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Bachelor's Thesis

Influence of Space Flight on Fascicle Dynamics and Aponeurosis Movements in the Medial Gastrocnemius Muscle

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Abstract

Muskelatrophie ist ein Thema, mit dem man sich aus verschiedenen Gründen befassen muss, z. B. um den Muskelabbau bei älteren und bettlägerigen Patienten zu verstehen und zu verringern.
Astronauten nach einem Weltraumflug sind interessante Studienteilnehmer, da sie Muskelschwund als Nebenwirkung der Schwerelosigkeit erleben und gleichzeitig gesunde Probanden sind.

Ziel dieser Arbeit war die Auswertung von Daten aus der Studie Sarcolab 3. In dieser Studie wurden Bewegungs- und Ultraschalldaten des Wadenmuskels *Gastrocnemius medialis* vor und nach dem Weltraumflug von fünf Astronauten aufgezeichnet. Diese Aufnahmen wurden am ruhenden Muskel und während der Kontraktion gemacht, im Gegensatz zu früheren Studien, die sich vor allem auf den Muskel in Ruhe konzentrierten.

Um die notwendigen Parameter aus den Daten zu gewinnen, wurde ein geeignetes Tool zur Auswertung gewählt. Die zu erfassenden Parameter waren die folgenden: Die Faszikellänge - die Länge eines Muskelfaserbündels zwischen den Bindegeweben, die den Muskel umgeben. Und der Fiederungswinkel, definiert als der Winkel zwischen Bindegewebe und dem Faserbündel. Beide wurden über die Dauer jedes Videos ausgewertet, das den ruhenden und den kontrahierten Zustand des Muskels zeigt.
Ferner wurden diese Parameter mit der entsprechenden Kraftkurve, die die Testperson während der Kontraktion entwickelte, super-positioniert.

Die anschließende Analyse ergab, dass sich die Faszikellängen während des gesamten Weltraumfluges um durchschnittlich -13,6 $\pm$ 8,1 % für die Ruhelängen verkürzt haben. Allgemeine Aussagen zu den Winkeln konnten nicht gemacht werden. Die Kraft der Probanden nahm während des Weltraumflugs um -10,4 $\pm$ 14,6 % ab, erholte sich jedoch und übertraf am Tag 30 nach der Landung die Werte vor dem Flug.

Bei der Auswertung traten Herausforderungen auf. Beispielsweise kann die Darstellung eines dreidimensionalen Merkmals, wie des Faszikels, auf dem 2D-Ultraschallbildschirm zu Ungenauigkeiten bei den Parametern führen. Dieses Problem könnte umgangen werden, wenn 3D-Aufnahmen verwendet würden, zum Beispiel Magnetresonanztomografie (MRT) oder 3D-Ultraschall.
Eine weitere Herausforderung war die Gewinnung reproduzierbarer Daten mit dem gewählten manuellen Tool. In Zukunft könnten automatisierte Algorithmen eingesetzt werden, um eine Auswertung nach objektiven Kriterien zu ermöglichen.
Außerdem war die Stichprobengröße mit fünf Teilnehmern klein, was an manchen Stellen zu Schwierigkeiten führte, klare Schlussfolgerungen zu ziehen. Astronauten sind eine seltene, aber hochinteressante Testgruppe und die begrenzten Daten sollten dennoch ausgewertet werden.

Abstract

Muscle atrophy is a topic that has to be dealt with for various reasons, for example, to understand and reduce muscle loss in elderly and bedridden patients.
Astronauts after a space flight are interesting study participants because they experience muscle atrophy as a side effect of weightlessness and are healthy subjects at the same time.

This thesis aimed to evaluate data from the study Sarcolab 3. This study recorded motion and ultrasound data of the calve muscle *gastrocnemius medialis* before and after the space flight of five astronauts. These recordings were taken at the resting muscle and during contraction, whereas earlier studies mainly focused on the relaxed muscle.

In order to obtain the necessary parameters from the data, a suitable tool for evaluation was chosen. The parameters to be collected were the following: Fascicle length - the length of a muscle fiber bundle between the connective tissue surrounding the muscle. And the pennation angle, defined as the angle between the connective tissue and the fiber bundle. Both were evaluated over the time of each video, containing the rested and the contracted state.
Further, these parameters were super-positioned with the corresponding strength developed by the subject during contraction.

The following analysis showed that the fascicle lengths have shortened throughout the spaceflight by an average of -13.6 $\pm$ 8.1 % for the rested lengths. General statements about the angles cannot be made. The subjects' strength decreased by -10.4 $\pm$ 14.6 % during space flight but recovered and exceeded values before space flight by day 30 after landing.

Some challenges were encountered during the evaluation. For example, displaying a three-dimensional feature such as the fascicle on the 2D ultrasound screen can lead to inaccuracies in the parameters. This problem could be circumvented if 3D recordings were used, for example, magnetic resonance imaging (MRI) or 3D ultrasound.
Another challenge was to retrieve reproducible data with the chosen manual tool. In the future automated algorithms could be used to allow evaluation under objective criteria.
Also, the sample size was small, with five participants, which sometimes led to difficulties drawing clear conclusions. However, astronauts are a rare but highly interesting test group, and the limited data should be evaluated nevertheless.

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Symbols and Formulas

Symbol	Unit	Description
R	-	reflection factor
r^2	-	coefficient of determination
$\bar{x}_{d,i}$	$mult$	mean mean value over all trials on recording day d for subject i
d	-	recording day $\in$ [PRE1, PRE2, R+3, R+6, R+15, R+30, R+60]
i	-	subject ID $\in$ [01, 02, 03, 04, 05]
$\bar{x}_d$	$mult$	value over all trials on recording day d
b_{inter}	$mult$	inter-subject baseline
$\bar{x}_{PRE,i}$	$mult$	values for subject i, averaged per recording day PRE1 or PRE2
$b_{intra,i}$	$mult$	baseline for subject i
δ_i	$mult$	difference of post-flight and baseline value for subject i
x_{POSTi}	$mult$	values after spaceflight for subject i, averaged per recording day
r_i	$mult$	relation of difference to baseline for subject i
$\bar{r}$	$mult$	averaged relative value for all subjects

$mult$: values have different units
depending on the parameter ($[cm]$, $[°]$, $[Nm]$, $[\%]$)

1 Introduction

Muscle atrophy - the loss of skeletal muscle, is an essential topic in research as it affects diverse populations. Be it muscle atrophy due to injury, bedriddenness, or simply aging. Astronauts are also severely affected by this, as weightlessness means that the muscles are not used to the usual extent. Research has been concerned with muscle loss in astronauts since the first long-term missions, as they form an exciting basis for investigation. Indeed, collecting data where muscles are immobilized is challenging to reproduce on Earth. Studies with diseased patients or older subjects are possible, but side effects cannot be excluded. In order to collect data on immobilized muscles on Earth, one would otherwise have to resort to complex bed rest studies.

For this reason, essential parameters of muscle atrophy are studied after space missions. On one hand, to reduce problems for astronauts post-flight and on the other hand to advance research for all areas related to muscle atrophy. The research often focuses on the calf muscle *gastrocnemius medialis* because it plays a significant role in the posture under gravity and is subject to particularly large effects if not used. The purpose of this thesis is to analyze data from the Sarcolab3 study with five astronauts as subjects. For the reasons mentioned above, the *gastrocnemius medialis* is also the subject of this study. Ultrasound videos before and after the flight are evaluated in relation to common parameters from the muscle architecture.

Previous studies can be divided into two classes. One type of study examined these relevant values before and after spaceflight at rest; the other compared rest and contraction values in general. The study Sarcolab3 combines these and evaluates values at rest and values at contraction before and after spaceflight. This was done in order to be able to make statements about muscle dynamics and not only about static values.

The aims of this thesis are to review the data of Sarcolab 3 and also an assessment of the measurement methods. Another goal is to find an evaluation tool to efficiently extract the wanted parameters from ultrasound videos. These parameters will be compared before and after spaceflight and conclusions will be drawn regarding the changes induced by weightlessness.

The questions to be answered primarily are: What are the effects of space flight on muscle parameters? And: Do these changes also show up under contraction?
Answering these questions may give us new insights into the dynamics and functionality of the musculature.

2 Background and Current State of Research

2.1 Background

This chapter gives background information to fully understand the questions, and statements made in this thesis.
Information will be given about the general structure of skeletal muscles and their functionality and the ultrasound sonography technology to understand the structures seen in ultrasound images. Also, results from previous studies will be covered to help classify results later.

2.1.1 Anatomy and Functionality of Muscle

Muscle fascicles, considered mainly in this thesis, are bundles of muscle fibers surrounded by connective tissue, called perimysium. Muscle fibers are multi-nucleated giant cells with a diameter of 10 to 100 µm and a length of up to 20 cm, built from contractile myofibrils. [4, p. 26]. The connective tissue has the purpose of carrying nerves, blood vessels, and muscle spindles [5, p. 310].
For a graphic scheme of the muscle structure see Figure 2–1.

Each myofibril is composed of sarcomeres, which in turn consist of approximately 3000 actin and 1000 myosin filaments, arranged in parallel, which results in the skeletal muscle looking cross-striated [6, p. 383].
Actin is anchored between the so-called z-discs, which are networks of linked protein filaments, and myosin is inserted between them, with actin and myosin overlapping. For a better understanding of the structure, one can look at Figure 2–2. During muscle contraction, the filaments are pushed into each other with a margin of only approximately one micrometer per sarcomere, which adds up to the total muscle contraction. A typical muscle can contract to 50% of its length. [6, p. 384]

Different kinds of contraction are distinguished. The two main forms are isometric and isotonic contraction. Isometric contraction is when the muscle contracts and builds up force but does not shorten, whereas, for isotonic contraction, the muscle shortens with constant force. [7, p. 112]
There are also hybrid forms of these two kinds of contractions. For example, one is called auxotonic contraction if the muscle simultaneously shortens and builds up force [7, p. 112]. For this thesis, the data provided by the Sarcolab 3 study was recorded during isometric contractions using the 'muscle atrophy research and exercise system' MARES.

