Feasibility of monitoring muscle health in microgravity environments using Myoton technology

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A B S T R A C T

Physical exercise is important for people living under extreme environmental conditions to stay healthy. Particularly in space, exercise can partially counteract the loss of muscle mass and muscle strength caused by microgravity. Monitoring the adaptation of the musculoskeletal system to assess muscle quality and devise individual training programmes is highly desirable but is restricted by practical, technical and time constraints on board the International Space Station (ISS). This study aimed to test the feasibility of using myometric measurements to monitor the mechanical properties of skeletal muscles and tendons in weightlessness during parabolic flights.

The mechanical properties (frequency, decrement, stiffness relaxation time and creep) of the m. gastrocnemius, m. erector spinae and Achilles tendon were assessed using the hand-held MyotonPRO device in 11 healthy participants (aged 47 +/- 9 years) in normal gravity as well as in microgravity during two parabolic flight campaigns. Results showed significant (p < .05-.001) changes in all mechanical properties of both muscles and the Achilles tendon, indicating a more relaxed tissue state in microgravity. Recordings from a phantom rubber material with the device in a test rig confirmed that the device itself was not affected by gravity, as changes between gravity conditions that were too small (<1%) to explain the changes observed in the tissues.

It is concluded that myometric measurements are a feasible, easy to use and non-invasive approach to monitor muscle health in extreme conditions that prohibit many other methods. Real-time assessment of the quality of a muscle being exposed to the negative effect of microgravity and also the positive effects of muscular training could be achieved using Myoton technology.

Keywords: parabolic flight; muscle; Myoton measurements; Myoton technology; myometry, microgravity

INTRODUCTION

Monitoring health while living in microgravity is important not only to improve mission success and mission safety but also to foresee and design adequate countermeasures to maintain physical and mental health. Furthermore, from an academic point of view, the adaptation, maladaptation or deconditioning of physiological systems, such as the musculoskeletal or cardiovascular system under microgravity, is an extremely interesting and challenging field of research providing important information, with relevance in the rehabilitation of patients after immobilization, e.g. due to prolonged bed rest or after surgery. It is widely accepted that an adequate exercise programme in space is required to prevent physiological deconditioning of the musculoskeletal system, helping astronauts and cosmonauts after long duration spaceflight to readapt to gravity on Earth, as rapidly as possible.

In general, the maladaptation of human muscles to microgravity is expressed as loss of muscle strength and muscle mass, with one or both being determined pre and post intervention e.g. space flight [9]. Although more frequent muscle health monitoring would be preferable, this is impeded by practical (e.g. subject positioning), technical (e.g. hardware not available) and/or temporal constraints (e.g. complex, time consuming procedures) during spaceflight. In contrast to purely objective measures, such as muscle mass and/or muscle volume, methods that involve voluntary effort, such as maximum strength testing, are known to be affected by the individuals' motivation, which can be affected during long-term isolation [23].

Examining the tone, biomechanical and viscoelastic properties of individual superficial skeletal muscles is proposed to be a complementary, non-invasive and cost-effective technology that enables real time assessment of muscle [6]. Changes in muscle tone and properties could be used to assess effects of pathology [7, 12], sport-related injury [25] or therapeutic intervention [14, 20]. Such assessments could be performed at regular intervals to monitor the stage of the pathological processes of muscles [19] and for assessing efficacy of therapeutic interventions [14, 20].

Altered muscle tone is a problem in several neurological conditions, such as stroke, Parkinson's disease, multiple sclerosis and spinal cord injury, but techniques for non-invasive objective assessment are lacking. The modified Ashworth scale (MAS) is a subjective technique, assessing muscular resistance to passive movements and is the most commonly used method for determining the tone of a muscle group, i.e. hypo or hypertonic. However, MAS results lack objective grading and reliability [5, 16] and are not well correlated with muscle stiffness after stroke [3]. To meet this need, a novel approach, myometric measurement, has been introduced showing reliable [2, 4, 15], objective and user independent measurements [1, 10, 22] for healthy adults [8] as well as for various patient populations, including Parkinson's disease [13, 21] and stroke [6] patients. As well as being used to evaluate the tone and biomechanical properties of muscles and tendons (e.g. frequency, stiffness and elasticity), myometric measurements have also shown an almost linear relationship with electromyographic (EMG) activity and therefore provide an indirect measure of changes in the muscle force generating capacity [4, 10].

Within space science, an approach is required that allows non-invasive, time-efficient and easy to use assessment of muscle health and quality for research purposes, as well as for health monitoring, providing an objective analysis of the deconditioning of muscle under prolonged unloading, as occurs during weightlessness in microgravity environments. A study of Myoton technology in an extreme environment is necessary before it could be considered for use in space.

The present study aimed to use the microgravity phase of parabolic flights to (1) confirm that changes in mechanical properties of skeletal muscles are directly affected by weightlessness, reflecting the change in contractile state of muscle that is known to occur; (2) demonstrate that performance of the MyotonPRO device is not affected by gravity, by making recordings from phantom test material to confirm that measurements from muscle reflect true changes in its state of tension and mechanical properties and therefore (3) determine the technical feasibility of performing myometric measurements on astronauts in weightlessness, as a surrogate for measuring muscle strength. It was hypothesized that muscle tone, stiffness and elasticity would decrease in microgravity during a parabolic flight, reflecting the reduction in contractile state that is known to occur in the absence of loading force caused by gravity and leads to muscle weakness when prolonged.

