Diplomarbeit

MOLECULAR MECHANISMS OF MUSCLE LOSS DURING BEDREST

eingereicht von

Tristan Jaskolla

Geb.Dat.: 27.10.1985

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Institut für Physiologie

unter der Anleitung von Betreuer

Prof. Dr. Nandu Goswami

Graz, 26.06.2014

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 26.06.2014

Table Of Contents

List Of Abbreviations

HSD11B1	Homo sapiens hydroxysteroid (11-beta) dehydrogenase 1
CNTF	Homo sapiens ciliary neurotrophic factor
TRIM63	Homo sapiens tripartite motif containing 63
CAV3	Homo sapiens caveolin 3
HSPB3	Homo sapiens heat shock 27kDa protein 3
KCNH2	Homo sapiens potassium voltage-gated channel member 2
SBF2	Homo sapiens SET binding factor 2
NOS1	Homo sapiens nitric oxide synthase 1
GDAP1	Homo sapiens ganglioside-induced differentiation-associated protein 1
ESA	European Space Agency
MEDES	Institute for Space Medicine and Physiology
SD	Standard Deviation
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
P	Phosphorus
Na	Sodium
K	Potassium
Ca	Calcium
Mg	Magnesium
CO	Cardiac output
SV	Stroke volume
HFr	Frequency of the heart
EDV	End diastolic volume
ESV	End systolic volume
MHC	Myosin heavy chain
MLC	Myosin light chain
DNA	Deoxyribonucleic acid
cDNA	Complementary deoxyribonucleic acid
RNA	Ribonucleic acid
cRNA	Coding ribonucleic acid
VO2	Maximal oxygen consumption
EKG	Electrocardiogram

List Of Figures

Abstract In German

Einleitung: Wie allgemein bekannt führt Immobilisation in Folge von Bettruhe zu schwerem Muskelabbau. Die genauen Ursachen hierfür sind jedoch noch nicht verstanden. Die vorliegende Diplomarbeit bietet einen Überblick über die Muskelphysiologie, Molekularbiologie und die physiologischen Grundlagen der Immobilisation. Die Daten, die eine Studie der ESA über Bettruhe lieferte, werden einer gründlichen Auswertung unterworfen, wobei die molekularen Mechanismen die zu muskulärer Atrophie und (Dys-) Funktion beitragen besonders berücksichtigt werden. Die Ergebnisse dieser Studie sind anwendbar im Bereich der Immobilisation, der Geriatrie sowie der Kardiologie.

Zielsetzungen: Diese Diplomarbeit untersucht die Auswirkungen von Immobilisation in Folge von Bettruhe auf die Muskelfunktion, besonders in Hinblick auf die molekularen Mechanismen die bei dem dadurch ausgelösten Muskelverlust beteiligt sind. Wir untersuchten die Hypothese, dass die *Funktion und Leistungsfähigkeit des Muskels durch die Bewegungseinschränkung bei Bettruhe beeinträchtigt werden und sich dies in den beobachteten molekularen Veränderungen widerspiegelt.*

Methodologie: Die Studie über Bettruhe wurde in MEDES, Toulouse durchgeführt. Die Studie, bei der die Probanden mit um 6 Grad gesenktem Kopfende in Bettruhe gehalten wurden dauerte 21 Tage, hatte 12 Teilnehmer mit einem durchschnittlichen Alter von 34 ± 8 Jahren, einer durchschnittlichen Größe von 1.76 ± 6 cm und einem durchschnittlichen Gewicht von 69.8 ± 8 kg (Durchschnitt ± SD). Die Auswirkungen der Immobilisation bei Bettruhe auf die Expression von Genen, die für die Skelettmuskulatur relevant sind, wurden untersucht, indem die vor und nach Bettruhe abgenommenen Blutproben verglichen wurden.

Ergebnisse: Bei 9 für die Skelettmuskulatur relevanten Genen wurde eine Änderung der Expression mit einem Fold Change von ≥ +1.5 oder ≤ -1.5 gefunden. Im Vergleich zu den Werten vor der Bettruhe war nach der Bettruhe die Expression der Gene HSD11B1, CNTF, TRIM63, CAV3, und HSPB3 hochreguliert und die Expression der Gene KCNH2, SBF2, NOS1, und GDAP1 herunterreguliert. Die Änderungen in der Expression wurden

daraufhin in Hinblick auf die existierende Literatur über Muskelfunktionsänderungen während Immobilisation durch Bettruhe überprüft.

Schlussfolgerungen: Die in der Studie beobachteten Änderungen der Genexpression, die der Muskelfunktion zugeordnet werden können, sind vereinbar mit dem während Bettruhe beobachteten Muskelverlust. Den zeitlichen Verlauf dieser Expressionsänderungen zu untersuchen könnte in Zukunft dabei helfen, Gegenmaßnahmen zu entwickeln, die zum richtigen Zeitpunkt eingesetzt den Muskelverlust verhindern könnten, noch bevor dessen Folgen klinisch sichtbar werden.

Abstract

Introduction: Bedrest immobilization is known to lead to severe muscle loss. Though bedridden persons have significant muscle loss, it is not yet known why this occurs. This dissertation provides an overview of muscle physiology, molecular biology and immobilization physiology. An in-depth examination of the data obtained during a bedrest campaign of the ESA will be done, with special emphasis on the molecular mechanisms that contribute to muscle atrophy and (dys-) function. The results of this work will be applicable in the areas of immobilization, geriatrics as well as cardiology.

Aims and objectives: This dissertation examines the effects of bedrest immobilization on muscle function, with regards to the molecular mechanisms that are involved in muscle loss during bedrest. We tested the hypothesis that *muscle function and strength will be affected by the bedrest immobilization and that this will be reflected in the molecular changes that were observed*.

Methodology: Bedrest immobilization was carried out at MEDES, Toulouse. The 6-degree head down bedrest of 21-days duration was performed on 12 male volunteers with an average age of 34 ± 8 years, an average height of 1.76 ± 6 cm and an average weight of 69.8 ± 8 kg (mean ± SD). The effects of bedrest immobilization on gene expression relevant for skeletal muscle function were examined by comparing pre- and post-bedrest immobilization blood samples.

Results: The expression of 9 genes involved in muscle physiology was found to have changed with a fold change ≥ +1.5 or ≤ -1.5. In comparison to pre-bedrest, post-bedrest gene expression of HSD11B1, CNTF, TRIM63, CAV3, and HSPB3 was up regulated while the gene expression of KCNH2, SBF2, NOS1, and GDAP1 was down regulated. These changes were then examined in relation to what is known in the literature regarding muscle function during bedrest immobilization.

Conclusions: The results of gene expression related to muscle function are consistent with muscle loss that occurs during immobilization. In the future, examining the time course of these changes may allow us to develop countermeasures at appropriate times, even before the muscle loss can be seen physiologically.

I Introduction

In this work I will focus on the current knowledge regarding the physiological basis of muscle function. As immobilization may lead to dizziness upon standing up, the role of the muscle pump, which is primarily responsible for post-immobilization orthostatic intolerance will also be explored.

The aim of the current dissertation is to provide an update of existing literature related to muscle function, bedrest immobilization and to how bedrest leads to deterioration in muscle structure, muscle properties and function.

I.1 Muscle Physiology

Due to their mass allotment of around 40% of the body's mass, muscles are the major tissue of the human body (1). All movements that relate to larger cellular compounds have their origin in the activity of muscles. This chapter mainly shall explain the muscle's macroscopic and microscopic components and structure (due to their relevance for understanding the muscle's physiology), and the physiology of muscular contraction.

I.1.1 Muscle Types

There are three distinct groups of muscles: The skeletal muscle, the cardiac muscle (which both are called striated muscles) and the smooth muscle. "Striated" and "smooth" derives from their microscopic structure.

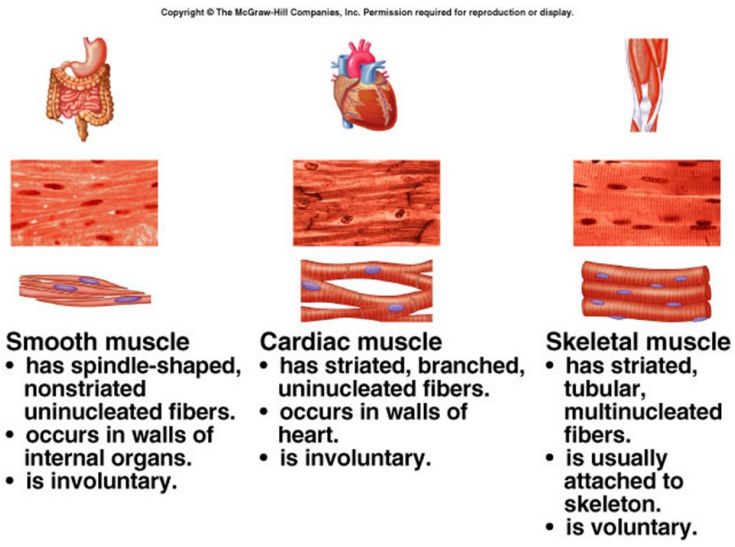

Figure 1: The different types of muscle tissue and their main characteristics. This figure was obtained from http://faculty.pasadena.edu/dkwon/chapt_11/images/image3.png

All muscles that enable voluntary movements are summarized as skeletal muscles - although most but not all of them have skeletal origins or insertions. (For example, the muscles within the tongue have no direct contact to bones). They are also called striated skeletal muscles and add up to around 400 muscles. They are linked to the motoric parts of the brain by α Moto neurons.

Except for the muscles in the heart, all involuntary muscles belong to the group of smooth muscles. They can be found in most of the human organs, for example in the walls of the gastrointestinal tract, the urogenital system, the respiratory tract, the blood vessels, the male and female reproduction systems.

The cardiac muscles only exist in the heart and differ both physiologically and histologically from the two other muscular types. Also, their mechanisms of regulation, activation and deactivation are different and perfectly match the needs of the body's blood pump (2).

I.1.2 The Types Of Striated Muscle

The skeletal muscle fibres can be divided into two different types. The first type comprises "slow twitch" fibres (Type I). They move in a relatively slow way, but they are most resistant against fatigue. Their metabolism is oxidative, therefore they have more mitochondria and much myoglobin. Also, they have a less active lactate dehydrogenase. Their colour is red.

The second type consists of "fast twitch" fibres (Type II), which can be divided into a Type IIa (fast resistant) and a type IIb (fast fatigue). Type IIb is the fastest, but easiest type to exhaust. It has a glycolytic metabolism, few mitochondria and myoglobin (and is of white colour), but a highly active lactate dehydrogenase. Type IIa has a mixed metabolism and ranks between Type I and IIb, regarding mitochondria, myoglobin, colour and the activity of its lactate dehydrogenase (3).

I.1.3 Extracellular Structure Of The Striated Muscle

The skeletal muscle has a quite orderly structure. This is made clear when starting macroscopically and then going into the microscopic detail more and more, as shown in the figure below.

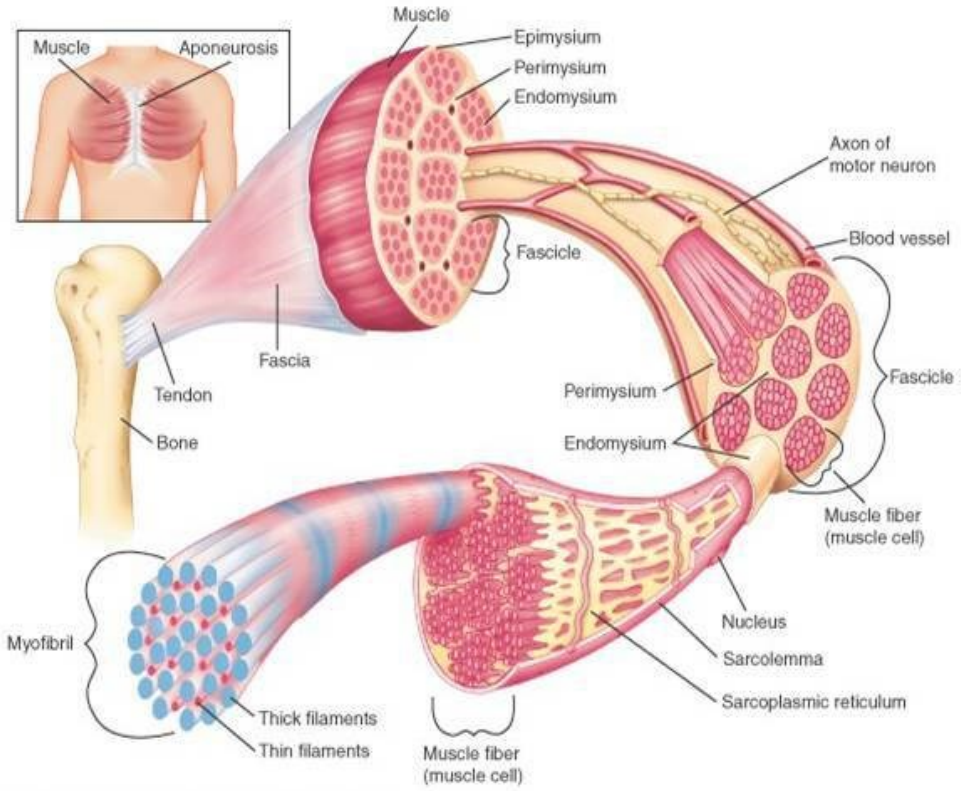

Figure 2: A staged insight into the anatomical composition of a striated muscle. This figure was obtained from http://thesportsscienceundergrad.files.wordpress.com/2012/10/skeletal_muscle_structure.jpg?w=577&h=480

Normally the muscle is completely surrounded by a fascia, a sheath of tight connective tissue, which either ends in the tendon thus connecting to the skeleton or directly arises out of its bone. It is not an active part of the muscle but transfers the forces generated by the muscles to the bones. Underneath there is the epimysium, a loose connective tissue conjoining the fascia and the inlying muscle, which is departed by the perimysium (originating from the epimysium) into several smaller segments, called the “fascicles”. The perimysium also contains blood vessels nourishing the muscle and neural axons, sending action potentials to the cells causing contraction or relaxation. The fascicle itself is a bundle of muscle fibres, which are divided and surrounded by endomysium just as the fascicles are covered by perimysium and which also contains small vessels. Muscle fibres (also called myofibers, which are long cellular tubes of diameters of about 40 to 80 micrometres and of several centimetres in length) are the genuine cells of the muscle (4).

I.1.4 The Striated Muscle Cell

The muscle fibres arise early in development by fusing of large numbers of progenitor cells called the myoblasts. Among others, they contain the remaining cell nuclei of the myoblasts embedded in a continuous cytoplasm, called the sarcoplasm. The cell nuclei are displaced laterally, near the cellular membrane, called the sarcolemma. The myofibrils are essential and are described below. The sarcoplasm also contains large sets of mitochondria, which provide the cell's energy by creating adenosine triphosphate (ATP), which is needed for contraction; and it contains the sarcoplasmic reticulum, a huge network which is a modification of the ordinary smooth endoplasmic reticulum and stores calcium (2).

I.1.5 Intracellular Structure Of The Striated Muscle

Microscopically, the myofibrils are sarcomeres attached in rows. A sarcomere consists of two lateral actin filament bundles and one myosin filament bundle in the middle. In the area where actin and myosin overlap, they are arranged in hexagons when viewed in cross section: each myosin is surrounded by six actin filaments. The myofibril structure appears as lines and bands (causing the skeletal muscle to look striated - microscopically), exactly as in the Figure below.

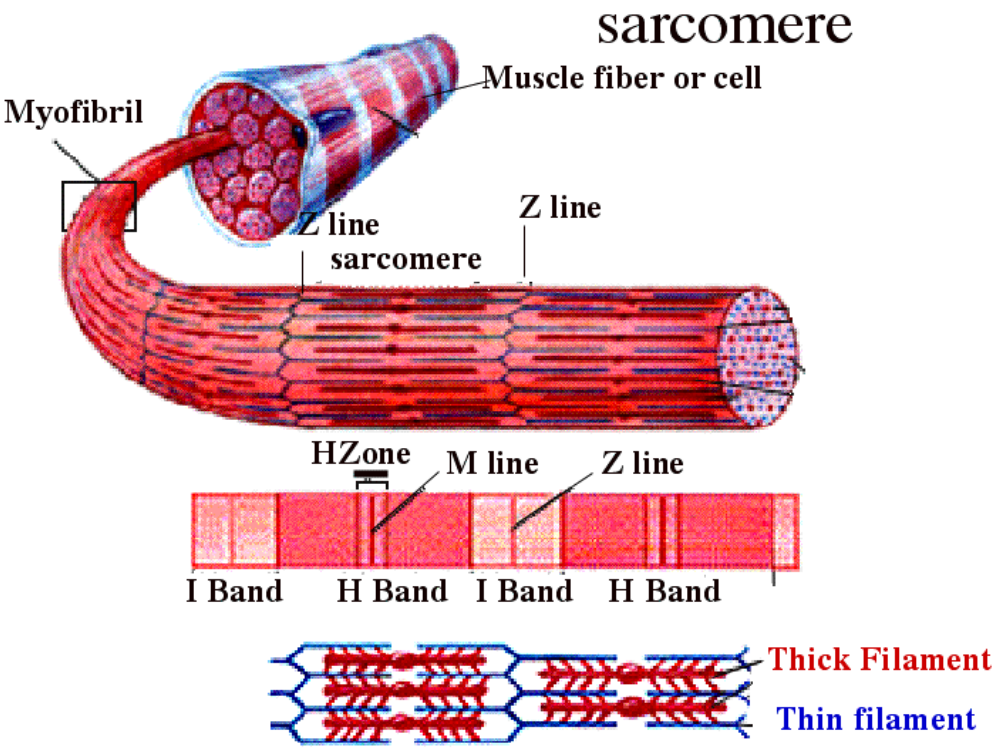

Figure 3: The microscopic appearance and composition of a striated muscle's sarcomere

This figure was obtained from http://www.ucl.ac.uk/~sjjgsca/MuscleSarcomere.gif

The striation is mainly created by three complexes: the thin filament, the thick filament and the Z disc. The Z lines (also called Z discs) are the junctions between the sarcomeres. Attached to the Z discs are the (+) ends of actin filaments (also called the thin filaments due to their small diameter), which create the I bands (I for isotropic) next to the Z lines. The actin filaments are directed towards the middle of the sarcomere. Here, their ends overlap with the ends of the myosin filaments, which are located in the centre of the sarcomere (also called the thick filaments due to their large diameter).
The myosin containing part in the middle of the sarcomere is called the A band (A for anisotropic).
The area in which the myosin is free of actin filaments is called the H zone.
In the middle of the sarcomere is the M line, where the adjacent myosin filaments are grounded (4)(5)(3)(2)(6).

I.1.6 Physiology Of The Contraction

When an electrical impulse reaches the muscular cell, this causes contraction in the sarcomeres by an electromechanical pathway. Due to its function, this system is called the *electromechanical coupling of the muscular cell*.

When activated, actin and myosin repeatedly interact always in the same manner. They glide into each other and thus shorten the myofibril. This is called the *cycle of contraction*.

The Electromechanical Coupling:

When an action potential arrives at *the cellular membrane*, acetylcholine is released from neuromuscular junctions (connected to the central nervous system) and is flooding to the membrane of the muscular cell. There, it activates nicotinic acetylcholine receptors (6), which open and mainly allow Na+ (sodium) ions to pass through, which flood into the cell and depolarize the membrane. This depolarisation is conveyed along the sarcolemma by voltage sensitive Na+ and K+ channels, similar to the expansion of depolarisations in neurons. In regular intervals, the membrane folds itself into the sarcoplasm, building transversal tubes, called T-Tubules. Being a part of the membrane they also depolarize. They extend to the surface of the myofibrils abutting upon the end parts of the sarcoplasmic reticulum (called the terminal cisterna of the sarcoplasmic reticulum). Their histological arrangement can be seen in the Figure below.

