Institute of Cardiovascular Research and Sports Medicine

Department of Molecular and Cellular Sport Medicine

German Sport University Cologne

Head of Department: Prof. Dr. Wilhelm Bloch

Impact of altered muscle perfusion through lower body negative pressure on acute cardiovascular, physiological and molecular responses to resistive leg press exercise

Doctoral thesis accepted for the degree

Doktorin der Naturwissenschaft

by

Dajana Parganlija

from

Zenica, Bosnia and Herzegovina

Cologne (2021)

First reviewer: Prof. Dr. Wilhelm Bloch

Second reviewer: PD Dr. Jochen Zange

Chair of the doctoral committee: Prof. Dr. Mario Thevis

Thesis defended on: 10th August, 2021

Affidavits following §7 section 2 No. 4 and 5 of the doctoral regulations from the German Sport University Cologne, February 20th 2013:

Hereby I declare:

The work presented in this thesis is the original work of the author except where acknowledged in the text. This material has not been submitted either in whole or in part for a degree at this or any other institution. Those parts or single sentences, which have been taken verbatim from other sources, are identified as citations.

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

10.09.2021 

Date, Signature

Table of contents

List of abbreviations

AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ARED	Advanced resistive exercise device
ATP	Adenosine triphosphate
EMG	Electromyography
HGF	Hepatocyte growth factor
iRED	Interim resistive exercise device
ISS	International space station
LBNP	Lower body negative pressure
MHC	Myosin heavy chain
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
NASA	National aeronautics and space administration
NIRS	Near infrared spectroscopy
P-AMPK	Phospho-AMPK
RCL	Robotically controlled leg press
RM	Repetition maximum
RPE	Rating of perceived exertion
TPR	Total peripheral resistance
TSI	Tissue oxygen saturation index
$V'O_2$	Respiratory oxygen uptake

1. Introduction

Human physiology is finely tuned to the requirements of its natural surroundings on Earth. Thus, when humans leave their natural habitat to explore the exciting, but unaccustomed space beyond, they encounter different challenges stemming from various sources, among them the lack of gravity. Space exploration is vital not only for understanding the origins of the universe, but also for shaping our future role in it. As we strive to access ever more of the vast space beyond Earth, and to spend ever longer periods of time outside of our home planet, it is vital we ensure that we can properly cope with the physical challenges that come with these aspirations. In addition, microgravity-induced adaptations during long duration space flight, such as musculoskeletal deconditioning, bare similarities to those that may occur with aging or disease (Hargens et al. 2013). Understanding the underlying mechanisms would therefore not only further the development of effective countermeasures enabling a prolonged stay in space, but might also broaden the general understanding of biology and promote the development of new medical technologies to address some of the issues humans face on Earth, such as disuse-induced atrophy in bed-ridden patients or sarcopenia in older individuals (Narici and Boer 2011).

1.1 Physiological challenges during space flight

Space flight involves exposure to an environment with a particular constellation of factors including microgravity, exposure to radiation, 90-min light/dark cycles and confinement that induce a myriad of adaptive responses affecting practically all aspects of human physiology, among them musculoskeletal, cardiovascular, immune, metabolic and central nervous system effects (Laurens et al. 2019, Aubert et al. 2005, Lang et al. 2017). Focus of the present thesis lies primarily on adaptive responses relevant for muscle deconditioning due to weightlessness, with highlighting established or potential impact on astronaut wellbeing and performance, the key prerequisites of any safe and successful space mission.

Background and impact of microgravity-induced fluid shift

A particular challenge astronauts face during space flight is the redistribution of fluids toward their upper body that occurs due to the lack of gravity (Hargens et al. 2013, Hargens and Richardson 2009, Parganlija et al. 2019, Parganlija et al. 2020, Aubert et al. 2005, Baker et al. 2008). In upright posture on Earth, blood pressure increases progressively from head to foot due to the hydrostatic pressure gradient, with the mean arterial pressure of the head at 70 mmHg increasing to approximately 100 mmHg at the level of the heart and further to 200 mmHg at the feet (Hargens and Richardson 2009). Gravitational influence and hydrostatic pressure being absent in space consequently leads to a redistribution of blood within the arteriovenous system solely based on the compliance of the vessels involved, effectively causing a headward fluid shift and evening out the blood pressure levels throughout the body (Baisch 1993, Hargens and Richardson 2009). This phenomenon results in a reduced blood supply to the lower limbs, leaving the legs appearing thinner and facial features more swollen compared to the situation under ordinary gravity conditions. Crew members report in this regard a feeling of fullness in the head and associated discomfort, which particularly affects the area behind the eyes and the maxillary and frontal sinus, and whose subjective perception reportedly resembles that of hanging upside down on Earth (Baker et al. 2008). The sensation is reported to occur within a few minutes to a few hours of entering weightlessness and can persist for a period of

few hours to several days. Based on the changes in tissue thickness of the tibia and the forehead, it was estimated that the fluid shift from the superficial tissue of the lower limbs amounts to around 200 ml, out of which 50 ml accumulate in the tissues of the head otherwise kept largely free of fluids by the influence of gravity (Aubert et al. 2005). However, the overall fluid shift from the lower to the upper body induced by weightlessness amounts to approximately 2 liters (Garrett-Bakelman et al. 2019). Redistribution of fluids is caused by the absence of the gravity component of hydrostatic forces in the vascular system, leading to a constant shift in the balance between the hydrostatic and oncotic pressure gradient, which determines the fluid passage across the capillary endothelium (Baisch et al. 2000b). In addition, range of cardiovascular control reduces through exposure to microgravity. Cardiovascular adaptations include an initial decrease in central venous pressure below the level detected at supine body position on Earth, due to microgravity-induced expansion of the thorax leading to a decrease in interpleural pressure (Norsk 2020). Transmural central venous pressure is consequently increased, indicating elevated cardiac preload. Initial increase in stroke volume and cardiac output relative to upright posture on Earth by approximately 18-26% during the first few weeks of space flight expands to 35-56% during the subsequent months of a space mission. Mean arterial pressure decreases, suggesting lower systemic vascular resistance. Observed systemic vasodilation is, however, not rooted in altered sympathetic nerve activity, as the latter in not suppressed under microgravity. Prolonged occurrence of the fluid shift during long duration space flight is apparently associated with increased retinal thickness sometimes leading to optical disc oedema. Other associated factors of concern have also been observed, such as ocular and cerebral structural changes, left atrial size increase and reduced flow accompanied by thrombi formation in the left internal jugular vein.

In addition, plasma volume is known to decrease during space flight (Baisch et al. 2000b). Reduction of plasma and blood volume falls in the range of approximately 10-15% (Watenpaugh 2001). Impaired functioning of large muscle groups has been suggested as a contributing factor in this regard, as the return of water to the vascular compartments is partially mediated by the massaging action of contracting muscles facilitating the lymph flow (Aubert et al. 2005). The cardiovascular system copes with this reduced vascular volume by increasing peripheral resistance directly following the return to Earth. This adaptive response is not always able to fully compensate for the volume loss; approximately 30-50% of astronauts will suffer from orthostatic intolerance when standing up for the first time after landing back on Earth, with the estimated proportion even amounting to 64% depending on the literature source and exact definition of orthostatic intolerance (Baisch et al. 2000b, Aubert et al. 2005). One of the potential hazards associated with occurrence of orthostatic intolerance is impaired evacuation from a spacecraft, especially in case of an emergency following a difficult landing. Orthostatic intolerance does not, however, only affect astronauts upon their return from space. It is a more widespread issue that impacts the general population as well, so understanding its causes and providing remedies would have wide medical significance. In addition, reduced performance due to muscle deconditioning further contributes to the potential risks of evacuation from a spacecraft. Impaired performance is illustrated by the example of a crew recovery from a Soyuz re-entry vehicle in Kazakhstan following a 5 ½ - month space mission, in which safety concerns dictated that the crew leave the landing capsule unassisted. The evacuation, which should have been a 30 min task, took the crew 5 h to accomplish due to severely impaired performance (Stein 2013).

Musculoskeletal deconditioning in space

Space flight leads to a loss in muscle force and power, and an increase in muscle fatigue and abnormal reflex patterns (Aubert et al. 2005, Lang et al. 2017, Fitts et al. 2000). Providing adequate loading, which is essential for maintaining musculoskeletal functionality, represents a substantial challenge in the space flight environment. Relative unweighting experienced by various skeletal muscles in space results in structural and functional adaptations proportional to the respective loading reduction (Adams et al. 2003). Along with the above-described blood redistribution impacting the delivery of oxygen and nutrients, skeletal muscles are faced with a difficult challenge that leads to structural alterations and a loss of muscle mass, particularly in the lower limbs. Even after short duration space missions, astronauts often experience muscle fatigue, weakness, impaired coordination during movement and muscle soreness (Stein 2013).

Skeletal muscle deconditioning in space has been a concern since the early Gemini, Soyuz and Skylab missions (Convertino 1990, Narici and Boer 2011, Baker et al. 2008, Shackelford 2019). Within the Apollo program as well as the Russian space flight missions, many of the basic responses to space flight have been identified that remain at the core of human adaptation to weightlessness, such as plasma volume reduction and decreased postflight orthostatic and exercise tolerance (Baker et al. 2008). Given the substantial impact of these responses, the goals of subsequent missions were expanded to further characterizing the mentioned findings, uncovering the underlying mechanisms and developing protective countermeasures that would enable the duration of space missions to be safely extended. Systematic evaluation of skeletal muscle adaptations related to space flight began with the Skylab missions in the 1970s, which represented the first three US long duration space flight missions lasting approximately between 28 and 84 days and testing initial aerobic and resistive exercise countermeasures (Adams et al. 2003, Baker et al. 2008). By mid 1970s, Russian and American space missions yielded the knowledge that humans could live and work effectively under weightlessness for up to 3 months, and that even longer space missions are feasible provided that adequate countermeasures become available (Baker et al. 2008). Though much progress has been accomplished since, exact form of these appropriate countermeasures remains the subject of ongoing research, particularly as space flight duration increases.

Beyond the obvious hazard of impaired performance in common tasks associated with space flight, skeletal muscle deconditioning bares further, profound risks due to its potential impact on the homeostasis of other organ systems, e.g. circulatory function related to blood pressure regulation or the integrity of soft and hard connective tissue, such as tendon and bone (Adams et al. 2003). A study involving 26 MIR cosmonauts revealed that one month of space flight induces bone mineral density loss of a magnitude typically observed in postmenopausal women over a one year period (Lang et al. 2017). Bone mineral loss primarily affects weightbearing bones of the lower extremities, lower pelvis and lumbar spine (Baker et al. 2008). Particular concern is raised by the observation that some astronauts achieve only a 50% restoration of bone mass even 9 months into their return from space (Davis and Davis 2012). Full recovery of the lost bone mass was achieved by most MIR astronauts within 3 years after the completion of their long duration space missions (Shackelford 2019).

In a review published in 2011, Narici and Boer stated based on results from multiple studies on the effect of actual and simulated microgravity on muscle size that, irrespective of the type of in-flight countermeasure, losses of lower limb muscle cross-sectional area, or volume, of between 6% and 24% should be expected for space flight durations spanning from 8 to 197 days (Narici and Boer

2011). Even short-duration space flight has a substantial impact on muscle volume and strength, despite the use of exercise countermeasures, which are, for example based on NASA flight rules, obligatory on missions lasting longer than 10 days (Lang et al. 2017, Adams et al. 2003). Space flight in duration of 8 days already induces a 6% decline in the quadriceps muscle volume as determined by MRI (LeBlanc et al. 1995). Following a 17-day space flight, 10% decrease in muscle strength was determined through various tests of the knee extensor muscle function (Tesch et al. 2005). In addition, the cross-sectional area of the knee extensor muscles, which also include the vastus lateralis muscle, was found to have decreased by 8%. Data from short-term missions ranging from 8 to 17 days generally demonstrate a 5-15% decline in the cross-sectional area or volume of muscles from the quadriceps group (Adams et al. 2003). Furthermore, it has been established that this decline affects the size of all fiber types in the vastus lateralis muscle after 11 days of space flight, the decline being most prominent in the type IIb, followed by type IIa and type I fibers, with a determined respective size reduction of 36%, 23% and 16% (Edgerton et al. 1995). Within the same study, the postflight biopsies of vastus lateralis were also found to have 6-8% fewer type I fibers as well as a 24% lower number of capillaries per fiber with the overall number of capillaries in the muscle tissue cross-sectional area having remained unchanged. The slow-to-fast shift in myosin isoforms might have compensatory significance, as it can minimize the functional consequences of muscle atrophy, such as the reduction in maximal power, at moderate-to-high velocities (Adams et al. 2003). Different levels of impact on various muscle fiber types are possibly attributable to their size (e.g., type IIa fibers are slightly larger than type I fibers), as a greater pre-flight fiber size is apparently consistently linked to a greater degree of atrophy post-flight (Fitts et al. 2000, Edgerton and Roy 1994). This is further supported by a study following a 17-day space flight, in which the greatest type I fiber atrophy was observed in the astronaut with the largest pre-flight average fiber size (Widrick et al. 1999).

As the muscle volume loss varies between astronauts, so too does the decline in muscle strength. Data of 17 crew members from Space Shuttle missions lasting up to 16 days demonstrate for example an average 12% loss in the concentric and 7% loss in the eccentric muscle strength of the quadriceps, which was the third most affected muscle group after back and abdominal muscles (Baker et al. 2008). However, a substantially higher impact can occur even if exposure to weightlessness is extended by only a few days, as one astronaut was shown to have developed a lower limb maximal power decrease of 54% after 21 days of space flight (Antonutto et al. 1998). Similarly to the bone mineral loss described further above, loss of muscle strength also predominantly affects muscles of the trunk and the lower body, which have a role in maintaining posture and are otherwise active under regular gravity conditions (Baker et al. 2008, Lang et al. 2017, Stein 2013, Fitts et al. 2000). The impact of weightlessness can conceivably progress with longer exposure, as also illustrated by the example at the beginning of this paragraph, though the magnitude of change established in different studies on space flight somewhat varies depending on the target muscle and exact nature of the conducted tests. Greatest muscle loss generally occurs early on during space flight, after which the decline continues at a lower rate (Stein 2013). Data from Skylab 2, Skylab 3 and MIR indicate that the decline in muscle torque initially occurs more rapidly in the lower limb extensors compared to flexors, with the reduction balancing out over time and minimizing by 4 months of space flight (Fitts et al. 2000). Nevertheless, the overall magnitude of strength loss affecting crew members can be substantial. Measurements five days into the return from a 28-day and a 56-day Skylab mission uncovered a 20% decline in the strength of the knee flexors (Adams et al. 2003). In addition, longer space flight of 90-180 days was found to induce a 17% decrease in maximal isometric torque of the plantar flexors,

accompanied by a 39% reduction in maximal muscle activation evaluated during isokinetic tests (Lambertz et al. 2001). Postflight recovery of muscle force is also related to the duration of the respective mission, e.g. peak force of both legs was shown to recover 6 days into the return from a 31-day space flight, whereas a peak force reduction of 12-22% was still present 26 days after missions with a duration of 169-180 days (Fitts et al. 2000).

Exercise devices available to astronauts onboard the ISS for countermeasure activities include a cycle ergometer, treadmill and a resistance exercise device (Petersen et al. 2016). Resistance exercise was originally performed on the interim resistive exercise device (iRED), which provided loads ranging from 5 to 136 kg. This device was replaced by its successor, the advanced resistive exercise device (ARED) in 2009, whose loads range between 2.2 and 272 kg. With the introduction of ARED, relative contribution of resistance exercise to overall in-flight exercise countermeasures increased from 33% to 46%, achieving a more balanced approach between resistive and cardiovascular exercise countermeasures. Beyond those primary sources of resistance exercise, one of the cycle ergometers aboard the ISS was also equipped with force generating, motor-driven cords that provided loads of up to 30 kg. Apart from its obvious role in counteracting musculoskeletal deconditioning, regular training is also known to increase blood volume and might therefore be beneficial for mitigating the volume loss during space flight and consequently protecting exercise capacity, as indicated by results from bed rest studies (Watenpaugh 2001). Furthermore, it has been suggested that an ideal program to counteract muscle deconditioning should include high-intensity concentric and eccentric exercise (Fitts et al. 2000).

However, the issue of muscle deconditioning in space could not be adequately addressed with the exercise countermeasures available thus far. Longer missions lasting 16 to 28 weeks lead to a 12% reduction in the quadriceps muscle volume, with other lower limb muscles being even more affected (LeBlanc et al. 2000). Astronauts with a 6 month stay aboard the ISS showed a significant decline in calf muscle mass and performance despite having utilized the available exercise countermeasures (Trappe et al. 2009). Although the exercise regimen somewhat varied among the astronauts, they all accomplished multiple hours of exercise per week (approximately 5 h/week of moderate intensity aerobic exercise and 3-6 days/week of resistance training incorporating multiple types of lower leg exercise). Nevertheless, their calf muscle volume decreased on average by approximately 13%, accompanied by a reduction in peak power of 32% and 20-29% decline in force-velocity characteristics across the velocity spectrum. Force-velocity relationship is indeed a particularly important determinant, as it illustrates the functional capacity of a muscle across a broad spectrum of loading conditions and not only dictates the maximal force that can be generated at any given shortening velocity but also defines the limit of work and power that can be produced under any loading condition (Adams et al. 2003). Another previously mentioned typical impact of unloading was also confirmed by this study, namely a slow-to-fast fiber type transition detectable as a 12-17% shift in the myosin heavy chain (MHC) phenotype. Although data from one of the later Skylab missions, Skylab 4 suggest that enhanced exercise and dietary measures can partially ameliorate the decline in muscle strength and volume, these issues nevertheless remained insufficiently resolved, particularly in terms of longer duration space flight, as also corroborated by data from the MIR missions. Decline in plantar flexor muscle volume was found to range between 6% and 20% subsequent to the MIR missions, with one of the cosmonauts having spent a month and three other cosmonauts 6 months in space (Zange et al. 1997). While maximum voluntary contraction was not affected by the one-month mission, its relative decrease following 6 months of space flight amounted

to 20-48% and thereby exceeded the decrease in muscle volume. In the same sample, maximal explosive power of the lower limbs determined using force platforms was shown to have reduced to 67% after the one-month mission and to 45% after 6 months of space flight, while the maximal cycling power decreased to 75% in both cases (Antonutto et al. 1999). Interestingly, contraction velocity has consistently been shown to increase both after short- and long duration space flight, partially compensating for the loss of muscle mass in order to preserve muscle power (Baker et al. 2008). Furthermore, a study of 37 ISS crew members with an average flight length of 163 days, out of whom 2/5 had used ARED, also revealed a significant reduction in isokinetic strength of between 8% and 17% across the entire sample (English et al. 2015). Although a trend was observed toward lower mean strength loss in the ARED subpopulation, the difference was not statistically significant. The authors concluded that the isokinetic strength data from the first 10 years of long duration space flight onboard the ISS show moderate strength loss in the locomotor and postural muscles of the knee, ankle and trunk, even with the available exercise countermeasures.

Despite indications of a positive development in terms of partially maintained bone mass upon ARED use in combination with adequate energy intake, skeletal muscle deconditioning thus remains a challenge yet to be fully tackled, which is particularly important when considering long duration space missions, such as travelling to a different planet (Thornton and Bonato 2017, Narici and Boer 2011, Aubert et al. 2005, Adams et al. 2003, Lang et al. 2017, Smith et al. 2012). Risk of developing osteoporosis due to extended exposure to weightlessness and partial gravity associated with an exploration mission to Mars (6 months of single direction space flight and 18 months on the Martian surface) has for example been raised as a substantial concern, since unabated bone loss can reduce bone density to such an extent that architectural changes occur and the loss cannot be fully recovered (Shackelford 2019). In addition, decline of muscle strength and endurance could also prove severely limiting and possibly hazardous during geological exploration of Mars.

1.2 Molecular adaptations in space flight and its ground-based models

Although more than 500 humans have flown into space since Yuri Gagarin's flight in 1961 and considerable knowledge has been gained in space physiology, the exact mechanisms behind microgravity-induced muscle atrophy still need to be fully elucidated (Narici and Boer 2011, Aubert et al. 2005). It has thus far been established that adaptation of metabolic and mechanical properties of muscle in response to space flight can largely be attributed to a loss of protein content and altered relative proportion of muscle proteins particularly affecting muscles with an antigravity function under normal loading conditions (Edgerton and Roy 1994). Primary mechanism for the loss of protein content seems to be a decline in protein synthesis (Fitts et al. 2000). This notion is supported by the finding of a 45% reduced whole body protein synthesis rate following prolonged exposure to weightlessness in a duration of more than 3 months onboard the MIR, which was accompanied by estimated equivalent reduction of whole body protein breakdown (Stein et al. 1999, Fitts et al. 2000). On the molecular level, acute decrease in the circulating levels of a number of free amino acids has been described that is suggested to contribute resources for muscle tissue and possibly also blood oncotic pressure recovery (Kashirina et al. 2019). This acute response is not yet fully understood, but apparently involves a fine balance of proteolytic enzyme regulation, as an increase of proteases as well as their inhibitors was described within this context. Furthermore, there are indications of a fiber type specific change in energy substrate utilization induced by space flight, as an apparent increase in myofibrillar ATPase activity only in type II but not in type I fibers of the vastus lateralis muscle

suggests (Edgerton et al. 1995). Activity of glycerol-3-phosphate dehydrogenase, an enzyme involved in oxidative metabolism, was concomitantly found to be increased by approximately 80% in type I fibers. On the other hand, the ratio of myofibrillar ATPase to succinate dehydrogenase, another oxidative enzyme, was found to be higher in type II fibers following space flight, indicating that fast fibers are more susceptible to fatigue after exposure to weightlessness. These findings are supported by further research into biopsies of other lower limb muscles, which also determined an increase in oxidative enzymes following space flight, with or without a concomitant increase of glycolytic enzymes, depending on the muscle involved (Fitts et al. 2000).

Due to a limited capacity for in-flight assessments, most research into the metabolic and molecular impact of space flight is limited to comparing the pre- with the postflight status. Changes potentially occurring solely during space flight and any timeline that might characterize them would thus inevitably be overlooked. Furthermore, physical challenges associated with the return flight and readaptation to the gravitational influence might potentially distort the research results. For instance, it has been suggested that loading of hypotrophic muscles during the acute period of readaptation to terrestrial gravity initiates an inflammatory response and activation of angiogenic processes (Kashirina et al. 2019). Recently, however, important progress was achieved within this research area. In its study involving various measurements pre, during and post space flight, NASA assessed one of its astronauts on a 340-day mission onboard the ISS (Garrett-Bakelman et al. 2019). An extracellular flux assay performed within this study by treating muscle cells with plasma obtained from the astronaut suggested an in-flight increase in ATP-linked respiration and a decrease of non-mitochondrial respiration, not accompanied by any changes in maximum respiration. Increase in the lactic acid / pyruvic acid ratio determined by urine metabolomics also indicated a shift from aerobic to anaerobic metabolism during the mission. In-flight gene expression of blood cells showed altered patterns in pathways related to oxygen and reactive species metabolic processes, mitochondrial transport, hypoxia and apoptotic mitochondrial changes. Although a larger sample would be required to confirm and further characterize the apparent metabolic changes, these results provide valuable first insights into the complex molecular responses induced during prolonged exposure to microgravity.