METHODS

Participants and setup

This study was conducted during six parabolic flight days conducted during the 55th and 57th ESA Parabolic Flight Campaigns using the MyotonPRO on eleven healthy individuals, two female (mean (SD) age: 38 (5) years; BMI: 21.1 (0.6) kg/m^2) and nine male (age: 42 (9); BMI 26.2 (3.6)). This study was approved by the Research Ethics Committee of the German Sport University and all participants underwent aviation medical screening and provided written informed consent. Apart from the aviation medical screening, the only other exclusion was skin disorders at the testing sites. Participats were recruited from the authors institutions.

European Space Agency (ESA) parabolic flights are conducted from Bordeaux International Airport (France), aboard the Airbus A300 ZeroG. This airbus A300 ZeroG is designed for microgravity research allowing a rapid and easy access to a microgravity environment, e.g. to prepare experiments planned for conducting on the International Space Station (ISS). A parabolic flight manoeuvre is characterized by gravitational changes from 1 to 1.8 gravitational constants (G) (20 seconds) to 0G (20 seconds) to 1.8G to 1G (20 seconds, Figure 1). One campaign consists of three to four parabolic flights, with each flight occurring on a separate day. One parabolic flight, i.e. one flight day, consists of 30 parabolas. A so called zero test-parabola precedes the first experimental parabola on each flight day so that every flight participant can become accustomed to the sensation of microgravity. Data for this study were recorded during parabolas 1-15 whereas parabolas 16-30 were reserved for a different experiment unrelated to the present study.

For all measurements participants were lying in a supine position on their abdomen with their arms rested beside the body. To avoid movements in the 0G-phase of the flight, participants were strapped to the floor of the aircraft without the straps touching the tissues being measured. The right leg was resting on a foam roll to ensure that Achilles tendon as well as gastrocnemius were in a neutral, unloaded position, which was checked by palpation of the muscle. Participants were trained and instructed to concentrate on full relaxation in order to avoid any muscular activity. This setup was simulated in the lab beforehand.

Equipment: Myoton Technology

The measurement method applied in the MyotonPRO is based on exertion of a quick released single mechanical impulse (time 15ms, force 0.4N) under constant pre-compression force (0.18N) of the subcutaneous tissue layer above the muscle/tendon being measured. Mechanical deformation is delivered by the device testing-end (d=3mm), held perpendicular to the skin surface. After a short mechanical impulse, the muscle or tendon responds in the form of a damped oscillation, which is registered by an acceleration sensor attached to the device's frictionless measuring mechanism. The properties of the sensor are as follow: amplitude range of ±8g in full range; resolution of < 0.001g; output data rate and bandwidth 3200Hz; sensitivity of < 20mg/LSB for each axis; sensitivity ±0.1% due to the temperature change; bias level of each axis 100mg; noise performance of each < 1.5 LSB rms; operating temperature -10 to + 50C.

From the oscillation acceleration signal (Figure 2) the following five parameters were computed simultaneously in real time:

***f* – Oscillation frequency [Hz]**

Oscillation frequency indicates the tone (that is, intrinsic tension) of a muscle in its testing state, which can be at rest without any voluntary contraction (EMG silent) or contracted. The higher the value the higher the tone or state of tension.

S – Dynamic stiffness [N/m]

Dynamic stiffness is the biomechanical property of a muscle that characterizes the resistance to a contraction or to a external force that deforms its initial shape. The higher the value, the higher the stiffness.

D – Logarithmic decrement:

The logarithmic decrement of a muscle's natural oscillation indicates the muscle's elasticity and dissipation of mechanical energy when tissue recovers its shape from being deformed. Elasticity is the biomechanical property of a muscle that characterizes the ability to recover its initial shape after a contraction or removal of an external force. The higher the value of logarithmic decrement, the lower the elasticity and the higher the dissipation of mechanical energy, as the tissue recovers its shape.

R – Mechanical stress relaxation time [ms]

Mechanical stress relaxation time is the time for a muscle to restore its shape from deformation after a voluntary contraction or an external force is removed. The higher the value, the longer the time of recovery from maximum deformation to full recovery of the shape.

C – Indication of creep [Deborah number]

Creep is the gradual elongation of a muscle over time when placed under a constant tensile stress. This is the ratio of the relaxation and deformation time of the muscle. The smaller the difference of Relaxation and Deformation time the higher the value of the C parameter indicating the Creep. Younger and healthier muscles have a smaller value of the C parameter.

It is well known that unloading (regardless whether it is caused by gravity shift or any other external physical loading force) will cause the same physical changes in biological soft tissues as in other non-biological materials or structures. While stiffness, tone and elasticity are the most commonly reported parameters using Myoton devices in the literature [2, 4, 15], we additionally investigated mechanical stress relaxation time and indication of creep in the present study to document the behaviour of all five parameters and relative changes to one another under the known influence of unloading in weightlessness.

During data collection immediately before each registration of the muscle oscillation in the form of an acceleration signal, the MyotonPRO device records the device's acceleration in X, Y and Z axes (Gx, Gy, Gz). This helps when processing the raw data to distinguish which measurements were taken in an unstable condition (turbulence) at 1G or zero gravity. Based on the recorded G values for each measurement, only stable 1G or zero gravity measurements were included in the statistical analysis.

In order to test for any effects of gravity on the device during the parabolic flights, performance of the MyotonPRO device was tested in a specially designed test rig.