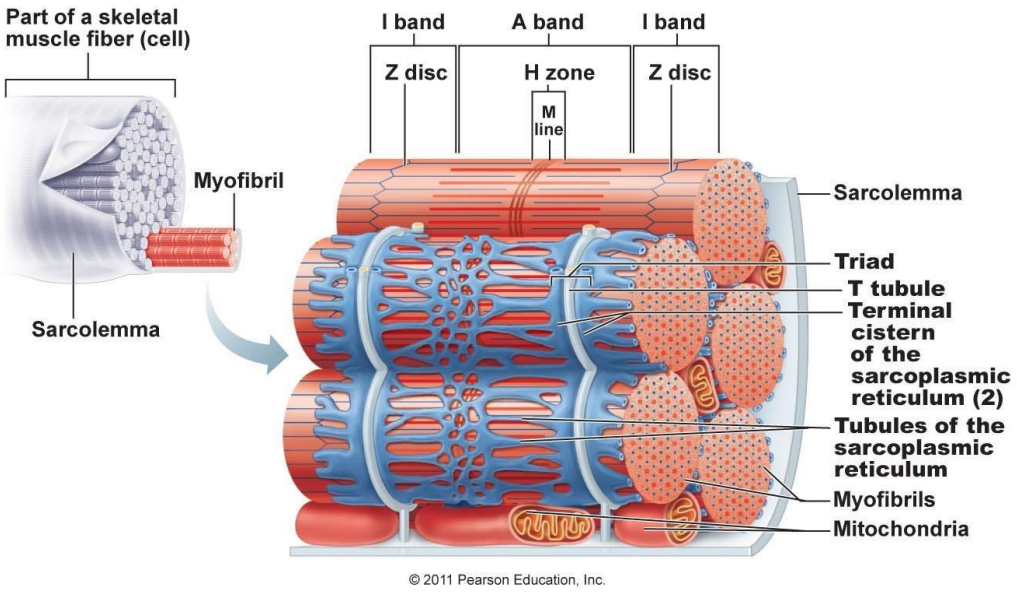

Figure 4: An insight into one single muscle fibre: Here can be seen, how the sarcoplasmic reticulum, the myofibrils and the mitochondria are attached together. This figure was obtained from http://classconnection.s3.amazonaws.com/811/flashcards/141811/jpg/sr1320422142082.jpg

There, the membrane of the T-tubules contain a voltage sensitive Ca2+ (calcium) channel, called the “dihydropyridine receptor” and the membrane of the sarcoplasmic reticulum contains a Ca2+ channel, which is called the “ryanodine receptor”(5). This type of ryanodine receptor is specific for skeletal muscles and is large enough to bypass the gap between the sarcoplasmic reticule and the T-tubules. Because of that, the dihydropyridine receptor, when activated by a depolarisation, likewise becomes permeable for Ca2+ ions and immediately activates the ryanodine receptor, which also opens for Ca2+. Because of the large quantity of calcium in the sarcoplasmic reticulum (flooding into the sarcoplasm), the concentration of Ca2+ ions in the sarcoplasm increases by the factor 100.
The calcium is flooding to the myofibrils and thus enables the cycle of contraction (2).

The Cycle Of Contraction

The contraction of the skeletal muscle is caused by the shortening of its sarcomeres, which itself is caused by the interaction between actin and myosin. Each non contracted

sarcomere in the striated muscle has a length of approximately 2.2 micrometres. During contraction, it is shortened up to about 70 percent (3).

The arrangement of actin and myosin was already explained in chapter I.1.5. The following figure gives a review:

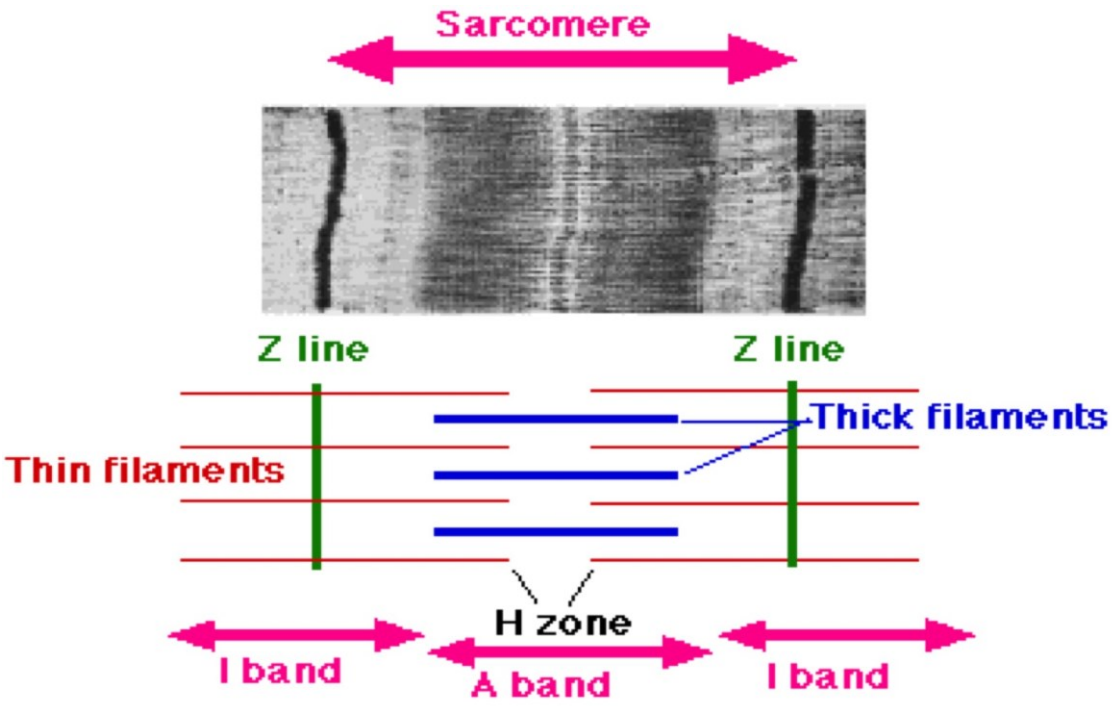

Figure 5: The thick filaments (containing myosin, here in blue) are located in the middle between two bunches of thin filaments (the actin filaments, here in red). In interaction with adenosine triphosphate (ATP) and calcium, the myosin filaments actively slide into the empty spaces between the actin filaments and, like that, shorten the whole actin – myosin complex. This figure was obtained from http://image.sciencenet.cn/olddata/kexue.com.cn/photo/upload/bigimg/20101113112311786.png

The interaction of actin and myosin can be divided into several steps, as can be seen in the Figure below. All steps together are called the contraction cycle:

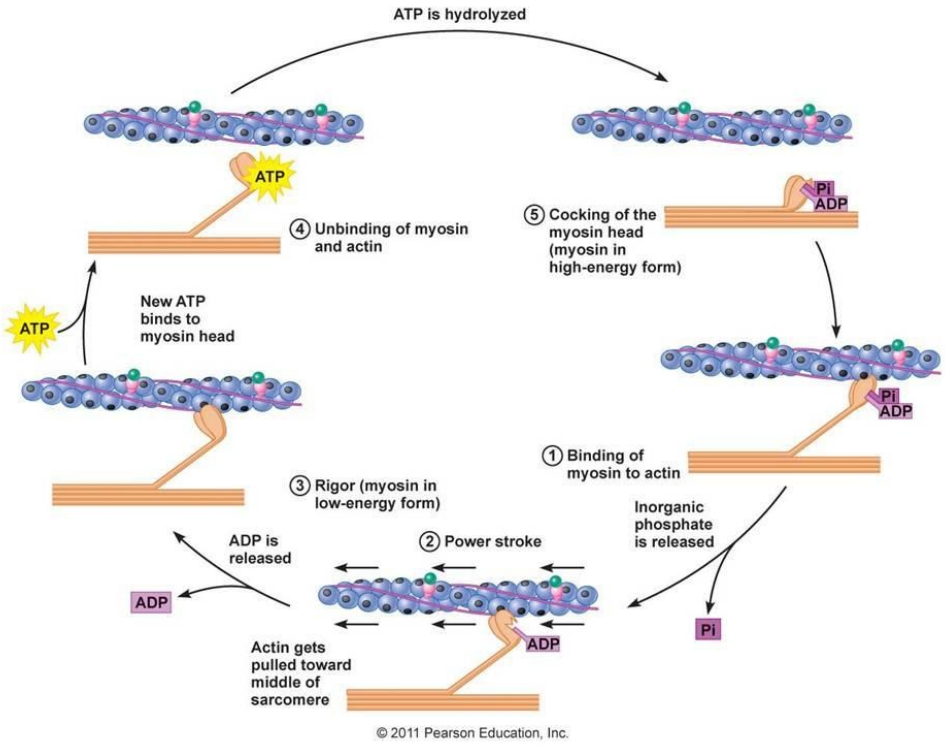

Figure 6: The principle of the cycle of contraction: The tense myosin head (holding ADP and P) attaches to actin [1] Then the myosin head tilts and thereby pulls itself towards the Z-line, releasing P and ADP [2] Myosin is still bound to actin [3] until another ATP connects to the head, which unbinds actin and myosin [4] The hydrolysis of ATP to ADP and P supplies enough energy to the head to tilt it again into its tense position (which stores the energy of the ATP). This figure was obtained from http://classconnection.s3.amazonaws.com/548/flashcards/1531548/jpg/cc1336593783725.jpg

The myosin binding sites of actin that show a high affinity to myosin are not accessible to the myosin head in an inactive myofibril. Actin is covered by tropomyosin. It is blocking the myosin head, which therefore is not able to attach to its binding site on actin. Myosin only can acquire a binding of low affinity to actin. Tropomyosin is connected to three more globular molecules called troponin T, troponin I and troponin C.

To start of the contraction cycle calcium ions are necessary. They are flooding into the actin myosin area from the sarcoplasmic reticulum during the process of electromechanical coupling. The Ca binds to Troponin C. Thereby the Troponin/Tropomyosin complex is moving away from the highly attractive myosin binding sites, so that the myosin head can now bind there (2).

The myosin head is able to work as an ATPase, but not without additional help. At first, it has to bind one molecule ATP. To do this a Mg2+ (magnesium) ion.is necessary (6). In the energetic ground state the myosin binding head and the tail of the actin molecule form an angle of 45°.When Ca2+ binds to the Troponin/Tropomyosin complex, the myosin head is able to split ATP to ADP and P (phosphorus). By the energy released by this process, the myosin head switches to an excited state, a configuration showing an angle of 90° with its tail and the energy obtained from ATP is stored in this tense position of the head. Now, the myosin head binds to the low-affinity myosin binding site, and afterwards to the high-affinity myosin binding site (which is accessible for myosin when tropomyosin shifted away under the influence of Ca2+) (2).

Now, the myosin head releases the phosphorus and tilts back to an angle of 50° with its tail, using the prior stored power from the split ATP. This step is also called the "power stroke". Next the head releases the remaining ADP and tilts a little bit more, to an angle of 45°. These two strokes physically shove the whole myosin molecule towards the Z line of the thin filaments.

The connection between the myosin head and actin remains stable, until another ATP binds to the myosin head, which causes myosin to set actin free again. The circle of contraction can restart (7).

Stopping the contraction

When calcium is released into the sarcoplasm, it is constantly transferred back into the sarcoplasmic reticulum by active ATP dependent Ca2+ transporters, called the Ca ATPase (3)(8). Using one ATP molecule they can transport 2 Ca2+ ions into the sarcoplasmic reticulum. Furthermore Na/ Ca antiporters in the sarcolemma transport Ca out into the extracellular space.

So the calcium concentration lowers as soon as the amount of calcium shifted out by these transporters surpasses the quantity released by electromechanical coupling. This makes more and more calcium getting free from the troponin C molecules and hence the contraction will stop. This happens when the calcium concentration in the sarcoplasm gets less than 10^-7 mol/l (2).

Providing Energy and O2

Mitochondria and special substances as creatine kinase are providing energy, and myoglobin provides O2. They are described in chapter I.1.8 "Muscle related proteins".

I.1.7 Muscle Pump

The muscle pump primarily is responsible for post-immobilization orthostatic intolerance. When standing upright, a certain amount of blood floods into the vessels of the legs and fills them, causing less blood supply for the rest of the body. When the muscles of the lower limbs contract, they compress the vessels of the lower limbs, pressing the blood back up again. The following explains in more detail in which way orthostatic intolerance is caused and how it is prevented by the muscle pump:

Standing up means severe stress for the cardiovascular system of the human body and is a harder challenge than one thinks of at a first glance. When standing upright, the heart has to provide enough blood to all organs, especially to the brain. To do so it must generate a certain output (cardiac output) (9). This output of the heart is generated as follows:

Cardiac output (CO) = stroke volume (SV) times frequency of the heart (HFr):

$$CO = SV * HFr$$

The stroke volume itself can be calculated by the formula:

SV = end diastolic volume (EDV) minus end systolic volume (ESV) (10)

$$SV = EDV – ESV$$

Since the end systolic volume cannot become infinitely small, the end diastolic volume has to be high enough for securing a sufficient stroke volume.

This is also described by the Frank-Starling-Mechanism: A higher EDV generates a higher SV of the heart. The EDV itself is dependent on the preload, that is the end diastolic pressure of the blood on the heart chamber, which is contingent upon the amount of blood flooding back to the heart (6). If the preload is not high enough, the SV decreases; to maintain a sufficient CO, the heart will raise its HFr for a while. *If all this is not sufficient, the patient will collapse.*

A high preload requires a high backflow of blood to the heart.

In a horizontal position the heart and the rest of the body are at the same level, and a high backflow is not problematic, because the blood simply can flow back.

When standing up, the blood will follow gravity flow into the lower limbs and therefore has to be transported up to the heart again to preserve the preload. If this is not possible within seconds, the patient will collapse - this is described by the term ***orthostatic intolerance****.*

In the lower limbs this can be prevented by contraction of the venous and/or arterial vessels, and the muscle pump. But the effect of vasoconstriction in the legs on the blood volume gushing into them seems to be small (11).

The muscle pump of the lower limbs is also called the musculovenous pump and supports the backflow of blood to the heart by pressing it out of the lower limbs, as the Figure below shows.

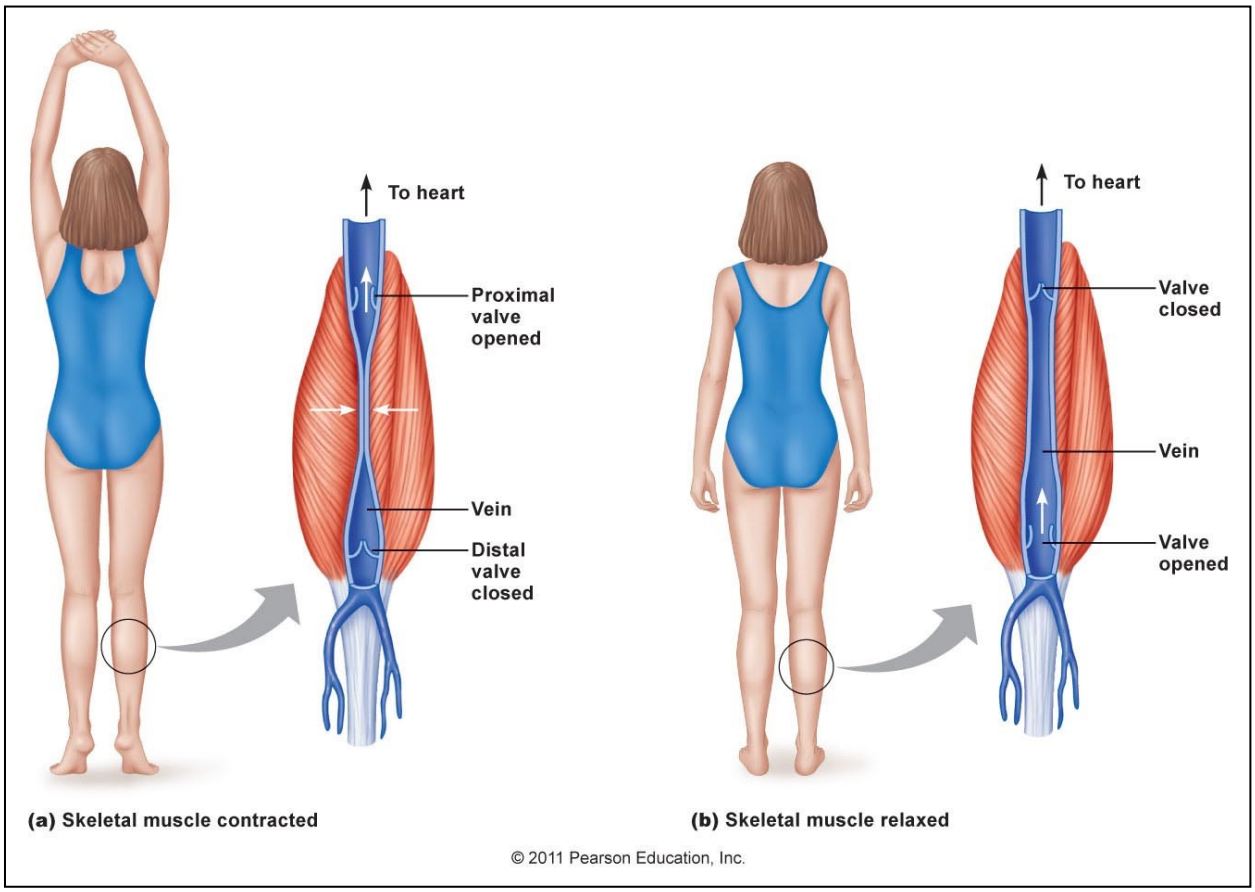

Figure 7: The principles of the muscle pump: The contracted muscle compresses the veins and presses the blood back into the system. This figure was obtained from: http://1.2.3.11/bmi/droualb.faculty.mjc.edu/Course%20Materials/Physiology%20101/Chapter%20Notes/Fall%202007/figure_14_22_labeled.jpg

The muscles of the lower limbs do not have much space to spread when they contract, because they are contained in their fascial compartments (12). Because of that, the contraction of muscles increases the pressure inside their fascial compartment (if they were not fixed in their compartments, the muscles / vessels could simply shift). Veins inside the compartment will therefore be compressed and the blood contained is squeezed out of the compartment. Due to the veins' valves that only allow a blood flow towards the heart, the ejected blood volume is transported upwards. (The pressure produced this way can even be high enough to lead to a compartment syndrome in rare cases (13))

The importance and the limits of the muscle pump

But the role of the musculovenous pump for maintaining orthostatic tolerance also has its limits:

- The amount of blood flooding into the legs when standing up is limited. Dependent on temperature, it is a matter of 400 to 650 ml blood gushing into the lower limbs (2).
- The leg's muscle pump only can support the backflow of blood in vessels located below the pelvis. The blood transport in the upper veins in the abdominal region is higher than in the lower limbs, representing about 20 to 30 of the total blood volume (14)(11). Compressing the abdominal region with elastic binding will cause a rise in blood pressure of about 15/6 mmHg syst./diast. and is the best way to counteract the lowering of blood pressure in cases of orthostatic intolerance (11)(15). This mechanism is not affected directly by the muscle pump. But maybe, it is affected indirectly: When we stand up, the muscles of our abdominal wall contract and thus raise the intra - abdominal pressure. By compressing the vascular beds, especially the vena cava inferior, and pushing the blood contained there towards the heart, this could enhance the effect of the muscle pump of the lower limbs by adding a "muscle pump of the abdomen".
- Some quantity of blood has to flow into the veins of the lower limbs and fill them for the muscle pump to be able to work. Therefore the muscle pump is ineffective during the first seconds after standing up (16).
- There are a lot of other mechanisms the body uses to maintain its cardiovascular stability. Besides the muscle pump, there are the neurovascular and neurohumoral regulation and the cerebral blood flow regulation (17).
- Standing up will activate the muscle pump and the adrenergic system, too, and thus is supposed to stabilize the blood pressure by neurohumoral activation. This might partially explain the effect of blood pressure stabilization effect of the muscle pump has. This is called in doubt by studies showing that the total peripheral resistance during muscle pump manoeuvers did not change. The manoeuvers only seemed to influence the cardiac output (18).
- There still is a scientific dispute whether the muscle pump is important for the human blood flow regulation or not (19).

> Some scientists assume a “trans mural transmission of energy from arteries to veins, i.e., the arteriovenous pump (AVP)” (18) instead of a muscle pump

Nevertheless, there are some studies showing evidence for the importance of the muscle pump for counteracting cardiovascular intolerance. Patients with orthostatic intolerance show a higher amount of blood gushing into the calf veins while standing up than healthy people do (20). For patients with blood phobia, manoeuvers that activate the muscle pump are effective in preventing cardiovascular instability (21).
Hypotension patients show severe symptoms during immobilization. By activating the muscle pump (by crossing the legs or similar exercises), they improve their cardiovascular stability (11). In tilt table tests, the blood pressure and cardiac output raised after the patients had performed muscle pump manoeuvers (18). Leg crossing increased the cardiac output and the blood pressure in patients suffering from orthostatic hypotension (while tiptoeing increased them in healthy participants) (22). “Lower limb compression garments” (that press the blood out of the lower limb, similar to the muscle pump) and leg exercises stabilized the blood pressure in orthostatic intolerant athletes (23).

I.1.8 Muscle Related Proteins

This chapter shall provide a short survey of important muscle proteins, which add up to around 20 % of the total muscle mass (1). It is not comprehensive. Especially the major regulatory proteins and the receptors are only described in form of a short summary here, because they also show up in chapter I.1.6 where their function concerning the muscular contraction is explained in more detail.
Understanding the protein’s function in the muscle is decisive for the analysis of our bedrest studies’ pre- and post bedrest data, as this data is about the quantity of RNA encoding muscle proteins. The muscle proteins can be classified (among others according to Makovický et al (24), Obinata et al (25)) :

- Contractile proteins: basically actin and myosin
- Regulatory proteins, which can be divided in
    - Major regulatory proteins: basically troponin and tropomyosin
    - Minor regulatory proteins
- Proteins of the Z disc
- Sarcoplasmic proteins
- Proteins of the sarcolemma and inner membranes

- Proteins that are present, but not specific for muscle cells: as there are collagen, elastin, lactate dehydrogenase and many others

The contractile proteins:

Actin is a protein family, whose isotypes are not only emerging in muscular cells (where it accounts for approximately half of the proteins) but also in all other cells of the human body. Their various functions include shape and stability creation in cells, intracellular conveying of vesicles and more. There are three groups of actin isoforms: the alpha actins (4 isoforms), beta and gamma actins (26). The alpha actin group can be divided into skeletal muscle specific, smooth muscle specific and cardiac muscle specific isoforms (27). Alpha-Skeletal-actin is the specific skeletal muscular actin of the contractile apparatus (28).