2.1.1.1 Gastrocnemius medialis

The calve muscle *gastrocnemius medialis* considered specifically in this thesis is a fusiform muscle. Its strength is proportional to the diameter of the muscle. Also, this muscle is a pennated muscle, meaning that its fascicles are shaped feather-like. More

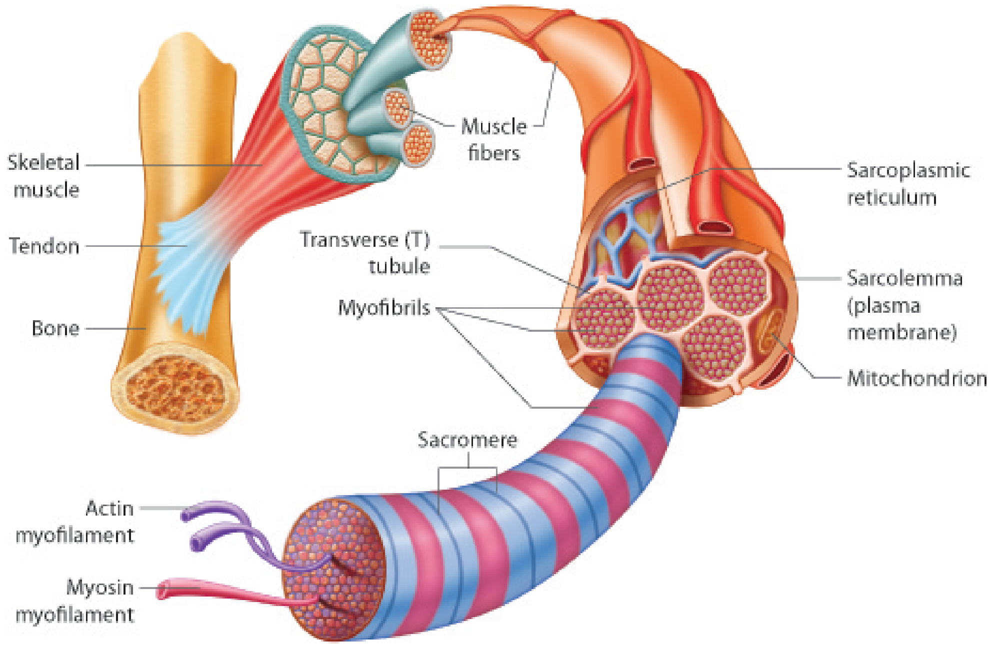


Fig. 2–1: Schematic representation of a muscle fiber [1]

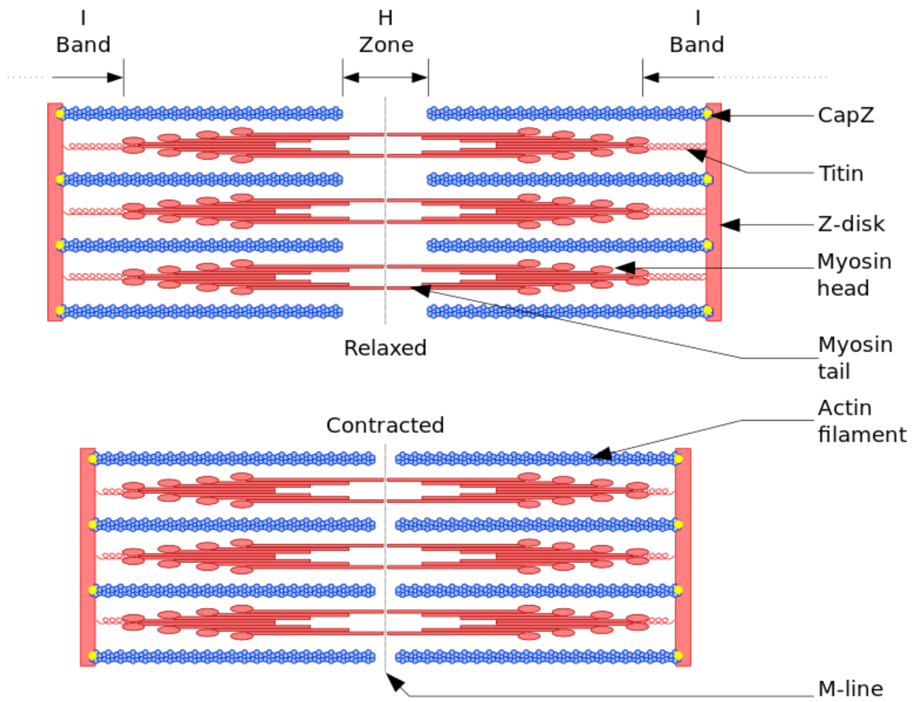


Fig. 2–2: Schematic representation of the myofilament apparatus [2]

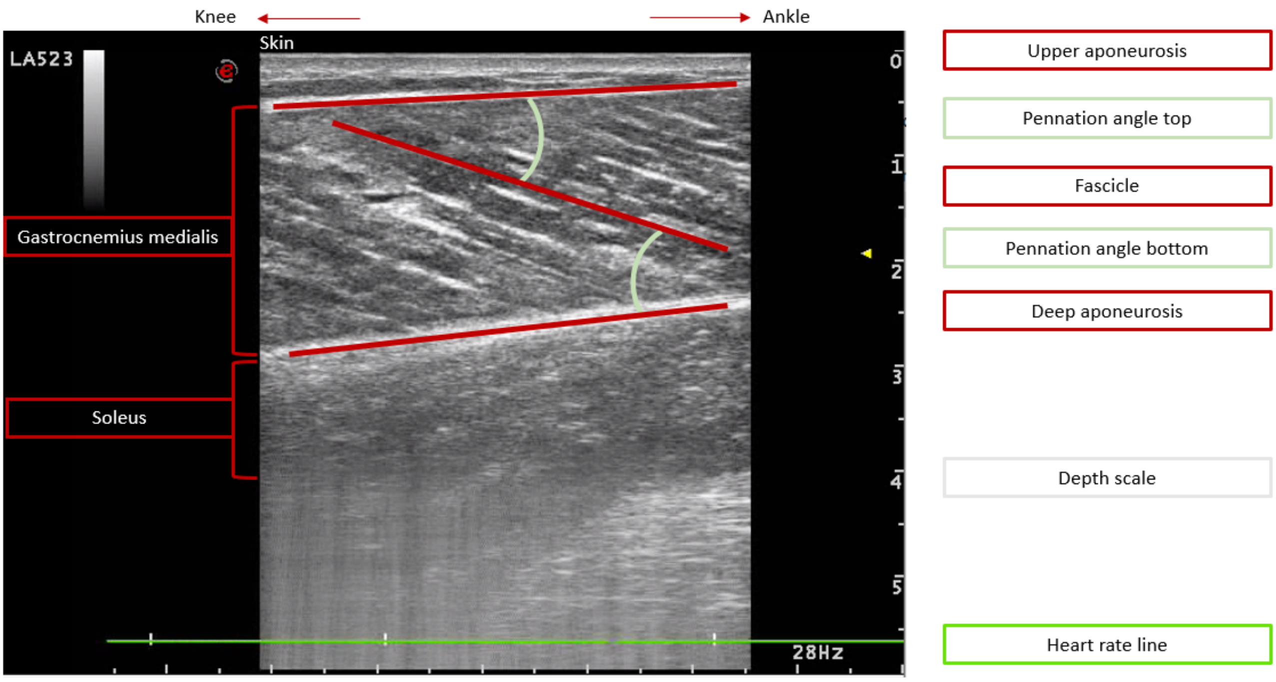


Fig. 2–3: Ultrasound picture marked with important features

specifically, it is uni-pennate, so all fascicles run in the same direction between the connective tissues, here between upper and deep aponeurosis. [5, p. 310]
Fascicle and pennation angle can be seen in the screenshot of one of the ultrasound videos taken in the study. (see Figure 2–3) The pennation angle is defined as the fascicle angle relative to the force-generating axis [8, p. 1649], in our case, as the angle between the fascicle and deep aponeurosis, also to be seen in 2–3. During contraction, the muscle increases in thickness, leading to shortening of the muscle fibers and enlargement of the pennation angle [9, p. 238].

2.1.2 Ultrasound Sonography

Ultrasound sonography is a popular imaging method, as it is non-invasive, does not expose the patient to radiation, and provides cross-sectional imaging [10, p. 316].

2.1.2.1 Physical Principle

Ultrasound sonography works with the principle of sound waves being reflected at interfaces with different sound impedance. Some tissues deflect the waves while others transmit them. Interfaces between soft tissues lead to only weak reflection, whereas, for example, the interface between soft tissue and air or soft tissue and bone reflect strongly and are therefore visible in the image. [11, p. 245]
The ideal proportion of reflection is about 1% which is represented by a reflection factor of $R = 0.01$. This factor is defined from 0 and 1 with $R = 0$ standing for no reflection and $R = 1$ being total reflection. It is calculated from the wave impedances of the media building the interface.
One problem can be air between probe and skin, leading to unclear images. This can

be explained by air having a wave impedance that deviates strongly from human tissue leading to a reflection factor of $R = 1$ and therefore to total reflection, making the image behind an air bubble not accessible. For a good image, ultrasound gel with a good sound conductivity is used to ensure a reflection factor of $R = 0$ at the skin. What cannot be ruled out are sound shadows behind internal air bubbles. [10, p. 317]

2.1.2.2 Sound wave generation

The sound waves are generated using specific crystals, which are subject to the so-called piezoelectric effect. If voltage is applied to the crystal, it dilates or contracts respectively, dependent on the voltage polarization. By applying a high-frequency alternating voltage, the frequency of ultrasound waves can be achieved. The imaging works the other way round. Reflected sound waves are received in the crystals and converted to electric current, translated into images later. [10, p. 318]
The selected frequency determines penetration depth and image resolution. Higher frequencies lead to shallower depths as the waves interact more strongly with the tissue leading to a higher energy loss. On the other hand, the resolution is better than with a lower frequency. The choice of frequency is, therefore, a compromise as the resolution is proportional, and penetration depth is inversely proportional to the frequency. [10, p. 319]

2.1.2.3 Imaging modes

In ultrasound imaging, different modes are differentiated. Two of them are called A-mode from 'amplitude mode' and B-mode from 'brightness mode'. In A-mode, the signal is plotted over the depth from where the signal came. Impulses from deeper points can be distinguished from shallower points due to the different run times of the signals. The peaks in the A-mode diagram can also vary in amplitude, showing the reflection factor of the interface. A-mode is rarely used in today's diagnostics. B-mode is similar to A-mode with the difference that amplitudes are now shown as brightness in image points, leading to a row of differently bright points showing a picture of the tissue. Today's diagnostics are using this mode showing the brightness of different points digitized in different gray values on a screen. [10, p. 323]

2.1.3 Spaceflight - History of manned missions

The first manned spaceflight took place in 1961, executed by Russia when Yuri Gagarin flew into space on Vostok I, followed by several manned Vostok missions. The first American manned mission happened only one month later when Alan Shepard flew into space with the Mercury spacecraft. Several milestones such as the first lunar landing with Apollo 11 and launches of the first space stations followed. [12, p. 13-31]
The beginning of the first space stations also heralded the beginning of the first long-term missions and the international space station ISS started as an idea in the early 1980s by the united states. Other nationalities joined the program, and in 1998 the first element of ISS - Zarya was launched. [12, p. 45-51]
Before the invention of spacestations, the longest stay in space took 18 days, but with

the space stations coming up, the space flight endurance record of 14 months was set in 1995 on the Russian space station Mir [13].
Until March 2020, the ISS hosted 170 long-duration crewmember flights with durations from 48 to 340 days. The majority of these flights took between five to seven months. [13]

2.2 Current State of Research

This chapter summarizes the results of previous studies on fascicle length, pennation angle and plantar flexor strength changes in relation to muscle atrophy.

2.2.1 Pennation angles

A study from 1998 by Narici et al. [14] aimed to investigate changes resulting from disuse atrophy after injury. The pennation angle decreased by 16.42 $\pm$ 2.8 % for patients with lower limb atrophy compared to healthy subjects. [14]
A different study from 2003 compared younger (20-39 yrs) to older subjects (60-85 yrs). Among other muscles, they also examined the *gastrocnemius medialis* and found no significant changes in pennation angles between younger and elderly subjects. [15, p. 125]
Another study by Kawakami in 1993 did not deal with atrophied but hypertrophied muscles. They found significant differences between normal subjects' and bodybuilders' triceps regarding muscle thickness and pennation angle and a significant correlation between muscle thickness and pennation angles. These results would suggest hypertrophy to lead to increasing pennation angles. [16]
One study took MRI images from 18 patients with their lower leg immobilized in a cast because of ankle fracture. Pictures were taken on days 5, 8, 15, 29, and 43 after casting. The fiber pennation angle was measured for the *gastrocnemius medialis*. They found a decrease in pennation angle by 3.7° compared to the leg not cast on day 29. [17, p. 690]
Ramsay's study from 2014 compared ten healthy subjects to ten post-stroke patients. These patients experience muscle weakness contralateral to the brain lesion. Pennation angles were compared between paretic (partially paralyzed) and non-paretic limbs in the patients and the healthy control group. For the pennation angle in *gastrocnemius medialis*, a significant decrease was found in the paretic limb compared to nonparetic and healthy. [18]
In a bed rest study from 2008 where ten healthy male subjects participated in 5 weeks horizontal bedrest, a decrease in pennation angle of 14.3 $\pm$ 6.8 % in the *gastrocnemius medialis* was found [19, p. 401].
In the pilot study Sarcolab for Sarcolab3, data of two astronauts was evaluated regarding plantar flexor muscle size and architecture. Both crew members stayed in space for six months. The subjects differed in the extent of training during space flight. Subject B trained more than subject A. The pennation angle in medial gastrocnemius decreased for both, but to a larger extent for subject A, which trained less. [20, p. 2]
Another study with muscle atrophy after space flight had eight male cosmonauts that stayed in space 213 days on average. This study found a decrease in pennation angle

in the *gastrocnemius medialis* from 15 to 26 % depending on the ankle position. Ankle positions ranged from -15° (dorsiflexion, ”pulling the foot”) to 30° (plantar flexion, ”pushing the foot”). The biggest decrease in pennation angle of 26 % was found for the 30° angle in the ankle. [21, p. 880]
In summary, it can be said that most mentioned studies find a decrease in pennation angle in the case of atrophy.