Different ground-based models, such as head-down tilt bedrest, dry immersion or lower limb immobilization, are able to successfully simulate some of the challenges encountered during space flight in terms of blood redistribution and altered muscle loading. These types of simulation also induce adaptive responses similar to the ones observed in astronauts. For instance, prolonged unilateral leg immobilization of more than 30 days was shown to diminish mean quadriceps fiber volume by 10.6% and reduce the muscle thigh volume by 8.3% (Gibson et al. 1987). Reduced rate of protein synthesis is also an adaptive response observed in different ground-based models including bed rest and leg immobilization (Ferrando et al. 2002, Gibson et al. 1987, Fitts et al. 2000). Bed rest has been used as an analogue of microgravity-associated unloading since the 1960s and is known to facilitate bone and muscle loss similar in pattern to ones induced by space flight, but differing in magnitude, since bed rest only alters the gravitational influence without completely abolishing the associated loading (Shackelford 2019). Proteomic analysis revealed common factors in the adaptational responses induced by space flight and two of its ground-based models, head-down tilt bed rest and dry immersion, with some of the factors involved in regulation of proteolysis, immune and stress response (Brzhozovskiy et al. 2019). Nevertheless, the intricacies of the molecular mechanisms underlying these adaptive responses remain unknown. Understanding these mechanisms

would support the optimization of current countermeasure programs for astronauts and could even steer the way for the development of new countermeasures. Ongoing research into simulated microgravity and countermeasure interventions therefore remains essential for any possible, future expansion of space exploration efforts.

1.3 Use of LBNP in countermeasures for muscle loss in space

Mode of action and relevant aspects of LBNP application

Lower body negative pressure (LBNP) is defined as the application of subatmospheric pressure to the lower portion of the body in order to pool blood in the lower extremities (Campbell and Charles 2015). Level of applied negative pressure can range between 5 mmHg and 100 mmHg, and the duration of exposure can vary between several minutes to hours. In light of the associated fluid shift toward the lower limbs, LBNP enables simulating the cardiovascular and physiological effects of gravity, and has previously been used to assess the function of the cardiovascular system before, during and after space flight (Baisch et al. 2000a, Hargens et al. 2013, Hargens and Richardson 2009, Baisch et al. 2000b, Baker et al. 2008). Exposure to LBNP causes a marked increase of blood volume in the lower body that correlates with the ensuing increase in leg volume (Baisch et al. 2000b). Highly elastic veins located between the muscles are filled and blood plasma filtered into the interstitial space. Plasma is most probably collected in the lymphatic system of the skin and musculature, which is also expanded under LBNP. Exposure to 40 mmHg LBNP was found to be sufficient to force filtration into the interstitial space and was estimated to redistribute an equal amount of blood (i.e., filter the same amount of plasma) as a transition from supine to upright body position (Baisch et al. 2000b). Estimated reduction of central blood volume at this particular level of LBNP amounts to 400 ml. LBNP of 40 mmHg also induces responses in the central circulation similar to those during upright standing, with one notable exception: while LBNP uniformly reduces venous pressure in the exposed regions, upright posture leads to a linear rise in venous pressure with increasing distance along the longitudinal axis of the body.

The principal physiological response to LBNP, and the underlying reason for its abovementioned effects, is reduced pressure in the exposed tissue which results in an elevated transmural pressure gradient across the vessel walls, enhances local vascular volume and increases filtration in accordance with the applied level of LBNP and the duration of exposure. Different vascular and tissue compartments are involved successively with increasing levels of LBNP (skin, musculature, lymphatic system, venous system, capacitance vessels and finally the microcirculation of the terminal flow). Blood shift to the lower extremities reduces venous return and cardiac filling, and consequently leads to various cardiovascular responses (Baisch et al. 2000b). Prolonged exposure to LBNP therefore warrants monitoring for signs of imminent syncope, which is characterized by a decrease in blood pressure and heart rate (Goswami et al. 2019a). Fluid deficit in the upper body is detected by its vascular pressure and stretch receptors triggering a systemic response and consequently serves as a test of orthostatic intolerance (Campbell and Charles 2015). Approaching the threshold of orthostatic tolerance leads to unstable circulation with fluctuations in heart rate and arterial blood pressure (Baisch et al. 2000b). Treating astronauts in space and presumably also bedridden patients on Earth with LBNP would expectedly redistribute plasma into the interstitial space and hence recover the interstitial volume in the lower extremities necessary for maintaining the dynamic range of cardiovascular control in upright posture. Indeed, LBNP has been shown to maintain plasma

volume and prevent orthostatic intolerance in bed rest trials lasting up to one month (Campbell and Charles 2015). Moreover, inflight LBNP response predicted the individual degree of postflight orthostatic intolerance in crew members of Skylab and Soviet missions. It is nevertheless important to note that there is substantial interindividual variability regarding the responses to LBNP, largely attributable to differences in the elasticity of tissue, skin and vessel walls, and consequently the blood volume shifted during exposure to LBNP, i.e. the same level of LBNP can displace different blood volumes depending on the individual venous tone (Baisch et al. 2000b). Certain gender differences have also become apparent, as women were reported to have lower tolerance of higher levels of LBNP compared to men, possibly due to lower total peripheral resistance as well as lower adrenergic response to orthostasis (Campbell and Charles 2015, Waters et al. 2002).

LBNP utilization in medicine and space flight

Application of negative pressure has a long history within the medical field (Baisch et al. 2000b). Exposing distinct areas of skin to subatmospheric pressure dates back to the year 1692. Clinical experience with the method was presented before the Academy of Science in Paris in 1834 and it subsequently enjoyed great popularity and widespread use throughout Europe and America in the treatment of a variety of medical conditions as well as for inducing syncope occasionally used for surgical anesthesia. Further interest in terms of physiological research was sparked in the 1960s and has since continued to grow, especially in the area of aerospace medicine due to the vast potential for training and conditioning in the context of space missions. Furthermore, in contrast to other methods such as the tilt table, cardiovascular responses induced by LBNP are independent of the Earth's gravitational field, making it particularly attractive for in-flight applications. First scientific paper on LBNP was published in 1965 and showed LBNP to be an excellent test for orthostatic intolerance, with advantages over the tilt table in terms of more controlled application without direct impact on the cerebral blood flow (Campbell and Charles 2015). Since then, LBNP has been used as an experimental tool for studying hemodynamic and neurohormonal responses following central hypovolemia as well as pharmacological interventions, and beyond that serves as an investigative as well as cardiovascular training tool for deconditioning induced by bed rest or space flight (Goswami et al. 2019a). Outside of its use within different research areas, LBNP has also proven to be valuable for various applications within the medical field, e.g. for assessing the autonomic system function in patients or as a test for screening pilots.

First prototype of an LBNP device designed for research related to space flight was reportedly built in the early 1960s by Dr. Duane Gavelin (Goswami et al. 2019a). Initial use of LBNP in the context of space missions involved routine pre- and postflight testing with graded LBNP up to the level of 50 mmHg within the Apollo Program (Campbell and Charles 2015). It was introduced due to the possibility of incremental application as well as easier standardization compared to tilt testing. Inflight application of LBNP followed within American as well as Soviet space missions (Baisch et al. 2000b, Campbell and Charles 2015). The Skylab Program utilized the same LBNP test protocol as the Apollo missions, expanding it to inflight use in addition to pre- and postflight testing (Campbell and Charles 2015). Within the Skylab missions, LBNP was primarily utilized for monitoring microgravity-induced deconditioning (Baisch et al. 2000b). Venous vessels of the lower limbs being more depleted due to the microgravity-induced fluid shift allows greater blood pooling during exposure to LBNP under weightlessness, which manifests as a notable increase of astronauts' leg volume; it was established that the inflight volume increase under LBNP exceeded that of its pre-

and postflight application (Campbell and Charles 2015, Baisch et al. 2000b). LBNP was also used during the Shuttle Program for investigating inflight cardiovascular responses as well as a countermeasure for postflight orthostatic intolerance (Campbell and Charles 2015). Within the Soviet missions, LBNP was rather used for substituting the missing gravitational load and thus counteracting the adverse circulatory effects of weightlessness (Baisch et al. 2000b). Soviet missions were the first to include inflight use of LBNP, with the introduction of the Veter LBNP device on Salyut-1 in 1971, two years prior to the first manned Skylab mission (Campbell and Charles 2015). Subsequently, the so called "Chibis suit" was developed and used to apply negative pressure to the lower body below the iliac crest (Baisch et al. 2000b). The German Aerospace Center has also been involved for a number of years in developing an LBNP device for use onboard the ISS. Initial design involved the application of LBNP at rest with the possibility of performing various physiological measurements. The robotically controlled leg press employed within the present thesis represents an advanced model enabling LBNP to be combined with exercise. Its design was inspired by the work of Watenpaugh et al., who created a vertical treadmill placed within an LBNP-chamber, with negative pressure generated by a high-capacity vacuum cleaner (Watenpaugh et al. 2000). Watenpaugh et al. had also previously described a leg press-type exercise device they had developed, with self-generating negative pressure within a collapsible chamber (Campbell and Charles 2015, Watenpaugh et al. 1999). Originally intended as an orthostatic countermeasure device, this concept has subsequently been included in the facilities of the Chinese space station.

In terms of responses associated with its inflight application, LBNP has been described as causing greater heart rate increase and a similar or higher sympathetic response, with overall findings suggesting that, notwithstanding the hypovolemia they experience, astronauts still respond normally to simulated orthostatic stress and are able to maintain normal levels of arterial pressure (Ertl et al. 2002). Elevated total peripheral resistance and increased left ventricular ejection time compared to preflight values have also been described (Campbell and Charles 2015). Single subject measurements during space flight have demonstrated that the loss of interstitial fluid at the thigh can amount to roughly 40% within a 4-day exposure to microgravity and that LBNP application can refill the interstitial spaces and counteract the associated cardiovascular deterioration (Baisch 1993). Hormonal responses to LBNP also seem to either remain similar or become exaggerated under microgravity and to be followed by particular changes in hormone levels during the readaptation period following space flight (Grigor'ev et al. 1998, Hinghofer-Szalkay et al. 1993, Hinghofer-Szalkay et al. 1999).

1.4 Benefits of combining LBNP with exercise for future space flight countermeasures

Loading the lower extremities during exposure to LBNP increases muscle tone and decreases venous capacitance, thereby providing a protective effect to the fluid shift induced by LBNP (Campbell and Charles 2015). For instance, use of the Chibis suit is due to its design associated with a certain level of lower limb muscle activity and leads to a lower increase in heart rate compared to another LBNP device utilized in the early American space missions, in which a saddle supports the user and abolishes any muscle contraction associated with sliding to the bottom of the LBNP chamber. In terms of its combination with exercise, LBNP has proved beneficial in several studies involving different methods of simulating the fluid shift otherwise occurring under microgravity, e.g. supine body position or head-down tilt bed rest. Use of LBNP combined with exercise is a proposed

countermeasure against deconditioning in long-duration space flight (Murthy et al. 1994). Combination of LBNP and exercise during space flight would be expected to produce Earth-like musculoskeletal loads while also providing the necessary cardiovascular stimulus to maintain adaptation as found under Earth's gravity.

Beneficial effects of LBNP superimposed on exercise include replication of the physiological responses (oxygen consumption and heart rate levels) and ground reaction forces of upright gait during supine walking and running on a vertical treadmill (Boda et al. 2000). LBNP also enhanced performance during incremental load supine dynamic leg exercise until exhaustion and was determined to be a suitable model for upright exercise (Eiken 1988). In addition, various benefits of exercise combined with LBNP were found in bed rest studies. Daily supine LBNP treadmill exercise was shown to maintain key exercise fitness parameters including peak oxygen consumption at the levels recorded prior to head-down tilt bed rest (Watenpaugh et al. 2000). Countermeasure regime for bed rest-induced deconditioning involving supine treadmill exercise with LBNP and maximal concentric and eccentric supine leg- and calf-press exercise was found to be beneficial for knee extensor strength and endurance, ankle strength and leg lean mass (Lee et al. 2014). Beyond its advantages for orthostatic tolerance, treadmill exercise with LBNP also benefits lumbar spine compressibility and back muscle strength, and decreases bed rest-induced bone loss (Macias et al. 2007, Smith et al. 2003, Watenpaugh et al. 2007).

Previous research regarding LBNP superimposed on exercise has primarily focused on dynamic or endurance-type exercise. Resistance exercise, however, also presents an interesting option to explore, as it stimulates muscle hypertrophy and angiogenesis and has not only been shown to maintain strength and prevent atrophy of chronically unloaded lower limb muscles, but also to have the potential of promoting their hypertrophy (Parganlija et al. 2019, Tesch et al. 2004, Holloway et al. 2018, Hargreaves and Spriet 2020). Indeed, results of various ground-based studies illustrate the benefits of resistance exercise for maintaining bone and muscle mass. For example, resistance exercise has been shown to maintain muscle protein synthesis rate and prevent muscle atrophy induced by bed rest (Alkner and Tesch 2004, Narici and Boer 2011). High-load exercise was shown to successfully prevent bone loss in the risk group of postmenopausal women, while plyometric and lower body resistance exercise were shown to increase the bone mineral density in premenopausal women (Davis and Davis 2012). Intense resistance exercise was also shown to counteract the bone mineral density loss otherwise occurring during 17-week horizontal bed rest and was thus concluded to be a potentially useful countermeasure for the prevention of deleterious skeletal changes associated with extended exposure to weightlessness (Shackelford et al. 2004). High-load resistance exercise with LBNP might therefore prove particularly beneficial in the development of countermeasures for future long duration space flight. Exact degree of musculoskeletal loading will be a relevant factor to explore, as it represents a direct determinant of muscle strength, mass and morphology (Shackelford 2019). It is important to note that neither constant compression using a full body-loading suit, otherwise known as the "penguin" suit, nor light to moderate resistance exercise appear to be effective countermeasures for deconditioning during bed rest or space flight (Shackelford 2019). Taken together, in light of the previously described impact of weightlessness on lower limb muscles, the positive effects of resistance exercise and the benefits of superimposing LBNP on lower body exercise, the present thesis focuses on high-load resistive leg press exercise combined with LBNP, which to the author's knowledge has so far not yet been explored from a biomedical perspective.

Relevant determinants of the exercise protocol in the present thesis

When examining the perfusion effects of exercise with LBNP, it is important to consider that exercise itself also impacts muscle blood flow, with periods of high forces during muscle contractions generally promoting ischemia and subsequent relaxation restoring muscle perfusion. This effect is attributable to the rise in intramuscular pressure through muscle contractions, leading to a compression of venous vessels (Laughlin 1987). Rhythmic contractions consequently generate a pumping action resulting from fluctuations in the pressure exerted onto the venous vessels, which facilitates venous outflow during a contraction and refill through arterial inflow during subsequent muscle relaxation. Contracting muscles therefore enter a somewhat paradoxical situation, as increasing force not only facilitates the oxygen demand, but also elevates intramuscular pressure that hinders blood flow, particularly during shortening contractions (Hendrickse and Degens 2019). Elevated intramuscular pressure can even result in muscle deoxygenation during contractions, which has been shown to occur with as little as 25% of maximal torque capacity in isometric contractions of the vastus lateralis (Ruiter et al. 2007).

Under appropriate exercise conditions, however, muscle perfusion can also benefit from the muscle pump activity (Parganlija et al. 2019). Indeed, the muscle pump has previously been suggested as a contributing factor to the early increase in skeletal muscle blood supply known to occur less than 5 s after the onset of exercise (Sarelius and Pohl 2010). Muscle blood flow during dynamic exercise is ultimately determined by a combination of factors including skeletal muscle vascular conductance, the perfusion pressure gradient and the efficacy of the muscle pump (Laughlin 1987). Within this constellation, muscle contractions have the potential of increasing or reducing blood flow and/or the apparent peak vascular conductance depending on the experimental setting and the exact type of muscle contractions. Certain rhythmic contractions even appear to induce blood flow and conductance greater than those observed under maximal vasodilation at rest. In an upright body position, perfusion of working leg muscles is hence facilitated by an increase in the arterio-venous blood pressure difference induced by the muscle pump in a mechanism involving a gravity-dependent pressure component on the arterial side and an outflow-induced pressure reduction on the venous side, supported by the prevention of backflow through venous valves (Parganlija et al. 2019, Laughlin and Schrage 1999, Pollack et al. 1949, Pollack and Wood 1949). Skeletal muscle perfusion increases during exercise in direct proportion to its metabolic needs, with studies involving altered arterial oxygen content suggesting oxygen delivery is the relevant determinant being matched to the metabolic demand (Mortensen and Saltin 2014). Under appropriate exercise conditions, complex regulatory mechanisms involving the muscle pump activity and vasodilation can altogether accomplish a 100-fold increase in skeletal muscle blood flow relative to resting conditions and achieve perfusion rates of 300-400 ml/(min x kg) (Mortensen and Saltin 2014, Hendrickse and Degens 2019). Tissue displacement arising from increased muscular tissue stiffness during contractions ensures lymph formation and propulsion through lymphatic vessels draining the skeletal muscles (Negrini and Moriondo 2011). The lymphatic system removes excess interstitial fluid, solutes and cells and returns them to the bloodstream, thus maintaining physiological plasma and interstitial fluid volume as well as solute concentration, and ultimately preventing tissue oedema (Negrini and Moriondo 2011, Weid 2019). Increased tissue pressure during muscle contractions reduces the net filtration forces across the capillary wall. Impact of muscle pump on the fluid balance might contribute to higher peak blood flows compared to maximal vasodilation at rest (Laughlin 1987). Lymph flow is regulated by the active (intrinsic) and passive (extrinsic) lymph pump. Active

lymph pump consists of phasic contractions of sequentially ordered chambers called lymphangions, which represent subunits of lymphatic vessels located between two adjacent lymphatic valves (Gashev and Zawieja 2016). Passive lymph pump includes various extrinsic lymph-driving factors such as skeletal muscle contractions, fluctuations in central venous pressure, respiratory movements and gravitational force influence (Gashev 2002, Gashev and Zawieja 2016, Ronald J. Korthuis 2011, Schmid-Schönbein 1990). Lymphangions also possess stretch- and shear-dependent autoregulatory mechanisms (Gashev and Zawieja 2016). At rest, approximately 1/3 of lymph transport in the lower extremities originates extrinsically from compression due to skeletal muscle contractions, and 2/3 stem from the active, intrinsic pumping of the lymph vessel network (Scallan et al. 2016). Overall, the lymph pump is subject to a complex regulatory system comprised of intrinsic, extrinsic, neural and humoral controllers, including lymph formation and flow rate, local compression, interstitial fluid pressure and inflammatory mediators (Gashev and Zawieja 2016, Kesler et al. 2013, Scallan et al. 2016, Zawieja 2009), which can be impacted by muscle contractions as well as LBNP.

Since relaxation periods involve continued arterial inflow, but do not involve femoral venous outflow, different venous segments do not refill immediately (Laughlin 1987). Indeed, a sequential refill occurs, with an initial wave directly following the contraction. Complete refill requires a relaxation period of sufficient duration, i.e. if another contraction follows before complete refill could be achieved, then venous pressure cannot recover and remains reduced. This illustrates the significance of adequate relaxation periods during exercise, which is an aspect also examined in one of the studies performed as part of the present thesis (Parganlija et al. 2019). Low-intensity resistance exercise with slow motion was shown to enhance basal femoral blood flow, promoting muscle perfusion (Tanimoto et al. 2009). Furthermore, the muscle pump also elevates central venous pressure and consequently increases cardiac stroke volume (Parganlija et al. 2019, Leyk et al. 1994, Rowland 2001, Laughlin and Schrage 1999). Fluctuations in central venous pressure in turn impact the passive lymph flow (Gashev 2002, Gashev and Zawieja 2016). Influence of the muscle pump on blood and lymph flow regulation as well as the physiological and cardiovascular effects of the given exercise protocol should hence be considered when examining potential exercise countermeasures with superimposed LBNP. Studies conducted within the present thesis therefore involve control sessions performed with identical exercise protocol under ambient pressure, to enable distinguishing between the effects of superimposed LBNP and those of the performed exercise.

Distribution of blood within the body, lymph flow and action of the muscle pump are naturally impacted by a change of body position (Olszewski et al. 1977, Petersen et al. 2014, Rasmussen et al. 2020). However, altered gravitational influence associated with a change in posture can also affect the pattern of muscle activation by neuromuscular control (Parganlija et al. 2019, Goswami et al. 2019a). In consequence, identical exercise performed at different body positions might not lead to identical muscle action, which complicates discerning the exact origin of any effects on muscle perfusion as well as their overall comparability. In light of this issue, the German Aerospace Center in Cologne developed in cooperation with the companies Sensodrive G.m.b.H (Weßling, Germany) and S.E.A. Datentechnik GmbH (Troisdorf, Germany) a robotically controlled leg press (RCL) within an LBNP-chamber, which can be used for exercise in a supine position (Parganlija et al. 2020, Parganlija et al. 2019). This unique device allows studying the effects of a blood volume shift toward the lower body, such as under orthostasis, without changing the biomechanical properties of exercise, as would occur in case of an actual change of body position. The linear drive of the RCL is controlled by an electromotor, which enables safe exercise without the need for energy storage in weights or

springs as well as a more controlled exercise environment with fully customizable force-distance profiles accommodating individual requirements. The LBNP-chamber of the RCL was designed analogously to a previously described device involving a treadmill (Watenpaugh et al. 2000). It is sealed through a neoprene skirt worn by the subjects with the seal placed around their hips, in order to maintain stable negative pressure within the chamber. Subjects' upper body rests outside of the LBNP-chamber on a backrest elevated at a 30° angle. Feet are placed on pedals attached to the linear drive, whose height is adjusted to the length of the subjects' lower extremities.

As the RCL only monitors horizontally directed forces, possible forces of other directionality were minimized in the present work by defining the individual range of motion based on a knee angle of 80° to 125° (Parganlija et al. 2020, Parganlija et al. 2019). In addition, the rotational axes of the pedals were placed under the tibiotalar joint in order to avoid loading of the plantar and dorsiflexor muscles. A screen attached to the front of the LBNP-chamber provides visual feedback to the subjects regarding their actual and target leg position, the difference in forces between both feet and the rotation angle of each foot. This feedback enabled the subjects to ensure they maintain a neutral foot angle and conduct the exercise within the required parameters. As adaptive responses to resistance training can be enhanced by reduced velocity of contractions (Schuenke et al. 2012), subjects were instructed to perform the exercise with a relatively low velocity of 8 s per repetition (4 s in each direction within the individual range of motion). Maintaining this target velocity was additionally supported by the use of a metronome and vocal instructions. The force-distance profile was designed in such a way as to limit the high force period to the middle of the contraction cycle. Specifically, the force was set to 6% of the subjects' one-repetition maximum (1-RM) at knee flexion, after which it linearly increased during the first half of concentric knee extension. The target force of 60% of the given 1-RM was reached midway through the individual range of motion and thereafter remained constant up to the terminal point of extension. The force-distance profile during the subsequent eccentric work phase consequently mirrored that of the concentric phase. Integrating the described low force period was advantageous both for exercise performance as well as for the intended research targets. On the one hand, it helped the subjects to control the movement and maintain the required velocity, as well as neutral foot position in case of a pause during knee flexion. Beyond that, it also limited the period of ischemia induced by high muscle forces and thereby somewhat contributed to promoting muscle perfusion. Varying force within an exercise protocol might have additional relevance regarding the development of countermeasures for muscle loss in space, as it has been previously reported that leg press training with a constant load could not prevent isokinetic strength losses induced by bed rest (Bamman and Caruso 2000). In accordance with previously reported suitability of this particular level of negative pressure for simulating orthostasis (described in dissertation chapter 1.3), the LBNP level was set to 40 ± 1 mmHg, starting and ending with a ramp of 4 mmHg/s. Location of the LBNP seal is known to affect the extent of central blood volume decrease, with standard position of the seal being at the iliac crest, as such application of LBNP does not result in mechanical compression of the bladder or intestine and consequently does not compromise splanchnic blood flow (Goswami et al. 2019a). This commonly utilized position of the LBNP seal was also applied in the studies conducted for the present thesis. Further details on the exercise protocol are available in the corresponding publications (dissertation chapter 2.1).