The actin filament itself resembles a rope made of two smaller strings wound around each other.

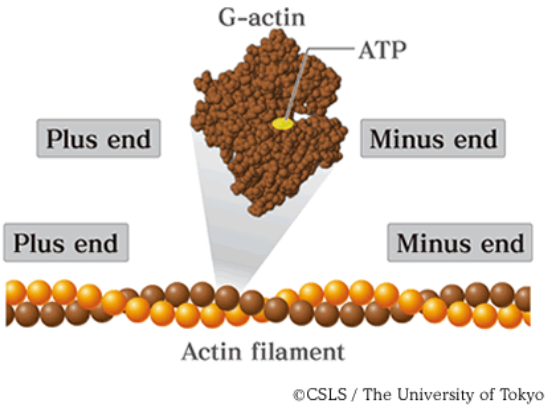

Figure 8: Actin (at the molecular level). This figure was obtained from http://csls-text.c.u-tokyo.ac.jp/images/fig/fig06_1_l.gif

It is also called F-actin and consists of a huge amount of single globular actin proteins called G-actin. The G-Actin proteins are put together in two helical compounded chains, forming the F-actin. As each of the G-actin particles has two different ends that are called the + (barbed) and the – (pointed) end, the F- actin also has two different ends. Construction and degradation of the F-actin are done by polymerisation (mainly at the + end, using ATP) and depolymerisation (mainly at the - end). The specific muscular actin filament has a diameter of 7 nanometres and a length of one micrometre. Due to its smaller diameter (compared to myosin) it is also called the “thin filament” (8)(29).

Myosin

Being able of active movements, the myosin protein is more complex than actin. Every muscular myosin protein consists of a tail, a head with ATPase activity and affinity to certain regions of the actin filament and an intermediate tilt able neck with two joints. The essential of this arrangement is shown in the Figure below.

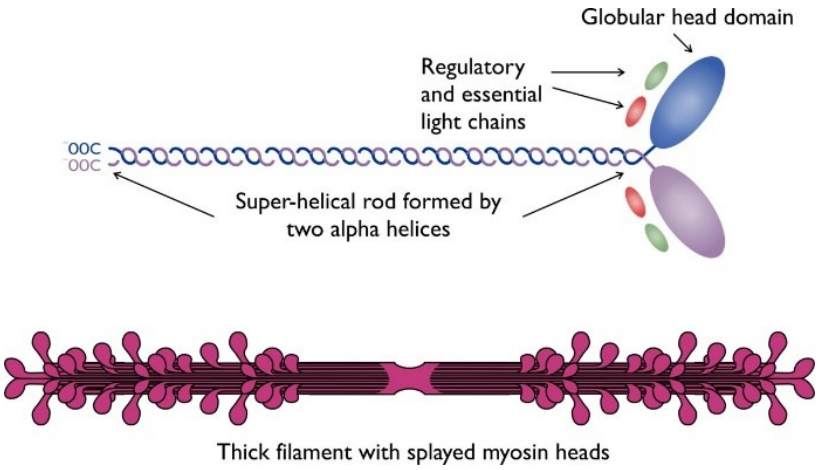

Figure 9: Myosin at the molecular level: one single molecule of myosin II ahead, one single myosin filament out of several hundred myosin molecules below. This figure was obtained from http://elte.prompt.hu/sites/default/files/tananyagok/practical_biochemistry/images/4aa9de13.jpg

Additionally each neck and head (called the MHC = myosin heavy chain) carry two myosin light chains (also called the MLC) that are called the “regulatory light chain” and the “essential light chain”. The muscular myosin filament is mainly accountable for the muscular contraction. However, it cannot contract by itself but needs a number of other proteins (as actin) to make the muscle contract and thus produce movement. It has a long stem with a diameter of approximately 15 nanometres and a length of 1.5 micrometres and is shaped laterally reversed, as it can be seen in the Figure below. Due to its wider diameter (compared to actin) it is also called the “thick filament”. The stem is formed of approximately 300 up to 400 myosin proteins that are aligned with their tails towards the middle of the myosin filament. The myosin heads jut out of in on each sides, (logically except the middle, where are only tails and no heads).

Myosin does not only appear in muscular cells. One can distinguish about 40 isoforms and three main classes. Each class seems to have a different physiological functioning. The sarcomeres of the muscle (and also some non-muscular cells) contain isotypes of the myosin type II class. 4 different MHC isoforms and 3 MLC isoforms can be found in the striated muscle. Dependent on their composition in the muscle cell, 4 major muscle fibre types and several minor "hybrid fibre types" can be divided (2)(3)(8)(30).

Major regulatory proteins

The following figure shows the principal configuration, form and location of relevant muscle proteins in the sarcomere**:**

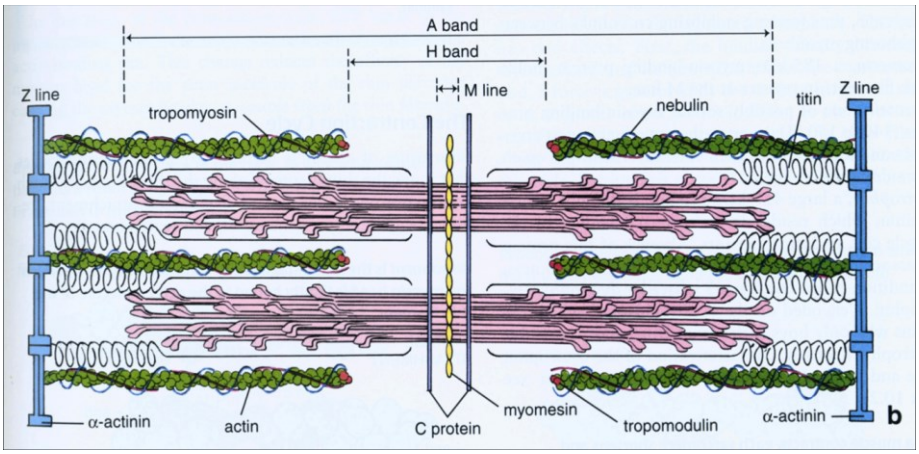

Figure 10: Different Proteins in the Sarcomere. This figure was obtained from http://classconnection.s3.amazonaws.com/117/flashcards/693117/png/untitled1316197336316.png

Tropomyosin is formed out of 2 protein chains that are wound α helical around each other. Each tropomyosin molecule adds up with one troponin complex and 7 actin monomers. Two tropomyosin strands are lying in the rims of one actin filament, following its windings. There, they are functioning as chaperons by blocking the myosin binding sites from the myosin head (2).

But it also stabilizes the actin filaments and protects them from degradation by the muscle call's ADF/cofilin-AIP1 actin disassembly system (31).

There are more than 20 known (non muscle specific and muscle specific) isoforms of tropomyosin (32). The skeletal muscle specific beta-tropomyosin derives from the same gene as the other isoforms (33).

The **Troponin** group can be divided into **Troponin T** (T stands for tropomyosin, because troponin T is the molecule connecting to tropomyosin), **Troponin I** (I stands for Inhibition, it is inhibiting the myosin head, especially the actinomyosin-ATPase) and **Troponin C** (C stands for calcium, it has calcium binding sites) (2)(34)(35).

Minor regulatory proteins:

The **Myomesin** family can be divided into myomesin 1 and 2 (which is also called **M - Protein**). These proteins can be found in the M line and mainly do bind myosin and titin (36), building a web structure and holding the myosin complex in position (resp. bring it back into position after contraction). Studies on their transcriptions factor MEF2C have shown that they are relevant for the sarcomere's structural integrity and their loss leads to sarcomere damage and in consequence is lethal (37).

C–protein, also called MyBP-C (myosin binding protein C) can be found in the A band. In adults, the slow skeletal, the fast skeletal and the cardiac isoform can be distinguished. MyBP-C binds to myosin and titin (thus stabilizing the sarcomere's structural arrangement) and provides the formation of long, compact and structured myosin filaments. Although, it does not seem to be essential (38).

Beta actin and **Gamma actin** are not specific for the striated muscle. They are part of the cytoskeleton and are essential for intracellular movements (28).

Tropomodulin is able to block the actin filament's elongation and degradation by binding to the filament's pointed ends (called "capping"). If the actin filament is free of tropomyosin, the block is incomplete. If actin and tropomyosin are present, tropomodulin also binds to tropomyosin, and the block is complete (39).

Myomesin

Several isoforms of Myomesin can be found in the sarcomere's M band of cardiac and skeletal muscles. Its extensibility contributes to the muscular elasticity (40)(41). It seems to restore the structural integrity of the M band after being stretched (42).

Proteins of the Z disc:

Actinin (alpha and beta)

Actinin alpha has several isoforms; some can be found in other cells than the striated muscle (43). The isoforms in the skeletal muscle are alpha actinin 2 and 3. When exercising, the expression of alpha actinin 2 increases, while alpha actinin 3 does not respond (44). In the striated muscle cell, alpha aktinin is the main protein of the Z line and is an antiparallel longish dimer with actin binding sites at both ends. It connects actin at its (+) ends to the Z line and helps arranging it (45).

Actinin beta mainly inhibits the growth, acceleration and interconnection of f – actin fragments and possibly binds to the pointed end of the actin filament as an ending factor (46)(47). It is also called Cap Z protein (48).

Titin also called **connectin**, is a very large (> 1 micrometre) polypeptide with many isoforms. It spans from the M line to the Z line and has a spring - like elastic region at height of the I band. It holds myosin fixed in its position in the sarcomere and creates a muscular resting tension through its elastic region (49)(50). Interacting with many other molecules, it also creates a structure in the sarcomere. Also, its importance for the active contraction and a role in stress signalling of the muscle cell is discussed (51).

Nebulin is a large, thin, inextensible filament with an α helical Structure. It interacts with Actin and Tropomyosin and destines the length of the thin filament (52)(53). But it also plays a role in the muscle contraction and the calcium homeostasis (54).

Telethonin is linked to titin in the Z line. There, it seems to fix titin to the Z disc and positions and stabilizes it. As a titin – telethonin complex, it is essential for the first phase of muscle growth (55).

Myotilin, a dimeric protein in the Z disc that crosslinks actin filaments together and without α actinin, and protects actin from degradation via Latrunculin A.
Mutations of myotilin lead to muscular dystrophy. But according to mouse models, it does not lead to changes in muscle mass, muscle fibre strength and thickness (56).
It interacts with several other proteins as α actinin, filamin A/B/C, f-actin, and FATZ (57).

FATZ 1 is also called **calsarcin 2** or **myozenin 1**. Together with myotilin, it is involved in the Z disc's assembly and stabilization (58).

Filamin exists in three isoforms, called **FLNa, FLNb** and **FLNc** (= **gamma filamin**). While FLNa and b are common in non-muscle cells, FLNc is a mainly muscle specific, Z disc associated cytoskeletal protein. Filamins are involved in crosslinking actin filaments, the adhesion of the cytoskeleton to the cellular membrane and intracellular protein and signal dispatching (59). FLNc binds to FATZ and myotilin and interacts with sarcoglycans of the dystrophin associated glycoprotein complex. It also is relevant for signalling pathways beneath the sarcolemma, interacting with the protein LL5ß (60).

ZASP interacts with actin filaments and several z disc proteins as myotilin and alpha actinin. Mutations of it lead to disruption of the Z disc and thus to a form of myopathy (61).

Sarcoplasmic proteins:

Myoglobin can be found in the sarcoplasm and is able to carry oxygen. Especially in the cardiac muscle cells and in the striated muscle cells that have to contract for a long time, the mitochondria have a high oxygen uptake. Myoglobin is a globin molecule as haemoglobin, but with a larger affinity to oxygen than haemoglobin, and a less affinity than that of the molecules in the mitochondrial respiratory chain. That is why oxygen shifts from the blood's haemoglobin to the intracellular myoglobin and then to the mitochondria, where it is used for ATP production (62)(6).

The **Myogen** group consists mainly of glycolytic enzymes (63).

Creatine Kinase

When the muscle is not able to produce the amount of ATP needed in an aerobic way, it can meet its demand by anaerobic glycolysis, but this takes some time (ca. half a minute). To avoid shortcuts, the muscle has an energy buffer, the phosphocreatine. By the muscular creatine kinase, it reversibly can be dephosphorylated to creatine. By this reaction, the phosphoric group is attached to ADP, so that ATP is created.
When the ATP shortage is over, creatine can be phosphorylated to phosphocreatine again by creatine kinase, using ATP.

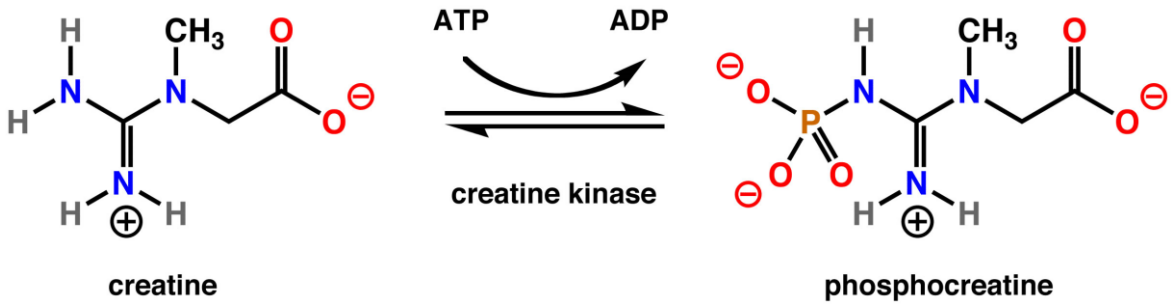

Figure 11: A chemical sketch showing the functioning of the creatine kinase together with its substrates ADP+P, ATP; creatine and phosphocreatine. This figure was obtained from http://upload.wikimedia.org/wikipedia/commons/c/c0/Creatine_kinase_rxn.png

There are several isoforms of creatine kinase in different tissues of the human body. The creatine kinase of the striated muscle is called MM-CK. There also exists a specific kinase in the mitochondria of the skeletal muscle cells, the MIb-CK (64).

Desmin, also called **skeletin** (65)**,** can be found next to the Z discs in all types of muscle cells. It is essential for the mechanic stability of the contractile apparatus as well as for muscular regeneration, muscular maturation, and the stability of the neuromuscular junctions (66). Its missing leads to skeletal and cardiac myopathy (67).

Proteins of the sarcolemma and inner membranes:

Dystrophin is existent in smooth and striated muscle cells. Lacking it causes Duchenne muscular dystrophy (68). It lies beneath the sarcolemma and stabilizes it. Increasing the sarcolemma's durability, it protects the muscle cell from rupturing during its contraction (69). Associating with dystrophin, **dystroglycans** and **sarcoglycans** form the **DAGC** (= Dystrophin associated glycoprotein complex) and link the intracellular Actin fibres and the cytoskeleton to the extracellular laminin, stabilizing the cellular membrane during the muscle's contraction (70).

The **Nicotinic Acetylcholine Receptor** is a protein bedded into the sarcolemma at the neuromuscular junction and is part of the "ligand-gated ion-channel superfamily". It has a variety of subtypes, deriving from different isotypes of their 5 trans-membrane subunits. There are also nicotinic Ach Receptors in neurons, epithelium, macrophages and other cells (71). The muscular nicotinic Ach Receptor consists of the subunits α1,α1,β1,γ,δ (72).

The **Dihydropyridine Receptor** is an L-type calcium channel consisting of 5 subunits called α1, α2, β, γ, δ. The α1 subunit only exists in skeletal muscle, and interacts with the ryanodine receptor (73)(74)(75).

The **Ryanodine Receptor** is a homotetrameric protein bedded into the membrane of the sarcoplasmic reticulum. There, it serves as a calcium channel. It also exists in parts of the brain's neurons (76).

I.2 Molecular biology

Molecular biology is the science of biology applied to a molecular level. It comprises the exploration and manipulation of molecular mechanisms and their molecular components. That basically comprises biological pathways, proteins and genetics (DNA, RNA, etc.) (77). Although the field of molecular biology is relatively new, it lately got expended by the "omics" sciences. With their help, diseases as muscular dysfunctions can be better measured, screened and understood (78).
The study of this dissertation particularly used the technologies of transcriptomics and bloodomics.

I.2.1 Omics

By the suffix „OMICS", methods designed to test large quantities of related biological molecules are summarized (79).
The field of OMICS technologies is divided into genomics, transcriptomics, proteomics and metabolomics (80), as exemplified in the Figure below:

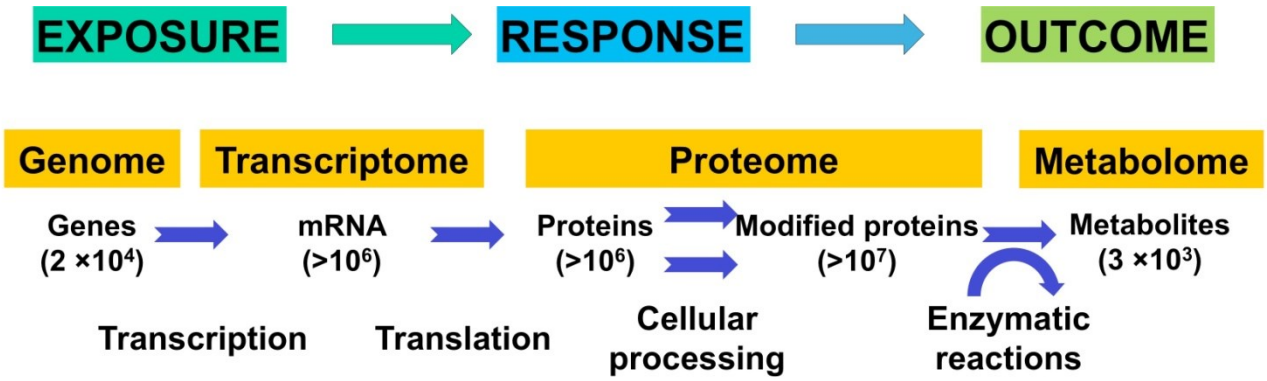

Figure 12: The connection between the different OMICS technologies and their biological basis. It also shows the correlation of the different OMICS technologies among each other

I.2.2 Genomics

Genomics is the tool to explore the human genome by decoding large parts of it. It is based on testing the DNA (81).

The Human Gene Project was a relevant step towards Genomics, allowing a better understanding of the human genetic configuration by revealing the number of genes as well as the type of the human genome (79).

As the individual genome does not actively change during lifetime (except the shortening of the chromosomes telomeres), genomics is only a static tool. That is to say, it is not applicable for testing on temporal changes or current disease status, because current events do not influence the genome.

Only the transcription of the genome can show variations over time by epigenomic processes, which are mainly based on DNA methylation and histone modification (79). The study of these processes of regulating DNA transcription is called the Epigenomics.

I.2.3 Transcriptomics

Transcriptomics, means scanning of large quantities - ideally of all of the mRNA available. The mRNA - m stands for messenger - is conveying genetic information necessary for the production of cellular proteins by the ribosomes - and hence crucial for all biological processes that are including proteins. Therefore, it is essential for the organism that the transcription of DNA to mRNA is regulated accurately and adapted to all internal and external requirements of the human body. That is to say, the structure of the respective mRNA molecule is variable over time and among individuals, and is even depending on the cellular system observed. The variability over time is also enforced by the mRNA turnover rate. Environmental changes as well as changes in internal and external biological processes (both physiological and pathological) implicate changes in the mRNA transcription, which therefore are closely related to them.

The mRNA consists of RNA strands copied from the cellular DNA (By RNA polymerase II through the steps of initiation, elongation and termination, optional re-initiation. These steps are regulated in various ways, including positive and negative regulatory factors and inhibitory signals at the gene promoter (82)) and processed in several steps: during the splicing step, intron parts are cut out (83)(84). Afterwards, the RNA tails are modified by capping the 5’end and adding a poly A tail at the 3’ end to enable an effective translation (85).

The processed mRNA leaves the cellular core and can now be translated into proteins by ribosomes (86).
All those steps (transcription and its regulation, modification of the RNA, translation, posttranslational modification) regulate the rate of gene expression (87).
The pattern created by different mRNA strands and their frequency in one sample are recorded by transcriptomics tools (88). It is therefore temporally variable and sensible to external influences exerted by environment and disease. Changes in these two influencing factors will lead to changes in the mRNA pattern and so allow conclusions about the state of the patient providing the sample.
Thus, transcriptomics indirectly allow statements about the present state of a person's health. It has already been proved, that different medical conditions lead to characteristic mRNA patterns (89).
But Transcriptomics can also be used to observe the changes in mRNA patterns due to intentionally precipitated external changes. The information gained by those experiments can help to acquire a better understanding of human physiology and pathology.

I.2.4 Proteomics

All proteins existing in one sample (regardless of them being modified or not [see Figure above], or whether they are extracellular or intracellular) are called its Proteome.
Proteomics focuses on identifying their type and number (80). As the proteins are derived from their mRNA, the patterns of protein types and the quantities are temporally changeable and sensible to external influences, similar to the Transcriptome.
Because of that, by scanning the quantity and quality of certain proteins, illnesses can be indicated already at this early stage. An example is Creatine kinase -MB, whose quantity in the blood normally exceeds a certain level after a myocardial infarction and even could be a "predictor of infarct size" under certain circumstances (90).

I.2.5 Metabolomics

Metabolomics means the scientific measuring of the aggregate of the metabolites emerging in the human system, the metabolome (91).
As cellular biochemical pathways adapt themselves in consequence of changing external influences as nutrition, health issues and more, metabolites offer a well-known possibility to draw conclusions from their appearance and prevalence.