2.2.2 Fascicle lengths

The study already mentioned above by Narici also compared fascicle lengths in the injured and healthy subjects. They found a decrease of the fascicle length by 12.7 $\pm$ 1.9 % because of atrophy caused by disuse. [14]
In the study comparing healthy subjects to post-stroke patients, no statistical differences for fascicle lengths were found between affected and non-affected extremity as well as for healthy subjects [18].
Another study compared 15 young males (aged around 25 y) and 12 elderly males (aged around 74 y) in order to investigate muscle architecture under sarcopenia (muscle loss because of aging or immobility). A difference in fascicle lengths of 16% was found with the elderly having shorter fascicles than the younger. [22]
A study from 2019 evaluated data from eleven patients with functional deficit two years after Achilles tendon rupture. They found a significant decrease in fascicle length in *gastrocnemius medialis* compared to the uninjured side by 18 %. [23]
The mentioned five-week bed rest study with ten healthy male subjects by De Boer et al. found a decrease in fascicle length of 4.8 mm compared to lengths before bed rest [19, p. 404].
Another bed rest with six males also researched changes in fascicle lengths in the *gastrocnemius medialis*. They found a decrease of fascicle length of 9$\pm$ 3% after 90 days of bed rest. [24]
In the Sarcolab pilot study with two astronauts, fascicle lengths in medial gastrocnemius were reduced for both subjects. The difference was larger for the crew member, that trained less. [20, p. 2]
The study with eight male cosmonauts mentioned above in which the subjects stayed in space for approximately 213 days found a decrease in fascicle length from 43 to 25 mm for the medial gastrocnemius [21, p. 880].
In summary, it can be said that, except for one, all studies mentioned above found a decrease in fascicle length related to muscle atrophy.

2.2.3 Plantar flexor strength

The mentioned 90 days bed rest study by Reeves also evaluated plantarflexion force before and after immobilization. Strength was reduced by 55 $\pm$ 19% after the bed rest. [24]
The pilot study for Sarcolab3 comparing two participants with different amounts of training during their stay on ISS also evaluated muscle strength. They found a reduction in plantar flexor muscle strength by 30.6 % in the crew member, which was the one training less but no change for the crew member that trained during space flight. [20,

p. 2]
The study by Koryak with eight male cosmonauts that stayed in space for around 213 days found a decrease in maximum voluntary contraction (MVC) from 520 $\pm$ 67N to 303.1 $\pm$ 50N, which equivalates to a change of -42 %. [21, p. 884]
A pilot study before the just mentioned study with only one cosmonaut found a decline in plantar flexor force by 26 %. The measurements were taken 30 days before and 5-7 days after a 180-day space flight. [25]
An earlier study from 2001 investigated effects on long-term space flight, here defined with a duration of 90 to 180 days. Parameters of plantar flexor muscles from 14 cosmonauts were measured before and 2-3 days after landing. A decrease in MVC was found for 12 subjects. The decrease ranged from 2 to 37 % for these 12 subjects, whereas for two subjects, a slight increase was found on 0.2% and 0.8%. [26, p. 182]
In total, most of the studies above found a decrease in plantar flexor strength related to atrophy after space flight. One result was a similar strength for a crew member before and after space flight. The subject with this result trained intensively. One study even found a slight increase.

3 Material and Methods

The following chapter explains the steps that have been taken in order to retrieve the wanted parameters from ultrasound videos and corresponding movement data.
It is explained, how the ultrasound and motion recordings were made. Further, the tool selection for analyzing the videos and the synchronization of the resulting values and the rest of the data is addressed. At the end it will be explained which data was suitable for further analysis and which needed to be discarded for what reasons.

3.1 Data Collection

Data was collected as ultrasound videos and machine logs from MARES (muscle atrophy research and exercise system) from five healthy subjects participating in a space mission to ISS. The evaluated muscle was the calve muscle *gastrocnemius medialis*.
The procedure contained taking steady ultrasound pictures of the muscle architecture, then recording ultrasound videos performing a maximal voluntary contraction (MVC) of the muscle belly and the tendon during the contraction. In this thesis, only the videos of the muscle belly during MVC are about to be evaluated.
The procedure was followed two times before spaceflight and several days after the subjects' return to the ground.

3.1.1 MARES Data

MARES consists of five main elements: the main box with motor and control electronics, adapters that support the different exercise movements, and provide the possibility to isolate a single muscle and a chair. Additional is a laptop with the experiment software that provides the experiment protocol and functions as an interface to the subject. [27]
In the Sarcolab3 study, the *gastrocnemius medialis* was observed. Therefore, the subjects were restrained at the upper body, thighs, and knee. The foot was attached to a footplate. Before the test, the ankle joint range of motion was determined. A schematic representation of the subject placed in MARES can be seen in Figure 3–1.
The calve muscle was then contracted by pushing the ball of the foot forward, following the instructions by the software. It indicates the force that the subject should push using visual representation and shows when to rest the muscle. The subject is supposed to push to maximal contraction and hold this force for several seconds.

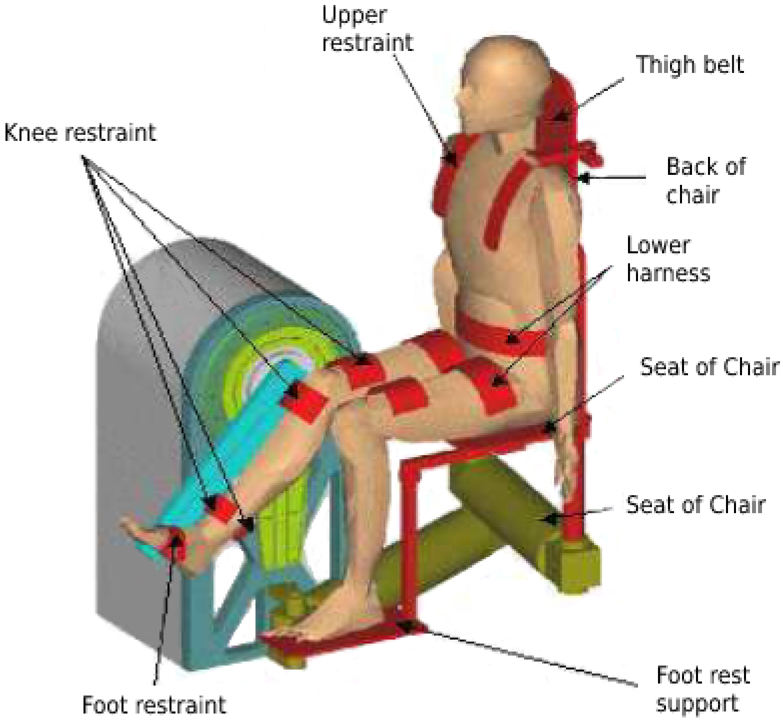


Fig. 3–1: Schematic representation of MARES [3]

3.1.2 Ultrasound Data

The ultrasound videos were taken using an ultrasound device by Esaote Mylab. The probe was placed on the thickest part of the muscle in the sagittal plane, parallel to the course of muscle fibers. The probe was fixed to the subjects calve, and the MVC program was performed with MARES. In this program, the device guides the subject to contract the muscle as strongly as possible.

3.2 Data Evaluation

3.2.1 Choice of Evaluation Tool

After data was collected in the form of ultrasound videos, the aim was to derive specific parameters from the videos. Interesting factors are, for example, the change in length of the fascicles or the pennation angle of the muscle. Extracting this data used to be a time-consuming procedure in muscle mechanics research. Because of this, many teams worked on automated or semi-automated solutions often available to use as open source [28, 29, 30, 31].

3.2.1.1 PointTracking algorithm

The first tested tool was an algorithm developed by Drazan et al. [28] from the University of Pennsylvania, for simplicity after this referred to as PointTracking algorithm.
This automatic fascicle tracking tool uses the Kanade-Lucas-Tomasi feature tracking

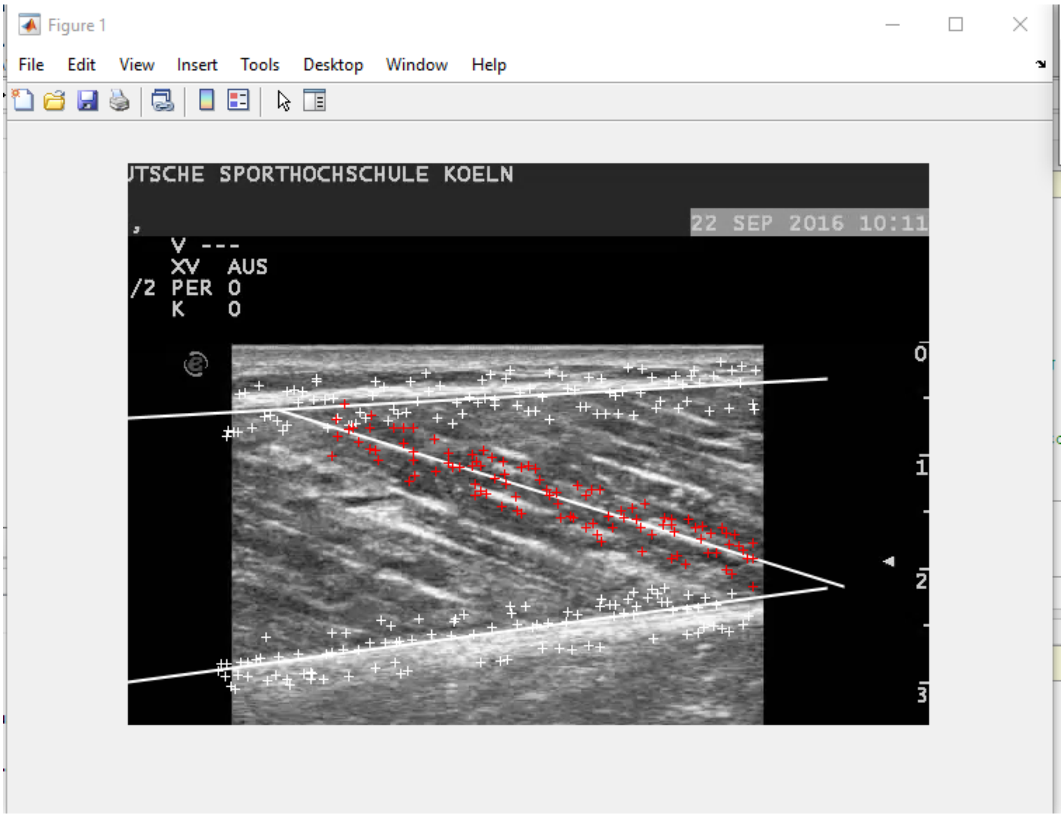


Fig. 3–2: Confirmation of aponeuroses and fascicle

algorithm implemented in MATLAB using the Computer Vision Toolbox. A study by the developing team described it as strongly repeatable and reproducible. [28]
It is supposed to work as follows: The user registers its video that has been converted from DICOM to mp4. With running the main script, the program opens the first frame of the video and asks the examiner to define the three features tracked later on: Deep aponeurosis, upper aponeurosis, and one of the visible fascicles. The algorithm then draws a rectangular region of interest (ROI) around each feature and seeds it with a pre-defined number of points evenly distributed in the ROI. The point cloud is fitted using linear regression to obtain the orientation and position of each feature. [28]
The drawn points and lines are presented to the examiner, who is then asked to confirm the proper placement. A screenshot of the first frame after selecting features and the algorithm asking for confirmation can be seen in Figure 3–2.