As part of this doctoral work, two studies with healthy male participants were conducted with the aim of examining the cardiovascular, physiological and molecular biological effects of LBNP superimposed on resistance exercise performed on the above-described robotically controlled leg

press. Details on their research questions, methodology and results are presented in two peer-reviewed scientific publications covered by chapter 2.1 of this dissertation.

1.5 Physiological and molecular responses to LBNP

Present work explores general cardiovascular responses to LBNP as well as physiological and molecular biological responses associated with its impact on the local perfusion of the working muscle. LBNP induces a distinct set of responses, whose occurrence and magnitude are dependent on the exact level of LBNP and the duration of exposure (Goswami et al. 2019a). Shorter exposure to LBNP (3-5 min) generally triggers a response from the autonomic nervous system, whereas longer exposure additionally involves neurohumoral responses. Short-term LBNP application is therefore advantageous for research specifically into autonomic responses to LBNP. Physiological responses to LBNP have thus far been extensively investigated both at rest as well as in connection with certain forms of exercise. Exposure to LBNP causes a reduction in venous return to the heart due to blood pooling in lower parts of the body leading to central hypovolemia (Goswami et al. 2019a). Resulting hypotensive activation of arterial and cardiopulmonary baroreflexes triggers compensatory neurohumoral-mediated increases in heart rate and vasoconstriction in order to maintain adequate arterial pressure and cerebral perfusion and thereby protect tissue oxygenation. Systemic effects of LBNP consequently include a decrease in central venous pressure accompanied by a reduction in stroke volume leading to diminished cardiac output (Eiken 1988, Parganlija et al. 2019, Baisch et al. 2000b). Decrease in central venous pressure and cardiac output not only occur in parallel but also progressively, when increasing levels of 10, 20 and 40 mmHg LBNP are applied (Berdeaux et al. 1992). Heart rate promptly increases, while the total peripheral resistance slowly rises under LBNP, thereby stabilizing the blood pressure (Berdeaux et al. 1992). These compensatory cardiovascular responses are similar to those induced by increased gravitational load (Goswami et al. 2019a). Significant increase in heart rate and decrease in pulse pressure were only observed at 40 mmHg LBNP, which indicates selective deactivation of cardiopulmonary baroreflexes down to 20 mmHg LBNP, and deactivation of both the cardiopulmonary and arterial baroreflexes at 40 mmHg LBNP (Berdeaux et al. 1992). Considering the prominent role of cardiopulmonary baroreceptor stimulation in cutaneous, muscular and renal vasoconstriction, and the impact of arterial baroreceptors on mesenteric perfusion, it is apparent that LBNP elicits a complex pattern of reactions aimed at stabilizing blood pressure (Goswami et al. 2019a). Cardiac contractility itself is, however, not impacted by LBNP (Campbell and Charles 2015).

Similarly to a transition towards upright posture, exposure to 40 mmHg LBNP also causes distinct changes in lung perfusion ultimately affecting cardiorespiratory parameters (Baisch et al. 2000b). Oxygen uptake is not impacted by such exposure to LBNP, as higher rate of oxygen removal is compensated by lower cardiac output. Nevertheless, there is a notable impact on pulmonary function in light of the caudal diaphragm shift, pulmonary hypoperfusion as well as the increase in forced vital capacity, forced expiratory volume and functional residual capacity without accompanying alterations in pulmonary compliance (Goswami et al. 2019a). Beyond elevated ventilation associated with the LBNP-induced reduction in central blood volume, sympathetically mediated vasoconstriction reduces tissue perfusion consequently leading to lower tissue oxygen content. Enhanced respiration in response to LBNP rather reflects hyperventilation due to lower central blood volume than increased requirement for gas exchange, considering the reduced end-tidal CO_2 without changes in oxygen uptake, CO_2 production, arterial oxygen saturation or blood lactate. The overall

respiratory response might represent a protective mechanism involving faster and/or deeper breathing in order to optimize cardiac filling in light of the occurring central hypovolemic hypotension. Furthermore, reduced intrathoracic pressure associated with deeper inspirations also translates into reduced intracranial pressure and thereby supports the maintenance of adequate cerebral blood flow, a mechanism which was shown to be associated with delayed onset of syncope and increased tolerance to LBNP.

In relaxed leg muscles, LBNP facilitates an increase of the tissue hemoglobin index and deoxygenated blood, suggesting an occurrence of venous blood pooling (Blaber et al. 2013, Bartels et al. 2011). Combined with supine dynamic leg exercise, LBNP was found to enhance the local perfusion pressure and consequently muscle blood flow in the lower extremities (Eiken 1988). Significant increase in the level of total and oxygenated hemoglobin in the calf muscle and improved fatigue resistance have also been described in the context of intense dynamic exercise with LBNP (Zange et al. 2008). Aerobic ATP resynthesis is known to correlate with the magnitude of skeletal muscle perfusion, i.e. the availability of oxygen in the muscle (Toussaint et al. 1996). Enhanced muscle perfusion is thus beneficial for the energy generating oxidative metabolism and might facilitate the maintenance of energy levels during prolonged activity. Present thesis therefore examines some of the above-described physiological responses to LBNP in a novel context of resistance exercise.

Molecular responses to LBNP have thus far been investigated for certain parameters including catecholamines, volume regulating hormones, and markers of renal function and blood coagulation (Parganlija et al. 2020, Baily et al. 1991, van Helmond et al. 2015, Cvirn et al. 2019, Goswami et al. 2019b, Gasiorowska et al. 2005, Maillet et al. 1996, Goswami et al. 2019a). Typical humoral responses to LBNP include increasing levels of norepinephrine, plasma renin activity and antidiuretic hormones (Baisch 1993). Exercise in upright body position on a cycle ergometer within an LBNP box has been shown to increase the plasma levels of norepinephrine and epinephrine without any accompanying changes in lactate levels (Bonde-Petersen et al. 1984). LBNP superimposed on supine exercise on a cycle ergometer also attenuated any exercise-induced increase in lactate levels, which was considered attributable to altered contribution of anaerobic metabolism towards energy production associated with pressure-dependent variations in muscle blood flow (Eiken 1987). During maximum dynamic exercise at almost horizontal body position, LBNP facilitated the resynthesis of phosphocreatine ultimately promoting fatigue resistance (Zange et al. 2008). Supine exercise on a treadmill with LBNP complemented by additional resistive exercise during head-down tilt bed rest has been shown to affect the nitric oxide signaling and proteolysis in female skeletal muscle and to promote bone formation (Salanova et al. 2008, Smith et al. 2008). Nevertheless, molecular responses to LBNP are not yet fully understood, particularly regarding its combination with resistance exercise. Above-described effects on lactate levels associated with changes in energy metabolism give rise to the question of a possible impact on the energy-sensing AMP (adenosine monophosphate)-activated protein kinase. Furthermore, the well described influence of LBNP on muscle perfusion makes angiogenic factors interesting research targets. Energy sensing and angiogenesis can both also be impacted by exercise alone, which additionally raises a relevant issue of if and how this impact is modified by exposure to LBNP. These open questions are addressed in the present work.

1.6 Molecular targets explored in the present thesis

AMP-activated kinase

AMPK is an abundant, phylogenetically conserved energy-sensing regulator activated by an increase in the cellular AMP content that can occur due to curbed ATP production under glucose deprivation or hypoxia, or through increased energy expenditure such as during muscle contractions (Parganlija et al. 2020, Winder 2001, Kahn et al. 2005, Richter and Ruderman 2009). AMPK is involved in the regulation of various cell processes affecting core functions not only of individual tissues and organs, but also of the entire human body. These processes include cell polarity, autophagy, mitochondrial biogenesis, angiogenesis, skeletal muscle function and even the systemic energy expenditure regulated by the hypothalamus (Richter and Ruderman 2009, Nagata et al. 2003, Minokoshi et al. 2004, Ouchi et al. 2005, Jäger et al. 2007, Klaus et al. 2012, Hardie 2011, Vilchinskaya et al. 2018). Dysregulation of AMPK therefore potentially plays a role in a variety of medical conditions and has been of particular interest for research into obesity, type II diabetes and the metabolic syndrome (Richter and Ruderman 2009, Winder 2001, Kahn et al. 2005). It is also suggested that AMPK substrates would constitute good candidates for muscle wasting therapy (Sanchez et al. 2012).

AMPK is a heterotrimeric kinase consisting of a single catalytic α subunit and two regulatory subunits termed β and γ (Winder 2001, Jørgensen et al. 2006, Richter and Ruderman 2009, Hardie 2011). The α subunit contains the kinase domain, which transfers a phosphate from ATP onto the target protein. Different subunits present with up to three isoforms (two in case of the α and β subunit, and three for the γ subunit). The α1 and α2 isoform of the catalytic subunit are expressed in skeletal muscle, with the α2 isoform known to be more sensitive to AMP (Winder 2001). The α3 isoform, on the other hand, is more abundant and accounts for 80% of the total AMPK activity. As its name suggests, activation of AMPK is dependent on the cellular AMP content, which increases in accordance with duration and intensity of exercise due to ongoing ATP utilization during muscle contractions (Richter and Ruderman 2009, Jørgensen et al. 2006). The underlying mechanism involves binding of AMP to the regulatory γ subunit of AMPK, causing not only allosteric activation of the kinase, but also further facilitating its activation by rendering it a better substrate for phosphorylation through upstream kinases and concurrently less accessible to phosphatases. AMP is therefore an essential regulator of AMPK, responsible both for triggering and maintenance of its activity. Combined effect of the two different modes of activation, phosphorylation of threonine 172 (Thr172) and allosteric regulation, leads to more than a 1000-fold increase in AMPK activity, making AMPK highly sensitive to changes in the cell's energy status (Vilchinskaya et al. 2018). Activation of AMPK through AMP also benefits from a decrease in creatine phosphate during muscle work, as creatine phosphate and ATP act as inhibiting factors within this context (Winder 2001). In addition, another important aspect of AMPK regulation is the allosteric inhibition through glycogen, which hinders the Thr172 phosphorylation by upstream kinases (Sanchez et al. 2012). Once activated, AMPK assumes the role of a master switch adjusting cellular metabolism to the altered energy state in order to prevent high-energy phosphate depletion (Parganlija et al. 2020). This is accomplished by triggering energy-generating processes such as fatty-acid oxidation, glucose uptake and glycolysis, as well as by downregulating energy-expending processes not acutely required for cell survival, e.g. lipid and protein synthesis and pathways involved in cell growth and proliferation (Richter and Ruderman 2009, Hardie 2011, Kjøbsted et al. 2018). AMPK functions as a multisubstrate serine/threonine protein kinase (Jørgensen

et al. 2006). Downstream targets of AMPK are involved in the regulation of acute responses as well as long term adaptation to exercise (Winder 2001).

Exercise is characterized by a substantial increase in energy turnover (> 100-fold), which poses a major energy challenge for the muscle fibers (Richter and Ruderman 2009, Jørgensen et al. 2006). Activation of AMPK in response to an exercise stimulus occurs in an isoform-specific manner that also appears to be dependent on the duration and intensity of exercise. Indeed, a certain threshold seems to exist for the activation of AMPK, as it apparently occurs acutely at exercise intensities exceeding 60% of maximal aerobic capacity or otherwise during prolonged exercise at lower intensities (Richter and Ruderman 2009). For purposes of the present doctoral work, biopsies of the vastus lateralis muscle following intense exercise at 60% of 1-repetition maximum (1-RM) were used to detect AMPK and its activated, phosphorylated form Phospho-AMPK (P-AMPK). The antibodies used for protein detection target both the α1 and α2 isoforms of the catalytic subunit, in case of the activated kinase with phosphorylation of Thr172, enabling the present work to build up on existing knowledge regarding the activation of AMPK, which has previously been shown to respond to different exercise stimuli. For example, cycle ergometer exercise has been shown to activate the α2 AMPK isoform in the vastus lateralis muscle in accordance with its duration and level of intensity (Fujii et al. 2000). With exercise at 70% of the maximum rate of oxygen uptake (VO_2max), AMPK α2 activity significantly increased at 20 and 60 min of exercise, and remained elevated 30 min into the recovery, without any accompanying significant changes in the activity of the α1 isoform. However, in the same subject pool, 20 min exercise at 50% VO_2max produced no effect on AMPK activity. These findings correspond to those of another study, in which AMPK α2 activity was shown to increase in the vastus lateralis directly following high intensity bicycle exercise at approximately 75% VO_2max for 60 min, without any changes in the AMPK α1 activity (Wojtaszewski et al. 2000). This study also revealed the activation of AMPK α2 to be reversed 3 h following the exercise. Furthermore, in the same study, low intensity exercise at approximately 50% VO_2max for 90 min did not induce any changes in the activity of AMPK α1 or α2. Intensity of the exercise stimulus thus seems be the primary determinant of AMPK activation, as lower intensity endurance exercise did not translate into increased AMPK activity even at extended duration. Incremental load cycling, on the other hand, was shown to facilitate an increase in the activity of both AMPK isoforms, although the response of AMPK α2 was stronger, as would be expected considering the abovementioned research results (Chen et al. 2003). Research findings also show that regulation of AMPK activity in skeletal muscle depends on the training status, i.e. activation of skeletal muscle AMPK triggered by acute exercise performed at identical absolute and relative intensity will be reduced after a period of exercise training (Kjøbsted et al. 2018). In addition, AMPK abundance itself is apparently impacted by endurance training, as trained individuals show higher expression of AMPK α1 (Richter and Ruderman 2009). As illustrated by all these previous findings, AMPK is well known as responding to an endurance exercise stimulus of adequate intensity. Present thesis hence expands on existing knowledge by investigating AMPK activation in the context of high-load resistance exercise.

Beyond the impact of exercise on the activation of AMPK, other factors relevant for the present thesis also play a role within this context. Ischemia and hypoxia, for instance, have been identified as triggers of AMPK activation (Mu et al. 2001, Jørgensen et al. 2006, Sanchez et al. 2012). In addition, mechanical unloading due to injury or under experimental setting of ground-based models for microgravity also impacts the phosphorylation of AMPK (Vilchinskaya et al. 2018). Abundance of the AMPK α2 subunit was found to be reduced by 25% during the first year following a complete

cervical spinal cord injury, without significant changes in the AMPK α1 content. In the vastus lateralis muscle, AMPK phosphorylation on Thr172 was also significantly decreased in the first year after a spinal cord injury. Furthermore, a short-term, 3-day gravitational unloading via dry immersion was shown to significantly reduce the level of AMPK Thr172 phosphorylation in the human soleus muscle (Vilchinskaya et al. 2015), underlying the significance of AMPK activation for muscle function.

Angiogenic factors (matrix metalloproteinases and endostatin)

Matrix metalloproteinases (MMPs) represent a large and heterogeneous family of zinc-dependent endopeptidases, whose basic function is to degrade proteins of the extracellular matrix by cleaving internal peptide bonds (Lo Presti et al. 2017). MMPs participate in multiple biological processes essential for the formation and repair of tissue, including cell migration, growth and differentiation, and tissue remodeling (Parganlija et al. 2020). Proteolysis performed by MMPs fulfills various functions, from creating space for cell migration to producing particular substrate cleavage fragments with independent biological activity, regulating tissue architecture through effects on the extracellular matrix and intercellular junctions, as well as directly or indirectly activating, deactivating or modifying the activity of signaling molecules (Page-McCaw et al. 2007). MMPs accordingly have a physiological function in diverse tissues and a wide range of substrates including peptide growth factors, tyrosine kinase receptors, cell-adhesion molecules, cytokines and chemokines, as well as other MMPs and unrelated proteases. MMPs are secreted as inactive propeptides and subsequently activated, among other mechanisms, by the cleavage of their regulatory peptide sequence, accomplished by plasmin and other MMPs (Prior et al. 2004, Jaoude and Koh 2016). Activation of MMP-2 and that of MMP-9 in particular seem to constitute important proteolytic events promoting angiogenesis, i.e. formation of new capillaries from existing ones (Prior et al. 2004).

Extracellular matrix of the skeletal muscle is known to acutely respond to increased loading (Kjaer 2004). Furthermore, immobilization increases pre- and posttranslational MMP expression, suggestive of accelerated collagen breakdown. MMPs are located in the extracellular matrix of the skeletal muscle tissue and mediate its adaptive responses to exercise, including angiogenesis (Kjaer 2004, Suhr et al. 2007, Ross et al. 2014). Capillarity is substantially increased in active muscle, particularly in response to endurance-type training, but also following high-resistance training involving repetitive contractions (Prior et al. 2004, Hudlicka et al. 1992, Leuchtmann et al., Verdijk et al. 2016). Increased capillarity is in turn associated with elevated maximal oxygen consumption of the muscle. Significant increase in capillary content is detectable already after four weeks of training (Kissane and Egginton 2019). Remodeling of the extracellular matrix through MMPs principally promotes angiogenesis by making way for the expansion of capillaries (Prior et al. 2004). However, proteolysis of collagen 18 releases endostatin, which acts as an anti-angiogenic signaling molecule (Page-McCaw et al. 2007, Lee et al. 2002, Walia et al. 2015). Studies investigating the response of endostatin to exercise stimuli have thus far reported differing results, possibly influenced by the exact type of stimulus and physical fitness of the study participants (Parganlija et al. 2020). Although some studies show endostatin levels are influenced both by endurance and resistance exercise (Suhr et al. 2010, Beijer et al. 2013), there is also research that suggests endostatin is not impacted by exercise (Rullman et al. 2007). Endostatin response seems to therefore only be triggered by certain exercise stimuli.

MMP-2 and MMP-9, known as gelatinases due to their affinity toward denatured collagen (gelatin), are able to hydrolyze the components of the basal lamina surrounding the myofiber sarcolemma and thus play a particularly important role in muscle growth, development and repair (Parganlija et al. 2020, Nascimento et al. 2015, Lo Presti et al. 2017). MMP-2 is constitutively expressed on the surface of myofibers and fibroblasts in skeletal muscle and can also be detected within skeletal muscle fibers by immunochemistry (Lo Presti et al. 2017). MMP-9, on the other hand, is secreted by various cell types including endothelial and satellite cells, neutrophils and monocyte macrophages. Its release from intracellular secretory granules can be induced by different exogenous stimuli such as cytokines, growth factors and altered cell-matrix contacts. Aside from its effect on angiogenesis, exercise also has the potential of inducing muscle damage (Pascual-Fernández et al. 2020). Gelatinases are associated with both of these impacts of exercise. Muscle damage can occur due to high intensity and particularly acute exhausting exercise, with its extent depending on various factors such as individual physical fitness, type and intensity of exercise as well as oxygen supply (Lo Presti et al. 2017). MMPs are released at the site in need of reparation by damaged myofibers or immune cells recruited by the occurrence of muscle injury (Fu et al. 2015).

Activation of satellite cells located between the sarcolemma and the basal lamina of myofibers and involved in muscle tissue repair is triggered when their anchor sites are disrupted through the impact of muscle injury (Fu et al. 2015). Satellite cells are additionally activated by the hepatocyte growth factor (HGF) released from the extracellular matrix through the action of gelatinases as well as secreted by vascular endothelial cells, as satellite cells are commonly located in close proximity to capillaries (Parganlija et al. 2020, Fu et al. 2015, Montarras et al. 2013, Pascual-Fernández et al. 2020). Other growth factors released by vascular endothelial cells are also involved in signaling to satellite cells and promoting their proliferation after muscle injury (Montarras et al. 2013, Pannérec et al. 2012). Satellite cells in turn reciprocate within a signaling loop back to vascular endothelial cells, which facilitates angiogenesis, and have also been shown to secret MMP-2 upon muscle injury (Montarras et al. 2013). In addition, satellite cells can migrate along the muscle fiber and show more presence close to the neuromuscular junction, specifically in case of slow muscle fibers, where they might be directly influenced by neurotrophins released by the nerve (Montarras et al. 2013). Reciprocal signaling is maintained also with neurons and interstitial cells (Montarras et al. 2013), apparently allowing satellite cells to both be influenced by and actively shape their environment. Facilitated in part by the superficial location of skeletal muscle tissue, subtle myofiber injuries occur routinely during daily activity, making satellite cells vital for skeletal muscle homeostasis and regeneration throughout life (Yablonka-Reuveni 2011, Ceafalan et al. 2014). Satellite cells are rare in uninjured muscles, typically accounting for 2-5% of identifiable nuclei in adult muscle, but have a remarkable proliferative potential and can efficiently repair even severely damaged muscles (Biressi and Rando 2010, Ceafalan et al. 2014, Yin et al. 2013). Number of satellite cells also varies depending on gender and muscle fiber type (Horwath et al. 2021). During muscle regeneration, satellite cells come out of their quiescence and undergo proliferative expansion, thereby generating myoblasts and, with exception of those that ultimately replenish the satellite cell pool, terminally differentiate by fusing with one another or with damaged fibers (Biressi and Rando 2010). Only other stimuli beyond muscle injury leading to activation of quiescent satellite cells in adult muscle are degenerative muscle diseases such as Duchenne muscular dystrophy (Montarras et al. 2013). Reduced activity of satellite cells caused by defects in HGF signaling is as contributing factor in the occurrence of sarcopenia, i.e. loss of muscle strength associated with aging (Pascual-Fernández et al. 2020). Transcripts for

most genes encoding metalloproteinases and their inhibitors are expressed in quiescent satellite cells, and MMP-2 activity is known to be elevated in activated satellite cells (Montarras et al. 2013, Fu et al. 2015).

Both resistance and endurance exercise induce a response from various MMPs, including the gelatinases. A single bout of cycling exercise with a duration of 65 min was shown to activate the MMP-9 protein in the vastus lateralis muscle, without any accompanying changes in MMP-2 (Rullman et al. 2007). The same study determined that endostatin levels in the muscle tissue are neither altered immediately nor 120 min following this particular exercise stimulus. Interestingly, venous and arterial endostatin concentration was elevated during the exercise intervention and subsequently not altered at the end of the 120 min recovery period. Furthermore, a certain temporal pattern in the response of gelatinases became apparent within the mentioned study, as MMP-9 activity increased already following a single 45min bout of cycling, whereas the MMP-2 activity only became elevated after multiple days of training, suggesting a role for MMP-9 in early, acute response to this type of exercise. An acute stimulus involving resistance exercise, however, was shown to produce a different response. An acute resistance exercise test was immediately followed by an increase in the serum MMP-2 concentration, whereas a substantial increase in MMP-9 was observed 15 min later; in addition, both effects seemed to be reversed following 8 weeks of resistance training (Urso et al. 2009). Serum concentration of both MMP-2 and MMP-9 was also elevated 10 min following a single bout of muscular endurance resistance exercise, with the MMP-9 response being substantially higher (Ross et al. 2014). Difference in the acute responses of MMPs depending on the exact exercise stimulus was further confirmed in a study involving short- and long-track runners (Suhr et al. 2010). At the gene expression level, acute exercise was shown to induce a similar increase of both pro- and antiangiogenic factors in untrained and trained muscle (Hoier et al. 2012). Regarding the response of circulating gelatinases to acute exercise, a recent systematic review of Lo Presti et al. concluded based on evidence from human studies that the most commonly observed effect of a single bout of exercise was an increase in the MMP-9 level (Lo Presti et al. 2017). The results on MMP-2 were less consistent, as the types of changes in its circulating levels varied greatly between different studies. In addition, response of circulating MMP-2 and MMP-9 to a single bout of high-intensity exercise appears to be dependent on the exercise mode and may facilitate training-specific adaptations (Nascimento et al. 2015).