The purpose of taking measurements in the test rig during the horizontal flight at 1G and the parabolas at 0G, was to investigate whether the device's gravity compensation system works efficiently and whether the measurements from signals resulting from impulses applied to the phantom material at 0G differ significantly from those recorded at 1G.

In order to conduct the test, the lightest possible phantom material with elastic properties was identified (Nitrile Butadiene Rubber, dimensions: d=30mm, h=7mm, total weight 0.72g, estimated oscillation mass – max 0.07g). The most important criterion when selecting the phantom was its weight [17, 18], because the lighter the phantom, the less its mechanical properties are affected by the gravity shift from 1G to 0G.

During the flights, data were collected on six different phantoms during five parabolas (i.e. 1G and 0G phases) each. As described above, values for each Myoton parameter were the mean of 12 single measurements within each series, calculated automatically by the device.

Measurement of muscle properties

Measurements were performed 30 minutes before take off (pre-flight, #1), during the horizontal flight prior to the parabola zero (before 0, #2), during the 1G phase before parabolas (inflight 1G, #3) and in the 0G phase (inflight 0G, #4) of each parabola. A final measurement was performed on ground 30 minutes (post-flight, #5). During and after parabolas 1-5, myometric measurements were performed on the right Achilles tendon. During and after parabolas 6-10, measurements were taken on the lateral belly of the right m. gastrocnemius lateralis, followed by measurements on the right sided m. erector spinae during parabolas 11-15 (Figure 3). The paraspinal and gastrocnemius muscles are important postural muslces. As the spine and lower extremities, especially the m. gastrocnemius, suffer most from unloading during prolonged microgravity [9] and recommendations exist also to address (de-)adaptation processes in the stabilizing muscles in more detail, these two muscles were selected.

In order to avoid transition effects, myometric measurements were started two seconds after a robust gravity level (1G or 0G) had been reached.

Each muscle/tendon of interest was measured 5 times (each measurement consisted of 12 mechanical impulses at 1 second intervals) in every flight phase. Measurements of oscillations elicited by each impulse contained 12 single measurements, conducted automatically by the device with an interval of 1 second. The mean of the 12 measurements within each series was used in the analysis.

Coefficient of Variation was used to assess the variability of measurements within each series of 12 impulses in order to document how stable the sets of measurements obtained were.

Statistical Analysis

In total, 60 measurements were recorded during each phase (pre-flight, before 0, inflight 1G, inflight 0G, post-flight) for each of the testing sites on the body (Achilles tendon, m. gastrocnemius, m. erector spinae). For statistical analysis all 60 measurements were averaged and repeated measures analysis of variance (ANOVA) with the intra-individual factor MEASUREMENT TIME were performed for each of the measurement points. Fisher's Least Significant Difference (LSD) test served as a post-hoc test if applicable. The same statistical analyses were applied for the phantom material in the test rig stand.

Two-tailed level of significance was set at $p < 0.05$. Data in the text are presented as means +/- standard deviation. All statistical analyses were performed by Statistica 7.1 (StatSoft, Tulsa, USA).

RESULTS

Achilles Tendon

Figure 4 illustrates the pattern of change in each parameter. For the first two measurement periods (pre-flight and before flight on board), there were no significant changes in any of the parameters after which, changes occurred inflight at 1G and zero G, before returning close to pre-flight values post-flight. Repeated measures ANOVA showed a clear effect of gravity over time for all five parameters (Oscillation frequency: $F_{(4, 40)} = 6.56$, $p < .001$; Logarithmic Decrement: $F_{(4, 40)} = 3.05$, $p < .05$; Dynamic stiffness: $F_{(4, 40)} = 10.72$, $p < .001$; Indication of creep: $F_{(4, 40)} = 16.74$, $p < .001$; Mechanical stress relaxation time: $F_{(4, 40)} = 16.47$, $p <. 001$).
Post-hoc analysis revealed that all five parameters were significantly different between pre-flight and zero G ($p<.05$). There were also significant differences observed between inflight conditions, with a decrease of Dynamic stiffness from 1G to 0G inflight ($p < .05$, Figure 4c), as well as related increases in Indication of creep ($p < .05$, Figure 4d) and Mechanical stress relaxation time ($p < .01$, Figure 4e).

M erector spinae

Unlike the pattern over time for the Achilles tendon, Figure 5 illustrates that there were no significant changes in any of the parameters at 1G, either before or during flight. ANOVA results revealed a clear effect of gravity over time for all five parameters (Oscillation frequency: $F_{(4, 40)} = 19.35$, $p < .001$; Logarithmic Decrement: $F_{(4, 40)} = 9.87$, $p < .001$; Dynamic stiffness: $F_{(4, 40)} = 14.36$, $p < .001$ Indication of creep: $F_{(4, 40)} = 15.32$, $p < .001$; Mechanical stress relaxation time: $F_{(4, 40)} = 16.27$, $p <. 001$; Figure 5).
Post-hoc analysis confirmed a significant difference of the 0G inflight data compared to all other data collection points ($p < .01$), i.e. a decrease in tone (Oscillation frequency, Figure 5a) and stiffness (Figure 5c), with an increase in Logarithmic decrement (i.e. decrease in elasticity, Figure 5b), Indication of creep (figure 5d) and Mechanical stress relaxation time (Figure 5e). Furthermore, only the Logarithmic decrement showed a significant increase post-flight to pre-flight (Figure 5).

