehneneinheit herangezogen. Diese besteht aus kontraktilen und serienelastischen Elementen deren Länge mittels eines Muskel-Sehnen-Modells berechnet wurden.

Die Ergebnisse wurden in drei Publikationen präsentiert, die in folgende drei Themen aufgeteilt sind: Rehabilitation von Patienten auf der Erde, Sporttraining auf der ISS, und Sporttraining während zukünftiger planetarer Missionen. Die erste Publikation weist im Wesentlichen darauf hin, dass das Verhalten von Muskelfaserbündeln und Sehnen während des <u>Gehens</u> auf dem AlterG mit 30 % Entlastung, zusätzlich zur Gangkinematik, erhalten bleibt. Dies ist für eine Wiederherstellung "natürlicher" Gangmuster wesentlich und unterstützt die empfohlene Dosis von 30% Entlastung. Im Gegensatz dazu zeigen die Ergebnisse der zweiten und dritten Publikation signifikante Veränderungen im Faserbündel- und Sehnenverhalten sowie in der Gangkinematik zwischen dem <u>Laufen</u> bei 1g und dem Laufen auf dem VTF bei simulierten 0,7g (Publikation 2), sowie bei simulierten 0,38g und 0,16g (Publikation 3). Je geringer die Schwerkraft ist, desto größer wird die Modulation der muskulären Arbeitsweise im Vergleich zu 1g. Zum Beispiel nehmen mit abnehmender simulierter Schwerkraft die Länge der Sehne und der Muskel-Sehnen-Einheit sowie der Fiederungswinkel und die Muskel-Verkürzungsgeschwindigkeit ab, während die Faserbündellänge zunimmt.

Dies deutet darauf hin, dass das Laufen an Bord der ISS bei einer maximalen Belastung von ~0,7g keine exakte Replikation eines erdähnlichen kontraktilen Verhaltens darstellt. Ob diese funktionelle Anpassung an das Laufen mit Entlastung eine muskuläre Degeneration verursacht, muss weiter untersucht werden. Dennoch kann nicht ausgeschlossen werden, dass die beobachteten Veränderungen im kontraktilen Verhalten, wenn sie nicht anderweitig kompensiert Arbeitskapazität des Muskels beeinflussen. wenn werden. Schwerkraftbelastung ausgesetzt wird. Dies könnte nicht nur während der Rehabilitationsphase zurück auf der Erde relevant sein, sondern auch bei der Bewältigung missionsspezifischer Aufgaben nach der Landung auf dem Mond und auf dem Mars. Darüber hinaus deuten die Ergebnisse darauf hin, dass das Faserbündel- und Sehnenverhalten sehr empfindlich auch auf kleine absolute Veränderungen des Schwerkraftniveaus reagiert, was die 1:1-Übertragbarkeit von Prozeduren zwischen Mond und Mars in Frage stellt. Es kann schlussgefolgert werden, dass, um die Muskelmasse und -funktion des GM während Raumfahrtmissionen zu erhalten, Trainingsmaßnahmen wie das Laufen für die jeweiligen individuellen Gravitationsbedingungen jeder Mission optimiert werden sollten.

ACKNOWLEDGEMENTS

First, I would like to express my sincere gratitude to my supervisors Prof. Dr. Kirsten Albracht and Dr. David A. Green for their inspiration and continuous support of my doctoral study and the related publications. Your guidance and incredible amount of advice helped me tremendously in completing this project successfully. I know, it was not always easy, for either of us. However, without you and also the valuable feedback of all my fantastic co-authors I wouldn't have succeeded nearly as efficiently.

Furthermore, I would like to thank my colleagues from the Institute of Movement and Neurosciences, the Institute of Biomechanics and Orthopaedics at the German Sport University, the Department of Medical Engineering and Technomathematics at the University of Applied Sciences Aachen for all their support and thoughtful discussions. Your doors were always open, and whenever I ran into problems you helped me to work through them. Special thanks go to my direct colleagues of the neuromechanics research group: Benjamin Stäudle, Fabian Göll, René Lingscheidt, Björn Braunstein and Constance Badali. We have shared a lot of ups and downs, laughs and tears, but in the end it was always fun to work and hang out with you guys. You have made the laboratories and offices we shared a special place to work.

This work was further supported by the European Space Agency (ESA) and its program "Spaceship EAC", which is a great initiative investigating innovative technological and operational concepts in support of ESA's exploration strategy. The EAC Space Medicine Team, and especially Dr. Tobias Weber deserve a special thanks for their support throughout the project. Tobi, you have always been a great mentor for me, and truly fun to work with. I hope that many collaborative projects with you will follow in the future. Likewise, I would like to thank Prof. Dr. Dietrich Manzey for his open ear and thoughtful but always friendly words in times where I have needed it most.

I would also like to express my appreciation to the study's participants. Without your dedication and motivation to participate in the MoLo study, this cumulative dissertation would not exist.

Moreover, I wish to acknowledge the many other individuals who have contributed to this work, be it logistically, technically or financially, e.g., through the PhD scholarship I have gratefully received from the FH Aachen. Or through the provision of the "Studierzimmer", through the thoughtful review comments on this dissertation that have strongly improved its quality, or by any other kind of motivational support.

A huge thanks goes to all my friends who unselfishly supported me, and more importantly always

believed in me. Your pleasant company during all kinds of sports activities, scout trips, dinner

parties, night-long talks and your repeated words of encouragement paired with bad jokes made

me see the light at the end of the tunnel.

Finally and foremost, I would like to express my greatest thankfulness to my incredibly supportive

relatives and family, especially my beloved parents Reinhard and Monika, the most-fantastic

brother I could ask for, Bernhard, and my genius sisters Kay-Sölve, Silja and Antje. Thanks for

always being there for me and supporting me in any matter. I could not have done this without

you.

Alex, thank you so much for inspiring, encouraging and supporting me to reach for the stars. You

showed me that it is worth to venture beyond my horizons. I cannot imagine anyone else at my

side with whom I would rather be part of what is arguably the greatest scientific experiment, life.

Thank you, vielen Dank!

Cologne, 24.06.2021

Charlotte Robiter

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