Different stimuli associated with exercise contribute to facilitating angiogenesis. Capillary shear stress and/or wall tension stemming from an increase in muscle blood flow and mechanical stress caused by sarcomere length changes during muscle contraction and subsequent relaxation are apparently closely linked to angiogenesis (Parganlija et al. 2020, Prior et al. 2004, Hudlicka et al. 1992, Green et al. 2017). The role of blood flow as a trigger is illustrated in different models investigating the impact of shear stress on angiogenesis. Passive leg movement, for example, which causes a certain increase in blood flow (hyperemia) and a longitudinal stretch of myofibrils without the typical metabolic demand of active exercise, is associated with mechanoreceptor-driven angiogenesis (Kissane and Egginton 2019). Furthermore, 4 weeks of treatment with a vasodilator was found to result in a 12.5% increase in capillary-to-fiber ratio and 24% increase in capillary density in the vastus lateralis muscle (Kissane and Egginton 2019, Mortensen et al. 2017). In exercise trained muscles, increased blood flow seems to be one of the more probable stimuli of angiogenesis (Brown and Hudlicka 2003, Hudlicka et al. 1992). In this regard, it has been established that capillaries start to grow around oxidative fibers and that blood flow during exercise increases more in those parts of

the muscle that are composed of oxidative rather than glycolytic fibers. However, on the molecular level, contrasting results have been reported so far as to the impact of blood flow. In the context of dynamic constant-load knee-extension exercise, for instance, blood flow restriction was shown not to impact the activity of MMP-2 or MMP-9 in vastus lateralis (Rullman et al. 2009). On the other hand, passive training on a motor-driven knee extensor device was reported to induce an increase in MMP-2 and MMP-9 mRNA (Høier et al. 2010). Beyond its above-described effect on satellite cell activation, exercise is also known to enhance the number of satellite cells, even in old age, which can otherwise be associated with a drastic decline of satellite cells (Yablonka-Reuveni 2011). Resistance exercise in particular has been associated with increased satellite cell content and is known to facilitate their activation and differentiation (Karlsen et al. 2020, Moore et al. 2018, Luk et al. 2019, Pugh et al. 2018, Snijders et al. 2019, Nederveen et al. 2020). Proliferation and differentiation of satellite cells during muscle regeneration are profoundly influenced by innervation, vasculature, hormones, nutrition and the extent of muscle injury (Yin et al. 2013). Lactate has been shown to act as a signaling molecule affecting the fate of satellite cells (Nalbandian et al. 2020). Oxygen availability is another relevant factor, as exercise in a hypoxic environment may improve or impair muscle remodeling induced by contractile activity, depending on the duration of hypoxia and mediated by satellite cells (van Doorslaer de Ten Ryen et al. 2021, Britto et al. 2020). Furthermore, increased satellite cell content and activation following exercise have been found to correlate with muscle fiber capillarization (Snijders et al. 2019, Nederveen et al. 2018). From the above-described current state of knowledge, open questions arise regarding the impact of resistance exercise with varying load and the additional influence of LBNP-induced blood flow changes on angiogenic factors. These research questions are addressed in the present thesis.

2. Scientific conferences and publications

Oral presentations were given in several conferences by the author of the present dissertation. Based on the vote of the scientific audience, the presentation given at the 5th Symposium of Young Physiologists in Jülich, Germany was honored with one of three, equally ranked Best Talk Awards of the conference. As part of this award, the author was extended the opportunity to give a presentation at the Annual Meeting of the German Physiological Society the following year and co-organize its 6th Symposium of Young Physiologists.

Oral presentations were given on the following abstracts, cited in the language of each conference:

- Parganlija D, Rittweger J, Bloch W, Zange J: Lower body negative pressure (LBNP) enhances the oxygen supply of the knee extensor muscles during highly intensive resistive strength training in supine position. 96th Annual Meeting of the German Physiological Society, Greifswald, Germany, March 2017
- Parganlija D, Rittweger J, Bloch W, Zange J: Die Wirkung eines Unterkörper-Unterdrucks (LBNP) bei intensivem Training auf einer Beinpresse auf die Hb-Oxygenierung und die AMPK in den Kniestreckern. 51. Atmungs- und Leistungs-Physiologische Arbeitstagung, Cologne, Germany, Jan. 2017
- Parganlija D, Rittweger J, Bloch W, Zange J: Lower body negative pressure (LBNP) enhances the oxygen supply of the knee extensor muscles during highly intensive resistive strength training in supine position. 5. Symposium der Jungen Physiologen, Jülich, Germany, Sept. 2016

- Parganlija D, Herrera F, Rittweger J, Bloch W, Zange J: Unterkörper-Unterdruck verbessert die Oxygenierung der Kniestreckermuskeln während eines hochintensiven Krafttrainings. 50. Atmungs- und Leistungs-Physiologische Arbeitstagung, Hannover, Germany, Jan. 2016
- Parganlija D, Krause VM, Sauer M, Herrera F, Rittweger J, Bloch W, Zange J: During highly intensive resistive strength training in supine position, lower body negative pressure (LBNP) enhances the oxygen availability of the knee extensor muscles. 36th Annual Meeting of the International Society for Gravitational Physiology, Ljubljana, Slovenia, June 2015

Present doctoral work resulted in two scientific publications, described in chapter 2.1.

2.1 Description of the scientific publications

Results of the two human studies conducted as part of the present doctoral thesis were each published in a peer-reviewed scientific paper:

- Parganlija D, Nieberg V, Sauer M, Rittweger J, Bloch W, Zange J: Lower body negative pressure enhances oxygen availability in the knee extensor muscles during intense resistive exercise in supine position. European Journal of Applied Physiology 119:1289–1303, March 2019. https://doi.org/10.1007/s00421-019-04113-w
- Parganlija D, Gehlert S, Herrera F, Rittweger J, Bloch W, Zange J: Enhanced blood supply through lower body negative pressure during slow-paced, high load leg press exercise alters the response of muscle AMPK and circulating angiogenic factors. Frontiers in Physiology 11:781., July 2020. doi: 10.3389/fphys.2020.00781

First study conducted on the robotically controlled leg press within the present doctoral work was performed in the time period of March to July 2014, with the aim of establishing the merits of the training protocol developed during pretrials. Nine male subjects completed this study investigating the effects of LBNP superimposed on intense resistive leg press exercise in supine position, in a permutated crossover design of exercise sessions without or with LBNP and a short pause between the repetitions (Parganlija et al. 2019). Main hypothesis of the study was that LBNP would promote oxygen availability in the lower extremities during exercise, potentially benefiting energy levels and performance of the working muscles. In addition, it was presumed that muscle relaxation between contractions accomplished by the introduction of a short pause would further benefit the blood flow originating from the combined efforts of LBNP and the muscle pump activity. As previous research on exercise with LBNP primarily focused on endurance-type exercise and the effects of reduced movement velocity still remained to be investigated, an additional aim was to verify the impact of simulated orthostasis on muscle oxygenation and performance during slow-paced resistive exercise. Subjects performed series of concentric and eccentric contractions, each in a comparatively slow pace of 4 s, without or with 40 mmHg LBNP and 4 s pause between the repetitions. Force applied at knee flexion amounted to 6% of the one-repetition maximum (1-RM), gradually increased to 60% 1-RM within the first half of the individual range of motion and subsequently remained constant until the terminal point of extension. Further details on the experimental setting and exercise protocol are available in chapter 1.4 of the present thesis and the respective publication, Parganlija et al. 2019. Non-invasive measurements were conducted to characterize acute cardiovascular responses and leg muscle oxygenation and performance. Results of the study revealed that these acute responses as well as the effects of superimposed LBNP are substantially influenced by the exercise mode, i.e. continuous movement (without pause) or intermittent exercise (with pause). Using the example of

cardiovascular responses, higher heart rate increase and stroke volume decrease were observed during continuous exercise under ambient pressure as well as under LBNP in comparison to respective intermittent exercise. Furthermore, certain effects of LBNP were only present with continuous, but not with intermittent exercise, e.g. reduced cardiac output and elevated total peripheral resistance. In terms of local physiological responses on the level of the working muscle, LBNP promoted blood refill during the low force periods of exercise as suggested by the increase in total hemoglobin and oxyhemoglobin in the vastus lateralis, with a substantially higher margin between maximal levels under LBNP vs. ambient pressure during continuous as compared to intermittent exercise. Concomitant increase in respiratory oxygen uptake indicated enhanced muscle energy turnover. However, energy provision was possibly at least partially secured by anaerobic metabolism, as higher lactate levels in blood obtained from the ear lobe after exercise with LBNP suggested. While intermittent exercise led to a markedly higher EMG amplitude under LBNP, continuous exercise with LBNP was found to be associated with a trend toward a lower EMG amplitude increment compared to the control setting, indicating possible impact on fatigue appearing in later stages of the investigated exercise protocol which could potentially manifest with physiological relevance under a prolonged exercise stimulus. Taken together, it was demonstrated that LBNP superimposed on intense, slow-paced resistive leg press exercise can enhance blood volume and oxygenation of vastus lateralis, suggesting facilitated muscle perfusion, particularly during uninterrupted exercise. Within the context of research efforts at the German Aerospace Center in Cologne, this study was a step within a research program aimed at understanding whether LBNP can improve the effectiveness of strength training in the microgravity environment of a spaceship (Parganlija et al. 2019). For purposes of the present thesis, it provided vital information by clarifying that resting periods are not necessary within the investigated type of exercise in order to elicit potentially beneficial perfusion effects of LBNP, as continuous exercise proved to be more favorable in that regard. On this basis, the exercise protocol without pause during flexion was chosen for additional investigation of molecular responses within a subsequent study.

Second study was performed from February to April 2015 and had two principal objectives. On the one hand, the exercise protocol established in the previous cross-over study was to be examined with independent subject groups in a non-crossover design. Furthermore, muscle biopsies and blood samples were to be investigated in addition to the previously employed physiological measurements, in order to characterize the underlying molecular responses behind the observed effects of LBNP (Parganlija et al. 2020). Physiological responses observed with LBNP in the previous study were expected to be associated with acute molecular responses affecting energy sensing and angiogenesis. Circulating levels of angiogenic factors MMP-2, MMP-9 and endostatin involved in muscle tissue remodeling and capillary growth were examined in venous blood samples. Muscle biopsies of vastus lateralis were used to detect the energy sensor AMPK, a vital regulator of cell metabolism known to be impacted by exercise as well as muscle unloading and hypoxia. Activation of AMPK was assessed through detection of its phosphorylated form. Relevant characteristics of function and known responses of these molecular targets are presented in chapter 1.6 of this dissertation. Effects of LBNP observed in the previous study were partially reproduced, in particular regarding the heart rate increase, elevated total hemoglobin in the vastus lateralis muscle and increased overall oxygen uptake. However, some elements of the cardiovascular response pattern differed from those found in the preceding study, as cardiac output was elevated, total peripheral resistance reduced, and stroke volume not significantly altered under LBNP. Oxyhemoglobin levels were now gradually declining

over the course of the exercise under LBNP, with a notably high margin between maxima and minima, and were accompanied by steadily increasing deoxyhemoglobin levels. In light of the elevated oxygen uptake and a trend toward lower post-exercise lactate levels, these results indicated higher oxygen consumption and reliance on oxidative metabolism under LBNP. Energy provision was, however, apparently insufficient to cover the needs of the working muscle, as higher EMG amplitude increment was observed under LBNP, with some subjects even displaying clear signs of fatigue reflected in strained performance during final repetitions of the leg press exercise. Superimposed LBNP resulted in more pronounced post-exercise reduction of MMP-2 and also abolished the early post-exercise increase in endostatin consequently observed only under ambient pressure. While post-exercise AMPK and P-AMPK levels were reduced under ambient pressure, no significant reduction was observed under LBNP. Combining exercise with LBNP also counteracted the reduction of AMPK activation reflected in the lower post-exercise ratio of P-AMPK to AMPK found in the control group. These novel insights into the molecular responses to LBNP suggest that simulated orthostasis modifies the effects of intense resistance exercise on the local metabolism of the working muscle and its surrounding extracellular matrix, with potential impact on aerobic energy provision and muscle growth. LBNP might promote beneficial structural adaptations of skeletal muscles during resistance exercise and contribute to astronauts achieving increased muscle strength and endurance during space flight. Continued research into simulated orthostasis therefore remains of high significance for the optimization of countermeasures for muscle loss induced by space flight.

3. Summarizing discussion

Lower body exercise holds great significance for countermeasure efforts, as postural and lower limb muscles tend to be particularly affected by space flight induced loss of muscle mass and function. Present thesis introduces a novel combination of a robotically controlled leg press (RCL) with an LBNP chamber as an experimental model with promising results for eliciting a blood shift toward the lower body similar to the one generated by Earth's gravity, potentially benefiting the muscle response to exercise and opening new possibilities for the optimization of countermeasures for space flight related skeletal muscle impairments. RCL is a unique device that enables resistance exercise without any need for gravity-related energy storage and could thus be a model for future inflight exercise devices (see also thesis chapter 1.4). Furthermore, the RCL allows customization of all relevant exercise parameters and use of varying force-distance profiles, thereby enabling highly customizable training and optimized effectiveness based on individual needs. As a research tool, the RCL enables studying the influence of simulated orthostasis on leg muscle perfusion without the additional impact associated with an actual change of body position (Parganlija et al. 2019). Applying LBNP of 40 mmHg in supine body position results in a fluid shift toward the lower body to a volume otherwise observed in upright posture due to gravitational influence (Boda et al. 2000, Baisch et al. 2000a, Parganlija et al. 2019). Equal fluid shift can hence be achieved without the application of LBNP by a change from supine to upright body position. However, such a change elicits a complex pattern of physiological reactions associated with vestibular sensing and altered biomechanical properties resulting in diverse cardiovascular and neuromuscular responses. In such a network of interconnected responses to different stimuli, studying the effects of the fluid shift alone would not be possible. LBNP has the advantage of generating a fluid shift without the need for a gravitational stimulus and therefore, under appropriate conditions, enables studying the specific effects of the fluid

shift itself, for the purposes of the present doctoral work superimposed on a particular type of exercise.

Previous research on exercise combined with LBNP was primarily focused on endurance-type exercise with a constant load within a given contraction (see chapter 1.4). However, resistance exercise has previously been linked with benefits for maintaining muscle mass and function as well as for muscle growth, and constant load leg press exercise reportedly could not prevent muscle strength loss induced by bed rest, a form of ground-based simulation for space flight induced muscle atrophy (Bamman and Caruso 2000, Tesch et al. 2004, Parganlija et al. 2019). Impact of reduced exercise velocity remained to be investigated, and the effects of added resting periods were only addressed in other simulations of enhanced perfusion involving postural changes rather than LBNP. In addition, among molecular responses to LBNP, energy sensing and angiogenesis, mechanisms conceivably associated with enhanced provision of oxygenated blood, were yet to be explored (see chapter 1.5). Present doctoral work addresses these scientific questions by exploring the physiological and molecular effects of LBNP superimposed on intense resistive, slow-paced leg press exercise with a varying muscle load featuring gradually increasing force during the first half of the concentric and consequently the second half of the eccentric contractions. Potential benefits of resting periods during flexion for the facilitation of muscle perfusion through LBNP were additionally investigated in the first, cross-over study conducted within the present doctoral work. As described in the preceding chapter, continuous exercise without such resting periods was determined to be more effective in enhancing the blood content and oxygenation of the working muscles and was therefore utilized in a subsequent non-crossover study additionally investigating acute molecular responses to exercise with LBNP. Although cardiovascular responses to the intervention somewhat differed between the two studies, enhanced muscle perfusion was overall found to be accompanied by increased oxygen uptake and changes in the muscle hemoglobin content indicative of enhanced oxidative energy turnover. Furthermore, these physiological effects were, as expected, accompanied by acute molecular responses of the energy sensor AMPK and angiogenic factors. Current section offers a summarized discussion of relevant aspects and results from the two conducted studies, highlighting similarities and differences in their findings.

Exercise performance and EMG amplitude increment

In both studies of the present thesis, various parameters characterizing the exercise were assessed in order to ensure equivalent performance was accomplished in the LBNP and control sessions. Average test force at the turning point between extension and flexion (± 1 s), mean force, average work distance, and the concentric and eccentric velocity and power were all comparable between different exercise sessions within each study, suggesting equivalent performance in the various test conditions (Parganlija et al. 2019, Parganlija et al. 2020). Rating of perceived exertion did not significantly differ between the LBNP and corresponding control sessions, indicating comparable exercise intensity. Furthermore, bilateral EMG measurements of the vastus lateralis provided equivalent signal patterns, suggesting that the exercise was performed with equal contribution from both limbs. These findings altogether exclude any hypothetical influence of uneven mechanical performance on further results of the two studies.

Rising EMG amplitude during exercise has previously been described as an indicator of the motor unit recruitment in the working muscle (Zange et al. 2003). Background of this response is that certain muscle fibers reach metabolic depletion early on during exercise, which manifests as a reduction in

the adenine nucleotide pool. Remaining motor units, which have retained a high-energy state, are hence progressively recruited in order to compensate for muscle fatigue. First study within the present thesis recorded a steady increase of the EMG amplitude in all test conditions, with an expectedly higher increase during continuous as compared to intermittent exercise (Parganlija et al. 2019). However, the effect of added LBNP was notably different depending on the type of exercise. While LBNP facilitated a higher increase in the EMG amplitude during intermittent exercise compared to control, it had no significant impact during continuous exercise. Tendency toward lower EMG amplitude during continuous exercise with LBNP was suggestive of a possible benefit in terms of a delayed onset of muscle fatigue, which could potentially manifest with physiological relevance during a longer exercise session. Findings on intermittent exercise stood in contrast with previous research showing postural effects on leg muscle fatigue during intermittent calf muscle contractions, with lower fatigue occurring in an inclined head-up compared to supine position (Egaña and Green 2007). Described postural effects on calf muscle fatigue manifested at moderate to very high forces, a range that corresponds well to the target force investigated within the present thesis. Differing findings could therefore potentially stem from other aspects of the study design. Based on previous estimates, 40 mmHg LBNP leads to a volume shift equivalent to a transition from supine to upright position (Baisch et al. 2000b). Its benefit for muscle perfusion should hence exceed the one of the 67° inclination used by Egaña and Green. However, muscle perfusion is also influenced by the activity of the muscle pump, which is in turn impacted by the exact type of contractions. Study of Egaña and Green involved isometric contractions of 2 s duration, whereas the present work focused on 4 s concentric and eccentric contractions. Furthermore, intermittent exercise in both studies involved 4 s periods of relaxation, making the ratio of relaxation to contraction duration substantially higher in the study of Egaña and Green. Higher EMG amplitude during intermittent exercise with LBNP as indicator of progressive compensation for muscle fatigue might be attributable to reduced energy content due to higher venous pooling and resulting insufficient oxygenation of the muscle tissue.

In contrast to the abovementioned result of the first study, the EMG measurement in the second study within the present thesis rather showed a steeper amplitude increase under LBNP indicative of higher compensation for muscle fatigue occurring during continuous exercise (Parganlija et al. 2020). As mentioned further above, all exercise parameters were comparable and suggested no difference between the LBNP and the control setting. Nevertheless, 3 out of 9 subjects were unable to fully perform the exercise set and terminated the exercise shortly before completing their final repetition, substantiating the EMG-related indications of increased muscle fatigue. Although interindividual differences in LBNP tolerance may have played a role in the subjects' response, differing findings on hemoglobin and lactate levels in the two studies suggest the muscle fatigue was likely of a metabolic origin. First study showed LBNP to result in elevated oxyhemoglobin levels in the vastus lateralis accompanied by higher oxygen uptake as well as higher lactate levels, indicative of both aerobic and anaerobic contribution to energy provision. The second study also found indications of aerobic energy production, however rather accompanied by a tendency toward reduced lactate levels. It is therefore conceivable that this different metabolic state could not adequately meet the rising energy demands over the course of the exercise, ultimately resulting in muscle fatigue. This kind of metabolic imbalance is indeed known to cause muscle fatigue during highly intense exercise, in which growing ATP requirements cannot be met by increases in oxygen delivery (Wan et al. 2017). In the present thesis, occurrence of metabolically induced fatigue is further supported by elevated P-

AMPK under LBNP, indicating elevated AMP levels and correspondingly depleted energy in the working muscle. Furthermore, activation of muscle AMPK has previously been described as promoting glycogen storage and enhancing muscle endurance as part of a positive feedback mechanism for resisting muscle fatigue (Wan et al. 2017). Observed elevated levels of P-AMPK therefore may have been part of an initial compensatory mechanism.

Although metabolic limitations are likely the primary source of muscle fatigue within the constellation of present findings, other factors could also contribute and are well worth exploring. Due to the intense nature of the applied exercise protocol, non-metabolic fatigue is conceivable, which occurs due to disruption of internal structures facilitated by high forces during exercise and is particularly pronounced following eccentric muscle activity, which was part of the present exercise protocol (Green, H. J. 1997). However, non-metabolic fatigue is characterized by myofibrillar disorientation and damage to the cytoskeletal framework in absence of any metabolic disturbance. Due to the observed impact on the energy sensor AMPK exerted by the present exercise protocol, a certain degree of metabolic disturbance is likely. Another aspect to consider is central fatigue decreasing the neural drive to the muscle, which is characterized by slowing or cessation of motor unit firing (Wan et al. 2017). Muscle fatigue during intense exercise has previously been linked to excitation-coupling failure and potentially a reduced nervous drive due to reflex inhibition at the spinal level (Bangsbo 1997). Towards the end of exercise sessions with LBNP, the EMG amplitude indeed displayed a steep decrease. As changes in the EMG amplitude during submaximal contractions are dependent on the number of active motor units, their size and firing rates (Suzuki et al. 2002), this finding could indicate a contribution from centrally induced fatigue. Higher recruitment of motor units reflected by the preceding steady rise of the EMG amplitude may have increased the scope for gradual fatigue, as less fatigue resistant muscle fibers become increasingly engaged (Wright et al. 1999). In light of the conditions attached to participation in the studies within the present thesis, residual fatigue from other physical activity prior to the exercise sessions can largely be excluded. Finally, morphology and fiber type composition of the vastus lateralis are known to vary, which could account for different sensitivity to fatigue among subjects (Staron et al. 2000, Willan et al. 1990, Wright et al. 1999).

It is important to note that previous research had demonstrated enhanced work performance during supine incremental load dynamic leg exercise on a cycle ergometer combined with LBNP (Eiken 1988). In addition, increased fatigue resistance was observed in a cross-over study on ten bouts of 15 s maximum dynamic exercise in the form of load lifting intermitted by 9 periods of 45 s rest (Zange et al. 2008). However, this setting of concentric muscle work strongly differs from the high-load concentric and eccentric contractions investigated in the present thesis. Since electrical activation dominates in eccentric rather than comparably induced concentric contractions (Newham et al. 1983), this difference in findings could at least partly be attributed to distinct exercise protocols. Tendency toward lower EMG amplitude observed during the first study of the present thesis nevertheless suggests certain individuals might potentially benefit from improved muscle fatigue during intense resistance exercise with LBNP.