M. gastrocnemius lateralis

A similar pattern to that seen for m. erector spinae was found for m. gastrocnemius, with no changes occurring until 0G and then a return to pre-flight values post-flight (Figure 6). The ANOVA results revealed significant effects over time (Oscillation frequency: $F_{(4, 40)} = 3.92$, $p < .01$; Logarithmic Decrement: $F_{(4, 40)} = 7.81$, $p < .001$; Dynamic stiffness: $F_{(4, 40)} = 7.83$, $p < .001$; Indication of creep: $F_{(4, 40)} = 12.11$, $p < .001$; Mechanical stress relaxation time: $F_{(4, 40)} = 10.77$, p <. 001; Figure 6). Post-hoc analysis found a significant ($p<.05$) decrease in tone (Oscillation frequency) and Dynamic stiffness (Figure 6a and c), as well as an increase in Logarithmic decrement (reduction in elasticity), Indication of creep and Mechanical stress relaxation time (Figure 6b, d and e) in the 0G inflight condition. Furthermore, a significant difference in Oscillation frequency, Logarithmic decrement, Dynamic stiffness, Indication of creep and Mechanical stress relaxation time occurred between the 1G inflight data and the data obtained before parabola 0 and post-flight (Figure 6).

<u>Coefficient of Variation within sets of 12 impulses</u>

Coefficient of Variation (CV, standard deviation/mean; Table 1) was significantly increased ($p < .05$) during the inflight measurements compared with pre-flight CV but statistical analysis did not reveal any differences in the CV for parameters between 1G inflight and 0G inflight. All CV values were below 9%. Those pre-flight in muscles were 1.4-3.1% and tendon 2.0-4.1%; those inflight (1G and 0G) for muscles were 1.9-6.3% and tendon 4.4-8.7%; and post-flight muscles 1.1-3% and tendon 2.2-3.4%.

<u>Test rig stand</u>

Statistical analysis revealed a significant, albeit minor decrease of 0.80%, for Oscillation frequency ($p < .001$) and Dynamic stiffness ($p < .001$) under 0G (Table 2). No effect was found for Logarithmic decrement, Mechanical stress relaxation time or Indication of creep.

D I S C U S S I O N

Regular exercise is important for those living in space to help counteract the loss of muscle mass and muscle force caused by microgravity [9]. Measuring the adaptation of the musculoskeletal system in order to monitor muscle quality and to develop individual training programmes, based on the biomechanical properties of the muscle, is highly desirable. However, such monitoring is restricted by practical, technical and time constraints on board of the International Space Station (ISS). The present study aimed to demonstrate that measurements using Myoton technology are a feasible method to monitor the tone, biomechanical and viscoelastic properties of the superficial skeletal muscles and tendons in a microgravity environment. Parabolic flights offer the opportunity for such feasibility testing.

M. gastrocnemius lateralis, m. erector spinae, as well as the Achilles tendon, showed a clear change in all five parameters measured (Oscillation frequency, Logarithmic decrement, Dynamic stiffness, Mechanical stress relaxation time and Indication of creep) when tested under microgravity conditions.

The present study showed similar results to previous studies on the ground for the muscles testes and therefore demonstrates the robustness of data and procedures [2, 15]. The fact that Oscillation frequency and Dynamic stiffness were found to decrease whereas Logarithmic decrement, Mechanical stress relaxation time and Indication of creep increased indicates that both muscles and the Achilles tendon were significantly more relaxed under microgravity conditions. It appears that microgravity affects the oscillation characteristics of the muscles and tendons, most probably caused by an unloading effect. Any tensioning force including gravity is tensioning any materials and tissues. When the external (i.e. gravity) tensioning factor is suddenly altered (removed), the materials and tissues will lose that part of the tension immediately and accordingly will oscillate slower when mechanically perturbed. One could also argue that these changes are due to haemodynamic changes and a redistribution of blood volume increasing/decreasing tissue mass, which might influence the responding oscillation of the underlying tissue. Nevertheless this can be ruled out as participants have been in a horizontal position lying on their abdomen and accordingly hemodynamic changes can be neglected [11].

Although the test rig results showed that the embedded gravity correction (compensation system) of the MyotonPRO showed slight differences when measuring a phantom rubber under normal gravity and microgravity, the slight inaccuracy of less than 1% (although statistically significant) cannot be considered responsible for the changes observed in m. gastrocnemius lateralis, m. erector spinae or Achilles tendon. The changes in tissue parameters were of much greater magnitude than those on the phantom material.

Interestingly the Achilles tendon, as the most rigid structure, showed a significant relaxation not only in the microgravity phase of the parabolas but also during the 1G phase in between the parabolas(Figure 4), whereas m. erector spinae (Figure 5) and m. gastrocnemius lateralis (Figure 6) showed stable recordings for all tests conducted at 1G and significant changes occurred only at 0G. The Achilles tendon observations could be explained by a general relaxation during the flight. In general, participants of a parabolic flight are initially quite nervous before the flight and exhausted after the flight [24]. Once they have experienced the effect of weightlessness, a general relaxation can be assumed, especially as participants were allowed to take anti-nausea medication. The fact that similar effects could not be obtained for m. gastrocnemius and m erector spinae requires further studies examining the effect of stress on different tissue material. It is known that muscle tone increases with psychological stress [26], but perhaps this is easier to identify with more rigid tissue such as tendons. We attempted to assess muscle tone of the gastrocnemius by EMG on three participants during two flights, but due to the relaxed position, only ambient noise could be obtained.