Automatic feature tracking is then performed. The MATLAB pointTracker function follows the position of each seeded point through the video using the Kanade-Lucas-Tomasi (KLT) algorithm to calculate the optical flow of a region to determine the movement of an object between two frames. Point validity is reviewed by tracking the point from the current frame back to the previous frame. If the distance between backtracked and current point is greater than a pre-defined bi-directional error, the point is figured invalid as well as if the point moved out of frame. If more than 10% of the points were considered invalid, 100 new points are set in the current frame. [28]
During the entire automatic tracking process, the examiner can watch the point clouds

and structures being tracked. After completion, the examiner is asked to confirm the tracking. Fascicle length is defined as the distance between intersections of fascicle with the aponeuroses and pennation angle as the angle between deep aponeurosis and fascicle line. [28]
These values are saved to a file if the examiner confirmed a correct process.
Problems using this algorithm occurred first with registering videos. The built-in function was written to record videos in combination with the Matlab script, saving important parameters and the video during the process. As our videos were recorded beforehand, necessary parameters were missing. These parameters are normally stored in a file, that is later on needed during tracking. As it was not possible to create this file for foreign videos, a solution was found to open the videos using the parameter files from example videos which were part of the download of the PointTracking algorithm. As the parameters did not match the video, they were changed manually. This turned out to be a time consuming task. It could be managed to find parameters that worked as far that the algorithm was able to open one of our videos and start the process of the examiner selecting the features as described above.
Watching the automatic tracking it could be seen that the features were not correctly tracked over the duration of the video. An example of this false tracking of the aponeuroses can be seen in Figure 3–3, whereas a mismatching fascicle line is depicted in 3–4.
This mismatching leads to incorrect values for calculated fascicle lengths and pennation angles. Even if the right parameters could have been found in order to run the examination correctly, the process of adjusting the parameters would have needed to be repeated several times, as our videos change because of being recorded with different ultra sound devices.
This would have required time-consuming manual work without promising correct results with a high degree of certainty. For the reasons mentioned above, it was therefore decided not to use this algorithm for the evaluation.

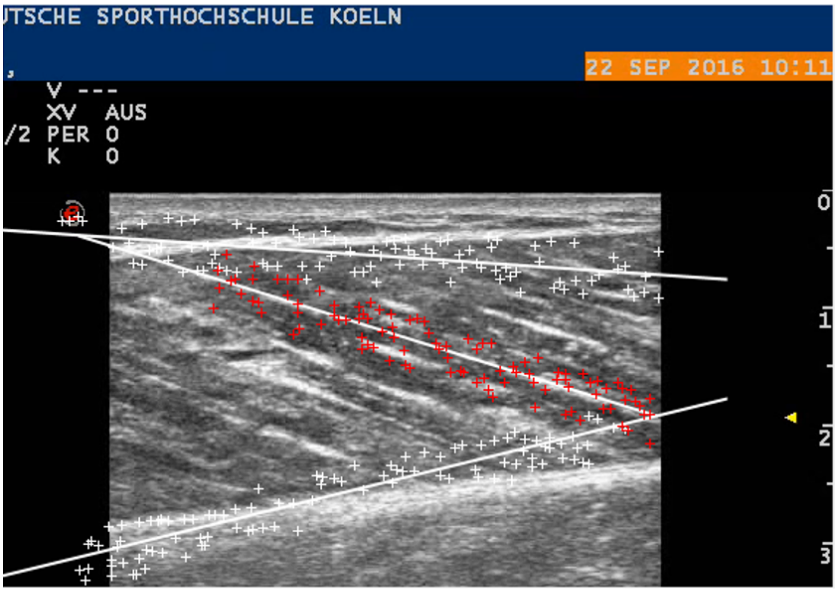


Fig. 3–3: Mismatching aponeuroses lines

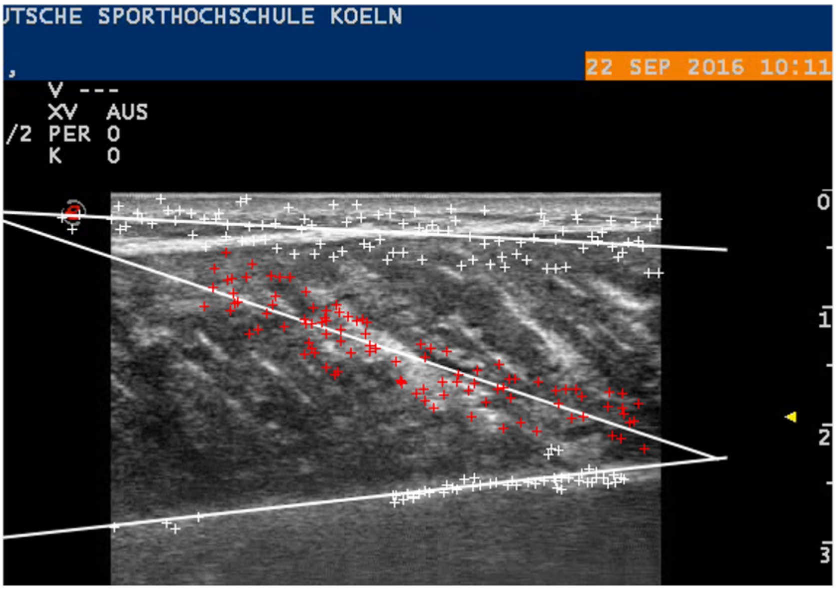


Fig. 3–4: Mismatching fascicle line

3.2.1.2 USA Tool

The tool finally used to receive the needed values such as fascicle length and pennation angle was an in-house written program called 'Ultra Sound Analysis Tool', here referred to as 'USA Tool'. This tool is less automated than the previously tested algorithm but therefore less error-prone. This tool reads a table created in advance, containing the path to the current avi video and the frames the user wants to evaluate.
For the evaluation, frame steps were chosen adapted to video length and contraction event. For example, for a video with average length, which would be approximately 11 seconds, every 20th frame was viewed outside and every 10th frame during the contraction. Frame steps were altered if videos were particularly long or short.
The tool then opens the frames according to this list and asks the user to pick two points each to draw the following lines: upper and deep aponeurosis, primary and secondary fascicle. The secondary fascicle was introduced as earlier studies often found curved fascicles during contraction instead of straight. The assumed form of a fascicle - a straight line - is not valid in this case. There fore, the option to draw a second fascicle allows the user calculate alternative pennation angles.
Also, the tool automatically calculates the scaling factor between pixels and centimeters using the scale on the right of the ultrasound image. This scaling factor is, later on, necessary to correctly calculate fascicle lengths in centimeters.
The script calculates the parameters, saves them into the results file, and loads the next frame as soon as the user confirms the correct markings. The results file contains coordinates of all points the user selected, the pennation angle at upper and lower aponeurosis, as well as the alternative bottom pennation angle for the secondary fascicle. Further on, it contains the fascicle length in pixel and centimeters and the scale factor used to convert one into the other. For better illustration, a screenshot of a marked frame in the USA tool is visible in Figure 3–5.

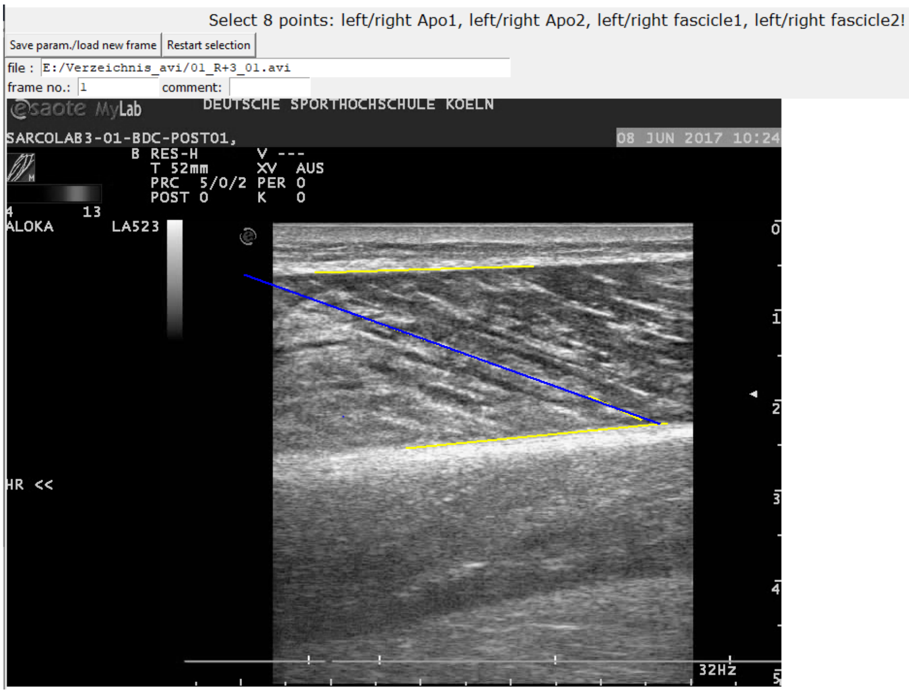


Fig. 3–5: Screenshot of USA tool after marking

3.2.2 Synchronization of MARES and ultrasound data

The following requirement was to synchronize MARES data with the data read out from the USA tool. To do this, time differences were calculated between recorded contractions in MARES data as well as time differences between beginnings of recorded videos. Since the entire procedure for a subject consists of fixed sequences, characteristic time differences can be detected.
The individual contractions were thus assigned to the videos. In both data sets, steep ascending edges can be observed after every contraction. This fact was used to accurately synchronize torque data from MARES with fascicle length or pennation angle changes from the USA tool. Torque is zero at rest and yields an average of approximately -200Nm during maximal contraction. To find this ascending edge in the torque data, a threshold was introduced. The threshold was applied as soon as torque exceeded -40Nm after contraction. For the USA tool data, fascicle length was differentiated, and the maximum of the differentiated data was sought to find the steepest point in the ascending edge. The frame corresponding to this steepest point was saved into a file as 'decontraction frame'. An example of this identification can be seen in Figure 3–6.
The mentioned file also contained further information needed for the final synchronization. For example, this information was paths to corresponding MARES data for each video, the time stamp for the specific contraction during MARES recording, and framerates of the specific video.
All this information was then used to match torque data with corresponding fascicle lengths and pennation angles, respectively.

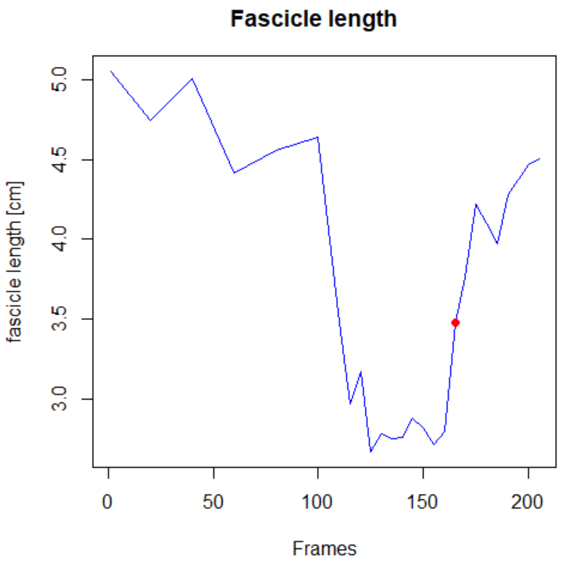


Fig. 3–6: Identifying ascending edge

3.2.3 Data selection

For various reasons, not all videos could be used in the final statistical evaluation. A representation of why and how many videos got rejected for what reasons can be found in table 3–1.