Cardiovascular responses

In terms of its cardiovascular effects, LBNP is known to elevate resting heart rate and to augment the heart rate increase during bicycle and treadmill exercise (Eiken and Bjurstedt 1985, Eiken et al. 1986, Nishiyasu et al. 1999, Bonde-Petersen et al. 1984, Groppo et al. 2005). Analogous observations were

made within the present thesis on intense resistive leg press exercise. In addition, previous research on dynamic leg exercise in supine position showed steady-state heart rate not to be significantly impacted by LBNP, which was also established regarding continuous exercise without pause in the first study of the present doctoral work (Eiken and Bjurstedt 1985, Eiken et al. 1986, Parganlija et al. 2019). Second study showed a differing result, with heart rate values under LBNP consistently higher than in the control condition (Parganlija et al. 2020). However, heart rate values in the second study notably also only began to stabilize much later, towards the end of the exercise session. Difference in the findings of the two studies could therefore be attributed to earlier stabilization and accomplishment of an actual steady state during the first study. In terms of further cardiovascular effects, LBNP has also been shown to reduce the stroke volume and cardiac output as well as to attenuate exercise-induced increase in cardiac output during supine, incremental load exercise on a cycle ergometer (Eiken and Bjurstedt 1985, Eiken 1987, 1988). Initial observations from the first study within the present doctoral work supported these previous findings within the context of continuous exercise, in which LBNP led to lower cardiac output and stroke volume as well as elevated total peripheral resistance compared to ambient pressure. However, the second study on exercise without pause rather revealed elevated cardiac output and reduced total peripheral resistance under LBNP accompanied by a stroke volume reduction comparable to the one of the control setting. As decreased stroke volume as well as reduced cardiac output have previously been mentioned as signs of reduced venous return at rest as well as under exercise with LBNP, these contrasting findings indicate a higher rate of venous pooling under LBNP in the first study (Bonde-Petersen et al. 1984, Eiken and Bjurstedt 1985, Blaber et al. 2013, Parganlija et al. 2020). In addition, assessing the cardiovascular data in a broader context of other findings from both studies, in particular the hemoglobin and lactate levels, reveals further indications of higher venous return within the second study. For instance, the minima of total hemoglobin were higher from the exercise onset and progressively increased during exercise with LBNP in the first study, whereas they were comparable with the ones of the control sessions in the second study (see also related parts of the discussion further below). Since the exercise protocols of both studies were identical, it would be plausible to assume the underlying causes are at least partly attributable to different subject pools. Mean age of the subjects was relatively comparable (27 and 23 years in the first and second study, respectively) and is therefore unlikely to have had a major impact. However, there were certain differences in terms of subjects' physical activity. More subjects of the second study were sport students, some of whom performed regular leg muscle resistance training. Average test and mean force recorded during the exercise sessions were somewhat higher during the second study (Parganlija et al. 2019, Parganlija et al. 2020). Heart rate increase is known to be lower in trained individuals (Williams and Williams 1983), and the heart rate increase during the control sessions was somewhat lower in the second compared to the first study (similar assessment for the LBNP sessions would incur possible interference from the effects of LBNP and is therefore refrained from). Abovementioned findings altogether suggest that the subjects of the second study may have been more accustomed to leg muscle resistance training, which was possibly associated with structural and functional adaptations of leg muscles promoting the activity of the muscle pump and facilitating venous return.

Prolonged phases of low muscle force through resting periods during flexion expectedly promoted muscle relaxation and apparently somewhat facilitated venous return consequently resulting in higher cardiac output and lower total peripheral resistance compared to continuous exercise. Nevertheless, compared to the corresponding control sessions, intermittent exercise with LBNP showed decreased

stroke volume without significant impact on cardiac output or total peripheral resistance (Parganlija et al. 2019). These findings indicate intermittent exercise leads to lower venous return under LBNP compared to ambient pressure, probably due to interrupted action of the muscle pump, which is known to facilitate blood outflow. Brief pauses during flexion also resulted in a lower heart rate increase both with LBNP and under ambient pressure compared to continuous exercise, which was apparently also associated with a faster stabilization of heart rate values. Taken together with the lower EMG amplitude increment and lower rate of perceived exertion also observed for intermittent exercise, these findings altogether indicate that this exercise protocol was, as would be expected, comparatively less strenuous than exercise without pause.

Muscle hemoglobin content

Blood volume shift associated with the application of LBNP has previously been verified through near infrared spectroscopy, e. g. by detection of decreased tissue hemoglobin index in the forearm accompanied by its simultaneous elevation in the calf muscle (Bartels et al. 2011). Near infrared spectroscopy was also utilized in the present thesis, and the elevated levels of total hemoglobin in the vastus lateralis observed under LBNP in both its studies are consistent with previous findings at rest and during exercise (Nishiyasu et al. 1999, Hachiya et al. 2004, Bartels et al. 2011, Zange et al. 2008). First study conducted within the present doctoral work also revealed elevated levels of oxyhemoglobin and tissue oxygen saturation, particularly during continuous exercise, indicative of enhanced presence of oxygenated blood in the working muscle (Parganlija et al. 2019). These findings can be attributed to superimposed LBNP, since resistive leg exercise has been shown to reduce the oxygen saturation of muscle tissue (Downs et al. 2014). While the total and oxyhemoglobin levels were gradually increasing during continuous exercise with LBNP, levels of deoxyhemoglobin remained comparable to the ones of the control sessions. Furthermore, minimal values of total hemoglobin were higher with LBNP than under ambient pressure from the beginning of the exercise set and continued to increase over the course of the exercise. In light of the crossover design of the study and the gradually increasing reduction of stroke volume and cardiac output accompanied by a rise of total peripheral resistance compared to control sessions, this finding suggests venous pooling was present from exercise onset and that venous return progressively declined over the course of the exercise with LBNP. Given the higher oxygen uptake and lactate levels in the LBNP sessions, the observed changes in hemoglobin levels also suggest that higher supply of oxygenated blood was not matched by higher oxygen exploitation. Abovementioned increase in the levels of total and oxygenated hemoglobin was also present during intermittent exercise, but to a notably smaller extent. Resting periods after exercise have been shown to coincide with increased blood flow and recovery of muscle oxygenation (Downs et al. 2014). Furthermore, relaxation periods associated with intermittent contractions have previously been linked to elevated blood flow (Hendrickse and Degens 2019). It is thus conceivable that, while blood vessels were operating under maximal filling capacity with LBNP, resting periods facilitated a rise of hemoglobin levels under ambient pressure, thereby reducing the margin between LBNP and control otherwise observed under continuous exercise. Contrary to the results of the first study, the second study within the present thesis rather showed elevated total hemoglobin to be accompanied by lower maxima of oxyhemoglobin and a gradually declining tissue oxygen saturation index (Parganlija et al. 2020). However, the margin between minima and maxima of oxyhemoglobin was now notably higher under LBNP compared to ambient pressure. Moreover, the margin between minimal levels of oxyhemoglobin under LBNP compared to control progressively increased during exercise. Coupled

with gradually rising levels of deoxyhemoglobin under LBNP as well as the tendency toward lower lactate levels under LBNP, and in light of the somewhat higher oxygen uptake compared to the first study, these findings suggest higher exploitation of supplied oxygen with gradual stimulation of oxidative metabolism over the course of the exercise. Notion of a progressive shift to oxidative metabolism correlates well with current knowledge on energy provision during exercise, which states that during very intense efforts lasting only seconds most ATP is derived from the breakdown of phosphocreatine and glycogen to lactate, while oxidative phosphorylation becomes the major ATP-generating pathway only when exercise duration extends beyond approximately 1 min (Hargreaves and Spriet 2020).

Oxygen uptake

In accordance with the previously described increased oxygen consumption under LBNP as well as the known benefit of LBNP combined with supine treadmill exercise in maintaining peak oxygen consumption during bed rest, higher oxygen uptake was found during exercise with LBNP in both studies within the present thesis (Groppo et al. 2005, Watenpaugh et al. 2000, Lee et al. 2007, 2009). However, there were some notable differences between the findings for intermittent and continuous exercise, i.e. the oxygen uptake was somewhat higher with pauses during flexion, indicating possibly higher metabolic power despite pauses reducing the mechanical power by providing a longer time period to perform identical exercise (Parganlija et al. 2019). First study enabled collecting data on the recovery following exercise, which was not possible during the second study, as the subjects had to be transported to the biopsy room. Data on the post-exercise recovery show higher oxygen uptake after continuous exercise, indicating higher oxygen debt compared to intermittent exercise. Furthermore, continuous exercise resulted in higher respiratory exchange ratio during the recovery, supporting the notion of a larger contribution of anaerobic energy metabolism during this form of exercise, as also indicated by the above-discussed further results from the first study. It is important to note that elevated oxygen uptake might also at least partially correlate with the cardiovascular responses to LBNP, which are prone to interindividual variation. In light of elevated heart rate, cardiac output and systolic blood pressure observed in the second study within the present thesis, it is conceivable that increased cardiac work rate may have contributed to the higher oxygen uptake with LBNP (Parganlija et al. 2020).

Circulating levels of lactate and angiogenic factors

Blood lactate levels were found to increase less, when supine incremental load dynamic leg exercise on a cycle ergometer is performed with LBNP (Eiken 1988). Results of the present thesis partially support this finding. Its first study revealed increased lactate levels in capillary blood obtained from the ear lobe following continuous exercise (Parganlija et al. 2019). Lactate levels after intermittent exercise were similar in the LBNP and the control sessions, and also expectedly lower than those following continuous exercise. These findings originally led to the assumption that the underlying cause could lay in the higher frequency and lower loading of leg muscles during cycling compared to the slow-paced, high-load resistance exercise on the leg press which might potentially limit the oxidative metabolism by contraction-related inhibition of blood inflow and oxygen delivery. In addition, the slow exercise rhythm coupled with a gradually increasing force could have benefited the lactate washout and led to higher lactate levels detected in capillary blood. Pauses during flexion would in that case allow earlier lactate washout and thereby potentially result in a detection of lower

lactate values. Due to delays in the transport of lactate and its consumption in other parts of the body, lactate levels in the capillary blood obtained from the ear lobe only qualitatively reflect anaerobic metabolism in the working muscle. Nevertheless, capillary blood lactate has been shown to correlate well with muscle lactate content following leg press exercise (Gorostiaga et al. 2014). In light of this previous finding as well as the remaining results of the present thesis that suggest a contribution of anaerobic metabolism during its first study, it is plausible that the measured lactate levels accurately reflected the metabolic state of the working muscle. In contrast to data of the first study, the second study rather showed comparable lactate levels between the LBNP and control group measured in venous blood samples, with a notable, but not statistically significant tendency toward lower lactate levels under LBNP (Parganlija et al. 2020). Second study thus revealed, in the constellation of the overall results including the changes in the hemoglobin levels, that a more oxidative metabolism is possible under the current exercise protocol. Increased muscle blood flow efficiency resulting from elevated local perfusion pressure has previously been discussed as a contributing factor to the enhanced work performance observed during the abovementioned supine exercise on a cycle ergometer (Eiken 1988). This hypothesis correlates well with the indications of enhanced peripheral blood supply and facilitated venous return in the second study of the present thesis.

Gelatinases are released by various cell types into the bloodstream and their circulating levels are dependent on the ratio between their release and resorption (Lo Presti et al. 2017). Circulating levels of gelatinases ultimately reflect a systemic response to exercise, including a contribution from the working muscle. Previous studies have generally shown that exercise of sufficient intensity triggers an acute release of MMP-9, which is confirmed by the elevated post-exercise levels of MMP-9 determined in the present thesis (Parganlija et al. 2020). Activation of MMP-9 seems to generally occur irrespective of exact nature of the exercise stimulus, in contrast to the less consistent findings on the response of MMP-2, apparently also depending on type of samples utilized for the measurement. For example, cycling and dynamic knee extension exercise have both been shown to activate MMP-9 without affecting MMP-2 in the skeletal muscle tissue, whereas a single bout of muscular endurance resistance exercise led to elevated circulating levels of MMP-9 and MMP-2, albeit with a higher response from MMP-9 (Rullman et al. 2007, Ross et al. 2014, Rullman et al. 2009). Cycling has also been shown not to affect skeletal muscle endostatin levels (Rullman et al. 2007). These previous findings are partially supported by the results of the present thesis, which show that intense leg press exercise also leads to elevated levels of circulating MMP-9. However, findings of the present thesis rather demonstrate differential regulation of the two angiogenic factors, since elevated levels of MMP-9 were accompanied by a reduction in MMP-2, in support of previous research showing that serum MMP-2 decreases after prolonged cycling at submaximal intensity (Nourshahi et al. 2012). Furthermore, the observed increase in endostatin directly following exercise under ambient pressure indicates early suppression of angiogenesis that did not occur under LBNP (Parganlija et al. 2020). Possible explanation for this finding lies with reduced activation of AMPK in the control group, as the AMPK signaling cascade is known to facilitate VEGF production in muscle and promote angiogenesis in response to ischemic injury (Ouchi et al. 2005). Notably, a comparable increase in MMP-9 activity has previously been observed in the vastus lateralis muscle following a single bout of supine dynamic constant-load knee extension exercise without and with blood flow restriction (Rullman et al. 2009). Results of the present thesis are consistent with this observation, as they demonstrate similar increase in circulating MMP-9 without and with LBNP. Substantial decrease of MMP-2 observed under LBNP likely suggests stronger suppression of the

angiogenic stimulus compared to exercise under ambient pressure (Parganlija et al. 2020). This early and progressive response of MMP-2 is apparently balanced out by subsequent activation of MMP-9. However, it is important to note that local perfusion and cardiovascular effects of LBNP can conceivably impact the release of gelatinases from storage within tissue, their transport within the bloodstream and consequently their measurable levels at the site of detection. Increased transmural pressure (reduced interstitial pressure) under LBNP facilitates capillary filtration, leading to an increase in interstitial volume accompanied by a concomitant decrease in blood plasma volume (Aratow et al. 1993, Goswami et al. 2019a). This filtered volume is hence returned to the bloodstream via the lymphatics with considerable delay (Goswami et al. 2019a). During this transient volume retention, proteins otherwise supplied to the bloodstream are consequently also temporarily contained within the interstitial fluid. Lymph pump control includes various physiological, humoral and cardiovascular elements potentially impacted by LBNP, resulting in complex regulation and substantial possibilities for influence of LBNP on the lymph flow (see chapter 1.4). Delayed entry into the bloodstream due to a decrease in lymphatic transport induced by short-term immobilization has previously been shown to result in a blunted response of the creatine kinase (Sayers and Clarkson 2003). Although the experimental setting and target proteins differ from those of the present thesis, the common factor of delayed entry into the circulation suggests a possible analogous impact of LBNP in the present case. Capillary filtration facilitated by LBNP could thus affect protein levels measured in blood samples as well as the time course of their detected changes.

Muscle AMPK content and phosphorylation

AMPK phosphorylation is known to decrease in the early stages of muscle unloading (Parganlija et al. 2020, Vilchinskaya et al. 2015, Vilchinskaya et al. 2018). Equivalent response was simulated within the present thesis, as muscle biopsies of the control group demonstrated an initial decrease in AMPK, P-AMPK and the P-AMPK/AMPK ratio, with the protein levels recovering 1 h following the exercise. Two conclusions can be inferred from this finding with regard to the impact of muscle perfusion and exercise on AMPK phosphorylation. On the one hand, reduced muscle perfusion associated with supine body position seems to be a facilitating factor for decreased AMPK phosphorylation. In addition, the mentioned impact of supine position as a simplified model of altered perfusion comparable to microgravity is not abolished by intense resistance exercise (Parganlija et al. 2020). Considering the role of AMPK in downregulating protein synthesis, this result is consistent with previous research showing that minimal resistance exercise preserves muscle protein synthesis throughout bed rest (Ferrando et al. 2002). It is important to note that the reduction in AMPK phosphorylation may have been modulated by the present resistance exercise intervention, which could, however, only be detected if an appropriate comparison group occupying the same supine position without performing the exercise would be included in future investigations. Since resistance exercise with LBNP did not alter the AMPK or P-AMPK levels in the working muscle compared to their respective baseline, superimposed LBNP can apparently counteract their otherwise occurring initial decrease, further substantiating the conclusion on a possible association between muscle perfusion and regulation of AMPK abundance and activity.

Cycling is known to activate AMPK, with progressive phosphorylation occurring during continued exercise (Parganlija et al. 2020, Wojtaszewski et al. 2000, Wojtaszewski et al. 2002, Stephens et al. 2002, Rose et al. 2005). Findings of the present thesis partially support this previous research on endurance exercise, as they demonstrate that resistance exercise can also lead to increased AMPK

activation, reflected in an elevated ratio of P-AMPK to AMPK 10 min and 30 min after exercise with LBNP. These results suggest that a short bout of intense resistance exercise can lead to rapid increase in AMPK activity, provided a sufficient blood supply to the working muscle (Parganlija et al. 2020). In contrast, previous research has shown that low-load resistance exercise in the form of bilateral knee extension with or without blood flow restriction does not impact AMPK phosphorylation in the vastus lateralis 2 h and 4 h post exercise (Ferguson et al. 2018). Differing outcomes might, however, stem from a different time frame for muscle biopsy collection, which occurred notably earlier during the present thesis, and/or an insufficient exercise stimulus due to a comparatively low load of 20% 1-RM vs. 60% 1-RM in the present thesis. Indeed, as the authors themselves discuss, blood flow restriction was expected to provide a metabolic stimulus, but most probably failed to do so, as the exercise protocol may not have afforded a sufficient metabolic challenge (Ferguson et al. 2018). Appropriate combination of an exercise and a perfusion stimulus might therefore constitute a likely trigger for the modulation of AMPK activity. However, depending on the type and duration of exercise, an exercise stimulus alone could also suffice for changing, even facilitating AMPK activation, as has been demonstrated for leg extension exercise, with 10 sets of 10 repetitions at 60-70% 1-RM increasing AMPKα2 activity (Dreyer et al. 2006). Such aspects should optimally be given consideration in future studies. While reflecting on the findings of the present thesis in the general context of optimizing countermeasures for muscle loss in astronauts, particularly in terms of an extended mission such as travelling to a different planet, it is also important to consider that exercise-induced AMPK activation does not interfere with muscle hypertrophy in response to resistance training (Lundberg et al. 2014). Interpreting the results on AMPK in the context of other findings of this thesis provides clues as to their possible physiological background. In light of the higher EMG amplitude under LBNP, it is conceivable that elevated levels of AMPK and P-AMPK compared to the control group might perhaps be the result of a cumulative effect stemming from increasing numbers of active motor units rather than elevated expression and phosphorylation in individual muscle fibers (Parganlija et al. 2020). Enhanced recruitment of motor units would in turn result in higher oxygen exploitation, a notion supported by the observed increase in total hemoglobin accompanied by gradually declining tissue oxygen saturation under LBNP contrasting the largely stable tissue oxygen saturation during exercise under ambient pressure. Since hypoxia is one of the known triggers of AMPK activation (Mu et al. 2001, Jørgensen et al. 2006), transient hypoxia induced by higher oxygen exploitation in the working muscle and subsequent reduced oxygen delivery due to the absence of LBNP after exercise may also have initially contributed to the higher P-AMPK to AMPK ratio compared to the control group (Parganlija et al. 2020). In addition, elevated AMPK activation may have facilitated initial maintaining of angiogenesis, supported by the lack of an early increase in endostatin under LBNP in contrast to the control group.

Summary and outlook

In summary, the experimental setting described in the present thesis involves a novel, robotically controlled leg press that allows customized training specifications including varying loads and enables even exercise performance with equivalent contribution from both limbs, while also providing an additional benefit of enhanced muscle perfusion through superimposed LBNP. Results of the two studies conducted within the present thesis indicate that combining uninterrupted, slow-pace and high-intensity resistance exercise with LBNP facilitates oxygenation of the working muscle which could, provided sufficient LBNP tolerance and appropriate individual exercise conditions, stimulate oxidative energy provision and potentially result in a positive impact on muscle

performance (Parganlija et al. 2019, Parganlija et al. 2020). Training customization allowed by the RCL would expectedly be of great value in achieving this goal. As results of the present thesis confirm, individual differences not only in LBNP tolerance but also in the overall fitness and activity profile of the subjects will be relevant aspects to consider within said endeavor. Indications of higher oxygen exploitation in the second versus the first study suggest this potentially beneficial response might rather be reserved for more trained individuals, which might be associated with the transition from low oxidative type IIb fibers to high oxidative type IIa fibers induced by high resistance training (Goreham, C. et al. 1999). Nevertheless, occurrence of elevated muscle fatigue in contrast to the first study also indicates that not all subjects equally profit from enhanced muscle oxygen supply under an identical exercise protocol, substantiating the need for further research into training optimization. Molecular responses observed within the present thesis suggest that superimposed LBNP modifies the impact of intense resistance exercise on the local metabolism of the working muscle and its surrounding extracellular matrix (Parganlija et al. 2020). Beyond their relevance in terms of aerobic energy production, these molecular responses are further potentially advantageous for muscle growth, since angiogenesis has been found to accompany hypertrophy (Holloway et al. 2018). Distinct response patterns observed for the energy sensor AMPK and the angiogenic factors MMP-2, MMP-9 and endostatin not only make the associated molecular pathways interesting targets for future research into mechanisms underlying the LBNP response but might also uncover potentially valuable molecular markers for the progress assessment and optimization of future training interventions with LBNP. In addition, the associated metabolic processes including oxidative metabolism, protein synthesis and angiogenesis being affected by space flight and readaptation to Earth's gravity gives further relevance to studying these pathways in the context of countermeasure activities. Considering the value of resistance exercise for muscle mass enhancement and strength development (Tesch et al. 2004), especially when involving concentric and eccentric movement (Dudley et al. 1991), and the benefits of added LBNP, continued research into this combined intervention could advance the development of countermeasures for muscle loss in astronauts as well as ground-based medical technology (Parganlija et al. 2019, Parganlija et al. 2020). With accessibility of published work within the scientific community in mind, it is the author's hope that the results of the present thesis might contribute a small piece towards solving such scientific puzzles and perhaps also afford a slight impulse for future research endeavors.