Variability of measurements, analysed using coefficient of variation, was increased for all three measurement points in the inflight condition. This is due to small air turbulences as they occur typically during flight. With respect to the fact that no differences in variability were found between the 1G- and the 0G-inflight conditions, it can be assumed that the observed changes in the mechanical properties of the muscle are not related to an increase in variability.

This study is limited by the fact that only the impact of short periods of microgravity on the mechanical properties of the Achilles tendon and two muscles could be obtained. Moreover it would also be very important to identify the impact of countermeasures during microgravity on the mechanical properties of biological tissue. Further studies are now warranted to monitor the long-term adaptation of musculoskeletal tissues to: (1) prolonged exposure to weightlessness, which is known to result in a loss of muscle mass and force; and (2) adequate exercise programmes to counteract this loss, and restore muscle function and maintain joint health. The results from this study suggest that myometric measurements are an accurate and reliable tool to monitor muscle quality and to aid development of individual training programmes informed by the muscles' mechanical properties.

CONCLUSIONS

The present findings demonstrated that myometric measurements, using Myoton technology, are an accurate, non-invasive and easy to use approach to assess the tone, biomechanical and viscoelastic properties of the muscle under microgravity. As expected, these properties change in zero gravity, causing the measured tissues, muscles as well as tendons, to be in a more relaxed state.

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CONFLICT OF INTEREST STATEMENT

One of the authors of this manuscript, namely Aleko Peipsi, is acting CEO of MYOTON AS, Estonia. There are no other conflicts of interest.

Table 1: Coefficient of variation (CV) for each of the five measurement points and muscle properties.

Object	Phase	CV of frequency (%)	CV of decrement (%)	CV of stiffness (%)	CV of relaxation (%)	CV of creep (%)
Achilles Tendon	pre-flight	2.6	4.1	2.0	2.6	2.6
	before 0	3.0	4.8	2.8	3.1	3.0
	inflight 1G	5.3*	8.7*	4.7*	5.2*	4.7*
	inflight 0G	4.3*	7.7*	4.4*	4.7*	4.4*
	post-flight	2.2	3.4	2.5	3.0	2.9
Gastroc-nemius	pre-flight	1.6	2.2	1.5	1.4	1.3
	before 0	6.1*	7.3*	4.1*	3.4*	3.2*
	inflight 1G	4.3*	6.3*	2.8*	2.8*	2.7*
	inflight 0G	3.8*	6.9*	1.9	2.8*	2.9*
	post-flight	1.1	2.2	1.5	1.2	1.2
Erector spinae	pre-flight	1.5	3.1	2.2	2.2	2.1
	before 0	2.3	4.4*	3.2*	3.2	3.1
	inflight 1G	2.7*	4.7*	4.0*	4.2*	4.1*
	inflight 0G	3.4*	5.8*	4.3*	4.%*	4.5*
	post-flight	1.5	3.0	2.1	1.9	1.8

* indicates $p < .05$ to pre-flight and to post-flight.

Table 2: Results from the test-stand: (mean +/- SD) averaged over 5 parabolas from 6 different phantoms in 1G and 0G as well as the resulting difference in % and the corresponding p-values

	1G	0G	Difference [%[	P-value
Frequency (Hz)	25.08 +/- 0.13	24.88 +/- 0.13	0.80%	< .001
Stiffness (N/m)	466.05 +/- 4.35	462.27 +/- 3.92	0.81%	< .001
Decrement (log)	0.5 +/- 0.01	0.5 +/- 0.01	n/a	n.s.
Relaxation (ms)	9.97 +/- 0.05	10.00 +/- 0.01	0.30%	n.s.
Creep	0.75 +/- 0.05	0.72 +/-0.04	4%	n.s.

Displayed are results (mean +/- SD) averaged over 5 parabolas from 6 different phantoms in 1G and 0G as well as the resulting difference in % and the corresponding p-values.