Tab. 3–1: Data rejection reasons

missing R+ Data	no contraction	synchronization failed	MARES data unclear
13	14	12	4

The first reason was missing R+ data, meaning data after the subjects return to earth. PRE data, meaning before spaceflight, was recorded for eight subjects in total as preparation. Only five of those eight subjects eventually participated in the mission later on. Therefore, data of not participating subjects were rejected due to lack of comparative data. 13 videos were declined for this reason.
The second cause was when no contraction could be seen in the video. Reasons for that are difficult to reconstruct. It could be, for example, that the subject did not follow the instructions to contract at the right time. Furthermore, it is possible that the video was started or ended falsely so that the contraction could not be recorded. It trivially explains that these 14 videos were rejected because the desired process of contraction was not recorded.
The synchronization mentioned above was not realizable for some videos if the sought characteristic time differences could not be identified with certainty. Twelve videos were therefore excluded from analyses requiring associated torque data but can still be used only regarding fascicle lengths and pennation angles.

For four videos, the MARES data did not appear as expected. This error is also challenging to reconstruct, but again, as mentioned above, data on fascicle length and pennation angle can still be used.
From 115 videos at the beginning, 42 videos were rejected for the mentioned reasons.

4 Results

This chapter presents the results of the thesis. First, examples for the synchronization are given and it is explained, how a quality criterion was introduced to filter failed synchronizations.
Further, plots containing the development of the relevant parameters over the research period are shown and the numerical values are presented in tables.

4.1 Graphical Representation of Synchronization

After the sychronization, first plots were created to graphically represent the matching torque with its corresponding angles and fascicle lengths. It could be seen that for most data sets the curves matched well like in Figure 4–1. The fascicle length de- and increases synchronously with the torque and reaches a plateau at maximal contraction with only small fluctuations.
But also curves as in Figure 4–2 were observed. Torque and fascicle length do not change synchronously and neither of the curves reaches a plateau. This indicates that the subject did not hold the maximum contraction as instructed by MARES. After the start of the maximal contraction, the fascicle length gets larger again around frame 70 even though the force increases which contradicts the expectation of fascicle shortening resulting in force generation.
Data sets like the one shown in Figure 4–2 should not contribute to the further analyses, because the unsynchronous pattern indicates an error during the recording.

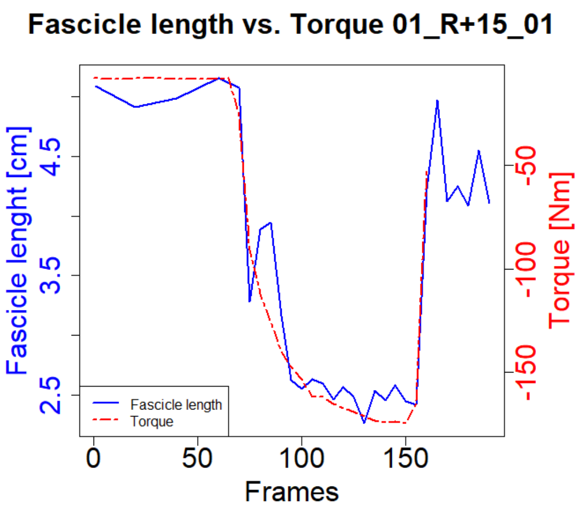


Fig. 4–1: Fascicle length vs. torque
Subj 1 - Day R+15 - TrialNo 1

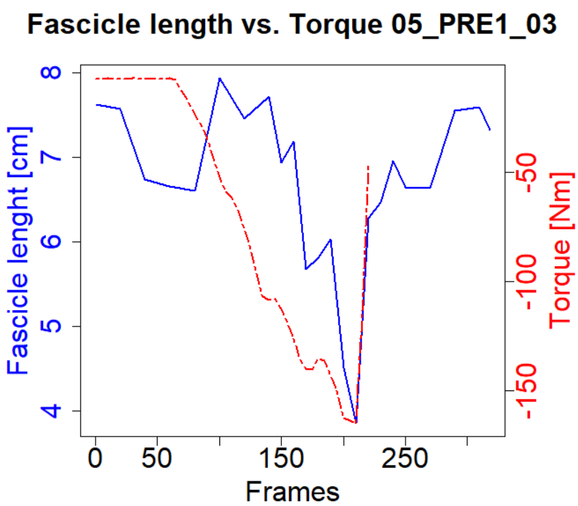


Fig. 4–2: Fascicle length vs. torque
Subj 5 - Day PRE1 - TrialNo 3

4.2 Linear Regression Analysis

A linear regression analysis was performed, in order to find an objective criterion to reduce the data set by videos, which were subjectively considered poorly recorded. Subjective criteria for rejection would be, for example, a strong deviation of the two curves or not reaching a plateau. Fascicle lengths were assigned to their respective torques and plotted into a scatter plot with torque on the x-axis and the fascicle length or pennation angle on the y-axis. Then the line was fitted, and the parameters r^2, constant, and slope were calculated.
This regression shows the linearity of correlation of force and fascicle length or pennation angle. The constant represents the angle or length at 0 Nm. The slope stands for the change corresponding to an increasing force of 1 Nm. r^2 functions as measurement for linearity of the linear model, whereas $r^2 = 1$ means perfectly linear and $r^2 = 0$ stands for non-linear behavior. r^2 was used as a quality criterion to clean the data from 'bad' plots.

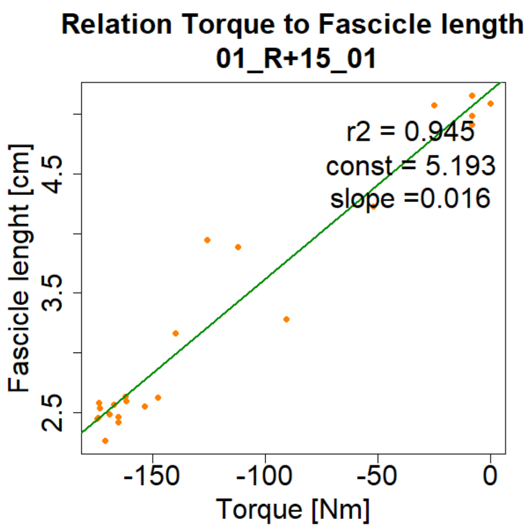


Fig. 4–3: Linear regression model Subj 1 - Day R+15 - TrialNo 1

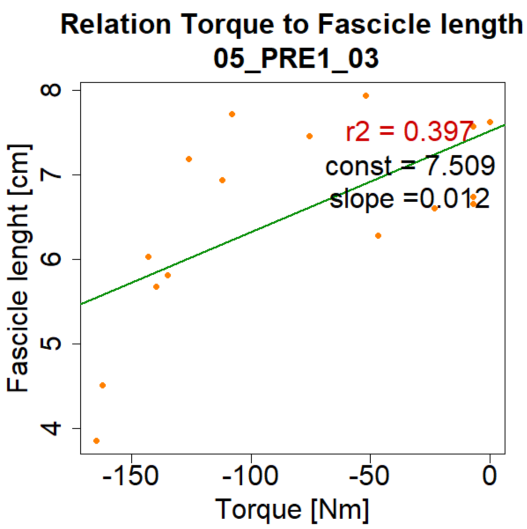


Fig. 4–4: Linear regression model Subj 5 - Day PRE1 - TrialNo 3

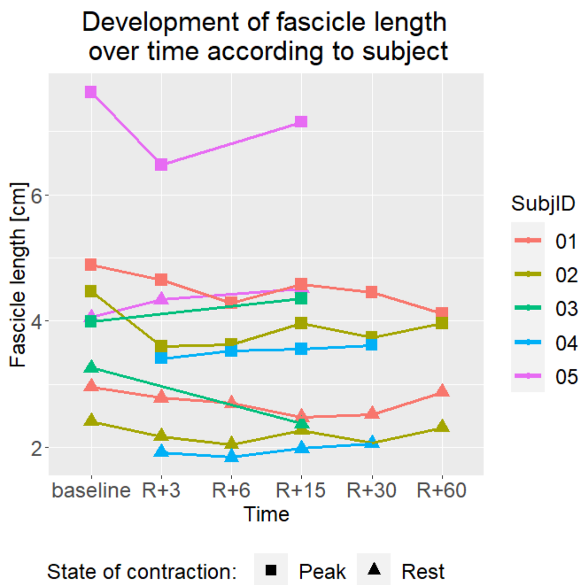
For example, for subject 01 at recording day R+15 (Figure 4–3), the fascicle length at a torque of 0 Nm was 5.193 cm, and fascicle length decreased by 0.16 cm with each Nm increasing force. The correlation of fascicle length and torque is highly linear with an r^2 of 0.945. This reinforces the impression of the plot in Figure 4–1 being well recorded data which should be used in the ongoing analyses.
In contrast to this r^2 in Figure 4–4 for subject 5 at day PRE1 only reaches $r^2 = 0.397$, suggesting non-linear correlation between torque and fascicle length. Also here the value of r^2 underlines the subjective impression of poorly recorded data from Figure 4–2.
The important part of the contraction is the maximum and building force. This is why after discussion, we decided to repeat the linear regression analysis, this time only to the end of the maximum, and cut the relaxation of the muscle.

Data were excluded if r^2 was < 0.7 in regression analysis for either fascicle length or pennation angle or both.

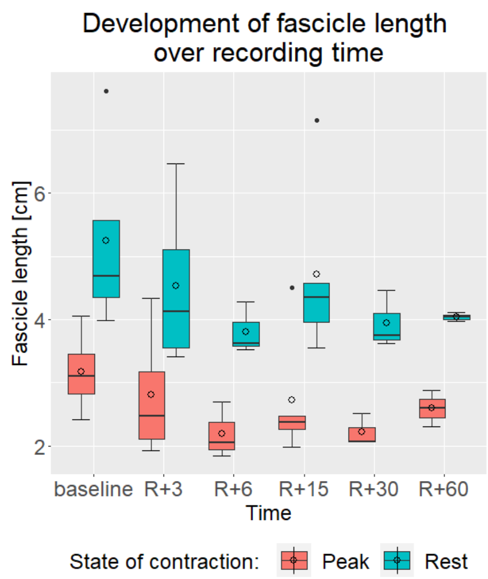
4.3 Resulting Plots

After the described data rejection, 59 videos remained for final analysis. From all this data, the mean value was determined over all trials on one day for each subject. Point line plots and boxplots were created from these averaged values, showing the development of fascicle length and pennation angles pre- and post-flight. These plots are shown and described below.
The point line-plot shows all averaged data points for each subject. The values are plotted as points and connected with a line for each subject. The triangle resembles the rest data, whereas the rectangle stands for the peak of contraction data. On the x-axis, the measurement points in time can be found; on the y-axis, the parameter's value. A specific color represents each subject.
The boxplots are to the right of each point line plot and summarize the data subjects for the specific recording day. The values are grouped into peak, standing for values at maximal contraction and rest. Peak values are the red boxes, whereas the rest values are in blue. The median is resembled by a black bar inside the box, whereas the mean is displayed as an unfilled circle.
The whiskers represent 1.5 interquartile range (IQR). If values exceed 1.5 IQR, they are visualized as outlier with a single point.