References

Adams GR, Caiozzo VJ, Baldwin KM (2003) Skeletal muscle unweighting: spaceflight and ground-based models. Journal of applied physiology (Bethesda, Md. : 1985) 95(6):2185–2201. doi: 10.1152/japplphysiol.00346.2003

Alkner BA, Tesch PA (2004) Efficacy of a gravity-independent resistance exercise device as a countermeasure to muscle atrophy during 29-day bed rest. Acta physiologica Scandinavica 181(3):345–357. doi: 10.1111/j.1365-201X.2004.01293.x

Antonutto G, Bodem F, Zamparo P, Di Prampero PE (1998) Maximal power and EMG of lower limbs after 21 days spaceflight in one astronaut. Journal of gravitational physiology : a journal of the International Society for Gravitational Physiology 5(1):P63-6

Antonutto G, Capelli C, Girardis M, Zamparo P, di PPE (1999) Effects of microgravity on maximal power of lower limbs during very short efforts in humans. Journal of applied physiology (Bethesda, Md. : 1985) 86(1). doi: 10.1152/jappl.1999.86.1.85

Aratow M, Fortney SM, Watenpaugh DE, Crenshaw AG, Hargens AR (1993) Transcapillary fluid responses to lower body negative pressure. Journal of applied physiology (Bethesda, Md. : 1985) 74(6):2763–2770. doi: 10.1152/jappl.1993.74.6.2763

Aubert AE, Beckers F, Verheyden B (2005) Cardiovascular function and basics of physiology in microgravity. Acta cardiologica 60(2):129–151. doi: 10.2143/AC.60.2.2005024

Baily RG, Leuenberger U, Leaman G, Silber D, Sinoway LI (1991) Norepinephrine kinetics and cardiac output during nonhypotensive lower body negative pressure. Am J Physiol 260(5 Pt 2):H1708-12

Baisch F, Beck L, Blomqvist G, Wolfram G, Drescher J, Rome JL, Drummer C (2000a) Cardiovascular response to lower body negative pressure stimulation before, during, and after space flight. Eur.J.Clin.Invest 30(12):1055–1065

Baisch FJ (1993) Body fluid distribution in man in space and effect of lower body negative pressure treatment. The Clinical investigator 71(9):690–699. doi: 10.1007/BF00209722

Baisch JF, Wolfram G, Beck L, Drummer C, Störmer I, Buckey J, Blomqvist G (2000b) Orthostatic stress is necessary to maintain the dynamic range of cardiovascular control in space. Pflugers Archiv : European journal of physiology 441(2-3 Suppl):R52-61. doi: 10.1007/s004240000381

Baker ES, Barratt MR, Wear ML (2008) Human Response to Space Flight. In: Barratt MR, Pool SL (eds) Principles of Clinical Medicine for Space Flight. SPRINGER-VERLAG NEW YORK, New York, NY, pp 27–57

Bamman MM, Caruso JF (2000) Resistance exercise countermeasures for space flight. implications of training specificity. J.Strength.Cond.Res. 14(1):45–49

Bangsbo J (1997) Physiology of Muscle Fatigue during Intense Exercise. ne Clinical Pharmacology of Sport and Exercise:123–133

Bartels SA, Bezemer R, Vries FJ, Milstein DM, Lima A, Cherpanath TG, van den Meiracker, A. H., van BJ, Heger M, Karemaker JM, Ince C (2011) Multi-site and multi-depth near-infrared

spectroscopy in a model of simulated (central) hypovolemia. lower body negative pressure. Intensive Care Med 37(4):671–677

Beijer Å, Rosenberger A, Bölck B, Suhr F, Rittweger J, Bloch W (2013) Whole-body vibrations do not elevate the angiogenic stimulus when applied during resistance exercise. PloS one 8(11):e80143. doi: 10.1371/journal.pone.0080143

Berdeaux A, Duranteau J, Pussard E, Edouard A, Giudicelli JF (1992) Baroreflex control of regional vascular resistances during simulated orthostatism. Kidney Int.Suppl. 37:S29-S33

Biressi S, Rando TA (2010) Heterogeneity in the muscle satellite cell population. Seminars in cell & developmental biology 21(8):845–854. doi: 10.1016/j.semcdb.2010.09.003

Blaber AP, Hinghofer-Szalkay H, Goswami N (2013) Blood volume redistribution during hypovolemia. Aviation, space, and environmental medicine 84(1):59–64

Boda WL, Watenpaugh DE, Ballard RE, Hargens AR (2000) Supine lower body negative pressure exercise simulates metabolic and kinetic features of upright exercise. J.Appl.Physiol. 89(2):649–654

Bonde-Petersen F, Suzuki M, Christensen NJ (1984) Cardiovascular and hormonal responses to bicycle exercise during lower body negative pressure. Advances in space research : the official journal of the Committee on Space Research (COSPAR) 4(12):31–33

Britto FA, Gnimassou O, Groote E de, Balan E, Warnier G, Everard A, Cani PD, Deldicque L (2020) Acute environmental hypoxia potentiates satellite cell-dependent myogenesis in response to resistance exercise through the inflammation pathway in human. The FASEB Journal 34(1):1885–1900. doi: 10.1096/fj.201902244R

Brown MD, Hudlicka O (2003) Modulation of physiological angiogenesis in skeletal muscle by mechanical forces: involvement of VEGF and metalloproteinases. Angiogenesis 6(1):1–14

Brzhozovskiy AG, Kononikhin AS, Pastushkova LC, Kashirina DN, Indeykina MI, Popov IA, Custaud M-A, Larina IM, Nikolaev EN (2019) The Effects of Spaceflight Factors on the Human Plasma Proteome, Including Both Real Space Missions and Ground-Based Experiments. International journal of molecular sciences 20(13). doi: 10.3390/ijms20133194

Campbell MR, Charles JB (2015) Historical Review of Lower Body Negative Pressure Research in Space Medicine. Aerospace medicine and human performance 86(7):633–640. doi: 10.3357/AMHP.4246.2015

Ceafalan LC, Popescu BO, Hinescu ME (2014) Cellular players in skeletal muscle regeneration. BioMed research international 2014:957014. doi: 10.1155/2014/957014

Chen Z-P, Stephens TJ, Murthy S, Canny BJ, Hargreaves M, Witters LA, Kemp BE, McConell GK (2003) Effect of exercise intensity on skeletal muscle AMPK signaling in humans. Diabetes 52(9):2205–2212. doi: 10.2337/diabetes.52.9.2205

Convertino VA (1990) Physiological adaptations to weightlessness: effects on exercise and work performance. Exercise and sport sciences reviews 18:119–166

Cvirn G, Waha JE, Brix B, Rössler A, Jantscher A, Schlagenhauf A, Koestenberger M, Wonisch W, Wagner T, Goswami N (2019) Coagulation changes induced by lower-body negative

pressure in men and women. Journal of applied physiology (Bethesda, Md. : 1985) 126(5):1214–1222. doi: 10.1152/japplphysiol.00940.2018

Davis SA, Davis BL (2012) Exercise equipment used in microgravity: challenges and opportunities. Current sports medicine reports 11(3):142–147. doi: 10.1249/JSR.0b013e3182578fc3

Downs ME, Hackney KJ, Martin D, Caine TL, Cunningham D, O'Connor DP, Ploutz-Snyder LL (2014) Acute vascular and cardiovascular responses to blood flow-restricted exercise. Medicine and science in sports and exercise 46(8):1489–1497. doi: 10.1249/MSS.0000000000000253

Dreyer HC, Fujita S, Cadenas JG, Chinkes DL, Volpi E, Rasmussen BB (2006) Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. The Journal of physiology 576(Pt 2):613–624. doi: 10.1113/jphysiol.2006.113175

Dudley GA, Tesch PA, Harris RT, Golden CL, Buchanan P (1991) Influence of eccentric actions on the metabolic cost of resistance exercise. Aviat.Space Environ.Med. 62(7):678–682

Edgerton VR, Roy RR (1994) Chapter 2 Neuromuscular Adaptation to Actual and Simulated Weightlessness. In: Bonting SL (ed) Advances in space biology and medicine, vol 4. [JAI Press], [Place of publication not identified], pp 33–67

Edgerton VR, Zhou MY, Ohira Y, Klitgaard H, Jiang B, Bell G, Harris B, Saltin B, Gollnick PD, Roy RR (1995) Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. Journal of applied physiology (Bethesda, Md. : 1985) 78(5). doi: 10.1152/jappl.1995.78.5.1733

Egaña M, Green S (2007) Intensity-dependent effect of body tilt angle on calf muscle fatigue in humans. Eur J Appl Physiol 99(1):1–9

Eiken O (1987) Responses to dynamic leg exercise in man as influenced by changes in muscle perfusion pressure. Acta physiologica Scandinavica. Supplementum 566:1–37

Eiken O (1988) Effects of increased muscle perfusion pressure on responses to dynamic leg exercise in man. European Journal of Applied Physiology and Occupational Physiology 57(6):772–776. doi: 10.1007/bf01076002

Eiken O, Bjurstedt H (1985) Cardiac responses to lower body negative pressure and dynamic leg exercise. European Journal of Applied Physiology and Occupational Physiology 54(5):451–455. doi: 10.1007/bf00422950

Eiken O, Lind F, Bjurstedt H (1986) Effects of blood volume distribution on ventilatory variables at rest and during exercise. Acta physiologica Scandinavica 127(4):507–512. doi: 10.1111/j.1748-1716.1986.tb07935.x

English KL, Lee SMC, Loehr JA, Ploutz-Snyder RJ, Ploutz-Snyder LL (2015) Isokinetic Strength Changes Following Long-Duration Spaceflight on the ISS. Aerospace medicine and human performance 86(12 Suppl):A68-A77. doi: 10.3357/AMHP.EC09.2015

Ertl AC, Diedrich A, Biaggioni I, Levine BD, Robertson RM, Cox JF, Zuckerman JH, Pawelczyk JA, Ray CA, Buckey JC, Lane LD, Shiavi R, Gaffney FA, Costa F, Holt C, Blomqvist CG, Eckberg DL, Baisch FJ, Robertson D (2002) Human muscle sympathetic nerve activity and plasma noradrenaline kinetics in space. The Journal of physiology 538(Pt 1):321–329. doi: 10.1113/jphysiol.2001.012576

Ferguson RA, Hunt JEA, Lewis MP, Martin NRW, Player DJ, Stangier C, Taylor CW, Turner MC (2018) The acute angiogenic signalling response to low-load resistance exercise with blood flow restriction. European journal of sport science:397–406. doi: 10.1080/17461391.2017.1422281

Ferrando AA, Paddon-Jones D, Wolfe RR (2002) Alterations in protein metabolism during space flight and inactivity. Nutrition 18(10):837–841. doi: 10.1016/S0899-9007(02)00930-9

Fitts RH, Riley DR, Widrick JJ (2000) Physiology of a microgravity environment invited review: microgravity and skeletal muscle. Journal of applied physiology (Bethesda, Md. : 1985) 89(2):823–839. doi: 10.1152/jappl.2000.89.2.823

Fu X, Wang H, Hu P (2015) Stem cell activation in skeletal muscle regeneration. Cellular and molecular life sciences : CMLS 72(9):1663–1677. doi: 10.1007/s00018-014-1819-5

Fujii N, Hayashi T, Hirshman MF, Smith JT, Habinowski SA, Kaijser L, Mu J, Ljungqvist O, Birnbaum MJ, Witters LA, Thorell A, Goodyear LJ (2000) Exercise induces isoform-specific increase in 5'AMP-activated protein kinase activity in human skeletal muscle. Biochemical and biophysical research communications 273(3):1150–1155. doi: 10.1006/bbrc.2000.3073

Garrett-Bakelman FE, Darshi M, Green SJ, Gur RC, Lin L, Macias BR, McKenna MJ, Meydan C, Mishra T, Nasrini J, Piening BD, Rizzardi LF, Sharma K, Siamwala JH, Taylor L, Vitaterna MH, Afkarian M, Afshinnekoo E, Ahadi S, Ambati A, Arya M, Bezdan D, Callahan CM, Chen S, Choi AMK, Chlipala GE, Contrepois K, Covington M, Crucian BE, Vivo I de, Dinges DF, Ebert DJ, Feinberg JI, Gandara JA, George KA, Goutsias J, Grills GS, Hargens AR, Heer M, Hillary RP, Hoofnagle AN, Hook VYH, Jenkinson G, Jiang P, Keshavarzian A, Laurie SS, Lee-McMullen B, Lumpkins SB, MacKay M, Maienschein-Cline MG, Melnick AM, Moore TM, Nakahira K, Patel HH, Pietrzyk R, Rao V, Saito R, Salins DN, Schilling JM, Sears DD, Sheridan CK, Stenger MB, Tryggvadottir R, Urban AE, Vaisar T, van Espen B, Zhang J, Ziegler MG, Zwart SR, Charles JB, Kundrot CE, Scott GBI, Bailey SM, Basner M, Feinberg AP, Lee SMC, Mason CE, Mignot E, Rana BK, Smith SM, Snyder MP, Turek FW (2019) The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight. Science (New York, N.Y.) 364(6436). doi: 10.1126/science.aau8650

Gashev AA (2002) Physiologic aspects of lymphatic contractile function: current perspectives. Annals of the New York Academy of Sciences 979:178-87; discussion 188-96. doi: 10.1111/j.1749-6632.2002.tb04878.x

Gashev AA, Zawieja DC (2016) Lymph Transport and Lymphatic System. In: Vohr H-W (ed) Encyclopedia of immunotoxicology. With 108 tables, 2. ed. Springer, Heidelberg, New York, NY, Dordrecht, London, Berlin, pp 547–549

Gasiorowska A, Nazar K, Mikulski T, Cybulski G, Niewiadomski W, Smorawinski J, Krzeminski K, Porta S, Kaciuba-Uscilko H (2005) Hemodynamic and neuroendocrine predictors of lower body negative pressure (LBNP) intolerance in healthy young men. J Physiol Pharmacol. 56(2):179–193

Gibson JN, Halliday D, Morrison WL, Stoward PJ, Hornsby GA, Watt PW, Murdoch G, Rennie MJ (1987) Decrease in human quadriceps muscle protein turnover consequent upon leg immobilization. Clinical science (London, England : 1979) 72(4):503–509. doi: 10.1042/cs0720503

Goreham, C., Green, H. J., Ball-Burnett, M., Ranney, D. (1999) High-resistance training and muscle metabolism during prolonged exercise. The American journal of physiology 276(3):E489-96. doi: 10.1152/ajpendo.1999.276.3.E489

Gorostiaga EM, Navarro-Amezqueta I, Calbet JAL, Sanchez-Medina L, Cusso R, Guerrero M, Granados C, Gonzalez-Izal M, Ibanez J, Izquierdo M (2014) Blood ammonia and lactate as markers of muscle metabolites during leg press exercise. Journal of strength and conditioning research / National Strength & Conditioning Association 28(10):2775–2785. doi: 10.1519/JSC.0000000000000496

Goswami N, Blaber AP, Hinghofer-Szalkay H, Convertino VA (2019a) Lower Body Negative Pressure: Physiological Effects, Applications, and Implementation. Physiological Reviews 99(1):807–851. doi: 10.1152/physrev.00006.2018

Goswami N, Reichmuth J, Di Mise A, Brix B, Roessler A, Centrone M, Ranieri M, Russo A, Santo NG de, Tamma G, Sasso FC, Valenti G (2019b) Comparison between men and women of volume regulating hormones and aquaporin-2 excretion following graded central hypovolemia. European Journal of Applied Physiology and Occupational Physiology 119(3):633–643. doi: 10.1007/s00421-018-4053-2

Green DJ, Hopman MTE, Padilla J, Laughlin MH, Thijssen DHJ (2017) Vascular Adaptation to Exercise in Humans: Role of Hemodynamic Stimuli. Physiological Reviews 97(2):495–528. doi: 10.1152/physrev.00014.2016

Green, H. J. (1997) Mechanisms of muscle fatigue in intense exercise. Journal of sports sciences 15(3). doi: 10.1080/026404197367254

Grigor'ev AI, Noskov VB, Poliakov VV, Vorob'ev DV, Nichiporuk IA, Hinghofer-Szalkay G, Rossler A, Kvetnianski R, Macho L (1998) Dinamika reaktivnosti sistemy gormonal'noĭ reguliatsii pri vozdeĭstvii ODNT vo vremia dlitel'nogo kosmicheskogo poleta (Dynamic changes in the reactivity of the hormonal system regulation with the impact by LBNP sessions in long-term space mission). Aviakosmicheskaia i ekologicheskaia meditsina = Aerospace and environmental medicine 32(3):18–23

Groppo ER, Eastlack RK, Mahar A, Hargens AR, Pedowitz RA (2005) Simulated hypergravity running increases skeletal and cardiovascular loads. Medicine and science in sports and exercise 37(2):262–266. doi: 10.1249/01.mss.0000152732.46744.4f

Hachiya T, Blaber AP, Saito M (2004) Changes in superficial blood distribution in thigh muscle during LBNP assessed by NIRS. Aviat.Space Environ.Med 75(2):118–122

Hardie DG (2011) AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes & development 25(18):1895–1908. doi: 10.1101/gad.17420111

Hargens AR, Bhattacharya R, Schneider SM (2013) Space physiology VI. exercise, artificial gravity, and countermeasure development for prolonged space flight. European journal of applied physiology 113(9):2183–2192. doi: 10.1007/s00421-012-2523-5

Hargens AR, Richardson S (2009) Cardiovascular adaptations, fluid shifts, and countermeasures related to space flight. Respiratory physiology & neurobiology 169 Suppl 1:S30-3. doi: 10.1016/j.resp.2009.07.005

Hargreaves M, Spriet LL (2020) Skeletal muscle energy metabolism during exercise. https://www.nature.com/articles/s42255-020-0251-4. Accessed 12 Dec 2020.678Z

Hendrickse P, Degens H (2019) The role of the microcirculation in muscle function and plasticity. J Muscle Res Cell Motil 40(2):127–140. doi: 10.1007/s10974-019-09520-2

Hinghofer-Szalkay HG, Noskov V, Jezova D, Sauseng-Fellegger G, Fueger GF, Sukhanov Y, König EM, Zambo-Polz C, Barowitsch C, Viehböck F (1993) Hormonal changes with lower body negative pressure on the 6th day in microgravity in one cosmonaut. Aviation, space, and environmental medicine 64(11):1000–1005

Hinghofer-Szalkay HG, Noskov VB, Rössler A, Grigoriev AI, Kvetnansky R, Polyakow VV (1999) Endocrine status and LBNP-induced hormone changes during a 438-day spaceflight: a case study. Aviation, space, and environmental medicine 70(1):1–5

Hoier B, Nordsborg N, Andersen S, Jensen L, Nybo L, Bangsbo J, Hellsten Y (2012) Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. The Journal of physiology 590(3):595–606. doi: 10.1113/jphysiol.2011.216135

Høier B, Rufener N, Bojsen-Møller J, Bangsbo J, Hellsten Y (2010) The effect of passive movement training on angiogenic factors and capillary growth in human skeletal muscle. The Journal of physiology 588(Pt 19):3833–3845. doi: 10.1113/jphysiol.2010.190439

Holloway TM, Snijders T, van Kranenburg J, van Loon LJC, Verdijk LB (2018) Temporal Response of Angiogenesis and Hypertrophy to Resistance Training in Young Men. Medicine and science in sports and exercise 50(1):36–45. doi: 10.1249/MSS.0000000000001409

Horwath O, Moberg M, Larsen FJ, Philp A, Apró W, Ekblom B (2021) Influence of sex and fiber type on the satellite cell pool in human skeletal muscle. Scandinavian journal of medicine & science in sports 31(2):303–312. doi: 10.1111/sms.13848

Hudlicka O, Brown M, Egginton S (1992) Angiogenesis in skeletal and cardiac muscle. Physiological Reviews 72(2):369–417. doi: 10.1152/physrev.1992.72.2.369

Jäger S, Handschin C, St-Pierre J, Spiegelman BM (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proceedings of the National Academy of Sciences of the United States of America 104(29):12017–12022. doi: 10.1073/pnas.0705070104

Jaoude J, Koh Y (2016) Matrix metalloproteinases in exercise and obesity. Vascular health and risk management 12:287–295. doi: 10.2147/VHRM.S103877

Jørgensen SB, Richter EA, Wojtaszewski JFP (2006) Role of AMPK in skeletal muscle metabolic regulation and adaptation in relation to exercise. The Journal of physiology 574(Pt 1):17–31. doi: 10.1113/jphysiol.2006.109942

Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell metabolism 1(1):15–25. doi: 10.1016/j.cmet.2004.12.003

Karlsen A, Soendenbroe C, Malmgaard-Clausen NM, Wagener F, Moeller CE, Senhaji Z, Damberg K, Andersen JL, Schjerling P, Kjaer M, Mackey AL (2020) Preserved capacity for satellite cell

proliferation, regeneration, and hypertrophy in the skeletal muscle of healthy elderly men. The FASEB Journal 34(5):6418–6436. doi: 10.1096/fj.202000196R

Kashirina DN, Percy AJ, Pastushkova LK, Borchers CH, Kireev KS, Ivanisenko VA, Kononikhin AS, Nikolaev EN, Larina IM (2019) The molecular mechanisms driving physiological changes after long duration space flights revealed by quantitative analysis of human blood proteins. BMC medical genomics 12(Suppl 2):45. doi: 10.1186/s12920-019-0490-y

Kesler CT, Liao S, Munn LL, Padera TP (2013) LYMPHATIC VESSELS IN HEALTH AND DISEASE. Wiley interdisciplinary reviews. Systems biology and medicine 5(1):111–124. doi: 10.1002/wsbm.1201

Kissane RWP, Egginton S (2019) Exercise-mediated angiogenesis. Current Opinion in Physiology 10:193–201. doi: 10.1016/j.cophys.2019.06.005

Kjaer M (2004) Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiological Reviews 84(2):649–698. doi: 10.1152/physrev.00031.2003

Kjøbsted R, Hingst JR, Fentz J, Foretz M, Sanz M-N, Pehmøller C, Shum M, Marette A, Mounier R, Treebak JT, Wojtaszewski JFP, Viollet B, Lantier L (2018) AMPK in skeletal muscle function and metabolism. The FASEB Journal 32(4):1741–1777. doi: 10.1096/fj.201700442R

Klaus S, Keipert S, Rossmeisl M, Kopecky J (2012) Augmenting energy expenditure by mitochondrial uncoupling: a role of AMP-activated protein kinase. Genes & nutrition 7(3):369–386. doi: 10.1007/s12263-011-0260-8

Lambertz D, Pérot C, Kaspranski R, Goubel F (2001) Effects of long-term spaceflight on mechanical properties of muscles in humans. Journal of applied physiology (Bethesda, Md. : 1985) 90(1). doi: 10.1152/jappl.2001.90.1.179

Lang T, Van Loon JJWA, Bloomfield S, Vico L, Chopard A, Rittweger J, Kyparos A, Blottner D, Vuori I, Gerzer R, Cavanagh PR (2017) Towards human exploration of space: the THESEUS review series on muscle and bone research priorities. npj Microgravity 3(1):1–10. doi: 10.1038/s41526-017-0013-0

Laughlin MH (1987) Skeletal muscle blood flow capacity. role of muscle pump in exercise hyperemia. The American journal of physiology 253(5 Pt 2):H993-1004

Laughlin MH, Schrage WG (1999) Effects of muscle contraction on skeletal muscle blood flow. when is there a muscle pump? Medicine and science in sports and exercise 31(7):1027–1035

Laurens C, Simon C, Vernikos J, Gauquelin-Koch G, Blanc S, Bergouignan A (2019) Revisiting the Role of Exercise Countermeasure on the Regulation of Energy Balance During Space Flight. Frontiers in physiology 10:321. doi: 10.3389/fphys.2019.00321

LeBlanc A, Lin C, Shackelford L, Sinitsyn V, Evans H, Belichenko O, Schenkman B, Kozlovskaya I, Oganov V, Bakulin A, Hedrick T, Feeback D (2000) Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. Journal of applied physiology (Bethesda, Md. : 1985) 89(6):2158–2164. doi: 10.1152/jappl.2000.89.6.2158

LeBlanc A, Rowe R, Schneider V, Evans H, Hedrick T (1995) Regional muscle loss after short duration spaceflight. Aviation, space, and environmental medicine 66(12):1151–1154

Lee S-J, Jang J-W, Kim Y-M, Lee HI, Jeon JY, Kwon Y-G, Lee S-T (2002) Endostatin binds to the catalytic domain of matrix metalloproteinase-2. FEBS Letters 519(1):147–152. doi: 10.1016/S0014-5793(02)02742-4

Lee SMC, Schneider SM, Boda WL, Watenpaugh DE, Macias BR, Meyer RS, Hargens AR (2007) Supine LBNP exercise maintains exercise capacity in male twins during 30-d bed rest. Medicine and science in sports and exercise 39(8):1315–1326. doi: 10.1249/mss.0b013e31806463d9

Lee SMC, Schneider SM, Boda WL, Watenpaugh DE, Macias BR, Meyer RS, Hargens AR (2009) LBNP exercise protects aerobic capacity and sprint speed of female twins during 30 days of bed rest. Journal of applied physiology (Bethesda, Md. : 1985) 106(3):919–928. doi: 10.1152/japplphysiol.91502.2008

Lee SMC, Schneider SM, Feiveson AH, Macias BR, Smith SM, Watenpaugh DE, Hargens AR (2014) WISE-2005: Countermeasures to prevent muscle deconditioning during bed rest in women. Journal of applied physiology (Bethesda, Md. : 1985) 116(6):654–667. doi: 10.1152/japplphysiol.00590.2013

Leuchtmann AB, Mueller SM, Aguayo D, Petersen JA, Ligon-Auer M, Flück M, Jung HH, Toigo M Resistance training preserves high-intensity interval training induced improvements in skeletal muscle capillarization of healthy old men: a randomized controlled trial. Sci Rep 10(1):1–10. doi: 10.1038/s41598-020-63490-x

Leyk D, Essfeld D, Baum K, Stegemann J (1994) Early leg blood flow adjustment during dynamic foot plantarflexions in upright and supine body position. Int.J Sports Med 15(8):447–452

Lo Presti R, Hopps E, Caimi G (2017) Gelatinases and physical exercise: A systematic review of evidence from human studies. Medicine 96(37):e8072. doi: 10.1097/MD.0000000000008072

Luk H-Y, Levitt DE, Boyett JC, Rojas S, Flader SM, McFarlin BK, Vingren JL (2019) Resistance exercise-induced hormonal response promotes satellite cell proliferation in untrained men but not in women. American journal of physiology. Endocrinology and metabolism 317(2):E421-E432. doi: 10.1152/ajpendo.00473.2018