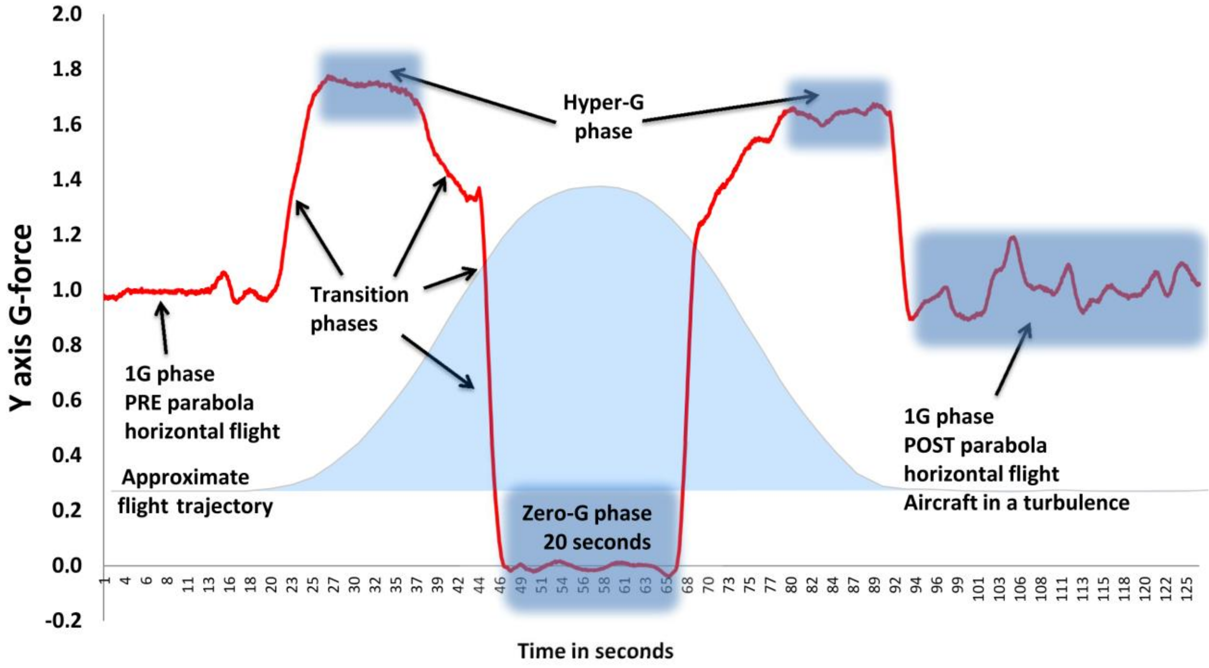
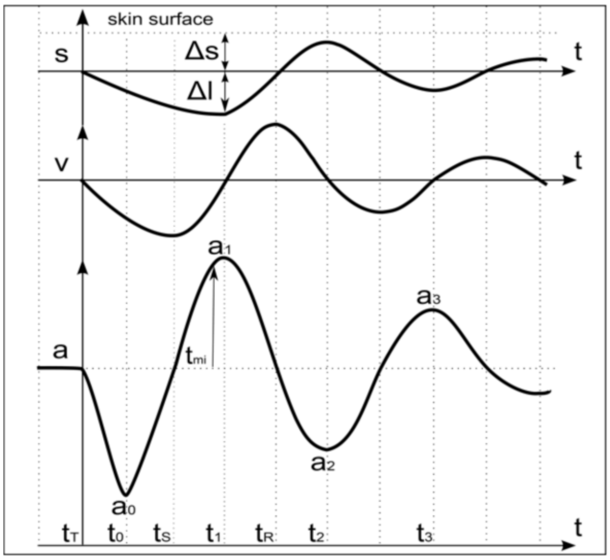


Figure 1: G-force diagram of a typical parabola, generated from the aircraft's accelerometer data. During hypergravity, gravity (G) values reach approximately 1.8G. During the 20 second microgravity phase (Zero-G), gravity remains below 0.05G.



Symbol	Description
a	Acceleration of the damped oscillation – unit: mG
a_0	Maximum acceleration
a_1	Maximum displacement i.e. maximum tissue resistance measured in mG
a_2	Maximum opposite displacement due to the residual inertia of the tissue oscillation
a_3	Maximum displacement of the second period of oscillation which takes place due to the recuperation of stored residual mechanical energy in the tissue
ΔS	Pre-compression of subcutaneous tissues above the muscle being measured
Δl	Maximum displacement of the tissue
m_{probe}	Mass of the measurement mechanism
S	Displacement (tissue oscillation) – unit: mm
t	Time – unit: ms
t_{mi}	The end of mechanical impulse
t_0	The time when maximum acceleration was reached
t_T	Start of the mechanical impulse
t_r	The time when tissue returns to its initial shape
t_s	Zero acceleration and maximum velocity due to the force of deformation
$t_t - t_0$	The time from zero to maximum acceleration
$t_0 - t_s$	The time from maximum back to zero acceleration
$t_s - t_1$	The time from maximum velocity to zero velocity of deformation
$t_1 - t_r$	The time of acceleration from maximum displacement of the tissue to return to its initial shape. It represents the tissue Mechanical Stress Relaxation time
$t_r - t_2$	The time of acceleration from restored initial shape of the tissue to its maximum opposite displacement
t_1	The time when maximum displacement was reached
t_2	The time when maximum opposite displacement was reached
t_3	The time when maximum displacement of the second period of oscillation was reached
$t_2 - t_3$	The time of two consecutive displacements
V	Oscillation velocity – unit: m/s

Frequency [Hz]: $F = f_{max}$ **Dynamic Stiffness [N/m]:** $S = a_1 \cdot m_{probe} / \Delta l$ **Creep (Deborah number):** $C = R / (t_1 - t_T)$ **Logarithmic Decrement:** $D = \ln (a_1 / a_3)$ **Mechanical Stress Relaxation time [ms]:** $R = t_R - t_1$

Figure 2: Description of the relationship of the displacement oscillation (S) and oscillation velocity (V) in relation to the oscillation acceleration (a), from which the four parameters were derived: frequency (Hz), dynamic stiffness (N/m), logarithmic decrement and relaxation time (ms)

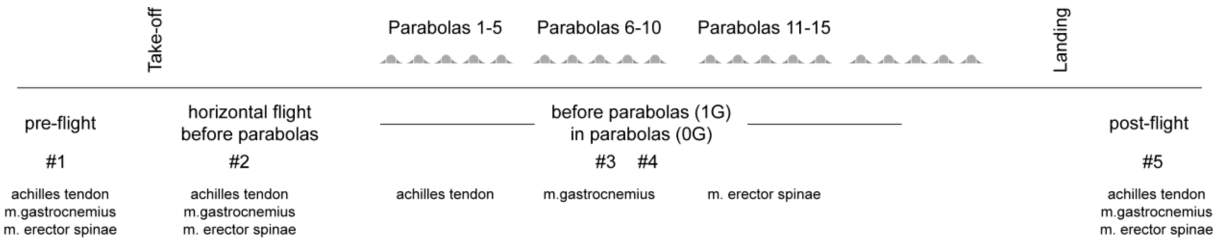


Figure 3: Timeline of the experimental procedures. Measurements were taken pre-flight, during the horizontal flight prior to start of the parabolas, during the 1G phase and the 0G phase during each parabola, as well as post-flight.