The most interesting change is between baseline and R+3, showing the difference of the specific value before and directly after space flight. Behavior in recovery between measurement days R+3 to R+60 is another crucial part looked at in the following sections.
Due to data reduction of various reasons as described in chapter 'Data selection' (chapter 3.2.3) and by the linear regression analysis, measurement points are missing for some subjects. These are days R+3, R+6, R+30, and R+60 for subject 03; baseline data and R+60 measurements for subject 04 and days R+6, R+30, and R+60 for subject 05. Data sets for subjects 01 and 02 are complete. Missing values will not be explicitly mentioned for every plot in the following descriptions.



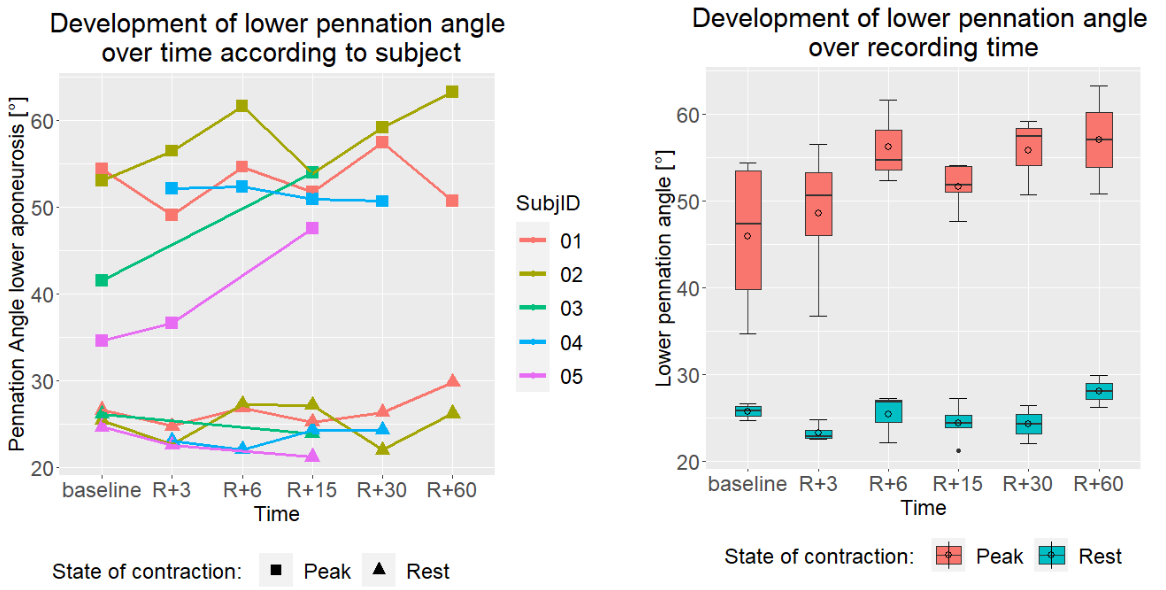
(a) Point line plot



(b) Boxplot

Fig. 4–5: Plots regarding fascicle lengths

First, fascicle lengths (4–5 (a)) at rest will be described. These can be found as points marked with a triangle. Subjects 01 and 02 show a decrease in rest fascicle length directly after space mission and have similar recovery patterns except for a decreasing rest fascicle length in subject 01 from days R+6 to R+15, whereas in this time, subject 02 experiences an increase. For subject 04, the recovery pattern is comparable to the one of 02. Subject 03 shows a similar tendency as subjects 01 and 02, with a slightly stronger expression. Subject 05 stands out first for its longer fascicle lengths compared to all other four. Also, subject 05 shows an increase in rest fascicle length from before to after space flight, contradicting patterns of subjects 01 and 02.
Contracted fascicle lengths (Peak) for subjects 01 and 02 decrease like the corresponding rest fascicle lengths. Also, here their recovery patterns are similar. Subject 03 shows a different tendency as contracted fascicle length is longer on day R+15 compared to baseline. This differs from subjects 01 and 02, where fascicle lengths also increase again between R+6 and R+15 but do not exceed baseline values. Subject 04 does not show big differences over recovery in contracted fascicle lengths just as in rest fascicle lengths. Values for subject 05 are again noticeable due to their length exceeding all others. Unlike in resting fascicle lengths, the tendencies of 05 match the patterns of subjects 01 and 02 with a decrease between baseline and R+3 and a renewed rise to R+15.
The boxplot on the right (4–5 (b)) as a summary of the point line plot shows the overall tendency of contracted as well as rest fascicle lengths. Fascicle lengths tend to decrease from before space flight to R+3 and continue the decrease until R+6. For day R+15, an increase can be seen, followed by a renewed drop in values to R+30. At the last measurement point, the values increase again but do not reach baseline equivalent.



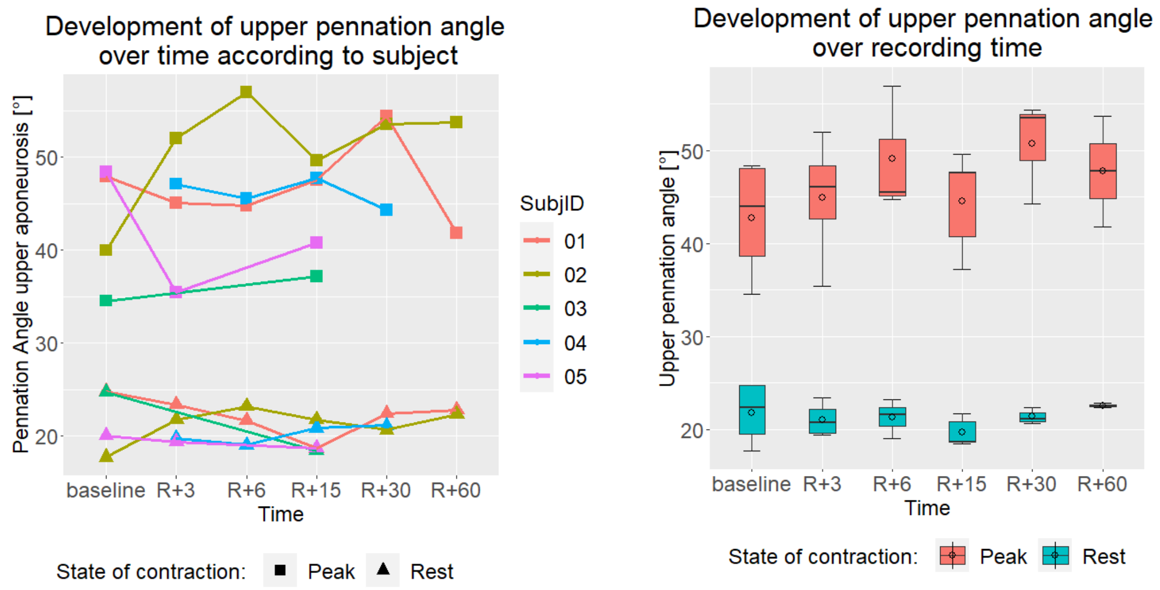
(a) Point line plot **(b) Boxplot**

Fig. 4–6: Plots regarding lower pennation angle

Starting again with the rested lower pennation angles (4–6 (a)), one can see that baseline values are very similar for all subjects, around approximately 25° to 27°. They decrease from baseline to R+3 in all subjects and continue to stay within a narrow range. At R+6 lower pennation angle continues to decrease for subject 04 but increases for subjects 01 and 02. This increase can be seen in subject 04, one measurement point later at day R+15. Value for subject 02 does not change compared to R+6, whereas they decrease for subject 01. For R+30, values of subject 04 are now invariant, whereas a decrease can be seen for subject 02 and an increase for subject 01. From R+30 to R+60, values for both subjects 01 and 02 increase and exceed their baseline value, respectively. Values for 05 behave differently as they show a straight decrease from baseline to R+15.

Contraction pennation angles at lower aponeurosis show a tendency to increase from baseline to R+3 except subject 01. For subject 01, the pennation angle decreased over the time of space flight. Besides that, subjects 01 and 02 show similar recovery patterns with de- and increase alternating. Both have similar values at day R+30, after which values for subject 02 increase to R+60 and values for 01 decrease, resulting in pennation angle of 02 exceeding its baseline value and last measured angle in 01 falling below the baseline. Subject 03 shows an increase of lower pennation angle between baseline and R+15, and subject 04 steadily decreases during recovery. Subject 05 shows smaller pennation angles than the other subjects and also a decrease from baseline to R+15.

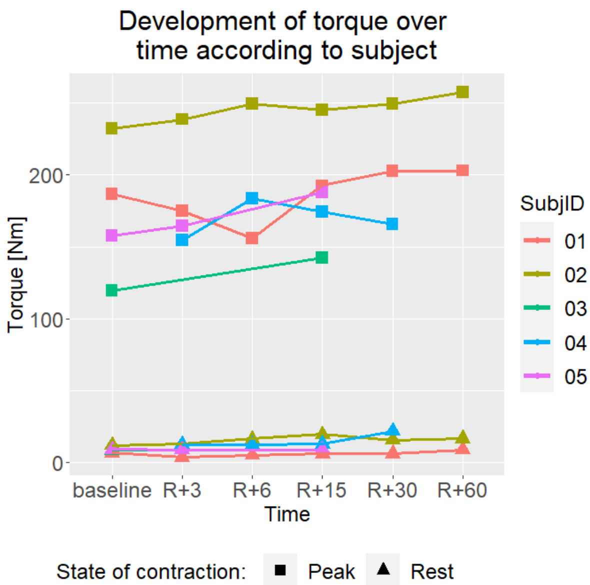
The boxplot (4–6 (b)) for the lower pennation angles shows an alternating pattern for resting pennation angles. It also shows an overall increase for contracted lower pennation angles between baseline and measurement day R+6, followed by a decrease on R+15. After that, the contracted angle increases again and exceeds the baseline value at the last measurement point.



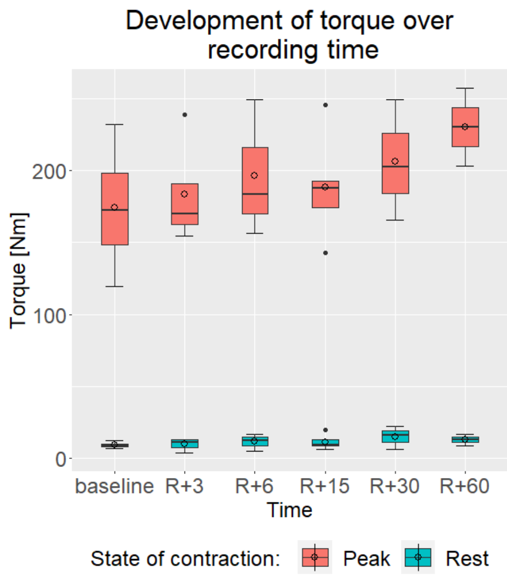
(a) Point line plot **(b) Boxplot**

Fig. 4–7: Plots regarding upper pennation angle

Rested upper pennation angles (4–7 (a)) for subjects 01 and 02 show divergent behavior. Rest pennation angles increase for subject 02 until day R+6, whereas they decrease for subject 01 until day R+15. For subject 04, a slight increase can be noticed between R+3 and R+30. Overall, no clear pattern can be observed.
This also applies to the contracted upper pennation angle. Also, here values for subject 02 increase between baseline and R+3 and exceed base values at the last measurement point. Opposite to that, subject 01 shows a decrease postflight, then strongly increases between R+15 and R+30 but closes with an undershoot of the initial value. Angles for subject 03 increase slightly between baseline and day R+15, and values for subject 05 show a drop between baseline and measurement point R+3, followed by an increase until day R+15.
The corresponding boxplot (4–7 (b)) shows an overall decrease of the rested angle of the upper aponeurosis over the time of the flight. Afterward, the values alternated between de- and increase. For contracted upper pennation angles, one can see a strong variance, especially for baseline. In summary, the angles increase between baseline and R+6, followed by a decrease on day R+15, after which the increase continues. At the last measurement point, the angles exceed their corresponding baseline values.