Lundberg TR, Fernandez-Gonzalo R, Tesch PA (2014) Exercise-induced AMPK activation does not interfere with muscle hypertrophy in response to resistance training in men. Journal of applied physiology (Bethesda, Md. : 1985) 116(6):611–620. doi: 10.1152/japplphysiol.01082.2013

Macias BR, Cao P, Watenpaugh DE, Hargens AR (2007) LBNP treadmill exercise maintains spine function and muscle strength in identical twins during 28-day simulated microgravity. Journal of applied physiology (Bethesda, Md. : 1985) 102(6):2274–2278. doi: 10.1152/japplphysiol.00541.2006

Maillet A, Fagette S, Allevard AM, Pavy-Le Traon A, Guell A, Gharib C, Gauquelin G (1996) Cardiovascular and hormonal response during a 4-week head-down tilt with and without exercise and LBNP countermeasures. Journal of gravitational physiology : a journal of the International Society for Gravitational Physiology 3(1):37–48

Minokoshi Y, Alquier T, Furukawa N, Kim Y-B, Lee A, Xue B, Mu J, Foufelle F, Ferré P, Birnbaum MJ, Stuck BJ, Kahn BB (2004) AMP-kinase regulates food intake by responding to

hormonal and nutrient signals in the hypothalamus. Nature 428(6982):569–574. doi: 10.1038/nature02440

Montarras D, L'honoré A, Buckingham M (2013) Lying low but ready for action: the quiescent muscle satellite cell. The FEBS journal 280(17):4036–4050. doi: 10.1111/febs.12372

Moore DR, Kelly RP, Devries MC, Churchward-Venne TA, Phillips SM, Parise G, Johnston AP (2018) Low-load resistance exercise during inactivity is associated with greater fibre area and satellite cell expression in older skeletal muscle. Journal of cachexia, sarcopenia and muscle 9(4):747–754. doi: 10.1002/jcsm.12306

Mortensen SP, Egginton S, Madsen M, Hansen JB, Munch GDW, Iepsen UW, Åkerström T, Pedersen BK, Hellsten Y (2017) Alpha adrenergic receptor blockade increases capillarization and fractional O2 extraction and lowers blood flow in contracting human skeletal muscle. Acta Physiologica 221(1):32–43. doi: 10.1111/apha.12857

Mortensen SP, Saltin B (2014) Regulation of the skeletal muscle blood flow in humans. Experimental physiology 99(12):1552–1558. doi: 10.1113/expphysiol.2014.081620

Mu J, Brozinick JT, Valladares O, Bucan M, Birnbaum MJ (2001) A Role for AMP-Activated Protein Kinase in Contraction- and Hypoxia-Regulated Glucose Transport in Skeletal Muscle. Molecular Cell 7(5):1085–1094. doi: 10.1016/S1097-2765(01)00251-9

Murthy G, Watenpaugh DE, Ballard RE, Hargens AR (1994) Exercise against lower body negative pressure as a countermeasure for cardiovascular and musculoskeletal deconditioning. Acta astronautica 33:89–96

Nagata D, Mogi M, Walsh K (2003) AMP-activated protein kinase (AMPK) signaling in endothelial cells is essential for angiogenesis in response to hypoxic stress. The Journal of biological chemistry 278(33):31000–31006. doi: 10.1074/jbc.M300643200

Nalbandian M, Radak Z, Takeda M (2020) Lactate Metabolism and Satellite Cell Fate. Frontiers in physiology 11:610983. doi: 10.3389/fphys.2020.610983

Narici MV, Boer MD de (2011) Disuse of the musculo-skeletal system in space and on earth. European journal of applied physiology 111(3):403–420. doi: 10.1007/s00421-010-1556-x

Nascimento DdC, Durigan RdCM, Tibana RA, Durigan JLQ, Navalta JW, Prestes J (2015) The response of matrix metalloproteinase-9 and -2 to exercise. Sports medicine (Auckland, N.Z.) 45(2):269–278. doi: 10.1007/s40279-014-0265-8

Nederveen JP, Joanisse S, Snijders T, Thomas ACQ, Kumbhare D, Parise G (2018) The influence of capillarization on satellite cell pool expansion and activation following exercise-induced muscle damage in healthy young men. The Journal of physiology 596(6):1063–1078. doi: 10.1113/JP275155

Nederveen JP, Joanisse S, Thomas ACQ, Snijders T, Manta K, Bell KE, Phillips SM, Kumbhare D, Parise G (2020) Age-related changes to the satellite cell niche are associated with reduced activation following exercise. The FASEB Journal 34(7):8975–8989. doi: 10.1096/fj.201900787R

Negrini D, Moriondo A (2011) Lymphatic anatomy and biomechanics. The Journal of physiology 589(Pt 12):2927–2934. doi: 10.1113/jphysiol.2011.206672

Newham DJ, Mills KR, Quigley BM, Edwards RH (1983) Pain and fatigue after concentric and eccentric muscle contractions. Clinical science (London, England : 1979) 64(1):55–62. doi: 10.1042/cs0640055

Nishiyasu T, Tan N, Kondo N, Nishiyasu M, Ikegami H (1999) Near-infrared monitoring of tissue oxygenation during application of lower body pressure at rest and during dynamical exercise in humans. Acta physiologica Scandinavica 166(2):123–130. doi: 10.1046/j.1365-201x.1999.00548.x

Norsk P (2020) Adaptation of the cardiovascular system to weightlessness: Surprises, paradoxes and implications for deep space missions. Acta Physiologica 228(3):e13434. doi: 10.1111/apha.13434

Nourshahi M, Hedayati M, Ranjbar K (2012) The correlation between resting serum leptin and serum angiogenic indices at rest and after submaximal exercise. Regulatory peptides 173(1-3):6–12. doi: 10.1016/j.regpep.2011.08.008

Olszewski WL, Engeset A, Sokolowski J (1977) Lymph flow and protein in the normal male leg during lying, getting up, and walking. Lymphology 10(3)

Ouchi N, Shibata R, Walsh K (2005) AMP-activated protein kinase signaling stimulates VEGF expression and angiogenesis in skeletal muscle. Circulation research 96(8):838–846. doi: 10.1161/01.RES.0000163633.10240.3b

Page-McCaw A, Ewald AJ, Werb Z (2007) Matrix metalloproteinases and the regulation of tissue remodelling. Nature reviews. Molecular cell biology 8(3):221–233. doi: 10.1038/nrm2125

Pannérec A, Marazzi G, Sassoon D (2012) Stem cells in the hood: the skeletal muscle niche. Trends in molecular medicine 18(10):599–606. doi: 10.1016/j.molmed.2012.07.004

Parganlija D, Gehlert S, Herrera F, Rittweger J, Bloch W, Zange J (2020) Enhanced Blood Supply Through Lower Body Negative Pressure During Slow-Paced, High Load Leg Press Exercise Alters the Response of Muscle AMPK and Circulating Angiogenic Factors. Front. Physiol. 11. doi: 10.3389/fphys.2020.00781

Parganlija D, Nieberg V, Sauer M, Rittweger J, Bloch W, Zange J (2019) Lower body negative pressure enhances oxygen availability in the knee extensor muscles during intense resistive exercise in supine position. European Journal of Applied Physiology and Occupational Physiology 119(6):1289–1303. doi: 10.1007/s00421-019-04113-w

Pascual-Fernández J, Fernández-Montero A, Córdova-Martínez A, Pastor D, Martínez-Rodríguez A, Roche E (2020) Sarcopenia: Molecular Pathways and Potential Targets for Intervention. International journal of molecular sciences 21(22). doi: 10.3390/ijms21228844

Petersen LG, Carlsen JF, Nielsen MB, Damgaard M, Secher NH (2014) The hydrostatic pressure indifference point underestimates orthostatic redistribution of blood in humans. Journal of applied physiology (Bethesda, Md. : 1985) 116(7):730–735. doi: 10.1152/japplphysiol.01175.2013

Petersen N, Jaekel P, Rosenberger A, Weber T, Scott J, Castrucci F, Lambrecht G, Ploutz-Snyder L, Damann V, Kozlovskaya I, Mester J (2016) Exercise in space: the European Space Agency

approach to in-flight exercise countermeasures for long-duration missions on ISS. Extreme Physiology & Medicine 5. doi: 10.1186/s13728-016-0050-4

Pollack AA, Taylor BE, Myers TT, Wood EH (1949) The Effect of Exercise and Body Position on the Venous Pressure at the Ankle in Patients Having Venous Valvular Defects. J Clin Invest 28(3):559–563. doi: 10.1172/JCI102105

Pollack AA, Wood EH (1949) Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. J Appl Physiol 1(9):649–662

Prior BM, Yang HT, Terjung RL (2004) What makes vessels grow with exercise training? Journal of applied physiology (Bethesda, Md. : 1985) 97(3):1119–1128. doi: 10.1152/japplphysiol.00035.2004

Pugh JK, Faulkner SH, Turner MC, Nimmo MA (2018) Satellite cell response to concurrent resistance exercise and high-intensity interval training in sedentary, overweight/obese, middle-aged individuals. Eur J Appl Physiol 118(2):225–238. doi: 10.1007/s00421-017-3721-y

Rasmussen JC, Kwon S, Pinal A, Bareis A, Velasquez FC, Janssen CF, Morrow JR, Fife CE, Karni RJ, Sevick-Muraca EM (2020) Assessing lymphatic route of CSF outflow and peripheral lymphatic contractile activity during head-down tilt using near-infrared fluorescence imaging. Physiological reports 8(4):e14375. doi: 10.14814/phy2.14375

Richter EA, Ruderman NB (2009) AMPK and the biochemistry of exercise: implications for human health and disease. The Biochemical journal 418(2):261–275. doi: 10.1042/BJ20082055

Ronald J. Korthuis (2011) Anatomy of Skeletal Muscle and Its Vascular Supply. In: Korthuis RJ (ed) Skeletal Muscle Circulation. Morgan & Claypool Life Sciences

Rose AJ, Broholm C, Kiillerich K, Finn SG, Proud CG, Rider MH, Richter EA, Kiens B (2005) Exercise rapidly increases eukaryotic elongation factor 2 phosphorylation in skeletal muscle of men. The Journal of physiology 569(Pt 1):223–228. doi: 10.1113/jphysiol.2005.097154

Ross MD, Wekesa AL, Phelan JP, Harrison M (2014) Resistance exercise increases endothelial progenitor cells and angiogenic factors. Medicine and science in sports and exercise 46(1):16–23. doi: 10.1249/MSS.0b013e3182a142da

Rowland TW (2001) The circulatory response to exercise. role of the peripheral pump. Int.J Sports Med 22(8):558–565

Ruiter CJ de, Goudsmit JFA, van Tricht JA, Haan A de (2007) The isometric torque at which knee-extensor muscle reoxygenation stops. Medicine and science in sports and exercise 39(3):443–453. doi: 10.1249/mss.0b013e31802dd3cc

Rullman E, Norrbom J, Strömberg A, Wågsäter D, Rundqvist H, Haas T, Gustafsson T (2009) Endurance exercise activates matrix metalloproteinases in human skeletal muscle. Journal of applied physiology (Bethesda, Md. : 1985) 106(3):804–812. doi: 10.1152/japplphysiol.90872.2008

Rullman E, Rundqvist H, Wågsäter D, Fischer H, Eriksson P, Sundberg CJ, Jansson E, Gustafsson T (2007) A single bout of exercise activates matrix metalloproteinase in human skeletal muscle. Journal of applied physiology (Bethesda, Md. : 1985) 102(6):2346–2351. doi: 10.1152/japplphysiol.00822.2006

Salanova M, Schiffl G, Püttmann B, Schoser BG, Blottner D (2008) Molecular biomarkers monitoring human skeletal muscle fibres and microvasculature following long-term bed rest with and without countermeasures. Journal of anatomy 212(3):306–318. doi: 10.1111/j.1469-7580.2008.00854.x

Sanchez AMJ, Candau RB, Csibi A, Pagano AF, Raibon A, Bernardi H (2012) The role of AMP-activated protein kinase in the coordination of skeletal muscle turnover and energy homeostasis. American journal of physiology. Cell physiology 303(5):C475-85. doi: 10.1152/ajpcell.00125.2012

Sarelius I, Pohl U (2010) Control of muscle blood flow during exercise: local factors and integrative mechanisms. Acta physiologica (Oxford, England) 199(4):349–365. doi: 10.1111/j.1748-1716.2010.02129.x

Sayers SP, Clarkson PM (2003) Short-term immobilization after eccentric exercise. Part II: creatine kinase and myoglobin. Medicine and science in sports and exercise 35(5):762–768. doi: 10.1249/01.MSS.0000064933.43824.ED

Scallan JP, Zawieja SD, Castorena-Gonzalez JA, Davis MJ (2016) Lymphatic pumping: mechanics, mechanisms and malfunction. The Journal of physiology 594(20):5749–5768. doi: 10.1113/JP272088

Schmid-Schönbein GW (1990) Microlymphatics and lymph flow. Physiological Reviews 70(4):987–1028. doi: 10.1152/physrev.1990.70.4.987

Schuenke MD, Herman JR, Gliders RM, Hagerman FC, Hikida RS, Rana SR, Ragg KE, Staron RS (2012) Early-phase muscular adaptations in response to slow-speed versus traditional resistance-training regimens. European journal of applied physiology 112(10):3585–3595. doi: 10.1007/s00421-012-2339-3

Shackelford LC (2019) Musculoskeletal Response to Space Flight. In: Barratt MR, Baker ES, Pool SL (eds) Principles of clinical medicine for space flight. SPRINGER-VERLAG NEW YORK, [Place of publication not identified], pp 581–607

Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, Spector E, Feeback DL, Lai D (2004) Resistance exercise as a countermeasure to disuse-induced bone loss. Journal of applied physiology (Bethesda, Md. : 1985) 97(1):119–129. doi: 10.1152/japplphysiol.00741.2003

Smith SM, Davis-Street JE, Fesperman JV, Calkins DS, Bawa M, Macias BR, Meyer RS, Hargens AR (2003) Evaluation of treadmill exercise in a lower body negative pressure chamber as a countermeasure for weightlessness-induced bone loss: a bed rest study with identical twins. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 18(12):2223–2230. doi: 10.1359/jbmr.2003.18.12.2223

Smith SM, Heer MA, Shackelford LC, Sibonga JD, Ploutz-Snyder L, Zwart, SR (2012) Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: Evidence from biochemistry and densitometry. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 27(9). doi: 10.1002/jbmr.1647

Smith SM, Zwart SR, Heer M, Lee SMC, Baecker N, Meuche S, Macias BR, Shackelford LC, Schneider S, Hargens AR (2008) WISE-2005: supine treadmill exercise within lower body

negative pressure and flywheel resistive exercise as a countermeasure to bed rest-induced bone loss in women during 60-day simulated microgravity. Bone 42(3):572–581. doi: 10.1016/j.bone.2007.11.015

Snijders T, Nederveen JP, Bell KE, Lau SW, Mazara N, Kumbhare DA, Phillips SM, Parise G (2019) Prolonged exercise training improves the acute type II muscle fibre satellite cell response in healthy older men. The Journal of physiology 597(1):105–119. doi: 10.1113/JP276260

Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, Ragg KE, Toma K (2000) Fiber type composition of the vastus lateralis muscle of young men and women. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 48(5):623–629. doi: 10.1177/002215540004800506

Stein TP (2013) Weight, muscle and bone loss during space flight: another perspective. European journal of applied physiology 113(9):2171–2181. doi: 10.1007/s00421-012-2548-9

Stein TP, Leskiw MJ, Schluter MD, Donaldson MR, Larina I (1999) Protein kinetics during and after long-duration spaceflight on MIR. The American journal of physiology 276(6 Pt 1):E1014-21. doi: 10.1152/ajpendo.1999.276.6.e1014

Stephens TJ, Chen Z-P, Canny BJ, Michell BJ, Kemp BE, McConell GK (2002) Progressive increase in human skeletal muscle AMPKalpha2 activity and ACC phosphorylation during exercise. American journal of physiology. Endocrinology and metabolism 282(3):E688-94. doi: 10.1152/ajpendo.00101.2001

Suhr F, Brixius K, Marées M de, Bölck B, Kleinöder H, Achtzehn S, Bloch W, Mester J (2007) Effects of short-term vibration and hypoxia during high-intensity cycling exercise on circulating levels of angiogenic regulators in humans. Journal of applied physiology (Bethesda, Md. : 1985) 103(2):474–483. doi: 10.1152/japplphysiol.01160.2006

Suhr F, Rosenwick C, Vassiliadis A, Bloch W, Brixius K (2010) Regulation of extracellular matrix compounds involved in angiogenic processes in short- and long-track elite runners. Scandinavian journal of medicine & science in sports 20(3):441–448. doi: 10.1111/j.1600-0838.2009.00960.x

Suzuki H, Conwit RA, Stashuk D, Santarsiero L, Metter EJ (2002) Relationships between surface-detected EMG signals and motor unit activation. Medicine and science in sports and exercise 34(9):1509–1517. doi: 10.1097/00005768-200209000-00018

Tanimoto M, Kawano H, Gando Y, Sanada K, Yamamoto K, Ishii N, Tabata I, Miyachi M (2009) Low-intensity resistance training with slow movement and tonic force generation increases basal limb blood flow. Clinical physiology and functional imaging 29(2):128–135. doi: 10.1111/j.1475-097X.2008.00847.x

Tesch PA, Berg HE, Bring D, Evans HJ, LeBlanc AD (2005) Effects of 17-day spaceflight on knee extensor muscle function and size. European journal of applied physiology 93(4):463–468. doi: 10.1007/s00421-004-1236-9

Tesch PA, Trieschmann JT, Ekberg A (2004) Hypertrophy of chronically unloaded muscle subjected to resistance exercise. Journal of applied physiology (Bethesda, Md. : 1985) 96(4):1451–1458. doi: 10.1152/japplphysiol.01051.2003

Thornton W, Bonato F (2017) Countermeasures to Loss of Muscle and Bone During Spaceflight. In: Thornton WE, Bonato F (eds) The human body and weightlessness. Operational effects, problems and countermeasures, vol 86. Springer, Cham, Switzerland, pp 177–221

Toussaint J-F, Kwong KK, M'Kparu F, Weisskoff RM, Laraia PJ, Kantor HL (1996) Interrelationship of oxidative metabolism and local perfusion demonstrated by NMR in human skeletal muscle. the American Physiological Society 0161-7567/96

Trappe S, Costill D, Gallagher P, Creer A, Peters JR, Evans H, Riley DA, Fitts RH (2009) Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. Journal of applied physiology (Bethesda, Md. : 1985) 106(4):1159–1168. doi: 10.1152/japplphysiol.91578.2008

Urso ML, Pierce JR, Alemany JA, Harman EA, Nindl BC (2009) Effects of exercise training on the matrix metalloprotease response to acute exercise. European journal of applied physiology 106(5):655–663. doi: 10.1007/s00421-009-1063-0

van Doorslaer de Ten Ryen S, Francaux M, Deldicque L (2021) Regulation of satellite cells by exercise in hypoxic conditions: a narrative review. Eur J Appl Physiol. doi: 10.1007/s00421-021-04641-4

van Helmond N, Johnson BD, Curry TB, Cap AP, Convertino VA, Joyner MJ (2015) Coagulation changes during lower body negative pressure and blood loss in humans. American journal of physiology. Heart and circulatory physiology 309(9):H1591-7. doi: 10.1152/ajpheart.00435.2015

Verdijk LB, Snijders T, Holloway TM, van Kranenburg J, van Loon LJC (2016) Resistance Training Increases Skeletal Muscle Capillarization in Healthy Older Men. Medicine and science in sports and exercise 48(11):2157–2164. doi: 10.1249/MSS.0000000000001019

Vilchinskaya NA, Krivoi II, Shenkman BS (2018) AMP-Activated Protein Kinase as a Key Trigger for the Disuse-Induced Skeletal Muscle Remodeling. International journal of molecular sciences 19(11). doi: 10.3390/ijms19113558

Vilchinskaya NA, Mirzoev TM, Lomonosova YN, Kozlovskaya IB, Shenkman BS (2015) Human muscle signaling responses to 3-day head-out dry immersion. Journal of Musculoskeletal & Neuronal Interactions 15(3):286–293

Walia A, Yang JF, Huang Y-H, Rosenblatt MI, Chang J-H, Azar DT (2015) Endostatin's emerging roles in angiogenesis, lymphangiogenesis, disease, and clinical applications. Biochimica et biophysica acta 1850(12):2422–2438. doi: 10.1016/j.bbagen.2015.09.007

Wan J-J, Qin Z, Wang P-Y, Sun Y, Liu X (2017) Muscle fatigue: general understanding and treatment. Experimental & molecular medicine 49(10):e384. doi: 10.1038/emm.2017.194

Watenpaugh DE (2001) Fluid volume control during short-term space flight and implications for human performance. The Journal of experimental biology 204(Pt 18):3209–3215

Watenpaugh DE, Ballard RE, Breit GA, Hargens AR (1999) Self-generated lower body negative pressure exercise. Aviation, space, and environmental medicine 70(5):522–526

Watenpaugh DE, Ballard RE, Schneider SM, Lee SM, Ertl AC, William JM, Boda WL, Hutchinson KJ, Hargens AR (2000) Supine lower body negative pressure exercise during bed rest

maintains upright exercise capacity. Journal of applied physiology (Bethesda, Md. : 1985) 89(1):218–227. doi: 10.1152/jappl.2000.89.1.218

Watenpaugh DE, O'Leary DD, Schneider SM, Lee SMC, Macias BR, Tanaka K, Hughson RL, Hargens AR (2007) Lower body negative pressure exercise plus brief postexercise lower body negative pressure improve post-bed rest orthostatic tolerance. Journal of applied physiology (Bethesda, Md. : 1985) 103(6):1964–1972. doi: 10.1152/japplphysiol.00132.2007

Waters WW, Ziegler MG, Meck JV (2002) Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance. Journal of applied physiology (Bethesda, Md. : 1985) 92(2). doi: 10.1152/japplphysiol.00544.2001

Weid P-Y von der (2019) Lymphatic Vessel Pumping. Advances in experimental medicine and biology 1124:357–377. doi: 10.1007/978-981-13-5895-1_15

Widrick JJ, Knuth ST, Norenberg KM, Romatowski JG, Bain JLW, Riley DA, Karhanek M, Trappe SW, Trappe TA, Costill DL, Fitts RH (1999) Effect of a 17 day spaceflight on contractile properties of human soleus muscle fibres. The Journal of physiology 516(Pt 3):915–930. doi: 10.1111/j.1469-7793.1999.0915u.x

Willan PL, Mahon M, Golland JA (1990) Morphological variations of the human vastus lateralis muscle. Journal of anatomy 168:235–239

Williams DH, Williams C (1983) Cardiovascular and metabolic responses of trained and untrained middle-aged men to a graded treadmill walking test. British Journal of Sports Medicine 17(2):110–116

Winder WW (2001) Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. Journal of applied physiology (Bethesda, Md. : 1985) 91(3):1017–1028. doi: 10.1152/jappl.2001.91.3.1017

Wojtaszewski JF, Nielsen P, Hansen BF, Richter EA, Kiens B (2000) Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle. The Journal of physiology 528 Pt 1:221–226. doi: 10.1111/j.1469-7793.2000.t01-1-00221.x

Wojtaszewski JFP, Mourtzakis M, Hillig T, Saltin B, Pilegaard H (2002) Dissociation of AMPK activity and ACCβ phosphorylation in human muscle during prolonged exercise. Biochemical and biophysical research communications 298(3):309–316. doi: 10.1016/S0006-291X(02)02465-8

Wright JR, McCloskey DI, Fitzpatrick RC (1999) Effects of muscle perfusion pressure on fatigue and systemic arterial pressure in human subjects. Journal of applied physiology (Bethesda, Md. : 1985) 86(3):845–851. doi: 10.1152/jappl.1999.86.3.845

Yablonka-Reuveni Z (2011) The skeletal muscle satellite cell: still young and fascinating at 50. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 59(12):1041–1059. doi: 10.1369/0022155411426780

Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. Physiological Reviews 93(1):23–67. doi: 10.1152/physrev.00043.2011

Zange J, Beisteiner M, Müller K, Shushakov V, Maassen N (2008) Energy metabolism in intensively exercising calf muscle under a simulated orthostasis. Pflugers Arch. 455(6):1153–1163

Zange J, Grehl T, Disselhorst-Klug C, Rau G, Müller K, Schröder R, Tegenthoff M, Malin JP, Vorgerd M (2003) Breakdown of adenine nucleotide pool in fatiguing skeletal muscle in McArdle's disease. A noninvasive 31P-MRS and EMG study. Muscle Nerve 27(6):728–736

Zange J, Müller K, Schuber M, Wackerhage H, Hoffmann U, Günther RW, Adam G, Neuerburg JM, Sinitsyn VE, Bacharev AO, Belichenko OI (1997) Changes in calf muscle performance, energy metabolism, and muscle volume caused by long-term stay on space station MIR. International journal of sports medicine 18 Suppl 4:S308-9. doi: 10.1055/s-2007-972738

Zawieja DC (2009) Contractile physiology of lymphatics. Lymphatic research and biology 7(2):87–96. doi: 10.1089/lrb.2009.0007

Summary

Space flight is associated with various physical challenges, among them the loss of muscle mass and strength which particularly affects postural and lower limb muscles and is yet to be adequately tackled by countermeasures. Beyond the lack of the accustomed gravitational load, reduced blood supply through the headward fluid shift astronauts experience, resulting in lower availability of oxygen and nutrients, is another likely contributing factor to the deconditioning of lower limb muscles. Adequate solution for this issue might therefore be found in the combination of an appropriate form of exercise with lower body negative pressure (LBNP), which generates a fluid shift toward the lower limbs, and can thus counteract the microgravity-induced blood volume reduction in the lower limbs and produce physiological responses equivalent to orthostasis independently of body position or the presence of gravity. Combination of LBNP and exercise should expectedly produce musculoskeletal loads and the cardiovascular stimulus needed to maintain gravity-like adaptation and has been proposed as a countermeasure against deconditioning during long-duration space flight.