I.2.6 Bloodomics

To examine certain organic systems, it is normally inevitable to acquire tissue by biopsy of the relevant region, what makes the examination not only difficult and often painful, but also brings about a relevant increase of hazards. Therefore the application of biopsy dependent methods in the everyday clinical use is shunned or even is excluded completely.

As methods to avoid the hazards of applying biopsies were explored, the blood's ability to reflect the state of various tissues was found, leading to the designation of blood as a „surrogate for biopsy tissue" (89).
Bloodomics is a coinage, indicating the relevance of the blood for diagnostics in the „OMICS" technologies (89).
It stands for scanning the blood's contents – by using „OMICS" technology – in order to gather information about distinct parts and organs of the body. This is possible because of the blood's capability to reflect the physiological and pathological status of different tissues: changes in organs or organic systems lead to similar changes in the ingredients of the blood.
The mechanisms are like that:
Dying cells or tissues often shed inner proteins, which consequently emerge in the blood and can be tracked down by Proteomics or Metabolomics tools. If they lose free genetic material as mRNA or even DNA fragments, this may be measured by Transcriptomics technologies.
But Transcriptomics technologies also disclose a significant similarity of about 80 percent between the Transcriptomes of tested tissue cells and of peripheral blood cells (89).
It is assumed, that regulation systems for distinct cellular transcriptomes not only have an effect within the targeted cells but also systemic effects on other cellular types. Many regulatory systems, even of cellular systems that not directly interact seem to be linked, too, so that changes in one system can be perceived in the other.

I.3 Immobilization Physiology

I.3.1 Physical Inactivity

Immobilization is defined by abated movements of the immobilized object. If the object is a particular part of the body, the immobilization mostly serves to protect from physical forces. A person may be physically immobilized by various reasons. Often, immobilization

supports or enables the recovery of strength of people weakened by age and/or diseases. Furthermore it can be necessary to fixate people that would possibly injure themselves (for example agile but disorientated people) or that are highly infective (in this case, partial immobilization, the quarantine may be necessary). Obviously, there are many possible reasons, the most common one yet not mentioned is the unintentional immobilization caused by weakness of the patient. Probably this is the most problematic form of immobilization, because it cannot or hardly can be terminated, and consequently it persists for a long time, so that labile persons are struck by this kind of immobilization by more complications. In most cases, the immobilization of a person means bedrest.

I.3.2 Bedrest Immobilization

Apparently, people forced to bedrest undergo similar biological effects as astronauts in environments with decreased gravity. Because of that, ESA, the European Space Agency says: “These medical studies offer great opportunities to investigate the human body and physiological reactions in a controlled environment over long periods” (92).
Immobilized people also seemingly suffer from “accelerated aging” and similar effects can be seen in their bodies: Cardiovascular changeover even after a short period of time, loss of bone and muscle mass, only to name some of the most apparent changes.
To lie horizontally resp. with a slightly lowered head in bed seems to be comparable to loss of gravity. This is the reason why the ESA uses bedrest in slightly lowered head positions as a model for weightlessness in spaceflight.
But these findings will not only be helpful for future manned space programs. To assure a better understanding of the biology of the various processes occurring in the human body during long periods of bedrest could improve the treatment of immobilized people in many ways. These are suffering from various deterioration of their physical condition.

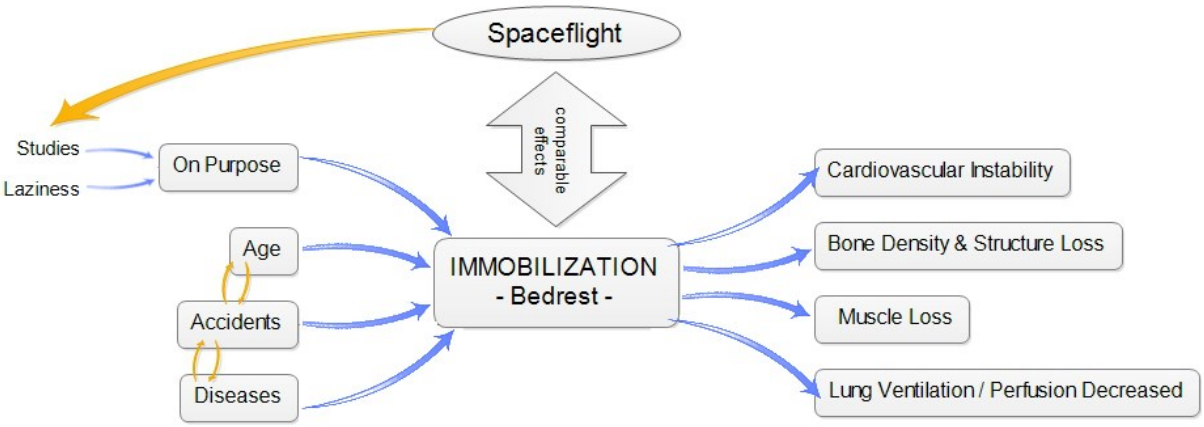

Figure 13: A short summary of the main reasons and effects of Immobilization and how spaceflight studies are involved; Age, Accidents and Diseases are influencing each other leading to Immobilization – which leads to "accelerated aging" itself

Of course, this sketch only covers the main reasons and consequences of bedrest immobilization. Other consequences to mention would be loss of connection tissue, shortening of tendons, lowered flexibility of joints, ulcers and skin infections from getting bedsore and much more.

The loss of bone density and structure is one of the most serious impacts of both spaceflight and bedrest for various reasons. First, this loss occurs and is advancing very fast. Second, it is difficult to counteract and up to now, no sufficient countermeasures have been developed. In addition, the consequences of instable bones – fractures – are severe and may cause months of regeneration, the exit of space missions or even death. Finally, the regeneration of the humans' bones needs much more time than its loss. Studies show, that after 10 days of bedrest, healthy persons need 4 months to regain the bone density they had before. On the one hand, this can be explained by missing weight load, which is one of the main augmentation triggers for the human bone. On the other hand, it is supposed that the negative nitrogen balance (caused by muscle degradation) people usually evolve during bedrest amplifies the speed of the degradation of the bone mass (93). The calcium loss is associated with a reduced gastrointestinal calcium uptake and an increased urinary calcium excretion (94).

Other complications increasing significantly with age, like disorientation (in studies, nearly one third of young people suffer from disorientation after 2,5 hours of enforced bedrest

(93) – and lack of gravity disables the human's vestibular system, especially the gravity dependent macula of saccule, leading to disorientation in every astronaut) or difficulties in properly releasing urine and faeces (due to the additional barriers in the bed and space). Also, the lung ventilation decreases by laying down simply because supine patients have higher closing volumes (93) – like old people, too. But these last mentioned effects only have minor priority due to their immediate reversibility after returning from space or after standing up.

Additionally studies show that bedrest leads to cardiovascular instability. This has several reasons.

Among others, this is due to a decrease of plasma volume of about 600 ml (93), caused by a reflex called the "Gauer-Henry-reflex"(95). When standing up about half a litre of blood flows down into the legs' vessels. When laying down the blood to shifts back into the centre of the body, where a gain of plasma volume can be registered by atrial mechanoreceptors. Those receptors are linked to the central nervous system by the vagus nerve; the central nervous system reacts to the registered plasma overload by decreasing the amount of ADH released by the posterior lobe of the pituitary gland. Also, the renin – angiotensin – aldosterone system is shut down and the secretion of ANP increases (96). This causes the kidneys nephrons to excrete more sodium and water – thus diminishing the volume of the blood plasma.

But studies also found evidence that changes of the sensitivity of the body's baroreflex receptors and lowered norepinephrine may be responsible or are at least accompanied by orthostatic intolerance (97).

A decreased cardiac output also seems to be responsible for post – immobilization orthostatic intolerance. Studies found, that this effect is more problematic for patients with a high arterial compliance (98).

Additionally, "loss of vascular tone" (99) seems to be one of the main reasons for cardiovascular instability. This loss of vascular tone can be explained by the degradation of the muscle pump.

I.3.3 Muscle Loss During Bedrest Immobilization

By every week of immobilization the strength of the affected muscles is decreased by about 10 to 15 percent. Especially the muscle groups involved in counteracting gravity (muscle groups located in the lower limb and the trunk) are degrading when immobilized. This was shown by an immobilization study wherein the degrading of different muscle

groups was measured. The gastrocnemius and soleus, main plantar flexors of the ankle and required for counteracting gravity were severely reduced in mass and strength, while its antagonists, the dorsal flexors, hardly showed degrading after all (100). The consequences of this can be observed in all patients that were forced to bedrest immobilization and then experienced difficulties in standing up and walking. The immobilization of skeletal muscles results in their atrophy and loss of strength even after a short time. Another study showed, that even after 5 days of immobilization of the knee a reduction of muscle mass in the quadriceps muscle of 3,5 % (referring to its cross sectional area) and a loss of strength of 9,0 % was observed. After 14 days, the level of decline had more than doubled (101). A study observing muscle fibres from the vastus lateralis muscle found evidence that the strength of the fibre's contractions decreased significantly after 4 days of immobilization (102). The age of the affected individual seems to be influential, too : There was observed a faster loss of muscle mass in younger persons (103). Another study showed a faster loss of muscle mass in older persons accompanied with a larger loss in strength and in aerobic capacity. While younger patients lost less than half a kilogram of muscle mass after 28 days of bed rest, older patients lost one kilogram within 10 days (104). Besides the decline of the muscle strength, the recruitment patterns of the muscle fibres changed (105).

Reasons for muscle loss during immobilization

At cellular level, immobilization related loss of muscle mass and volume derive from a volume loss of the muscle fibres, while their number does not change (96). Until now, the molecular causes and the mechanisms of muscle loss during immobilization are not clearly understood. However, it is assumed that muscle loss has its origin in a lowered build-up of muscle proteins in combination with an accelerated loss of muscle proteins, so that the degradation exceeds the rebuilding (104). But until now, no one knows what contributes more. Animal studies indicate that both processes take place (less building and increased degradation of proteins), but the accelerated degradation of muscle proteins seems to be of major importance. In contrary to that, studies on humans indicate that the decreased building of proteins is more important (103). According to studies by Shangraw et al. and Ferrando et al., the loss of muscle mass during bedrest, which is accompanied by nitrogen excretion, is mainly caused by lowered muscle protein synthesis rates, while degrading of muscle proteins was not affected (104). During space flight, a regression of protein building of - 15 % was measured (96).

There also seems to be a difference between short term and long term muscle loss. During periods of immobilization of more than 10 days, the building of muscle proteins (especially the postprandial building) goes back, while the degrading of muscle proteins does not show distinguishable changes. In periods of immobilization of less than 10 days, protein degrading could have a certain influence, too (106).

Consequences of muscle loss during immobilization

Because the primarily affected muscles are those needed most to cope with gravity, all activities dependent on these will get more complicated. This includes standing up, walking or even sitting in an upright position. But after long periods of bedrest, when other muscle groups are affected as well, all physical activities will cause problems.

Moreover, muscle atrophy is associated with arthritis and is functionally influencing it (107).

Periods of immobilization during a person's lifetime, even short ones, are also supposed to add up and to result in sarcopenia, the accelerated muscle loss of elderly people (106).

Loosing skeletal muscle mass may also lead to an aggravation of the immobilization (by causing fatigue and inactivity of the patient (104)), which gives rise to even more loss of muscle mass.

In cachectic patients, the loss of muscle mass leads to elevated rates of mortality (104).

Nevertheless, there is some evidence that, at least in elderly patients, the muscle strength is not lowering even after long periods of hospitalisation (108). But the question is left open in this study, whether the patients in this study were more immobilized during their hospitalisation than at home.

The loss of muscle mass in the lower limbs is also weakening the function of the muscle pump, because the latter is dependent on the pressure onto the veins built up by these muscles. It has been shown that the muscle pump in legs with less muscle mass attains a lower ejection of blood volume out of its veins. A study found a linear correlation between the muscle mass in the lower limbs and the volume of blood lifted by the muscle pump (109). This may lead to an aggravation of post - bedrest orthostatic intolerance. Experiments with head up tilt tables support this theory: When the function of the muscle pump is inhibited by tilting a healthy person that had been strained before into an upright position by a tilt table without letting the person stand actively (so that the muscles of the

lower limbs are relaxed and the muscle pump cannot work), in 50 to 80 % of the cases pre-syncope is brought about within 15 minutes (110). Due to another study, after one hour nearly all healthy participants experienced syncopation, if their muscle pump was inactivated (9). All the same it is assumed that the pre-syncopes result from a combination of neural syncope and loss of muscle pump power (110).

There was found evidence too, that a weakened muscle pump leads to orthostatic intolerance can also be found in patients with paralysis of the lower limbs. This inhibits the muscle pump, thus causing cardiovascular instability in these individuals (111).

I.3.4 Spaceflight Induced Deconditioning And Its Relationship With Immobilization

Spaceflight induced deconditioning is similar to what occurs during immobilization. It is known that the lack of gravity imposes major problems on the human physiology. Without gravity, the astronaut's body mainly suffers from catabolic physiological modifications that often are described as accelerated aging. The ESA describes it as follows: "Stay in a tilted bed for weeks with your head at the lower end and your body starts to change as if it were ageing prematurely or living in space" (112).

Physiological deconditioning in space occurs in multiple systems. One of the most obvious modifications are a **cardiovascular changeover** that causes astronauts to collapse when standing up after returning from space flights taking longer than a few days. Thus returning astronauts cannot stand up without collapsing on first attempt. They have to wait until their cardiovascular system has adapted to the earth's gravity again. This even gets worse in longer absence of gravity and is due to the shift of fluids (a quantity of 1 to 2 litres (113)), mainly blood, from the lower body parts (where blood accumulates when the standing person is exposed to gravity) to the heart in a micro – gravity environment. The oversupply of blood volume now sensed activates the "Gauer Henry Reflex", as explained in chapter I.3.2 "Bedrest immobilization". About 17 % of the plasma volume gets lost within the first day, (96) (113) and even 22% after 2 days (114).

On earth, the 6 degrees head down tilt is used to create cardiovascular effects in a human's body similar to the effects that would occur in a micro – gravity environment.
The head down tilt also induces the "Gauer Henry Reflex" due to fluid shifts and leads to a decrease in blood volume. But the cardiovascular changeover in persons in space and those in 6 degrees head-down tilt differs in some way indicating that the head-down tilt is not an exact model for microgravity. In fact, it is only an approach.(96)

It is known that the reduction of plasma volume which increases the blood's haematocrit, is responsible for the measured decrease of erythropoietin. With lower levels of EPO, the blood production goes down, leading to low levels of red blood cells in the astronaut. Also, young red blood cells go into lyse (114), resulting in an anaemia (113) - like a reduction of the total blood volume of about 10 % (96).

But the cardiovascular system is not only stressed by a loss of **body fluids**. The less work the **cardiac muscle** does in spaceflight leads to reduction in cardiac muscle mass and performance, and cardiac arrhythmia are more common (115). This can be observed in bedrest immobilization, too. After two weeks of bedrest, the cardiac muscle mass was reduced by 5% on average and the performance was significantly lowered: the stroke volume was reduced of about 12 %, which perhaps contributes to post immobilization orthostatic intolerance (116). Other studies showed decreasing left ventricular mass and wall thickness after 6 weeks with enhancing after 12 weeks during bed rest. Similar findings were observed during space flight (117).

A loss of **bone mass** is another obstacle for a long stay in space. If not being strained by gravitational forces, human bones reduce in mass and structural strength, leading to instability and, in the worst case, to fractures (118). After two days in space, the quantity of serum ionized calcium increases by 30 % and the PTH levels increase by 50 % (114). A 3 month space flight study revealed that astronauts in space lose 250 mg bone calcium/day on average (119). Bones that under normal circumstances carry the body-weight lose 1 – 2 % of their bone density per month, and that the quantity of calcium excreted in the urine and the faeces increases of about 60 – 70 % (96). Demineralization mainly occurs in the lower body parts, that lack the load of the body's mass (heel, femur, hip, lower spine (113)); the bones of the upper extremities are less affected (118).
Bedrest seems to be a good model for micro gravity environments according to the levels of bone demineralization and calcium loss, which are quite similar (96)(118).
The high rate of calcium excretion in the urine even can lead to renal stones in some cases (118).

Problematic as well is the loss of **muscle mass** during long periods of reduced gravity. After some time in space, the unoccupied muscles in the astronaut's body begin to

degenerate as they do not have to stand gravity any more. The muscle mass drops, the muscles weaken and lose tenacity (115). After two weeks, astronauts will have lost about 20 % of their muscle mass if no counteractions are made; and after 3 to 6 month, the loss is up to 30 %. Measurements of knee extension strength showed 12 to 31 % (and even – 50 % in single cases) strength loss dependent on the duration of the space journey (but not correlating with the loss of volume; the reason for this is not clear yet).
There was also observed that muscle fibres of type 2 changed to type 1, producing faster, but less tenacious fibres (96)(113). This mechanism can also be observed on injured limbs that are forced to stay motionless for long periods of time, for example in broken and therefore immobilized legs: after weeks or months the affected limbs are discernibly thinner and have lost substantial parts of their former muscle mass. Returning to earth after a long lasting mission in space, astronauts can hardly stand on their feet even after regaining their cardiovascular capabilities and need hard long-term training programs to regain their original strength (96).

The immune system is also affected by the environment in space. During the Apollo missions, half of the astronauts got ill due to infections, and several alterations were observed in the astronauts' immune system, as to lowered lymphocyte and T cell activation, lowered granulocyte and natural killer cell function, and alterations in cytokines, antibodies and more (96)(114). Although the causes of these alterations of the immune system are not yet known, it is supposed that they occur due to stress (which is able to release more stress hormones like glucocorticoids, that suppress the immune system), elevated radiation in space and loss of gravity (In studies, gravity is shown to be important for T cell activation). As exposing astronauts to artificial gravity during space missions has an improving effect on their immune system, micro – gravity seems to play an important role in suppressing it (96).

The impact of radiation exposure is only observed is space but not in bedrest studies, of course (especially high – energy radiation). The radiation levels in earth orbit are about 10 times higher than on earth. Due to a lack of long time tests, the effects of long lasting exposures to this special type of radiation are not clear (113). However, development of cancer, DNA damages and neurological impairments are assumed (115).

As a result, 6 degrees head down tilt bedrest is mainly used as an easy way to create approximately similar physiological reactions in people regarding the bone and muscle loss, and the cardiovascular changes (120). It is not the only model for space flight weightlessness. Other models like whole body water immersion or chair - sitting for several days were also used, but showed problems by implementing them for a long period (94).

II Aims And Objectives

T**he pivotal question is how the muscular system is affected by bedrest immobilization.** Though bedridden persons have significant muscle loss, it is not yet known why this occurs. An in-depth examination of the data obtained during a bedrest campaign will be done, with special emphasis on the **molecular mechanisms that contribute to muscle atrophy and (dys-) function**. The results of this work will be applicable in the areas of immobilization, geriatrics as well as cardiology.

We tested the hypothesis that muscle function and strength will be affected by the bedrest immobilization and that this will be reflected in the molecular changes that were observed.

The main objective of the study was to examine the microscopic molecular changes in the cell (resp. the changes in RNA expression that lead to different RNA end products and in consequence to changes in biological pathways all over the body, following the hypothesis that all physical processes have their origin in genetic control/ transcription and translation) that result in the macroscopic changes observed in the patient's body by collecting blood samples and examining the changes in the cells' gene expression (in form of cRNA). Differences in the expression of genes (expressing different genes or expressing the same gene in different quantities) lead to different gene products, for example enzymes, structural proteins or hormones, that lead to changes in the physiological and pathological pathways whose results are open to macroscopic examination.
On the other hand external influences like stress factors, differences in nutrition, sleep, environment, or – as in our ESA Bedrest study- immobilization should affect the intracellular expression of genes. This way , adaption to external changes may be explained: External changes give rise to changes in intracellular gene expression, which now leads to changes in the cellular gene products (structural proteins, enzymes etc.) which result in alterations in the cell (pathway changes, building/degradation of cellular components, cellular atrophy or hypertrophy, mitosis or apoptosis etc.). It also may cause changes in the whole body, if intracellular substances pass the cellular membrane and flood into the intercellular region. There, they may be detected by other cells of the same cell compartment or even of completely different cellular compartments, if the substances have entered the bloodstream and have been transported all over the body. Thus, if some of these

that had entered the bloodstream have a hormonal function, they can produce changes in cells all over the body. Ideally, those changes make the organism adapt to its new environment.

By monitoring the transcriptome- all transcripted genes that are trackable in their mRNA form at the particular moment- before, during and after the environmental change, the observer should be able to draw conclusions about the way the body is adapting to its new environment. For example: If it is known, which proteins are present in different pathways, and an increase is detected of mRNA coding for proteins known to be important for a certain pathway, it's up-regulation may be presumed.

In our case, the environmental change was immobilization of the human body and the observed changes were above all different forms of degrading (see chapter Immobilization physiology). As this dissertation is about muscle loss, the observation of the RNA status **before** and **after bedrest** sessions shall help in understanding the complexity of the muscular degradation following bed rest immobilization.