(a) Point line plot



(b) Boxplot

Fig. 4–8: Plots regarding torque

Resting torques (see 4–8(a)) are relatively stable close to zero, but because the foot exerts a force on the footplate by its weight, the values are not exactly zero.
For torques during contraction, the last measured values for subjects 01, 02, and 04 exceeded the corresponding baseline value. Subjects 01 and 05 show a steady increase in their points. Contrary to that, subject 01 shows a decrease in torque from baseline until day R+6 followed by an increase and an exceeded value at day R+60 compared to baseline. Torque values during contraction increase for subject 04 from day R+3 to R+6, then decrease until R+30.
The boxplot (4–8(b)) as a summary shows a slight increase in mean for torques during maximal contraction comparing baseline and R+3 data. After that, torques continue to rise until the last measured day, R+60.

4.3.1 Endpoint tables

Table 4–1 summarizes all data graphically represented by the boxplots seen above plus additional endpoints such as angle and fascicle length range of motions and differences between upper and lower angles. Range of motion was calculated as the difference between rested and contracted values. Each row shows one specific parameter, whereas the columns represent the different recording days.
The values in this table were calculated as described in equations 4–1 to 4–3.

$$\bar{x}_{d,i} = \frac{1}{n_{d,i}} \sum_{k=1}^{n_{d,i}} x_{d,i,k} \qquad n_{d,i} = \text{number of trials on day d for subject i} \tag{4–1}$$

$$\bar{x}_{d} = \frac{1}{m} \sum_{i=1}^{m} \bar{x}_{d,i} \qquad m = \text{number of subjects} \tag{4–2}$$

$$b_{inter} = \frac{1}{2}\left(\bar{x}_{PRE1} + \bar{x}_{PRE2}\right) \tag{4–3}$$

where

$\bar{x}_{d,i}$ = mean value over all trials on recording day d for subject i
d = recording day $\in$ [PRE1, PRE2, R+3, R+6, R+15, R+30, R+60]
i = subject ID $\in$ [01, 02, 03, 04, 05]
$\bar{x}_{d}$ = mean value over all trials on recording day d
b_{inter} = inter-subject baseline

First, the average over all recorded trials on one day d for one subject i were calculated (eq. 4–1). These values were then averaged over all subjects (eq. 4–2). Baseline values are here calculated as the mean of both PRE measurements. (eq. 4–3)
It has to be noted that for calculations, the mean was used instead of the median. This is why the values correspond to the unfilled circles in the boxplots and not the bars. Additionally, standard deviations are given for each mean value.
Range of motion is here defined as the difference between angles at rest and maximal contraction.
Differences between upper and lower angles can appear if aponeuroses are not parallel or if fascicles are not straight.

Tab. 4–1: Endpoint table - total mean values

	unit	PRE1	PRE2	R+3	R+6	R+15	R+30	R+60	baseline
Fascicle length at rest	$[cm]$	5.6 ± 1.8	5.3 ± 1.6	4.5 ± 1.4	3.8 ± 0.4	4.7 ± 1.4	3.9 ± 0.5	4.0 ± 0.1	5.4
Fascicle length at max	$[cm]$	3.2 ± 0.7	3.2 ± 0.9	2.8 ± 1.1	2.2 ± 0.4	2.7 ± 1.0	2.2 ± 0.3	2.6 ± 0.4	3.2
Lower pennation angle at rest	$[^\circ]$	26.9 ± 0.4	24.8 ± 1.8	23.3 ± 1.0	25.4 ± 2.9	24.4 ± 2.2	24.3 ± 2.2	28.1 ± 2.6	25.8
Lower pennation angle at max	$[^\circ]$	49.2 ± 10.4	44.6 ± 9.8	48.6 ± 8.5	56.2 ± 4.8	51.7 ± 2.6	55.8 ± 4.5	57.0 ± 8.9	46.9
Upper pennation angle at rest	$[^\circ]$	21.9 ± 4.0	21.1 ± 3.7	21.1 ± 1.9	21.3 ± 2.1	19.7 ± 1.5	21.4 ± 0.9	22.6 ± 0.3	21.5
Upper pennation angle at max	$[^\circ]$	45.6 ± 6.0	42.6 ± 6.2	44.9 ± 6.9	49.1 ± 6.8	44.6 ± 5.3	50.7 ± 5.6	47.8 ± 8.4	44.1
Torque at rest	$[Nm]$	-1.2 ± 2.5	8.7 ± 3.2	-9.7 ± 4.3	-11.5 ± 5.8	-11.4 ± 5.2	-14.7 ± 7.9	-12.9 ± 5.5	-9.4
Torque at max	$[Nm]$	-211.6 ± 19.9	-159.3 ± 52.2	-183.1 ± 37.8	-196.1 ± 47.8	-188.4 ± 37.2	-205.9 ± 42.0	-230.1 ± 38.5	-185.4
Range of motion fascicle lengths	$[cm]$	2.4	2.1	1.7	1.6	2.0	1.7	1.4	2.2
Range of motion lower pennation angle	$[^\circ]$	22.2	19.8	25.3	30.8	27.3	31.5	29.0	21.0
Range of motion upper pennation angle	$[^\circ]$	23.7	21.6	23.8	27.8	24.9	29.3	25.2	22.6
Difference upper and lower angle at rest	$[^\circ]$	5.1	3.7	2.2	4.1	4.74	2.9	5.5	4.4
Difference upper and lower angle at max	$[^\circ]$	3.6	2.0	3.7	7.1	7.1	5.0	9.2	2.8

Table 4–3 shows changes compared to baseline. The values $\bar{x}_{d,i}$ for each day d and subject i were calculated the same way as for table 4–1. (equations 4–1 and 4–2). The baseline was calculated for each subject, then the intra-subject percentual change, which was then averaged over all values. This results in more reliable values, as a subject-specific baseline is used.

$$\bar{x}_{PRE1,i} = \frac{1}{n}\sum_{k=1}^{n} \bar{x}_{PRE1,i,k} \qquad n = \text{number of trials on PRE1} \tag{4–4}$$

$$\bar{x}_{PRE2,i} = \frac{1}{n}\sum_{k=1}^{n} \bar{x}_{PRE2,i,k} \qquad n = \text{number of trials on PRE2} \tag{4–5}$$

$$b_{intra,i} = \frac{1}{2}\left(\bar{x}_{PRE1,i} + \bar{x}_{PRE2,i}\right) \tag{4–6}$$

$$\delta_i = \bar{x}_{d,i} - b_{intra,i} \tag{4–7}$$

$$r_i = \frac{\delta_i}{b_{intra,i}} \tag{4–8}$$

$$\bar{r} = \frac{1}{m}\sum_{i=1}^{m} r_i \qquad m = \text{number of subjects} \tag{4–9}$$

where:

$\bar{x}_{PRE,i}$ = values for subject i, averaged per recording day PRE1 or PRE2
$b_{intra,i}$ = baseline for subject i
δ_i = difference of post-flight and baseline value for subject i
x_{POSTi} = values after spaceflight for subject i, averaged per recording day
r_i = relation of difference to baseline for subject i
$\bar{r}$ = averaged relative value for all subjects

First, for each subject i the mean over all trials on days PRE1 and PRE2 was calculated. (equations 4–4 and 4–5) Then the subject specific baseline $b_{intra,i}$ was calculated as mean between values for PRE1 and PRE2. (eq. 4–6) Next, the baseline for subject i was subtracted from the value on post-flight day d (eq. 4–7). This difference was set in relation to baseline for fascicle lengths and strength. (eq. 4–8) Angles were kept as difference without applying equation (4–8), as their differences are more informative than the relative value. In a final step, the relative values r_i were averaged over all subjects (eq. 4–9).

The baseline $b_{intra,i}$ used in table 4–3 can be found in table 4–2 as average over all subjects.

Tab. 4–2: Baseline values with standard deviation

	baseline	unit
Fascicle length at rest	5.7 ± 1.8	$[cm]$
Fascicle length at max	3.0 ± 0.7	$[cm]$
Lower pennation angle at rest	26.5 ± 1.0	$[°]$
Lower pennation angle at max	49.7 ± 7.3	$[°]$
Upper pennation angle at rest	20.9 ± 3.6	$[°]$
Upper pennation angle at max	47.0 ± 6.3	$[°]$
Torque at max	-214.8 ± 19.5	$[Nm]$
Range of motion fascicle lengths	2.7 ± 1.1	$[cm]$
Range of motion lower pennation angle	23.2 ± 8.1	$[°]$
Range of motion upper pennation angle	26.1 ± 5.4	$[°]$
Difference upper and lower pennation angle at rest	5.6 ± 3.3	$[°]$
Difference upper and lower pennation angle at max	2.7 ± 12.4	$[°]$

Tab. 4–3: Endpoint table - relative mean values

	unit	R+3	R+6	R+15	R+30	R+60	Type of calculation
Fascicle length at rest	[%]	-13.6 ± 8.1	-15.4 ± 4.9	-8.2 ± 2.7	-12.3 ± 5.5	-13.4 ± 3.1	Relative difference to baseline
Fascicle length at max	[%]	-0.2 ± 12.9	-11.4 ± 5.1	0.6 ± 17.8	-14.0 ± 0.5	-2.9 ± 1.7	Relative difference to baseline
Lower pennation angle at rest	[°]	-3.1 ± 1.6	1.1 ± 1.0	-1.9 ± 4.0	-1.8 ± 2.3	2.1 ± 1.8	Total difference in relation to baseline
Lower pennation angle at max	[°]	-2.3 ± 4.9	4.2 ± 6.1	1.4 ± 4.6	4.4 ± 2.3	3.1 ± 10.0	Total difference in relation to baseline
Upper pennation angle at rest	[°]	0.6 ± 3.0	1.2 ± 6.1	-1.2 ± 5.1	0.3 ± 3.8	1.3 ± 4.7	Total difference in relation to baseline
Upper pennation angle at max	[°]	-2.8 ± 14.5	6.6 ± 14.7	-1.0 ± 10.6	9.7 ± 5.5	3.5 ± 14.5	Total difference in relation to baseline
Torque at max	[%]	-10.4 ± 14.6	-5.2 ± 17.9	-2.9 ± 11.4	7.2 ± 0.6	8.9 ± 2.9	Relative difference to baseline
Range of motion fascicle lengths	[%]	-26.9 ± 21.7	-20.8 ± 3.7	-14.8 ± 21.7	-9.5 ± 12.6	-28.0 ± 11.9	Relative difference to baseline
Range of motion lower pennation angle	[°]	0.8 ± 5.0	3.1 ± 5.0	3.3 ± 8.0	6.2 ± 4.7	1.1 ± 11.8	Total difference in relation to baseline
Range of motion upper pennation angle	[°]	-5.9 ± 3.4	-2.4 ± 8.7	-5.0 ± 3.5	-5.3 ± 3.1	-0.4 ± 4.5	Total difference in relation to baseline
Difference upper and lower pennation angle at rest	[°]	3.7 ± 3.2	0.1 ± 5.1	0.7 ± 5.0	2.0 ± 6.1	-0.7 ± 6.4	Total difference in relation to baseline
Difference upper and lower pennation angle at max	[°]	-0.5 ± 10.7	2.4 ± 8.7	-2.4 ± 13.8	5.3 ± 3.1	0.4 ± 4.5	Total difference in relation to baseline

Fascicle lengths shortened compared to baseline, what can be seen at the negative values. Lengths continued shortening after landing until day R+6.
Lower pennation angles at rest fluctuate around the baseline. At maximal contraction they show a decrease for day R+3 but an increase for all following days.
Upper pennation angles at rest show a slight increase for most days. For upper pennation angle during contraction no statement can be made, as they fluctuate around baseline.
The lower pennation angles show lower standard deviations than upper angles.
Torques at maximal contraction decreased by 10% compared to baseline, recover until day R+15 and exceed the baseline value on days R+30 and R+60.
The fascicle range of motion decreased by almost 27 %, recovers until day R+30 but drops again at day R+60.
Range of motion for the upper pennation angle decreased, but increased for the lower pennation angle.