Ground-based simulations of deconditioning related to space flight, such as bed rest, have shown benefits of combining LBNP and exercise for counteracting the loss of muscle strength and endurance. However, utilizing such countermeasures in long-duration space flight would require the corresponding equipment to be suitable for use under microgravity conditions and an exercise stimulus powerful enough to induce appropriate muscle responses. Resistance exercise is known to maintain the strength and prevent atrophy of chronically unloaded lower limb muscles, even showing potential for promoting their hypertrophy, and it has previously been suggested that an ideal countermeasure program for astronauts should include high-intensity concentric and eccentric exercise. In addition, adaptive responses to resistance training can be enhanced by reduced contraction velocity, and training with a constant load could not prevent isokinetic strength losses induced by bed rest. Present thesis addresses these issues by exploring the benefits of LBNP applied during intense, slow-paced resistance exercise with varying force on a novel, robotically controlled leg press (RCL) developed at the German Aerospace Center in Cologne, which operates without any gravity-dependent energy storage. It was hypothesized that LBNP would enhance the blood volume and oxygen availability in the leg muscles, consequently stimulating oxidative energy metabolism and potentially benefiting muscle energy levels and performance. These physiological effects were expected to be accompanied by acute molecular responses involving the energy sensor AMPK and angiogenic factors.

Two studies with healthy male subjects were conducted within the present thesis. First study involved 9 subjects performing a series of 15 slow-paced concentric (4 s) and eccentric contractions (4 s) in a permutated crossover study design, without or with 40 mmHg LBNP and 4 s pause between repetitions. Beyond exploring the effects of LBNP, a further aim of this study was to investigate possible benefits of additional relaxation periods between repetitions for muscle perfusion. Exercise was performed with a varying load, starting with a force corresponding to 6% of the one-repetition maximum (1-RM) at knee flexion and gradually increasing to 60% of 1-RM in the first half of the individual range of motion, thereafter remaining constant until full extension. Non-invasive measurements were used to characterize acute cardiovascular responses and leg muscle oxygenation and performance. As continuous exercise proved to have more favorable effects, it was applied during a second, parallel group study with 18 subjects, additionally investigating acute molecular responses of the energy sensor AMPK in the vastus lateralis and circulating angiogenic factors.

Results of the crossover study revealed that LBNP superimposed on exercise induces various acute physiological and cardiovascular responses, which are substantially influenced by the exercise mode (i.e., presence or absence of relaxation periods between contractions). Heart rate increase and stroke volume decrease were more apparent during continuous exercise under ambient pressure or LBNP compared to respective intermittent exercise. Furthermore, reduced cardiac output and elevated total peripheral resistance were only present with continuous exercise. LBNP facilitated the blood refill during low force periods of the contractions, detected as increase in the total hemoglobin and oxyhemoglobin content of the vastus lateralis, with a substantially higher margin between maximal levels under LBNP vs. control during continuous than during intermittent exercise. Elevated respiratory oxygen uptake and post-exercise lactate levels with LBNP indicated enhanced muscle energy turnover with possible contributions from oxidative as well as anaerobic metabolism. EMG amplitude increment was substantially higher during intermittent exercise with LBNP. In contrast, continuous exercise with LBNP showed a trend toward lower EMG amplitude increase compared to control, indicating possible benefits regarding muscle fatigue, which could potentially manifest during prolonged exercise. The outlined findings on continuous exercise were partially reproduced in the parallel group study, particularly regarding the increase in heart rate, oxygen uptake and total muscle hemoglobin. However, oxyhemoglobin levels were found to rather gradually decline during exercise with LBNP, accompanied by steadily rising deoxyhemoglobin content. Taken together with the elevated oxygen uptake and a trend toward lower post-exercise lactate levels, these findings indicated higher oxygen consumption and reliance on oxidative metabolism under LBNP. Higher EMG amplitude increment under LBNP nevertheless suggested insufficient energy provision and ensuing metabolically induced fatigue. LBNP enhanced the post-exercise reduction in MMP-2 and abolished the increase in endostatin as well as the reduction of P-AMPK, AMPK and their ratio observed under ambient pressure.

Taken together, it was demonstrated that LBNP superimposed on continuous, slow-pace and high-intensity leg press exercise enhances blood volume and oxygenation of the vastus lateralis, suggesting facilitated muscle perfusion. Furthermore, enhanced peripheral blood supply and higher oxygen exploitation due to LBNP modify the effects of intense resistance exercise, resulting in activation of the energy sensor AMPK and distinct regulation of angiogenic factors involved in muscle tissue remodeling and capillary growth. Adequate LBNP tolerance and exercise conditions addressing individual needs provided, combining resistance exercise with LBNP could stimulate oxidative energy provision and potentially facilitate muscle performance. LBNP might therefore promote beneficial structural adaptations of skeletal muscles during resistance exercise and contribute to astronauts achieving increased muscle strength and endurance with corresponding countermeasure activities during space flight. Continued research into simulated orthostasis remains of high significance for the optimization of countermeasures for muscle loss induced by space flight.

Zusammenfassung

Raumfahrt setzt den menschlichen Körper besonderen physiologischen Herausforderungen aus, darunter einem Verlust der Muskelmasse und -kraft, welcher insbesondere die Haltungs- und Beinmuskulatur betrifft und bislang von Gegenmaßnahmen nicht adäquat adressiert werden konnte. Über das Fehlen der gewohnten gravitationsbedingten Muskelbelastung hinaus wird diese Dekonditionierung potenziell auch durch die verminderte Durchblutung der Beinmuskulatur gefördert, welche durch die kopfwärts gerichtete Flüssigkeitsverschiebung unter Schwerelosigkeit entsteht und in einer geringeren Verfügbarkeit von Sauerstoff und Nährstoffen resultiert. Eine adäquate Lösung für diese Herausforderung könnte sich daher in der Kombination aus einem geeigneten Training und Unterkörper-Unterdruck (Englisch: “lower body negative pressure”, LBNP) finden, welcher eine Flüssigkeitsverschiebung in Richtung der unteren Extremitäten hervorruft. LBNP kann somit der Schwerelosigkeit-bedingten Reduktion des Blutvolumens in den Beinen entgegenwirken und physiologische Reaktionen ähnlich wie bei einer Orthostase herbeiführen, unabhängig von der Körperposition oder Präsenz der Schwerkraft. Eine Kombination aus körperlicher Übung und LBNP würde erwartungsgemäß die Muskelbelastung und den kardiovaskulären Stimulus gewährleisten, welche für die Aufrechterhaltung einer gravitationsähnlichen Adaptation notwendig sind, und wurde bereits als Gegenmaßnahme für die Dekonditionierung bei Langzeit-Raumflügen vorgeschlagen.

Vorteilhafte Effekte eines Trainings mit LBNP als Gegenmaßnahme für den Verlust an Muskelkraft und -ausdauer sind aus erdbasierten Simulationen der Raumfahrt-assoziierten Dekonditionierung, etwa aus Bettruhestudien, bekannt. Allerdings hätte die Einführung derartiger Gegenmaßnahmen bei Langzeit-Raumflügen als Voraussetzung, dass die zugehörige Apparatur für die Nutzung unter Schwerelosigkeit geeignet ist, sowie dass der angewandte Trainingsstimulus eine geeignete Muskeladaptation wirksam auslösen kann. Krafttraining kann bekannterweise dem Kraftverlust und der Atrophie chronisch unbelasteter Beinmuskulatur vorbeugen, sogar mit Anzeichen für eine mögliche Förderung deren Hypertrophie. In Bezug auf Gegenmaßnahmen für die Dekonditionierung bei Astronauten wurde bereits empfohlen, dass diese idealerweise konzentrische und exzentrische Übungen mit einer hohen Intensität einbeziehen sollten. Ferner kann durch ein Krafttraining ausgelöste Muskeladaptation nachgewiesenermaßen von einer verminderten Kontraktionsgeschwindigkeit profitieren, und ein Training mit konstanter Kraft kann dem Bettruhe-bedingten isokinetischen Kraftverlust nicht entgegenwirken. In der vorliegenden Promotionsarbeit werden diese relevanten Aspekte adressiert, indem mögliche Vorteile einer Anwendung von LBNP bei intensiven Kraftübungen mit reduzierter Geschwindigkeit und variierender Kraft an einer neuartigen, Robotik-gesteuerten Beinpresse (Englisch: „robotically controlled leg press“, RCL) erforscht werden. Die RCL wurde bei dem Deutschen Zentrum für Luft- und Raumfahrt in Köln entwickelt und wird ohne gravitationsabhängige Energiespeicherung bedient. Es wurde von der Hypothese ausgegangen, dass LBNP das Blutvolumen und die Sauerstoffverfügbarkeit in der Beinmuskulatur erhöhen würde, resultierend in einer Stimulation des oxidativen Energiestoffwechsels und potenziell in einer positiven Auswirkung auf den Energiezustand und die Leistung der Muskulatur. Darüber hinaus wurde angenommen, dass diese physiologischen Effekte mit molekularen Akutreaktionen seitens des Energiesensors AMPK und der Angiogenese-Faktoren einhergehen würden.

Zwei Studien mit gesunden männlichen Probanden wurden im Rahmen der vorliegenden Promotionsarbeit durchgeführt. In der ersten Studie haben 9 Probanden 15 konzentrische und exzentrische Kontraktionen in einem langsamen Tempo von jeweils 4 s geleistet, bei einem permutierten Crossover-Studiendesign ohne oder mit 40 mmHg LBNP und 4 s Pause zwischen den Wiederholungen. Neben der Erforschung von LBNP-bedingten Akutreaktionen war ein weiteres Ziel dieser Studie, mögliche Vorteile von zusätzlichen Relaxationsperioden zwischen den Wiederholungen für die Muskelperfusion zu untersuchen. Die Übungen waren von einer variierenden Kraft gekennzeichnet, beginnend mit 6% der maximalen Kraft für eine Wiederholung (Englisch: „one-repetition maximum“, 1-RM) bei der Kniebeugung. Daraufhin wurde die Kraft in der ersten Hälfte der individuellen Bewegungsdistanz allmählich auf 60% des 1-RM gesteigert und schließlich bis in die volle Beinextension konstant gehalten. Zur Charakterisierung der akuten kardiovaskulären Reaktionen sowie der Oxygenierung und Leistung der Beinmuskulatur wurden nicht-invasive Messmethoden angewandt. Da das Übungsprotokoll ohne Pausen zwischen den Wiederholungen vielversprechendere Effekte zeigte, wurde dieses daraufhin in einer Parallelgruppenstudie mit 18 Probanden angewandt, in welcher zusätzlich molekulare Akutreaktionen des Energiesensor-Proteins AMPK im Musculus vastus lateralis und der Angiogenese-Faktoren im Blutserum untersucht wurden.

Die Ergebnisse der Crossover-Studie zeigten, dass diverse akute physiologische und kardiovaskuläre Reaktionen auf die Anwendung von LBNP von der genauen Durchführung der Beinpressübungen (ohne oder mit Pausen zwischen den Wiederholungen) bedeutsam beeinflusst werden. So waren die Erhöhung der Herzfrequenz und die Reduktion des Schlagvolumens im Laufe einer durchgehenden Leistung unter Umgebungsdruck oder LBNP auffälliger als bei respektiven Übungen mit Pause. Zudem wurden eine Verminderung des Herzminutenvolumens sowie eine Erhöhung des totalen peripheren Widerstands nur bei den Übungen ohne Pause beobachtet. LBNP förderte offenbar den Bluteinstrom im Laufe von geringeren Kräften gekennzeichneter Kontraktionsphasen, nachgewiesen als Erhöhung des Total- und Oxyhämoglobins im Vastus lateralis, und zwar mit einer bemerkenswert höheren Differenz zwischen den Maximalwerten unter LBNP vs. Kontrolle während ununterbrochener Arbeit im Vergleich zu den Übungen mit Pausen. Höhere Sauerstoffaufnahme durch die Atmung und höhere Laktatspiegel nach den Übungen mit LBNP deuteten auf einen erhöhten Energieumsatz der Muskulatur hin, mit potenzieller Beteiligung des oxidativen sowie des anaeroben Stoffwechsels. Der Anstieg der EMG-Amplitude war wesentlich höher bei den Übungen mit LBNP und Pausen zwischen den Wiederholungen. Im Gegensatz hierzu zeigte durchgehende Muskelleistung mit LBNP (ohne Pausen) einen gegenüber der Kontrolle tendenziell geringeren Anstieg der EMG-Amplitude, als Anzeichen möglicher Vorteile für die Muskelermüdung, die sich potenziell im Laufe einer längeren Beinpressübung manifestieren könnten. Die oben dargestellten Ergebnisse zu den Beinpressübungen ohne Pausen konnten in der Parallelgruppenstudie zum Teil reproduziert werden, insbesondere in Bezug auf die Erhöhung der Herzfrequenz, der Sauerstoffaufnahme und des Totalhämoglobins in der Muskulatur. Allerdings zeigte der Oxyhämoglobinspiegel im Laufe der Beinpressübungen mit LBNP nun eher eine allmähliche Abnahme, begleitet von einer fortschreitenden Zunahme des Desoxyhämoglobingehalts. Angesichts der höheren Sauerstoffaufnahme und der tendenziell niedrigeren Laktatspiegel nach den Übungen mit LBNP deuteten diese Ergebnisse insgesamt auf einen erhöhten Sauerstoffverbrauch und höhere Leistung des oxidativen Metabolismus unter LBNP. Nichtsdestotrotz zeugte der höhere Anstieg der EMG-Amplitude unter LBNP von einer unzureichenden Energieversorgung und daraus

resultierender, metabolisch bedingter Muskelermüdung. LBNP steigerte zusätzlich die nach den Beinpressübungen aufgetretene Reduktion des MMP-2 Spiegels im Blutserum. Zudem wirkte LBNP der Endostatin-Erhöhung und der Reduktion von AMPK, P-AMPK und des Verhältnisses von P-AMPK zu AMPK entgegen, die folglich nur ohne LBNP beobachtet wurden.

Zusammenfassend wurde festgestellt, dass die Anwendung von LBNP während ununterbrochener, von einer hohen Intensität und einem verhältnismäßig langsamen Tempo gekennzeichneter Beinpressübungen das Blutvolumen und die Oxygenierung des Vastus lateralis fördern kann, die als Anzeichen für eine erhöhte Muskelperfusion gelten. Weiterhin modifizieren die erhöhte periphere Blutzufuhr und der höhere Sauerstoffverbrauch unter LBNP durch Kraftübungen ausgelöste Akutreaktionen, mit Aktivierung des Energiesensors AMPK und geänderter Regulation an der Remodellierung des Muskelgewebes und an dem Wachstum von Kapillaren beteiligter Angiogenese-Faktoren als Folge. Adäquate LBNP-Toleranz und auf individuelle Bedürfnisse ausgerichtete Übungsbedingungen vorausgesetzt, könnte die Kombination aus LBNP und Kraftübungen die oxidative Energieproduktion stimulieren und unter Umständen die Muskelleistung fördern. Somit könnte LBNP vorteilhafte strukturelle Adaptationen der Skelettmuskulatur während eines Krafttrainings begünstigen und zusammen mit entsprechenden Gegenmaßnahmen für die Dekonditionierung unter Schwerelosigkeit der Erreichung einer höheren Muskelkraft und -ausdauer bei Astronauten beitragen. Forschung an mittels LBNP simulierter Orthostase bleibt von hoher Relevanz für die Optimierung von Gegenmaßnahmen zur Bekämpfung der Muskeldekonditionierung in der Raumfahrt.

Master student supervision

Parallel performance of multiple measurements during the two human studies completed as part of the present doctoral thesis was accomplished with support of multiple on-site personnel and students. Under my supervision, three master students supported implementation of the studies: Vita Nieberg, Marc Sauer and Frankyn Herrera. In acknowledgment of their support, the students were granted access to some of the generated raw data with permission to use them in their master theses and were also included as co-authors on respective publications cited in the present thesis. Results presented in the mentioned master theses are hence based on some of the raw data originating from the studies that were conceptualized by the author of the present doctoral thesis and implemented by the same with support of multiple staff and students. For the present doctoral thesis, all raw data were analyzed and interpreted independently and thus without reference to the abovementioned master theses. Entirety of the present doctoral thesis, including therein described studies, data analyses, scientific interpretation and accompanying publications, represents original and independent research work of the author.

Grants

I hereby state to have been the recipient of a Helmholtz Space Life Sciences Research School (SpaceLife) Ph.D. scholarship, beginning from November 2012 and expiring in October 2016. The scholarship was funded in equal parts by the Helmholtz Association (Grant No. VH-KO-300) and the German Aerospace Center (DLR, Cologne). Conference attendance and publishing of scientific papers generated as part of my thesis were funded by DLR, Cologne.

Acknowledgments

Looking back to the beginning of my doctoral journey, I realize just how many people have been very kind to get me started on my way, provide all sorts of valued support on the long and sometimes challenging road, and cheer all the way to the final destination. Therefore, I find it important to enclose a chapter, in which these valued contributions and cheers are reflected in my acknowledgment.

I was fortunate to have conducted my doctoral work as part of the Helmholtz Space Life Sciences Research School (SpaceLife), within a collaboration of the German Sport University and the German Aerospace Center (DLR), Cologne. SpaceLife offered a comprehensive program addressing various aspects of scientific and personal development. Moreover, it provided a framework supporting the development of doctoral projects and facilitated exchange between students and senior scientists. It was complemented well by courses at the German Sport University, among them the Interdisciplinary Research Colloquium headed by Prof. Dr. med. Dr. Sportwiss. Christine Joisten, in which I gained valuable insights into research projects and their presentation. I would like to acknowledge the Helmholtz Association and the DLR for making SpaceLife possible, together with the efforts of the SpaceLife spokesperson, PD Dr. Christine Hellweg and coordinator Dr. Luis Spitta. I would also like to thank PD Dr. Hellweg for her kind support and responsiveness both during and after my SpaceLife scholarship days.

I greatly appreciate the valuable mentorship from Prof. Dr. Bloch and PD Dr. Zange throughout my doctoral work, whose suggestions and input have advanced my scientific knowledge and helped shape my thesis. Since our first encounter, they have impressed with their openness, scientific flair and approachability, which left no questions unanswered. They have provided precious guidance and support through my scholarship days and beyond up to the completion of my thesis, for which I would like to express my gratitude.

I thank Prof. Dr. Sebastian Gehlert for his scientific input and support particularly with regard to molecular analyses and Prof. Dr. Jörn Rittweger for his support during my time at the DLR. Thanks also to Prof. Dr. Rupert Gerzer for his valued endorsement, Dr. Pettra Rettberg for her guidance as well as to Dr. Krassimira Ivanova for her encouragement and constructive suggestions on scientific and practical aspects of my doctoral work. Dr. Klaus Müller I thank for his open, supportive attitude and hands-on contribution during the studies carried out within my doctoral work.

Valuable assistance related to my experimental work was provided by multiple staff at the former Space Physiology Department of the DLR and the Department of Molecular and Cellular Sport Medicine at the German Sport University, who are acknowledged in respective publications. To all the subjects, who participated in my studies and bravely endured the intense experimental setting, I am grateful for the trust they placed upon me and my teams to safely bring them through their sessions. They were all committed to completing the studies to the best of their abilities and cooperated well with our staff. Their interest in science and motivation at the chance of adding value through their effort are noteworthy.

I would like to acknowledge my teachers, who supported and nurtured my early academic and extracurricular interests during primary education in “Osnovna škola Meša Selimović” and secondary education in “Prva gimnazija”, in my hometown Zenica, Bosnia and Herzegovina. Dedication with

which they practiced their profession, as well as the interest and kindness with which they approached their students was commendable. I would also like to acknowledge my professors at the Faculty of Biological Sciences of the Goethe University in Frankfurt, whose classes and practical courses expanded my horizons and further reinforced my interest in research, and the Friedrich Ebert Foundation, which provided much appreciated support during my graduate studies.

I thank my friends and work colleagues for their interest in my doctorate, their friendship and for cheering me along the way.

To my family, I extend appreciation and thanks for all they have done and continue to do for me:

To my brother Denis for his faith in me, his support during my graduate studies and his encouragement and advice particularly during the initial years of my doctoral work.

To my grandmother Dobrila, who started working at a very young age, whose interest in her own craft endured up to her final days with us, and who in different ways encouraged and supported me.

To my mother Ljiljana, now retired from her long career in emergency medicine, one of the toughest and most demanding professions I know of, which she pursued with dedication and utmost care for her patients. It is the positive impact she made through her devoted approach to medicine and her patients and the familiarity I gained with medical topics through my conversations with her that had sparked my interest and inspired me to pursue my academic path. She was not only a dedicated doctor, but has been and continues to be a dedicated mother, who has enabled me to study abroad, encouraged me to pursue my doctorate and set an enduring positive example for me, all of which I am grateful for.

To my husband Vivek, my loving partner in all things, who has brightened my days and enriched my life ever since we first met. Pursuing my doctorate for an extended period of time outside of another, completely separate career has not been without its challenges and I was blessed to have him at my side, keeping me grounded and yet lifting me up, steadily cheering me on and holding the fort, when I was not available to help. His understanding, encouragement and support have been immensely valuable on my doctoral journey and continue to be in all that we share. He inspires and brings out the best in me every day, for which I am grateful.

To my extended family from Bosnia and Herzegovina and India, who have taken interest in my doctorate and have offered kind words of support and encouragement, which I appreciate.

Hvala (“thank you” in Bosnian)!