For this purpose, healthy volunteers underwent complete bedrest for several weeks and were examined before and during the campaign and also afterwards. This included taking blood samples from them at defined times. Those were processed in order to acquire the cells "active" coding RNA, the cRNA (which, in contrary to the non-coding RNA is coding for proteins. This is particularly the mRNA, the messenger RNA (121)). This way the changes in the RNA expression that lead to physiological and pathological changes in the cell and subsequently in the human body could be examined.

III Methodology

This study was carried out at MEDES, Toulouse, France. It was organized by the European Space Agency. The MEDES team also was responsible for the recruiting and selection process in cooperation with the ESA

The tests performed on the candidates were administered at the MEDES Space Clinic and in specialized Departments of the Rangueil and Larrey Hospitals (Toulouse).

The study is legal and conform to the 1989 WMH declaration of Helsinki. Also the Ethic Boards of MEDES (the Institute for Space Medicine and Physiology in Toulouse, France) in Toulouse gave countenance.

III.1 Subject Inclusion And Exclusion Criteria

14 male participants were selected: The 12 volunteers participating in the study and 2 volunteers as a reserve to compensate any drop outs.
Due to the large list of difficulties that possibly could affect the study, the participants had to be checked with regard to several inclusion and exclusion criteria. These were:

Inclusion criteria:

- Males
- healthy
- between 20 and 45 years old
- BMI between 20 and 26
- height between 158 and 190 centimetres
- free of drug consumption of any form, as cigarettes, alcohol, etc.
- no concurrent medical treatment
- the participant and his family are free of acute or chronic diseases
- the participant and his family have no psychological disturbances
- no cardiovascular, orthopaedic, musculoskeletal disorders
- in a social security system
- Fitness level within the following limits (measured in the maximal oxygen consumption test):
  Max. VO2 between 35ml/min/kg and 60 ml/min/kg in men under 35 years or

Max. VO2 between 30 ml/min/kg and 60 ml/min/kg in men over 35 years

- Not engaged at the time of the tests periods
- Informed consent signed

Exclusion criteria:

- health problems, including:
    - chronic back pain
    - gastro oesophageal reflux
    - hiatus hernia
    - history of migraine
    - history of diabetes
    - history of genetic bone diseases
    - history of genetic muscular diseases
    - history of renal stones
    - history of thyroid dysfunction
    - cardiac rhythm disorders
    - food allergies
    - lactose intolerance
- psychological disorders
- Orthostatic intolerant
- Low bone mineral density (evaluated by quantifying the bone mineral density: the T-score had to be at least -1.5)
- osteosynthesis materials, former broken legs, knee problems, joint surgeries or metallic implants
- Prior cases of thrombophlebitis or thrombosis (in the patient or in the family) or positive thrombosis screens or coagulation defects
- abnormal lower limbs echo doppler findings
- positive hepatitis and/or HIV test results
- Claustrophobia
- lack of vitamin D which could not be corrected before the start of the study
- volunteers having problems with blood sampling
- evaluated as likely to be difficult (during the performed tests)

- not compliant or not able to comply (for example because of difficulties with the language) in any way
- volunteer not allowing to contact his general practitioner
- volunteers undergoing diets, vegetarians, vegans
- Inadequate thoracic acoustic windows found during the echocardiography
- Volunteers not mental capable/mature to join the experiment
- Participating in another clinical research at the particular time or having received more than 4500 euro for taking part in other research projects within the last year
- Blood samples of more than 8ml/kg donated during the last eight weeks.
- People confined or guarded (guardianship, trusteeship)

III.2 Pre-screening Tests

The candidates got an Information and Consent Paper, received all information needed and demanded and had to undergo several medical and psychological tests.

The medical tests included:

- a medical/surgical anamnesis,
- drug consumption (as alcohol, smoking, coffee, medication)
- a physical examination
- several tests necessary for clarifying the application of the inclusion and exclusion criteria These included:
    - several laboratory tests
    - a 12 lead EKG
    - an orthostatic hypotension test (including measurements of systolic/diastolic blood pressure and heart rate in horizontal and vertical body position)
    - bone density measuring
    - a maximal oxygen consumption test
    - an alcohol breath test
    - a doppler sonography of the legs
    - a cardiac sonography
    - a chest radiography
    - an examination by a psychologist
    - psychological tests, screening for mental instabilities

The laboratory tests were mainly blood tests including:

- hepatitis and HIV markers
- blood cell counts
- markers of coagulation and phlebitis
- vitamin (especially vitamin D, any lack of it had to be corrected before the start of the study)
- mineral and electrolyte status
- blood protein/ fat/ cholesterol/glucose/ urea/ creatinine values
- liver and thyroid related values

Also urine tests were administered to screen for drugs, to measure the urines pH and density and to screen for glucose, ketone bodies, protein, bilirubin, urobilinogen, nitrites and erythrocytes and leucocytes in the urine.

The physiological examination was performed by a psychologist who tested the candidates' psychological appropriateness, and they included standardized personality tests, personal conversations and written personality tests (about the candidate's life and history). Finally the remaining candidates were listed due to their likelihood of being specially qualified for successfully participating in this study.

The participants got information and consent document and were precisely informed about the study. All remaining questions were answered, before they had to sign informed consent. They were allowed to exit the study without giving reason and at any time. Ethics approval was obtained before the study was started.

III.3 The Study's Design

As only a few small similar test had been performed before, it was designed as a pilot study. The study had a cross-over design (see figure below):

Figure 14: A Visualization of the Timetable for a better understanding

Three groups had 4 persons each. All participants had to lay down with 6 degrees head downwards position, like in the figure below. This was to simulate spaceflight induced fluid shifts.

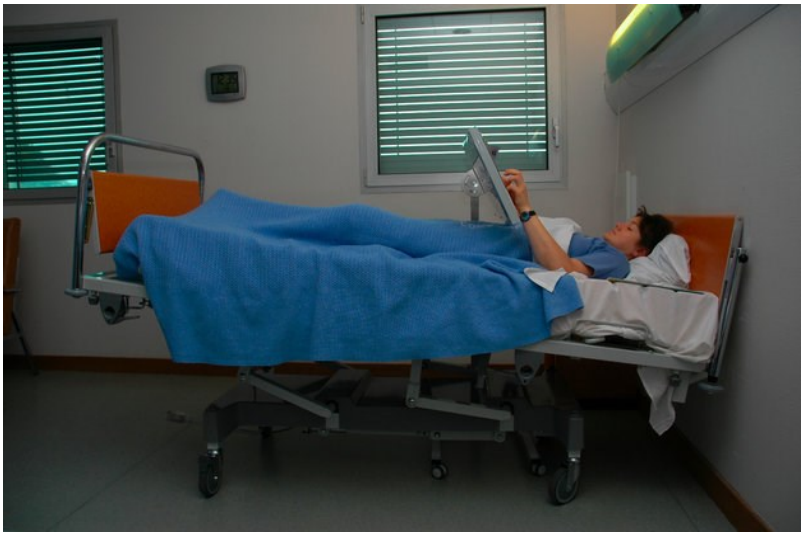

Figure 15: An ESA volunteer lying in bed. This figure was obtained from http://1.2.3.12/bmi/spaceinimages.esa.int/var/esa/storage/images/esa_multimedia/images/2012/11/bedrest/12097643-2-eng-GB/Bedrest_node_full_image.jpg

The second immobilized group additionally had to perform exercises intended to preserve their physical condition.
The third group did perform exercises AND got daily doses of additional proteins, while still being immobilized. According to the finding, that bodybuilders taking protein shakes are gaining muscle mass faster, administering proteins to immobilized test persons may preserve their muscles. Apart from that, everyone got the same meals in order to assure the same setup for each participant.
Each test session took 21 days. To avoid distortion of the test results during the session, the volunteers should only perform minor sports within 10 days before the start of the study. In addition, stimulating substances as coffee were not to be consumed two days before. After the 21 days of head down tilt, the volunteers got 4 months of normal life in order to regain their original physical condition.
After this resting period, all persons switched the group – the participants from the first group switched to the second group, the ones from the second group switched to the third group and those from the third group switched to the first group (randomized cross over study). This was done twice, so that 3 sessions were held.

III.4 Blood Sampling

Every morning during the bedrest phases, the participants got medical control (weight measure, blood pressure and Pulse) by the medical staff of the MEDES.
The medical staff of the MEDES took the blood samples from the volunteers.
Blood samples were taken from 07:00 to 08:30 during the marked days:

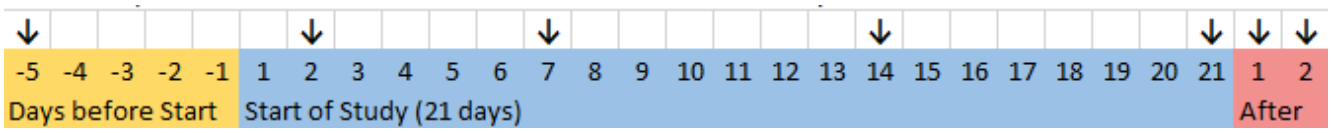

Figure 16: The dates of blood sampling prior, during and after one bedrest session

In this dissertation, only the transcriptomics data of two samples (prior bedrest and directly post bedrest) was taken. Also, it was taken from only 9 participants (3 per group) due to financial issues.
The following list shows the steps that had to be performed to record the data suitable for analysis, extracted from the volunteers' blood:

Step	Domain	Procedure	Used Resources / Tools
1	**Getting Blood samples**	Taking blood	Butterfly needle,6 ml Tempus™ Blood RNA tubes with stabilizing reagent form Applied Biosystems, Halle, Belgium
2		Cooling RNA Samples down to -80 ° -20° C → storing	Freezer
3	**RNA Extraction**	RNA extraction, following the manual	Tempus Spin RNA Isolation kit from Applied Biosystems
4		Testing the gained RNA	NanoDrop Spectrophotometer from Isogen Liefe Science, PW De Meern, the Netherlands
5		Depleting the globin mRNA from the total RNA, following the manual	Ambion Globinclear kit from Applied Biosystems
6		Determining the purity of the residually globin-depleted RNA	Agilent 2100 Bioanalyzer using RNA 6000 Chips from Agilent Technologies, Belgium
7		Storing at -80° C	Freezer
8	**RNA Amplification and Classification**	Transforming globin-depleted RNA to c (complementary) RNA	One Colour Low Input Quick Amp Labelling kit from Agilent Technologies
9		Reverse Transcription of 100 to 200 ng of cRNA into cDNA	T7-promotor primer, MMLV reverse transcriptase
10		Transcription of cDNA to cRNA → conceiving single stranded labelled cRNA	Cyanine 5 dye-CTP for post bedrest sample hybridization, Cy3 dye for pre bedrest sample hybridization
11		Purification of the cRNA	Qiagen's Rneasy mini spin columns from Qiagen, KJ Venlo, Netherlands
12		Testing quantity, specific activity	NanoDrop Spectophotometer

13	**Microarray analysis**	Handling microarray slides: Putting 1.65 µg cRNA to 4x44 microarray slides for 17 hours, following the manual	Tecan HS 4800™ Pro Microarray Hybridization Station,Agilent Whole Human Genome Microarray Slides
14		Scanning microarrays	Agilent DNA microarray scanner (G2565BA)
15		Saving Data	Agilent Feature Extraction Software Version 10.7
16		Converting .tiff files to text files	Agilent protocol GE-10.7-SEP09; gProcessedsignal for analysis
17		Quantile normalizing and log2-transforming signals; keeping only 41000 unique probes; Filtering to the intenseness of gene expression; matching it to an approved HUGO gene symbol	Agilents's GeneSpring 12.1 software
18	**Statistical process**		Paired Student t-tests per probe
19		P - Value multiplicity tests; with significance positive for P - Values < 0,05 AND absolute fold change > 2	Benjamini-Hochberg false discovery rate

By these steps, data plots of expression values of 41000 probes were generated.
The data was sent to Belgium and got assessed there: Using Ingenuity Pathway Analysis (http://www.ingenuity.com, application build 220217) and the ontology term "muscular process" in the Muscular System Development and Function tree, a list of **220 probes that relate to muscle wasting** was extracted. Comparing the relation of gene expression level after bedrest to gene expression level prior bedrest, the fold change of each gene was calculated (if levels are equal: fold change = 1; negative fold change means: down regulation, positive fold change means: up regulation).
This smaller gene pool of 220 genes and their fold change was used for this dissertation.

IV Results

This study (pilot study) was completed by 12 male volunteers that did meet the criteria described in the results part. They had an average age of 34 ± 8 years, an average height of 1.76 ± 6 cm and an average weight of 69.8 ± 8 kg.

The transcriptomics data assessed from the whole blood samples taken from all 9 participants (taken prior and post bedrest) can be seen in the following figure:

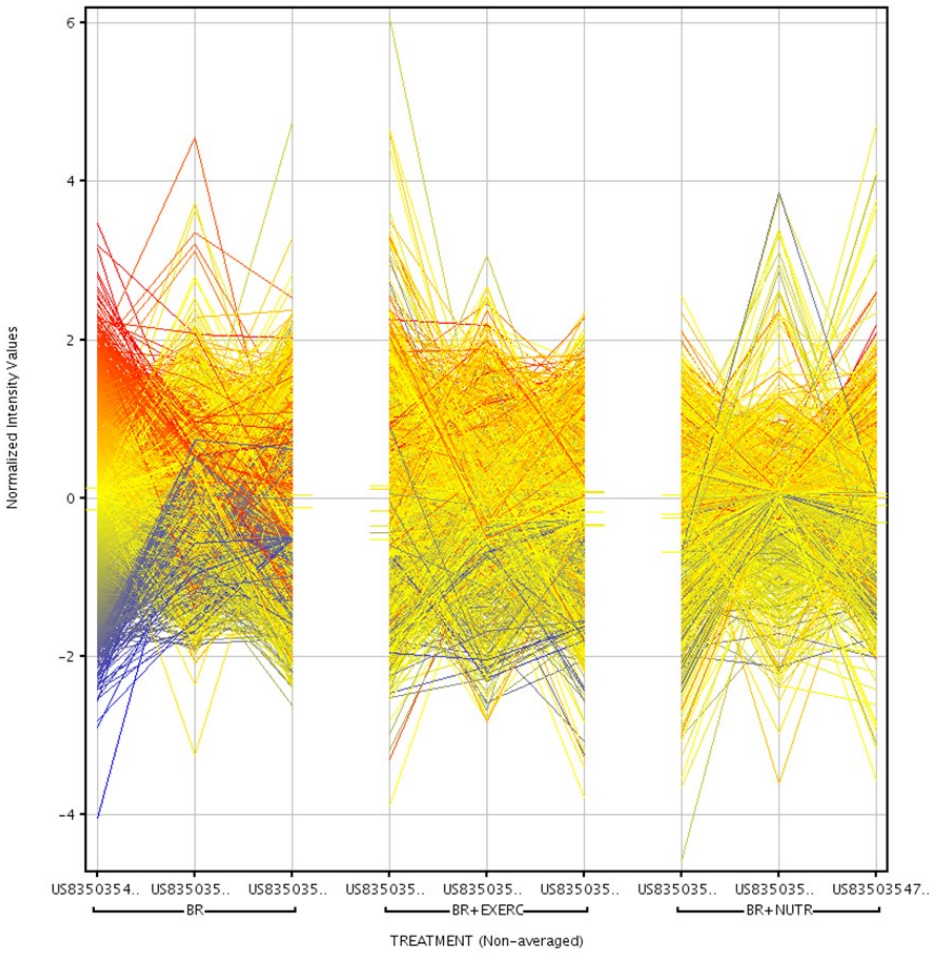

Figure 17: Reaction in gene expression: This figure shows the unsorted data of **all** obtained genes (not only the muscle specific). The numbers on the horizontal axis (US…) denominate the participants. As they were divided into three groups depending on the particular treatments during the study, BR stands for the bedrest group (group 1: the group relevant for this dissertation; their fold change data were used for acquiring the results (see below)), BR+EXERC stands for the second group undergoing bedrest and exercise training, BR+NUTR stands for the third group receiving additional nutrition. On the vertical axis, the normalized intensity values of each gene were inserted. That means

values above zero for up regulation and values below zero for down regulation of the particular gene expression after treatment. The intensity of same genes in different participants were connected by lines between them. Therefore, flat lines describe the same reaction in gene expression by different participants.

A list of 220 genes coding for muscular proteins was sorted out using Ingenuity Pathway Analysis (http://www.ingenuity.com, application build 220217). Their fold change is visualized in the figure below:

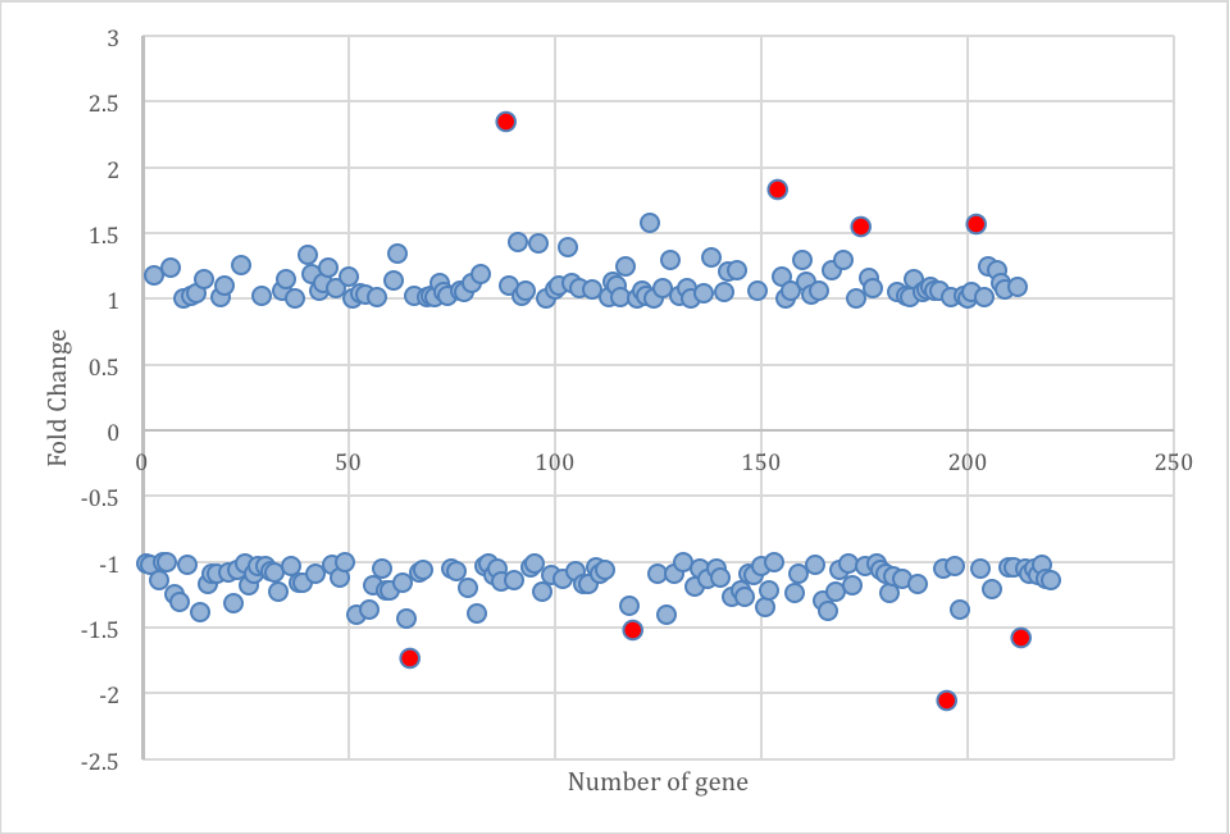

Figure 18: The fold changes of the obtained 220 genes coding for muscular proteins. Fold changes meeting the significance criteria are marked red.

Their fold change was calculated and the cut offs for the fold change being significant were set to -1.5 (meaning, that the gene was down regulated after bedrest) and 1.5 (meaning, that the gene was up regulated after bedrest). 9 genes did meet the cut off criteria, the rest was sorted out.

The following table shows the remaining genes

Agilent ID	Fold Change	Gene Name
A_23_P63209	2,344603	HSD11B1
A_23_P36100	1,8305221	CNTF
A_23_P114983	1,5776052	TRIM63
A_24_P319989	1,564108	CAV3
A_23_P92730	1,5470045	HSPB3
A_23_P168403	-1,5205417	KCNH2
A_24_P324630	-1,5772822	SBF2
A_23_P204791	-1,7334913	NOS1
A_23_P421587	-2,059072	GDAP1

These genes will be discussed in detail under discussion.

Using the diseases and functions tool in the “exercise analyse” section of the ingenuity pathway analysis tool, the 5 genes HSD11B1, TRIM63, CAV3, NOS1, and KCNH2 were recognized as involved in / triggering muscular changes.

A canonical diagram showing how these genes affect the skeletal muscle was generated using the ingenuity software (see figure 19).