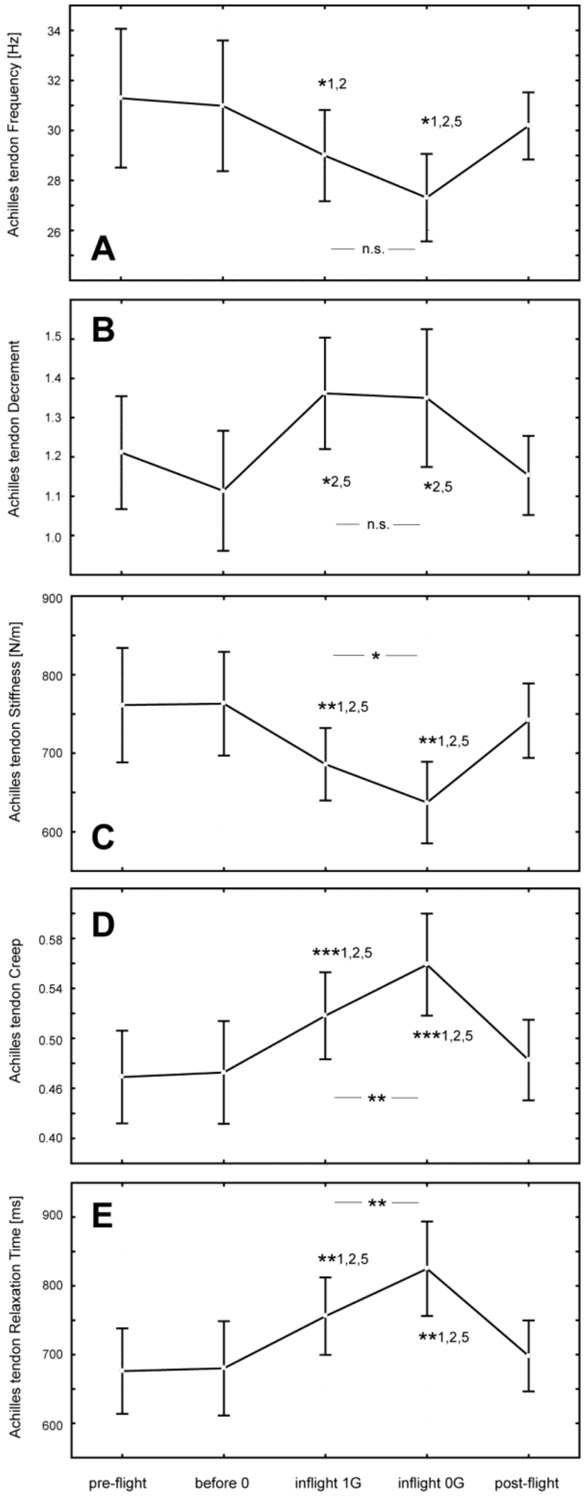


Figure 4: Changes in Achilles tendon Frequency (A), Decrement (B), Stiffness (C), Creep (D) and Relaxation time (E) (top down) for the five measurement points (1:pre-flight; 2:before parabola 0; 3:inflight 1G; 4:inflight 0G; 5:post-flight). * mark $p < .05$, ** $p < .01$, *** $p < .001$. Numbers next to * mark significant differences to measurements 1-5. Significant differences between the 1G and 0G inflight condition are marked separately.