5 Discussion

In this chapter, the results from the previous chapter will be critically discussed and put in relation to previous findings of research. First, the main findings are discussed, followed by the validity of some of these findings. Limitations of the methods are stated, and Conclusion and Future Work summarize which achievements were made in this thesis and what improvements could be made for future research.

5.1 Main findings

5.1.1 Synchronization

The first finding was that the synchronization did not lead to matching curves of fascicle lengths or pennation angles and torque for some videos. Reasons for the curves not matching to this extent can be various. Problems could have been with the synchronization method itself, with the USA tool's evaluation or could also have their origin already during recording. For example, it could be that the subject was able to keep up the applied force while relaxing the targeted muscle, compensating with other muscles. This could result in spontaneous fascicle length increase in the *gastrocnemius medialis*. Another possibility is a discontinuity in the ultrasound video recording. For example, the ultrasound probe could have been moved between specific frames. Even small changes in the set-up angle of the ultrasound probe on the skin can cause severe changes in the recorded parameters like fascicle length even though the actual lengths did not change.

5.1.2 Boxplots

It needs to be noted that all boxplots have to be interpreted with caution as missing values partly lead to changes in the boxes not visualizing real changes in parameters. This is why all boxplots should always be seen in combination with the corresponding point line plots. Only this way, real changes can be distinguished from apparent changes.

5.1.3 Changes in fascicle lengths

Changes for fascicle lengths in the plots and table 4–1 behave quite similar over time during rest and maximal contraction if curves for each subject are compared. The overall tendency of a decrease in length comparing pre and post-flight at day R+3 corresponds to the results from previous studies [14, 22, 23, 19, 24, 20], mentioned in chapter **??**
Also the values from table 4–3 show an overall decrease in fascicle length, in rested as well as in the contracted muscle.
It also can be seen that in most subjects, a recovery of fascicle length can be noted in form of an increase after the decrease. It seems like the effect of weightlessness on fascicle length continues past landing and reduced at day R+15.

However fascicle length changes must be seen critically as the positioning of the ultrasound probe can have a strong impact on the resulting value. Because of the 2D properties of ultrasound, the seen fascicle is only a projection of the real object. A slight inclination of the displayed plane can thus lead to changes of the measured length.
Values for subject 05 stand out from the rest. Fascicles for this subject are longer than for the others, at rest as well as during maximal contraction. It is also noticeable that it is the only subject for which fascicles during rest elongated comparing pre and post-flight values. This contradicts findings among other subjects as well as findings from previous studies. Evaluation of subject 05 will be discussed separately later on.

5.1.4 Changes in pennation angles

In order to produce more comparable insights, more importance should be attributed to the lower angle, as most studies discussed in the Background chapter only look at the angle at the deep aponeurosis. Also, the accuracy of the angle strongly depends on the evaluation tool. For example, if the fascicle during evaluation is drawn close to the lower aponeurosis, the lower calculated angle represents reality, whereas the upper can show deviations as fascicles tend to be not a perfectly straight line. During the usage of USA tool for this thesis, fascicles were drawn closer to the lower aponeurosis, which makes lower angles here more reliable than upper angles.
For the upper pennation angle at rest as well as for maximal contraction, no clear tendencies can be seen neither in table 4–1 nor in table 4–3. This could be because the values for the upper pennation angle are not as reliable due to the choice of placement of the drawn fascicle.
For lower pennation angles in table 4–1, one must distinguish between angles at rest and angles at maximal contraction since it is difficult to make uniform statements otherwise. The lower pennation angle at rest shows a decrease from pre to post-flight in all recorded subjects. This finding corresponds to those of earlier studies looking at pennation angles in relation to muscle atrophy. After day R+3, recovery patterns differ across subjects. However, they show that most subjects' rested angle recovers over the time of recording.
For lower pennation angle during maximal contraction, four of five subjects show an increase between baseline and a certain recovery day with exception of subject 01. This finding contradicts findings from earlier studies but has to be seen critically as measurement points for certain days are missing for some subjects.
From table 4–3 no clear tendency can be seen neither for rested nor for contracted pennation angles. Angles at rest seems to have slightly increased but keep close to zero.

5.1.5 Changes in plantar flexor strength

Torques at rest are not of big interest as they should be zero.
The only subject having contracted values in table 4–1 that match with values from earlier studies is subject 01. Subject 01 shows a decrease in strength on days R+3 and R+6 compared to baseline. In contrast, subjects 02, 03, and 05 do not show the expected decrease in strength after space flight. Also, subject 01 shows that its strength

exceeds its baseline value by the time of day R+60. It has to be noted that subject 02 reported pain in its foot during baseline recordings. This could be an explanation for his increase in strength after spaceflight. However also subjects 03 and 05 show a similar increase in strength.
Values from table 4–3 calculated with the subject-specific baseline show a different tendency. Values from this table are more coherent with findings from earlier studies as they report a decrease in strength compared to baseline for days R+3 to R+15. After that, values again exceed baseline values, matching the results from table 4–1. Because of subject-specific calculation of the baseline for table 4–3, these values should carry more weight than values from table 4–1.
A reason for increasing strength could be strict training after space flight, compensating effects of weightlessness. However, no data was available on the exact workload of the training, so that no firm statements can be made on this.

5.1.6 Range of motion pennation angles

As seen in table 4–3, the range of motion of the lower pennation angle increased, whereas it decreased for the upper pennation angle. A possible reason for this may be the measurement method, which outputs more accurate values for the lower angle as discussed in chapter 5.1.4. Another possibility could be the difference between the lower and upper aponeurosis. The lower aponeurosis is connected with the underlying soleus muscle, while the upper one is the last connective tissue under the skin. This could lead to different behavior of the connective tissue and thus to changes in the range of motion.

5.1.7 Standard deviations

The standard deviations must be interpreted critically, as they are subject to the number of values used. This varies as not for all days, values for all subjects were available. The standard deviations are therefore higher on the days where more subjects could be included, for example R+3 and R+15. Nevertheless, statements can be made for some values. For example, the larger standard deviations for angles during contraction for the upper angles compared to the lower angles during contraction in table 4–3. This fact underlines the assumption that the measurement method with the USA Tool is less accurate for upper angles than for lower ones.

5.2 Validity

5.2.1 Subject 05

Values for subject 05 stand out compared to the others, especially for its longer fascicle lengths and smaller pennation angles in the contracted state. It should be noted, that from the videos it is subjectively noticeable that this subject has a thicker *gastrocnemius medialis* than others. Also, it is the only subject showing slightly curved fascicles during contraction, which may have led to deviations in its values. In addition, the quality of the images for subject 05 does not match that of the others, as more prominent blood vessels are repeatedly seen. This fact makes it more challenging to draw in the correct fascicles. It was decided to evaluate the videos nevertheless as best as possible.

5.2.2 Pennation angles at rest vs. pennation angles during contraction

In summary, it must be discussed whether the pennation angles during contraction do not depend more on the daily form of the subject than on influences of the space flight. The contraction itself might underlie various factors such as calorie intake, sleep cycle, and also the motivation to build up as much strength as possible provided by the research team.
A counter-argument to the theory that the deflection of the angle under maximum contraction is subject to some fluctuations is that the torque curves hardly show the same fluctuations. This could lead to the assumption that the changes over time in upper angles might result from the data evaluation with the USA tool, as discussed above. (chapter 5.1.4)

5.3 Limitations

5.3.1 Data situation

A limitation to the data situation of this thesis is first the sample size. With only five subjects, it is not possible to obtain significant data. Still, it should be noted that a study with astronauts as subjects has the problem of not being able to easily recruit more participants, as experiments on the ISS require a great deal of lead time and planning.
Another limitation is the missing data, for example, for later follow-up days such as day R+30 or R+60. This, in combination with small sample size, leads to incomplete data after evaluation.

5.3.2 Evaluation tool

Another crucial point is the quality of the evaluation of the parameters. As already described above, the evaluation with a manual tool such as the USA tool is strongly dependent on the user's input. For this reason, it would make sense to use automated evaluations to increase reproducibility.
However, automated solutions need to take into account that ultrasound recordings are made using different devices, different ultrasound probes, different frame rates and depths. A universal tool should be independent of the exact parameters of an ultrasound device and it should be possible to insert the boundary conditions defined by the device and probe used.

5.3.3 Synchronization

Also, the method of synchronization chosen here can be responsible for deviating results. It is a time-consuming and error-prone process to calculate and match the time difference patterns of both data series.

5.3.4 Force measurement

It is also to discuss if the method of measuring strength using the ergometer MARES is the most reliable. The excecution of a maximum voluntary contraction strongly depends on various factors, as already discussed above. (chapter 5.2.2) It also is hard to reproduce for the subject on one specific day due to fatigue of the muscle. Another point is the learnability of the process. It could be that the more measurements were made, the better the subjects understood how exactly to contract their *gastrocnemius medialis*. This could also lead to the result of plantar flexor strength increasing over the time of recovery.

5.4 Conclusion and Future Work

5.4.1 Conclusion

Concluding one can say, that ultrasound recordings of muscle mechanics before and after space flight show some challenges in their evaluation, especially if contraction is considered as well. These challenges include:

- 2D properties of ultrasound images and associated distortions
- reproducible, but efficient evaluation of the relevant parameters
- synchronization of ultrasound recording and corresponding data
- reproducible strength measurement
- small sample size due to astronauts as participants

5.4.2 Future Work

A solution for problems with the 2D properties of ultrasound images could be to take 3D images instead. This would cancel out problems such as fascicles only being projections of the actual object. Having 3D images, measured fascicle lengths and pennation angles would depict values close to reality. A possibility for 3D images would be magnetic resonance imaging (MRI). Disadvantage of this technology is the long duration of recording the image. This results in recordings during contraction not being possible. However it could be a solution for fascicle lengths and pennation angles at rest.
Also 3D ultrasound solutions have been developed for example by Weide et al. [32]. This team constructs 3D images from 2D B-mode images which are positioned into a voxel array. The image is taken by a standard ultrasound probe combined with a cluster marker to track position and orientation of the US probe. [32]
Technologies like this could be an alternative to MRI but recordings still have to be taken at the resting muscle.
Regarding a reproducible but also efficient evaluation of parameters, algorithms provide various advantages. They are able to select aponeuroses and fascicles under objective aspects and therefore provide highly reproducible results. Disadvantages were seen during evaluation of this thesis, for example in problems with varying video formats or fascicle directions. For the future, algorithms would have to be developed

that are less sensitive to parameters such as video format or size.
For an unambiguous synchronization, unique identifiers of a point in time would be needed in all relevant data series. This would make it possible to synchronize the data precisely.
In order to produce more reproducible measurements of strength, an alternative to the ergometer MARES could be used. For example a vertical jump test could be conceivable. Different variants exist for the exact execution of this test. In general the take-off power of the test person is determined and standardized via his body weight. A disadvantage of this test is the difficulty of taking ultrasound videos during the jump because of its short duration and also because of the forces acting on the ultrasound probe. Also this test is currently not executable during weightlessness. However, devices such as exoskeletons are under development to make such implementations possible in the future.

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