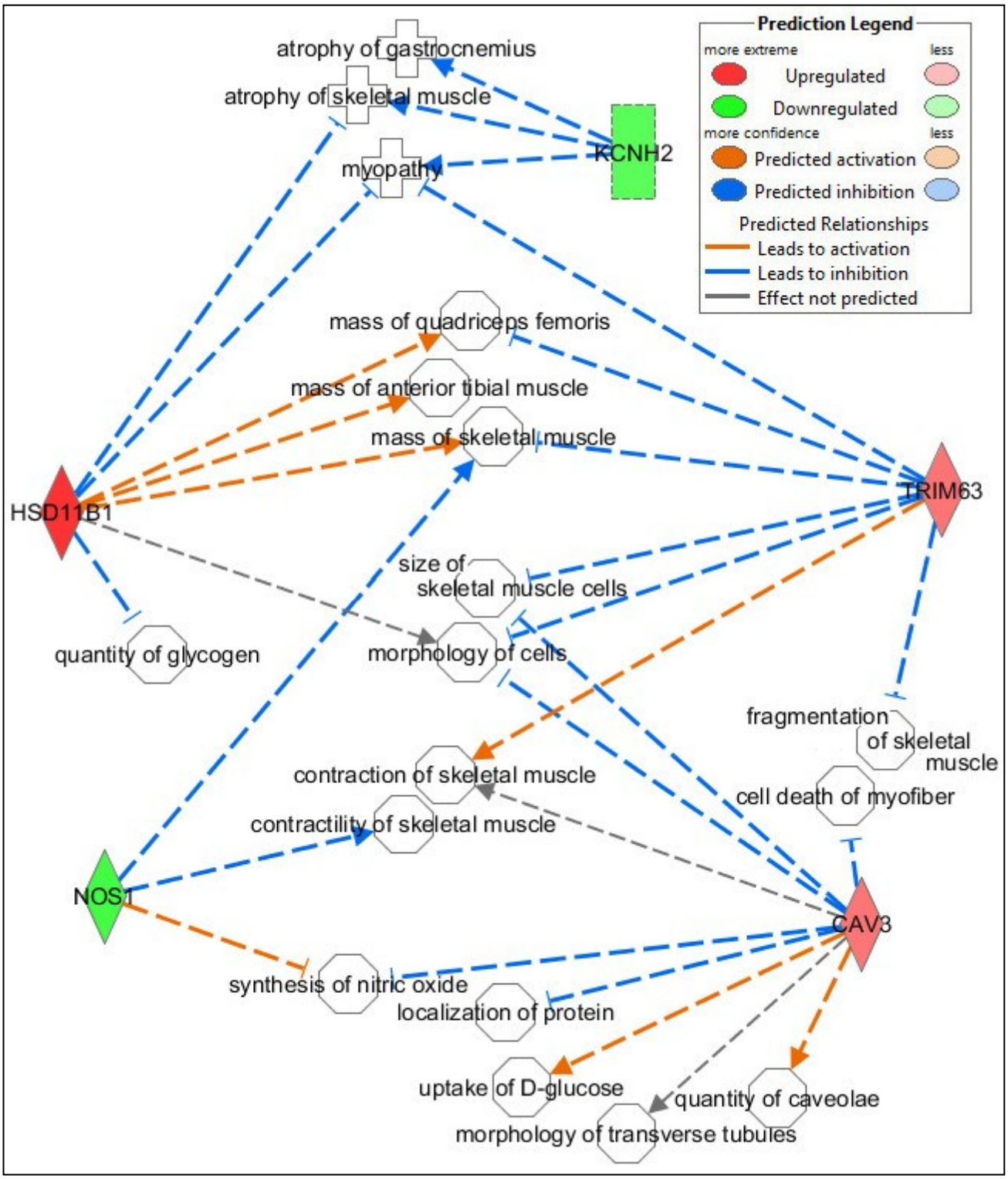

Figure 19: Effects of the genes HSD11B1, TRIM63, CAV3, NOS1, and KCNH2 on skeletal muscle. This figure was obtained from Ingenuity pathway analysis

V Discussion

The findings presented in the results are mainly consistent with the clinical changes observed on the participants' skeletal muscle system. Not all genes were covered by figure 19. The following list discusses the 9 genes that were sorted as relevant for skeletal muscle and that did meet the cut off (see results):

KCNH2 has the gene name **Homo sapiens potassium voltage-gated channel, subfamily H** (eag-related), member 2 (KCNH2), transcript variant 1, mRNA [NM_000238]. It codes for the alpha subunit (called **Kv11.1**) of a voltage-activated potassium channel. Its mutation mainly can lead to long QT syndrome of the heart (122). Mouse studies show, that an expression of the potassium channel causes skeletal muscle atrophy in mice (by activation of the ubiquitin proteasome pathway), whereas an inhibition of the channel inhibits the atrophy (123). *Thus, an expression of the KCNH2 gene is associated with skeletal muscle atrophy. The down regulation of the gene should slow down skeletal muscle atrophy occurring by bedrest.* This is consistent with the findings of the Ingenuity pathway program:
According to Ingenuity pathway analysis (see figure 19), the gene **KCNH2** has a role in atrophy of skeletal muscle, especially known for gastrocnemius, meaning a decline in volume. Causing atrophy, it can severe myopathy. As it becomes *down regulated*, this should *conserve the volume of skeletal muscle / gastrocnemius* and *protect from myopathy*. The ingenuity analysis software gave following information about the relation of the gene to skeletal muscle:

- **atrophy of gastrocnemius** and thus **atrophy of skeletal muscle** undergoes less atrophy in dominant negative mutant mice
- **myopathy** is attenuated in dominant negative mutant mice through protection from muscle atrophy

HSD11B1 has the gene name **Homo sapiens hydroxysteroid (11-beta) dehydrogenase 1** (HSD11B1), transcript variant 2, mRNA [NM_181755]. It codes for a protein (**Hydroxysteroid (11-Beta) Dehydrogenase 1**) *mainly relevant for transforming cortisol and cortisone into each other* (124). It is also relevant for glucose metabolism and mutations of the gene are associated with diabetes type 2 (125). As seen in figure 19, it is also relevant for skeletal muscle preservation according to ingenuity pathway analysis. In

contrast to that, the study on HSD11B1 deficient mice (knockout) did NOT reveal relevant muscular atrophy (126) (-> see chapter "skeletal myopathy" and figure 3A; based on this study, ingenuity pathway analysis evaluated the gene HSD11B1). *Considering this contradicting data, it is unclear whether the gene HSD11B1 has relevant impact on muscle mass / atrophy.* Another study suggests a possible involvement in growth and differentiation of skeletal muscles. The dehydrogenase also seems to accelerate the cortisone-induced protein degradation in muscle and thus *could lead to skeletal muscle atrophy together with the glucocorticoid* (127).
According to Ingenuity pathway analysis (see figure 19), the gene **HSD11B1** is known to have a role in the inhibition of atrophy of skeletal muscle / gastrocnemius. It also seems to be important for skeletal muscle mass, especially with regard to the mass of the quadriceps femoris and the anterior tibial muscle. (As it becomes *up regulated*, this should *conserve the volume and mass of skeletal muscle, protect from myopathy, and conserve the mass of quadriceps femoris and the anterior tibial muscle.)* The gene *lowers the quantity of glycogen in the skeletal muscle cell*, and it is relevant for the transverse tubules and thus *affects the morphology of the muscle cell*. The ingenuity analysis software gave following information about the relation of the gene to skeletal muscle:

- increases **atrophy of skeletal muscle** if mutated (knockout mouse)
- severes **myopathy**, because atrophy is increased if mutated (knockout mouse)
- decreases **mass of quadriceps femoris** if mutated (knockout mouse)
- decreases **mass of anterior tibial muscle** if mutated (knockout mouse)
- decreases **mass of skeletal muscle** if mutated (knockout mouse)
- affects **morphology of cells** by alteration of transverse tubules if mutated (knockout mouse)
- increases **quantity of glycogen** in skeletal muscle if mutated (knockout mouse)

TRIM63 has the gene name **Homo sapiens tripartite motif containing 63** (TRIM63), mRNA [NM_032588]. It is also called **MURF1** and codes for a protein called **E3 ubiquitin ligase** (belonging to the RING zinc finger protein family), which is found in the iris and the skeletal muscle. It is localized at the Z line and the M line and mainly relevant for the disassembly of myosin light and heavy chains, myosin binding protein and the muscular kreatine kinase. *Thus it is involved in degradation of skeletal muscle* (128). In studies on mice with acute lung injury (a disease, which is accompanied with high MURF1

protein rates) it was shown that mice with elevated MURF1 levels developed skeletal muscle atrophy with losses in muscle fibre diameter of more than 25%, while an inhibition of the gene nearly neutralized this effect (129). *Thus, it is highly relevant for muscular atrophy.* This is consistent with the findings of the Ingenuity pathway program:
According to Ingenuity pathway analysis (see figure 19), the gene **TRIM63** is known to have a decreasing influence on skeletal muscle mass, especially known for the quadriceps femoris and the skeletal muscle cells themselves (-> which affects the sellular morphology), causing the muscle not only to shrink at a macroscopic level, but also at a cellular level. On the other hand, TRIM63 has importance for the skeletal muscle's strength of contraction, and has a protecting role against myopathy and fragmentation of skeletal muscle. *It becomes up regulated. That should reduce muscle mass, but have a positive influence on contraction strength of skeletal muscle*. The ingenuity analysis software gave following information about the relation of the gene to skeletal muscle:

- decreases **contraction of skeletal muscle** if mutated (knockout mouse)
- changes **morphology of cells** through the increased myofiber size if mutated (knockout mouse)
- increases **mass of quadrizeps femoris** if mutated (knockout mouse)
- increases **mass of skeletal muscle** if mutated (knockout mouse)
- increases **fragmentation of skeletal muscle** if mutated (knockout mouse)
- increases **myopathy of skeletal muscle** if mutated (knockout mouse)
- increases **size of skeletal muscle cells** if mutated (knockout mouse)

CAV3 has the gene name **Homo sapiens caveolin 3** (CAV3), transcript variant 1, mRNA [NM_033337]. It codes for a protein of the caveolin family, **caveolin 3**, which is a scaffolding protein in the caveolae of cellular membranes of most cells. Mutations lead to forms of muscular dystrophy (130), but can also lead to skeletal myopathy (131). In homozygous CAV3 mutant (deficient) mice, skeletal muscle degeneration, but no strength impairment was observed after 8 week (132). *Thus, the up regulation of the gene should have a protective effect against skeletal dystrophy/myopathy – it may protect against atrophy.*
According to Ingenuity pathway analysis (see figure 19), the gene **CAV3** is known to *be relevant for the surviving of the myofiber*, and it *increases the variability of size of skeletal muscle cells, the insulin triggered D-glucose uptake, the quantity of caveolae in the*

skeletal muscle, and affects the morphology of the skeletal muscle's transverse tubules and thus of the skeletal muscle itself, the localisation of glycosylphosphatidylinositol bound protein and is involved in the contraction of the skeletal muscle. It is *up regulated*. The ingenuity analysis software gave following information about the relation of the gene to skeletal muscle:

- affects skeletal muscle **morphology of transverse tubules** if mutated (knockout mouse)
- decreases insulin-triggered **uptake of D-Glucose** in skeletal muscle if mutated (knockout mouse)
- increases **quantity of caveolae** in skeletal muscle (transgenic mouse)
- **localization of protein** bound to glycosylphosphatidylinositol: it is shifted from skeletal muscle fibres into the intracellular space (knockout mouse)
- increased **cell death of myofibres** if mutated (knockout mouse)
- decreases muscular **synthesis of nitric oxide** by binding to nNO-synthase
- the **size of skeletal muscle cells** increases in variability if mutated (knockout mouse)
- involved in the **contraction of skeletal muscle**
- **morphology of skeletal muscle cells** is affected if mutated (knockout mouse)

NOS1 has the gene name **Homo sapiens nitric oxide synthase 1** (neuronal) (NOS1), transcript variant 1, mRNA [NM_000620]. It codes for a protein of the nitric oxide synthase family, **nNOSμ** (skeletal muscle and heart specific) which creates nitric oxide using L arginine. Nitric oxide synthases can be found all over the body, but skeletal muscles have especially high concentrations of it (133). As seen in figure 19, it is also relevant for skeletal muscle. The NO produced by the muscular nitric oxide synthase is involved in the regulation of muscular contraction (in the electromechanical coupling), the regeneration of skeletal muscle cells (134), the differentiation of myocytes, allocation of glucose, and autonomic blood flow regulation in the muscle (135). The pathways and role of the nNOSμ in the muscle can be seen in the figure below:

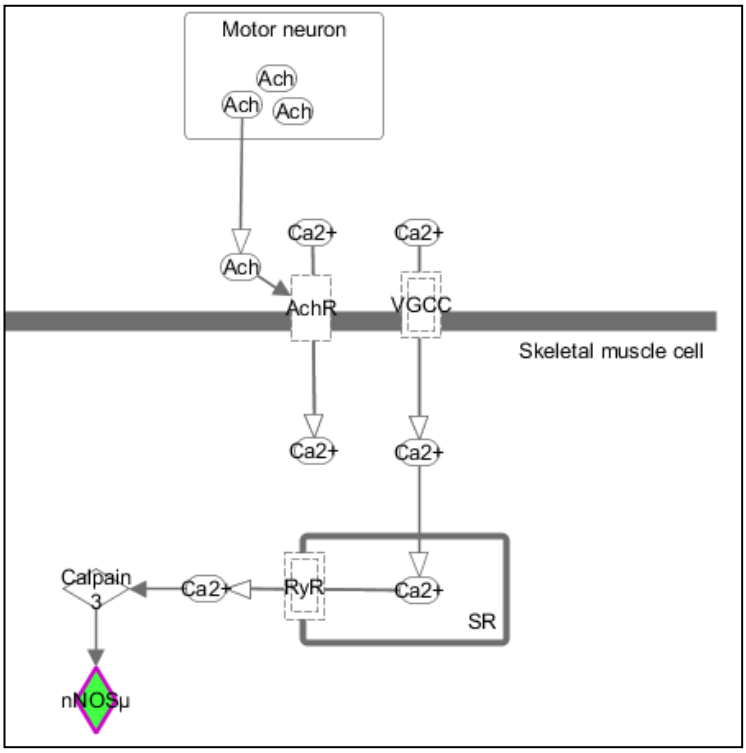

Figure 20: The role of the Homo sapiens nitric oxide synthase 1 in the skeletal muscle cell. In the figure, the right part shows the pathway from activation of the skeletal muscle cell via Ach to release of calcium out of the sarcoplasmic reticule into the sarcoplasm. For further information about this pathway see chapter I.1.6 "Physiology Of The Contraction". The sarcoplasmic calcium activates the calpain 3 protein, which then increases the degradation of the nNOSμ. This figure was obtained from Ingenuity pathway analysis

In rat models, inhibition of NO synthases induced a reduction of muscle mass and muscle fibre cross sectional area of 40% after 22 days. *Thus, muscle mass preservation seems to be highly dependent on muscular NO synthases* (136). This is consistent with the findings of the Ingenuity pathway program:

According to Ingenuity pathway analysis (see figure 19), the gene **NOS1** is known to influence the muscle mass and the contractility of the skeletal muscle positively. It is coding for a nitric oxide synthase, which produces nitric oxide, which is of relevance in various muscular processes. *As it is down regulated, this should cause a reduction of skeletal muscle mass and decrease the skeletal muscle's contractility*. The ingenuity analysis software gave following information about the relation of the gene to skeletal muscle:

- decreases **mass of skeletal muscle** if mutated (knockout mouse)

- decreases **contractility of skeletal muscle** if mutated (knockout mouse)
- relevant for **synthesis of nitric oxide**; Caveolin 3 binds to the gene product nNOSynthase and inactivates it.

HSPB3 has the gene name **Homo sapiens heat shock 27kDa protein 3** (HSPB3), mRNA [NM_006308]. It codes for a muscle specific heat shock protein, called **heat shock 27kDa protein 3**. Its mutation leads to autosomal dominant distal hereditary motor neuropathy type 2 C (137). It is mainly present in cardiac muscle and in low quantities in skeletal muscle (138). Although it is not well explored, it is known to bind to HSPB2 during muscle differentiation, forming a complex which is relevant for *preservation of muscle. Its up regulation may inhibit skeletal muscle atrophy, although it is questionable whether the complex is relevant in differentiated muscle cells, too (or only in muscle cells during their differentiation).* Mutations of HSPB3 lead to congenital myopathy (139).

CNTF has the gene name **Homo sapiens ciliary neurotrophic factor** (CNTF), mRNA [NM_000614]. It codes for a protein (also called **ciliary neurotrophic factor CNTF**) mainly relevant for growth of neuritis and synthesis of neurotransmitters in neurons (140) (141). Studies show, that the gene has influence on muscular strength in humans: people with different gene types develop different muscle strength and function after training, but it is not clear, whether muscle mass is also influenced by this gene, studies are contradicting each other (142). Also, no difference in skeletal muscle cross-sectional area was found (143). Rat studies show, that by administration of CNTF to rats, the strength of their skeletal muscles increased (144). *This suggests a positive correlation between CNTF and muscular strength. As the gene is up regulated, this would mean an improvement of skeletal muscle strength.*

Concerning the 2 remaining genes, no linkage to skeletal muscle could be found. It is assumed, that these genes are not directly relevant for muscle loss during bedrest.

GDAP1 has the gene name **Homo sapiens ganglioside-induced differentiation-associated protein 1** (GDAP1), transcript variant 1, mRNA [NM_018972]. It codes for a protein relevant for signal pathways in early neuronal evolvement (145). A mutation in this gene causes Charcot–Marie–Tooth disease Type 4A, which causes motor neuropathy. This leads to muscular atrophy of the muscles linked to these motor neurons, because they are

not activated any more. Thus, this gene is indirectly linked to muscular atrophy, but not of interest concerning atrophy during bedrest (146).

SBF2 has the gene name **SET binding factor 2** [Source:HGNC Symbol;Acc:2135] [ENST00000526353]. It codes for a protein called Set binding factor 2. Its function is unknown, although it is assumed that it plays a role in Schwann cell development (147). It is a membrane associated pseudo phosphatase, which is found to be mutated in Charcot–Marie–Tooth disease Type 4B (148).

Both genes, GDAP1 and SBF2, are known to be mutated in different types of Charcot–Marie–Tooth disease, in which peripheral neurons are affected and degenerate. This causes atrophy of the skeletal muscle cells activated by these neurons (149). Principally, the same happens during bedrest: The skeletal muscle cells are less activated by their motor neurons and degrade in mass and strength. If this observed muscular degradation is (at least partially) caused by functional losses of their motor neurons, this could mean a positive linkage between these genes and the observed muscle atrophy after bedrest. *Thus, the genes GDAP1 and SBF2 could be of importance for muscular atrophy. Their down regulation during bedrest could cause muscle loss* by similar mechanisms muscle loss is caused in Charcot–Marie–Tooth disease Type 4A and 4B.

Conclusions and clinical application

Our results show that bedrest immobilization causes **muscle loss** and this is reflected by the genes that are affected by bedrest. For example, genes such as HSD11B1, CNTF, TRIM63, CAV3, HSPB3 are up regulated and GDAP1, NOS1, SBF2, KCNH2 are down regulated. The genes that are up regulated are known to be associated with muscle loss (TRIM63), but also with muscle preservation (CAV3, HSPB3) and muscle strength (CNTF). Concerning the gene HSD11B1, the effects on muscle building and loss are not clear. The genes that are down regulated are known to be associated with muscle building (NOS1), with preservation of muscle (GDAP1, SBF2), but also with muscle loss (KCNH2). The findings of TRIM63, NOS1, GDAP1 and SBF2 are consistent with the clinical observation concerning muscle loss during bedrest.

At a first glance, the findings about the influence of the genes KCNH2, HSPB3, CNTF, CAV3 seem to contradict the clinical observation of decreased skeletal muscle volume,

muscle mass and muscle strength in immobilized people. But they could be part of the human body system's countermeasures against exaggerated muscle loss. Maybe, this is a cellular reaction to muscle loss and thus is not directly caused by immobilization, but by muscle loss itself (preventing too much loss). It could be a counter-regulation of the muscle fibres to compensate for the loss of muscle mass during bedrest. This would apply to the genes KCNH2, CAV3, and HSPB3. The up regulation of CNTF could be interpreted as a body reaction to maximize the strength of the remaining muscle mass in order to avoid larger loss of total muscle strength. *If this assumption is correct, steps to avoid down regulating the expression of KCNH2 (and/or steps to stimulate further up regulation of CAV3 and HSPB3) might initiate a possible future therapy against muscle loss during immobilization.*

Another reason for the clinically observed loss of skeletal muscle mass despite the findings concerning the genes KCNH2, HSPB3, CAV3 may be the superior influence of the genes NOS1 and TRIM63 on muscle mass and atrophy (as explained above). *Due to that, up regulating the expression of NOS1 and down regulating the expression of TRIM63 might be primary therapeutically steps in a future therapy to counteract muscle loss during immobilization. Also, up regulating the expression of GDAP1 and SBF2 might have a protecting influence on muscle mass.*

No relevant studies connected to the current subject were found which are testing the whole transcriptome for changes in the expression of muscle genes in humans. Existing studies mainly cover the function of single genes (150)(151) or small groups of genes (152)(153)(154) during immobilization or are focussing on animals (155). The genes examined in these studies were for example the myosin heavy-chain (MyoHC) gene, Atp5g3, TOM22, INrf2, Slc25a4, Hdac6, Tpm1, Sv2b, Podxl, Myh1, Surf1, Akt, mTOR, S6k, 4E-BP1, GSK3β, Ubiquitin, MURF1, Atrogin-1, MURF1, FOXO1, Collagen (COL1A1/3A1), insulin-like growth factors (IGF-1Ea/Ec), lysyl oxidase (LOX), matrix metalloproteases (MMP-2 and MMP-9), decorin, tenascin-C protein ubiquitination, ubiquitin ligase.