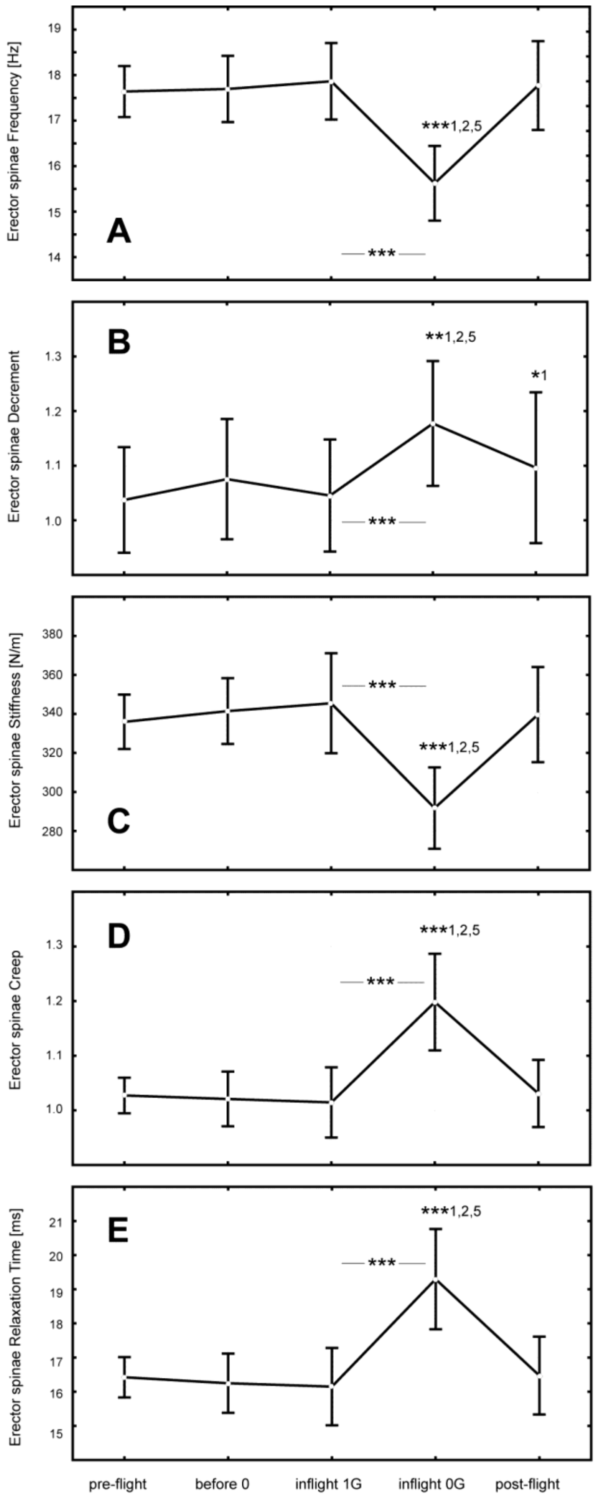


Figure 5: Changes in m. erector spinae Frequency (A), Decrement (B), Stiffness (C), Creep (D) and Relaxation time (E) (top down) for the five measurement points (1:pre-flight; 2:before parabola 0; 3:inflight 1G; 4:inflight 0G; 5:post-flight). * mark $p < .05$, ** $p < .01$, *** $p < .001$. Numbers next to * mark significant differences to measurements 1-5. Significant differences between the 1G and 0G inflight condition are marked separately.

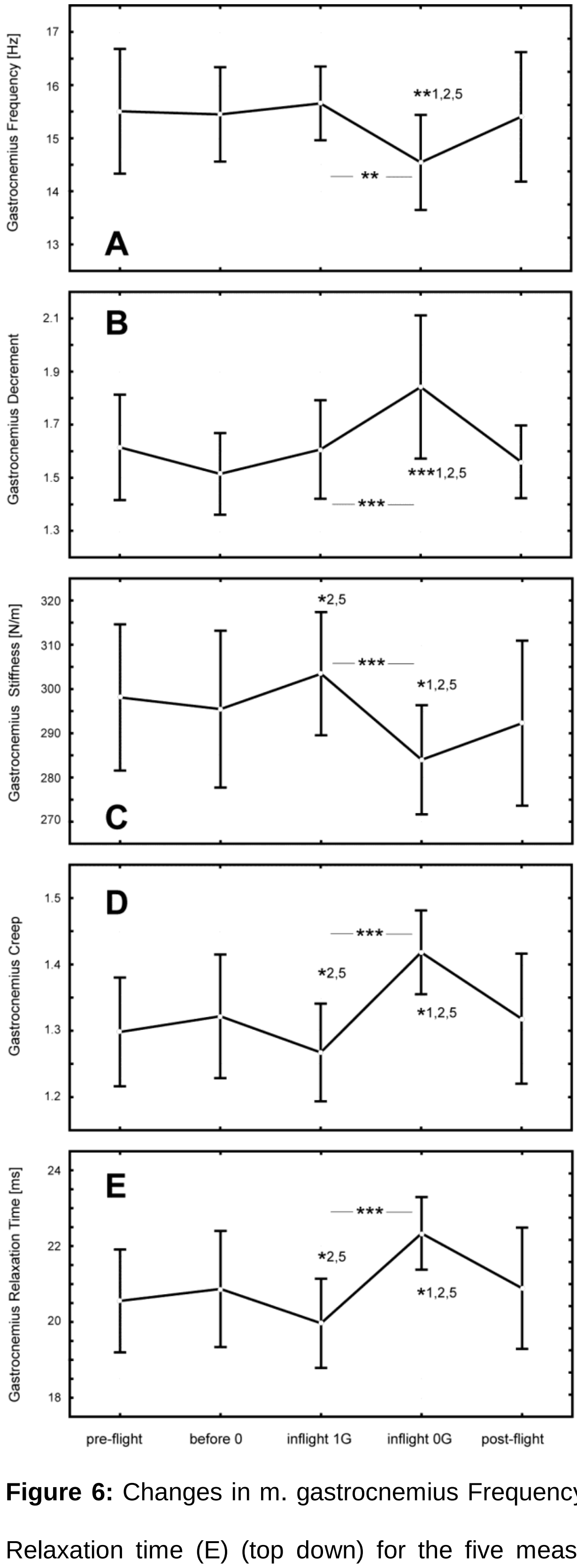


Figure 6: Changes in m. gastrocnemius Frequency (A), Decrement (B), Stiffness (C), Creep (D) and Relaxation time (E) (top down) for the five measurement points (1:pre-flight; 2:before parabola 0; 3:inflight 1G; 4:inflight 0G; 5:post-flight). * mark $p < .05$,. ** $p < .01$, *** $p < .001$. Numbers next to * mark significant differences to measurements 1-5. Significant differences between the 1G and 0G inflight condition are marked separately.

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