The question remains, how far the findings of this study are comparable to findings to be observed in clinical studies. Being a space agency, the ESA used inclusion and exclusion criteria not consistent with the typical hospitalized persons that are mostly elderly ones. Its

primary goal is to find countermeasures against physical deterioration of astronauts in space in contrast to most hospitalized people. Thus, the results of this study may not match those that would be found in typical patients due to their different conditions, especially in health and age. It cannot be guaranteed that the results can be generalized, although the principles of the major physiological processes should stay the same or at least be comparable regarding hospitalized patients versus young healthy people. Often other pathways are activated in different cellular compartments during illness, and in case of aged persons some organs only have left a reduced capacity to react to external stress factors, and many physiological processes in the human body are diminished by age and disease. Therefore it is difficult to directly apply the results of this study to those of hospitalized patients. But being a pilot study, supplying reliable facts is not its primary goal - it rather works out data that can be confirmed in later and larger studies.

VI References

1. Protein (biochemistry) : The muscle proteins -- Encyclopedia Britannica [Internet]. [cited 2014 Apr 25]. Available from: http://www.britannica.com/EBchecked/topic/479680/protein/72556/The-muscle-proteins

2. Klinke, Rainer; Pape, Hans-Christian; Kurtz, Armin; Silbernagl S. Physiologie. 6th ed. Thieme Verlag; 2010.

3. Behrends J, Bischofberger J, Deutzmann R. Duale Reihe Physiologie. 2nd ed. Thieme Verlag; 2012.

4. Lüllmann-Rauch R. Taschenlehrbuch Histologie. 2nd ed. Thieme Verlag; 2006.

5. Speckmann, Erwin-Josef Hescheler J, Köhling R. Physiologie. 5th ed. Urban & Fischer in Elsevier; 2008.

6. Silbernagl, Stefan; Despopoulos A. Taschenatlas Physiologie. 7th ed. Thieme Verlag; 2007.

7. Warum sind Ca++ und ATP bei der Muskelkontraktion so wichtig? 2009 Apr 14 [cited 2014 May 4]; Available from: http://vmrz0100.vm.ruhr-uni-bochum.de/spomedial/content/e866/e2442/e4687/e4692/e4775/e4804/index_ger.html

8. Lodish, Berk, Kaiser, Krieger, Scott, Bretscher, et al. Molecular Cell Biology. 6th ed. W.H. Freeman and Company; 2008.

9. Van Lieshout JJ, Wieling W, Karemaker JM, Secher NH. Syncope, cerebral perfusion, and oxygenation. J Appl Physiol [Internet]. 2003 Mar [cited 2014 Feb 15];94(3):833–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12571122

10. Siegenthaler W, E. BH. Klinische Pathophysiologie [Internet]. Georg Thieme Verlag; 2006 [cited 2014 Feb 12]. Available from: http://books.google.com/books?id=360YvLkY_5cC&pgis=1

11. Smit AAJ, Halliwill JR, Low PA, Wieling W. Pathophysiological basis of orthostatic hypotension in autonomic failure. J Physiol [Internet]. 1999 Aug [cited 2014 Feb 13];519(1):1–10. Available from: http://doi.wiley.com/10.1111/j.1469-7793.1999.0001o.x

12. Wurzinger LJ. Duale Reihe Anatomie. 2nd ed. Thieme Verlag; 2010.

13. Reneman RS. The anterior and the lateral compartmental syndrome of the leg due to intensive use of muscles. Clin Orthop Relat Res [Internet]. [cited 2014 Feb 13];(113):69–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1192677

14. Swope R, Adams A. Prevention and treatment of orthostatic hypotension in the orthopedic patient population. Orthopedics [Internet]. 2012 Jul 1 [cited 2014 Feb 13];35(7):600–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22784888

15. Smit AAJ, Wieling W, Fujimura J, Denq JC, Opfer-Gehrking TL, Akarriou M, et al. Use of lower abdominal compression to combat orthostatic hypotension in patients with autonomic dysfunction. Clin Auton Res [Internet]. 2004 Jun [cited 2014 Feb 13];14(3):167–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15241645

16. Stewart JM, Clarke D. "He's dizzy when he stands up": an introduction to initial orthostatic hypotension. J Pediatr [Internet]. 2011 Mar [cited 2014 Feb 13];158(3):499–504. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3029466&tool=pmcentrez&rendertype=abstract

17. Perlmuter LC, Sarda G, Casavant V, Mosnaim AD. A review of the etiology, asssociated comorbidities, and treatment of orthostatic hypotension. Am J Ther [Internet]. [cited 2014 Feb 15];20(3):279–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23656967

18. Pancheva M V, Panchev VS, Suvandjieva A V. Improved orthostatic tolerance by leg crossing and muscle tensing: indisputable evidence for the arteriovenous pump existence. J Appl Physiol [Internet]. American Physiological Society; 2006 Oct 1 [cited 2014 Feb 15];101(4):1271–2; author reply 1272. Available from: /han/pubmed/jap.physiology.org/content/101/4/1271.full

19. Panchev VS, Suvandjieva A V, Pancheva M V. The muscle pump is not an important determinant of muscle blood flow during exercise. J Appl Physiol [Internet]. American Physiological Society; 2005 Aug 1 [cited 2014 Feb 15];99(2):778. Available from: /han/pubmed/jap.physiology.org/content/99/2/778.full

20. Belin de Chantemèle E, Pascaud L, Custaud M-A, Capri A, Louisy F, Ferretti G, et al. Calf venous volume during stand-test after a 90-day bed-rest study with or without exercise countermeasure. J Physiol [Internet]. 2004 Dec 1 [cited 2014 Jan 28];561(Pt 2):611–22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1665364&tool=pmcentrez&rendertype=abstract

21. Peterson AL, Cigrang JA. Management of vasovagal syncope: controlling or aborting faints by leg crossing and muscle tensing. Circulation [Internet]. Lippincott Williams & Wilkins; 2003 May 27 [cited 2014 Feb 15];107(20):e198; author reply e198. Available from: /han/pubmed/circ.ahajournals.org/content/107/20/e198.full

22. Ten Harkel AD, van Lieshout JJ, Wieling W. Effects of leg muscle pumping and tensing on orthostatic arterial pressure: a study in normal subjects and patients with autonomic failure. Clin Sci (Lond) [Internet]. 1994 Nov [cited 2014 Feb 15];87(5):553–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7874844

23. Privett SE, George KP, Whyte GP, Cable NT. The effectiveness of compression garments and lower limb exercise on post-exercise blood pressure regulation in orthostatically intolerant athletes. Clin J Sport Med [Internet]. 2010 Sep [cited 2014 Feb 15];20(5):362–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20818194

24. Makovický P, Jílek F. Short review of some properties of muscular proteins. Cesk Fysiol [Internet]. 2008 Jan [cited 2014 May 2];57(1):10–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18630139

25. Obinata T, Maruyama K, Sugita H, Kohama K, Ebashi S. Dynamic aspects of structural proteins in vertebrate skeletal muscle. Muscle Nerve [Internet]. 1981 Jan 12 [cited 2014 May 2];4(6):456–88. Available from: http://europepmc.org/abstract/MED/7031467/reload=0

26. Perrin BJ, Ervasti JM. The actin gene family: function follows isoform. Cytoskeleton (Hoboken) [Internet]. 2010 Oct [cited 2014 May 12];67(10):630–4. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2949686&tool=pmcentrez&rendertype=abstract

27. Suurmeijer AJH, Clément S, Francesconi A, Bocchi L, Angelini A, Van Veldhuisen DJ, et al. Alpha-actin isoform distribution in normal and failing human heart: a morphological, morphometric, and biochemical study. J Pathol [Internet]. 2003 Mar [cited 2014 May 3];199(3):387–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12579541

28. ACTG1 actin, gamma 1 [Homo sapiens (human)] - Gene - NCBI [Internet]. [cited 2014 May 3]. Available from: http://www.ncbi.nlm.nih.gov/gene/71

29. Introduction: Actin Dynamics and Kinetics - Soft Matter Physics Division - University of Leipzig [Internet]. [cited 2014 Apr 25]. Available from: http://www.uni-leipzig.de/~pwm/web/?section=introduction&page=actin

30. Schiaffino S, Reggiani C. Myosin isoforms in mammalian skeletal muscle. J Appl Physiol [Internet]. 1994 Aug [cited 2014 May 12];77(2):493–501. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8002492

31. Yu R, Ono S. Dual roles of tropomyosin as an F-actin stabilizer and a regulator of muscle contraction in Caenorhabditis elegans body wall muscle. Cell Motil Cytoskeleton [Internet]. 2006 Nov [cited 2014 May 3];63(11):659–72. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1705952&tool=pmcentrez&rendertype=abstract

32. Perry SV. Vertebrate tropomyosin: distribution, properties and function. J Muscle Res Cell Motil [Internet]. Kluwer Academic Publishers; 2001 Jan 1 [cited 2014 May 3];22(1):5–49. Available from: http://link.springer.com/article/10.1023/A%3A1010303732441

33. Helfman DM, Cheley S, Kuismanen E, Finn LA, Yamawaki-Kataoka Y. Nonmuscle and muscle tropomyosin isoforms are expressed from a single gene by alternative RNA splicing and polyadenylation. Mol Cell Biol [Internet]. 1986 Nov [cited 2014 May 3];6(11):3582–95. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=367118&tool=pmcentrez&rendertype=abstract

34. Parmacek MS, Solaro RJ. Biology of the troponin complex in cardiac myocytes. Prog Cardiovasc Dis [Internet]. [cited 2014 Apr 29];47(3):159–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15736582

35. Bewegungsvorgänge - Chemgapedia [Internet]. [cited 2014 May 3]. Available from: http://www.chemgapedia.de/vsengine/vlu/vsc/de/ch/8/bc/vlu/proteine/bewegungsvorgaenge.vlu/Page/vsc/de/ch/8/bc/proteine/funktion_v_prot/bewegung/troponin.vscml.html

36. Myomesin-2 - MYOM2 - Homo sapiens (Human) [Internet]. [cited 2014 May 3]. Available from: http://www.uniprot.org/uniprot/P54296

37. Potthoff MJ, Arnold MA, McAnally J, Richardson JA, Bassel-Duby R, Olson EN. Regulation of skeletal muscle sarcomere integrity and postnatal muscle function by Mef2c. Mol Cell Biol [Internet]. 2007 Dec 1 [cited 2014 May 3];27(23):8143–51. Available from: http://mcb.asm.org/content/27/23/8143.full

38. Flashman E, Redwood C, Moolman-Smook J, Watkins H. Cardiac myosin binding protein C: its role in physiology and disease. Circ Res [Internet]. 2004 May 28 [cited 2014 Apr 29];94(10):1279–89. Available from: http://circres.ahajournals.org/content/94/10/1279.long

39. Weber A, Pennise CR, Babcock GG, Fowler VM. Tropomodulin caps the pointed ends of actin filaments. J Cell Biol [Internet]. 1994 Dec [cited 2014 May 3];127(6 Pt 1):1627–35. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2120308&tool=pmcentrez&rendertype=abstract

40. Bertoncini P, Schoenauer R, Agarkova I, Hegner M, Perriard J-C, Güntherodt H-J. Study of the mechanical properties of myomesin proteins using dynamic force spectroscopy. J Mol Biol [Internet]. 2005 May 20 [cited 2014 Apr 29];348(5):1127–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15854649

41. Tskhovrebova L, Trinick J. Making muscle elastic: the structural basis of myomesin stretching. PLoS Biol [Internet]. Public Library of Science; 2012 Mar [cited 2014 Apr 29];10(2):e1001264. Available from: http://dx.plos.org/10.1371/journal.pbio.1001264

42. Pinotsis N, Chatziefthimiou SD, Berkemeier F, Beuron F, Mavridis IM, Konarev P V, et al. Superhelical architecture of the myosin filament-linking protein myomesin with unusual elastic properties. Petsko GA, editor. PLoS Biol [Internet]. Public Library of Science; 2012 Mar [cited 2014 Apr 29];10(2):e1001261. Available from: http://dx.plos.org/10.1371/journal.pbio.1001261

43. Blanchard A, Ohanian V, Critchley D. The structure and function of α-actinin. J Muscle Res Cell Motil [Internet]. 1989 Aug [cited 2014 Apr 25];10(4):280–9. Available from: http://link.springer.com/10.1007/BF01758424

44. Ogura Y, Naito H, Kakigi R, Akema T, Sugiura T, Katamoto S, et al. Different adaptations of alpha-actinin isoforms to exercise training in rat skeletal muscles. Acta Physiol (Oxf) [Internet]. 2009 Jul [cited 2014 May 3];196(3):341–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19040707

45. Sjöblom B, Salmazo A, Djinović-Carugo K. Alpha-actinin structure and regulation. Cell Mol Life Sci [Internet]. 2008 Oct [cited 2014 Mar 31];65(17):2688–701. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18488141

46. Maruyama K, Kimura S, Ishi T, Kuroda M, Ohashi K. beta-actinin, a regulatory protein of muscle. Purification, characterization and function. J Biochem [Internet]. 1977 Jan [cited 2014 Apr 25];81(1):215–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/66229

47. Funatsu T, Asami Y, Ishiwata S. Beta-actinin: a capping protein at the pointed end of thin filaments in skeletal muscle. J Biochem [Internet]. 1988 Jan [cited 2014 May 4];103(1):61–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3360762

48. Maruyama K, Kurokawa H, Oosawa M, Shimaoka S, Yamamoto H, Ito M. Beta-actinin is equivalent to Cap Z protein. J Biol Chem [Internet]. 1990 May 25 [cited 2014 Apr 25];265(15):8712–5. Available from: http://www.jbc.org/content/265/15/8712.short

49. Labeit S, Kolmerer B. Titins: Giant Proteins in Charge of Muscle Ultrastructure and Elasticity. Science (80-) [Internet]. 1995 Oct 13 [cited 2014 Apr 29];270(5234):293–6. Available from: http://www.sciencemag.org/content/270/5234/293.short

50. Tskhovrebova L, Trinick J, Sleep JA, Simmons RM. Elasticity and unfolding of single molecules of the giant muscle protein titin. Nature [Internet]. 1997 May 15 [cited 2014 Apr 29];387(6630):308–12. Available from: http://dx.doi.org/10.1038/387308a0

51. Titin Research Roundup [Internet]. [cited 2014 Apr 29]. Available from: http://www.py.rub.de/kardp/respro/research3.html

52. Labeit S, Gibson T, Lakey A, Leonard K, Zeviani M, Knight P, et al. Evidence that nebulin is a protein-ruler in muscle thin filaments. FEBS Lett [Internet]. 1991 May [cited 2014 Apr 29];282(2):313–6. Available from: http://www.sciencedirect.com/science/article/pii/001457939180503U

53. Kruger M. Nebulin as a length regulator of thin filaments of vertebrate skeletal muscles: correlation of thin filament length, nebulin size, and epitope profile. J Cell Biol [Internet]. 1991 Oct 1 [cited 2014 Apr 29];115(1):97–107. Available from: http://jcb.rupress.org/content/115/1/97.abstract

54. Labeit S, Ottenheijm CAC, Granzier H. Nebulin, a major player in muscle health and disease. FASEB J [Internet]. 2011 Mar [cited 2014 Apr 29];25(3):822–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3042846&tool=pmcentrez&rendertype=abstract

55. The Titin/Telethonin Complex [Internet]. [cited 2014 Apr 29]. Available from: http://www.ks.uiuc.edu/Research/telethonin/

56. Ochala J, Carpén O, Larsson L. Maintenance of muscle mass, fiber size, and contractile function in mice lacking the Z-disc protein myotilin. Ups J Med Sci [Internet]. 2009 Jan [cited 2014 May 3];114(4):235–41. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2852774&tool=pmcentrez&rendertype=abstract

57. Salmikangas P, van der Ven PFM, Lalowski M, Taivainen A, Zhao F, Suila H, et al. Myotilin, the limb-girdle muscular dystrophy 1A (LGMD1A) protein, cross-links actin filaments and controls sarcomere assembly. Hum Mol Genet [Internet]. 2003 Jan 15 [cited 2014 May 3];12(2):189–203. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12499399

58. Gontier Y, Taivainen A, Fontao L, Sonnenberg A, van der Flier A, Carpen O, et al. The Z-disc proteins myotilin and FATZ-1 interact with each other and are connected to the sarcolemma via muscle-specific filamins. J Cell Sci [Internet]. 2005 Aug 15 [cited 2014 May 3];118(Pt 16):3739–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16076904

59. Krakow D, Robertson SP, King LM, Morgan T, Sebald ET, Bertolotto C, et al. Mutations in the gene encoding filamin B disrupt vertebral segmentation, joint formation and skeletogenesis. Nat Genet [Internet]. 2004 Apr [cited 2014 May 4];36(4):405–10. Available from: http://dx.doi.org/10.1038/ng1319

60. Murray JT, Campbell DG, Peggie M, Mora A, Alfonso M, Cohen P. Identification of filamin C as a new physiological substrate of PKBalpha using KESTREL. Biochem J [Internet]. 2004 Dec 15 [cited 2014 May 4];384(Pt 3):489–94. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1134134&tool=pmcentrez&rendertype=abstract

61. Lin X, Ruiz J, Bajraktari I, Ohman R, Banerjee S, Gribble K, et al. ZASP Mutations in Actin-binding Domain Cause Disruption of Skeletal Muscle Actin Filaments in Myofibrillar Myopathy. J Biol Chem [Internet]. 2014 Mar 25 [cited 2014 May 4];M114.550418–. Available from: http://www.jbc.org/content/early/2014/03/25/jbc.M114.550418

62. Wittenberg JB, Wittenberg BA. Myoglobin function reassessed. J Exp Biol [Internet]. 2003 Jun [cited 2014 May 3];206(Pt 12):2011–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12756283

63. Amberson WR, Bauer AC. Electrophoretic studies of muscle proteins. II. Complex formation between delta protein, myogen and myosin. J Cell Physiol [Internet]. 1971 Jun [cited 2014 May 3];77(3):281–300. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5104784

64. Wallimann T, Wyss M, Brdiczka D, Nicolayt K, Eppenberger HM. Intracellular compartmentation , structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands : the ' phosphocreatine circuit ' for cellular energy homeostasis. 1992;281:21–40.

65. Desmin [Internet]. [cited 2014 May 3]. Available from: http://www.hprd.org/summary?hprd_id=00514&isoform_id=00514_1&isoform_name=Isoform_1

66. Agbulut O, Li Z, Périé S, Ludosky MA, Paulin D, Cartaud J, et al. Lack of desmin results in abortive muscle regeneration and modifications in synaptic structure. Cell Motil Cytoskeleton [Internet]. 2001 Jun [cited 2014 May 3];49(2):51–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11443736

67. Paulin D, Li Z. Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. Exp Cell Res [Internet]. 2004 Nov 15 [cited 2014 May 3];301(1):1–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15501438

68. Hoffman EP, Brown RH, Kunkel LM. Dystrophin: The protein product of the duchenne muscular dystrophy locus. Cell [Internet]. Elsevier; 1987 Dec 24 [cited 2014 Apr 29];51(6):919–28. Available from: http://www.cell.com/article/0092867487905794/fulltext

69. Pasternak C, Wong S, Elson EL. Mechanical function of dystrophin in muscle cells. J Cell Biol [Internet]. 1995 Mar [cited 2014 Apr 29];128(3):355–61. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2120342&tool=pmcentrez&rendertype=abstract

70. Brown et al: dystroglycan complexes and their role in muscle development [Internet]. [cited 2014 May 4]. Available from: http://www.bio.unipd.it/bam/PDF/6-6/art.449-457.pdf

71. Kalamida D, Poulas K, Avramopoulou V, Fostieri E, Lagoumintzis G, Lazaridis K, et al. Muscle and neuronal nicotinic acetylcholine receptors. Structure, function and pathogenicity. FEBS J [Internet]. 2007 Aug [cited 2014 May 5];274(15):3799–845. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17651090

72. MICHAEL M. FRANCIS and ROGER L. PAPKE: Muscle-type Nicotinic Acetylcholine Receptor Delta Subunit Determines Sensitivity to Noncompetitive Inhibitors, While Gamma Subunit Regulates Divalent Permeability [Internet]. [cited 2014 May 5]. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.420.863&rep=rep1&type=pdf

73. Perez-Reyes E, Kim HS, Lacerda AE, Horne W, Wei XY, Rampe D, et al. Induction of calcium currents by the expression of the alpha 1-subunit of the dihydropyridine receptor from skeletal muscle. Nature [Internet]. 1989 Jul 20 [cited 2014 May 22];340(6230):233–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2474131

74. Wang M-C, Velarde G, Ford RC, Berrow NS, Dolphin AC, Kitmitto A. 3D Structure of the Skeletal Muscle Dihydropyridine Receptor. J Mol Biol [Internet]. 2002 Oct [cited 2014 May 22];323(1):85–98. Available from: http://www.sciencedirect.com/science/article/pii/S0022283602008902

75. Proenza C, O'Brien J, Nakai J, Mukherjee S, Allen PD, Beam KG. Identification of a region of RyR1 that participates in allosteric coupling with the alpha(1S) (Ca(V)1.1) II-III loop. J Biol Chem [Internet]. 2002 Feb 22 [cited 2014 May 22];277(8):6530–5. Available from: http://www.jbc.org/content/277/8/6530

76. Ryanodine receptor 1 - RYR1 - Homo sapiens (Human) [Internet]. [cited 2014 May 5]. Available from: http://www.uniprot.org/uniprot/P21817

77. Davis L. Basic Methods in Molecular Biology (Google eBook) [Internet]. 1986 [cited 2014 Apr 17]. Available from: http://books.google.com/books?hl=de&lr=&id=739enqOyW_UC&pgis=1

78. Huang NF, Kurpinski K, Fang Q, Lee RJ, Li S. Proteomic identification of biomarkers of vascular injury. Am J Transl Res [Internet]. 2011 Feb [cited 2014 Apr 17];3(2):139–48. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3056560&tool=pmcentrez&rendertype=abstract

79. Vlaanderen J, Moore LE, Smith MT, Lan Q, Zhang L, Skibola CF, et al. Application of OMICS technologies in occupational and environmental health research; current status and projections. Occup Environ Med [Internet]. 2010 Feb [cited 2014 Jan 27];67(2):136–43. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2910417&tool=pmcentrez&rendertype=abstract

80. Yadav SP. The wholeness in suffix -omics, -omes, and the word om. J Biomol Tech [Internet]. 2007 Dec [cited 2014 Feb 26];18(5):277. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2392988&tool=pmcentrez&rendertype=abstract

81. National Human Genome Research Institute. FAQ About Genetic and Genomic Science [Internet]. 2014 [cited 2014 Feb 26]. Available from: http://www.genome.gov/19016904

82. Shandilya J, Roberts SGE. The transcription cycle in eukaryotes: from productive initiation to RNA polymerase II recycling. Biochim Biophys Acta [Internet]. 2012 May [cited 2014 May 4];1819(5):391–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22306664

83. Transcription: Pre-mRNA Processing (Splicing) [Internet]. [cited 2014 May 4]. Available from: http://www.phschool.com/science/biology_place/biocoach/transcription/premrna.html

84. Pandya-Jones A. Pre-mRNA splicing during transcription in the mammalian system. Wiley Interdiscip Rev RNA [Internet]. [cited 2014 May 4];2(5):700–17. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3154077&tool=pmcentrez&rendertype=abstract

85. Gallie DR. The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. Genes Dev [Internet]. 1991 Nov [cited 2014 May 4];5(11):2108–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1682219

86. Knippers R. Molekulare Genetik [Internet]. Georg Thieme Verlag; 2006 [cited 2014 Feb 26]. Available from: http://books.google.com/books?id=FamuNgm33_oC&pgis=1

87. Day D. Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. J Endocrinol [Internet]. 1998 Jun 1 [cited 2014 May 1];157(3):361–71. Available from: http://joe.endocrinology-journals.org/content/157/3/361.short

88. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet [Internet]. 2009 Jan [cited 2014 Jan 21];10(1):57–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2949280&tool=pmcentrez&rendertype=abstract

89. Mohr S, Liew C-C. The peripheral-blood transcriptome: new insights into disease and risk assessment. Trends Mol Med [Internet]. Elsevier; 2007 Oct 1 [cited 2014 Jan 27];13(10):422–32. Available from: http://www.cell.com/trends/molecular-medicine/fulltext/S1471-4914(07)00165-7

90. Rakowski T, Dziewierz A, Legutko J, Kleczynski P, Brzozowska-Czarnek A, Siudak Z, et al. Creatine Kinase-MB Assessed in Patients with Acute Myocardial Infarction Correlates with Cardiac Magnetic Resonance Infarct Size at 6-Month Follow Up. Hellenic J Cardiol [Internet]. [cited 2014 Feb 11];55(1):4–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24491929

91. Nicholson JK, Lindon JC. Systems biology: Metabonomics. Nature [Internet]. 2008 Oct 23 [cited 2014 Feb 26];455(7216):1054–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18948945

92. Experiments wanted for the next-best-thing to human research in space / Research / Human Spaceflight / Our Activities / ESA [Internet]. [cited 2014 May 5]. Available from: http://www.esa.int/Our_Activities/Human_Spaceflight/Research/Experiments_wanted_for_the_next-best-thing_to_human_research_in_space

93. Creditor MC. Hazards of hospitalization of the elderly. Ann Intern Med [Internet]. 1993 Feb 1 [cited 2014 Feb 11];118(3):219–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8417639

94. Pavy-Le Traon A, Heer M, Narici M V, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986-2006). Eur J Appl Physiol [Internet]. 2007 Sep [cited 2014 Jan 22];101(2):143–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17661073

95. Sandler H. Inactivity: Physiological Effects (Google eBook) [Internet]. 1986 [cited 2014 Feb 11]. Available from: http://books.google.com/books?hl=de&lr=&id=Oy7ENVrxqX0C&pgis=1

96. Williams D, Kuipers A, Mukai C, Thirsk R. Acclimation during space flight: effects on human physiology. CMAJ [Internet]. 2009 Jun 23 [cited 2014 Feb 16];180(13):1317–23. Available from: http://www.cmaj.ca/content/180/13/1317.short

97. Engelke KA, Doerr DF, Crandall CG, Convertino VA. Application of acute maximal exercise to protect orthostatic tolerance after simulated microgravity. Am J Physiol [Internet]. 1996 Oct [cited 2014 Feb 11];271(4 Pt 2):R837–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8897972

98. Tuday EC, Meck J V, Nyhan D, Shoukas AA, Berkowitz DE. Microgravity-induced changes in aortic stiffness and their role in orthostatic intolerance. J Appl Physiol [Internet]. 2007 Mar [cited 2014 Feb 11];102(3):853–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17082368

99. Wieser M, Gisler S, Sarabadani A, Ruest RM, Buetler L, Vallery H, et al. Cardiovascular control and stabilization via inclination and mobilization during bed rest. Med Biol Eng Comput [Internet]. 2014 Jan [cited 2014 Feb 11];52(1):53–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24096595

100. Dittmer DK, Teasell R. Complications of immobilization and bed rest. Part 1: Musculoskeletal and cardiovascular complications. Can Fam Physician [Internet]. 1993 Jun;39:1428–32, 1435–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2379624&tool=pmcentrez&rendertype=abstract

101. Wall BT, Dirks ML, Snijders T, Senden JMG, Dolmans J, van Loon LJC. Substantial skeletal muscle loss occurs during only 5 days of disuse. Acta Physiol (Oxf) [Internet]. 2013 Oct 30 [cited 2014 Feb 12]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24168489

102. Hvid LG, Suetta C, Aagaard P, Kjaer M, Frandsen U, Ørtenblad N. Four days of muscle disuse impairs single fiber contractile function in young and old healthy men. Exp Gerontol [Internet]. 2013 Feb [cited 2014 Feb 12];48(2):154–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23220118

103. Suetta C, Frandsen U, Jensen L, Jensen MM, Jespersen JG, Hvid LG, et al. Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy. PLoS One [Internet]. 2012 Jan [cited 2014 Jan 22];7(12):e51238. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3526599&tool=pmcentrez&rendertype=abstract

104. Evans WJ. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. Am J Clin Nutr [Internet]. 2010 Apr 1 [cited 2014 Feb 10];91(4):1123S–1127S. Available from: http://ajcn.nutrition.org/content/91/4/1123S.short

105. Effects of Extended Bed Rest—Immobilization and Inactivity [Internet]. Demos Medical Publishing; 2004 [cited 2014 Feb 26]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK27213/

106. Wall BT, Dirks ML, van Loon LJC. Skeletal muscle atrophy during short-term disuse: Implications for age-related sarcopenia. Ageing Res Rev [Internet]. 2013 Sep [cited 2014 Jan 26];12(4):898–906. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23948422

107. De Oliveira Nunes Teixeira V, Filippin LI, Viacava PR, de Oliveira PG, Xavier RM. Muscle wasting in collagen-induced arthritis and disuse atrophy. Exp Biol Med (Maywood) [Internet]. 2013 Dec [cited 2014 Feb 13];238(12):1421–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24186267

108. Bodilsen AC, Pedersen MM, Petersen J, Beyer N, Andersen O, Smith LL, et al. Acute hospitalization of the older patient: changes in muscle strength and functional performance during hospitalization and 30 days after discharge. Am J Phys Med Rehabil [Internet]. 2013 Sep [cited 2014 Feb 15];92(9):789–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23552331

109. Stewart JM, Medow MS, Montgomery LD, McLeod K. Decreased skeletal muscle pump activity in patients with postural tachycardia syndrome and low peripheral blood flow. Am J Physiol Heart Circ Physiol [Internet]. 2004 Mar 1 [cited 2014 Feb 13];286(3):H1216–22. Available from: http://ajpheart.physiology.org/content/286/3/H1216#T2

110. Halliwill JR, Sieck DC, Romero SA, Buck TM, Ely MR. Blood pressure regulation X: what happens when the muscle pump is lost? Post-exercise hypotension and syncope. Eur J Appl Physiol [Internet]. 2013 Nov 7 [cited 2014 Feb 13]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24197081

111. Czell D, Schreier R, Rupp R, Eberhard S, Colombo G, Dietz V. Influence of passive leg movements on blood circulation on the tilt table in healthy adults. J Neuroeng Rehabil [Internet]. 2004 Oct 25 [cited 2014 Feb 13];1(1):4. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=544951&tool=pmcentrez&rendertype=abstract

112. ESA - European space agency. http://www.esa.int/Our_Activities/Human_Spaceflight/Bedrest_studies/Six_degrees_of_inclination. Six degrees of inclination.

113. White R. Weightlessness and the human body. Sci Am [Internet]. 1998 [cited 2014 Feb 24]; Available from: http://www.medicine.mcgill.ca/mnmsmi/white 1998.pdf

114. Hughes-Fulford M. To infinity ... and beyond! Human spaceflight and life science. FASEB J [Internet]. Federation of American Societies for Experimental Biology; 2011 Sep 1 [cited 2014 Feb 25];25(9):2858–64. Available from: /han/pubmed/www.fasebj.org/content/25/9/2858.full

115. White R, Averner M. Humans in space. Nature [Internet]. 2001 [cited 2014 Feb 24]; Available from: http://www.nature.com/nature/journal/v409/n6823/abs/4091115a0.html

116. Levine BD, Zuckerman JH, Pawelczyk JA. Cardiac atrophy after bed-rest deconditioning: a nonneural mechanism for orthostatic intolerance. Circulation [Internet]. 1997 Jul 15 [cited 2014 Feb 25];96(2):517–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9244220

117. Perhonen MA, Franco F, Lane LD, Buckey JC, Blomqvist CG, Zerwekh JE, et al. Cardiac atrophy after bed rest and spaceflight. J Appl Physiol [Internet]. American Physiological Society; 2001 Aug 1 [cited 2014 Feb 25];91(2):645–53. Available from: http://jap.physiology.org/content/91/2/645.full

118. Williams DR. The biomedical challenges of space flight. Annu Rev Med [Internet]. Annual Reviews 4139 El Camino Way, P.O. Box 10139, Palo Alto, CA 94303-0139, USA; 2003 Jan 28 [cited 2014 Feb 24];54:245–56. Available from: http://www.annualreviews.org/doi/abs/10.1146/annurev.med.54.101601.152215

119. Smith SM, Wastney ME, Morukov B V, Larina IM, Nyquist LE, Abrams SA, et al. Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. Am J Physiol [Internet]. 1999 Jul [cited 2014 Feb 16];277(1 Pt 2):R1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10409251

120. Schmitt D, Elmann-Larsen B. Bed-rest studies for the International Space Station. ESA Bull [Internet]. 2001 Nov [cited 2014 Feb 25];108:94–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15008207

121. Lodish H. Molekulare Zellbiologie. 4th ed. Spektrum Akademischer Verlag; 2001.

122. KCNH2 - potassium voltage-gated channel, subfamily H (eag-related), member 2. US National Library of Medicine, National Institutes of Health, Department of Health & Human Services; 2014 May 12 [cited 2014 May 14]; Available from: http://ghr.nlm.nih.gov/gene/KCNH2

123. Wang X, Hockerman GH, Green HW, Babbs CF, Mohammad SI, Gerrard D, et al. Merg1a K+ channel induces skeletal muscle atrophy by activating the ubiquitin proteasome pathway. FASEB J [Internet]. 2006 Jul [cited 2014 May 20];20(9):1531–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16723379

124. HSD11B1 hydroxysteroid (11-beta) dehydrogenase 1 [Homo sapiens (human)] - Gene - NCBI [Internet]. [cited 2014 May 14]. Available from: http://www.ncbi.nlm.nih.gov/gene/3290

125. Nair S, Lee YH, Lindsay RS, Walker BR, Tataranni PA, Bogardus C, et al. 11beta-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. Diabetologia [Internet]. 2004 Jun [cited 2014 May 20];47(6):1088–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15156315

126. Semjonous NM, Sherlock M, Jeyasuria P, Parker KL, Walker EA, Stewart PM, et al. Hexose-6-phosphate dehydrogenase contributes to skeletal muscle homeostasis independent of 11β-hydroxysteroid dehydrogenase type 1. Endocrinology [Internet]. 2011 Jan [cited 2014 May 20];152(1):93–102. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3219053&tool=pmcentrez&rendertype=abstract

127. Biedasek K, Andres J, Mai K, Adams S, Spuler S, Fielitz J, et al. Skeletal muscle 11beta-HSD1 controls glucocorticoid-induced proteolysis and expression of E3 ubiquitin ligases atrogin-1 and MuRF-1. Tomé D, editor. PLoS One [Internet]. Public Library of Science; 2011 Jan [cited 2014 May 22];6(1):e16674. Available from: http://dx.plos.org/10.1371/journal.pone.0016674

128. TRIM63 tripartite motif containing 63, E3 ubiquitin protein ligase [Homo sapiens (human)] - Gene - NCBI [Internet]. [cited 2014 May 14]. Available from: http://www.ncbi.nlm.nih.gov/gene/84676

129. Files DC, D'Alessio FR, Johnston LF, Kesari P, Aggarwal NR, Garibaldi BT, et al. A critical role for muscle ring finger-1 in acute lung injury-associated skeletal muscle wasting. Am J Respir Crit Care Med [Internet]. 2012 Apr 15 [cited 2014 May 20];185(8):825–34. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3360571&tool=pmcentrez&rendertype=abstract

130. CAV3 - caveolin 3. US National Library of Medicine, National Institutes of Health, Department of Health & Human Services; 2014 May 12 [cited 2014 May 14]; Available from: http://ghr.nlm.nih.gov/gene/CAV3

131. OMIM Entry - * 601253 - CAVEOLIN 3; CAV3 [Internet]. [cited 2014 May 20]. Available from: http://omim.org/entry/601253

132. Hagiwara Y, Sasaoka T, Araishi K, Imamura M, Yorifuji H, Nonaka I, et al. Caveolin-3 deficiency causes muscle degeneration in mice. Hum Mol Genet [Internet]. 2000 Dec 12 [cited 2014 May 20];9(20):3047–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11115849

133. NOS1 Gene - GeneCards | NOS1 Protein | NOS1 Antibody [Internet]. [cited 2014 May 20]. Available from: http://www.genecards.org/cgi-bin/carddisp.pl?gene=NOS1

134. Suhr F, Gehlert S, Grau M, Bloch W. Skeletal Muscle Function during Exercise-Fine-Tuning of Diverse Subsystems by Nitric Oxide. Int J Mol Sci [Internet]. Multidisciplinary Digital Publishing Institute; 2013 Jan 28 [cited 2014 May 13];14(4):7109–39. Available from: http://www.mdpi.com/1422-0067/14/4/7109/htm

135. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. Physiol Rev [Internet]. 2001 Jan [cited 2014 May 20];81(1):209–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11152758

136. Wang MX, Murrell DF, Szabo C, Warren RF, Sarris M, Murrell GA. Nitric oxide in skeletal muscle: inhibition of nitric oxide synthase inhibits walking speed in rats. Nitric Oxide [Internet]. 2001 Jun [cited 2014 May 20];5(3):219–32. Available from: http://www.sciencedirect.com/science/article/pii/S1089860301903489

137. HSPB3 - heat shock 27kDa protein 3. US National Library of Medicine, National Institutes of Health, Department of Health & Human Services; 2014 May 12 [cited 2014 May 14]; Available from: http://ghr.nlm.nih.gov/gene/HSPB3

138. Oshita SE. Inhibition of Caspase Activation by Small Heat Shock Protein, HspB2 [Internet]. ProQuest; 2007 [cited 2014 May 18]. Available from: http://books.google.com/books?id=ffO-9LmCzFwC&pgis=1

139. CHARACTERIZATION OF THE R7S MUTATION OF HEAT SHOCK PROTEIN HSPB3 AND OF TWO NOVEL MUTATIONS FOUND IN PATIENTS SUFFERING OF CONGENITAL MYOPATHY: UNDERSTANDING THE MECHANISMS LEADING TO DISEASE | Telethon [Internet]. [cited 2014 May 18]. Available from: http://www.telethon.it/en/funding-research/funded-projects/details/characterization-of-the-r7s-mutation-of-heat-shock-protein-hspb3-and-of-two-novel

140. National Center for Biotechnology Information [Internet]. [cited 2014 May 14]. Available from: http://www.ncbi.nlm.nih.gov/ http://www.ncbi.nlm.nih.gov/gene/4842 http://www.ncbi.nlm.nih.gov/gene/1270

141. CNTF Gene - GeneCards | CNTF Protein | CNTF Antibody [Internet]. [cited 2014 May 21]. Available from: http://www.genecards.org/cgi-bin/carddisp.pl?gene=CNTF

142. Roth SM, Schrager MA, Ferrell RE, Riechman SE, Metter EJ, Lynch NA, et al. CNTF genotype is associated with muscular strength and quality in humans across the adult age span. J Appl Physiol [Internet]. 2001 Apr 1 [cited 2014 May 21];90(4):1205–10. Available from: http://jap.physiology.org/content/90/4/1205#sec-8

143. Walsh S, Kelsey BK, Angelopoulos TJ, Clarkson PM, Gordon PM, Moyna NM, et al. CNTF 1357 G -> A polymorphism and the muscle strength response to resistance training. J Appl Physiol [Internet]. 2009 Oct [cited 2014 May 21];107(4):1235–40. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2763829&tool=pmcentrez&rendertype=abstract

144. Guillet C, Auguste P, Mayo W, Kreher P, Gascan H. Ciliary neurotrophic factor is a regulator of muscular strength in aging. J Neurosci [Internet]. 1999 Feb 15 [cited 2014 May 21];19(4):1257–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9952403

145. GDAP1 - ganglioside induced differentiation associated protein 1. US National Library of Medicine, National Institutes of Health, Department of Health & Human Services; 2014 May 12 [cited 2014 May 14]; Available from: http://ghr.nlm.nih.gov/gene/GDAP1

146. Charcot-Marie-Tooth Neuropathy Type 4A [Internet]. University of Washington, Seattle; 2013 [cited 2014 May 21]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1539/

147. SBF2 - SET binding factor 2. US National Library of Medicine, National Institutes of Health, Department of Health & Human Services; 2014 May 12 [cited 2014 May 14]; Available from: http://ghr.nlm.nih.gov/gene/SBF2

148. iHOP - Information Hyperlinked over Proteins [SBF2] [Internet]. [cited 2014 May 21]. Available from: http://www.ihop-net.org/UniPub/iHOP/gs/103618.html

149. Charcot-Marie-Tooth Disease Fact Sheet: National Institute of Neurological Disorders and Stroke (NINDS) [Internet]. [cited 2014 Jun 4]. Available from: http://www.ninds.nih.gov/disorders/charcot_marie_tooth/detail_charcot_marie_tooth.htm

150. Hortobagyi T, Dempsey L, Fraser D, Zheng D, Hamilton G, Lambert J, et al. Changes in muscle strength, muscle fibre size and myofibrillar gene expression after immobilization and retraining in humans. J Physiol [Internet]. 2000 Apr 1 [cited 2014 Jun 5];524(1):293–304. Available from: http://jp.physoc.org/content/524/1/293.full

151. J L Andersen TG-K. Bed rest increases the amount of mismatched fibers in human skeletal muscle. J Appl Physiol. 1999;86(2):455 – 60.

152. Nedergaard A, Jespersen JG, Pingel J, Christensen B, Sroczynski N, Langberg H, et al. Effects of 2 weeks lower limb immobilization and two separate rehabilitation regimens on gastrocnemius muscle protein turnover signaling and normalization genes. BMC Res Notes [Internet]. 2012 Jan [cited 2014 Jun 5];5(1):166. Available from: http://www.biomedcentral.com/1756-0500/5/166

153. Boesen AP, Dideriksen K, Couppé C, Magnusson SP, Schjerling P, Boesen M, et al. Tendon and skeletal muscle matrix gene expression and functional responses to immobilisation and rehabilitation in young males: effect of growth hormone administration. J Physiol [Internet]. 2013 Dec 1 [cited 2014 Jun 5];591(Pt 23):6039–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24081158

154. Ogawa T, Furochi H, Mameoka M, Hirasaka K, Onishi Y, Suzue N, et al. Ubiquitin ligase gene expression in healthy volunteers with 20-day bedrest. Muscle Nerve [Internet]. 2006 Oct [cited 2014 Jun 5];34(4):463–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16868939

155. Kim JW, Kwon OY, Kim MH. Differentially expressed genes and morphological changes during lengthened immobilization in rat soleus muscle. Differentiation [Internet]. 2007 Feb [cited 2014 Jun 5];75(2):147–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17316384